Foreword

Drug delivery is probably as aged an activity as is life on our planet. However, with all the discoveries and technology developments associated with treating illness which have occurred in our own lifetime, it is easy to overlook the roots of pharmacology and all the sciences we associate with it.

However, by learning from the experiences of our fore-fathers, and making use of all the available techniques, modern societies have pushed out the frontiers of medicine and related sciences to develop the array of available drugs and sophisticated medication technologies which so enhances our current lifestyle. Amongst those technologies is that of *controlled release*. The ability to control the administered dose of a drug over long time periods, and to direct the material to a preferred site of action in the body, has led to a variety of therapies which are more effective, use less drug, have fewer side-effects, increase patient compliance, ... and the list goes on!

But what, you might ask, has this to do with biological and pharmaceutical considerations of controlled release veterinary drug delivery, the topic of this book?

The answer is simple. Just as evolving societal structures have brought about improvements in drug delivery for humans, so our understanding of the 'symbiosis' between ourselves and our animals has grown, and the need to provide for their care and welfare has been recognized. Furthermore, rather than simply using animals as test or model subjects for the development of human medicines, we are now aware of the opportunities for applying some of these drugs and technologies directly to the animal for their own benefit.

When it came to the treatment of animals, primitive man probably did not worry about ethical issues (except perhaps for the occasional companion animal?), or disease transmission, or productivity. But as animal based agriculture evolved, any animal which was a potential food source or beast of burden became more valuable, and as such would be treated when ill. Over the years too, man has learned that animals can often (apparently) diagnose and treat themselves (e.g., cats eating grass, cattle eating bark of trees), and even that they have nutritional and health requirements not all that dissimilar to ourselves.

So it is not surprising then, that there is now an active community of scientists studying specifically the biological and pharmacological needs of many animal species, and attempting to match the advances in human medication with the requirements of other animals. Indeed, given the aforementioned dependency between man and many animal species, why is it that the effort and dollars spent on human drug delivery far outweighs that spent on veterinary issues? The answer is of course that we (usually) put far more value on human life than on that of any animal, despite the fact that those same animals are often central to our lifestyle (food), contribute signifi-

cantly to the ecological balance for the world we live in, or simply provide a companionship for which many of us yearn.

However, the preparation of this book is a real sign of the changes that are occurring. Michael J. Rathbone, Robert Gurney and their co-authors have compiled a wide-ranging overview of what has already been achieved in the application of controlled release technology to animals. The subject matter covered here-in is diverse, up-to-date and detailed. It deals with aspects of ruminant livestock through to companion animals, and with a variety of routes of administration. For those readers interested in commercialization of veterinary pharmaceutical products there are also chapters on testing procedures and regulatory aspects.

But more importantly, the reader is encouraged to read this book with an eye to the opportunities and challenges which exist for furthering this field. The perceived value of animals to human society is steadily increasing as we come to grips with the ecological sustainability of the world in which we live, and with the need for improved quality and quantity of food resources.

And as a final comment, let us not forget that we humans are also animals. Any pharmaceutical development in the veterinary field may also have application to human medicine. There has been a tendency for some 'human' pharmacologists, drug formulators and controlled release technologists to consider scientists in the veterinary field as their poorer cousins, or their science as not being quite at the cutting edge. It is to be hoped that this volume will help to rectify that misnomer, and that the 'animals' will rise to a position of equality allowing the biological and pharmaceutical considerations of the technology to be addressed for the benefit of all.

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Preface

Many controlled release veterinary drug delivery systems (CRVDDS) are presently in use, and in recent times we have seen the emergence of a host of new CRVDDS within veterinary medicine. The reason is that formulating a drug into a CRVDDS provides benefits to both the animal and the veterinarian/farmer/pet owner. Such benefits include the ability to integrate the administration of drug products around farm management practices, the reduction of pain and distress to the animal by decreasing the number of times the animal is handled and subjected to an administration procedure, financial benefits to the end user and improvements in drug therapy.

Although an onlooker may perceive that the challenges, and indeed the objectives, of controlled release veterinary drug delivery would be similar to those encountered in human medicine, they are in fact very different. This arises because the 'patients' contrast markedly in these two fields and the veterinary field more readily accepts novel approaches to the controlled delivery of drugs to animals. As a result, most controlled release drug delivery systems developed for animals are very different from those developed for their human counterparts.

The challenges of this area of drug delivery arise from the unique anatomy and physiology of the target animal, the cost constraints associated with the value of the animal being treated and the extended periods of time that delivery must be sustained for (often measured in months). To some these may be viewed as constraints, but to others, they are considered opportunities that allow the pharmaceutical scientist to develop innovative solutions to challenging delivery problems.

The purpose of this book is to introduce the reader to the unique opportunities and challenges of the field of CRVDDS and to explain and discuss the basic controlled release principles underlying the development of CRVDDS. Its aim is to provide an overview of many of the areas where CRVDDS have application, and to highlight the opportunities and prospects for controlled release technology in the veterinary field. It comprises chapters that provide workers in the field (and onlookers interested in this area) with information on the design, development and assessment of a variety of CRVDDS. The book contains chapters that describe the relevant animal physiological and anatomical considerations alongside descriptions of current and emerging controlled release delivery systems for a variety of routes for drug delivery. Other chapters are included that discuss the mechanisms of release from CRVDDS, describe the pharmacokinetic and biopharmaceutical principles of controlled release drug delivery, and present overviews on the physical and chemical assessment of veterinary controlled release delivery systems.

The first chapter of this book is written by Inskeep and Darrington and provides an overview of biopharmaceutical and pharmacokinetic principles that are essential in meeting the challenges facing innovative developers of controlled release veterinary

drug delivery systems. The chapter focuses on the two major veterinary markets, namely livestock and companion animals.

In the second chapter of this book Rathbone, Cardinal and Ogle describe the design principles behind various controlled release veterinary drug delivery systems, the mechanism of release, the physical model for drug release and the equations that describe drug release from such delivery systems. Overall, Chapter 2 demonstrates how an insight into the mechanism of release of drugs from controlled delivery systems assist in their design, development and optimization.

Cardinal, in Chapter 3, presents an overview of the anatomical and physical features of the ruminant stomach that make it such a unique and opportunistic site for controlled release drug delivery. The author describes several intraruminal drug delivery systems in depth providing the reader with an insight into the innovativeness of the workers in this field.

Chapter 4, written by Pell, Wu and Welch, outlines both the physiological and technical considerations encountered in the design of a post-ruminal delivery system. The authors describe and discuss how the ruminant digestive system differs from that of a single-stomached animal and how these differences affect the design of post-ruminal delivery systems. The formulation strategy for several rumen-stable systems are discussed as well as methods for in vitro and in vivo evaluation of post-ruminal delivery systems.

Baeyens et al. (Chapter 5) describe the principles behind the design and development of controlled release ocular veterinary drug delivery systems. This chapter provides some comparative anatomical and physiological features of the eye in the species most frequently encountered in veterinary practice alongside their essential ocular diseases and the most appropriate medications for such diseases. Currently available ocular drug delivery systems and recent developments in the field of drug delivery to the human eye together with their possible application to veterinary ophthalmology are also presented in this chapter.

Intravaginal veterinary drug delivery has a long history dating back to the early 1960s and today several commercially available intravaginal drug delivery systems are on the worlds market. Rathbone et al. critically review the literature in this area in order to document the available information relating to the design and development of intravaginal veterinary drug delivery systems. This chapter provides the basic information necessary for the design and assessment of intravaginal veterinary drug delivery systems.

The need for controlled release drug delivery systems in the field of estrous control of domesticated livestock arises due to the physicochemical and pharmacokinetic characteristics of the drugs that control the estrous cycle of such animals. In Chapter 7 Rathbone et al. describe the controlled release drug delivery systems which are currently available or in development for the control of the estrous cycle in sheep, cattle, pigs and horses. The chapter describes some of the formulation considerations for such products and highlights the recent advances which have occurred in this field of research.

In Chapter 8 Miller describes and discusses the area of ectoparacite control of livestock. In his chapter he points out that it is an area often ignored by even those

interested in veterinary applications of controlled release, which he surmises, is due to a perceived lack of market or profit potential. He points out however, that the variety of insects, ticks and mites that affect the well-being and productivity of livestock result in economic damage that far exceeds that expended for control. He indicates that there is a need to develop novel and improved delivery systems to (i) provide a means of minimizing the quantity of chemical needed for control of the pests, (ii) reduce the labor involved in herding and treating cattle at frequent intervals, and (iii) reducing the environmental hazards associated with chemical control. A clear opportunity for the innovative formulation scientist to exploit! In his chapter, Miller reviews the various CRVDDS that have been developed or are under development for the control of livestock ectoparasites focussing on a description of the products, their applications and their limitations.

Chapter 9, written by Witchey-Lakshmanan and Li, provides a perspective on CRVDDS for companion animals. The chapter highlights some of the reasons why controlled release drug delivery systems are developed for companion animals and describes the issues that are relevant to the design and development of controlled release products for various routes/clinical conditions in companion animals. Dogs and cats are the primary focus of the chapter, but other animals are included in their analysis of this area of research. The authors point out that the companion animal market is quite different from the food animal market and that the former is better able to support the development of a complex, expensive dosage form, in a fashion similar to human medicine.

Chapter 10 (Bowersock and Martin) discusses the delivery systems that assure sustained release of antigen and the induction of a protective immune response. The importance and current interest in this area of veterinary medicine arises due to the fact that infectious diseases cost livestock producers billions of dollars in losses annually, while prevention of infectious diseases has long been a primary source of veterinary visits for companion (dogs, cats, horses) animals. Delivery techniques and novel formulations have been identified as a means of overcoming the difficulties in delivering antigens to animals thereby offering many opportunities to the formulation scientist. The authors provide a comprehensive overview of this area and include detailed descriptions of the delivery systems currently being investigated alongside clinical results. Their chapter address advances in delivery systems that have the greatest potential for improving the efficacy of veterinary vaccines and addresses issues such as mode of delivery (sustained/pulsatile release of antigen), specificity of desired protective immune response, safety, and the desire to induce mucosal immunity.

CRVDDS are specifically designed to release their contents over an extended period at a predefined rate as a result of their formulation and the process conditions used during their manufacture. Rathbone et al. (Chapter 11) forward several reasons why it is desirable to develop an inexpensive, rapid, reproducible test that assesses the drug release characteristics of CRVDDS in vitro, which provides assurances to manufacturers and regulatory bodies of its batch-to-batch in vivo performance. Their chapter discusses some aspects of in vitro drug release test method development, optimization and validation for CRVDDS. Both compendial and non-compendial methods are

discussed, and suggestions to achieve sound, scientifically developed and validated methods are forwarded. The authors highlight the paucity of published literature on this area of veterinary research and point out that it is important to realize that each CRVDDS is unique and most likely requires considerations specific to that product that may or may not be covered by the ideas presented in their chapter.

In Chapter 12, Rathbone et al. discuss aspects of stability test programs for the assessment of a CRVDDS which falls within two specific stages of product development (pilot batch scale and final product full scale manufacture stage). The authors highlight that the design of such programs should be based on knowledge of the properties of the active ingredient, together with experience gained from stability studies conducted on product made during the development stage and taking into consideration any regulatory requirements. The requirements of a stability testing program can vary between products and regulatory authorities, however, the authors provide a useful list of documents, web site addresses and other sources of information in the public domain which provide a background on the principles and practices of conducting stability tests on CRVDDS.

In the final chapter of this book, Lindsey presents a brief overview of the US animal drug registration process. It is a highly legalistic process which is based upon the provisions of the US Food, Drug and Cosmetics Act (Act) which relegates enforcement responsibilities to the US Food and Drug Administration (FDA). Extensive and detailed regulations have been promulgated by the FDA pursuant to the authority of the Act. The key provisions of these regulations are described by Lyndsey in his chapter.

With the great strides being made to harmonize registration processes in the US, EU and Japan, it was a great disappointment to us not to be able to present an EU perspective on the registration of CRVDDS in our book; we were simply unable to secure an author to write a chapter on this topic. Any reader interested or involved in this area of veterinary drug delivery is advised to keep a watchful eye on the following websites to keep abreast of the developments in this area: Federation of Pharmaceutical Manufacturers Associations http://www.ifpma.org; Centre for Veterinary Medicine http://www.cvm.fda.gov/cvm. We were also unable to include a chapter on protein and peptide delivery to farmed animals. Any reader interested in this topic should be directed toward some very authoritative and recent texts by Cady[1], Fergusson[2] and Foster[3].

The veterinary area is abound with opportunities for the development of controlled release drug delivery technologies. It is an area of medicine that is open to the acceptance of novel drug delivery devices, and which readily encompasses the use of novel routes of administration. It is an area of many unmet needs, most of which offer opportunities and unique challenges for the innovative formulation scientist to provide solutions. We hope that this book will provide an insight into the biological, clinical and pharmaceutical challenges that face the formulation scientist in this interesting and diverse area of research.

We would like to express our sincere thanks to all the contributors for their efforts and for providing authoritative chapters that were based upon their extensive experi-

ences in their particular areas of expertise. We would also like to thank our reviewers for their timely and speedy reviewing of chapters.

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CHAPTER 1

Utilization of biopharmaceutical and pharmacokinetic principles in the development of veterinary controlled release drug delivery systems

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Overview

I. Introduction

The patient populations in veterinary medicine cover a broad range of species, body sizes, and drug absorption barriers, and therapies generally require long-acting agents that are administered infrequently. Consequently, the utilization of biopharmaceutical

and pharmacokinetic principles in the development of veterinary controlled release drug delivery systems is essential for a rationally designed product. Throughout the last 20 years, a wide variety of biopharmaceutical approaches have become available and, when applied with consideration of basic pharmacokinetic principles, allow for the development of veterinary medicines with greater efficacy and ease of administration.

The development of veterinary controlled release drug delivery systems requires the collaboration of several departments of an innovative pharmaceutical company. Once the appropriate product profile (e.g. patient population, disease indication, spectrum of activity, dosing route and frequency) has been established research biologists, chemists, biopharmaceutical and pharmacokinetic scientists interact to take advantage of the inherent dispositional properties of lead chemical candidates, and optimize delivery by formulation in an appropriate controlled release system.

This chapter will provide an overview of biopharmaceutical and pharmacokinetic principles that are essential in meeting the challenges facing innovative producers of veterinary controlled release products. In this chapter we focus on two major veterinary markets: (1) livestock, including cattle, swine, sheep, and poultry; and (2) companion animals, including cats, dogs and horses. The same general principles which are applied to pharmaceuticals for these major markets can be applied to pharmaceuticals for other markets, such as aquaculture, however, it should be noted that additional requirements or restrictions may need to be considered. We also have restricted this discussion to pharmaceutical agents and have excluded discussing biological agents such as vaccines.

II. Biopharmaceutics

Biopharmaceutics, first introduced by Levy and Wagner,[1] can be considered a study of the relationship between physical and chemical properties of a drug molecule and its formulation, and the pharmacokinetic and pharmacodynamic effects observed following administration of the drug in these formulations. When absorption is not rate limiting, the intrinsic physical and chemical properties of a drug molecule and the interplay of these properties with the drug delivery system, formulation, and physiological fluids, can convey significant control over the rate and duration of drug release. Hence, in the realm of veterinary controlled release drug delivery, consideration of the biopharmaceutical issues related to a drug and its delivery system can provide significant guidance towards the successful development of an effective and cost efficient formulation.

Prior to determination of acceptable routes for controlled delivery, numerous variables must first be considered such as target species(s), desired duration of release, release profile, cost and dose. Figure 1 illustrates the significance of required duration of release and species on limiting the number of feasible routes.[2] Once a list of acceptable potential routes has been identified, additional biopharmaceutical and pharmacokinetic information must be gathered in order to assess the feasibility of the desired route(s) as outlined in Table 1. While obtaining all of this information in

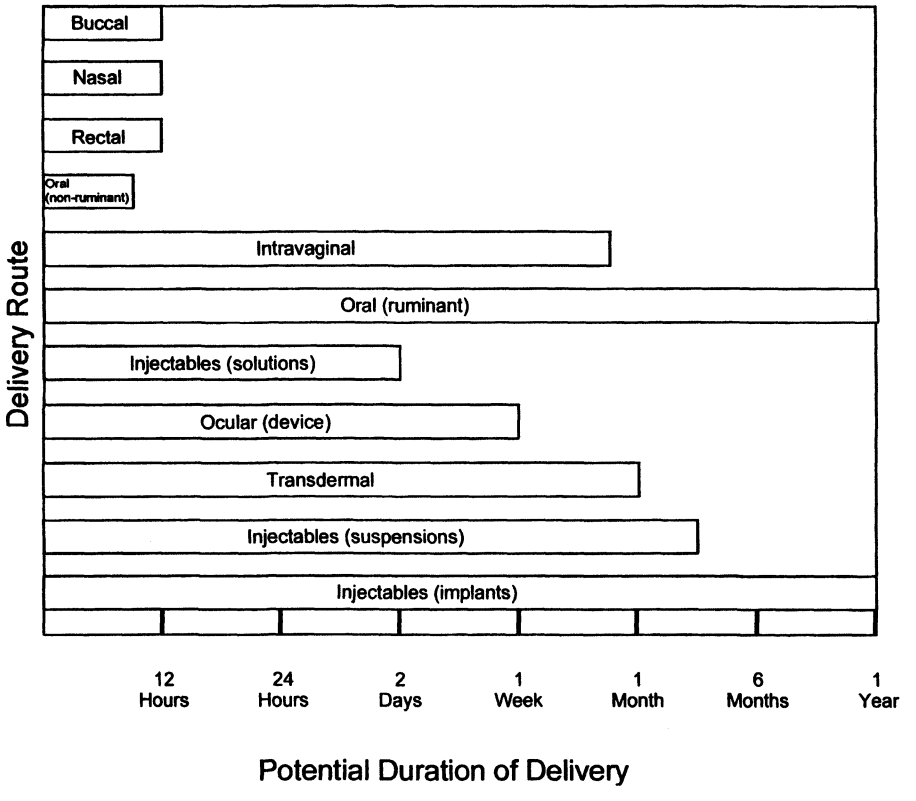

Fig. 1. Potential delivery duration as a function of administration route (adapted from Ref. 2).

Table 1
Necessary biopharmaceutical, physicochemical, and pharmacokinetic information for a drug molecule and target species in the assessment of appropriate delivery routes and controlled release delivery systems

Therapeutic window/desired profile
Physicochemical properties
pK_a(s)
pH solubility profile
pH stability profile
Solubility in non-aqueous vehicles
Stability in non-aqueous vehicles
Partition coefficient
Molecular weight
Body size
Absorption
Distribution
Metabolism
Excretion

the time frame normally allotted for the development of veterinary systems is often difficult, an early identification of the feasible routes of delivery can allow for focused efforts in obtaining the information necessary for a certain delivery route.

III. Pharmacokinetics

A detailed description of the basic principles of pharmacokinetics is beyond the scope of this chapter, and many excellent volumes on this topic are available.[3] Pharmacokinetics involve the assessment of absorption, distribution, metabolism, and excretion (ADME) of drugs by the animal. In general, these characteristics can be affected to varying degrees by manipulating the formulation of the drug, and often are critical in determining if controlled release is an option for a specific chemical entity. Because some of the ADME characteristics of a drug may be species specific, the pharmacokinetics of a drug intended for multiple species should be characterized in each target species before embarking upon a full development program.

When controlled release formulations are being developed, they are usually tested in the target species or a validated model species. The rate and extent of drug delivery to the systemic circulation are usually determined by measurement of drug in plasma at appropriate intervals after dosing. While this is a valid approach for examining the absorption of drug via the tested formulations, it does not confirm that the drug is distributed to the target tissue. It is theoretically possible for a controlled release formulation to have a much greater plasma AUC than a non-formulated form of the drug, but achieve much lower tissue concentrations. For this reason, it is prudent to examine the pharmacokinetic/pharmacodynamic relationship of the drug early in its development. If a quantitative relationship between plasma drug levels and tissue penetration can be established in prototype formulations, there will be a greater level of confidence that a controlled release approach will result in a formulation with the desired pharmacokinetic and pharmacological profile.

IV. Application of biopharmaceutic and pharmacokinetic principles in formulation development

The following discussion will attempt to provide the drug delivery scientist with 'biopharmaceutical points to consider' when developing a controlled release dosage form for veterinary applications.

IV.A. Therapeutic window/desired profile and dosing regimen

Veterinary controlled release systems (VCRS) allow for tight control of plasma levels and provide for prolonged delivery of drugs. Therefore they are ideally suited for long-term treatment when multiple dosing would be inconvenient and/or for drugs with narrow therapeutic windows. Caution is warranted however. Both oral and implantable VCRS run the risk of dose dumping which could result in potential toxicity. With oral VCRS, inter-subject, inter-species and fed/fasted variability may be exaggerated

relative to conventional delivery. Fed/fasted variability in the context of oral VCRS is often the result of changes in gastrointestinal pH. Basic drugs, which are ionized at low pH and not expected to be absorbed in the stomach, may be absorbed in the stomach to a greater extent after feeding. Acidic drugs, which are unionized and absorbed in the stomach under normal conditions may become ionized after feeding resulting in greatly reduced absorption. However, species differences may be observed. For example, gastric pH is much lower in monogastric animals than in ruminants.

The duration of oral controlled release systems is often dictated by the intended species. In general, oral VCRS are not useful in cats, dogs, and pigs with gastrointestinal transit times of approximately 3–8 h,[4,5] but are feasible for cattle, sheep, and horses with transit times approaching 3 days.[6–9] In addition, effective oral VCRS dosing in ruminants can be achieved using bolus devices that remain trapped in the rumen for several months duration.[10] However, oral administration of antimicrobial agents to ruminants may have unacceptable gastrointestinal effects resulting from alterations in the composition of gut microflora.

While parenteral (intramuscular and subcutaneous) routes provide minimal species to species variability and have the potential for months to a year of release, issues such as immunogenicity, local irritation as well as injection site drug residues must be considered. Transdermal delivery may be able to provide extended release without these complicating factors, although weather fastness and herd cleaning behavior may become issues. Revolution® and Frontline®, and Dectomax® pour-on are examples of well-tolerated, long-acting transdermal dosage forms for companion animals and livestock, respectively.

While the objective for controlled release formulations is to maintain pharmacological activity for long periods after dosing, a complicating factor for livestock products may be edible tissue residues of drug, formulation components and metabolites. Obtaining a practical balance between extended pharmacological activity and an acceptable withdrawal time for the product can be a formidable challenge in veterinary drug development.

IV.B. Physicochemical properties

The physicochemical properties of the target molecule can significantly impact the rate of release and in vivo absorption. Table 2 illustrates the impact of physicochemical variables on drug solubility and dissolution rate. The physicochemical properties listed below will play a role in the release and absorption characteristics, and can provide guidance on determining optimal formulation variables. Should the properties of the drug under development be unsuitable for the desired biopharmaceutical characteristics, prodrug approaches may be warranted.[11–14] While not a physicochemical property, it should be noted that particle size modification can be used to provide additional control over dissolution rates.[15,16]

IV.B.1. pK_a

Permeability in the gastrointestinal tract can be highly dependent on site, in part due to

Table 2
Impact of physicochemical variables on drug solubility and dissolution rate

Variable	Solubility/ dissolution range[a]	Advantages	Limitations	References
pK_a[b]	1000	Predictive solubility/dissolution behavior	Basically a prodrug approach Requires molecule redesign.	43
Salt	10 000	Large range of dissolution possible without changing molecular structure Some correlation between acid/base properties and properties of salt	Salts may be difficult to make.	44–49
Prodrug	10 000	Large range of dissolution rates possible Possible to customize properties	Requires molecule redesign Customization of properties not predictive Must consider rate of solid state decomposition of prodrug in vivo	11–14
Crystal energy heterogeneity/ coprecipitates	10 000	Large range of dissolution rates possible Good control over dissolution rate by careful control of energy or precipitate properties	Metastable system. Could convert to homogenous energy state over time.	50–55
Polymorph/ hydrates/solvates	10 000	Large range of dissolution rates possible	May be metastable. Polymorphs can convert, hydrates can become anhydrous or change hydration number, solvates can become anhydrous	56–60
Complexation	500[c]	Stable, no change in properties of drug molecule	Low range of dissolution rate. Limited choices of ligands minimizes customizability	43,61–65
Particle size	100[d]	Easy to control Predictive relationship Can combine with above variables for even greater control	Metastable. Particle size can increase over time (Oswald ripening) Small dissolution rate range	15,16

[a] Intrinsic dissolution rate can be correlated with solubility via the Noyes–Whitney equation.
[b] Based on the relationship $S_{acid} = S_o(1 + K_a/[H^+])$ or $S_{base} = S_o(1 + [H^+]/K_a)$ and assuming a physiological pH of 7.0 and a pK_a range of 4.0–10.0
[c] The relationship between solubility and dissolution rate is somewhat more complex than with a single system due to multiple diffusion coefficients[43].
[d] Not truly an equilibrium solubility value but will significantly impact dissolution rate[15,16,66].

the change in ionization state of the drug with changing pH. An understanding of the ionization behavior of the selected drug and of the changes in pH as a function of location along the gastrointestinal tract, in fed and fasted states, is critical to identifying the suitability of a drug for oral VCRS. Permeability is not just a matter of ionization state however. Numerous researchers have suggested that there is an interplay between solubility and permeability on the successful delivery of a drug via oral VCRS.[17] As a drug becomes ionized its solubility increases but its permeability decreases. Depending on which effect is dominant, the drug may or may not be orally bioavailable.

Changes in ionization state of drugs administered intravenously can result in injection site precipitation leading to local irritation, and in the case of highly insoluble or slowly dissolving precipitates, drug particles can be lodged in the capillary system resulting in organ failure and death. Subcutaneously, changes in ionization state resulting in precipitation at the injection site could conceivably be used to slow the delivery of a formulation solubilized drug into the systemic circulation or lymphatic system. Caution is warranted with this approach however, as variations in precipitate particle size may significantly impact the release profile and the potential for injection site irritation is significant.[15] As lymphatic versus systemic uptake of subcutaneously administered drugs is governed, in part, by the lipophilicity of the molecule, the ionization state of the molecule at physiological pH may impact the mechanism of uptake and hence release into the systemic circulation.[18–20]

IV.B.2. pH solubility profile

The ionization state of a molecule can play a significant role in the permeability of a drug via a solubility and permeability effect. The role of pH on solubility can readily be experimentally defined by a pH-solubility profile determination. While it is reasonable to assume that the solubility will increase as the molecule becomes more highly charged ($pH > pK_a$ for an acid and $pH < pK_a$ for a base), the impact on absorption cannot be fully defined without an understanding of the solubility of the unionized form as well as the solubility of salts of common physiological counter ions such as sodium (salt of an acid) and chloride (salt of a base). Once the solubility of a drug form is known, dissolution rate can be estimated and controlled via particle size or other methods such as including a diffusion step in the release system.[21] An understanding of the interplay between solubility, ionization state and absorption will allow the formulations scientist to evaluate the applicability of an oral or parenteral VCRS system.

IV.B.3. pH stability profile

Drugs in VCRS systems must be stable at physiological conditions for an extended period of time. While the shelf-life stability of aqueous based formulations can be readily optimized via pH control and/or lyophilization, the formulation scientist must be aware of the drug stability after administration. An early definition of the delivery route, species and desired duration will provide guidance as to the extent of the pH-stability assessment required.

IV.B.4. Solubility and stability in non-aqueous vehicles

Several controlled release technologies require processing or administration in non-aqueous vehicles. A criterion for the selection of an appropriate VCRS delivery system, therefore, requires an assessment of the solubility and stability of the molecule during manufacture and/or storage of these non-aqueous systems. The formulation scientist should be aware of the processing variables and the impact of these variables on the solubility and stability of the drug under development.

IV.B.5. Partition coefficient

The partition coefficient (P) is a measure of the drug's affinity for hydrophilic versus hydrophobic media and can be used to gain an understanding of the absorption behavior and distribution of the drug.[22] A log$P$ of approximately 2 has been suggested as optimal lipophilicity for transport across the mucosa of the stomach and a range for log$P$ of 2.5–3.3 for transport across the small intestine.[23] With ionizable compounds, the apparent partition coefficient, as defined by the concentration of drug in the aqueous phase relative to the concentration of drug in the organic phase at equilibrium will be a function of the pH of the aqueous phase. Thus partitioning of the molecule will be dependent on this pH-partitioning relationship. Brodie and Hogben first developed a pH-partition hypothesis which reasoned that only the unionized form of the drug passes through the biological barrier.[24] However, Ho and co-workers later showed that the pH-partition hypothesis is only approximate as small ionized drugs may be absorbed via aqueous pores.[25]

The partition coefficient can also provide guidance for selection of optimal vehicles used for the delivery system. For percutaneous absorption, proper selection of the formulation vehicle is crucial to the bioavailability as the rate of drug transport is proportional to the relative affinity of the drug between the skin and the vehicle, i.e. skin–vehicle partition coefficient.

IV.B.6. Molecular weight

Transport across biological membranes, particularly the stratum corneum is greatly reduced with increasing molecular weight. High molecular weight compounds are therefore not well suited for transdermal delivery.[26]

IV.C. Body size

An important consideration in developing a controlled release formulation is the body size or weight of the target species (Tables 3 and 4). The key consideration for body size of the animal is the appropriateness of the size and form of the dose for the animal. For example, extended release subcutaneous implants that are appropriate for livestock, may be too large for use in small companion animals. Another consideration for size of the target species is the appropriate measure of animal size for dosimetry, so that the appropriate dose is administered to animals of varying size. For example, drugs intended for livestock as well as companion animals must be able to deliver the efficacious dose systemically to animals that may range in total body weight from 1 to over 600 kg. Even within a species, for example the dog, body weight can range over

Table 3
Body weights of livestock species (values are means with *N* indicated in parentheses; table adapted from Ref. 67)

Species	Age	Body weight (kg)	
		Male	Female
Cattle, Holstein	Birth	45.5 (220)	40.0 (262)
	3 months	99.1 (145)	97.7 (256)
	6 months	190.5 (104)	181.4 (247)
	9 months	287.3 (65)	258.2 (244)
	12 months	370.5 (25)	320.0 (242)
	18 months	526.8 (4)	420.0 (233)
	2 years	640.9 (2)	522.3 (215)
Cattle, Ayrshire	Birth	36.8 (58)	35.9 (100)
	3 months	74.5 (23)	84.1 (81)
	6 months	140.9 (12)	155.9 (91)
	9 months	205.9 (4)	222.7 (75)
	12 months	289.1 (3)	266.4 (86)
Sheep, Hampshire[a]	Birth	4.4, 5.4	4.1, 5.1
	3 months	28.2, 29.5	26.0, 28.2
	6 months	40.6, 43.8	38.2, 40.4
	12 months	66.0, 66.8	53.6, 54.0
Swine, Duroc[b]	Birth	1.46 (1204)	1.46 (1204)
	21 days	5.29 (769)	5.29 (769)
	56 days	16.54 (741)	16.54 (741)
	154 days	85.63 (604)	85.63 (604)
Chicken, White Leghorn[c]	Hatched	0.036	0.036
	1 week	0.059	0.070
	3 weeks	0.191	0.190
	6 weeks	0.449	0.450
	12 weeks	1.243	0.960
	22 weeks	n/a	1.500
Turkey, Broad-Breasted[c]	1 week	0.123	0.123
	3 weeks	0.431	0.363
	6 weeks	1.135	0.908
	12 weeks	3.723	3.178
	24 weeks	11.441	7.854

[a] The first figure in the weight columns for sheep represents values for twin-birth animals, the second represents values for single-birth animals. *N* is not available.
[b] Values represent combined means of males (castrated and uncastrated) and females.
[c] *N* is not available.

two orders of magnitude. Because of these differences in size, the efficacious dose expressed as mg/kg for a large species may be ineffective or toxic for a smaller species. Although many recommendations have been made to base dosimetry on surface area rather than on body weight, body weight is much simpler to determine and continues to be the most commonly used measure of size.

IV.D. Absorption

The absorption of drugs is the one component of ADME that is most readily modified by formulation efforts. Many routes of administration of drugs for animals are available. Drugs can be administered in food or water, by tablet or capsule, by subcuta-

Table 4
Body weights of companion species (values for cats and dogs represent ranges; values for horses represent means with *N* indicated in parentheses; table adapted from Ref. 68)

Species	Age	Body weight (kg)	
		Male	Female
Cat, domestic ($N = 6$)	Birth	0.083–0.107	0.097–0.120
	1 week	0.083–0.196	0.097–0.212
	2 weeks	0.146–0.282	0.162–0.296
	4 weeks	0.266–0.487	0.330–0.475
	8 weeks	0.559–0.820	0.645–0.760
	12 weeks	1.200–1.347	0.902–1.216
	Adult	1.410–4.234	1.415–3.476
Dog, Beagle ($N = 39M, 31F$)	Birth	0.17–0.45	0.21–0.39
	1 week	0.37–0.73	0.32–0.72
	2 weeks	0.35–1.35	0.43–1.11
	4 weeks	0.52–2.08	0.77–1.75
	8 weeks	1.62–4.28	1.67–3.59
	12 weeks	2.60–7.00	2.87–5.81
	Adult	3.54–9.50	3.64–7.86
Dog, Cocker Spaniel	Birth	0.17–0.31	0.15–0.33
($N = 31M, 36–37F$)	1 week	0.27–0.61	0.24–0.58
	2 weeks	0.40–0.84	0.41–0.85
	4 weeks	0.64–1.44	0.66–1.44
	8 weeks	1.90–3.76	1.86–3.26
	12 weeks	3.55–6.21	3.33–5.21
	Adult	5.02–8.62	4.39–7.15
Dog, German Shepherd	Birth	0.34–0.68	n/a
($N = 20–22M, 15F$)	1 week	0.57–1.02	0.34–0.64
	2 weeks	1.14–1.70	0.57–1.02
	4 weeks	2.39–3.52	1.48–2.27
	8 weeks	7.0–16.0	8.0–18.0
	12 weeks	10.5–29.5	15.5–28.0
	16 weeks	13.5–43.0	16.5–38.0
Dog, Shetland Sheepdog	Birth	0.14–0.28	0.11–0.29
($N = 15M, 14F$)	1 week	0.23–0.55	0.16–0.56
	2 weeks	0.32–0.84	0.24–0.86
	4 weeks	0.42–1.66	0.42–1.52
	8 weeks	0.95–4.89	0.72–4.16
	12 weeks	1.56–8.36	0.98–7.10
	16 weeks	1.92–12.00	1.48–9.86
Dog, Wirehaired Fox Terrier	Birth	0.10–0.28	0.14–0.24
($N = 21M, 23F$)	1 week	0.22–0.52	0.22–0.54
	2 weeks	0.35–0.79	0.35–0.77
	4 weeks	0.58–1.44	0.63–1.29
	8 weeks	1.49–3.01	1.24–2.96
	12 weeks	2.26–5.20	2.22–4.62
	16 weeks	3.50–6.78	3.23–5.95
Horse, thoroughbred	3.1 days	52.45 (18)	n/a
	5.6 days	n/a	54.32 (19)
	33.5 days	93.89 (3)	n/a
	83 days	n/a	116.7 (4)
	Yearling	306.35 (5)	354.0 (1)
	2–3 years	433.92 (3)	408.50 (7)

neous or intramuscular injection, by intravascular injection, implantation, or by dermal, sublingual, vaginal, aural, or ocular application. Controlled release formulations may be applicable to most of these routes, but the maximal duration of delivery is dependent on the chosen route of administration (Figure 1).

For oral delivery of drugs, absorption must not be the rate limiting factor if a VCRS formulation is to be controllable. Drugs which are only poorly absorbed or exhibit site specific adsorption in the gastrointestinal tract will not be good candidates for oral VCRS systems.

Development of controlled release formulations, per se, for drugs administered in food or water is rare. Nevertheless, this route of administration can be considered controlled release because the drug input is relatively constant throughout the day, depending on the animals' eating or feeding habits. This approach is often taken with poultry because of the difficulties in administering drug through other routes. A key factor affecting the absorption of such drugs is the consumption rate of food or water. A relatively simple approach to calculate food and water consumption rates for any given species is possible using allometry. Food and water consumption have been correlated with body weight for a large number of animal species.[27] In general, a single equation for water consumption (and a separate equation for food consumption) provides an adequate estimation for all major veterinary species (Table 5). However, separate equations are presented for poultry because an adequate data base exists for poultry and this is the species for which dosing in food or water is most often used.

While often overlooked, absorption into the lymphatic system followed by slow release into systemic circulation may also result in a loss of dosage form control over the availability of a drug from a VCRS. However, for compounds which are rapidly taken up into the circulatory system after intramuscular or subcutaneous administration, a prodrug approach to change the mechanism of uptake to the lymphatic system followed by chemical breakdown of the prodrug and subsequent release into the circulation may provide the necessary release profile.[18]

IV.E. Distribution

Generally, distribution of drugs from the systemic circulation to target tissues is independent of drug characteristics that can be altered through biopharmaceutical approaches. Tissue distribution is governed by the physicochemical characteristics of the drug and barriers at biological membranes the drug must cross to reach target tissues. However, if transport of the drug into target tissues is dependent on the

Table 5
Allometric relationships for food and water consumption (adapted from Ref. 27)

Animal group	Allometric equation (r^2)	
	FC (kg/day)[a]	WC (l/day)
Chickens	$0.075\ BW^{0.8449}$ (0.97)	$0.13\ BW^{0.7555}$ (0.74)
All other species	$0.065\ BW^{0.7919}$ (0.95)	$0.11\ BW^{0.7872}$ (0.93)

[a] FC, food consumption; BW, body weight; WC, water consumption.

magnitude of the plasma/tissue drug concentration gradient, VCRS control of systemic drug concentrations may optimize penetration into tissue. As previously mentioned, rapid uptake by the tissue or lymphatic system followed by slower conversion and/or release into the circulatory system may provide formulation scientists with an additional means by which to achieve parenteral VCRS.[18–20] It should also be noted that disease states may affect the tissue distribution of drugs.[28]

IV.F. Metabolism

A detailed discussion of drug metabolism pathways is beyond the scope of this chapter, and several comprehensive reviews are available.[29,30] Drug metabolism occurs in virtually all tissues throughout the body, but the major drug metabolizing organs are the liver, gastrointestinal tract, kidney, and skin. Drug metabolism is generally described as occurring in two phases. In phase I pathways, oxidative or reductive processes are catalyzed by enzymes in the cytosol or endoplasmic reticulum that usually result in increased polarity or water solubility of the drug. In phase II pathways, conjugation of polar functional groups (either inherent to the drug or introduced via phase I metabolism) occurs aiding in the renal or biliary elimination of the drug. An important concept of metabolism is the first pass effect. When orally administered drugs are absorbed through the portal blood system, they must clear the high levels of drug metabolizing enzymes localized in the gastrointestinal tract and the liver. These drugs are characterized by much lower systemic plasma concentrations of drug after oral administration than after intravenous administration. Drugs exhibiting a major first pass effect can be candidates for controlled release parenteral formulations.

Drugs that are rapidly metabolized by the target species may only be suitable for controlled release formulations when very low systemic drug concentrations are efficacious. However, if persistence of edible tissue residues is a major issue for the drug, this may provide an opportunity to achieve extended activity with an acceptable withdrawal period, especially if the dosing formulation is one that can be removed from the animal (e.g. subcutaneous implant).

Drugs that undergo saturable metabolism in the gastrointestinal tract may not be suitable for oral VCRS delivery if the lower concentrations of drug resulting from controlled delivery fall below the level of saturation of the drug metabolizing enzymes. Recent advances in methods to block the P450 enzymes however, may provide a remedy to this limitation.[31–33] Gastrointestinal metabolism in veterinary medicine can be especially challenging, because gut microfloral metabolism may be significant among target species.[34]

Drug metabolism can be a complicating factor when a drug is intended for multiple species. While multiple pathways exist for the metabolism of drugs, the pathways are not equally distributed in all species. While acceptable pharmacokinetics with a given formulation may be observed for the primary species of interest, pharmacokinetics of the same formulation in a secondary species, because of species-specific metabolism, may be unacceptable. For oxidative pathways (phase I) of drug metabolism, there are few interspecies differences in the ability to carry out various oxidations. However, the rates of particular oxidative pathways may be dramatically different among species.[29]

Species differences in conjugation reactions (phase II) are also well documented. For example, dogs are deficient in *N*-acetylation reactions,[35] cats lack some glucuronidation reactions,[36] and swine appear to be deficient in some sulfate conjugation pathways, although this may reflect a preponderance of glucuronidation rather than a deficiency of specific sulfotransferases.[37]

Even within a single species, age-related differences in metabolism may complicate the drug development process. For example, very young animals often have attenuated expression of major drug metabolizing enzymes resulting in much longer residence times than seen in adults. For example, major changes in oxidative metabolic pathways are observed in cattle during early stages of development.[38–41] As animals age some of the enzymatic pathways become less efficient, also resulting in decreased clearance of drug. In addition, disease states can affect hepatic function.[42]

IV.G Excretion

Excretion of drugs is also a species-specific process that usually is not impacted by biopharmaceutical approaches. Ultimately, drugs are excreted in the urine or bile as either the parent molecule or as metabolite(s). Other minor excretory processes occur (e.g. salivation, perspiration), but usually have no impact on drug development. Urinary and biliary elimination are complex processes involving passive and active transport. When the predominant excretory pathway for a drug is dependent on systemic plasma concentrations, a controlled release formulation may affect the rate of excretion by maintaining lower plasma concentrations for a longer duration.

Excretion is sometimes affected by disease states.[28] Often, kidney function is affected resulting in less urinary elimination and longer residence time of the drug. If a drug is being developed for animals likely to suffer from such a disease (e.g. geriatric companion animals), the affect of the disease on potential drug candidates should be examined before embarking on an involved controlled release research project.

V. Summary

Development of controlled release pharmaceuticals for veterinary use is a complex process involving scientists spanning several disciplines. These scientists attempt to reach a balance among the pharmacological profile of the chemical entity, its immutable physicochemical characteristics, the delivery characteristics of the molecule which can be modified through biopharmaceutical approaches, and the biological (ADME) variability among various target species. A single strategy for developing extended release formulations is clearly not available. However, careful consideration and continuous review of the above principles of biopharmaceutics and pharmacokinetics as more information becomes available during a development program will provide a basis for advancement of promising agents or early discontinuation of agents that cannot achieve a suitable profile.

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CHAPTER 2

Mechanisms of drug release from veterinary drug delivery systems

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Overview

I. Introduction

In the veterinary industry, the incorporation of drugs into polymeric matrices in order to control drug release at a predefined and reproducible rate for prolonged periods, provides significant benefits over veterinary dosage forms in which the drug is immediately available for absorption. Such benefits include (1) the ability to tailor or integrate the administration of health and production oriented pharmaceuticals around the constraints of farm management systems, (2) reduction of pain and distress to the animal by decreasing the number of times the animal is handled and subjected to an administration procedure, (3) financial benefits to the end user resulting from reduced veterinarian cost, optimization of employees time by reducing time needed for the task of herding and administration, (4) increased cost/benefits to the end user and (5) improved therapeutic outcomes.

Many controlled release veterinary drug delivery systems are described in the literature. Their design dictates the manner in which the drug is released, and several mechanisms of release have been mathematically described to define how that release occurs. In this chapter an overview of controlled drug delivery system designs which exist in the veterinary literature and details of the mathematical models for the description of drug release from such systems are reviewed. The aim of the chapter is to describe models that aid in the understanding of drug release from veterinary drug delivery systems and to highlight how knowledge of the mechanism of release can be used to optimize such delivery systems. Details of the derivation of equations can usually be found in the associated referenced publications. In this chapter 'working equations' are presented which elucidate the mechanisms of drug release and permit comparison of the release data to mathematical models.

This chapter briefly describes the design principles of various veterinary drug delivery systems. In addition it describes the mechanism of release, defines the physical model for drug release, and details the kinetics of drug release from such delivery systems. Overall, the chapter demonstrates how an insight into the mechanism of release of drugs from controlled delivery systems will aid their design, development and optimization.

II. Mechanisms of drug release and plasma profiles

The aim of formulating a veterinary drug into a controlled release dosage form is to produce a product which releases drug at a predetermined rate and duration and that fulfills the therapeutic goals of the veterinarian. Definition of the clinical outcome (and preferably the plasma profiles required to achieve that therapeutic goal) should be stated at the beginning of the development process as this dictates the pattern of drug release and subsequent plasma profiles. An example of the effect of release mechanism on plasma profile was demonstrated by Chien[1,2] who compared the in vivo release profiles and plasma profiles of two norgestomet-containing ear implants (Hydron and the Microsealed Drug Delivery Technology implants) (Figure 1). The ear implant manufactured from Hydron exhibited a declining in vivo drug release profile with

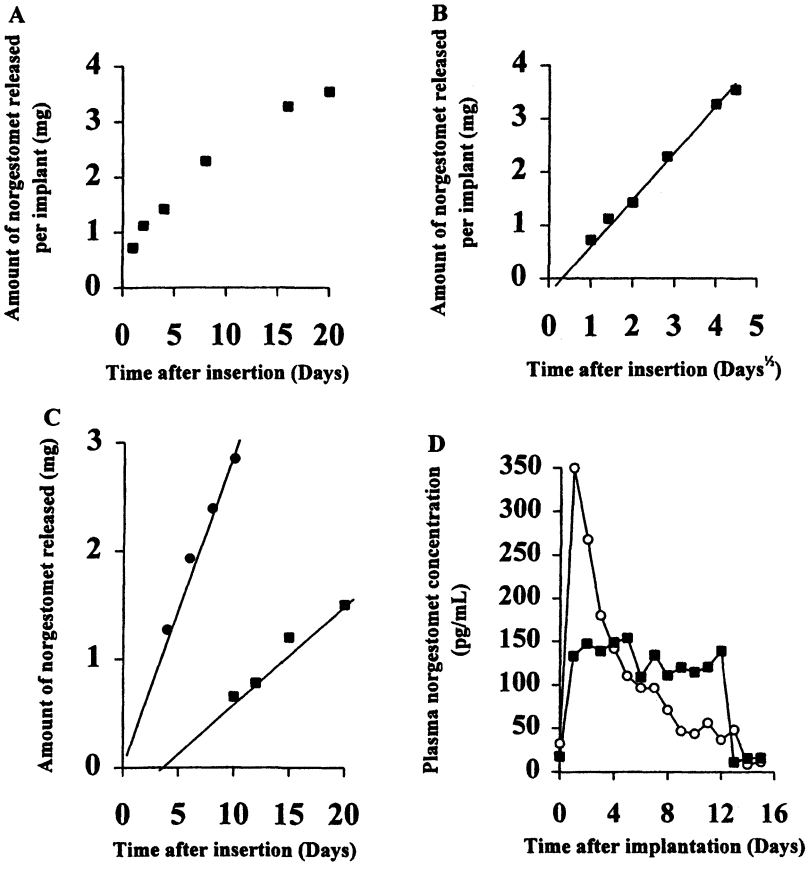

Fig. 1. Example of the effect of in vivo release profile on plasma levels. (A) Non linear dependence of the in vivo release of norgestomet from a Hydron implant (SYNCRO-MATE-B®) upon time showing dependence of release upon the square-root-of-time (B).[1,2] (C) Linear (zero-order) in vivo release of norgestomet as a function of time (days) for two differently formulated Microsealed Drug Delivery® ear implants[1,2] where (●) release of norgestomet following insertion into the ear of 16 heifers from MDD 445 implants for up to 10 days (■) release of norgestomet following insertion into the ear of 20 cattle from MDD 434 implants for up to 20 days. (D) Plasma profiles of norgestomet in ewes treated with Hydron implants (○) or MDD 445A (■) for 12 days in ewes.[1,2]

time (Figure 1A) (square-root-of-time dependence; Figure 1B).[1] In contrast the ear implant manufactured using the Microsealed Drug Delivery Technology exhibited a zero-order in vivo release profile (constant drug release with time) which occurred independent of initial load (Figure 1C). The different release profiles from these two ear implants resulted in quite different plasma profiles (Figure 1D). The Hydron ear implant was observed to produce very high plasma norgestomet concentrations over the first few days that steadily declined over the remaining days of implantation (open circles; Figure 1D). In contrast, the ear implant manufactured using the Microsealed Drug Delivery Technology produced plasma concentrations which rapidly reached steady-state concentrations that were maintained over the entire insertion period

(closed squares; Figure 1D). Such results demonstrate the benefits that can accrue from the formulation of veterinary drugs into controlled release delivery systems that exhibit zero-order release profiles. Potential benefits for the manufacturer and/or veterinarian include; optimum drug utilization, which includes the avoidance of either declining plasma profiles that fall below minimum effective concentrations or elevated plasma levels that exceed minimum toxic levels. The implication is that formulation scientists should strive to produce delivery systems that provide zero-order drug release. However, this goal should be considered in conjunction with the therapeutic aims and window. Such attributes of a drug delivery system would be very desirable when formulating veterinary drugs that have narrow therapeutic ranges. However, given that many veterinary pharmaceuticals possess a reasonably wide therapeutic index and that wide differences (20–40%) in plasma drug levels may not show a difference in biological response, the absolute need for zero-order release is questionable from an efficacy viewpoint. Furthermore, the variation encountered when experimentally determining a plasma profile (due to assay sensitivity, animal variation, etc.) may be such that differences between drug release mechanisms of controlled release drug delivery systems cannot be differentiated on the basis of their observed plasma levels.

To the formulation scientist the rate and pattern of release are the primary design criteria for the controlled release drug delivery system. This dictates the formulators selection of polymeric matrix material, need for, and type of, formulation additives, drug loading, formulation strategy (e.g. monolithic, membrane rate controlled, etc.) and manufacturing conditions. Each of these factors will influence (and ultimately their combined and interrelated effects will control) the rate and duration of drug delivery from the product. Empirical studies are one approach that allow the formulator to adjust the delivery profile to produce the intended therapeutic effect. However, the theoretical time-dependent pattern of drug release is determined from the mechanism by which the drug is released from the delivery system. Indeed, knowledge of the mechanism of release will allow the factors that influence drug release from that particular dosage form to be identified, and ultimately be used, to optimize the rate of drug delivery and duration of drug release through their rational modification.

III. General design principles for controlled release drug delivery systems

Veterinary controlled release drug delivery systems are, by necessity, innovative in their design and challenging to formulate because of the implicit drug delivery requirements or the environment in which they will ultimately find themselves.[3,4] Compared to human controlled release products, veterinary products are designed to deliver drugs over very extended time periods (weeks or months compared to hours or days). The environment in which the delivery system is placed can be harsh (e.g. rumen; contains abrasive components, elevated pressures, large volumes of fluid, etc.[4,5]) and must be exposed to the elevated temperature and moisture associated with that administration site for long periods.

The majority of veterinary drug delivery systems are fabricated from polymers which exhibit proven histories of biocompatibility, are biologically inert, have regulatory approval and are inexpensive. Such polymers can be either biodegradable or non degradable and include silicone (e.g. Compudose implant, CIDR-B, CIDR-G intravaginal insert, Crestar implant), polyurethane (e.g. intravaginal sponges, some collar technologies), poly(ethylene glycolmethacrylate) (e.g. SYNCRO-MATE-B ear implant), polyvinyl chloride (e.g. some collar technologies), ethylene vinylacetate copolymer, (e.g. some collar technologies, Paratect Flex Bolus), cellulose acetate (IVOMEC® SR Bolus) and various hydrogels such as hydroxyethyl methacrylate-methyl methacrylate copolymer, hydroxyethyl methacrylate homopolymer cross-linked with ethylene glycol dimethacrylate, polyvinyl alcohol, and polyethylene oxide. These polymers may be fabricated into delivery systems by a number of different manufacturing techniques. These include thermosetting (e.g. silicone), thermoplastic (e.g. polycaprolactone, ethylene vinylacetate copolymer, cellulose acetate), cross polymerization of monomers in the presence of a catalyst (e.g. hydroxyethyl methacrylate homopolymer cross-linked with ethylene glycol dimethacrylate), compression (e.g. elemental nutrients, hydroxypropyl cellulose), casting (e.g. D,L-lactide-co-glycolide), spray coating (e.g. ethylene vinylacetate), extrusion (e.g. polyvinyl chloride), amongst others.

The availability of these polymers and fabrication methods results in the ability to incorporate veterinary drugs into polymers in a number of ways (Table 1). Such delivery systems exhibit characteristic release profiles (Table 1; column 3). As pointed out earlier, it is knowledge of that release profile which allows veterinary drug delivery systems to be rationally designed and optimized.

IV. Classification of controlled release veterinary drug delivery systems

Controlled release veterinary drug delivery systems can be classified according to their mechanism of release (Table 2). This system is adopted in this chapter since it allows for a simple classification of currently available veterinary drug delivery systems.

Clearly a wide variety of options are available to the formulator to design a veterinary drug delivery system. Formulators are faced with making choices from a wide number of polymers, and many different design options. A number of factors affect the selection of polymers and subsequent properties of the controlled release drug delivery system. However, their selection also dictates the release kinetics of the drug from the delivery system. Such choices are therefore crucial since they affect the success and potential of the delivery system for achieving the therapeutic goals of the veterinarian. The factors that affect the selection of a polymer and the delivery system design are given in Table 3.

Table 1
Types of veterinary drug delivery systems classified by method of manufacture

Method of manufacture	Mechanism of release	Kinetics of release	Example of veterinary drug delivery system
Dispersion in a matrix comprising surface eroding polymers	Erosion	$t^{1/2}$	Spanbolet, Monensin RDD
Dispersion in a matrix comprising bulk eroding polymer	Diffusion	Complex because of simulatneous erosion and diffusion processes occurring simultaneously	Matrix systems manufactured from lactide/glycolide copolymers
Dispersion in the core of microcapsules manufactured using bulk eroding polymers	Diffusion	Zero order (provided microcapsule structure does not change during the delivery period)	Lutamate Plus (P+)
Dissolution in an oil or aqueous carrier	Diffusion	First order	Estradiol benzoate injection
Suspension in an oil or aqueous carrier	Dissolution/Diffusion	First order	Dexamethasone injection
Dispersion in a soluble matrix	Dissolution	First order	Soluble phosphate glass
Dispersion in a swelling matrix	Swelling (or gelation) of the polymer in the environmental fluid and subsequent diffusion of the active agent	Zero order/square-root-of-time	
Dispersion within a matrix of specific geometric configuration	Diffusion	Zero order	Paratect Flex Bolus
Homogenous dispersion throughout a matrix comprising non-eroding or non-dissolving polymers	Diffusion	$t^{1/2}$	CIDR-B, Compudose
Incorporation into mechanical pumps	Mechanical expulsion under microchip control	Zero order	Smartt 1
Incorporation into reservoirs which are surrounded by a rate controlling membrane	Diffusion across rate controlling membrane	Zero order	PARATECT bolus. Sponges developed by Chien et al.
Incorporation into reservoirs containing osmotic propellants and surrounded by semi-permeable membranes	Osmotic pressure	Zero order	ALZET osmotic minipumps
Dispersion throughout a porous matrix	Diffusion through fluid filled pores	$t^{1/2}$	Polyurethane sponges
Encapsulation of drug in microdispersion within polymer	Partitioning and diffusion	Zero order	Microsealed drug delivery system, SYNCRO-MATE-C
Chemical bonding of the active agent to form a complex	Diffusion controlled via bond cleavage of drug from polymer backbone	$t^{1/2}$	BST conjugated with BSA
Incorporation into an osmotic push-melt formulation	Expulsion of melted drug reservoir due to expansion of hydrogel following imbibement of water through a semi-permeable membrane	Zero order	IVOMEC® SR Bolus

Table 2
Classification of controlled release drug delivery systems

Classification	Veterinary example
1. Diffusion controlled systems	
1.A. Reservoir systems	
1.A.1. Dense membranes	Silicone capsules
1.A.2. Porous membranes	PARATECT bolus
1.B. Solid matrix systems	
1.B.1. Solute loading above solubility limit	CIDR-B Compudose
1.B.2. Solute loading below solubility limit	
1.C. Porous matrix systems	
1.C.1. Pore forming systems	
1.C.2. Pre-formed pore systems	Intravaginal sponges
2. Erosion controlled systems	
2.A. Bulk erosion	Lutamate Plus (P+)
2.B. Surface erosion	Poly(orthoester) matrices described by Wuthrich et al.
2.C. Physical erosion	Laby device
3. Solution or suspension systems	Estradiol benzoate
4. Chemically controlled systems	A suspended complex of BST/Bovine serum albumin
5. Osmotic controlled systems	ALZET osmotic minipump
6. Pulsatile systems	Holloway device
7. Novel approaches	
7.A. Geometrically configured diffusion-controlled systems	Paratect Flex Bolus
7.B. Corrosion of metal	Multidose 130
7.C. Microchip controlled	Smartt 1
7.D. Osmotic propellant and swelling hydrogel	IVOMEC SR bolus
7.E. Microsealed drug delivery technology	SYNCRO-MATE-C
7.F. Liposomes	
7.G. Dissolution controlled	Glass bolus
7.H. Hydrogel systems	

V. Veterinary drug delivery systems and mechanism of release

V.A. Diffusion controlled systems

The mathematical expressions that describe the release of drugs from matrix systems are based upon Fick's laws of diffusion.[6] Fick's first law states that the flux (J) or rate of drug transfer across a plane of unit area is

$$J = -D\frac{dC}{dx} \tag{1}$$

where dC/dx is the change in concentration, C, with respect to distance, x, and D is the

Table 3
Factors which affect selection of polymer and delivery system design for controlled release veterinary drug delivery systems

Required release rate
Duration of release rate
Physiochemical characteristics of the drug
Physiochemical characteristics of the polymer
Potential for interaction between the drug and polymer
Potential for interaction between the drug and other formulation excipients
Manufacturing conditions associated with the polymer
Chemical stability of drug
Geometry of the delivery system
Size of the delivery system
Route of administration

diffusion coefficient of the drug which is assumed to be independent of solute concentration. Fick's second law is given by

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} \tag{2}$$

where the rate of change of concentration with time, t, at a particular level is proportional to the rate of change of the concentration gradient at that level. It should be realized that in actuality the driving force for diffusion is the difference in thermodynamic activity of the drug. In this paper it is assumed that solutions are sufficiently dilute such that the concentration approximates the activity.

These equations form the basis of those defined in the remainder of this chapter for diffusion controlled drug delivery systems. The value to the formulator of mathematically modeling the release kinetics of a controlled release drug delivery system during the formulation and development stages is that such knowledge may be used to predict drug release rates from, and drug diffusion behavior through polymers, thus avoiding excessive experiments. The elucidation of the mechanisms of drug release from a drug delivery system need not be complicated. Indeed, sufficient information for formulation and development purposes may be obtained by simply comparing experimentally determined release data to published mathematical models and determining the appropriate model based on the best fit using, for example AIKAE criteria or examining residual plots. Knowledge of the release mechanism by this approach allows for a priori design and evaluations and will enable the formulator to optimize a drug delivery system through rational modifications to the delivery system.

V.A.1. Membrane-reservoir systems

Membrane-reservoir systems consist of a drug reservoir (a formulation comprising solid particles, or a suspension of solid particles) encapsulated within a polymer membrane. The drug reservoir provides a constant source of drug that is released from the delivery system by solution and diffusion through the encapsulating poly-

meric membrane. The polymeric membrane can be porous or dense, but in either case is of well defined thickness and possesses known physiochemical properties. The properties of the membrane and those of the drug control the rate of release from this type of delivery system.

Several examples of this approach are described in the veterinary literature. Silicone capsules for subcutaneous administration are described by Dzuik[7–9] and Chien[10] wherein each capsule contains solid drug within its lumen. Another approach involved the use of porous caps. The Paratect bolus for intraruminal administration utilized this approach and was designed to provide continuous administration of the anthelmintic morantel tartrate[11,12] to the rumen over 90 days. The delivery system comprised a stainless steel cylinder filled with a paste comprising drug and polyethylene glycol. The cylinder was capped at each end with a porous polyethylene disk impregnated with cellulose triacetate. The porous disks form the rate-limiting barrier to drug release.

Another similar system was detailed by Viswanathan and DePrince[13] who described subcutaneous implants in which somatotropin was placed inside the lumen of silicone tubes which had their ends covered by microporous polyethylene disks each having a 70 μm pore diameter.[13]

A further approach was described by Kabadi and Chien which comprised an unloaded polyurethane sponge onto which was adhered a laminate of silicone sheet (immediately adjacent to the sponge), drug loaded silicone sheet (drug reservoir) and silicone sheet (rate limiting membrane).[14–16]

A matrix sheet device was also designed for intraruminal administration comprising a trilaminate sheet.[17] The product comprised a highly porous ethylene vinylacetate copolymer drug reservoir coated on both sides with layers of ethylene vinylacetate copolymer which incorporated a water soluble compound such as starch or lactose. The highly water soluble ingredient dissolved when the device came in contact with ruminal fluids resulting in a membrane which possessed fluid filled pores. Drug partitioned from the central drug reservoir into the fluid filled pores and then diffused through them into the surrounding medium.

In addition to these approaches, various collar technologies for the delivery of actives against flies or ticks also utilized this approach.[18–23]

A close examination of these examples reveals that two types of membranes can be used to form membrane-reservoir systems; porous or dense. Porous membranes contain soluble components that are leached from the membrane upon placement in the receptor environment. Leaching of the solubilized material leads to small pores within the structure of the membrane. In situ these pores form a solvent filled connecting network of channels which runs from the inner to the outer surface. Dense membranes, in general, are homogenous polymer films that are above their glass transition temperatures in the environment of use.

V.A.1.a. Dense membranes The release of drug from membrane-reservoir systems manufactured using dense membranes is described by the physical model shown in Figure 2 and can be visualized as follows for drugs formulated as suspensions in the drug reservoir.[10] The solid drug particles entrapped within the reservoir of the delivery

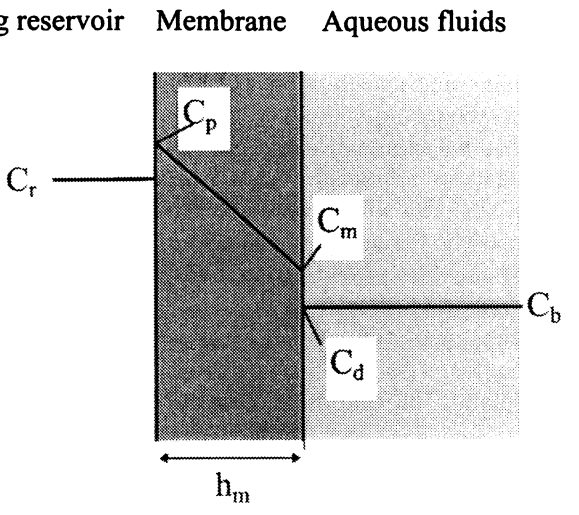

Fig. 2. Physical model describing drug release from membrane-reservoir systems manufactured using dense membranes. The model assumes that boundary layers are insignificant and that the drug reservoir comprises a suspension of drug. C_r is the solubility of drug in the drug reservoir; C_p is the solubility of drug in the polymer phase; C_m is the concentration of drug at the polymer/solution interface; C_d is the concentration of drug at the solution/polymer interface; C_b is the concentration of drug in the bulk of elution solution; and h_m is the thickness of capsule wall or rate controlling membrane.

system dissolve from the solid state into the medium in which they are suspended and then diffuse through the medium to the inner surface of the polymer membrane where they partition into the polymer membrane. The drug molecules then diffuse through the polymer membrane from the inner to the outer surface. When they reach the outer surface they pass from the polymer membrane into the fluid surrounding the drug delivery device by partitioning.

The kinetics of drug release from such systems is theoretically predictable from the following relationship

$$\frac{Q}{t} = \frac{C_p D_p}{h_m} \tag{3}$$

where Q is the cumulative amount of drug released per unit area, t is time, C_p is the solubility of drug in the polymeric membrane, D_p is the diffusion coefficient of drug in the polymer, and h_m is the thickness of the polymeric membrane.

Eq. (3) suggests that the rate of drug release is constant with respect to time i.e., zero-order. The rate of drug release is a linear function of both the polymer solubility (C_p) and polymeric membrane diffusivity (D_p) and is inversely proportional to the thickness of polymeric membrane wall (h_m). Therefore modification of the formulation (addition of excipients to the polymer) to increase the diffusion coefficient of the drug in the polymer, or to improve the drugs solubility in the polymer, can be utilized by the formulator to tailor the release profile of the drug delivery system. However, the most common means of modifying the release of drug from such a system is to manipulate

the thickness of the rate controlling membrane and/or alter the size of the delivery system thereby altering the available surface area for release.

V.A.1.b. Porous membranes The difference between porous and dense membrane systems is that in dense membrane systems drug diffusion occurs through the polymer itself and the solute diffusion coefficient refers to diffusion through the polymer phase. In contrast, in the porous membrane system drug diffusion occurs through fluid filled pores and the solute diffusion coefficient refers to diffusion through the entrapped fluid phase, rather than the polymer itself as this is generally the path of least resistance for the diffusing drug molecule.

Drug release from porous-type membrane systems can be described by the physical model depicted in Figure 3. The kinetics of drug release from such systems is theoretically predictable from the following relationship

$$\frac{Q}{t} = \frac{C_f D_f \epsilon}{h_m \tau} \tag{4}$$

where C_f is the solubility of the drug and D_f is the diffusion coefficient of drug in the fluid filling the pores, respectively, ε is the porosity, and τ is tortuosity of the porous membrane. The terms ε and τ, are the effective volume, and the effective length, respectively, of the tortuous pathway that the molecule must traverse within the polymer film.

Eq. (4) suggests that the rate of drug release is constant with respect to time i.e. zero-order. The rate of drug release is a linear function of both the solubility (C_f) and diffusivity of the drug in the fluid that fills the pores (D_f) and is inversely proportional

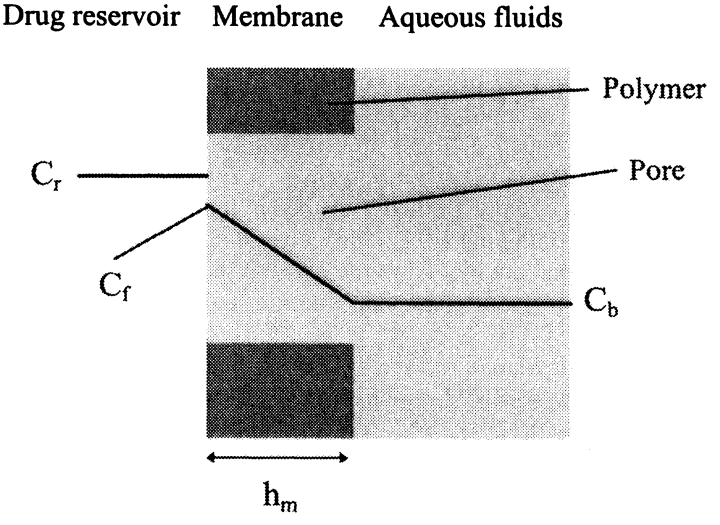

Fig. 3. Physical model describing drug release from porous membrane-type drug delivery systems. The model assumes that boundary layers are insignificant and that fluid within the pores remains unstirred. C_r is the solubility of drug in the drug reservoir; C_f is the solubility of drug in the fluids which fill the pores; C_b is the concentration of drug in the bulk of the fluid phase; and h_m is the thickness of the rate controlling membrane.

to the thickness of polymeric membrane wall (h_m). The most common means of modifying the release of drug from such a system is to either manipulate the thickness of the rate controlling porous membrane or alter the size and/or number of pores in the membrane (which effectively alters the porosity and tortuosity of the membrane).

V.A.2. Matrix systems

Within the context of this review, a matrix system is defined as one in which the drug is uniformly dispersed within a non-degradable polymer. Three possibilities exist; a *dissolved matrix system* which contains drug at or below the saturation solubility of the drug in the polymer; a *dispersed matrix system* which contains drug at a concentration that is above the saturation solubility of the drug but below that which induce the formation of continuous pores or channels in the matrix; a *porous matrix system* where continuous channels exist within the device. Details of these systems have been described including the appropriate mathematical treatment of the equations for drug release.[24] In all of these systems, drug is released via diffusion through the polymer matrix itself or via the pores which are inherent within the structure of the matrix.

Of the three types of matrix devices only dispersed and porous type devices have been used in the veterinary field. In the following section the mechanism of drug release from these two types of systems will be discussed in detail.

V.A.2.a. Loading of drug above its solubility limit (dispersed matrix system)

V.A.2.a.i. Veterinary examples of dispersed matrix system Several dispersed matrix systems have been formulated for veterinary applications and include the following.

- The PRID device comprises progesterone (1.55 g) uniformly suspended throughout silicone rubber which is cured as a uniform layer onto a stainless steel spiral coiled to produce a spiral shaped cylinder approximately 4 cm in diameter and with a length of 12 cm.[24–27]
- The CIDR-B consists of a pre-formed T-shaped nylon spine over which is molded a silicone rubber skin containing 1.9 g of progesterone.[24–27] One end of the device has two flattened wings that are hinged to the body of the device. The wings retain the device in the vagina by gently exerting pressure against the walls of the anterior vagina.[24–27]
- SYNCRO-MATE-B is a small rod shaped ear implant containing 6 mg norgestomet (=5% load) in the polymer Hydron which measures approximately 3 × 18 mm and weighs approximately 0.125 g.[24–27]
- Crestar is a small cylindrical shaped ear implant containing 3 mg norgestomet homogeneously dispersed throughout a silicone matrix.[24–27]
- The CIDR-G insert is a T-shaped intravaginal device comprising a pre-formed nylon spine coated with a layer of silicone impregnated with 9% w/w progesterone.[24–27]
- Sil-Estrus is a subcutaneous implant which comprises a solid silicone rod contain-

ing 375 mg of progesterone (10% w/w initial load), which measures 0.9 cm in diameter and 5.0 cm in length.[24–27]

V.A.2.a.ii. Drug release from dispersed matrix systems For a better understanding of the manner in which a drug is released from a dispersed matrix system (in which it is dispersed within the matrix above its solubility limit) one must assume the following:

1. The drug present is in excess of its solubility (the total amount of drug per unit volume of polymer is substantially greater than the solubility of the drug per unit volume of the polymer) and is uniformly suspended throughout the polymer matrix.
2. The drug is in a fine state such that the particles are much smaller in diameter than the thickness of the polymer matrix.
3. The polymer matrix does not swell or shrink once release has commenced.
4. Diffusion of drug through the polymer is the rate-limiting step to release.
5. The dissolution of the drug in the polymer is rapid relative to drug transport.
6. The receptor fluids provide a perfect sink.

The release of drug from a dispersed matrix system can be visualized as follows. The delivery system comprises drug dispersed homogeneously throughout the polymer matrix and exists both in solution in the polymer and as discrete solid particles which are evenly distributed throughout the polymer network. The solid drug particles are fixed in position during fabrication of the matrix system and cannot delocalize throughout the polymer following manufacture. As a result only dissolved drug can be released from the matrix (i.e. solid particles cannot be released from the matrix). The release of drug therefore occurs through a series of steps:

dissolution of the drug into the polymer that immediately surrounds the drug particle;
diffusion of the dissolved drug from the region of dissolution through the polymer to the surface of the matrix;
partitioning of dissolved drug from the surface of the polymer into the surrounding aqueous environment;
movement of released drug away from the surface of the polymer.[1]

As with any process which involves a series of sequential steps, any of those steps can be the rate-limiting step in the process (dependent upon the relative magnitude of the rate of each step). The slowest step in the whole process will be the one which ultimately controls the rate at which the overall process proceeds. In the case of the release of drug from matrix systems it is assumed that the diffusion of drug through the polymer matrix is the slowest step in the whole process.

This process results in a zone of depletion within the polymer matrix. Solid drug near the surface is eluted first. With time the interface between the drug dispersion zone and the drug depletion zone moves further into the body of the polymeric device and a concentration gradient exists across the distance from the interface to the outermost layer of the device. It should be noted that diffusion through the polymer occurs because the dissolved drug sets up a concentration gradient between where it has dissolved and the outer surface of the polymer. The magnitude of the concentration

gradient is dependent upon the inherent solubility of the drug in the polymer (i.e. its saturation solubility). However, as the depletion zone becomes larger, the distance that the dissolved drug must diffuse across to get to the interface becomes longer. It is this increase in the distance that dissolved drug must diffuse across to reach the interface that causes a non-linear dependence of drug release with time.

This description of drug release from a dispersed matrix system allows the reader to visualize the process. The physical model which describes the release from such systems is shown in Figure 4.[10] Using the physical model shown in Figure 4, and the assumptions stated earlier, a mathematical description of the release of drug from the polymer matrix has been derived which is commonly referred to as the Higuchi square-root-of-time model. This model suggests that the cumulative amount of drug released per unit area (Q) by a dispersed matrix system may be defined by

$$Q = \left[\left(2A - C_p\right)C_p D_p t\right]^{1/2} \tag{5}$$

where A is the initial amount of drug impregnated in a unit volume of polymer matrix, C_p is the solubility of drug in the polymer phase and D_p is the diffusion coefficient of the drug in the polymer phase.

If one assumes that $A \gg C_p$ then Eq. (5) reduces to

$$Q = \left[2AC_p D_p t\right]^{1/2} \tag{6}$$

Thus the release of drug from a solid matrix system is defined by

$$\frac{Q}{t^{1/2}} = \left[2AC_p D_p\right]^{1/2} \tag{7}$$

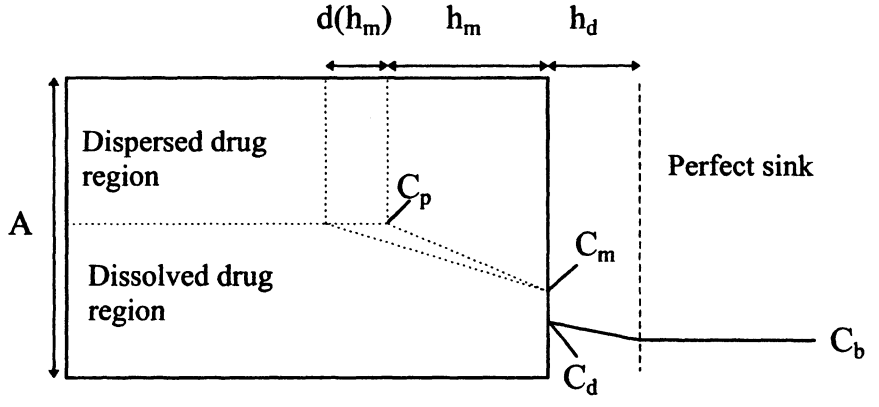

Fig. 4. Theoretical concentration profile in a dispersed matrix system in contact with a perfect sink. A is the initial amount of drug impregnated in a unit volume of polymer matrix; C_p is the solubility of drug in the polymer phase; C_m is the concentration of drug at the polymer/solution interface; C_d is the concentration of drug at the solution/polymer interface; C_b is the concentration of drug in the bulk of elution solution; h_d and h_m are the thicknesses of the hydrodynamic diffusion layer on the immediate surface of the device and of the depletion zone, respectively, and $d(h_m)$ is the differential thickness of the depletion zone when more solid drug is released.

Eq. (7) shows that drug release is dependent upon time, drug content and the magnitude of C_p and D_p. Thus a plot of Q versus square-root-of-time ($t^{1/2}$) should be linear with a slope equal to

$$\left[2AC_pD_p\right]^{1/2} \tag{8}$$

To optimize such a system, the addition of excipients to the polymer that alter C_p and D_p are options. D_p might also be manipulated by the degree of crosslinking or relative co-polymer ratios.[1] Drug load and surface area have the most dramatic effect upon release rates and are more commonly manipulated to produce the optimum formulation. The effect of drug load upon release rate from a dispersed matrix system is shown in Figure 5. This figure shows that the in vitro release rate from the CIDR-B (a dispersed matrix system containing progesterone uniformly dispersed throughout silicone) can be marked, dependent upon the initial progesterone load in the device. The square-root-of-time mechanism predicts that there should be a linear dependence of rate of release upon the square root of twice the drug load ($2A$). This dependence is shown in Figure 5.[28]

In addition to the above variables, the optimization of dispersed matrix systems can focus on optimizing the total drug load for the device. Elegant research which utilized knowledge of the mechanism of release of estradiol 17β from the Compudose implant for growth promotion and feed efficiency, resulted in the formulation of a dispersed matrix system which comprised a non-drug loaded inert core surrounded by a drug loaded outer layer[29] (Figure 6). The development process was reported by Ferguson et al.[29] and the reader is referred to this paper as it contains several useful equations which describe the mechanism of release of drug from such a system, for calculating drug depletion zone thickness and an expression for the time-dependent changes in the drug depletion zone. In addition, it reports on a useful method for experimentally determining the thickness of the final depletion zone following removal.

V.A.2.b. Loading of drug below its solubility limit (dissolved matrix system) When the initial drug load in a matrix system is equal to or less than the saturation solubility of the drug, the rate of release is dependent upon the diffusion coefficient of the drug in the polymer and the initial drug load in accord with the simple laws of diffusion.[6,24,30] The drug concentration and diffusion coefficient of the drug in the reservoir both affect release and can be used to optimize the formulation. The diffusion coefficient is dependent upon the inherent properties of the drug and the composition of the reservoir, and thus is altered via the addition of excipients to the reservoir. Since the total drug load in dissolved matrix systems is usually quite low, such designs are of little practical value for veterinary applications and will not be discussed further.

V.A.3. Porous matrix systems

5V.A.3.a. Pore forming systems

V.A.3.a.i. Veterinary examples of pore forming porous matrix systems Within the context of this review, a pore forming system is defined as one where the initial drug

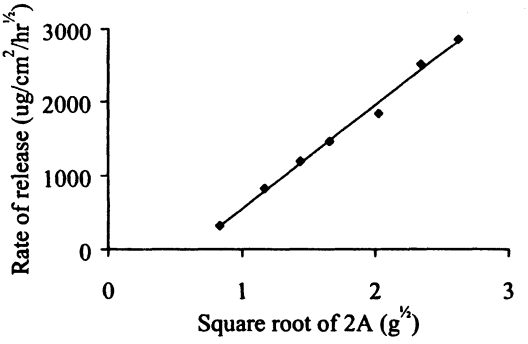

Fig. 5. Effect of initial drug load on the release rate of progesterone from a dispersed matrix system manufactured from silicone (CIDR-B) as a function of the square root of 2*A*.

load is increased in a matrix system, or alternatively, a quantity of highly water soluble ingredient is co-formulated into the system, to a point where the solid drug particles do not remain as discrete entities, but are effectively in contact with each other sufficiently to form a continuous network within the matrix (Figure 7). Under these conditions drug is released via diffusion through fluid filled pores which are formed within the structure of the matrix after administration, rather than through the polymer matrix itself.

Examples of this type of drug delivery system that have been reported in the

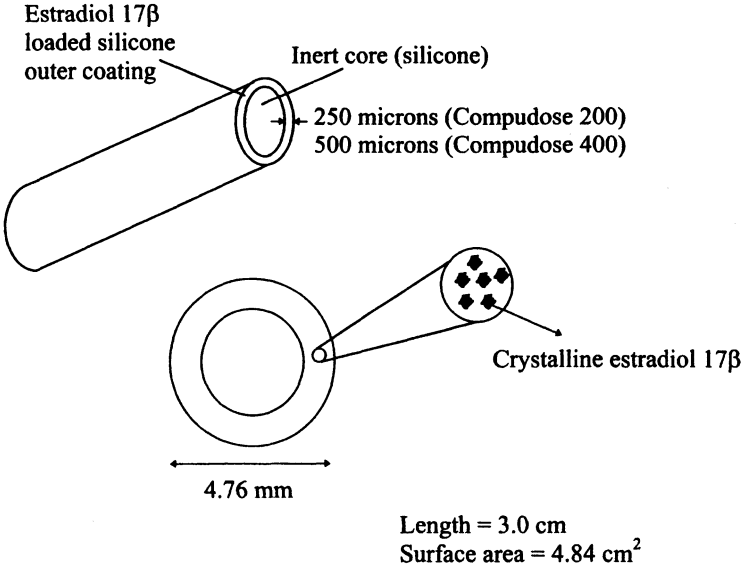

Fig. 6. Compudose 200 and 400 product design. The drug utilization was optimized through knowledge of the mechanism of release (square-root-of time) and determination of the drug depletion zone after insertion. The part of the inner core of the delivery system which does not participate in the release process was replaced by non-drug loaded inert silicone rubber.

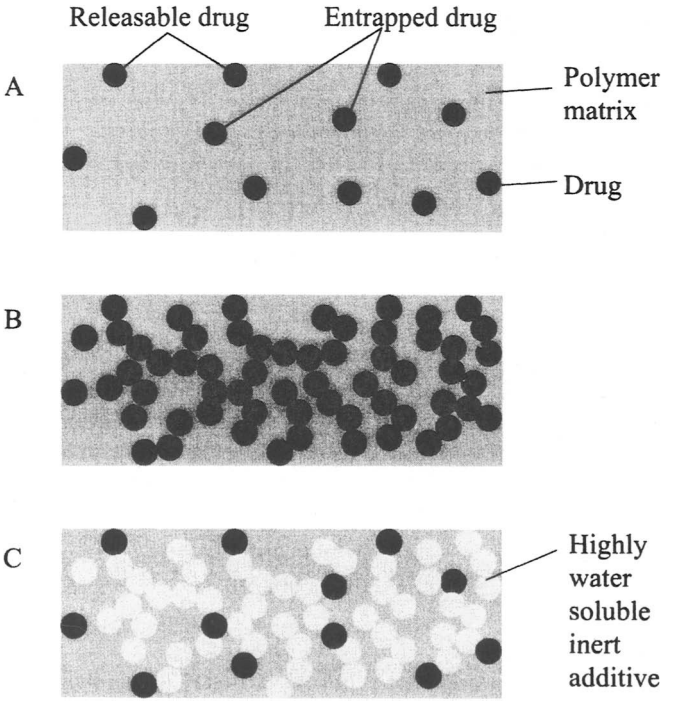

Fig. 7. Effect of loading (or addition of inert, highly water soluble additive) upon the formation of pores in an inert matrix. At low drug loading drug must release via diffusion through the matrix (A). At higher drug loading (B), or if an inert, highly water soluble additive is included in the formulation (C), then following the dissolution of the drug or inert additive after the gradual imbibement of water and formation of fluid filled pores, drug can become released via these pores.

veterinary literature include the Paratect Flex Bolus[31] and the device described by Brewer and Griffin.[17] Both systems utilize a porous core matrix of morantel tartrate and EVA. This core matrix is further coated to modify the release pattern. With the device of Brewer and Griffin, a second porous matrix is added to modify the drug release pattern from a slab-type porous matrix. The Paratect Flex Bolus has a unique design that alters its drug delivery profile and will be discussed separately in a later section of this chapter.

V.A.3.a.ii. Drug release from pore forming porous matrix systems When a porous matrix delivery system is administered the drug or water soluble ingredient dissolves (beginning at the interface and progressively entering the bulk of the matrix) resulting in the formation of fluid filled pores within the matrix. Under these circumstances the drug diffuses through the fluid-filled pores since this is the path of least resistance for the drug. In contrast to a dense matrix system, the rate of release will be governed by factors influencing the diffusion through these fluid filled pores (and not through the matrix material itself).

The equation describing the release of drug from a porous matrix system is shown in Eq. (9)

$$\frac{Q}{t^{1/2}} = \left[C_f D_f \frac{\varepsilon}{\tau}(2A - \varepsilon C_f) \right]^{1/2} \quad (9)$$

where the terms have the same meanings as previously described. Knowledge of the tortuosity is required to account for the tortuous nature of the pore network. It should be noted that the pores of the network must be of sufficient size so that diffusion of the drug is accomplished in the absence of interference or interaction with the polymeric material which makes up the walls of the pores. For this condition to exist, the pore size must usually be greater than 200–500 Å. In the lower limit of this range, hindered diffusion may occur, in which case a correction to the drug diffusion coefficient may have to be made to account for pore wall effects.

Eq. (9) shows that release of drug from such systems is not linearly related to time, but dependent upon the square-root-of-time. The rate of release is dependent upon transport within the fluid-filled pores or channels, therefore, in contrast to solid matrix systems, the release rate can be modified through alteration of either the porosity of the delivery system by altering the drug or water soluble substance load (which changes pore diameter or degree of tortuosity) or via changing drug-dependent properties such as the diffusion coefficient in the fluid which fills the pores (e.g. by incorporating a hydrogel into the formulation), initial drug load, or total surface area of the delivery system.

V.A.3.b. Pre-formed pore system Some veterinary drug delivery systems are manufactured from polymers which, as a result of their fabrication process, come with a randomly porous structure ready formed within the matrix. Intravaginal sponges which contain potent progestagens and are used for the synchronization of estrus in sheep and goats are an example of this type of delivery system. Such delivery systems are made from polymers such as polyurethane which are hydrophobic and impermeable to drug. The sponges come prefabricated and are loaded by the addition of drug in solution in a volatile solvent, which evaporates off leaving a deposit of fine crystals distributed over the inner walls of the porous network. Release can be envisaged to occur by imbibement of water into the pores, dissolution of the drug particles and then diffusion of drug through the aqueous environment of the fluid filled pores into the bulk fluid. Pore geometry is of such a size that it does not affect drug release. Transport occurs exclusively within water filled pores and release is dependent upon the square-root-of-time as described by Eq. (9).

V.B. Erosion controlled systems

Two basic types of controlled drug release devices can be manufactured from erodible polymers. One type involves a drug encapsulated within a rate-controlling erodible membrane, and the other type involves formulating the drug into an erodible matrix.

Drug release from the rate-controlling erodible membrane type involves the process of diffusion across the membrane which is of defined thickness and permeability. Essentially the system is designed to maintain its structural integrity during drug delivery. However, after it is depleted of drug, it erodes to prevent the need to remove

it from the body. If significant erosion of the membrane occurs during release then its thickness changes and alters the release profile. Indeed in the extreme case the membrane could rupture and the drug contained in the core could be released as a bolus.

In erodible matrix systems drug release can occur by either diffusion or erosion. The former occurs if the rate of diffusion of the drug through the polymer is greater than the rate of erosion of the polymer. Drug release will be controlled by erosion of the polymer if the rate of erosion of the polymer is greater than the rate of drug diffusion through it. Several factors must be considered if the rate of release from these types of systems is to be controlled by erosion. Firstly, the drug must be physically immobilized within the system, second diffusional release must be minimal and third, the rate of erosion must be relatively fast.

The erosion process can occur by bulk erosion (e.g. by hydrolysis) at a uniform rate throughout the matrix, or by surface erosion where the process of erosion is confined to the surface of the device. In the former the matrix will remain physically intact until all parts reach some critical degree of reaction, at which point the matrix will disintegrate. In the latter case only those layers which are in contact with an external fluid environment will become eroded while the interior remains intact.

In addition to these chemically-controlled erosion processes, in the veterinary field a third erosion mechanism can be utilized to advantage. This involves the principles of physical erosion whereby the environmental conditions the delivery system finds itself in results in the exposed surface of the system to be physically abraded and gradually eroded away.

V.B.1 Bulk erosion

Examples of polymers which undergo bulk erosion in aqueous conditions are polylactic acid and copolymers of glycolic and lactic acids. The drug can be formulated as a solid erodible matrix or as reservoirs encapsulated by a rate controlling membrane. Such delivery systems offer a distinct advantage over non-erodible implant formulations. Because the delivery system erodes after the delivery period has been completed, there is no need to surgically remove it after administration. Consequently drug release can be tailored to the desired rate and duration and the depleted polymer remains in the animal for varying periods of time after therapy has been completed during which time it erodes to its toxicologically innocuous products which can be eliminated by normal metabolic processes.

In the veterinary area there are several examples of delivery systems which use bulk eroding polymers to form a rate controlling membrane around the drug, which are then usually prepared for administration in the form of a subcutaneous or intramuscular injection. These include microspheres which have been prepared from lactide/glycolide copolymers (D,L-lactide 50:50) using an anatomization process in liquid nitrogen[32] or glyceryl tristearate of glyceryl distearate using a spray prilling technique[33]. A further example is an intramuscular injection comprising a microsphere formulation manufactured from poly (D,L-lactide). This product (P+) has been designed to deliver its entire contents of progesterone (1.25 g) and estradiol (100 mg) continuously for a duration of 12–14 days.[34–40]

Drug release from such delivery systems can be controlled by the thickness and/or size of the particles. Optimization of drug release can also be achieved via modification of the ratio of monomers which can significantly alter the degradation rate of the polymer. Indeed, this variable is used extensively to optimize drug delivery devices manufactured from glycolic and lactic acids.

The drug in the core of such delivery systems is released via diffusion through the rate controlling membrane. Provided the membrane properties do not change during the release process, i.e. the bulk erosion process occurs at a very much slower rate than the rate of diffusion, then drug is delivered from the delivery system in a zero-order fashion and can be approximated by Eq. (3).

In contrast, a detailed understanding of drug release from an erodible matrix type delivery system that is undergoing a bulk erosion process is complex, because it involves the combination of both diffusion and erosion. In addition, because the matrix properties will be continually changing due to the chemical process which are occurring (and which result in the bulk erosion of the matrix), the permeability of the polymer matrix to the drug will also change (increase) with time. Consequently, the rate of drug release from a matrix that is undergoing a bulk erosion process may initially follow the square-root-of-time relationship, but, as the polymer permeability gradually increases, the drug release profile will deviate from the square-root-of-time profile. Furthermore, if the matrix disintegrates before drug depletion, a large burst in rate of drug delivery will be observed.

V.B.2. Surface erosion

Given the complexities in predicting drug release from matrix-type delivery systems which are manufactured from bulk eroding polymers, a matrix-type delivery system which relies upon a surface erosion process for drug release is much more desirable to formulate. The drug in such devices is physically immobilized in the polymer and is only released following erosion of the polymer. In the ideal situation the polymer would only undergo surface erosion, releasing the drug at a rate proportional to the erosion rate. If correctly formulated, surface erosion delivery systems can deliver drug in a zero-order manner, however, this requires that diffusional release of the drug is minimal, the erosion process is constant and the overall surface area of the device (matrix geometry) does not change with time. Examples of surface eroding polymers include polyanhydride and poly(orthoesters). Such polymers have found application in the veterinary area for pulsing actives from implant formulations.[41,42]

Pulsatile release of a model protein (lysozyme) from a poly(orthoester) was described by Wuthrich et al.[41] A pulsatile release system was constructed using two acetate polymers of molecular weights 6000 and 12 000. The different molecular weight acetate poly(orthoesters) eroded at different rates and controlled the duration of the delivery. The authors demonstrated in vitro that the initial release occurred between 18 and 48 h after administration and the booster release occurred 72–144 h later.

Göpferich described a multilaminate matrix consisting of a core of drug loaded fast-eroding polyanhydride matrix (poly(1,3-bis[*p*–carboxyphenoxy]propane-co-sebaic acid) 20:80).[42] This core was then coated with drug-free polyanhydride, then a

drug-free layer of poly(D,L-lactic acid), then a layer of drug containing polyanhydride. The device was cylindrical in shape with a diameter of approximately 4.5 mm. The polyanhydride layers were prepared by compression while the poly(D,L-lactic acid) coat was applied by dip coating in a 20% w/v polymer solution in methylene chloride. The incorporation of the poly(D,L-lactic acid) layer reduced the final size of the device because of its slowly eroding nature; a much thinner layer of poly(D,L-lactic acid) was needed for an equivalent delay time compared to if the fast eroding polyanhydride had been used.[42]

In addition to the above examples, several other delivery systems have been developed for intraruminal use which release drug via surface erosion. However, in these cases the delivery systems do not utilize degradable polymers, but comprise an active material formulated with a mixture of waxes, surface active agents and a dense material to retain them in the rumen. For example an intraruminal drug delivery system comprising monostearin (5%), carnauba wax (10%) and barium sulfate (83%) has been described which delivered the active following slow surface erosion of the formulation.[43–46] Likewise, a rectangular-shaped surface eroding delivery system, approximately 8.75 × 1.25 cm in dimensions, was manufactured by compressing drug, carnauba wax, barium sulfate, polyethylene glycol and iron powder.[47]

Further examples which utilize surface erosion as the mechanism for drug release utilize copolymers of polylactic/polyglycolic acid. Although these copolymers degrade by bulk erosion, selection of appropriate molecular weights and innovative product designs can result in this polymer combination being utilized in delivery systems which exhibit surface erosion as the mechanism of release. For example monensin RDD is an intraruminal drug delivery system that releases monensin sodium following the controlled surface erosion of polylactic/polyglycolic acid (PLA/PGA). The delivery system comprised a low molecular weight PLA/PGA copolymer (average molecular weight of 3000–3800 g/mol which exhibited a relatively rapid degradation rate) formulated with the drug into a core matrix that was adhered to the inner surface of a metal cylinder.[48–50] The ends of the cylinder were then covered with plastic caps that prevented the mechanical abrasion of the drug/polymer mix and the cylinder was housed inside a plastic sheath. Following administration the delivery system primarily degraded by surface hydrolysis; bulk hydration of the polymer was minimized due to the hydrophobic nature of both the drug and polymer ratio used in the formulation. A similar delivery system has been described[51–53] comprising a stainless steel cylinder which was coated with a mixture of drug dispersed in a copolymer of lactic acid and glycolic acid.

The release of drug from such delivery systems is theoretically predictable from the following relationship

$$\frac{M_t}{M_\infty} = 1 - \left[1 - \frac{k_0 t}{C_0 a}\right]^n \tag{10}$$

where M_t is the amount of drug released at time, t, M_∞ is the amount of drug released at time infinity, k_0 is an erosion constant, C_0 is the initial drug concentration in matrix, a

is the radius of sphere or cylinder, or half the thickness of a slab and $n = 1$ for a slab, 2 for a cylinder and 3 for a sphere.

V.B.3. Physical erosion

Several veterinary drug delivery systems utilize the principles of physical erosion for drug release. Such systems are generally characterized by the encasement of the drug formulation (generally manufactured by compression) within a plastic casing which is impermeable to aqueous fluids. Laby[54–62] described an intraruminal delivery system composed of compressed tablets which were loaded into a hollow plastic cylinder that was sealed at one end, but was open to the environment at the other via a delivery orifice. The delivery system included a compressed spring that was separated from the tablets by a piston. During administration the compressed spring continually forced the tablets to remain in contact with the exterior environment via the delivery orifice. The drug release rate was controlled via the properties of the erodible tablet formulation.

An alternate method for encasement involves the application of a moisture resistant coating over the drug reservoir. A simple but effective formulation which utilizes this approach is the TimeCapsule which comprises a drug reservoir of compressed zinc oxide covered on all surfaces except one by a moisture resistant waxy coating. The zinc oxide erodes from the open end (it is prevented from eroding on all surfaces by the coating) while simultaneously the coating splits off as it becomes unsupported by the zinc oxide reservoir.

V.B.4. Issues with erosion devices in veterinary drug delivery

Three issues arise with the formulation of erosion devices for controlled drug release in veterinary drug delivery and all are associated with the rumen delivery route. First, the effect of erosion on the retention of the drug delivery system in the rumen. Riner et al.[63] manufactured erodible density-retained boluses with densities in the range of 1.2–2.4 which they administered to cattle and subsequently followed their location and fate over time. The outcome of their findings suggested that an erodible device which was dependent on density for retention must be designed such that the erosion process does not adversely affect its retention in the rumen. The second issue relates to the location of an erodible bolus and the resultant effect on drug release rate.[64] Byford et al. demonstrated that the release rate from an erodible drug delivery system was dependent upon the location of the delivery system in the stomach.[64] These authors noted that because of differences in the nature and extent of muscular contractions and/or the effects of abrasion arising from other ingested materials that tend to locate in the reticulum (e.g. metallic materials such as barbed wire) that delivery systems were eroded at a faster rate in the reticulum compared to other regions of the ruminants stomach.[64] This has the potential to result in variability in degradation rates of devices that depend upon abrasion for release.[65] The third issue relates to the types of drugs which are incorporated into intraruminal products which erode with time. Because of the first two issues, erodible devices may be best utilized for the delivery of nutrients, as opposed to drugs, where therapeutic indexes are usually much wider.

V.C. Suspensions and solutions

Suspension and solution controlled release dosage forms are formulations comprising drug in solution or suspension within an oily vehicle. They are designed for injection into subcutaneous or intramuscular tissue which results in the formation of a depot at the site of injection. This depot acts as a drug reservoir that releases the drug continuously at a rate determined by the characteristics of the formulation. Examples of this type of formulation in the veterinary literature include a dispersion of somatotropin in a biocompatible vegetable oil (e.g. sesame oil, peanut oil) co-formulated with thickening agents,[66] an estradiol benzoate injection, amongst others.

Drug release from solution-type injections generally follows a first-order release profile. Thus the following equation is generally applicable to describe the kinetics of drug release from such drug delivery systems

$$Q = Ce^{-kt} \tag{11}$$

where k is a rate constant and C is the drug concentration.

For suspension injections the following may be more appropriate:

$$\frac{Q}{t} = \frac{S_a D_s C_s}{h} \tag{12}$$

where S_a is the surface area of the drug particles in contact with the oil, D_s is the diffusion coefficient of drug molecules in the oil, C_s is the saturation solubility of the drug in the oil and h is the thickness of the diffusion layer surrounding each drug particle.

Predicting release from such systems is often complicated by the fact that surface area plays a large role in the amount of drug released from injectable systems which form variable shaped depots of different areas and this is dependent upon the administration technique.[66] Release rates from such formulations can be modified by the addition of excipients, changing the type of oil used to formulate the delivery system and varying the manufacturing process. For example, the viscosity of an oily formulation can be modified to increase the duration of release by the addition of viscosity inducing excipients (e.g. esters of glycerol) or thickening agents (e.g. aluminium monostearate, white beeswax, yellow beeswax). Solution injections can be modified to produce a longer duration by converting them to suspensions. For suspension injections, change in particle size can be used to optimize release rate since the larger the particle size the slower the dissolution rate and therefore the more prolonged the delivery period.

V.D. Chemically-controlled systems

Chemically-controlled systems include polymeric formulations in which drug diffusion is controlled by a chemical reaction such as cleavage of the drug from a polymer backbone. Only a few veterinary drug delivery systems utilize this approach, for example, suspended complexes of HGH and BST have been described which

comprise a conjugate of the proteins with bovine serum albumin using glutaraldehyde or with immunoglobulin using carbomide.[67]

V.E. Osmotically-controlled systems

Osmotically-controlled systems consist of an osmotic core containing the drug, a semi-permeable membrane which surrounds the osmotic core and allows only water to permeate through it and a delivery orifice through which drug is released. When an osmotically-controlled system is administered it imbibes water via the process of osmosis, which results in the release of an equal volume of drug solution through a delivery orifice.

The release of drug may be controlled by the osmotic pressure of the osmotic propellant, the permeability of the delivery system to water and the dimensions of the membrane as given by

$$\frac{dV}{dt} = \frac{L_p A}{h}(\sigma \Delta \pi - \Delta P) \tag{13}$$

where dV/dt is the volume flow of water, L_p is the filtration coefficient, σ is the reflection coefficient, $\Delta\pi$ is the difference in osmotic pressure, ΔP is the difference in hydrostatic pressure, and A and h represent the surface area and thickness of the membrane, respectively.

Several delivery systems which utilize the principles of osmosis are described in the veterinary literature. For example, ALZET osmotic minipumps have been used to deliver progesterone to various species including cattle and pigs.[68–70] ALZET osmotic minipumps were developed in the mid 1970s and are implantable systems which can be manufactured in various sizes. Each pump consists of the following components: an inert, impermeable, flexible drug reservoir open to the exterior via a single orifice; a thin sleeve of osmotic agent surrounding the reservoir, and a semi-permeable membrane surrounding the sleeve of the osmotic agent (Figure 8). The reservoir is filled with a solution or suspension of the active ingredient and a flow moderator is inserted into the orifice which restricts diffusion of the agent from the delivery orifice. Delivery of drugs from such solutions can be visualized as involving diffusion of water from the surrounding tissue through the semi-permeable membrane in response to the osmotic pressure difference across the membrane. The permeability characteristics of the semi-permeable membrane controls the rate at which water moves into the osmotic sleeve, and since the membrane is rigid it causes swelling of the osmotic propellant within the delivery system resulting in drug solution/suspension being forced out of the delivery orifice. The release of drug occurs in a zero order manner and can be described by the following equation

$$\frac{dV}{dt} = \frac{L_p A_m}{h_m}[\sigma(\pi_s - \pi_e) - (P_s - P_e)] \tag{14}$$

where L_p, A_m and h_m are the filtration coefficient, the effective surface area and the thickness of the semi-permeable housing, respectively, σ is the reflection coefficient of the membrane, $(\pi_s - \pi_e)$ which is the differential osmotic pressure between the drug

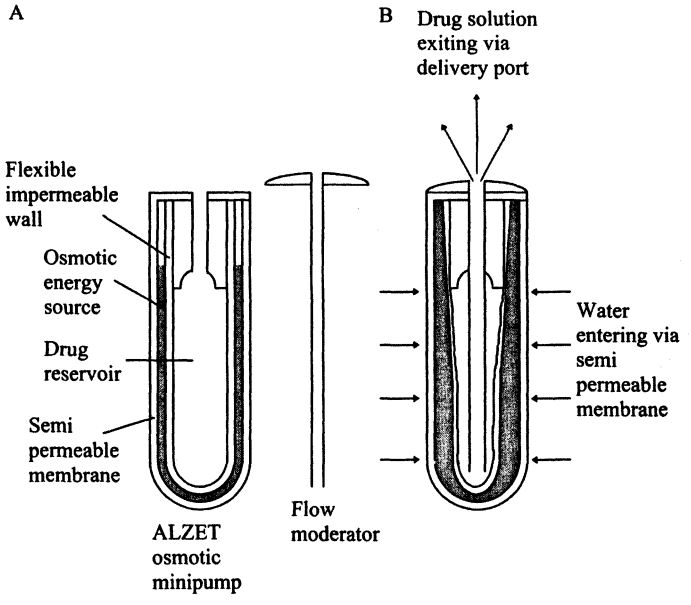

Fig. 8. ALZET osmotic minipump. (A) Major components; (B) osmotic pump in operation.

delivery system with an osmotic pressure of π_s and the environment with an osmotic pressure of π_e and $(P_s - P_e)$ is the differential hydrostatic pressure between the drug delivery system with a hydrostatic pressure of P_s and the environment with a hydrostatic pressure of P_e.

Another example of an osmotic pressure based systems can be found in the literature.[71–73] In this system a core formulation was developed comprising pellets of a swellable polymer such as PEG 600 or polyvinyl alcohol dispersed throughout an oil such as isopropyl myristate or soybean in which the drug was dissolved. This core formulation was contained within a metal cylinder which was capped at either end with microporous polyethylene membranes. One membrane was impregnated with cellulose triacetate and wetted with PEG 400, while the other was either left unimpregnated or treated to permit the membrane to be permeable to oil.

V.F. Pulsatile systems

Delivery systems which release multiple doses of drug at pre-programmed intervals have been termed pulsatile or intermittent release systems. In the veterinary field, several groups have described pulsatile release systems.[74–78] For example, Holloway described a delivery system that contained a series of degradable partitions that were manufactured from cellulosic materials.[74] The partitions separated compartments which either contained drug or inactive excipients. As each cellulosic partition degraded, the contents of the compartments were successively exposed to the external environment. The dosage regimen was controlled by the composition (drug or inactive

excipients) of each drug compartment and by the thickness and composition of the degradable partitions.

Bagnall and Gyurik[75] described a device that was composed of a series of cylinders containing drug. The drug formulation was retained within the cylinder by a plug which was expelled after an increase in the internal pressure within the cylinder which occurred following ignition of a chemical squib. The squib was electrically activated via a circuit that regulated the timing for the application of current. The delivery system was activated following its placement within the rumen fluids that acted to close the circuit.

Another device that utilized electrical forces to control the pulsed release of an active has been described by Whitehead and Shepherd[76]. This device was composed of five doses of oxfendazole placed in cups that were in turn placed on a magnesium rod. Drug was compressed to form 'lifesaver'-shaped rings. The cups were separated on the rod by spacers which prevented water penetration into the drug formulation until each successive cup was shed due to the degradation of the magnesium rod. The rod degraded due to the action of a galvanic couple element that was placed in the base of the device holding the rod. The base also provided the necessary weight to retain the device in the rumen. This system was commercialized as the Synanthic® Multidose 130 and delivered five doses of oxfendazole for grazing cattle over approximately 130 days.

Kwan and Steber[77,78] also described a system that enabled the pulsing of several doses. Each dose was contained within a formulation that comprised two layers which were held together with an adhesive; a drug containing layer and a layer that contained sufficient weight for retention of the system. Each formulation was then coated with a hydrophobic polymer that controlled the time that the drug became exposed to ruminal fluid. By coating the two layered formulations with different polymers that degraded at different rates the time of release of drug could be controlled.

Other approaches to achieve pulsatile delivery of drugs utilizing biodegradable polymers have been described in Section V.B.2.

V.G. Novel approaches

V.G.1. Geometrically configured diffusion controlled systems

As described previously the release characteristics of a drug that is homogeneously dispersed above its solubility limit throughout a polymer matrix generally follow square-root-of-time dependent kinetics. In such systems, it may be possible to produce a zero-order release profile by altering the geometry of the device. To achieve this the device must be configured to enable its surface area to change (increase) in order to offset the increase in diffusional distance the drug must diffuse within the polymer matrix as drug release occurs. If this can be achieved, although drug release occurs in accord with the mechanistic principles of a dispersed matrix system, the observed release profile will be near zero order. This theory has been exploited to advantage in the Paratect Flex Bolus. This delivery system comprises a central drug loaded ethyl vinyl acetate sheet which is coated on both sides with a polymer which is impermeable to drug[79] and which contains a series of holes which pass completely through the

trilaminate structure. Following administration, drug release occurs from both the outermost edges of the trilaminate and from the inner surface of each hole. Drug release from outer edges follows a square-root-of-time profile. However, the release from each hole results in a zero order profile because of the simultaneous increase in surface area and receding drug boundary. The release rate from such a system is described by the following equation[5,80,81]

$$M_r = N\left(\lambda^2 - n^2\right)L\pi\varepsilon\left[\rho - C_s + \frac{C_s}{2[h\varepsilon/n\tau + \ln(\lambda/n)]}\right] - \left[\frac{NL\pi\varepsilon C_s n^2}{[h\varepsilon/n\tau + \ln(\lambda/n)]}\ln\frac{\lambda}{n}\right]$$

$$+S\left[\frac{D\varepsilon(2A - \varepsilon C_s t)}{\tau}\right]^{1/2} \tag{15}$$

where M_r is the total amount released, t is the time, N is the number of perforations in the sheet, n is the radius of the perforation, λ is the radius of the depletion zone, L is the thickness of the core matrix, ε is the porosity of the core matrix, τ is the tortuosity of the core matrix, ρ is the density of the drug, C_s is the saturation solubility of the drug in the dissolution medium, D is the diffusion coefficient of the drug in the dissolution medium, h is the diffusion layer thickness, S is the surface area of the edges of the core matrix and A is the weight of drug per unit volume of matrix.[5] This design offers the advantage that drug release follows a nearly zero-order profile especially at longer times. The release rate was shown to be a function of the number and the diameter of the perforations and the usual parameters that control the rate of release from dispersed matrix systems. These parameters can be used to optimize the delivery profile.

V.G.2. Microchip controlled

The intelligent breeding device (IBD) is a single delivery system which is designed to deliver progesterone continuously over a 10 day period, and pulse a dose of estradiol 1 h after administration and prostaglandin 6 days after administration. The rate, duration and timing of release of each of these drugs is controlled via a programmable microchip. The IBD comprises an outer plastic sheath designed to protect the electronic chip board which controls the rate and time of release of the actives, four drug reservoirs (one large one at the base of the device and three smaller ones at the head of the device), a retention mechanism and a tail.[25–27] At the top of the large drug reservoir is a small orifice that is opened and closed by a switch mechanism operated by a solenoid. In the open position, drug solution is forced up a small bore stainless steel tube that opens to the exterior at the head of the device. The three smaller drug reservoirs are closed by tight fitting rubber seals and drug release occurs following the melting of a plastic tether which is holding a spring loaded plunger under tension. The melting of the plastic is controlled by the microchip board which operates the resistor over which the plastic cord is lying, causing it to heat up.

V.G.3. Osmotic push-melt system

As with delivery systems designed for human oral applications, there is a need in the field of veterinary drug delivery for delivery systems capable of delivering highly

water insoluble compounds to ruminants via the oral route. This need was met through the development of the 'Push-Melt' ruminal bolus[5,79,82–84] by scientists at ALZA and Merck. The product comprises a membrane cup that is extruded from cellulose acetate and various plasticizers which is filled with an osmotic hydrogel tablet. Placed above the tablet is a partition layer, a drug containing layer and a densifier. The densifier is made of sintered iron filings designed such that it has a crush strength similar to that of bone[82] to ensure that it will not harm the equipment used in rendering plants following slaughter of the animal. The centrally located port in the densifier is covered with a capscreen made from plastic and designed such that the drug suspension is forced through a series of small openings in the capscreen.[83] When administered the drug layer softens and is fluid enough to flow through the exit channel and exit port screen. The softening point of the partition layer is higher than that of the drug layer, consequently the partition layer serves as a piston to help direct the flow of the softened drug containing layer through the exit port in the densifier. The drug layer is released from the device following imbibement of water through the semi-permeable membrane which results in expansion of the hydrogel which forces the softened drug containing layer through the exit port.

V.G.4. Microsealed drug delivery technology

The microsealed drug delivery (MDD) Technology was patented in the mid 1970s by Chien and Lambert. The technology is used to fabricate drug delivery systems which exhibit a zero-order release profile. The technology has found application in both the human and veterinary pharmaceutical areas. In the latter area it has been fabricated into small cylindrical shaped subcutaneous implants containing norgestomet which are administered to the ear of farmed animals for the purpose of estrous synchronization.[1,2,85,86] The drug delivery system is manufactured by microdispersing a drug reservoir (norgestomet/40% v/v polyethylene glycol 400/water) into a biocompatible polymer (silicone) using a high torque mixer to form a homogenous dispersion. The drug-polymer dispersion is then cured to immobilize the drug reservoir in the cross-linked polymer matrix. Figure 9 shows the physical model associated with this technology. Drug molecules elute out of the MDD first by dissolution in the liquid drug reservoir, then by partitioning into and diffusion through the polymer matrix, and then partitioning into the surrounding aqueous environment. Drug is theoretically released from the MDD according to the following equation[1,2]

$$\frac{Q}{t} = \frac{D_\rho D_s \gamma' K}{D_\rho \delta_d + D_s \delta \gamma' K}\left[\beta S_p - \frac{D_l S_l (1-\beta)}{\delta_l}\left(\frac{1}{K_l} + \frac{1}{K_p}\right)\right] \tag{16}$$

where Q/t is the rate of drug release from a unit surface area of MDD; K is the partition coefficient for the interfacial partitioning of drug molecules from polymer coating membrane toward the elution solution; K_l is the partition coefficient for the interfacial partitioning of drug molecules from the microscopic liquid compartment toward the polymer matrix; K_p is the partition coefficient for the interfacial partitioning of drug molecules from the polymer matrix toward the polymer coating membrane; D_l is the diffusivity of the drug molecules in the microscopic liquid compartment; D_p is the

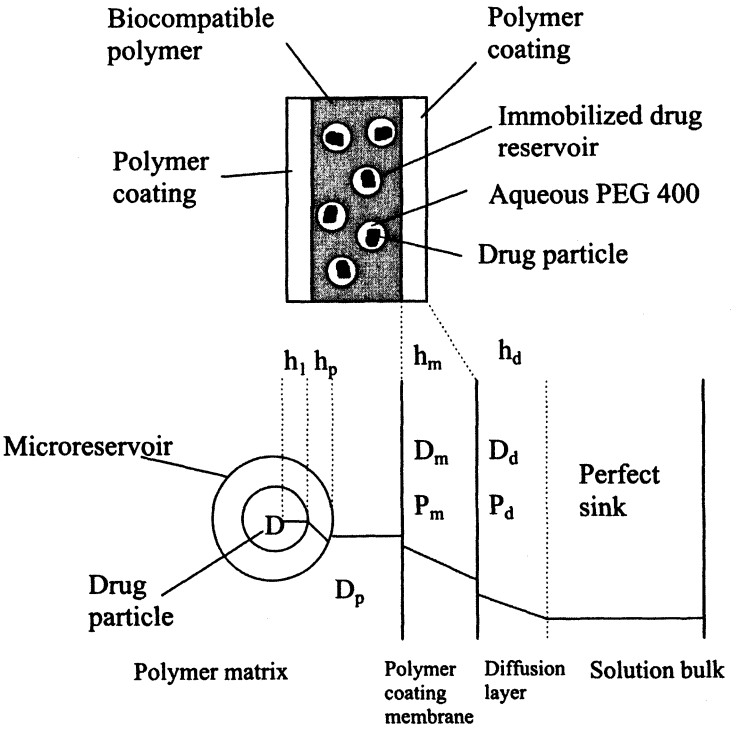

Fig. 9. Physical model of a microreservoir dissolution controlled drug delivery system (MDD). The microscopic liquid compartments, which encapsulate drug particles, are homogeneously dispersed as discrete, immobilized, unleachable spheres (with a diameter $\leq$ 30 μm) in cross-linked polymer matrix. *D*, *P* and *h* are the diffusivity, permeability and thickness, respectively, and the subscripts p, m and d denote the polymer matrix, polymer coating membrane and diffusion layer, respectively.

diffusivity of the drug molecules in the polymer coating membrane; D_s is the diffusivity of the drug molecules in the elution solution; S_l is the solubility of the drug molecules in the microscopic liquid compartment; S_p, the solubility of the drug molecules in the polymer matrix; δ_l is the thickness of the liquid layer around the drug particles, δ_p is the thickness of the polymer coating membrane around the polymer matrix, δ_d is the thickness of the hydrodynamic diffusion layer surrounding the polymer coating membranes; β is the ratio of drug concentration at the inner edge of the interfacial barrier over drug solubility in the polymer matrix; γ' is α'/β' in which α' is the ratio of drug concentration in the bulk of elution solution over drug solubility in the elution solution, and β' is the ratio of drug concentration at the outer edge of the polymer coating membrane over drug solubility in polymer coating membrane.[85] Since all terms on the right-hand side of Eq. (16) are constants during the drug delivery process the delivery rate of drugs from delivery systems fabricated using the MDD technology exhibit a zero-order release profile.

V.G.5. Liposomes

Liposomes are composed of phospholipids that spontaneously form multilamellar, concentric, bilayer vesicles, with layers of aqueous media separating the lipid layers. Several examples of liposomes as drug delivery systems appear in the veterinary literature. These include an egg phosphatidylcholine, ethanolomine and α-tocopheryl hemisuccinate and Tris salt liposomal delivery system,[87] and one comprising of hydrogenated soy phosphatidylcholine-cholesterol hemisuccinate.[88]

V.G.6. Dissolution controlled systems

Glass-based delivery systems are an example of a veterinary drug delivery system which releases drug via the process of dissolution.[89–95]

Glass exhibits a relatively high solubility in buffered aqueous solutions, and therefore has been used in the design of intraruminal drug delivery systems. Glass based delivery systems are formed by melting the glass and bioactives (nutrient components) at high temperatures (up to 700°C) and drawing the melt into the desired shape. Drug is generally released from such systems by dissolution of the glass, however, depending upon the composition of the glass and therefore the rate of chemical reaction (dissolution), drug may also be released by diffusion. If drug is released exclusively via dissolution, then the following expression may describe drug release for various shaped delivery systems

$$\frac{M_t}{M_\infty} = 1 - \left[1 - \frac{k_{\text{diss}} t}{C_0 a}\right]^n \tag{17}$$

where M_t is the amount of drug released at time, t, M_∞ is the amount of drug released at time infinity, k_{diss} is a dissolution rate constant, C_0 is the initial drug concentration in matrix, a is the radius of sphere or cylinder, or half the thickness of a slab and $n = 1$ for a slab, 2 for a cylinder and 3 for a sphere.

The rate of release from such systems can be modified by altering the dissolution rate of the glass by modifying its composition using additives such as P_2O_5, Na_2O and CaO. P_2O_5.[89]

V.G.7. Hydrogel systems

Hydrophilic materials have been used as excipients for the controlled delivery of veterinary drugs. Various hydrogel veterinary drug delivery systems have been described in the literature. Somatotropins have been suspended in silicone oil and placed into hydroxyethyl methacrylate-methyl methacrylate copolymer and hydroxyethyl methacrylate homopolymer cross-linked with ethylene glycol dimethacrylate to form a rate-limiting membrane over cylindrical drug reservoirs.[96] Small spheres of somatotropin-chitosan (60:40) which are coated with a hydrogel comprising polyvinyl alcohol have also been described.[97] Casey and Rosati describe a matrix system comprising biodegradable thermoplastic hydrogels consisting of ABA or AB block polymers where the block A is a glycolide and block B is polyethylene oxide, or in the case of an ABA block polymer, a glycolide and trimethylene carbonate.[98]

The manufacture of a simple hydrogel drug delivery system involves mixing the drug and hydrophilic material together and compressing it to form a tablet. Following administration and initial rapid release of drug is observed. However, the hydrophilic material hydrates and gels at the interface between the delivery system and aqueous environment. This results in the formation of a viscous gel which coats the surface of the delivery system and acts as a barrier to drug release. When this occurs, the drug is released at a slow rate, which is theoretically controlled by its rate of diffusion through the gel barrier. However, the release profile from such systems can be complex because of the dynamic nature of the gellation process. The release profile from such systems is dependent upon both the surface area and the rate of advance of the boundary separating the outer gelled portion from the unhydrated core. If the rate of advance of this boundary is constant then a zero-order release will be observed. However, drug release from such systems can also be observed to follow a square-root-of-time profile.

VI. Conclusions

The foregoing account has demonstrated that a large number of controlled release veterinary drug delivery systems have been developed using a variety of technologies that range from the relatively uncomplicated (e.g. a mixture of drug plus polymer) to the more complex (e.g. osmotic) systems. The mechanism of drug release varies with the technology used to manufacture the drug delivery system, which in turn influences the resultant plasma profiles and therefore its suitability for a particular health or production application. However, knowledge of the release mechanism allows for *a priori* design and evaluations and enables the formulator to optimize a drug delivery system through rational modifications to the size, geometric configuration and/or components which comprise the delivery system.

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CHAPTER 3

Intraruminal controlled release boluses

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Overview

I. Introduction

Ruminants such as cattle and sheep are commonly placed in remote locations on pasture to grow and mature for a full grazing season that normally will last for 4–6 months. The delivery of drugs to these animals can be an involved and costly process. In order to minimize the number of times these animals must be handled during grazing periods, numerous products have been devised to provide very long-term delivery of drugs to them.

In this chapter systems that have been designed to deliver drugs to the rumen of grazing animals will be reviewed and, in particular, focus will be placed on those product concepts that have been commercialized in various countries of the world. The mechanisms of drug delivery utilized to achieve these product concepts vary widely. In some systems the delivery rate is largely free of local environmental influences such as the osmotic mechanism associated with the IVOMEC® SR bolus. In others the rate is strongly dependent on the local environmental factors such as the erosion-based systems utilized in various nutritional and antibacterial delivery systems. In this chapter focus will be on the pharmaceutical and clinical performance of these products. The mathematical equations required to describe the rate of release from

these devices has been described for most of the relevant devices in Chapter 3 of this book and elsewhere[1–3] and will not be repeated here.

II. General design characteristics

All intraruminal boluses have several critical design features required to ensure proper delivery to, and retention within, the rumen of the animal.

Overall, the system must be of such a design that it can be administered orally with a balling gun or equivalent. A balling gun has a cup or suction type mechanism to hold the bolus in place, a long rod-like tool designed to move the device through the oral cavity, and a means to expel the bolus from the cup upon placement at the upper end of the esophagus. Several commercial designs of balling guns are available. Associated with this is the need to provide a bolus shape compatible with passage down the esophagus. Commonly, cylindrical or elongated capsule-shaped devices are used. An example of a device intended for use in cattle, the Paratect® flex bolus, is shown in Figure 1.[3] This device is approximately 10 cm long and 2.5 cm in diameter. Some general shape and volume restraints for devices for sheep or cattle have been described in a patent by Edwards et al.[4] Also shown in Figure 1 is a dosage form intended for human oral applications. The relative size difference is striking. In general, passage of the device down the esophagus is not an issue. However, if the device is of relatively low density and contains an open passageway through the long axis of the device, as is the case for the Paratect® flex diffuser, it may be essential to plug the passageway to ensure proper passage of the device. For this system end-caps were designed to plug the passageway during administration of the device.[5] The end-caps allow the animal to progress the device through the esophagus and into the reticulum. In the absence of the end-caps, devices were found to lodge in the esophagus.

An intraruminal bolus must provide some mechanism for long-term retention in the rumen of the animal. Two methods are commonly employed to retain devices in this site. The first involves the incorporation of components that provide an overall device density of greater than 2.00.[4,6] This condition ensures that the device will remain at the bottom of the reticulo–rumen cavity and will not be regurgitated. Alternatively, device retention can be achieved through the incorporation of a means to permit the overall dimensions of the device to expand following passage from the esophagus into the reticulo–rumen cavity.[7–14] This is normally accomplished using a design that leads to a significant expansion of at least one dimension of the device following its introduction into the reticulo–rumen cavity. Following these concepts, Laby[7] introduced the approach of incorporating polymeric 'wings' that are constrained by a water soluble tape or adhesive during administration. Upon passage into the rumen, the tape or adhesive dissolves and the wings expand thereby preventing regurgitation. The same objective was accomplished in devices designed by Boettner[8] and by Brewer and Griffin[9,10]. These devices are polymeric sheets that are coiled during manufacture to generate a cylindrical device (Figure 1). The device is maintained in this shape during packaging and storage through a piece of tape held in place by a water-soluble

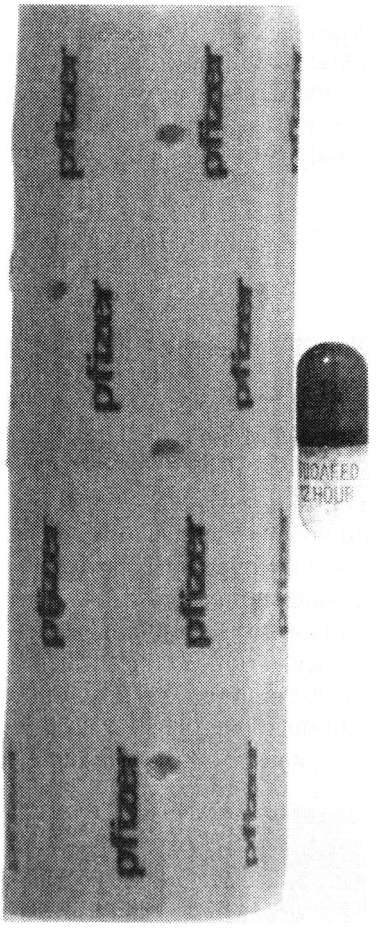

Fig. 1. Picture of Paratect® flex bolus and typical human oral capsule showing the relative size differences.[3]

adhesive. Upon administration and passage into the rumen, the adhesive dissolves and the cylindrical device expands so that its effective diameter is larger than the opening to the esophagus. In this way regurgitation of the device is prevented. Ranade and Curtiss[13,14] designed a specific tape to meet these design specifications for the Paratect® flex diffuser. Curtiss and Lo[12] described a modification of the latter system that enhances the degree to which the device expands following administration.

The final design criteria for an intraruminal bolus is the incorporation of a mechanism for the long-term release of the drug. These devices are usually designed for either relatively short-term delivery (3–5 days) of therapeutic agents or for very long-term delivery (up to 180 days) of growth promotents, nutrients or antiparasitic agents. For these applications drug delivery for an entire grazing season is desirable. Both the

amount of drug incorporated and the duration of delivery are substantially greater than that for human oral systems, and the release mechanisms must be appropriately tailored to meet these needs.

As noted intraruminal boluses are intended for administration via the oral route to the rumen of animals where the device remains until depletion of the incorporated drug. Depending upon the design, upon depletion of drug the device may erode away, or it may be composed of a polymeric or metallic shell that remains with the animal throughout its lifetime.

III. Rumen physiology

Ruminants such as cattle, sheep and goats have an alimentary canal that consists of the same organs as other animals, i.e. oral cavity, pharynx, esophagus, stomach, small intestine, large intestine and colon (Figure 2).[15] However, the stomach of a ruminant is large and complex. It is composed of four compartments, namely, the rumen, reticulum, omasum and abomasum. Taken together, the volume of these four compartments may reach 50–60 gallons in fully-grown cattle.[4] In sheep, the total size is about 10% of this. The rumen is the largest compartment and may contain from 20 to 50 gallons of fluid. The anatomical features of the remaining portions of the gastrointestinal tract are not markedly different from other animals.

Cattle secrete large amounts of saliva (up to 14 gallons/day) at a pH of approximately 8.2. The pH of the reticulo–rumen compartment is in the range of 5–7. The temperature of the rumen is in the range of 38–42°C. The conversion of foodstuffs into absorbable nutrients occurs through microbial fermentation, mainly by protozoa and bacteria. This process is accompanied by the evolution of large quantities of gas (up to

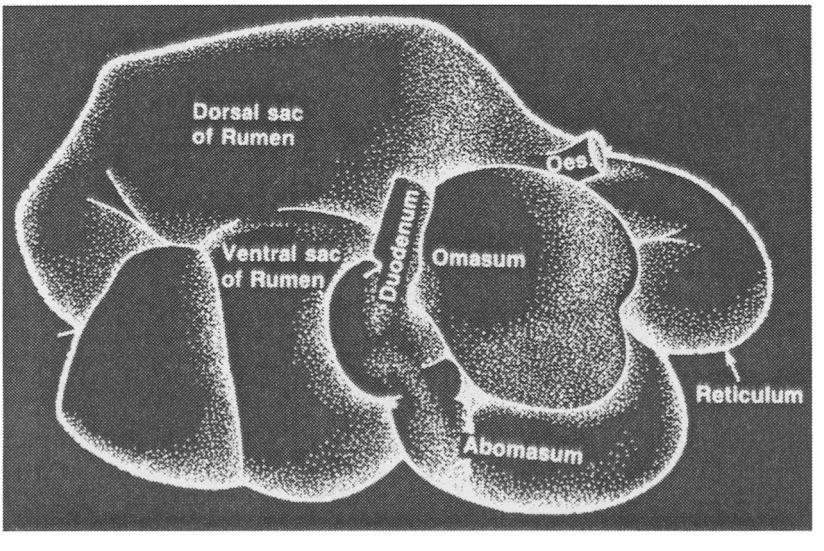

Fig. 2. Diagram showing the four compartments of the ruminant stomach.[15]

600 l/day); the composition of which is 40–70% carbon dioxide, 20–40% methane, 15–35% nitrogen, 0.1–7% oxygen, 0.1–0.5% hydrogen and 0.1–0.5% hydrogen sulfide.[16] The gas pressure within the rumen is approximately 1.1 atmosphere. The end result of fermentation is the production of absorbable nutrients, mainly short chain fatty acids such as acetic, propionic and butyric. The composition and density of ruminal materials varies with the location. At the bottom of the rumen, the material is relatively well-digested and mixed with copious amounts of fluid and is, therefore, of relatively high density. The upper layers of the rumen contain the newly ingested grasses which are much drier and of relatively low density. The total gastrointestinal transit time of ingested foodstuffs is in the range of 3–3.5 days with much of this time associated with the residence time in the rumen. The long gastrointestinal transit time will have significant consequences on the rate of absorption of oral dosage forms in ruminants. These pharmacokinetic issues were discussed by Dresback.[17]

IV. Intraruminal boluses

IV.A. Erodible systems

Perhaps the most common type of sustained release intraruminal bolus is the erodible system that is designed to dissolve or abrade due to the solution or mechanical action of the rumen. This approach was used in one of the earliest examples of an intraruminal controlled release device as described by Marston.[18] He developed an erodible bolus containing cobalt oxide and other excipients to produce a product that delivers cobalt for the full grazing season. Cobalt is a required trace nutrient for ruminants.

Some of the required characteristics of erodible boluses and the effects of compositional changes on the release characteristics were investigated by Riner et al.[7,19] They investigated the effects of the density on the retention and location of boluses in cattle. By varying the relative amounts of barium sulfate, carnuba wax, polyethylene glycol, and iron powder, boluses that ranged in density from 1.2 to 2.4 were obtained. These boluses were administered to cattle and their location and fate followed over time. The results are shown in Table 1. It can be seen that as the density of the device increases

Table 1
Effect of density on retention in the rumen

Density (g/cm^3)	Number of doses retained		
	Reticulum	Rumen	Missing
1.2	3	54	123
1.4	37	65	48
1.6	130	31	19
1.6	85	90	5
1.8	133	47	0
2.0	178	2	0
2.0	153	27	0
2.2	180	0	0
2.4	180	0	0

the extent of regurgitation decreases and the relative number of devices that locate in the reticulum increases. These data suggest that densities greater than 2.0 will be sufficient to prevent regurgitation of boluses.

Several authors have developed erodible boluses that are designed to release various drugs, trace elements or nutrients to grazing sheep or cattle.[20–35]

Of these, several have been commercialized for the delivery of sulfa drugs for the treatment of shipping fever in beef cattle and non-lactating dairy cattle. Examples of products available in the US include the Albon® S.R. bolus (sulfadimethoxine), the Sulfa Sustained release bolus (sulfamethazine), the Spanbolet II (sulfamethazine), the sulfa S-R bolus (sulfamethazine) and a sulfamethazine sustained release bolus.[20] In general, these products are designed to provide for the sustained delivery of the sulfa drug over a period of 72 h. An example of the plasma profiles obtained from these erodible boluses compared with the performance of a bolus designed for daily administration is shown in Figure 3. The data are taken from the freedom of information file available for each product as given in The Green Book.[21] Plasma concentrations of sulfamethazine obtained from the sulfamethazine sustained release bolus are shown in Figure 3. On day 0 each of ten healthy cross bred cattle were administered one bolus (containing 30 g of sulfamethazine) per 90 kg of body weight. The minimum effective therapeutic level for sulfamethazine is 80 ppm. From Figure 3 it can be seen that effective therapeutic levels were maintained from 6 to 72 h of treatment.

Another example is found in the work of Pierce et al.[22] They evaluated the performance characteristics of an erodible bolus containing S-methoprene for the control of hornfly designed by Miller et al.[23,24] The bolus contains 2% S-methoprene, 5% monostearin, 10% carnauba wax and 83% barium sulfate. The latter component provided the necessary density for the device to be retained in the rumen. The active is excreted in the feces and controls hornfly by eradicating the pupal stage of the hornfly in the

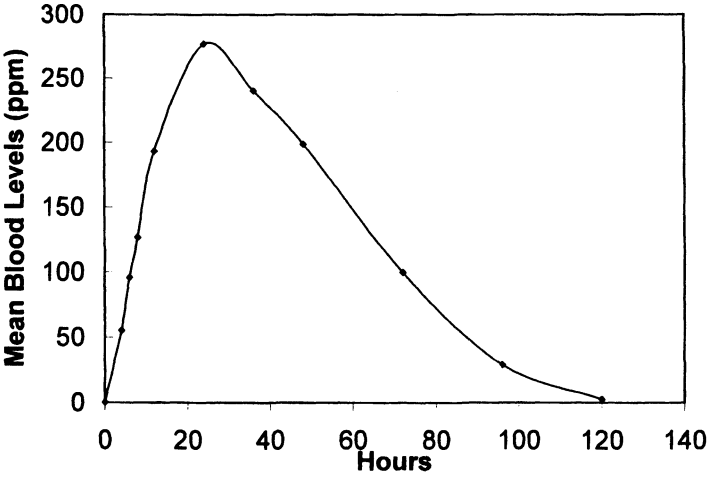

Fig. 3. Plasma levels for sulfametazine from the sulfametazine sustained release bolus.[20]

manure pat. Figure 4 shows the concentration of the active in the manure pat at various times during the grazing period.[22] It is apparent that the concentrations of the active vary widely but stay well above the minimum effective concentration. Figure 5 summarizes data obtained on the number of hornflies per animal for treated and control animals.[22] Clearly effective control of the horn fly is achieved in the treated animals for the full grazing season. Devices of similar design have been described for the season-long delivery of antiparasitacides.[25]

Another approach for the development of erodible boluses is the utilization of soluble glasses. This approach has been described by Drake et al.[26–32] These workers developed glasses that are soluble in buffered aqueous solutions to design systems of utility for ruminal boluses. For example glasses prepared from P_2O_5, Na_2O, and CaO, where P_2O_5 is the glass forming oxide can be utilized to design systems that deliver trace nutrients to grazing cattle for periods of up to 1 year.[26] Table 2 shows representative data[28] on plasma concentrations of copper taken from ewes at days 0, 64, and 128. The glasses were designed such that the solubility of glass B was twice that of glass A with the trace nutrients of copper, cobalt and selenium added in the form of their respective oxides. These glasses are formed by melting the components at 700°C followed by drawing the melt into the desired shape. For the field trial 100 ewes were selected and given five different treatments as outlined in Table 2. The ewes were grazed in fields known to be deficient in copper. Only the treatment group given two boluses of the more soluble glass maintained plasma copper concentrations at values similar to the initial control values.

Other systems have been designed to permit the addition of the active in a step that is subsequent to the formation of the glass. For example, the drug can be incorporated in the interstitial spaces via a pressure mediated sintering process[29] or via incorporation of drug into reservoirs formed in the glasses during processing. In the

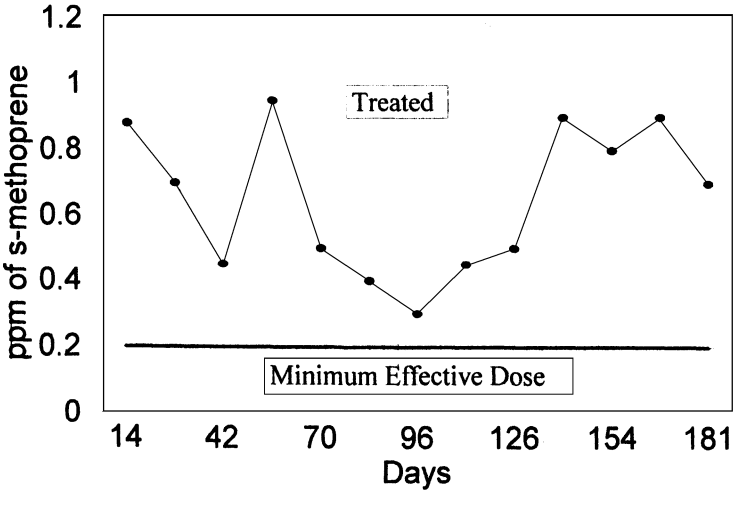

Fig. 4. Concentrations of S-methoprene in the manure pat compared with the minimum effective dose.[22]

Table 2
Plasma concentrations from soluble glass boluses

Treatment	Mean plasma concentration (μl/mole)		
	Day 0	Day 64	Day 128
Control	12.4	7.1	5.6
Glass Ax1	12.0	8.4	8.3
Glass Ax2	13.7	10.2	9.7
Glass Bx1	11.1	10.1	9.5
Glass Bx2	11.7	11.4	13.8

sintered boluses, drug release occurs by diffusion of drug from the device and/or by dissolution of the glass.[30,31] In the case of reservoir devices, drug was released as a bolus dose following rupture of one or more points in the wall of the glass reservoir. Delivery over a full grazing season was achieved through the formation of a series of boluses comprising glasses with varying dissolution rates. This approach was of value for the delivery of anthelmintic over a full grazing season via a series of four to six bolus doses delivered 30–45 days apart. Thus, therapeutic doses are released at regularly spaced intervals with drug free periods in between. This has the benefit that the drug is present at specific periods only, rather than continuously as with controlled release devices. This approach may offer some advantages regarding the prevention of drug resistance that may arise from continuous exposure to low levels of drug.

In the devices described above, all surfaces of the bolus are continuously exposed to ruminal fluids and the abrasive action of the rumen contents. Retention is due to the overall weight of the device. As noted earlier, Laby[7] was the first to advocate the use of polymeric wings to serve as a means to retain drug delivery devices within the rumen. He also introduced a means to control the surface area of the device that was exposed to the abrasive action of the rumen contents. The overall design advocated by

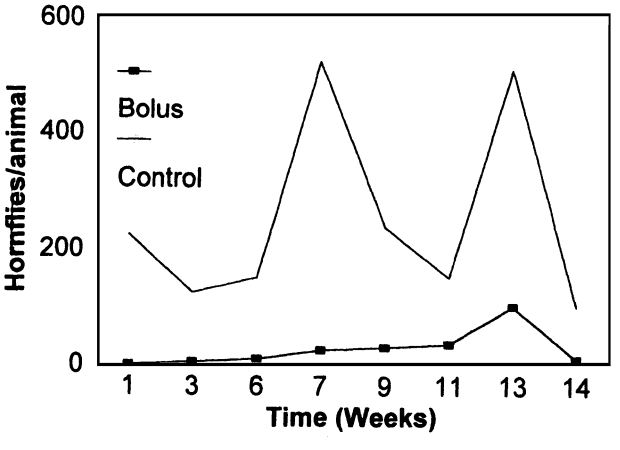

Fig. 5. Comparison of the number of hornflies per animal for controls and animals treated with a sustained release bolus.[22]

Laby and co-workers[7,33–36] is shown in Figure 6. The device is composed of a hollow cylinder capped at both ends. One end is closed and constrains a spring that moves a plunger that forces an erodible composition containing drug or nutrient against a second endcap. In practice the erodible composition can be uniform throughout or, for example, a series of compressed tablets that can be placed within the central compartment of the device. In the latter case the tablets can be made to contain active or placebo compositions, thus the drug release rate can be made to occur either in a continuous or a pulsatile fashion. The drug/nutrient release rate is controlled via the properties of the erodible composition. Attached to the cylinder are polymeric wings that are constrained to the side of the cylinder during administration by a piece of tape with a water-soluble adhesive. The wings expand and prevent regurgitation following administration and subsequent dissolution of the tape. This basic design has been the subject of extensive work that has appeared in the patent literature[33–42] which describes improvements in device retention mechanisms, core composition, piston/spring design and delivery of multiple drugs with a single device.

For example, Laby[34] defined a core formulation composed of a biologically acceptable wax and two or more biologically acceptable surfactants. The final formulation had a melting point in excess of 39°C and the capacity to absorb water at a controlled rate which in turn controlled the rate of delivery of drug. In patents Laby[35,36] also defines compositions that are capable of imbibing water at a controlled rate that have a softening point of >39°C, and a yield stress that is substantially less than the maximum driving force of the piston. Shepard[38] describes a device that utilizes the cylinder and piston design to deliver the drug, but utilizes weight rather than the polymeric wings to retain the device in the rumen. Furthermore, the system is designed such that the weight is expelled from the device at the end of the delivery period. This leads to the regurgitation of the device by the animal at the end of the delivery period thereby reducing the potential complications from retained devices either during the remainder

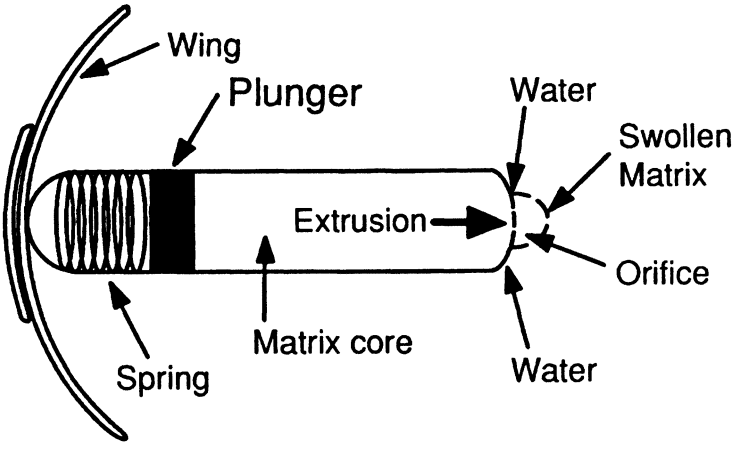

Fig. 6. Winged device of Laby.[7] The wings are held in place by a piece of tape during administration and passage down the esophagus.

of the life of the animal or during the processing of the animal in rendering plants. Lowe and McArthur[41,42] developed improvements to the sealing mechanisms to insure that ruminal fluids do not enter the device through the end of the device containing the piston. This helps to ensure more consistent delivery of the payload.

This 'winged-cylinder' design of Laby and others has been commercialized as the Extender 100 for the delivery of albendazole[37] to sheep. The device contains 3.85 g of drug that is released at a constant rate of 32.5 mg/day over 100 days. The devices are designed for sheep that weigh in the range of 35–65 kg. The performance of this system was detailed in a series of abstracts that appear in a publication entitled *Australian Advances in Veterinary Science*, published by the Australian Veterinary Association in 1988. In particular, one study compared the performance of treated ewes and lambs with either untreated controls or 'district' lambs that received a conventional regimen of a series of immediate release bolus doses. The treated animals had low worm burdens throughout the trial for those species that are sensitive to the albendazole. At the end of the trial period of about 1 year, the liveweights of the treated ewes were about 11% greater than controls and 4% greater than the district animals. The treated lambs were 24% heavier than controls and 16% heavier than district lambs. This study clearly demonstrated that continuous administration of drug was superior to conventional treatments where animals were exposed to drug on an intermittent basis.

This 'winged-cylinder' design has also been utilized by Elanco for a device termed the Rumensin ABC for the delivery of monensin sodium to cattle for the treatment of bloat and for the enhanced rate of growth that arises from the continuous administration of this polyether antibiotic.[43] The Rumensin ABC bolus is composed of a typical winged-cylinder design wherein the cylinder is about 16 cm long with a diameter of about 3 cm. The core is composed of monensin sodium dispersed in hexaglycerol distearate. The drug is delivered at the rate of about 300 mg/day for 100 days. The clinical effects of the administration of this system can be seen in Table 3 where the liveweight gains, and Table 4 where the bloat scores, for treated and control animals are summarized. It can be seen that the treated animals gained an average of 8.5 kg more than the untreated animals. Of greater importance is the significant increase in the number of treated animals that survive through the grazing season. These results highlight the important life saving benefits that can be gained through the use of these

Table 3
Summary of liveweight gain data for the Rumensin ABC

Treatment	Liveweight (kg)		Overall (93.7 days)	
	Initial	Final	Gain	ADG[a]
Control	294.4	402.7	108.3	1.156
Rumensin ABC	299.0	415.8	116.8	1.247
Treatment effect			+ 8.5 kg	+ 0.091 kg

[a] Average daily growth.

Table 4
Summary of bloat scores for the Rumensin ABC

Treatment	Average bloat score (scale 1–4)	Number of bloat deaths
Control	2.89	17
Rumensin ABC	1.21	3
Treatment effect/difference	1.68	14

long acting ruminal boluses. Also the fact that the animals actually gain more weight provides the economic benefit for their use in the absence of disease.

Another approach to an erodible device also completed at Elanco is that described by Conrad et al.[44–46] Their device also utilizes a cylinder to control the exposed surface area of the eroding material. In this system the release is controlled via the composition of a low molecular weight copolymer of polylactic/polyglycolic acid (PLA/PGA). This polymer hydrolyses upon exposure to an aqueous environment. Monensin sodium is dispersed within this copolymer. The overall design of their device, which is termed the Monensin RDD, is shown in Figure 7. The device is composed of a core matrix of the drug/PLA/PGA mixture that is placed within a metal cylinder. The drug/polymer mixture is held within the cylinder via an adhesive. The device is covered with plastic and the ends of the cylinder are protected with plastic caps that help prevent mechanical abrasion of the drug/polymer mix. The outside dimensions of the device are 11 cm in length and 3.7 cm in diameter.

In the Monensin RDD device the crystalline drug is dispersed at a 40% concentration by weight within a 80/20 (w/w) matrix of PLA/PGA. The polymer is biodegradable and undergoes surface hydrolysis in the presence of aqueous media. Low molecular weight PLA/PGA copolymers are utilized to achieve the desired release rates. This is shown in Figure 8 where it can be seen that the release rate increases as the average molecular weight of the copolymer decreases, and that after an induction

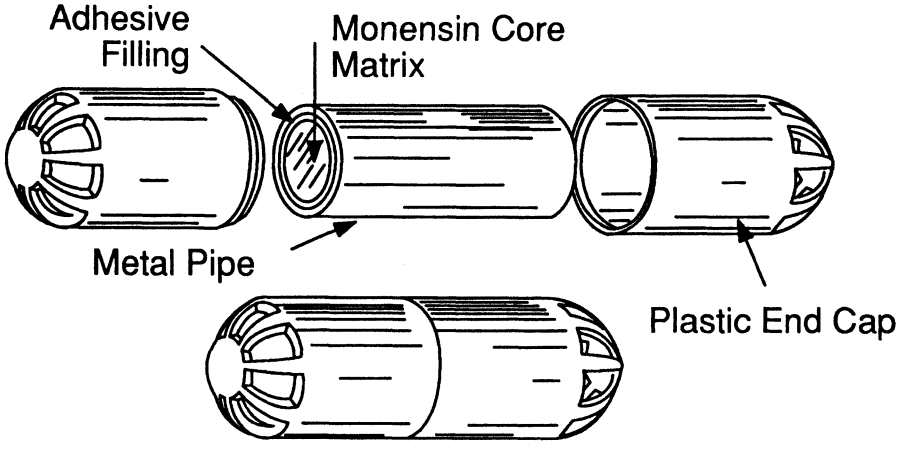

Fig. 7. Rumensin RDD.[44]

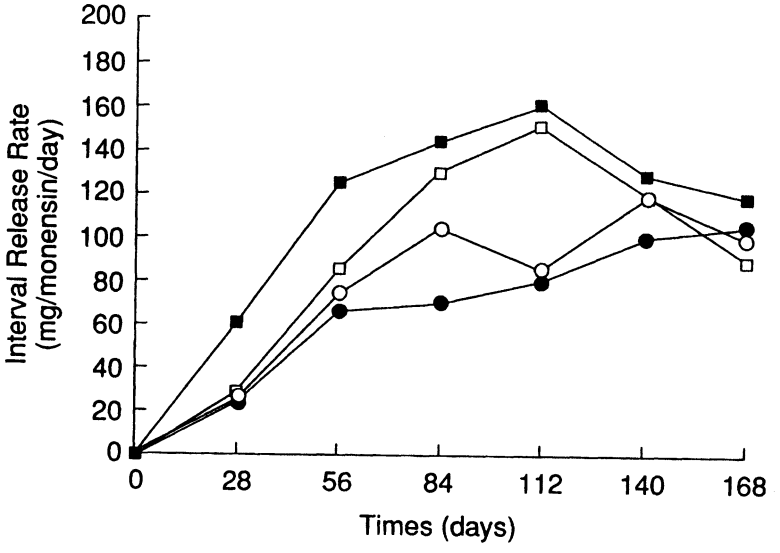

Fig. 8. PLA/PGA molecular weight effects on release of monensin. Key: number average molecular weight = 4480 g/mole (●), 3720 g/mole (○), 3000 g/mole (□), and 2320 g/mole (■).[44]

period of several weeks, a steady state release rate is achieved that is relatively constant over time.

Data on the clinical performance of the commercial device are shown in Tables 5 and 6.[43] The long-term administration of monensin is expected to enhance growth rates in grazing cattle. Based on prior experience with it in feed administration and with the Rumensin ABC described previously, a target delivery rate of 100 mg monensin per day for 150 days was established. The device contained 16.5 g of monensin with the copolymer of PLA/PGA having an average molecular weight of 3000–3800 g/mole. Average daily release rates determined from measurements of the residual drug amounts averaged from 89 to 121 mg/day depending on the trial. As seen in Table 5, the cumulative weight gains in the treated animals were greater than in the untreated controls with results depending upon the treatment condition. These treatments differed in time of the year and the nature of the grass the cattle were grazing.

Table 5
Effect of Rumensin RDD on the liveweight gain of grazing cattle

Trial number	Number of animals/trial	Total weight gain (kg)		Percent gain over control
		Control	RDD treated	
I	24	90.8	97.1	6.94
II	58	104.8	108.8	2.13
III	12	93.5	100.8	7.81
IV	14	135.8	142.6	5.01
V	12	69.6	79.6	14.4

Table 6
Comparison of the effects of an estradiol 17β implant and the Rumensin RDD on the daily gain of grazing cattle

Treatment	Number of animals/trial	Initial weight (kg)	Total weight gain (kg)	Percent gain over control
Control	29	238	100.1	
17β Estradiol	29	247	109.4	9.29
Rumensin RDD	29	244	102.5	2.40
RDD + estradiol	29	246	115.0	14.9

The range for the average daily gain increase relative to controls was 2.13–14.4%. A second trial compared the effects of the RDD versus an estradiol-17β implant. The greatest benefit occurred when the product was co-administered with an estradiol 17-β implant where weight gains of treated animals were about 15% greater than untreated controls (see Table 6). In this particular study the RDD alone showed a 2.1% improvement over the untreated controls.

IV.B. Reservoir systems

One of the earliest examples of a commercially successful product for once per season treatment of a parasitic infestation using a ruminal bolus is found in the work of Dresback and others at Pfizer.[47–49] This product, which is called the Paratect® bolus, was designed to provide continuous administration of morantel tartrate over a full grazing season. This drug is effective for the treatment of gastrointestional round worms in grazing cattle. This system is composed of a stainless steel cylinder which is about 10 cms in length and 2.5 cm in diameter. The cylinder is capped at each end with porous polyethylene disks impregnated with cellulose triacetate. This creates a reservoir within the cylinder. The reservoir is filled with a mixture of drug plus polyethylene glycol. Drug release occurs for approximately 90 days via diffusion of drug through the impregnated disks.

The Paratect® bolus was originally marketed in Europe and was highly effective in the season long control of gastrointestinal roundworms.[44–46] When untreated cattle are grazed in fields infected with larvae from these parasites, the cattle may be infected by the larvae which then mature and reproduce within the GI tract of the animal. Subsequently eggs are excreted in the feces to begin the lifecycle of the parasite again. Infected animals grow more slowly and, in severe cases, may die as a result of the infection. The disease is particularly devastating to animals during their first year of exposure to the parasite. Second year animals seem to attain some level of immunity to the parasite.

Typical data on the beneficial effects that arise from the administration of the Paratect® bolus to grazing cattle are shown in Figures 9 and 10. Figure 9 is a plot of the average number of eggs per gram (EPG) of feces for control and treated animals maintained in fields that were contaminated with susceptible parasites. Both control groups showed clinically significant levels of infestation throughout the grazing

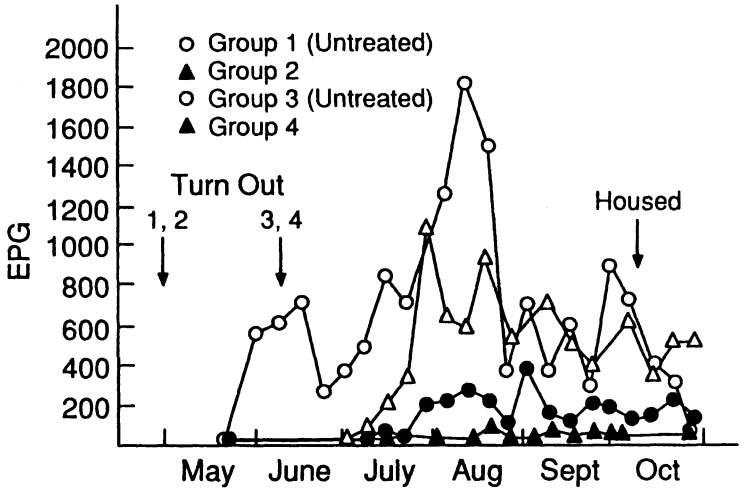

Fig. 9. Egg output per gram of feces (EPG) versus time for animals treated with the Paratect® bolus at turnout compared with untreated controls.[50]

season. In contrast the treated groups showed low levels of infestation through much of the grazing season. These differences in levels of infestation of the parasite dramatically affected the growth performance of the animals.[48–50] These results are shown in Figure 10 where the average weights of the treated and control animals for each treatment group are plotted against time. The dramatic effect that the bolus can

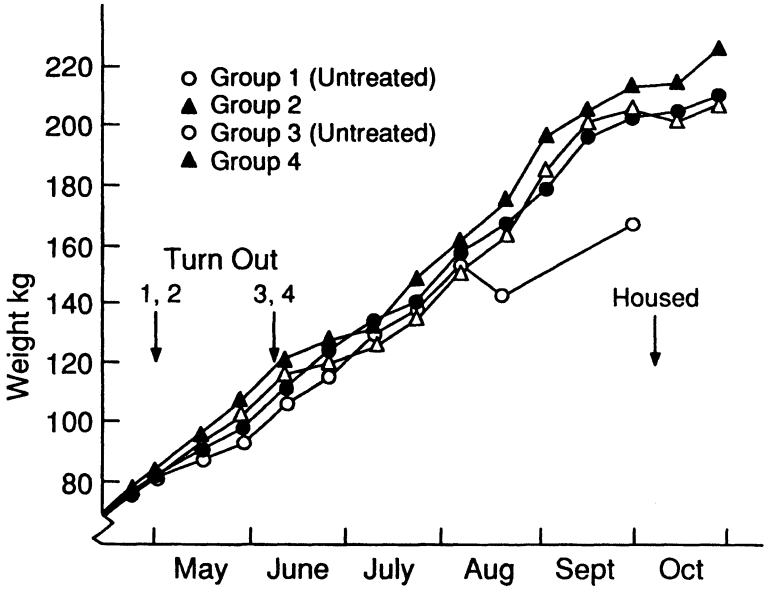

Fig. 10. Weight gain over a full grazing season for Paratect® bolus treated and control animals.[50]

have on the rate of growth of the animal is seen from a comparison of the treated and control groups for the animals placed at pasture in early May. In this study, high levels of pasture contamination existed as evidenced by the high EPG counts throughout the study in the control animals. The EPG counts reach a peak in August that translated to an actual weight decrease in the animals at this time. In contrast the treated animals from this same study showed a steady gain in weight. At the end of the trial the treated animals were heavier by approximately 50 kg or 33% greater in weight than the untreated controls. The relative difference in animal weights are not as striking in the second study, which was consistent with a lower level of infestation seen in the control animals.

Another feature of the graphs shown in Figures 9 and 10 is that the values for the EPG counts in the treated animals are not zero but rather the results suggest that the animals are subjected to a continuous low level of exposure to the parasite. This low level permits the animal to develop a natural resistance to the parasite such that it is not necessary to treat the animals during their second and subsequent seasons on pasture.

IV.C. Dispersed matrix systems

As described in Chapter 3, a dispersed matrix device contains solid drug particles uniformly dispersed within a non-biodegradable polymer matrix. Several authors have described dispersed matrix devices that are suitable for delivery of drug to ruminants.[8–14]

These devices are in the form of a large sheet that can be rolled up to form a cylinder. During packaging and storage, the rolled up devices are constrained by some type of tape with a water sensitive adhesive that dissolves and allows the device to unroll following administration. Under these circumstances the unrolled device will have dimensions that are greater than those of the esophageal channel thereby preventing device regurgitation.

The rate of drug release and release mechanism from these devices depends upon the individual design. In general, the release of drug from a planar sheet is described by the square-root time law as originally described by Higuchi.[51] This equation can be described as follows

$$M_t = \sqrt{\frac{D\varepsilon(2A - \varepsilon C_s)C_s t}{\tau}} \tag{1}$$

where M_t is the total amount of drug released, D is the diffusion coefficient of the drug in the diffusion matrix, ε is the porosity of the matrix, A is the weight of drug per unit volume in the matrix, C_s is the saturation solubility of the drug in the polymer, τ is the tortuosity of the matrix, and t is time. For devices whose delivery profile is described by this equation, a plot of the amount released versus $t^{1/2}$ will be linear. This implies that the rate falls continuously with time, which may not be desirable depending upon the application.

Griffin and Brewer[9] described a matrix device that utilizes a trilaminate sheet wherein the inner lamina is composed of a core matrix of drug dispersed in an ethylene–vinyl acetate (EVA) copolymer. The drug polymer ratio is in the range of

30–75% polymer, which yields a dispersed matrix device that has sufficient mechanical strength to serve as an effective drug delivery device. This inner lamina is coated on both sides with a layer that is either impervious to fluid (and drug) or as a dispersed matrix type material where a filler such as starch or lactose is added to control the overall drug permeability properties in the coating. Thus, in effect, this design is a dispersed matrix laminate with the outer layers being of lower permeability to drug than the core lamina. The drug release profile will follow the Higuchi equation and, therefore, the release rate decreases with time. This equation has been modified by numerous authors to account for various special cases of device shape or drug loading. These modifications have been reviewed by Cardinal.[52]

A second design of Griffin and Brewer[10] utilizes a similar device configuration but is composed of two lamina wherein one lamina is impermeable to drug. The second lamina is an erodible dispersed matrix of drug plus EVA. The drug release rate is controlled via the properties of the erodible matrix.

Cardinal et al.[8,11–14] utilized a device configuration that is similar in overall dimensions to that described above. Their device is a trilaminate sheet that is approximately 21 × 10 cm that is rolled into a cylinder and constrained with tape prepared with a water-soluble adhesive for administration.[13,14] The central lamina is approximately 0.19 cm thick and is formed by co-extrusion of the drug plus EVA to form a core matrix that functions as a dispersed matrix device. Both surfaces of the matrix are coated with a layer of EVA that renders the surface impermeable to drug (morantel tartrate). The edges of the core matrix can be coated but, in general, the edges are left uncoated. Drug release from the core matrix is achieved via the punching of a series of holes in the device that traverse the complete trilaminate. Under this configuration, drug is released from both the outer edges of the trilaminate and from the inner surface of each hole.

As configured the device is very resilient. Considerable force is required to roll the device into its final shape and, in turn, to retain it in this configuration during storage. This is achieved via a piece of tape that was specifically designed for this product.[13–14] The tape was composed of multiple layers, special water-soluble adhesives, and was also perforated to enhance the rate of water penetration once the device reaches the rumen. It is essential that the tape releases and permits the device to unroll within the first hour or two following administration to ensure that the device will not be regurgitated. For these reasons, the design of the tape was, in some ways, more difficult than was the design of the core matrix.

As noted above drug is released from this device via the incorporation of a series of holes that traverse the complete device, i.e. both the drug impermeable outer layers and the core matrix. Depending on whether the outer edges are coated or uncoated, drug may also be released from these edges. The kinetics of drug release from this device are novel. The release rate from the outer edges follows the typical $t^{1/2}$ kinetics as described by Higuchi.[50] The release from the perforated holes follows a model that can be essentially described as drug release from an inward-releasing cylinder. The equation necessary to describe the release profile was given by Lipper and Higuchi.[53] For the case of an inward-releasing cylinder, the surface area at the receding drug boundary increases with time. As drug release progresses a cylindrical surface is

formed at the zone of depletion that grows ever larger as the surface recedes. This factor should lead to an increasing drug release rate with time. However, simultaneous with this a drug depletion zone that is free of dispersed drug is created leading to an increase in the diffusional distance over which the drug must traverse prior to exit from the device. As noted by Higuchi[50] this factor leads to a declining release rate over time. Overall the release rate from a device where the release occurs only from the perforations can be described by the following equation[8,53]

$$M_t = N(\lambda^2 - n^2)L\pi\varepsilon\left[\rho - C_s + \frac{C_s}{2\left(\frac{h\varepsilon}{n\tau} + \ln\frac{\lambda}{n}\right)}\right] - \frac{NL\pi\varepsilon C_s n^2}{\left(\frac{h\varepsilon}{n\tau} + \ln\frac{\lambda}{n}\right)}\ln\frac{\lambda}{n} \tag{2}$$

and

$$t = \frac{\left(\frac{h\varepsilon}{n\tau} - \frac{1}{2}\right)\left(\lambda^2 - n^2\right)\rho\tau + \lambda^2\tau\rho\ln\frac{\lambda}{n}}{2DC_s} \tag{3}$$

where N is the number of perforations in the sheet, λ is the radius of the depletion zone, n is the radius of the perforation, L is the thickness of the core matrix, ρ is the density of the drug, C_s is the saturation solubility of the drug in the dissolution medium, and h is the diffusion layer thickness. This equation demonstrates that the total release rate is a function of the number and the diameter of the perforations and the usual parameters that control the rate of release from dispersed matrix devices. Several variables can be utilized to control the amount of drug released from these devices. For example, Figure 11 demonstrates the effects of the diameter of the hole on the overall release rate. From the shapes of the plots shown in this figure it is apparent that after an initial high release rate, the release rate is relatively constant with time. Furthermore, as the diameter of the perforation decreases the overall release rate is relatively more constant over time. The practical limit of the perforation diameter is about 4 mm in cattle since the holes tend to plug with rumen contents at hole sizes less than this value.

From a practical point of view, it is much easier to manufacture devices whose edges are not coated. Under these conditions the overall release rate will be a combination of the amount released from the perforations plus the amount released from the uncoated edges. The predicted amount released over time can be calculated from a linear combination of Eqs. (1) and (2) assuming that the zones of depletion do not overlap. The appropriate equation for the amount released is[8]

$$M_t = N(\lambda^2 - n^2)L\pi\varepsilon\left[\rho - C_s + \frac{C_s}{2\left(\frac{h\varepsilon}{n\tau} + \ln\frac{\lambda}{n}\right)}\right] - \frac{NL\pi\varepsilon C_s n^2}{\left(\frac{h\varepsilon}{n\tau} + \ln\frac{\lambda}{n}\right)}\ln\frac{\lambda}{n} + S\sqrt{\frac{D\varepsilon(2A - \varepsilon C_s)C_s t}{\tau}} \tag{4}$$

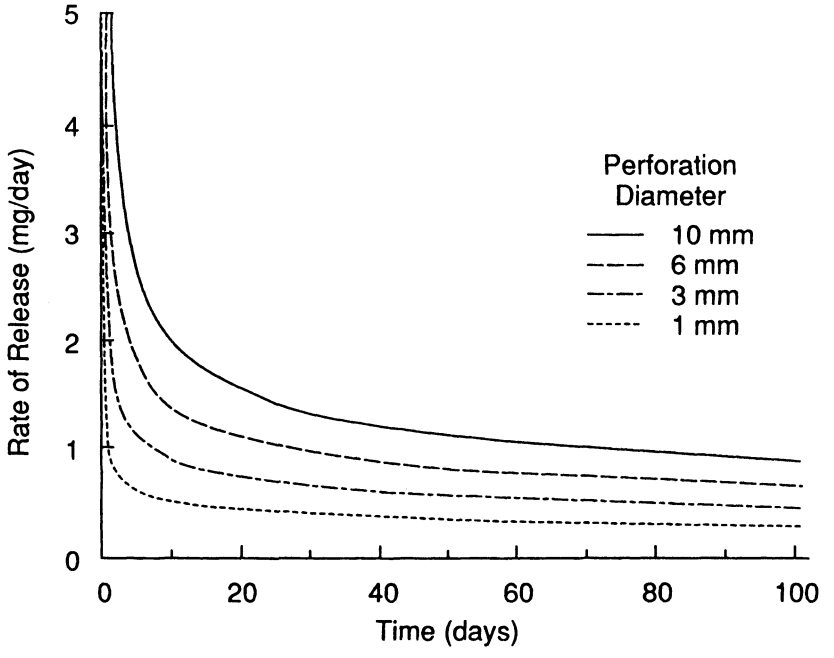

Fig. 11. Effect of hole diameter on the predicted rate of release of morantel tartrate from Paratect® flex diffuser assuming the device is 1 mm thick and with one hole.[8]

where S is the surface area of the perimeter edge. The value of t is calculated from Eq. (3). Utilizing this equation and Eq. (3), the predicted amount of drug released for a device with both edge release and release from perforations can be calculated. Utilizing Eqs. (2) and (3), the release from devices that contain only perforations can be calculated. This has been done in Figure 12 wherein the number of holes utilized in each calculation was chosen such that the calculated curves meet at approximately 90 days. While it is apparent that the overall release is somewhat more linear for the device with only the perforations, the shapes of the two curves are approximately the same. Figure 13 shows the amount released in vitro as a function of the number of perforations in the devices. Clearly the release rate increases and the duration of delivery decreases as the number of perforations increase. Figure 14 is a similar plot comparing the results obtained in vitro with those obtained in vivo. The agreement is excellent. This is not surprising given that the release rates are controlled by the diffusional processes occurring within the matrix that should be independent of the environment.

This technology has been commercialized and is sold in Europe and registered in the US under the trade name Paratect® flex diffuser. The clinical performance of this device was evaluated using trials[54,55] that were similar in design to those utilized for the Paratect® bolus described earlier. Typical results for the weight gain of treated animals versus controls are seen in Figure 15. As with the Paratect® bolus this product produces significant weight gains for the treated animals, especially when the levels of

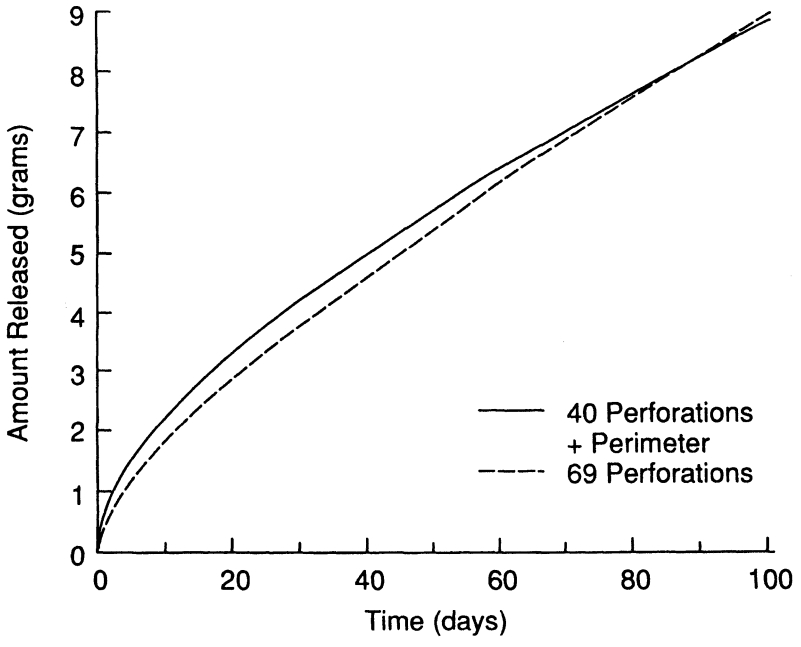

Fig. 12. Comparison of the predicted amount released versus time for the Paratect® flex diffuser made with coated and uncoated edges.[8]

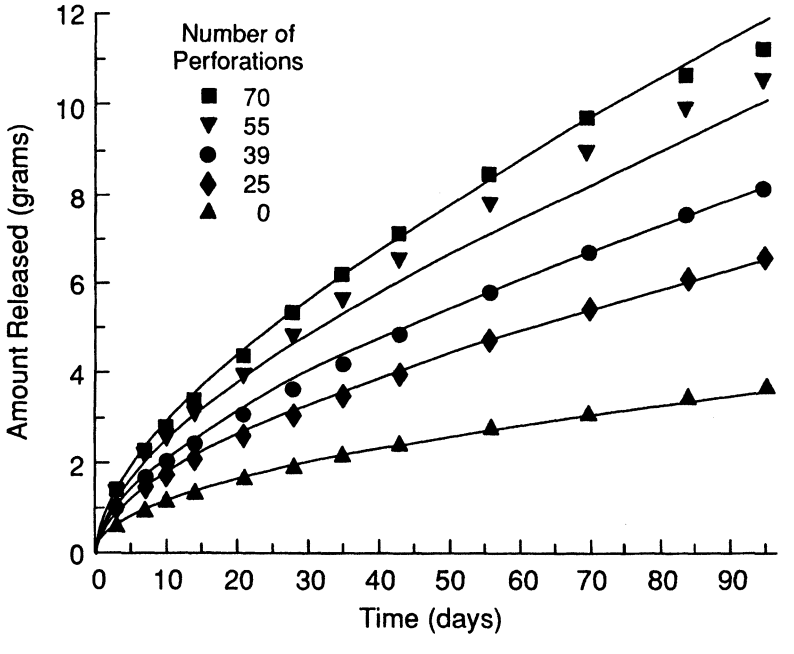

Fig. 13. In vitro and calculated amounts of drug released versus time for devices with 0, 25, 39, 55, and 70 holes.[8]

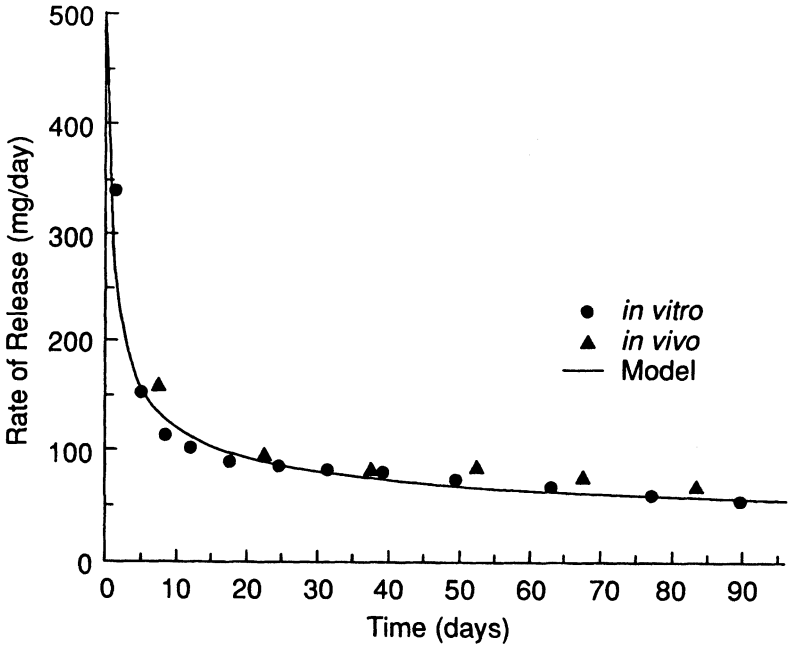

Fig. 14. In vitro/in vivo comparison of release of morantel tartrate from a device with 39 holes.[8]

infestation of the parasite are high. The clinical efficacy of the product against gastrointestional nematodes is shown in Table 7. The flex bolus offers the significant advantage that it does not impact the processing of the animals following slaughter. The Paratect® bolus had the disadvantage that the metal cylinder caused problems in rendering plants since the stainless steel cylinder is not removed by typical processes available for dealing with metals. In contrast, the Paratect® flex diffuser is made from a polymeric material that appears to degrade in the animals following the period of drug delivery. For example in an animal safety study, following the administration of devices to 45 cattle, ten animals were sacrificed at day 90 following administration of the device. Intact devices were found in all ten animals. However, at 12 months following administration, intact devices were found in only about 10% of the animals. Thus the Paratect® flex diffuser appears to remain intact through the delivery period but then degrades to smaller pieces that are ultimately eliminated from the animal during the period after release of the drug.

IV.D. Osmotic systems

As noted in the previous sections, erodible systems can be designed for either short or long duration products and, in general, their release rates are a function of both the nature and concentration of the drug in the system. Furthermore, their release rates are variable and, therefore, are best suited for actives that have wide therapeutic indices such as nutrients or mineral supplements. Reservoir and matrix devices provide very

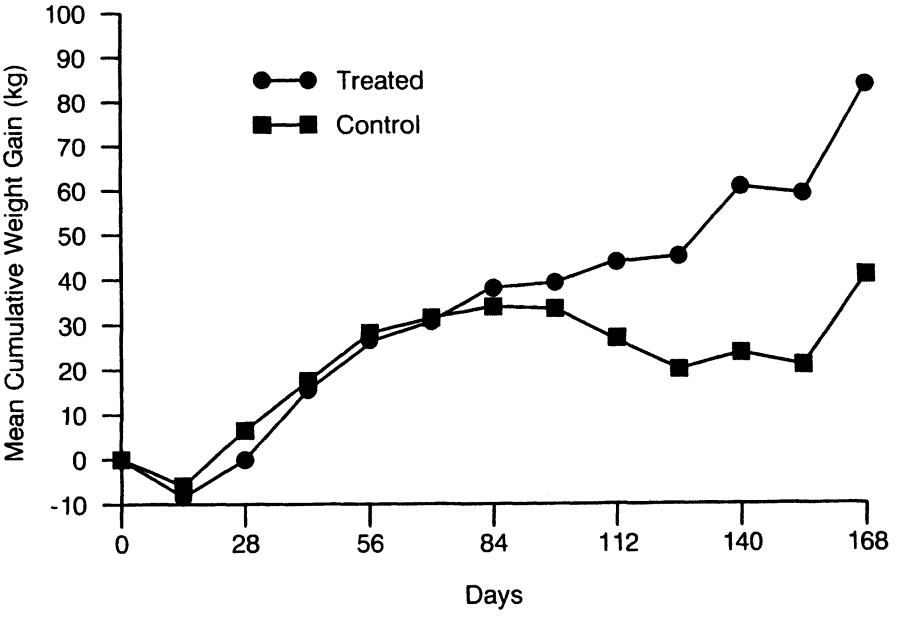

Fig. 15. Mean weight gain for treated and control animals for the Paratect® flex diffuser.[54]

good control of the delivery rate but the utility is generally limited to actives with relatively high aqueous solubility or low daily doses. Because of these limitations several groups have worked to develop ruminal systems where the release rate is largely independent of the aqueous solubility of the active and yet provides precise control of the delivery rate.

Early work by Thueewes and others established the utility of osmotic technologies to meet this goal. This group demonstrated that osmotic pressure based systems are effective for the delivery of both water soluble[56,57] and water insoluble compounds[58–60] for human oral applications. Their pioneering work on the OROS® and GITS (push–pull) systems established that the rate of water transport across a semipermeable membrane could be utilized as the principle determinant of the overall rate of active release from the drug delivery device.[60] Specific design characteristics of the GITS technology make it especially well suited for the delivery of highly water insoluble compounds and/or those with narrow therapeutic indices.

Table 7
Pooled efficacy data for the Paratect® flex diffuser

Parasite	Mean efficacy	Range
Ostertagia spp	70	63–80
Trichostrongylus axei	96	96–97
Cooperia spp	93	88–97
Oesophagostomum radiatum	96	86–100

The drug ivermectin is an example of a compound that has both of these attributes. The compound is highly insoluble in aqueous solution.[61] This drug is a highly potent and effective antiparasiticide useful for the treatment of both ecto- and endoparasites.[62,63] A system, termed the push–melt system, designed to provide treatment for a full grazing season has been developed through the cooperative effort of scientists at ALZA and Merck Animal Health.[61,64,65]

Early work by Egerton et al.[66] and Pope et al.[67] with the Alzet® Minipump established that a dose of ivermectin in the range of 12 mg/day protects fully grown cattle against a range of parasites including gastrointestinal nematodes, mange mites, sucking lice, cattle grubs, fly larvae and ticks. To ensure protection against these parasites for a full grazing season, a delivery duration of 135 days was established.

Figure 16 provides an outline of the primary design characteristics of the Ivomec SR bolus. The product is composed of a membrane cup that is extruded from cellulose acetate and various plastisizers. This cup is filled with an osmotic tablet that swells following imbibition of water through the membrane cup. Placed above the tablet is a partition layer, a drug containing layer, and a densifier. Both the partition layer and the

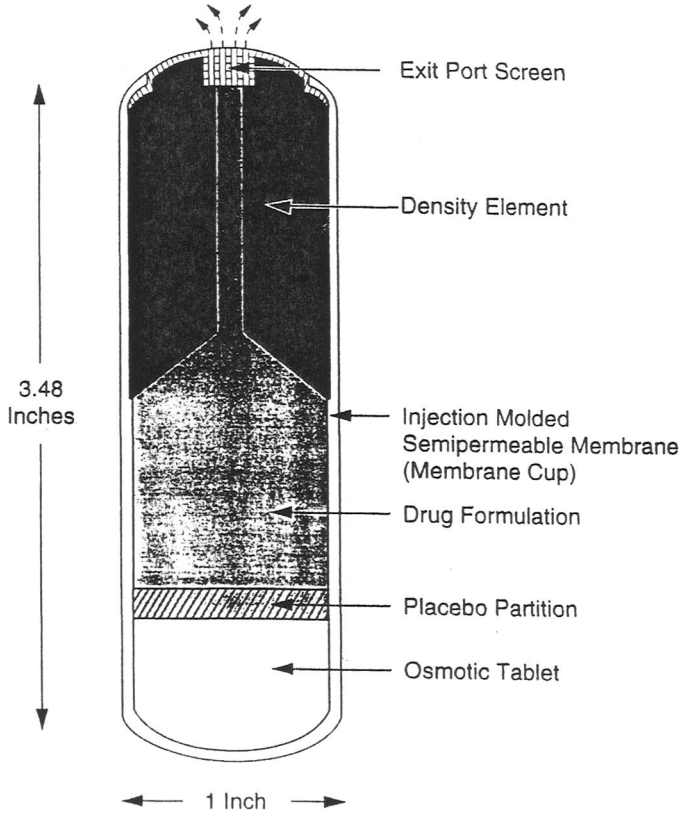

Fig. 16. Cross-sectional view of the IVOMEC® SR bolus.[61]

drug layer are prepared from paraffin waxes and Cab-O-Sil. Drug is suspended at a concentration of 22% in the drug layer. The softening point of the partition layer is somewhat higher than the drug layer so that the partition layer will basically serve as a piston to help direct the flow of the drug containing layer through the exit port channel located centrally in the densifier. The drug layer begins to soften in the range 31–35°C. Under these conditions the drug is maintained as a suspension during storage and delivery. The relatively high viscosity of the suspension prevents the drug from settling out during the delivery period. Also, since the suspension is below its softening point during storage, there is no concern with settling of drug during this period either. At temperatures greater than about 35°C the material has the requisite fluidity to flow through the exit channel and exit port screen. The central port in the densifier is covered with a capscreen made from plastic and designed such that the drug suspension is forced through a series of small openings in the capscreen.[68]

As noted above for the Paratect® bolus, metallic materials present in ruminal boluses may cause damage to the equipment utilized in rendering plants. For this reason, the density element was designed such that it would not damage this equipment. Several patents[69,70] describe the process utilized to manufacture this density element. In essence it was manufactured to provide a crush strength that was similar to that of bone. The process involves the sintering of iron filings to produce a highly porous material that is easily crushed. However, as noted below, the IVOMEC® SR bolus is hydrated in the package prior to release for sale. The high porosity of the metal also leads to a high potential for rusting of the metal during the hydration step. This requires that some means be incorporated to prevent rusting. A process for impregnating the density element with wax or other hydrophobic oils that help prevent corrosion of the density element during storage has been described.[70]

This system has several unique attributes that serve to substantially differentiate this system from those designed for human use. Some of these are implicit in the description provided above and are required due to the differences in the anatomy and physiology of ruminants. Several design features arose from observations of the release profiles obtained from earlier designs.[71] For example an early design was prepared without Cab-O-Sil® (a thickening agent) and the capscreen. A typical release profile from this type of device obtained in vivo is shown in Figure 17.[71] In Figure 17, the in vivo data was gathered using fistulated cattle and a collection device which sequestered the exit port and surrounding area from contact with ruminal fluids while permitting exposure of the portions of the bolus below the densifier to ruminal contents. In general boluses were collected at weekly intervals and the extruded wax collected and weighed to determine weekly outputs. From Figure 17, it is apparent that with this early design the average daily output varied dramatically from collection period to collection period. It was proposed that this highly variable release rate arose from the transport and subsequent expansion of dissolved gases into the device from rumen fluids. This proposal was consistent with the following three observations. First, devices placed in water, i.e. no significant source of dissolved gas, give relatively constant release profiles. Second, aberrant release behavior tends to occur at random times in any given bolus. This issue was felt to be associated with the random interaction of entrapped gas bubbles in the drug layer with the membrane.

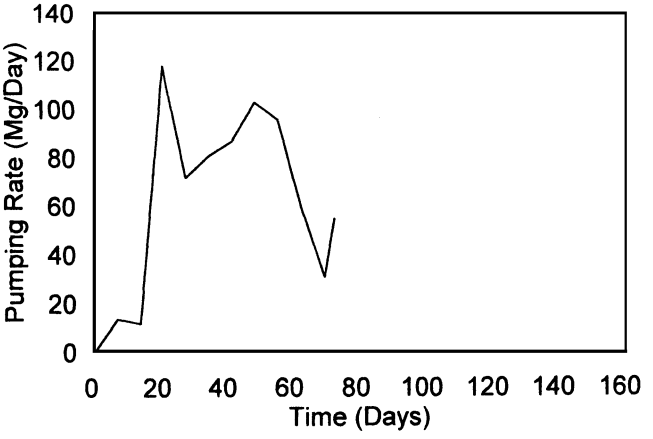

Fig. 17. In vivo rate of release versus time for early version of the IVOMEC® SR bolus. Device does not have the capscreen.[71]

Contact of a gas bubble with the membrane cup would be a prerequisite for gas transport to lead to a volume expansion. That is, the transported gas can only expand a preexisting void in the medium, it cannot create a void in the fluid due to the high cohesive energy density of the fluid. Third, the presence of dissolved gases in the rumen was an obvious source of the additional force that could lead to release rates that are significantly greater than those achieved from water transport.

The key to the final device design was to identify a means to prevent gas transport and expansion via the development of an internal pressure or resistance (frictional force) that was greater than the maximum pressure arising from transport of dissolved gas. This was accomplished through an increase in viscosity of the drug phase and the placement of a capscreen that effectively created and increased the frictional resistance within the bolus.[68,71–73] The viscosity of the drug phase was increased through the incorporation of Cab-O-Sil® as a filler. The capscreen effectively decreased the radius of the exit port thereby increasing the pressure drop across the interface as approximated by Poisuelle's law

$$\Delta P = \frac{Q 8 \eta L}{n \pi r^4} \tag{5}$$

where ΔP is the pressure drop across an interface, Q is the volume flow, η is its viscosity, n is the number of passageways, L is their length, and r is their effective radius. From this law one can infer that the frictional force can be increased in a linear fashion by increases in the viscosity and length of the tube. However, a decrease in the radius of the tube leads to a 4th power exponential increase in the pressure drop. With the capscreen in place the effective radius of the exit port decreased from about 0.5 to <0.1 cm which in turn led to a dramatic increase in the internal pressure. A common example of the effect of this type of change in the radius is the variations in distance that water can be sprayed from something like a garden hose by changes in the radius of the nozzle in the exit device.

The net effect of these changes on the release profile is shown in Figure 18. This figure provides data on the average daily output of drug from ten boluses placed either in vitro or in vivo with or without a prehydration step. Several points are of interest.

Irrespective of the prior treatment, all boluses provided a relatively constant release profile with a slight positive slope such that the average daily output increases from about 11 to about 13 mg/day over the duration of the study. The profiles show none of the aberrant behavior seen in Figure 17. These results confirm the hypothesis that an increase in the internal pressure prevents the high and variable release rates that arise from the transport of dissolved gases. The slight increase in the average daily output is thought to arise from two separate factors: (i) over time the osmotic gradient will decrease due to the dilution of the components of the osmotic tablet with water. This should decrease the average daily output over time; (ii) this effect is counterbalanced by a projected increase in the average daily output due to an effective increase in the average surface area available for water transport. The effective surface area for water transport must increase as the drug and partition layers move upward exposing more contact between the osmotic tablet and the membrane cup as drug suspension is delivered through the exit port.

Figure 18 shows the release curves for initially anhydrous boluses. Several observations can be made. First, for these boluses the onset of drug delivery from the devices is relatively slow compared to the results seen in Figure 17. This slow

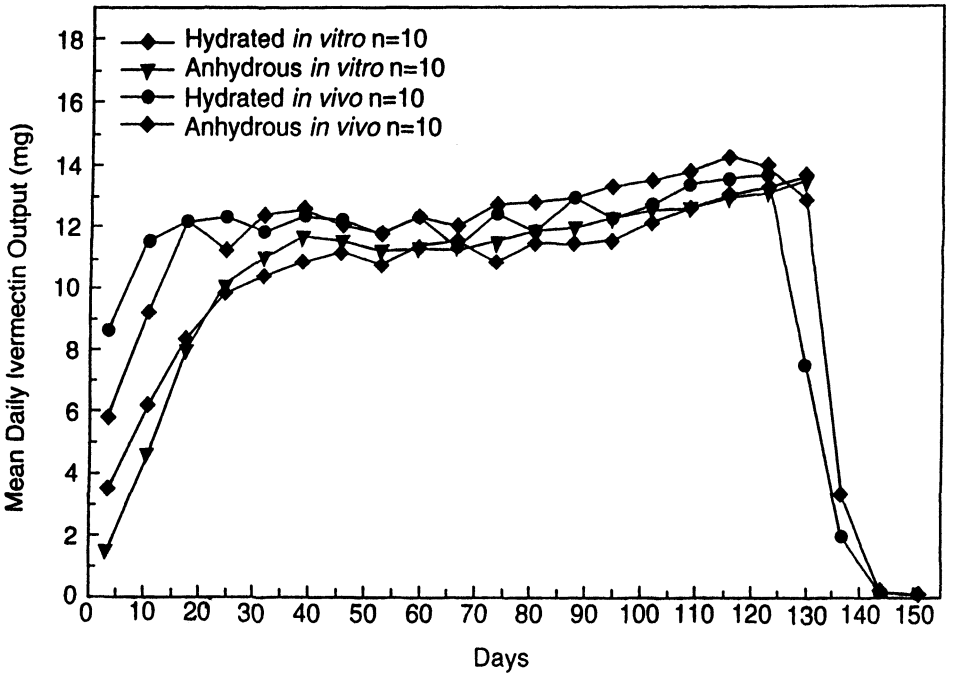

Fig. 18. Rate of release of ivermectin in vitro and in vivo from boluses hydrated with 1.3 ml of water and anhydrous boluses.[61]

onset arises due to the fact that a significant increase in the internal pressure of the bolus is required to overcome the frictional resistance caused by the capscreen and associated highly viscous materials. The increase in the internal pressure arises from the transport of water due to the osmotic gradients involved. Second, the onset of steady state delivery does not occur for about 3 weeks. This relatively slow onset is not desirable because of the slow onset of clinical activity that would likely follow. Third, from the legend it is apparent that the duration of delivery is somewhat greater than the established target of 135 days.

Because of the slow onset of delivery seen in anhydrous boluses, the aforementioned prehydration step was incorporated[71–73] wherein water is added to the package prior to administration. In general, boluses were hydrated for 60 days prior to testing. This prehydration of the boluses leads to a more rapid onset of delivery both in vitro and in vivo due to the increase in the hydrostatic pressure during the storage period. The duration of drug delivery for boluses prehydrated with 1.3 ml of water (Figure 18) is near the target of 135 days as determined by measurements of the drug contained in the wax output. The projected duration and profile of drug delivery for anhydrous or prehydrated boluses is nearly the same for boluses placed in vitro or in vivo. This consistency exists with essentially all data generated in the program. Also within a given lot, the coefficient of variation of the cumulative output was less than 5% which was independent of the site of the study. Thus a reasonable in vitro/in vivo correlation in the release profile exists. Shutdown of delivery from any given bolus occurred over a very short period, e.g. < 1 week and from any given lot of boluses e.g. < 2 weeks. This provided very effective control of the projected withdrawal period for the bolus.

The efficacy of this bolus against various internal and external parasites has been evaluated in trials conducted in both the US and international sites.[74,75] The results of this work has shown the IVOMEC® SR bolus is effective against established gastro-

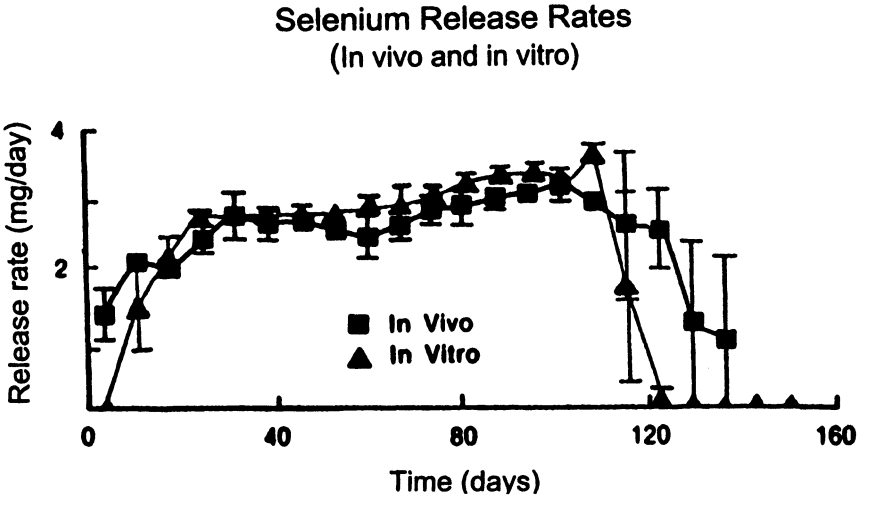

Fig. 19. Rate of release of selenium from push–melt ruminal bolus.

intestional and lung nematodes and will prevent the establishment of new infections for 135 days. Treatment with the bolus will also control established mange mites, sucking lice infections, and migrating grubs. For ticks, the bolus will interfere with the engorgement of blood and the completion of the life cycle. Thus the bolus is a highly effective product for the once per grazing treatment of the listed parasites.

This same push–melt system has been developed for the delivery of selenium to grazing cattle.[76] The system is similar in design to that described above except that the capscreen is not present, different waxes are used for the formulation and the system is not prehydrated. A typical release curve showing the in vitro and in vivo release rate for selenium from this system is shown in Figure 19. Note that the steady state release rate of approximately 3 mg/day is achieved after about 20 days of delivery and that approximately the same rate per day is seen both in vivo and in vitro. This system was compared in an in vivo study against an erodible system in the form of pellets that contain approximately 10% selenium.[77] The study demonstrated that the administration of two boluses, one on day 1 and one on day 120, leads to the effective delivery of this nutritional supplement for the proposed period and produced significantly higher plasma levels of selenium than was achieved with the pellets or untreated controls.

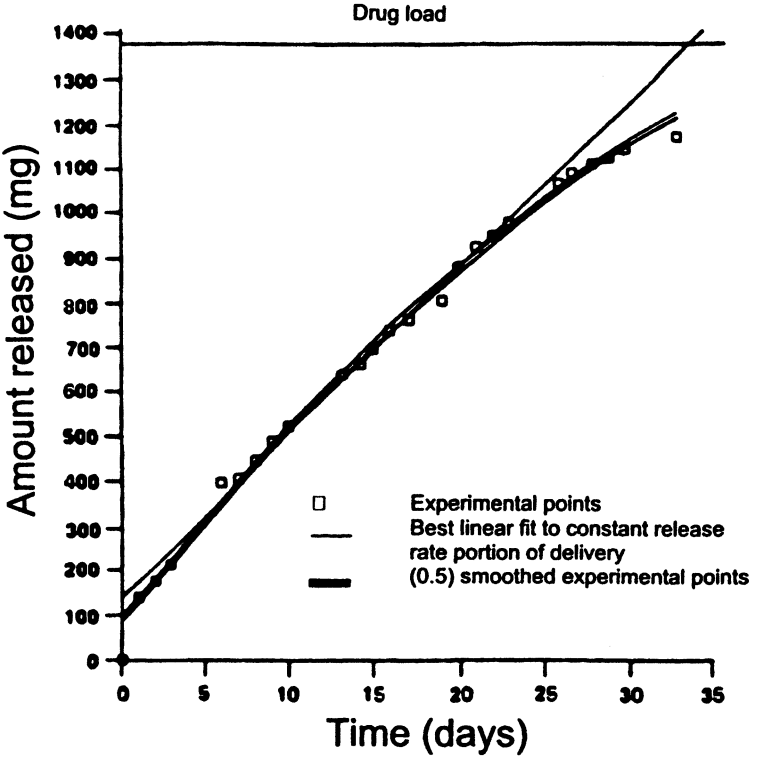

Fig. 20. Release of CP-53,607 from modified Paratect® bolus. The device contains 6 g of polyethylene oxide 600 pellets and 28 ml of a 5% solution of CP-53,607 in octyl alcohol. One membrane is sintered polyethylene; the other is impregnated with gelled cellulose triacetate and polyethylene glycol 400.[78]

Another example of an osmotic pressure based ruminal bolus is found in the work of Thombre et al.[78] Using a device similar in overall design to that of the Paratect® bolus,[47,48] Thombre et al. developed a novel osmotic technology suitable for the long-term delivery of drugs wherein the drug is delivered as a solution in oil. This system utilized both the cylinder and membranes of the Paratect® bolus as described earlier, however, the membranes and core formulations were suitably modified to convert the overall mechanism of drug delivery from diffusion control to an osmotic based mechanism.

In this system the core formulation is composed of an oil such as octanol, isopropyl myristate, or soybean oil and a swellable polymer such as PEG 600 or polyvinyl alcohol dispersed as pellets within the oil. The drug is dissolved within the oil phase. As with the Paratect® bolus, one microporous polyethylene membrane is impregnated with cellulose triacetate and wetted with PEG 400. The other microporous membrane is either unimpregnated or treated with various other options to permit the membrane to be permeable to oil.

Figure 20 shows some typical in vitro release data for a system prepared from 28 ml of a 5% solution of CP-53,607 (an ionophore with potential as a growth promotent in ruminants[79]) containing 6 g of pelletized PEG 600. One microporous membrane disk

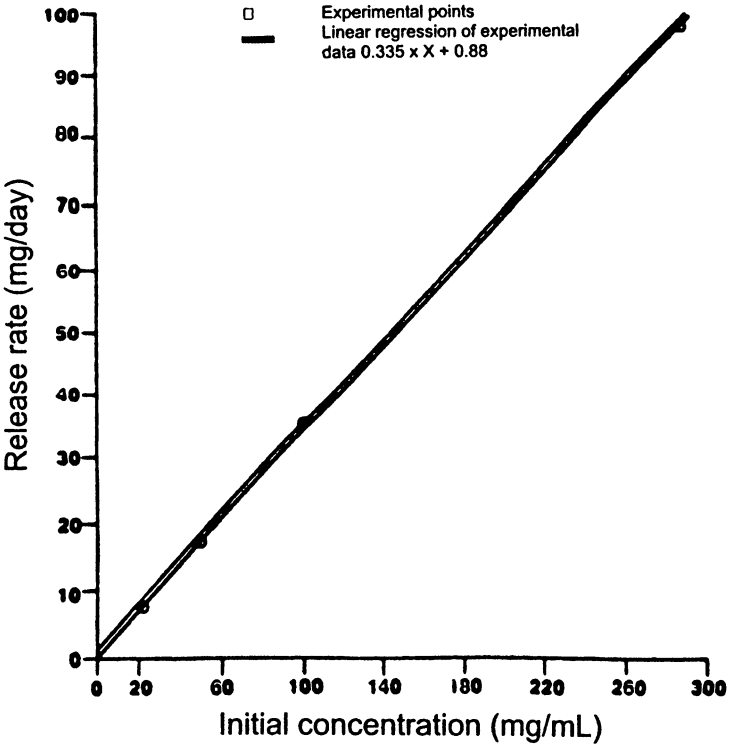

Fig. 21. Drug release rate versus initial drug concentration for the modified Paratect® bolus. The slope of the line is 0.34 ml/day and represents the volumetric flux of solution from the device.[78]

was impregnated with cellulose triacetate while the second was an unimpregnated microporous disk. Note that there is a 'burst effect' arising from release of drug initially associated with the unimpregnated disk. Following this burst, the release rate is nearly constant through >70% of the total delivery period. Figure 21 is a plot of the steady state release rate versus the initial drug concentration for a series of devices prepared with varying initial drug loads. Note the linearity of the plot. The slope of the line is 0.34 ml/day and represents the volumetric flux of fluid from the devices. This result is consistent with a mechanism where the rate of water transport across the cellulose triacetate membrane controls the overall release rate from these devices. Figure 22 provides further support for this where we see that the normalized percent drug release is independent of the nature of the oil used in the device preparation. Thus the overall rate of drug release from these devices will be dependent only upon those factors which control the water transport rate, i.e. the membrane permeability and surface area, and the activity of the swelling polymer. The described devices should have broad utility for the delivery of water insoluble/oil soluble compounds.

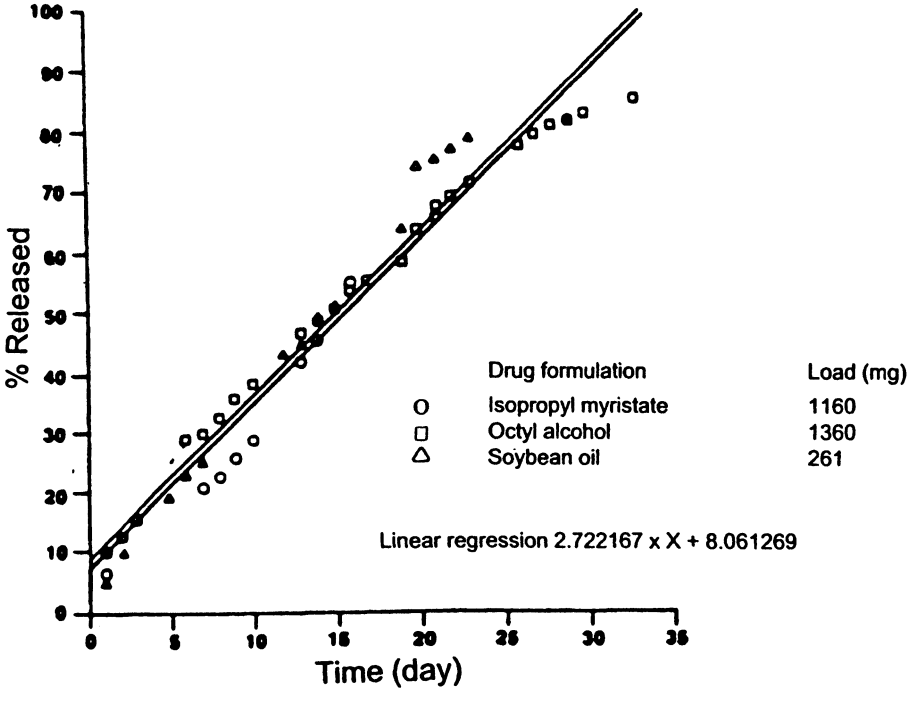

Fig. 22. Percent release versus time for the modified Paratect® bolus where various saturated solutions of CP-53,607 in different solvents were evaluated. The data demonstrate that release from this device is independent of the solvent.[78]

V. Conclusions

As with devices designed for human oral applications, numerous approaches have been developed for the controlled delivery of drugs to the reticulo–rumen cavity of ruminants. The duration of effective delivery from these systems ranges from a few days to approximately 6 months. The mechanistic approaches that have been tried for human oral systems have also been applied to the devices for ruminants. The distinguishing difference is duration of delivery and the need to incorporate a mechanism for the retention of the device in the cavity for that duration of delivery. The future challenges include the need to identify more predictable delivery profiles at lower overall cost of product manufacture. Ultimately these factors will govern the success of these systems in the marketplace.

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CHAPTER 4

Design parameters for post-ruminal drug delivery systems and rumen-stable products

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Overview

I. Introduction

Competitive livestock industries and concerns for animal well-being underlie the need for drug and nutrient delivery systems for ruminants that protect active ingredients from ruminal fermentation. In addition to delivering drugs or nutrients directly to the small intestine for absorption, commercially viable delivery systems must meet safety and cost criteria. Compared with products developed for human use, cost constraints have impeded the development of effective postruminal delivery systems and rumen-stable products. This chapter outlines both the physiological and technical considerations encountered in the design of effective post-ruminal delivery systems. The formulation strategy for a pH-dependent rumen-stable coating system will be discussed, as will methods for in vitro and in vivo evaluation of post-ruminal delivery systems. Before proceeding to these topics, it is necessary to discuss how the ruminant digestive system differs from that of a simple-stomached animal and how these differences affect the design of post-ruminal delivery systems.

II. Need for post-ruminal drug delivery systems and rumen-stable products

Ruminant animals, such as cattle, sheep and goats, are major sources of meat, milk, wool and leather and are important contributors to the agricultural economy. Ruminants play a particularly important role in food production because they utilize cellulose and non-protein nitrogen which are abundant in nature (straws, grasses, etc.) and which are either indigestible or are poorly utilized by other domestic animals and humans. Their ability to digest fiber is a function of their digestive physiology and a symbiotic relationship between the host animal and ruminal microbes including bacteria, protozoa and fungi.

Because of ruminal fermentation, some major developments in nutrition and veterinary science for simple-stomached farm animals cannot be applied directly to ruminants. For example, diets for poultry and swine have been routinely supplemented with limiting amino acids to improve productivity and feed efficiency. Dietary lipids are provided for poultry and swine to increase the level of some fatty acids in the tissue. Although highly productive ruminants may benefit from amino acid supplementation, direct addition of amino acids to the diet is not practical because ruminal microbes will utilize these amino acids before they reach absorption sites in the small intestine.[1] Ruminal fermentation may also destroy or modify nutrients and drugs administered to prevent or treat disease. For example, unprotected glucose or starch given orally to prevent or treat ketosis will be almost completely metabolized to short-chain fatty acids in the rumen. To be efficacious, potent growth promoters which improve feed efficiency must be delivered intact to the absorption site in the small intestine of the host animal, but these proteins will be digested by the microbes without adequate ruminal protection. An effective post-ruminal delivery system is a prerequisite to implementing some significant advances in animal nutrition and health in ruminants.

III. An overview of the ruminant digestive system

III.A. Digestive physiology

Unlike the single compartment of the human or pig stomach, the ruminant stomach has four compartments: the reticulum, rumen, omasum and abomasum (Figure 1).[2] The rumen and the reticulum are often considered together as the reticulorumen because they fulfil similar functions and are separated only by the reticuloruminal fold which permits free exchange of digesta between the two compartments. Almost all of the pregastric fermentative activity and absorption of the end products of this fermentation occurs in the reticulorumen.[3] The end products of anaerobic fermentation, largely acetate, propionate and butyrate, are important sources of energy for the animal. Greater detail on the importance and function of pregastric fermentation that enables ruminants to digest fiber without secreting fiber-degrading enzymes themselves and to utilize non-protein nitrogen is provided in the following references.[2-4]

Ruminal microbes are adapted to live at pH ranging from 5.5 to 7.0[5] but ruminal pH may be as low as pH 4 in animals with acidosis. Diet significantly affects ruminal pH. The ruminal pH of grain-fed ruminants such as feed lot cattle are at the lower end of the range (pH 5.5–5.8) while the pH of forage-fed animals typically exceeds 6.2.

Although the omasum is the least well understood of the four compartments of the ruminant stomach, it apparently filters coarse particles from passing to the lower gastrointestinal tract and absorbs water.[3] In contrast to the earlier theory that the

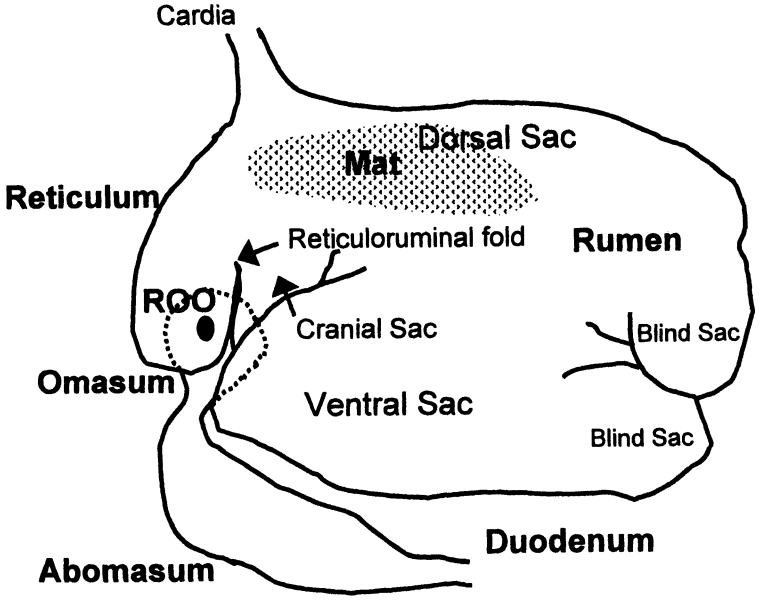

Fig. 1. Diagram of the ruminant stomach showing the compartments and important structures. ROO, reticulo-omasal orifice.

size of the opening of the reticulo-omasal orifice regulated the exit of large particles from the rumen,[6] more recent research has demonstrated that the opening of the orifice is too wide to act as an effective impediment to movement of particles ≤4 mm in cattle or ≤2 mm in sheep, the maximum size normally allowed to pass to the abomasum[7]. Estimates of the maximum size of the opening of the reticulo-omasal orifice have ranged from 10 × 45 mm[8] to 40 × 60 mm in cattle[9] while the opening in sheep is from 10 × 20 mm.[10] That the orifice is open 60–70% of the time in cattle is another indication that the reticulo-omasal orifice is not the primary regulator of passage of particles to the lower gastrointestinal tract.[10] The evidence that fluid flows in both directions through the omasum[11] has led to hypotheses that the reticulo-omasal orifice and the reticular groove regulate backflow to the reticulum. It is clear that sorting mechanisms other than the orifice itself are at work to prevent the passage of large particles to the abomasum.[7,10]

The functions of the abomasum are generally similar to those of the gastric stomach of other mammals. Secretion of hydrochloric acid maintains abomasal pH at approximately 2[5] so that there is a rapid decrease in the pH of the digesta as it moves from the reticulorumen and omasum to the abomasum despite the short abomasal residence time of 1–2 h.[3] This sharp reduction in pH provides the basis for pH-dependent controlled release strategies for abomasal delivery.

As in simple-stomached animals, the small intestine in ruminants is the primary site of digestion and absorption. Although the chyme is acidic as it leaves the abomasum, it is returned almost to neutrality by buffers secreted from the mucosa of the small intestine within a short distance of the pylorus.

III.B. Ruminal retention time

In the ruminant digestive system, understanding the dynamics of particle movement along the gastrointestinal tract is essential in the design of an effective post-ruminal delivery system. To effectively deliver solid active ingredients postruminally, the retention time of particles in the rumen and physical damage to encapsulated particles by rumination must be minimized. It is essential to consider particle dynamics in the rumen and optimize particle size, shape and specific gravity of the encapsulated particles.

Ruminal retention time depends not only on the feeds themselves, but also on the animal to which the feed is offered and, to a lesser extent, on the environment in which the animal is housed.[12,13] Ruminal nutrient availability is dependent on both digestion and passage rates (Figure 2). Ruminal digestibility of feed offered to a predominantly forage-fed water buffalo weighing 800 kg will be higher than the digestibility of the same feed in the rumen of a high-producing dairy cow weighing 600 kg. The rate of passage of digesta in the water buffalo will be slower than that of the dairy cow because of differences in diet, feed intake and animal size although the microbial digestion rates of the feeds probably will be similar. Longer rumen residence time increases ruminal availability, an advantage for fiber digestion but a disadvantage for ruminal protection. Ruminal availability of nutrients (R_d, kg/d) can be predicted using the following equation

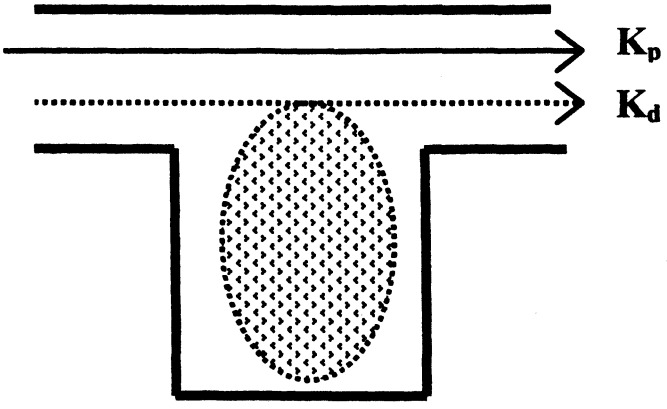

Fig. 2. Schematic showing relationship between rates of digestion and passage in determining ruminal availability. K_d and K_p are the fractional digestion and passage rates, respectively.[13]

$$R_d \text{ (kg/d)} = \text{Intake (kg/d)} \times (K_d/(K_d + K_p)) \quad (1)$$

where K_d and K_p are the fractional digestion and passage rates, respectively.[13] To accomplish the goal of post-ruminal delivery, the rate and extent of ruminal digestion of the protected compound must be minimized without affecting its intestinal availability or the digestibility of other fractions of the diet. This can be accomplished through manipulation of passage and digestion rates. It is difficult or undesirable to alter some of the variables that affect passage rate such as animal size, level of intake, and physiological status (e.g. lactation or pregnancy), but several feed characteristics which affect passage such as particle size and specific gravity can be manipulated.[14]

III.C. Anaerobic fermentation, fiber digestion and protein metabolism

Although there are many examples of non-ruminant herbivores such as horses, hippopotami, pandas and elephants, many of these animals compensate for their limited ability to extract energy from fiber by eating a large volume of forages.[3] Alternatively, smaller herbivores such as rodents and lagomorphs are highly selective in the feeds that they consume. In contrast, ruminants rely on pregastric fermentation to provide the fibrolytic enzymes needed to degrade plant material high in cellulose and hemicellulose to meet much of their energy requirement.

More than 99.5% of the ruminal microbes are strict anaerobes so that fermentation yields gas which is eructated, short chain fatty acids which provide energy to the host animal, and microbial mass. The 'bodies' of the ruminal microbes are used as sources of protein by the host animal. When ruminal microbes pass to the intestine from the forestomach and the abomasum, they are digested by enzymes produced by the host animal. The resulting amino acids and short peptides are absorbed to meet the animal's protein and amino acid requirements. As much as 3 kg/day of microbial protein has been produced daily by lactating cows[15] but, with poorly formulated rations, this number may be less than 0.9 kg daily.[16] The degree to which a feed provides the

profile of amino acids required by the animal is estimated by the amino acid index with a perfect match yielding a score of 100. In a study of the quality of protein supplements frequently fed to ruminants, the amino acid profile of ruminal microbes was 82 while the nearest competitor, soybean meal, had an index of 71.[17] Until the large, recent gains in animal productivity, ruminant nutritionists placed little emphasis on the adequacy of the amino acid supply because it was assumed that the microbial amino acids would meet the animals' requirements. Milk production by dairy cattle has increased dramatically in the last 30 years so that some herds of dairy cows now average more than 14 000 kg per lactation.[17] We can no longer rely on the studies conducted in the 1960s which showed that microbial protein could meet all of the amino acid requirements of lactating cows because the studies were conducted with cattle producing 4500 kg of milk annually.[17] At high levels of production, the assumed adequacy of the microbial amino acid supply is often not valid. To remedy this deficiency, farmers offer their animals more high protein feeds and are interested in supplementation of specific amino acids, especially lysine and methionine.[18]

Both economics and environmental concerns have stimulated interest in increasing the efficiency with which nitrogen (N) is used. If the amounts of N and amino acids supplied closely approximate the animal's requirements, N excretion into the environment will be decreased and farmers will be able to meet regulations proposed by the Environmental Protection Agency and other environmental regulatory groups.[19]

As we consider development of post-ruminal delivery systems, the active ingredient must be protected both from ruminal microbial activity and from physical disruption due to chewing. The rumen microbial ecosystem is complex with organisms with diverse enzymatic capabilities inhabiting varied niches. The actual number of species of ruminal bacteria is unknown as many new species are now being found as molecular biological techniques replace older culture-dependent methods.[20] Fungi and protozoa are also present in addition to the bacteria. The ability of the ruminal microbes to degrade starch, crystalline and amorphous cellulose, hemicellulose including xylans, pectin, lipids, protein, and sugars has been well documented.[21] Some of the ruminal bacteria can degrade phenolic monomers and hydrolyzable tannins even under the strictly anaerobic conditions of the rumen.[22–24] With this broad range of enzymatic activities and the possibility of synergism[25] among members of the microbial consortium, developing protective coatings that can protect active ingredient(s) from ruminal degradation poses a difficult challenge.

III.D. Rumination

Avoiding degradation by the ruminal microbiota is not the only challenge faced by a rumen-protected particle as it travels through the reticulorumen. Chewing during eating and rumination probably is the main cause of the premature release of the active ingredient from protected particles. In addition to the 4–6 h devoted to eating, forage-fed ruminants typically spend 7–9 h/day ruminating or cud-chewing to break down coarse fiber into small enough particles to permit passage from the reticulorumen. Thus, the protected pellets are often 'at risk' of destruction by chewing for more than half of the day. In both sheep and cattle, almost all reduction in particle size

occurs in the rumen.[26,27] When steers, lactating cows and young bulls were fed a variety of diets including pasture, long hay, chopped hay and grain, particles in the omasum ranged from 0.26 to 0.70 mm whereas those in the feces were 0.24–0.63 mm.[27] Almost 50% of the dry matter in the duodenum consisted of material that did not sediment after centrifugation for 30 min at 25 000 × *g*, strong evidence that the gastrointestinal tract is a small particle system after the rumen.[28]

During ingestion, minimal mastication occurs and feeds are mixed with just enough saliva to lubricate the feed to permit swallowing. Unpublished data from Welch's laboratory show that the hazard to the controlled release pellets is minimal at this stage with less than 5% of the pellets being destroyed during eating (Welch and Wu, unpublished data). When the recently-ingested feed arrives in the upper part of the rumen near the reticuloruminal fold, it is slowly mixed with the ruminal contents in the mat or raft of the rumen. The mat is a mass of floating digesta present under some dietary conditions in the dorsal rumen.[29] Most large particles in the rumen are in the mat,[30] but it also contains small particles. During the mixing process, coordinated reticuloruminal contractions continually force ruminal fluid containing approximately 10^9–10^{10} microbial cells/ml[21] up through the mat of digesta. As the contractions subside, the level of the ruminal fluid subsides. The continual cycle of mixing newly arrived long particles with the rest of the digesta and the vertical rise and fall of the rumen fluid ensures that all of the ingesta are repeatedly rinsed with microbe-rich ruminal fluid.

After ingestion, there is a sorting phase in which longer, lighter particles with a relatively low specific gravity are moved toward the dorsal sac of the rumen where they are likely to be regurgitated and ruminated.[11,30] Small, dense particles accumulate in the ventral rumen and the reticulum. Rumination boluses are formed from material in the dorsal sac and returned to the mouth. After intense lateral chewing by the molars and remoistening with saliva for about 1 min, each bolus is reswallowed. The 7–9 h of rumination per day poses a major hazard for coated particles. This danger can be averted if the particles are heavy enough to avoid being caught up in the rumination bolus, but they must not be so heavy that they sink to the bottom of the reticulorumen with a low probability of passage to the lower gastrointestinal tract. Adequate protection of particles in the rumen requires both protection from microbial degradation and from chewing to avoid premature release of the active ingredient(s).

IV. Ruminal protection, physical feed characteristics and ruminal dynamics

IV.A. Terminology and methodology

In the following section, the importance of the physical attributes of protected particles will be discussed. According to Kaske and Engelhardt,[31] particle size and specific gravity accounted for 28 and 59% of the variation in retention time of plastic particles in the reticulorumen of sheep. Given the importance of specific gravity on ruminal retention, the terminology used to describe specific gravity must be clarified. Specific

gravity is the weight of a substance divided by the weight of an equal volume of water at a specified temperature (usually 20°C). Unlike density (measured in mass per unit of volume), specific gravity is a ratio and thus dimensionless. When true specific gravity measurements are made, it is assumed that all gas- or air-filled voids in the material being measured are either eliminated or filled with the displacing solution. Functional specific gravity measurements are made with internal pore spaces intact and filled with gases or air. In the rumen, movement of particles is influenced by the density of particles with air and gas pockets, not by the true specific gravity of the forage. In most cases, protected pellets do not have large voids or gas pockets, so their true and functional specific gravities are similar, but this is not the case for forages. Initial functional specific gravity of dried forages usually is less than 0.8 and may increase to approximately 1.5 after 48 h of hydration[30,32] whereas the true specific gravity of the insoluble carbohydrates in forages exceeds 1.5.[33,34] The distinction between true and functional specific gravity is important as changes in functional specific gravity have profound effects on passage from the rumen.

Several papers have been published on the two basic approaches used to measure functional specific gravity of feed and digesta samples, pycnometers and flotation.[32,35,36] The pycnometric approach involves use of specialized bottles with constant volumes. The weight of water-filled pycnometers is compared to that of bottles filled with a known amount of sample and water at a controlled temperature.[37] The flotation method[36,38,39] involves mixing solutions of different specific gravities (usually 0.9–1.4). Sample is introduced into the solution with the lowest specific gravity and allowed to equilibrate. The fraction of the sample which sinks is recovered and placed in the next solution while the fraction which floats is dried and weighed. Using this approach it is possible to determine the fraction of the original sample over or under a given specific gravity.

IV.B. Particle dynamics, particle size and specific gravity

Most of the data on the effects of specific gravity on ruminal passage and digestibility have been obtained by feeding inert pellets (usually plastic or rubber) and recovering them either from the duodenum or the feces.[31,40,41] By varying the types of plastics used (polytheylene, polypropylene, nylon, acetal, polyvinylidene fluoride and teflon[40]) or by mixing barium sulfate with plastic,[31] the range of specific gravity can be varied from less than 1 to more than 2. Although recovery of the particles from the feces or digesta is laborious, it is possible to distinguish particles that have been masticated from those which have not been chewed.[42] This provides a good index of whether protected particles would have been destroyed by chewing, causing premature release of the protected compound. For studies of ruminal retention time of forages, plastic particles have the disadvantage that their specific gravity is constant while that of forages changes.[32,35] However, this is an advantage for evaluation of post-ruminal delivery systems with specific gravities that do not change due to ruminal exposure.

Data from sheep, beef cattle, water buffaloes and lactating dairy cows[31,42,43] all generally agree with the early results of King and Moore[44] showing that particles with a specific gravity of approximately 1.2 or more are less likely to be retained in

the rumen and be ruminated than are those which are lighter. Particles with a specific gravity of 1 or less are prone to rumination and are unlikely to pass from the rumen.[42] In cases when the specific gravity exceeds 1.5, the data are somewhat less clear. Particles with a specific gravity of 1.77 or higher remained in the rumen of forage-fed steers longer than those with a specific gravity less than 1.4 when pellets with specific gravities of 0.90, 0.96, 1.17, 1.42, 1.77 and 2.15 were fed.[42] All of the particles in this study had a diameter of 0.16 cm and were 0.5 cm long. In this study, it is was assumed that the heavy particles sank to the bottom of the reticulum and remained there. However, recovery of particles with specific gravities of 1.45 and 1.60 in dairy cows were similar (Welch, Pell and Wu, unpublished data; Figure 3). The threshold specific gravity for increased ruminal retention has not been clearly established and likely varies depending on the ruminal environment.

Recoveries of plastic pellets that have not been chewed from the rumen and omasum of four dairy cattle fed diets containing haylage, corn silage and commercial concentrate were similar to recoveries of ruminally protected lysine and methionine pellets with similar specific gravities (1.32) and particle size (2 mm) (Welch, Pell and Wu, unpublished data). However, when similar experiments were conducted with sheep fed hay and 100 g of concentrate, less than 30% of the plastic pellets fed were recovered in an identifiable form. These data underscore the need to evaluate rumen protection systems in different species of animals and in animals in different physiological states to ensure that the ruminal protection is adequate. Development of

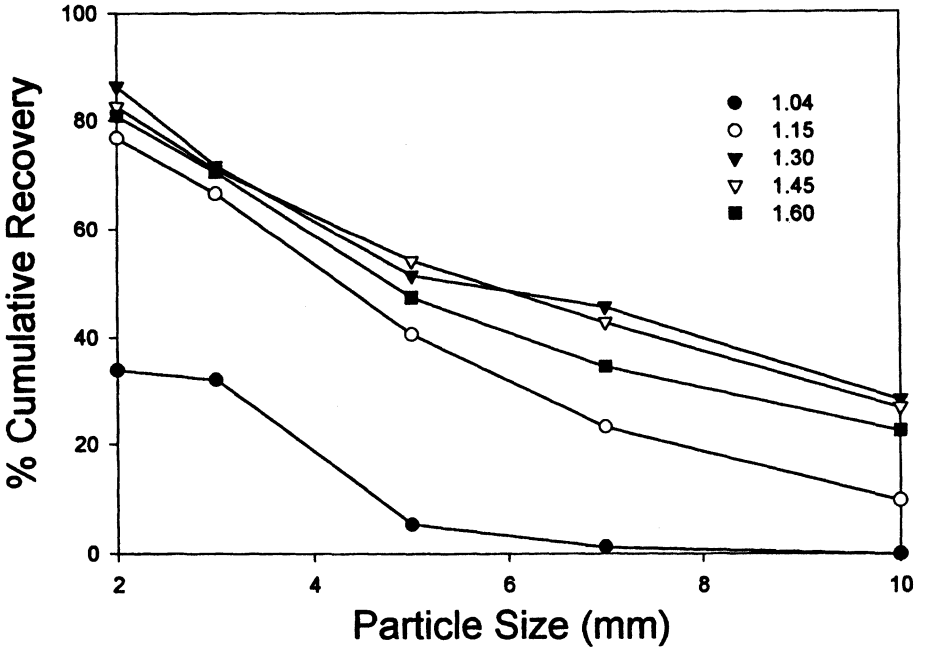

Fig. 3. Fecal recovery of non-ruminated particles of different particle sizes and specific gravity from dairy cattle 3 days after administration of particles.

effective post-ruminal delivery systems for sheep remains problematical because they ruminate their feed more extensively than do cattle causing destruction of the protected pellets in the rumen.

Based on the published data on specific gravity, the movement of particles through the gastrointestinal tract appears to be relatively simple: protected particles must be small and dense to maximize passage from the rumen and to minimize rumination. Unfortunately, the situation is considerably more complex. Ruminal retention time is variable depending on the size and physiological status of an animal. Fractional passage rates of 0.04–0.06/h which correspond to ruminal retention times of 16–24 h often are assumed for lactating dairy cattle but observed fractional passage rates vary from 0.01 to 0.19/h (ruminal retention time of 5.5–100 h) for specific feeds.[45] This wide range is due to both feed and animal variation, both of which are likely in field situations.

Although few large particles escape the rumen intact, the effects of dietary particle size on passage are varied. Sieving has been the primary means of measuring particle size[46] but, depending on the aspect ratio of the particles, particles as long as 5 mm passed through a 1.18 mm sieve.[47] Methodological problems are only part of the explanation, however. In a series of studies in which plastic particles were fed to beef and dairy cows that were fed diets typical of the feedlot and dairy industries, particle size was shown to be a more important determinant of passage in the dairy cows (Figure 4). The higher forage content of the dairy diet permitted formation of a more defined mat which modified the rate at which the plastic particles moved through the rumen.

When the physical characteristics of particles in the rumen are examined, 60–70% of the particles in the rumen have the particle size and specific gravity that makes their passage likely.[47] Entrapment of particles in the mat or raft of the rumen (Figure 1) must be included in the simple particle size-specific gravity model to predict ruminal passage.[11,14,48] Mat consistency affects the speed with which small particles work their way through the mat toward the reticulo-omasal orifice and exit from the reticulorumen.[14] Diet is an important factor in determining mat consistency[14,39] which may be determined by measuring the time required for a weight to ascend through the digesta in response to a counter-balancing mass.[14,49] The ascension time, measured 2 h after the morning feeding, of a 454 g weight from the bottom of the rumen varied from 21 s in steers fed alfalfa pellets to 900 s in the same animals fed long grass hay.[14] Intermediate values of 201 and 131 s were observed for animals fed corn silage or a high grain diet, respectively. Thus, the ruminal retention time of a small particle 'eligible' for passage but trapped in the mat differs greatly depending on diet composition. This has very practical implications in rumen protection schemes: systems that function well in animals fed high grain diets may work less well when fed to animals on high forage diets. In forage-fed animals, the protected particles may be retained in the mat increasing the probability of rumination, rather than sinking through the reticulum and passing quickly to the lower gastrointestinal tract.

The factors that determine the consistency and permeability of the mat are poorly understood. The specific gravity and buoyancy of feed, particle size and shape or aspect ratio all are involved. Different forages break down into particles of different

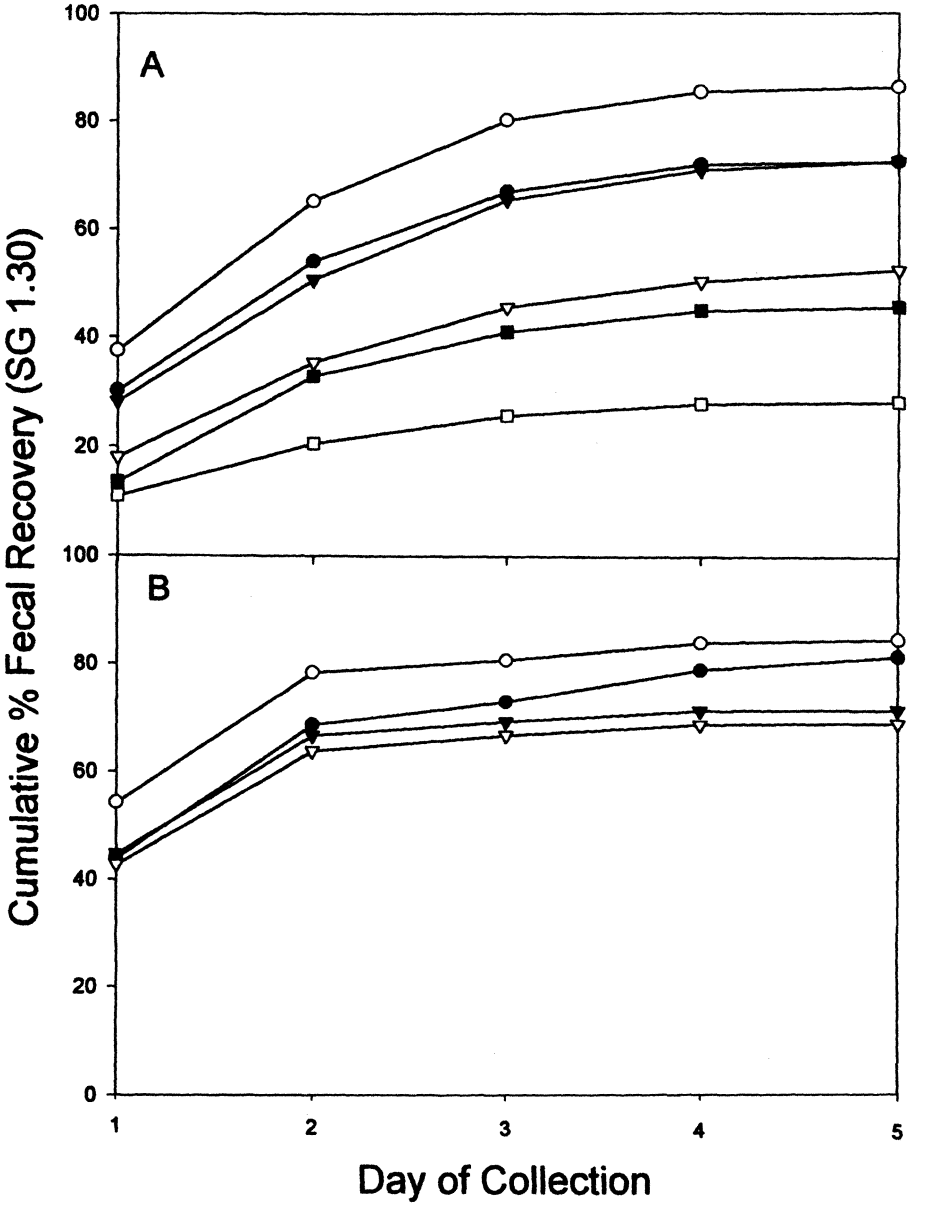

Fig. 4. Effect of particle size on fecal recovery of non-ruminated pellets with a specific gravity of 1.30 fed to lactating dairy cows (panel A) and growing steers (panel B). Symbols for particle sizes are as follows: 1 mm, ●; 2 mm, ○; 3 mm, ▼; 5 mm, ▽; 7 mm, ■; 10 mm, □. The 7 and 10 mm particles were not fed to the beef animals.

shapes with grasses tending to form long, needle-like particles while legumes are broken into round, stubby particles.[3] Differences in shape as well as particle size influence mat formation and passage of eligible particles from the rumen. Diet is an

important factor because concentrates like high moisture corn and corn gluten feed have an initial functional specific gravity greater than 1.4.[50] When animals are fed high grain diets typical of the beef feedlot industry, the rumen mats are poorly formed because the grain particles cannot float in the dorsal rumen. Physical processing also affects mat formation. For example, grinding roughage eliminated the rumen mat resulting in larger fecal particle size and shorter ruminal retention time.[51] Species and breed differences also influence mat structure: Hereford cattle had more distinct mats than did Brahman crosses.[48] Mat formation is less evident in sheep and goats than in cattle, possibly related to the faster rate of passage in small ruminants.

The quality of the ruminal mat has a second effect that is important for ruminal protection systems. Absence of a well-defined mat increases abomasal retention time. Figure 5 shows data from both beef and dairy cattle that were fed plastic particles of varying particle sizes (2 × 2, 3 × 3, 4 × 4 and 2 × 4 mm) and specific gravities (1.15–1.45). The color-coded pellets were administered 12, 24, 30, 36 and 48 h before the animals were slaughtered and particles were recovered from different segments of the gastrointestinal tract. This protocol permitted measurement of the rate at which pellets of different sizes and specific gravities moved along the gastrointestinal tract.

Comparisons of the beef and dairy data lead to four important conclusions for those interested in rumen protection systems.

1. Passage along the gastrointestinal tract was more rapid in lactating cows than in steers, presumably due to higher levels of intake. Twelve hours after dosing, only 14.6% of the 2 mm particles with a specific gravity of 1.30 remained in the rumens of the dairy cows while more than 30% of the same particles were still in the steers' rumens.
2. Particle size was not an important variable in predicting passage or extent of rumination in steers, but large particles were more likely to be ruminated by dairy cows than small ones. The 2 × 4 mm particles were as likely to be ruminated as the 2 × 2 mm particles suggesting that aspect ratio affects passage. The importance of particle size in dairy cows supports the hypothesis that dietary forage permits mat formation and that the mat is important in determining which particles are ruminated.
3. In the absence of a well-formed mat, the abomasum played an important role in sorting particles. Over 50% of the pellets with a specific gravity of 1.45 that were recovered from the steers were in the abomasum, approximately double the number of pellets recovered from that organ in dairy cattle. In steers, two sites of sorting were evident, the rumen and the abomasum. Light particles were retained in the rumen while the heavier particles were retained in the abomasum in the steers. The abomasal retention of heavy particles was not evident in the dairy cows, an important observation for ruminal protection. Due to the rapid rate of passage, exposure to the acid conditions of the rumen is reduced in dairy cows compared to steers.
4. Our data agree with those of Siciliano-Jones and Murphy[52] that particles with a low specific gravity moved faster than those which were heavier in the lower gastrointestinal tract.

These results suggest that it is more difficult to develop good ruminal protection

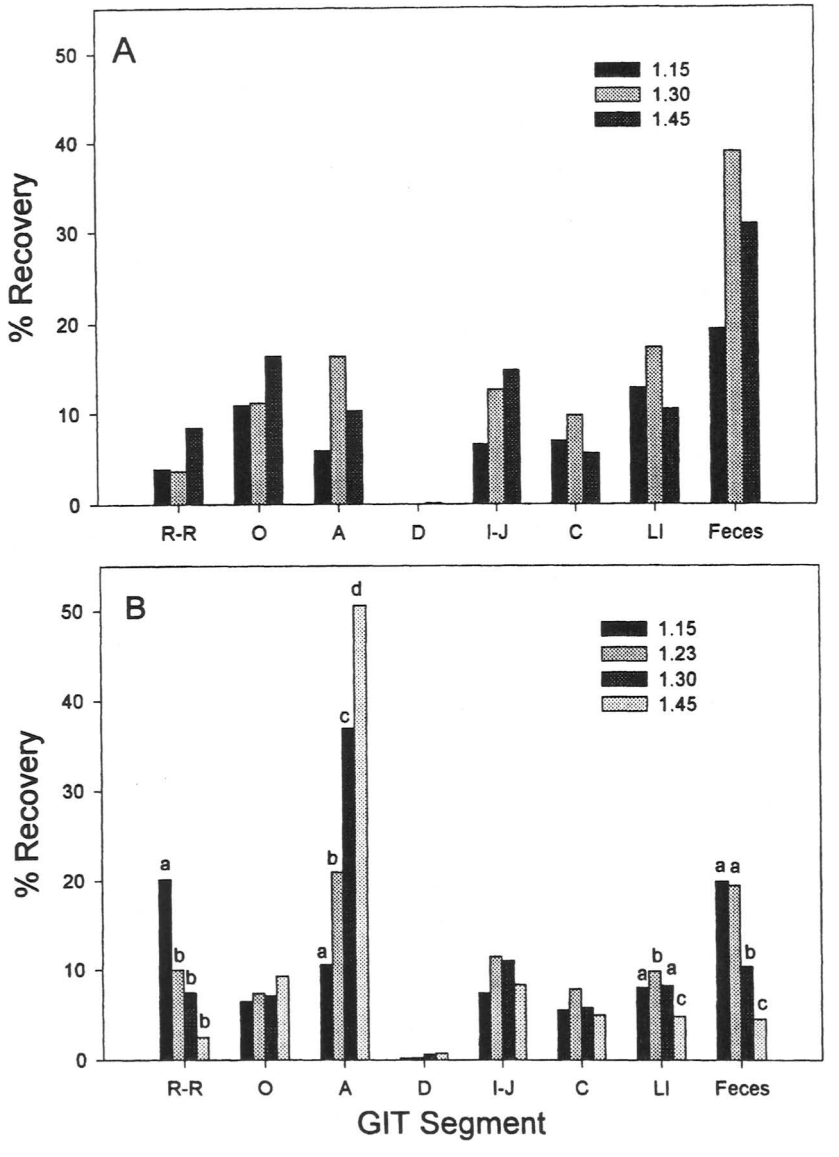

Fig. 5. Recovery of non-ruminated pellets administered 24 h before slaughter from different segments of the gastrointestinal tracts of dairy cows (panel A) and growing steers (panel B). The specific gravity of the particles was 1.15, 1.23, 1.30 or 1.45. The cylindrical particles were 2 mm long with a 2 mm diameter. The dairy cows did not receive particles with a specific gravity of 1.23. Segments of the gastrointestinal tract are abbreviated as follows: R-R, reticulo-rumen; O, omasum; A, abomasum; D, duodenum; I-J, ileum and jejunum; C, cecum; LI, large intestine and F, feces. Different letters indicate means within gastrointestinal tract segment differ ($P < 0.05$). There were no differences among means within segment in the dairy cows.

systems for dairy cows than for steers, because in dairy cows, the risk of rumination is higher and because exposure to acid in the abomasum may be brief, resulting in incomplete release of the protected material. These data underscore the importance of testing the ruminal protection system in the appropriate target animals fed typical diets for that class of animal. It is risky to use data from beef cattle to predict ruminal protection in dairy cows.

Sedimentation studies have been conducted to determine the relationship between passage from the rumen and sedimentation[11,28,48] but our understanding of the importance of this attribute is still very limited.

Although it is essential to consider several chemical and physical attributes of the diet and the class of animal in predicting ruminal protection, some desirable attributes of rumen-stable pellets are outlined in Table 1.

V. Requirements for a post-ruminal drug delivery system

Three areas must be considered in the development of an effective postruminal drug delivery system. These are physiological considerations, feeding practices and environmental concerns.

V.A. Physiological considerations

The delivery system should provide ruminal protection and post-ruminal delivery of the active ingredients. Ideally, nutrients, drugs and other active compounds should be fully protected in the rumen environment and then completely released so that they become available for absorption or to perform their intended postruminal function. Based on the discussion in Sections II and III, the essential attributes of a postruminal delivery system are:

- minimal degradation due to rumination;
- ability to withstand the chemical and microbiological conditions in the rumen;
- maximized rate of passage through the rumen.

The components used in formulating a postruminal delivery system must be safe for animals and humans. The chemical or other formulation processes must not generate undesirable residues in the finished product to cause safety concerns.

Table 1
Recommended attributes of particles for ruminal protection systems

Attribute	Recommendation
Particle diameter (mm)	<2.0
Particle shape	Short cylinder or sphere
Aspect ratio	1.0–1.25
Functional specific gravity	1.2–1.5

V.B. Feeding practices

A post-ruminal delivery system or rumen-stable product must be able to retain its integrity under ordinary feeding practices such as feeding of total mixed rations (TMR). Many commercial beef and dairy producers feed pre-mixed TMRs which include all dietary ingredients. This practice poses two dangers to protected particles: (1) mixing in specially designed mixer wagons may disrupt some of the delivery system, and (2) the pH of the silage in the TMR may solubilize the delivery system coating. After mixing, the TMR is placed in a feed bunk where it remains for up to 24 h. Feed may remain in the mixer wagon for 30–40 min during mixing and delivery during which time the feed is constantly agitated. The pH of well-fermented silage, a major component of many TMRs for dairy cattle, is 3.8[3] so the coating must either be protected from direct contact with silage, or be stable when in contact with silage. Optimally, the pellets should be strong enough to withstand the pelleting process in a grain mill which involves heat, pressure and mixing. If the protected pellets could withstand the pelleting process, they could be directly incorporated into commercial protein supplements. This would minimize the risk of solubilization of the pellets due to exposure to acid silage. Top-dressing, or adding the pellets on top of the TMR, is an option but it involves an additional step during feeding and some risk of pellet solubilization remains. While individual feeding of a small amount of protected pellets may sound attractive as a delivery system, beef and dairy producers have limited interest in products that do not permit group feeding of TMRs. Economics and sound nutrition are strong arguments in favor of these practices.

The ruminally protected product must be cost-effective in comparison with alternatives. For example, feeding of fish- or blood-meal increases the postruminal supply of methionine, lysine and other amino acids which are likely to be limiting. If rumen-protected methionine and/or lysine are supplemented, the response and cost must be comparable to competitive products, in this case, protein supplements high in undegradable protein. Consumer acceptance of a delivery system is less cost dependent when drugs rather than nutrients are the active compounds. However, injection with prolonged release formulations is an economic option as commercialization of bovine growth hormone has shown.

V.C. Environmental considerations

Any material that is being considered for use in ruminal protection systems must be safe for the target animal, the environment and for inclusion in the human food supply. Although there are many compounds that could be used in designing an effective post-ruminal delivery systems, they are of limited use because the compounds are either toxic or carcinogenic. The target animals for these products are food-producing animals and there are strict regulations on residues and safety. Most manure from livestock operations is spread on the land to fertilize the soil so the controlled release system must not contribute to soil contamination.

VI. Strategy for formulation of a pH-dependent post-ruminal drug delivery system

A variety of post-ruminal delivery systems have been developed using heat and chemical treatments, methionine analogs,[53] lipid-based formulations and a pH-sensitive polymer. The most sophisticated system, which was developed and commercialized by Rhône Poulenc (Smartamine®,[54]), was based on the use of a ruminally inert, pH-sensitive polymer. Initially, the system was developed to ruminally protect amino acids permitting release in the abomasum and amino acid absorption in the small intestine. The goal of this system was to permit amino acid supplementation directly to the small intestine to minimize microbial utilization of amino acids.[55,56] This polymeric coating system can also be used for encapsulation of many active ingredients formulated into particulate forms. In some pharmaceutical applications, the system has been referred to as a reverse-enteric coating system.[55]

The underlying principle of this coating system depends on the difference in pH between the rumen and the abomasum. The coating is composed of a basic polymer, a hydrophobic substance and a pigment material. The coating system can be applied to solid particles using an air-suspension coating method. The coated particles must meet the desirable design parameters for an effective rumen-stable products (Figure 6). The delivery system can be used to deliver micronutrients and pharmaceuticals postruminally to ruminants. In addition, it can also be combined with other controlled delivery devices or systems to enhance slow release or targeted delivery needs for ruminants. In this section, the development of this system will be used as a case study

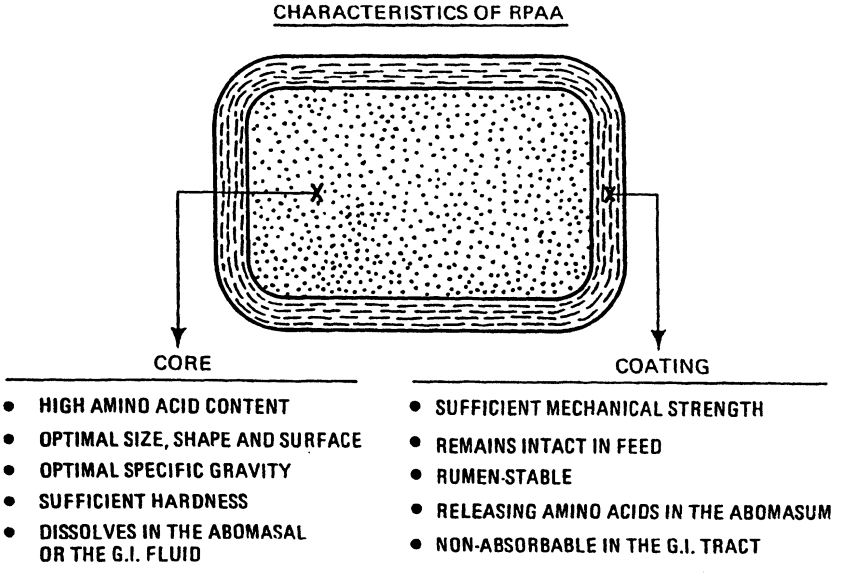

Fig. 6. Desirable characteristics of ruminally protected amino acid pellets.

to illustrate the factors involved in designing a pH-dependent rumen-stable delivery system.

VI.A. Formulation considerations

A polymer suitable for formulation of a pH-dependent rumen-stable delivery system must meet the following criteria

- Physiologically inert, non-absorbable, unchanged in excretions of target animals
- Non-mutagenic
- Produces no adverse effects with chronic feeding
- Thermally stable or non-degradable at temperatures encountered during processing and storage
- Insoluble in rumen fluid, but soluble in abomasal fluid
- Soluble in common volatile organic solvents for coating applications
- Safe for use in food-producing animals

In an early formulation, cellulose propionate 3-morpholinobutyrate (CPMB) was used as an acid-soluble polymer for coating amino acids such as methionine and lysine. This work established fundamental understanding of the essential steps and concerns in the development of an effective system: core composition, coating formulation, coating process, in vitro and in vivo evaluation including feeding trials.[56]

Subsequently, a more effective delivery system was developed using copolymers of vinylpyridine and styrene. The scheme for synthesis of this polymer is given in Figure 7. A series of patents relating to this technology have been awarded to the Eastman Chemical Company.[57] The coating system is composed of a basic polymer such as poly (2-vinylpyridine-co-styrene, 80/20) (2VP/ST, 80/20), a pigment material such as talc or aluminum and a hydrophobic substance such as stearic acid at a typical ratio of 31.5:63.5:5.0 by weight. The relationship of the average molecular weight (M_w) and the inherent viscosity of a typical acid soluble polymer, (2VP/ST, 80/20), in DMF as a solvent is shown in Figure 8. Methionine and glucose pellets were encapsulated with the coating by employing an air-suspension coating technique.

The protection of coated pellets in simulated rumen fluid was assessed by incubat-

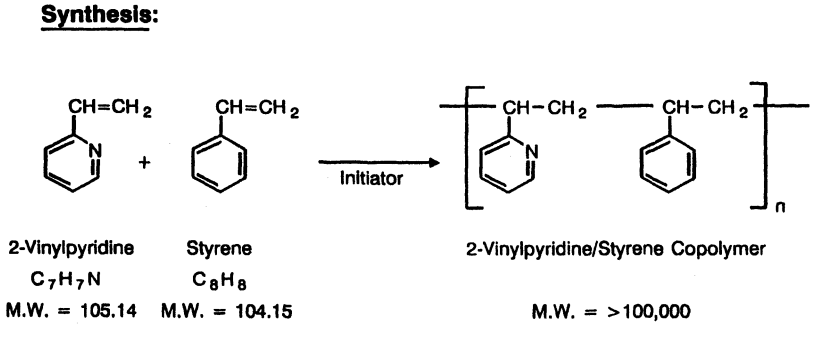

Fig. 7. Scheme for the synthesis of poly(2-vinylpyridine-co-styrene 80/20).

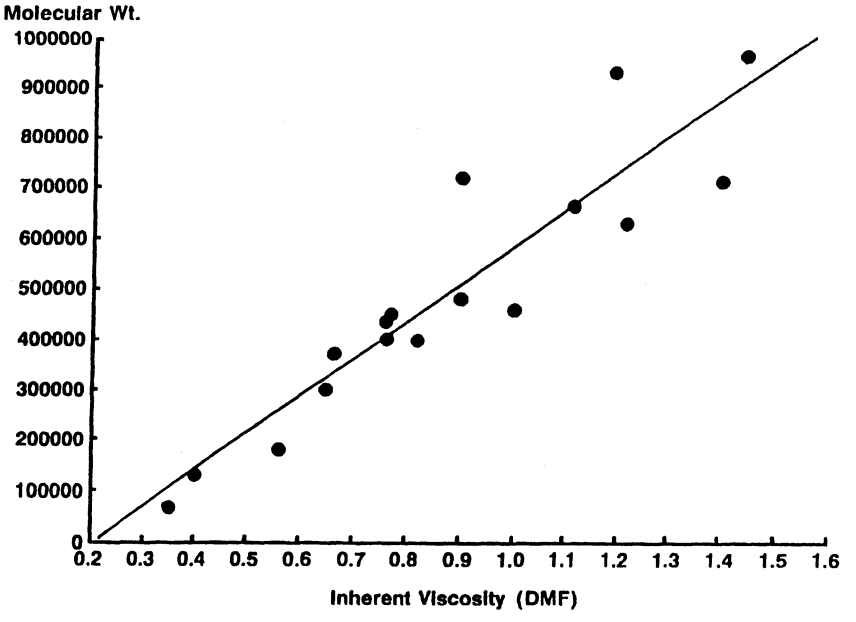

Fig. 8. Relationship between molecular weight and inherent viscosity of poly(2-vinylpyridine-co-styrene 80/20) using DMF as a solvent.

ing pellets in buffer (pH 5.4) for 24 h and measuring the amount of the active ingredient(s) remaining in the coated pellets. Abomasal release was evaluated by incubating the pellets in buffer (pH 2.9) for 1 h and measuring the amount of the active ingredient released. Figure 9 shows the average protection and release values for five batches of methionine and glucose encapsulated with the rumen-stable (or reverse-enteric) coating. The coating efficiency is highly dependent on the solubility of active ingredients, pellet size, and smoothness of pellet surfaces.[58] Figure 10 shows the release profile of coated methionine and lysine-HCl in pH 2.9 buffer as a function of time. Figure 11 shows the release characteristics of coated pellets after a brief (0.5–5 min) contact with pH 2.9 buffer and after immediate immersion in a buffer with a pH of 7.0 to mimic the transition from the abomasum to the duodenum. These results indicate that the pigmented polymeric coating serves as a pH-dependent gate for the core materials. An acidic medium generates pinholes instantaneously on the coating surface causing an irreversible rupture of the coating leading to release of the protected compound. Figure 12 shows the effect of polymer/pigment volume ratio on the protection of a very water-soluble active ingredient, lysine-HCl. The polymer suitable for the coating formulation must have a high molecular weight, usually more than 400 000 Da, or an inherent viscosity value of 0.5 or higher using DMF as the solvent. Results in Figure 13 suggest that a highly entangled network with considerable mechanical strength is required to retain a large amount of pigment and to provide sufficient mechanical strength for the coating. Scanning electron micrographs of cross sections of the coating revealed a lamellar structure with extremely tortuous paths for

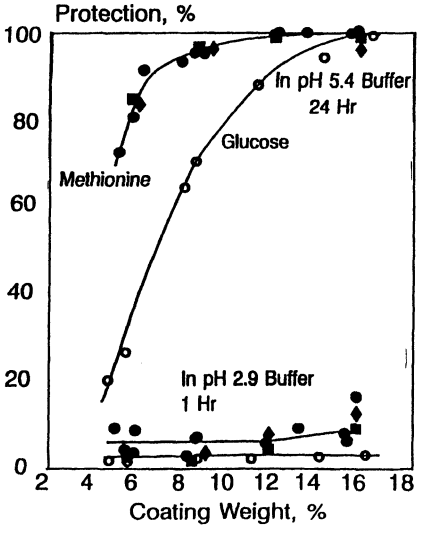

Fig. 9. In vitro ruminal protection and abomasal release of methionine and glucose pellets coated with a rumen-stable (reverse-enteric) coating.

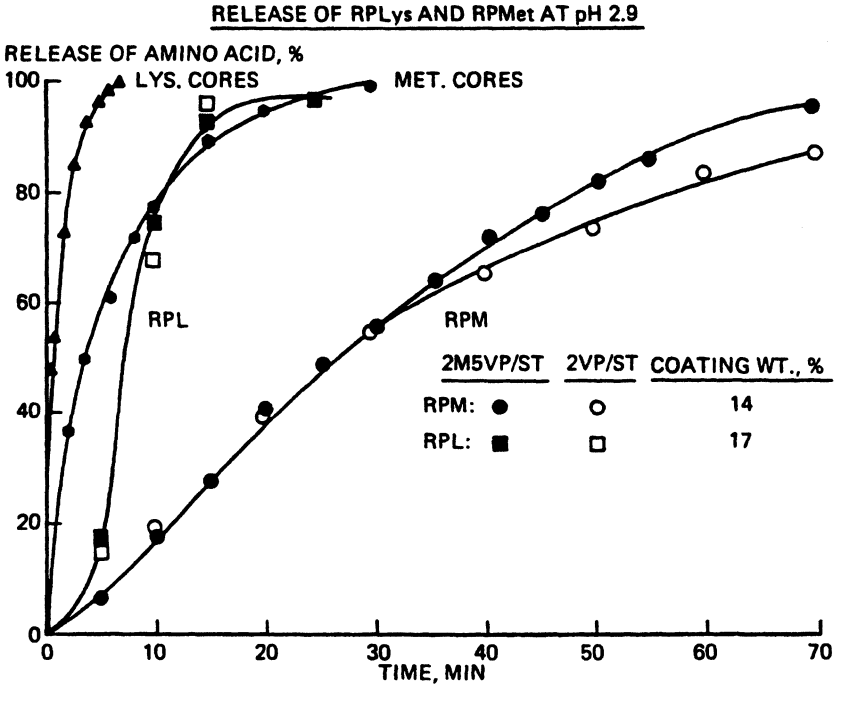

Fig. 10. Release profiles of coated methionine and lysine-HCl pellets incubated in pH 2.9 buffer.

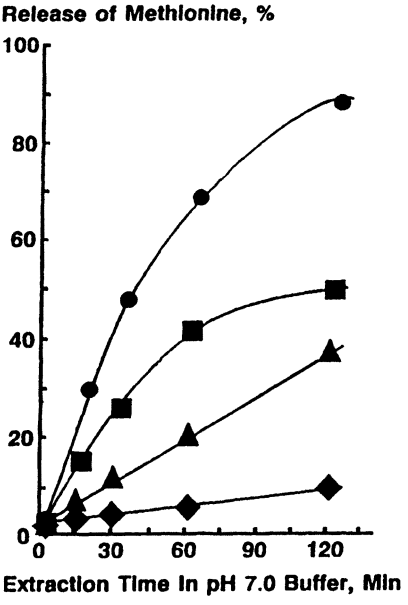

Fig. 11. Release of coated methionine pellets in pH 7 buffer after brief contact with pH 2.9 buffer for 0.5 (◆), 1 (▲), 2 (■), and 5 min (●).

fluid penetration.[53] With these results in mind, Wu proposed a 'brick-and-mortar' model for the delivery system as shown in Figure 14.[57] This system has been used successfully in delivering active ingredients such as antibiotics, growth hormone, growth promoter, peptides, and proteins postruminally. Smartamine® by Rhône Poulenc is an amino acid supplement based on this system.[54] Because the polymeric

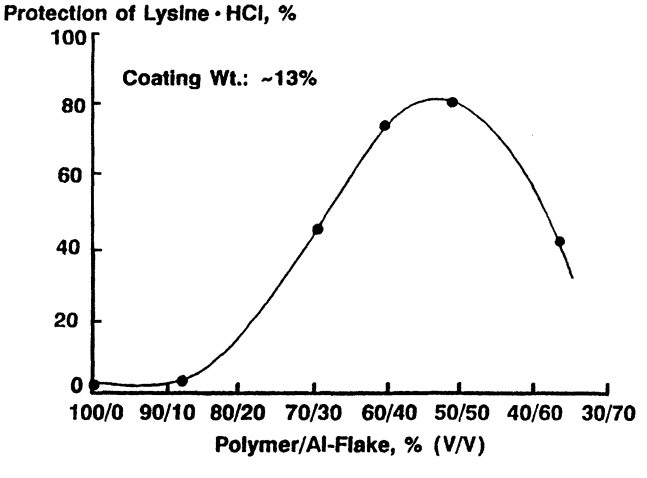

Fig. 12. Effect of the ratio of polymer:pigment volume on the ruminal protection efficiency of a rumen-stable coating applied to lysine-HCl pellets (coating weight ~13%). Al-flake was treated with stearic acid.

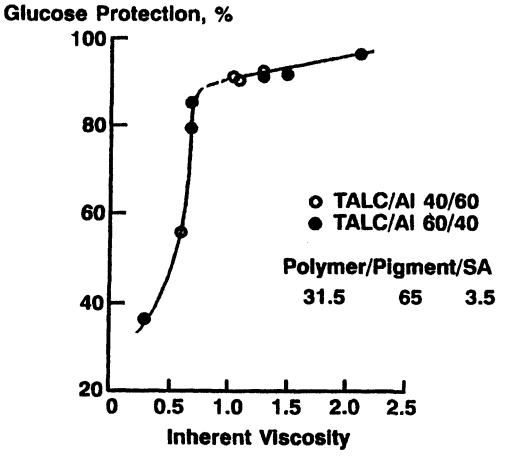

Fig. 13. Effect of polymer molecular weight (or inherent viscosity) on coating efficiency of a rumen stable coating applied to glucose.

coating is soluble in acid, the coating should not be in direct contact with acidic feed components such as silage. The coated particles should not be added along with other feed ingredients into a feed pelleting machine because the coating cannot survive a process that involves a lot of shear and abrasion.

VI.B. Pellet core formulation

In preparing rumen-stable amino acid pellets, it is essential to prepare suitable pellet cores prior to coating. The pellet cores must be fabricated in accordance with the criteria outlined in Section V.A. Physiological considerations to ensure (1) maximum

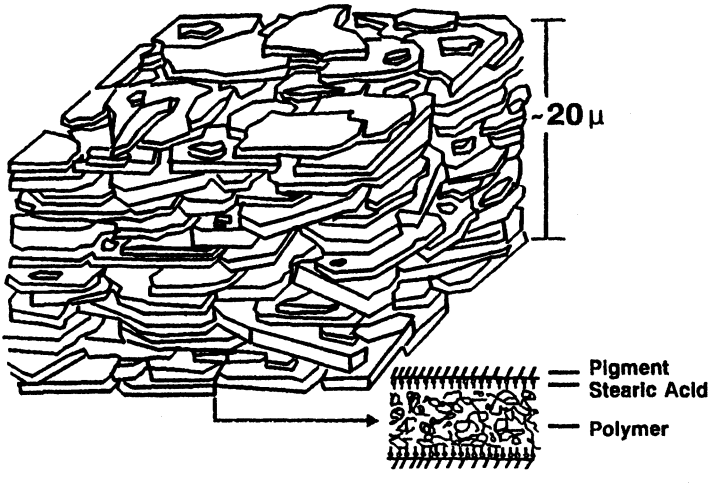

Fig. 14. A brick-and-mortar model for a pH-dependent rumen-stable (reverse enteric) coating.

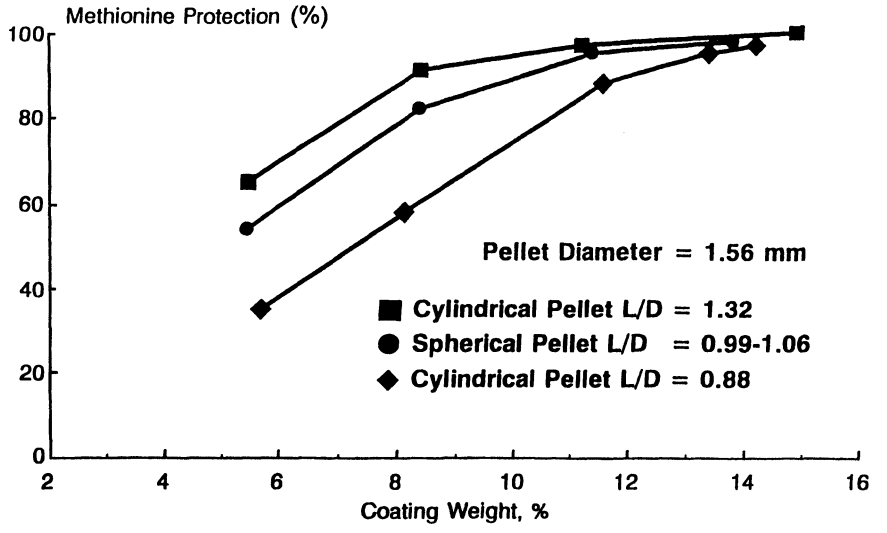

Fig. 15. The relationship between pellet geometry, % coating weight and ruminal protection of methionine.

delivery of the amino acid payload, (2) minimal degradation due to rumination and (3) minimal ruminal retention time. Wu[58] studied the effect of geometry of methionine cores on the efficiency of ruminal protection (Figure 15), the protection efficiency for two pellet sizes (Figure 16) and the effect of pellet rough edges on methionine protection for a given size of methionine pellets (Figure 17). Wu's results suggested that core smoothness is essential to maximize protection provided by the coating (Figures 18

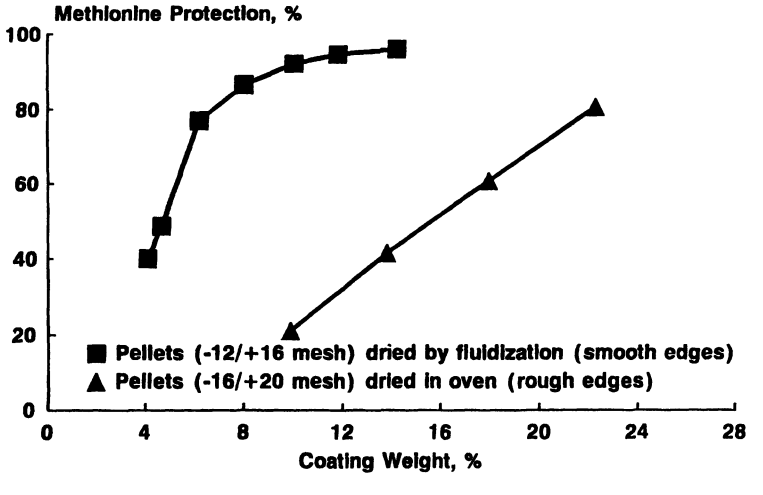

Fig. 16. Effect of pellet size on methionine protection.

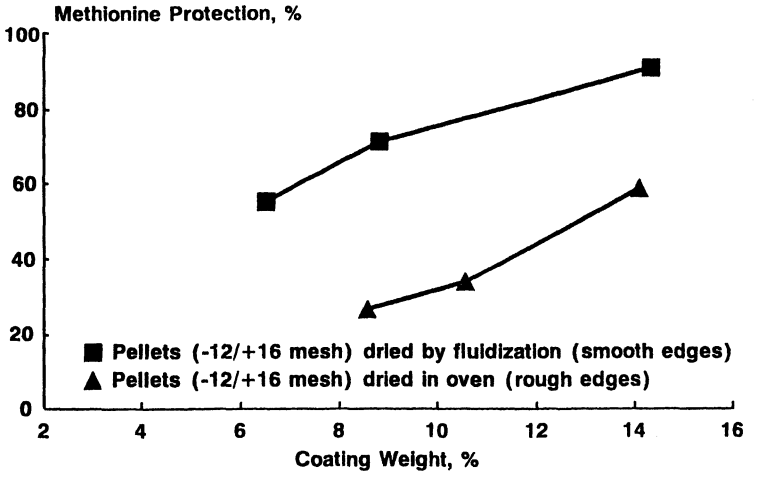

Fig. 17. Effect of rough edges of pellets on protection of methionine. Pellet roughness was altered by drying by fluidization (smooth) or in an oven (rough).

and 19).[58] The results of these studies suggest that the parameters listed in Table 1 are appropriate for formulation of a pellet core suitable for encapsulation with the rumen-stable coating formulation for cattle.[58]

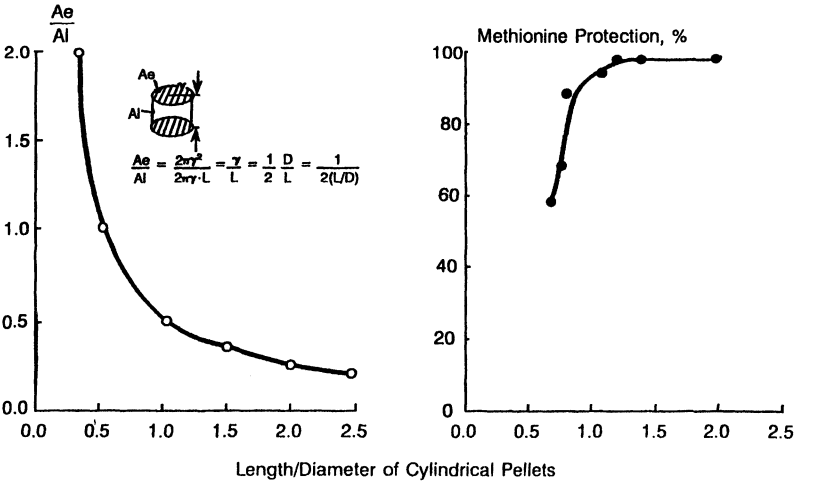

Fig. 18. Effect of pellet end-surface roughness on protection of methionine.

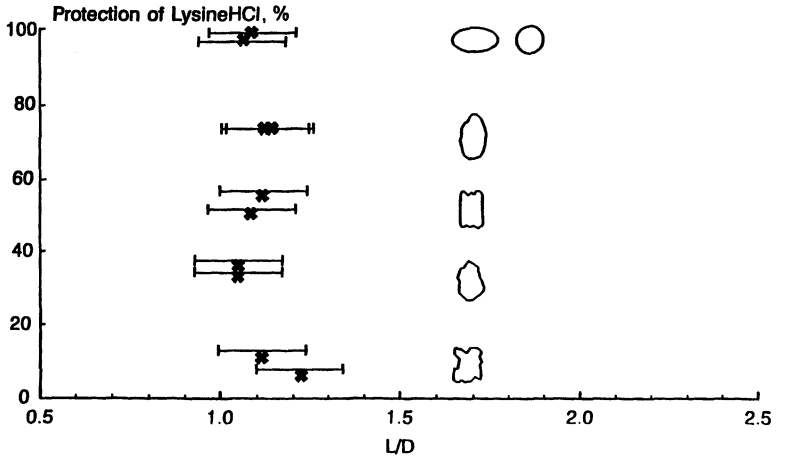

Fig. 19. Effect of pellet geometry on protection of lysine-HCl pellets at 14% coating weight. L/D is ratio of length to diameter (aspect ratio).

VII. Methods for evaluation of release and correlation of in vitro and in vivo results

Because direct evaluation of protection systems in animals is expensive and time consuming, several in vitro and in vivo methods have been developed or adapted from existing methods to evaluate post-ruminal delivery systems. A complete evaluation of a ruminal protection system involves two steps: (1) determination of whether the protected material is released into the rumen due to either microbial activity or physical disruption due to chewing and abrasion and (2) an assessment of whether the material is absorbed in the small intestine.

VII.A. Protection from ruminal fermentation

VII.A.1. In vitro buffer incubation to evaluate ruminal stability

An in vitro test which is particularly useful for screening systems composed of coated or matrix particles involves incubation of ruminally-protected pellets in phosphate buffer (pH 5.5) for 24 h at 40°C. These conditions mimic those found in the rumen. If required, the system may be stirred to simulate mixing of rumen contents. If the pellets dissolve under these conditions, it is unlikely that they will remain intact in the rumen. However, if the particles remain intact during the test, this does not guarantee resistance of a controlled release system to microbial fermentation in an active rumen environment. The primary advantages of this method are its low cost and ease of use. A limitation of this method is that it does not evaluate release of the protected compound from the delivery system.

VII.A.2. In vitro or in situ fermentation

In vitro[59,60] and in situ[61,62] methods have been developed for assessment of the rate or

extent of digestion and have proved to be useful for evaluation of ruminal protection. With the in vitro methods, samples of the protected compound are incubated with ruminal fluid collected from a fistulated cow and bicarbonate-phosphate buffers supplemented with trace minerals and vitamins. Although there are several different recipes for the in vitro medium,[60,63,64] all of the media have been designed to simulate conditions in the rumen. Despite many attempts to replace fresh ruminal fluid with a fecal inoculum, enzymatic treatments or freeze-dried ruminal fluid,[65–67] fresh ruminal fluid remains the standard inoculum. Recently, computerized systems that measure gas (CO_2 and CH_4) production from in vitro fermentations have been developed.[68–70] These systems may be useful in evaluating effectiveness of rumen protection systems, provided that the substrate cores contain energy-producing substrates such as glucose. If the pellets release the active, metabolizable ingredient, gas production will be greater in the bottles containing pellets than in the control. By using appropriate blanks, it should be possible to predict the amount of substrate released.

The in situ methods involve placing the test compound in nylon bags with a measured pore size (usually about 50 μm) and incubating the bags in the rumen of a fistulated animal. The pore openings must be large enough to permit entry of the ruminal microorganisms but small enough to retain feed particles and test material. With both the in vitro and in situ approaches, the test material can be recovered at different intervals to evaluate the effectiveness of the protection over time. The incubation time should exceed projected ruminal retention time (approximately 30 h for a dairy cow and up to 72 h for forage-fed animals at maintenance). The recovered samples then are analyzed for the active material in order to determine loss. These methods provide a reasonably accurate estimate of ruminal protection from microbial degradation, but they do not predict release of the protected compound for absorption. If a test material cannot be retained in a nylon bag (e.g. methionine analogs), in situ evaluation is not feasible unless a duodenally cannulated animal is used and small quantities of the test material can be measured from duodenal samples. This approach is far more complex than the standard in situ method and the investigator should explore in vitro approaches using analysis of the active material or its metabolites. Stern et al.[67] recently published an excellent review on methods to assess digestibility which should be useful not only to ruminant nutritionists, but also to developers of ruminal protection systems.

VII.B. Availability for absorption

VII.B.1. Simulated abomasal release test using buffers

Buffers and/or enzymes or actual digesta from the abomasum collected from a ruminally fistulated animal are used to assess abomasal release. Use of buffers simulating the abomasum environment are particularly useful in the evaluation of pH-sensitive coatings. These coatings are stable at ruminal pH (5.5–7.0) but disintegrate in the abomasum (pH 2.0). The second stage of the in vitro Tilley–Terry method[60] and the method developed by Calsamiglia et al.[71] both simulate abomasal digestion. Both of these systems involve incubation of the test material in an acidic ($pH < 2$) pepsin solution for 1–2.5 h at 39°C. Release of the protected compound is a good indicator of intestinal

availability for polymeric coatings but does not produce satisfactory results for systems relying on heat or chemical treatment or chemical derivatives or analogs.

VII.B.2. Abomasal in situ digestion

Mobile bag techniques[72–74] have been developed to assess intestinal digestibility of feed constituents, however, these techniques can also be used to assess the delivery of ruminally protected compounds. Small nylon bags (2 × 2 cm) can be placed either in the omasum or the abomasum of ruminally fistulated animals. These bags may either be recovered in the feces after passage through the gastrointestinal tract from the omasum or they can be tethered with string so that they remain in the abomasum to evaluate the effects of exposure to gastric acid and enzymes. Bags should be placed in the posterior abomasum because the anterior abomasum is less acidic, and be incubated 1–2 h, the mean abomasal residence time. When free-moving bags are used, some bags may be lost. This is probably due to back flow to the rumen and rumination.

VII.B.3. Blood response in ruminant animals

Absorption of the test material may be evaluated by measuring blood levels of the active material or of its metabolites after oral administration or placement in the rumen of a fistulated animal.[75] This approach requires dosing the animal with levels of the test compound known to cause an increase in blood levels. For nutrients like amino acids that have variable blood levels due to both fluctuations in supply and tissue demand, appropriate controls must be included in the experimental design. While increases in blood levels suggest that the delivery system is at least partially effective, one cannot conclude that the delivery system has failed if no increase is evident. Increased uptake by tissue or increased blood flow may explain why blood levels have not increased. Quantitative estimates of the effectiveness of the delivery system may be obtained by comparing blood levels with protected release administration with blood levels when similar amounts of the test compound are directly infused into the abomasum. This method is better suited for evaluation of individual compounds or well-defined mixtures than for the assessment of complex nutrients such as proteins and fats which are converted into variable amounts of many different metabolites. Because the end point in this test is blood level, it is not appropriate for compounds designed to function in the digestive system without being absorbed. For such compounds, the use of marker compounds and sampling of the digesta are recommended. This test is useful since it provides in vivo confirmation of in situ or in vitro data without the expense of efficacy studies.

VII.B.4. Efficacy responses

The final step in the testing process is to evaluate whether production increases, incidence of disease decreases or whether symptoms are alleviated. Taken in conjunction with the preliminary data, when the desired clinical outcomes are evident in an appropriate number of test animals, the formulation scientist can be confident that the delivery system is functional. Possible explanations for a lack of response include absence of need for the supplied compound, deficiency of other nutrients, stress,

disease or failure of the delivery system. The first four reasons cited above must be assessed and ruled out before it is assumed that the delivery system is ineffective.[75,76]

VII.C. Overall evaluation

Reasonable evaluation of a rumen delivery system requires use of several of the methods described above. In most cases, combinations of the above methods have been used. For example, the test material may be fed or placed in different locations in the gastrointestinal tract of ruminally or intestinely cannulated animals to assess the function of the delivery system in different locations. Alternatively, samples may be placed in nylon bags and evaluated in the rumen, abomasum, duodenum or other locations in the gastrointestinal tract.[72] Use of markers in combination with these qualitative techniques may provide a quantitative assessment of rumen protection and intestinal availability.

An effective delivery system must meet other important 'fitness-for-use' criteria. For example, if the protected compound is to be supplied in the feed, it must withstand necessary mixing and processing and remain stable during storage. For example, systems based on pH sensitive polymers cannot be exposed for long periods of time to acidic feeds such as silage. Damage to coated materials during chewing must be assessed: fragile materials may release the protected compound during initial mastication even if the particles are of a size and specific gravity to minimize rumination. These are major factors affecting commercial use of delivery systems and can be evaluated separately or using variants of methods discussed above.

VIII. Future developments

The future offers significant opportunities to improve production efficiency of ruminant animals through nutrition and health, and rumen-stable delivery systems provide a significant tool in the effort to optimize both rumen and post-ruminal function. This includes not only efficient production and supply of nutrients, but also control of other important parameters such as pH, microflora and prevention and treatment of disease.

There are currently two segments of the ruminal protection market and these will remain the focus of future developments in this area: (1) systems for the protection of relatively low cost nutrients like amino acids, and (2) targeted delivery of expensive drugs and peptides. The costs of nutrient protection will remain competitive with natural products like fish and blood meals. However, in the drug and bioactive peptide market, the emphasis will be on precise delivery of the protected compounds. In the future the development of these targeted delivery systems will depend both on the ability of chemists to develop new ruminal protection schemes and on their ability to take into account the digestive physiology of ruminants. The best protection system in the world will not work well if the particles are >4 mm and have a specific gravity close to 1 because they will be disrupted during rumination.

It is likely that future technical discoveries will occur in the development of new

delivery systems with enhanced protection and release characteristics. In the future, new products and delivery systems may include:

1. Commercially available rumen-stable tryptophan, threonine and phenylalanine. The existing coating technology will probably be used to protect these and other amino acids.
2. The delivery systems will become more sophisticated permitting delivery of nutrients, peptides or drugs to specific segments of the lower gastrointestinal tract. For example, high value peptides which have growth-hormone-like activity may be delivered directly to the small intestine instead of being released in the acidic abomasum, preventing degradation and inactivation. Similarly drugs which are sensitive to ruminal fermentation and the acidity of the abomasum may be targeted for the lower gut.
3. Systems designed for ruminal protection will be combined with other controlled release systems in order to maximize efficiency of delivery. This will enhance absorption from the gastrointestinal tract and post-absorptive pharmacological effects.
4. Improvements will be made in the environmental stability of the coatings so that pellets can be subjected to the mixing and exposure to acid that are inherent in the feed delivery systems for most dairy and beef operations

IX. Conclusion

Currently the major commercial application of post-ruminal delivery systems is for the delivery of amino acids, particularly methionine and lysine. These amino acids are used primarily as nutritional supplements for dairy and beef cattle in order to improve milk and/or milk protein production, reproductive performance, meat and/or wool production or growth. As with other nutrients, responses depend on the diet, physiological stage and other variables which must be taken into account when developing and assessing a rumen-stable or post-ruminal drug delivery system.

The technical and commercial feasibility of rumen-stable delivery systems is now being established. Cost constraints dictate that future efforts concentrate on protecting high value micronutrients and drugs, or on development of low cost protection schemes. With current technology, polymeric coatings provide better ruminal protection and postruminal release compared to the historical approaches of chemical and heat treatments or coating with a fat matrix.

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CHAPTER 5

Design and development of controlled release veterinary drug delivery systems to the eye

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Overview

I. Introduction

Over the past decade, the understanding of ocular physiopathology and pharmacokinetic and pharmacodynamic parameters of ophthalmic drugs has increased markedly and resulted in the development of new drugs and drug delivery systems for the human eye. In contrast, veterinary ocular pharmacology is a relatively new field of study and although pertinent information on the effects of ocular drugs and their proper use in small and large animal patients has increased, the veterinary-labeled ophthalmic drugs presently available in most countries consist primarily of topical antibiotics and antibiotic-corticosteroid combinations and there is a limited number of other drug categories for topical use. Thus, several valuable pharmacotherapeutic agents are not available with labels for veterinary use (like anti-glaucoma preparations, non-steroidal anti-inflammatory drugs, antifungal and antiviral agents) and it is an accepted practice to use drugs labeled for human use in treating certain ocular diseases in animals. This habit is also based partly on the fact that classical problems affecting the eye in animals are quite similar to conditions known to affect man (e.g. uveitis, glaucoma). Such an approach might be considered justified if more was known about the clinical pharmacologic features of these drugs as they are used in veterinary ophthalmology. On the contrary, there are no pharmacologic data available as to the kinetics, actions and possible side-effects for many of these drugs in animals, and because animals have unique diseases and may manifest specific responses to therapeutic agents it is wrong to assume that a drug product that works well in the human eye will necessarily be effective for the animal eye. These are all sufficient reasons for encouraging research and development of new drugs for veterinary ophthalmology and promoting evaluation of their therapeutic efficacy in field trials when naturally occurring ocular diseases are present.

Drug effectiveness apart, the success of ocular therapy in animals will also depend on the drug delivery system and the convenience of application of the ophthalmic medication by the animal's owner. For instance, in cats with herpetic keratitis or in dogs with pseudomonal keratitis, intensive topical therapy requires antiviral or antibiotic applications every 1–2 h in order to reach effective corneal drug concentrations. These frequent instillations are often inconvenient for the owner and compliance is

rather poor. To overcome such a problem, new delivery systems adapted from human ophthalmology, such as hydrogels, inserts, collagen shields and hydrophilic lenses have been tested to improve the contact-time of the drug with the ocular tissue and/or increase its penetration into the eye. Using such approaches constant levels of drug result and the frequency of administration can be reduced. Such an approach is more convenient, improves compliance of the owner and provides an insight into the process of adapting a human ophthalmic preparation for veterinary use.

This review will provide some comparative anatomical and physiological features of the eye in the species most frequently encountered in veterinary practice and present their essential ocular diseases and describe the most appropriate medications for such diseases. The application of currently available ocular systems to veterinary ophthalmology and recent developments in the field of drug delivery to the human eye and their possible application to veterinary ophthalmology will also be discussed.

II. Anatomical and physiological features of the eye in animals

The eye represents one of the most complex assemblies of specialized tissues and compartments in the body and it is known that physiological and anatomical features have a profound effect on the rate and extent of absorption of drugs after topical or systemic administration and influence drug concentrations at the desired site of action. The precorneal structural and functional features which can influence the ocular bioavailability of a topically applied drug include the tear flow and composition and lid activity. The tear film, which is a protective covering for the cornea and conjunctiva, represents the first structure encountered by a topically applied ocular medication. In mammals, it can be subdivided into three layers: the outermost oily layer, the central aqueous phase and a mucus layer covering the epithelium. The lipid layer is produced by the Meibomian glands located in the tarsal plate of each eyelid (20–25 glands in the human tarsal plate, 20–40 in the canine eyelids and 30–50 in the equine eyelids). The meibomian secretion is a low melting point mixture of polar and non-polar lipids[1] spread in the form of a thin layer on the surface of the aqueous phase by lid blinking. Its thickness between blinks varies from 0.013–0.581 μm in the dog[2] and is less than 0.089 μm in the cat.[3] The main functions of the meibomian lipid layer are to prevent evaporation of the aqueous phase and spillover of tears at the lid margin. The aqueous phase, which forms 90% of the tear film thickness, is constituted by the serous tear fluid layer secreted in humans by the lacrymal gland and the accessory glands of Krause and Wolfring. In animals, the aqueous part of the tear film is produced by the lacrimal gland and the gland of the third eyelid which are classic exocrine acinar glands. The aqueous layer of the tear film is a complex solution containing small molecular weight components, electrolytes and proteins including, in man, immunoglobulin A (IgA), lactoferrin, prealbumin, tear-specific protein and lysozyme. These proteins are involved in the local defense of the ocular surface. In the canine and equine lacrimal fluid, IgA is the main immunoglobulin with concentrations of 24 ± 3 mg/100 ml and 309 ± 33 mg/ml, respectively.[4,5] Lysozyme has not been detected in tears from cows, but is normally present at low levels in tears of dogs and is

found at higher concentrations in the lacrimal fluid of man, sheep and goats.[6] The normal tear pH range is 6.8–8.0 in dogs[7] and 6.5–7.6 in humans.[8] Basal tear secretion occurs at a rate of 1.2 μl/min in man,[9] 8–14 μl/15 min in the cat[10] and 32 μl/min in the cow.[11] In practice, the rate of tear production is estimated by use of the Schirmer tear test. Normal average values in the unanaesthetised cat, dog and horse are 16, 20 and 25 mm/min, respectively. The red phenol thread tear test is another diagnostic method for measuring tear production. This method was recently used to establish tear production values for the dog[12] and cat.[13] At the interface between the aqueous phase and the epithelium is the mucin layer, a material composed of the glycocalyx of the epithelial cells and tear-specific mucoproteins which are secreted by the conjunctival epithelial goblet cells. Among domestic animal species variations have been noted in the distribution and density of these cells in the conjunctiva.[14] The mucin secreted by the goblet cells serves as a corneal wetting agent and helps spread the aqueous phase over the lipid-bearing epithelial cells. The tear film break-up time (BUT) is a non invasive method of assessing preocular mucin function and is clinically used to infer the stability of the precorneal tear film. Normal BUT is 19 ± 5 s in the dog.[14] The tear film stability is also influenced by the lid activity since tears can function properly only if the tear film covers the entire ocular surface and is reestablished quickly and completely after blinking. Spontaneous blinking which ensures the spreading of the tear film occurs at a mean rate of 14 blinks/min (range: 5.8–25 blinks/min) in the dog[2] and 2.4 blinks/min (range: 0.9–4.0 blinks/min) in the cat.[3]

Tears constantly clean and moisten the ocular surface and by blinking are propelled toward the medial canthus where they enter the collecting portion of the lacrimal system through the upper and lower lacrimal puncta. These small openings, located just medial to the most nasal meibomian gland, are continued by the lacrimal canaliculi that join to form the lacrimal sac. The length of each canaliculus is 4–10 mm in cats and dogs, with a diameter of 0.7–1.2 mm,[15] and 15–20 mm long in horses.[16] The lacrimal sac is 1–5 mm long and 1.5–2 mm in diameter in dogs and cats.[15] This sac empties into the nasolacrimal duct whose length varies from 2.5–10 cm in the dog, 2.5–4 cm in the cat and 24–30 cm in the horse.[16] The nasolacrimal duct opens on the floor of the nasal cavity about 5–10 mm caudal to the end of the nares in dogs and cats. Some dogs have an accessory opening in the duct as it passes dorsal to the upper canine teeth.[17] In the horse, the nasal punctum is located on the floor of the vestibulum near the mucocutaneous junction and ranges from 3–10 mm in size.[16] The rabbit has only a lower lacrimal punctum and canaliculus.

Many domestic animals have a nictitating membrane, or third eyelid, that is a well developed semilunar fold of conjunctiva located at the medial canthus. The nictitating membrane is present in vestigal form as the plica semilunaris conjunctivae in man. The nictitating membrane is supported by a flat, T-shaped piece of elastic cartilage, the shaft of which is embedded in the nictitans gland located at its base.[18] The secretions of the gland reach the bulbar conjunctiva via multiple ducts opening between lymphatic nodules at the central posterior surface of the nictitating membrane.[19] In the dog, the nictitans gland has been estimated to contribute between 29–57% of the tear secretion.[20] In some species (e.g. pig, rodents, lagomorphs) a tubuloalveolar gland, called the harderian gland, is also associated with the nictitating membrane.[21] Its main

secretory product is lipid and its single duct opens on or near the nictitating membrane naso-ventrally. Movements of the nictitating membrane may be passive (e.g. retraction of the globe) or active. In the cat different smooth muscles are associated with protrusion or retraction of the nictitating membrane.[22] The position of the nictitating membrane is partially determined by sympathetic tone.[18] Gliding of the nictitating membrane mechanically protects the cornea, removes foreign bodies and assists in the distribution of precorneal tear film. From a pharmacologic point of view, it has been shown that the presence of a nictitating membrane does not affect significantly the ocular pharmacokinetics of topically applied drugs.[23]

The cornea and sclera together form the outer tunic of the eye and give the eye its shape. In humans the cornea occupies about 0.07 of the total area of the globe while this proportion is roughly 0.5 in most domestic animals. The shape of the cornea is almost circular in dogs and cats and oval in ungulates. In animals, as in man, the central region is thinner than the peripheral cornea. Average corneal thickness is 0.52 mm in man,[24] 0.56 and 0.58 mm in the dog and cat, respectively,[25,26] 0.6 mm in the horse and 0.8 mm in bovines and sheep.[27] The healthy cornea is avascular but richly supplied by sensory nerve fibers that are branches of the long ciliary nerves derived from the ophthalmic division of the trigeminal nerve. In addition to signaling sensory information, these fibers also exert trophic influence on the corneal epithelium and help maintain corneal transparency. The greatest sensitivity level is in the central region of the cornea but significant differences in sensitivity exist among species. Thus, it has been determined that the threshold of corneal sensitivity is higher for the dog (96 mg/0.0113 mm^2) than for other species including man.[28] Histologically, the cornea in most animals can be subdivided into four layers: the outermost layer is the epithelium and then come the stroma, Descemet's membrane and endothelium. Bowman's membrane, a modified portion of the stroma underlying the epithelium, is only present in birds and primates.[24] The epithelium is a layer of five to seven cells in dogs and cat and up to 20 cells in large animals.[27] It presents an effective barrier to fluid transport, which is achieved by extensive close contacts and junctional complexes between the columnar basal cells. The stroma, 90% of the corneal thickness, provides the strength, elasticity and form of the cornea. It is composed of type I collagen fibrils arranged in parallel lamellae with a glycosaminoglycan (GAG) extracellular matrix between them. Corneal fibroblasts, called keratocytes, are the source of stromal collagens and GAGs. Descemet's membrane forms the posterior surface of the stroma. It is an elastic sheet, composed of collagen fibers more densely packed than in the stroma, that is produced by the corneal endothelium throughout life. The endothelium lines the posterior surface of the cornea. It is a single layer of flattened hexagonal cells tightly packed in a mosaic-like pattern. The cells are 15–20 μm in diameter in humans, rabbits and dogs.[29] The mean endothelial cell density for dogs, cats and rabbits is around 2800 cells/mm^2 and decreases with age.[27,29–31] The endothelium plays an important role in maintaining the normal water content of the stroma by its active pump mechanism that generates a flux opposite in direction to the passive flux of water that crosses the endothelium from the aqueous humor to the stroma and causes it to swell. Thus, the endothelium maintains the relative state of stromal dehydration that is necessary for corneal transparency. The cornea may act as a path-

way, a barrier or a reservoir for topically applied drugs. It is a unique biological barrier that distinguishes itself from all other biobarriers by being composed of two hydrophobic layers (the epithelium and the endothelium) sandwiching the hydrophilic stroma. Therefore, transfer of substances through intact cornea is largely determined by phase solubility. The epithelium and the endothelium are most permeable to substances with a fat-soluble phase while the stroma is most permeable to water-soluble substances. Thus, drugs that are both lipid and water soluble pass through the cornea more readily. This is an important consideration for topical therapeutic agents which need to penetrate intra-ocularly. Following damage to the epithelium (through trauma, immune-mediated diseases or keratoconjunctivitis sicca) or endothelium (through iridocyclitis, glaucoma or dystrophy), vascularization, cellular infiltrate and edema can ensue and result in corneal changes that can modify drug penetration kinetics. In addition to providing an absorption barrier to topically applied drugs, the cornea is the location of drug-metabolizing enzymes which have gained prominence in prodrug research. For instance, it was recently established that both human and canine ocular tissues have a similar profile of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) prodrug hydrolysis.[32]

Following topical application, most ophthalmic drugs penetrate into the eye through the corneal and non corneal (conjunctiva and sclera) pathways and are distributed in the anterior chamber and surrounding tissues.[33] The anterior chamber is a crucial target area for drugs acting on the iris and the ciliary body such as mydriatics, corticosteroids or antiglaucoma agents. The anterior and posterior chambers are aqueous-filled cavities whose total volume is 200–300 μl in human and rabbit eyes and varies from 400–800 μl in canine and feline eyes. The ciliary epithelium is responsible for secretion of the aqueous humor, transferring solute and water from the stroma of the ciliary process into the posterior chamber.[9] In most species aqueous humor is produced at a rate of 1–2 μl/min, with the exception of the cat which exhibits a high value (15 μl/min).[34] The flow of aqueous humor is essential for transporting nutrients and oxygen to the surrounding tissues and for carrying away their waste matter. The portion of the instilled drug that penetrates the inner eye is distributed into, and eliminated through, the aqueous humor. Thus, turnover of the aqueous humor is a major factor influencing the therapeutic effect of a drug. Most often, the ocular clearance is higher than the aqueous humor turnover since additional mechanisms such as metabolism and systemic uptake by the iris vessels can occur. Conversely, binding to aqueous proteins (e.g. anterior uveitis) or tissue components such as uveal melanin can decrease ocular clearance. This binding is also able to reduce the aqueous concentration of free drug and decrease the pharmacological response.[35] The intraocular pressure (IOP) reflects a balance between the rates of entry and exit of the aqueous humor. Escape of aqueous humor from the anterior chamber occurs via two pathways: the trabecular meshwork (conventional outflow) and uveoscleral system (non-conventional outflow). In primates, the fluid returns from the eye to the vasculature largely through the trabecular meshwork and canal of Schlemm.[9] In non-primate mammals the canal of Schlemm is absent and aqueous humor leaves the anterior chamber through the pectinate ligament and trabecular meshwork, then drains into the trabecular veins which communicate with an extensive scleral venous plexus. The

comparative anatomy of the iridocorneal angle in carnivores and herbivores has been reviewed recently.[36] A variable proportion of aqueous humor may exit through the ciliary body musculature and thence into the suprachoroidal space to be absorbed by choroidal and scleral circulation. This pressure-independent uveoscleral outflow accounts for less than 20% of the total drainage in humans,[9] and 30–65% in cynomolgus monkeys.[37] This percentage is 3, 15 and 13% in normotensive cats,[38] dogs[39] and rabbits,[37] respectively. Perfusion with microspheres has shown that the equine eye has an extensive uveoscleral outflow system, but the percentage of the total outflow has not been estimated.[40] In domestic animals, quantitative determination of IOP may be achieved by the Schiotz indentation tonometer or an electronic applanation tonometer, such as the Mackay-Marg or Tonopen. Normal IOPs of domestic animals are 15–25 mmHg in dogs, 15–20 mmHg in cats and 25–35 mmHg in horses.[41]

III. Ocular pathologies in animals and available drugs[42–44]

III.A. Inflammation

Ocular inflammation is one of the most common eye disorders in animals.[45] The precise observation and interpretation of signs provides the basis for diagnosing the disease and its associated disorders, and for establishing their etiology (trauma, lid or lacrimal abnormalities, viral or bacterial infection, immune-related phenomena, corneal ulceration). Topical steroidal therapy in combination with non steroidal anti-inflammatory drugs, immunosuppressive agents such cyclosporine and azathioprine, antimicrobial agents, mydriatic-cycloplegic agents (atropine) and additional specific therapies are frequently prescribed to treat conjunctivitis, keratitis and uveitis (Table 1).[46,47] Topically applied steroids are in most cases used in combination with an antimicrobial agent. The reason is probably that the concomitant administration of steroid can increase the efficacy of antibiotics.[48] In addition, this association prevents a secondary infection that may occur after a corticosteroid treatment. Viral and fungal infections are generally treated with drugs that were developed for human use. Systemically or subconjunctivally administered corticosteroids are used to treat severe blepharitis, sleritis/episcleritis, uveitis, chorioretinitis, optic neuritis and orbital inflammatory diseases. Topical glucocorticoids are contraindicated in case of corneal ulceration because they delay corneal healing. Adult cat eyes develop a steroid-induced ocular hypertension which is reversible. Unilateral twice or thrice daily topical application of 10 μl of dexamethasone sodium phosphate 1% caused a gradual intraocular pressure increase which became significant after 2–3 weeks.[49] Non-steroidal anti-inflammatory drugs, including flurbiprofen, suprofen, ketorolac, diclofenac, indomethacin are also administered to animals which are about to undergo intraocular surgery to prevent prostaglandin (PG) synthesis.[50–53] New anti-inflammatory agents such as platelet-activating factor antagonist and leukotriene inhibitors are being developed for ophthalmic use.[54–57]

Table 1
Veterinary-labeled topical preparations available in United States, Australia, United Kingdom and France[58–60]

Active ingredients	Trade name (®)	Excipient	Dosage[a]	Species
Chloramphenicol	Chloromycetin Redidrops	Aqueous	8	Dog, cat
	Bemacol; Chloramphenicol 1%; Chloricol; Chlorbiotic	Oil	8	Dog, cat
	Ophtalon; Lacrybiotic	Oil	2	Dog, cat
	Chloromycetin Ophthalmic Ointment 1%	Ointment	2	Dog, cat
Cephalonium	Cepravin	Oil	1/48 h	Cattle
			1	Dog
Cloxacillin benzathine	Orbenin ophtalmic ointment	Ointment	1/24–72 h	All
	Opticlox eye ointment	Ointment	1/48 h	All
Chlorotetracycline HCl	Aureomycin ophthalmic ointment	Ointment	1	Cattle
			3	Dog, cat
Neomycin sulfate	Ophtalkan	Aqueous	2	Dog, cat
	Neobiotic eye ointment	Oil	2–4	Dog
Gentamycin sulfate	Gentocin	Aqueous	2–4	Dog, cat
		Oil	2–4	Dog, cat
Chloramphenicol + prednisolone acetate	Chlorasone	Oil	4–6	Dog, cat
Chloramphenicol + hydrocortisone acetate	Chloromycetin–Hydrocortisone–ophthalmic ointment	Oil	8	Dog, cat
Neomycin sulfate + hydrocortisone acetate	Neobiotic HC 15%; Neocortef 15%	Aqueous	3–6	Dog, cat
	Neo-Hydrops	Aqueous	3–4	All
Neomycin sulfate + bethamethasone	Betsolan	Aqueous	8	Dog, cat
	Vetsovate	Aqueous	4	Dog, cat
Neomycin sulfate + polymyxin B sulfate	Optiprime; Opthakote	Aqueous	4	Dog
	Tevemyxin	Aqueous	3–4	Dog, cat
		Oil	2	Dog, cat
Framycetin sulfate + dexamethasone sulfate	Fradexam	Aqueous	3–4	Dog, cat
		Oil	2	Dog, cat
Gentamycin sulfate + betamethasone	Beta-Septigen	Aqueous	6–8	Dog, cat
	Gentocin; Durafilm	Aqueous	3–4	Dog
Oxytetracycline HCl + polymyxin B sulfate	Terramycin	Oil	2–4	All
Prednisolone + vitamin A	VT CORT	Aqueous	4–6	Dog, cat
Ethylmorphine HCl + salicylic acid	Aminothionine	Oil	2	Dog, cat
Neomycin sulfate + flumethasone + polymyxin B sulfate	Anaprime; Opthakote	Aqueous	4	Dog
Neomycin sulfate + bacitracin zinc + polymyxin B sulfate	Neomycin–Bacitracin–Polymyxin	Oil	3–4	Dog, cat
	Mycitracin; Neobacimyx; Trioptic-P	Oil	3–4	Dog, cat
Neomycin sulfate + polymyxin B sulfate + neosynephrine HCl	Keratobiotic	Aqueous	2–4	All

Table 1 (*continued*)

Active ingredients	Trade name (®)	Excipient	Dosage[a]	Species
Neomycin sulfate + hydrocortisone acetate + tetracaine HCl	Neomycine–Hydrocortisone Vetoquinol	Oil	2	All
Neomycin sulfate + thiostrepton + triamcinolone acetonide	Panolog capsules	Oil	2	Dog, cat
Gentamycin sulfate + dexamethasone + THAM	Tiacil	Aqueous	2	Dog, cat
Framycetin sulfate + polymyxin B sulfate + synephrine HCl	Omnicol	Aqueous	3–6	Dog, cat
Neomycin sulfate + bacitracin zinc + polymyxin B sulfate + hydrocortisone acetate	Bacitracin–Neomycine–Polyxynin–Hydrocortisone; Trioptic-S; Neobacimyx-H	Oil	3–4	Dog, cat
Neomycin sulfate + sulfacetatmide + fluorocortisone acetate + lidocaine HCl	Fluokeratite	Oil	2	All
Idoxuridine	I.D.U. CUSI	Aqueous	6–10	Cat
Vidarabine	Vidarabine	Ointment	5	Cat
Trifluridine	TFT	Aqueous	6–10	Cat

[a] Number of daily applications.

III.B. Keratoconjunctivitis sicca

Keratoconjunctivitis sicca (KCS) is a deficiency of the precorneal tear film (PTF) and is a common and important disease in dogs.[61–63] A progressive increase in the number of cases recorded in veterinary schools from 0.04% in 1964 to more than 1.5% in 1988 has been reported.[64] The deficiency of the PTF produces pain and discomfort accompanied by blepharospasm and enophthalmos. As the KCS becomes chronic, a mucopurulent conjunctivitis occurs and may be associated with corneal ulceration, corneal vascularization and pigmentation, conjunctival erythema. The diagnosis of KCS is confirmed by the Schirmer tear test, a semiquantitative method of measuring production of the PTF. The etiology is various. It can be drug-induced, (e.g. phenazopyridine[65]), or surgically-induced after removal of prolapsed glands of the third eyelid. Lacrostimulants, like pilocarpine, because of their action as cholinergic agonists, may be used to increase lacrimal secretion.[66,67] The response depends upon whether functional lacrimal tissue is present. Lacromimetics, aqueous substitutes, mucinomimetics, and lipid replacements may serve as substitute for one or more tear component.[68] A large proportion of canine dry eye cases shows similarities to the KCS of human Sjörgen's syndrome, particularly in terms of immune-mediated destructive changes occurring in the lacrimal gland and hormonal involvement. Cyclosporine A, which is an immunomodulating drug, has been recently developed in topical form to treat canine KCS.[69–79] Topical 2% cyclosporine ointment has been demonstrated to improve 75–82% of idiopathic (presumed to be immune-mediated) cases of KCS in dogs. A six-week clinical trial of the efficacy of 0.2% cyclosporine ophthalmic oint-

ment (Optimmune®) for the treatment of chronic idiopathic KCS was carried out recently on 87 dogs in the United Kingdom, Germany and France.[80] The clinical response to the therapy was monitored after 7, 21 and 42 days and the results for the right and left eyes were analyzed separately. There was a statistically significant increase in lacrimal secretion throughout the study, with most of the increase occurring during the first week of treatment. Overall, 76% of the left eyes and 87% of the right eyes were considered to have improved at the end of the treatment. No serious adverse reactions were observed and only mild irritation was noticed by the owners immediately after the application of the ointment. Other symptomatic therapies, such as artificial tears, lubricant ointments and topical antibiotics are often used in association with topical cyclosporine. Cyclosporine has been used successfully to treat a variety of canine ocular conditions suspected of having immune-mediated basis, including nictitans plasmatic conjunctivitis,[78,79] and chronic superficial keratitis.[81]

III.C. Glaucoma

Glaucoma occurs in a large number of animal species, including dog, cat, rabbit, cow and horse, and results (as in the human) from the impairment of the aqueous humor outflow.[82] Elevation of the intraocular pressure, progressive optic neuropathy, visual impairment and blindness characterize glaucoma. The frequency in dogs in North America is about 0.5%.[83] The primary types are the breed-predisposed bilateral glaucoma (about 40 breeds such as beagles, cocker spaniel, basset hound). Secondary, post-uveitis, lens luxation or congenital glaucoma have been demonstrated in the canine population. Glaucoma occurs less frequently in cats than in dogs, and is often associated with anterior segment inflammation and lens luxation.[84] Siamese and Persian cats are reported to have a tendency to develop bilateral primary glaucoma.[85] Glaucoma in bovine species is rare and occurs mainly in case of severe infectious bovine keratoconjunctivitis. In horses, the major risk factors include age (over 21 years) anterior uveitis and the breed (Appaloosa). The intraocular pressure lowering action of these drugs is either by reducing the rate of aqueous humor secretion (beta-adrenergic agonists, carbonic anhydrase inhibitors) or by increasing aqueous humor outflow without affecting its formation (parasympathomimetic) or by affecting both its outflow and formation (adrenergic agonists). Unfortunately, animals are often taken to the veterinarian when the disease is already well advanced and medical treatment is then very difficult. Administration of medication (systemic administration of hyperosmotics or/and carbonic anhydrase inhibitors) combined with surgical procedures (transcleral cyclophotocoagulation, iridectomy, iridencleisis) is often necessary at advanced stages of the disease. Prophylactic treatment of the contralateral eye in dogs exhibiting unilateral glaucoma appears to delay the onset of glaucoma in this eye.

Numerous antiglaucoma drugs were evaluated by Gelatt for about 25 years on beagles which were bred with inherited open angle glaucoma.[86] Direct-acting parasympathomimetics such as pilocarpine were found to be active at 1, 2, and 4% concentrations. The response of decreasing intra ocular pressure and miosis appeared similar to that observed in man in terms of the effect observed and its time course. Side

effects reported in dogs include miosis, conjunctival irritation and blepharospasm. A 4% pilocarpine gel enhances the bioavailability of the drug, and only a once a day application is required to lower intraocular pressure and reduce the side effects.[87] Another parasympathomimetic agent, carbachol (0.75, 1.5, 2.25 and 3% concentrations) was reported to significantly lower intraocular pressure in glaucomatous beagles.[88] Cholinesterase inhibitors, such as demecarium bromide and echothiophate iodide are powerful and long-acting miotics that require less frequent installations than pilocarpine. Local irritation and systemic toxicity may develop after their topical application. In single-dose studies, demecarium bromide (0.125 and 0.5%) decreased intraocular pressure for 49 and 55 h, respectively. Echothiophate iodide (0.125 and 0.5%) reduced IOP for 25 and 53 h, respectively.[89] Adrenergic agonists such as epinephrine or its prodrug, dipivalyl epinephrine can be associated with parasympathomimetic drugs.[90–93] The clinical effectiveness of beta-blockers (timolol, betaxolol, carteolol, metipranolol, levobunolol, nadolol, pindolol) in lowering intraocular pressure by suppressing aqueous humor formation is well documented in human glaucoma. Unfortunately, the normally effective concentrations of timolol available (0.25 and 0.5%) are too low to induce a significant intraocular pressure lowering in dogs. The open angle glaucoma in beagles or cats requires 4 and 6% concentrations to significantly lower intraocular pressure (about 10–14 mmHg). These high doses cause a major decrease in the heart rate. Miosis was reported in cats after the topical administration of beta-blockers.[94–97] The differences in response to adrenergic agents between dogs, cats and humans suggest species differences in receptor density, subtypes or sensitivity.[98] The combination of timolol-pilocarpine is effective in dogs.[99] Also when topically applied to the eye apraclonidine (Iopidine) was reported to reduce intraocular pressure in both dogs and cats. Despite the potent ocular hypotensive effects, toxic effects (salivation and vomiting) make this formulation unsuitable for veterinary use on cats.[100] A topical carbonic anhydrase inhibitor, MK-927, was evaluated for its ocular hypotensive activity in normotensive and glaucomatous beagles. The 2% and 4% solutions of the topically applied anhydrase carbonic inhibitor significantly lowered intraocular pressure in glaucomatous beagles. New derivatives of $PGF_{2\alpha}$ have been tested on monkeys and humans and have demonstrated efficacy to lower intraocular pressure by increasing uveoscleral outflow.[101,102] Topical installations of 0.02, 0.2, and 0.4% $PGF_{2\alpha}$ isopropyl ester significantly decreased intraocular pressure in the treated eyes of glaucomatous beagles. The intraocular pressure remained lower than the baseline pressures 24 h post-treatment and the maximal change in intraocular pressure was of 19 mmHg. The side effects of $PGF_{2\alpha}$ isopropyl ester were miosis and mild conjunctival irritation.[103] The recently marketed topical carbonic anhydrase inhibitor, 2% dorzolamide and prostaglandin-related compounds latanoprost and unprostone should be a major advance in the management of animal glaucoma.

Research during the last decade has demonstrated that new therapeutics to treat human glaucoma will also have to protect or improve the ocular blood flow and prevent damage to the optic nerve. The use of calcium channels blockers, such as nifedipine, nicardipine, nimodipine for improving the ocular blood flow, and the use of growth factors or neuroprotectors for protecting axons within the optic nerve from

degeneration seem to be the right medical approaches for treating glaucoma in animals.[104,105]

III.D. Corneal ulceration

Corneal ulceration is a frequent disease in domestic animals. Trauma, foreign body, infectious keratitis, entropion or ectopic cilia are some of the causes of corneal ulceration. The use of vitamin A and of growth factors has been recommended, in combination with antibiotics and cycloplegic drugs such atropine.[106–110]

III.E. Infectious bovine keratoconjunctivitis

Infectious bovine keratoconjunctivitis (IBK) is one of the most common cattle diseases throughout the world. Approximately 20% (10 million) of the 48 million calves born annually develop IBK. The economical effects of IBK in beef and milk producing areas are that the animals do not gain weight, that they need to be fed during this time and that they require costly treatment.[111,112] Moraxella Bovis is considered to be the cause of IBK. The clinical signs are keratitis and conjunctivitis, intense lacrimation and epiphora, blepharospasm, central corneal opacity which enlarges and ulcerates. In severe cases, blindness may occur. Repeated topical administrations of antibiotics including penicillin, ampicillin, oxytetracycline, ormetropim-sulfadimethoxine, furazolidone, gentamicin, neomycin or erythromycin, are required to provide the high drug levels needed to treat the disease.[113] Ocular drug delivery systems, such as ocular inserts and contact lenses afford considerable advances in IBK treatment.[114,115]

IV. Currently investigated systems for ocular controlled drug delivery in animals

The most common dosage form in which drugs are formulated for use in the eye is the ophthalmic drop. However, a significant problem encountered with the administration of ophthalmic drops is an induced reflex lacrimation following instillation. This results in an initial high drug concentration in the eye associated with a short initial period of overdosing followed by its rapid removal and a long period of underdosing. This leads to the necessity for a frequent administration regime in order to maintain therapeutic levels. There is a need therefore in ocular drug delivery to develop delivery systems which prolong the time the drug is in contact with ocular tissue. This may be achieved through the development of ocular controlled drug delivery systems. The main objective of such systems is to retard the release of drug from the delivery system in order to prolong the contact time of the drug with the conjunctival tissue thereby ensuring a satisfactory topical or systemic treatment. In comparison with traditional ophthalmic preparations (e.g. eye drops), controlled drug delivery systems offer several advantages, including increased contact time of drug with the target tissues of the eye, prolonged drug release, reduction of systemic side effects and reduction of the number of administrations.

IV.A. Hydrogels

Kim et al.[116] defined hydrogels as polymers which have the ability to swell in water or in aqueous solvents, and induce a liquid-gel transition. However, in ophthalmic applications, it is difficult to draw a sharp line between actual hydrogels and highly viscosified solutions. According to Plazonnet et al.,[117] aqueous gels are the upper limit of viscous preparations and they are formed when high molecular weight polymers or high polymer concentrations are incorporated into a formulation.

Hydrogels can be classified into two distinct groups; preformed gels and in situ forming gels.[118] The preformed gels can be defined mainly as simple, highly viscous solutions which do not undergo further modification after administration (see Section IV.A.1.), whereas in situ gelling systems can be described as viscous liquids or suspensions that, upon exposure to physiological eye conditions (ionic strength, temperature or pH), will shift to a gel phase (see Section IV.A.2.). The latter formulations are superior to classical gels in that they allow administration of accurate and reproducible quantities which exhibit a high final viscosity. Preformed, as well as in situ forming gels, can be based on natural, semi-synthetic or synthetic polymers.

There are two main applications of hydrogels in veterinary drug delivery. These are as tear substitutes in, for example, diseases such as KCS (Table 2), or as drug delivery systems (Tables 3 and 4). The improvement in residence time of ophthalmic semisolid hydrogels is primarily based on an increase in ocular residence time as a result of a reduction in drainage rate through enhanced viscosity and mucoadhesive properties.

IV.A.1. Preformed hydrogels

The pioneering group of polymers used as components of ophthalmic preformed hydrogels is the family of cellulosic derivatives. Methylcellulose (MC) was first introduced into ophthalmic formulations in the 1940's as a viscosifyer.[119] Since then, cellulosic polymers have been extensively studied in human,[120,132–134] as well as in veterinary medicine,[121,134,135] for ocular administration. Most commonly used are MC, hydroxethylcellulose (HEC), hydroxypropylcellulose (HPC), hydroxypropyl methyl cellulose (HPMC) and carboxymethylcellulose sodium (CMC Na). These cellulosic polymers appear in several currently available commercial preparations such as Adsorbotear® (Alcon, Fort Worth, Texas), Lacril® (Allergan, Irvine, California) and Celluvisc® (Allergan, Irvine, California). Tolerance studies in humans have demonstrated that HEC was the best tolerated polymer among these cellulosic derivatives after topical administration.[120,136] At the concentrations studied for ocular purposes, all cellulosic polymers cited above are reported to be generally well accepted, but with the major drawback that they can induce blurring of vision and can sometimes lead to crusting eyelids.

Scientific interest has been directed toward using other viscosifying agents. Polyvinyl alcohol (PVA) was introduced in the early 1960's as a mean to increase solution viscosity and, hence, prolong precorneal residence time. The presence of PVA in ophthalmic preparations has been shown to significantly delay precorneal drainage of topically applied formulations and to increase drug bioavailability as well as pharmacological effects such as miotic response to pilocarpine exposure when compared

Table 2
Hydrogels for the treatment of keratoconjunctivitis sicca

Carrier	Remarks	Species[a]	Reference
Preformed hydrogels			
Methyl cellulose	Well tolerated; blurring vision; newtonian rheological behavior	R	119
Hydroxycellulose (Adsorbotear®)	No irritation and tolerable blurring vision; newtonian behavior	R/H	120
Hydroxypropylcellulose	Discomfort; blurred vision; lachrymation Newtonian behavior	R/H	120
Hydroxypropylmethyl cellulose (Lacril®)	As above; Newtonian behavior at low concentrations but pseudoplastic behavior at higher concentrations. Longer excretion time than polyvinyl alcohol	R/H	120–124
Polyvinyl alcohol (Hypotears®)	No blurring vision but discomfort at 42%; good adhesive quality; excellent contact time; very satisfactory wetting agent; newtonian behavior	R/H	125,126
Carbopol	No inflammation or discomfort; tolerable blurred vision; pseudoplastic behavior	H	122,127
Sodium hyaluronate	Better tolerated than HEC; blurring vision; shorter contact time in normal eye when compared to dry eye Good protective efficacy against dryness; pseudoplastic behavior	H	128
Xanthane	Growth of *P. aeruginosa* in presence of preservative; pseudoplastic behavior	R/H	122
Carraghenan	Well tolerated; pseudoplastic behavior	H	129
Dextran (Tears Naturale® II)	Well tolerated (preferred to PVA); pseudoplastic behavior	H	125
In situ forming hydrogels			
Gelrite	Excellent tolerance; no irritation Blurred vision; residues on eyelids; pseudoplastic behavior	R/H	130
Cellulose acetate phthalate latex	Good tolerance; pseudoplastic behavior	R	131
Pluronics	Improved tolerance when mixed with isotonic saline solution; pseudoplastic behavior	R	131

[a] H: human; R: rabbit.

with conventional saline.[137] Ludwig et al.[125] have established that PVA having a molecular weight of about 100 000 (Polyviol® W40/140) should not be used at

Table 3
Preformed hydrogels for ocular delivery of drugs

Drugs	Polymeric carrier	Remarks	Species[a]	Reference
Benzadac	Xanthan gum	Important decrease in systemic absorption	R	149
	Scleroglucan	See hydroxpropylmethylcellulose and xanthan gum	R	149
	Hydroxpropylmethylcellulose	A 10–100-fold increased viscosity produces only a modest increased aqueous bioavailability	R	149
Betaxolol	Carbopol®	Physical stability enhanced; ease of resuspendability; ocular comfort	R	150
Chloramphenicol	Carbopol®	Stinging; local discomfort	H	151
Fusidic acid	Carbopol®	72 h half-life elimination from lachrymal fluid; smarting and blurring sensation	H	152
		Useful antibiotic concentrations maintained up to 12 h; well tolerated	H	151
Gentamycin	Carbopol®	Drug penetration in aqueous humor only with non-neutralized polymer; relatively well tolerated, but irritancy due to low pH	R	153
	Hyaluronic acid	Increased bioavailability for 10 min compared to aqueous solutions	H	154
Pilocarpine	Carbopol® (Pilopine® HS)	High viscosity is not relevant in presence of mucoadhesive properties	R	155
		Significant increase ($P < 0.05$) in bioavailability as compared to polyvinyl alcohol and aqueous solution	R	137
		Optimal miosis duration at concentration of 3%, with a 15-fold increase	R	156
		45-fold clinical response, 2-fold AUC Not irritating	R/H	157
	Hyaluronic acid	Prolongs precornal residence time by direct interaction with corneal tissue, at low concentrations	R/H	158
		Increased bioavailability compared to aqueous and polyvinyl alcohol solutions	R	155
	Hydroxypropylcellulose	Autoclaving is preferred to gamma-irradiating	R	159
	Methylcellulose	Excellent linearity between increased ocular bioavailability and decreased drainage rate	R/H	160
	Polyvinyl alcohol	Activity significantly greater in humans than in rabbits	R/H	161
		Significant increased bioavailability ($P < 005$) compared to control	R	137
	Polyvinyl pyrrolidone	1–5-fold increased activity compared to aqueous solution	R/H	161
	Xanthane	Increased miotic response compared to aqueous and hydroxyethylcellulose solutions, but to a smaller extent than Gelrite	R/H	145
	Hydroxyethylcellulose (Piloplex®)	Intraocular pressure reduction; reduced visual disturbance	R/H	162,163
Pilocarpine pro-drug	Polyvinyl alcohol	Eye irritation of pilocarpine pro-drug eliminated with polyvinyl alcohol solution without impairing ocular absorption	R	164
Prednisolone	Carbopol®	45-fold higher AUC than solution	R	143

Table 3 (*continued*)

Drugs	Polymeric carrier	Remarks	Species[a]	Reference
Timolol	Carbopol®	Equivalent clinical effect with only one fifth of the timolol dose is compared to aqueous solution; less blurred vision due to timolol administration with the gel	H	142
	Carboxymethylcellulose sodium	Possible cardiac effects reduced by phenylephrine co-administration and increased solution viscosity, while keeping equal to increased concentrations in ocular tissues	R	165,166
	Hydroyethylcellulose	Good surface spreading, similar to polyvinyl alcohol	R	167
		In vitro release rates similar to gelrite when isoviscous	R	168
Timolol/Phenylephrine	Carbopol®	Reduced systemic absorption	R	167
Tropicamide	Polyvinyl alcohol	15-fold increased duration effect	R/H	168
	Hydroxypropylcellulose	Increased miotic effect; rabbits are less sensitive than human to increased viscosity and different rheological properties due to reduced blinking rate	R/H	169,170

[a] R: Rabbit; H: Human.

Table 4
Currently investigated in situ forming hydrogels for ocular delivery of drugs

Drugs	Polymeric carrier	Remarks	Species[a]	Reference
Methyl prednisolone	Gelrite®	2–6-fold higher AUC compared to the suspension; no irritation; opaque gel; ease of administration	R	174
Pilocarpine	Cellulose acetate phthalate latex	No prolongation of residence time	R/H	158
		Marked increase in corneal residence time compared to aqueous solutions	R	164,187
	Polyvinyl pyrrolidine	Pseudo-latex induces a greater intensity of the biological response; no objective sign of damage	R /H	188
	Pluronics®	1–2-fold increased residence time compared to aqueous solution;	R/H	189
		1–9-fold increased bioavailability compared to aqueous solution; mucomimetic properties; low toxicity	R	190
		Prolongation of residence time compared to saline solutions	R/H	158
		No significant improvement of miotic response duration	R	159
Timolol	Gelrite®	Increased AUC in vivo when compared to hydroxypropyl cellulose 0.5%, whereas similar in vitro release; excellent tolerance	R	191,192

[a] H: human; R: rabbit.

concentrations above 4.2% (w/w) for tolerance reasons. Furthermore, Patton et al.[138] pointed out the uselessness of increasing solution viscosity indefinitely since an optimal viscosity range (12–15 cps) exists for PVA formulations (Figure 1), which corresponds to a polymeric concentration of between 2.5 and 3%.

The actual trend in ocular delivery is to use sodium salt of hyaluronic acid (SH) and carbomer, patented as Healon® (Kabi Pharmacia, Sweden) and Carbopol® (B.F. Goodrich Chemical Company, Cleveland, USA), respectively. The former is a natural polysaccharide found in skin, connective tissues, vitreous body and aqueous humor, whereas the latter is a synthetic polymer. The main advantages of SH are its excellent biocompatibility, mucoadhesiveness as well as its pseudoplastic and viscoelastic behavior. The use of SH in the ophthalmological field was reviewed a few years ago by Bernatchez et al.[139] This polysaccharide is frequently proposed as a vehicle of choice in tear substitutes since it has been reported to possess a desirable protective effect against damage caused by benzalkonium chloride, a compound commonly added as a preservative in multiple dosage forms.[140] In addition, SH has been

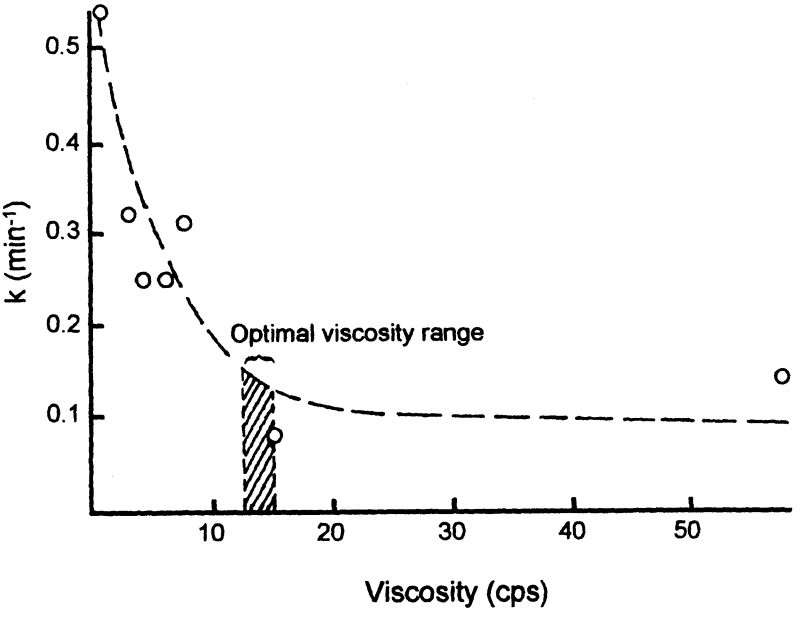

Fig. 1. Drainage rate of various polyvinyl alcohol solutions as a function of solution viscosity (adapted from Ref. 138).

shown to provide conclusive results in the treatment of KCS, being effective in reducing common symptoms such as blurring vision, pain and photophobia. Some commercial products containing SH are currently available being mostly indicated (for example Healon® and Viscoat®) for use as surgical aids in anterior segment procedures such as cataract extraction or intraocular lens implantation rather than for topical administration. The superiority of Carbopol® over simple saline and suspensions in enhancing precorneal residence time[141] and drug bioavailability[142,143] has been demonstrated by several authors, as shown by the example in Figure 2.

Some new polymers in the ophthalmic field such as xanthan gum or chitosan are currently under investigation for topical administration. Evaluating transcorneal delivery of pilocarpine from several ophthalmic formulations, Saettone et al.[144] demonstrated that the presence of 1.5% of xanthan gum induced a significant improvement of the pharmacokinetic parameters of the drug such as area under the curve (AUC), half-life time of elimination and the mean residence time in aqueous humor. Another study was conducted by Messeguer et al.[145] which compared an in situ forming gel (see Section IV.A.2.) based on Gelrite® and xanthan gum. Precorneal residence time showed that the former gel was more efficient in slowing down elimination from the eye surface.[145] However, the ability of these gels to enhance miotic activity parameters of pilocarpine were equivalent, and both were superior to a reference solution and HEC based formulations.

The use of chitosan as a vehicle for the ophthalmic route is also being investigated, although studies are at a preliminary stage. Chitosan has been studied most often as

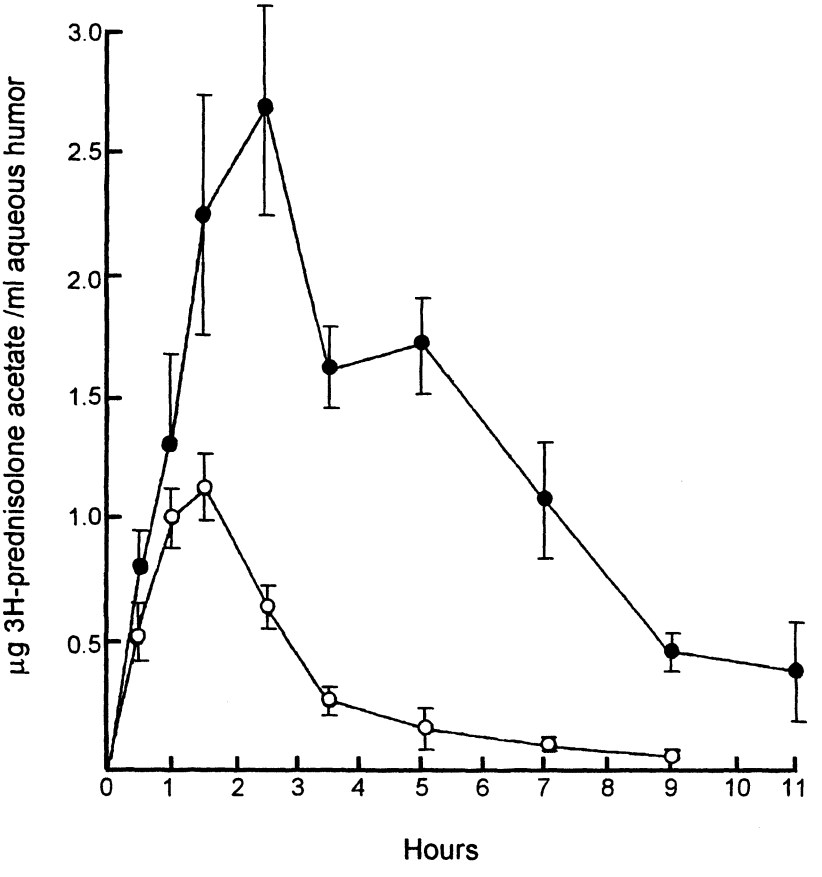

Fig. 2. Mean prednisolone acetate concentration in the aqueous humor of rabbits ($n = 6$) after dosing with 50 µl of (●) 1% Carbopol-940 gel or (○) aqueous suspension.[143]

dispersed forms, micro- or nanoparticles[146,147] rather than as a hydrogel. However, some experiments refer to the use of viscosified solutions based on chitosan for topical administration to the eye. It has been shown that chitosan is very well tolerated by using a reliable and reproducible method based on the combination of fluorescein staining and examination of the eye with a confocal laser scanning ophthalmoscope.[148] Promising results have been obtained, demonstrating that chitosan formulations remained significantly longer on the corneal surface when compared with a conventional commercial solution.[148]

IV.A.2. In situ forming gels

IV.A.2.a. In situ forming gels influenced by ionic strength Gellan gum is an anionic polysaccharide produced by the bacterium *Pseudomonas elodea*[171] which, when dispersed in aqueous solutions, undergoes a liquid-gel transition under the influence

of an increase in ionic strength.[172] As a consequence, after in vivo administration, the usual reflex lacrimation which would normally be expected to result in the dilution of viscous solutions, further enhances the viscosity of the formulation due to the cation concentration of normal tears.[130] The gellation increases proportionally to the amount of either monovalent or divalent cations present in the lacrimal fluid and in vitro experiments[173] have demonstrated that divalent cations are more efficient in promoting sol-gel transition than monovalent ions. However, the in vivo conditions (i.e. the concentration of sodium in tears) is sufficient to induce the gellation process. This gellation phenomenon is primarily a result of the formation of an ordered state of gellan chains.[174] Comparing Gelrite® 0.6% with HEC solutions of similar pseudoplastic behavior as well as comparable viscosities, Rozier et al.[168] have demonstrated that gellan gum induced significantly higher concentrations of timolol in different ocular segments such as in the cornea, the aqueous humor and in the iris and the ciliary body (Figure 3).

Recently a group of water soluble sulphated galactans, namely carrageenans, have been proposed as vehicles for ocular delivery showing similar rheological properties (pseudoplasticity, viscoelasticity) to Gelrite®.[129] Furthermore, the authors suggested that since these compounds are strong polyelectrolytes, they will have an identical gelling mechanism to gellan gum.

IV.A.2.b. In situ forming gels influenced by temperature Poloxamers are polyols which exhibit gelling properties when they are heated. Their concentration is chosen in accordance with the desired liquid-gel transition. At concentrations above 20%w/w, poloxamers exhibit the phenomenon of reverse thermal gellation, that is,

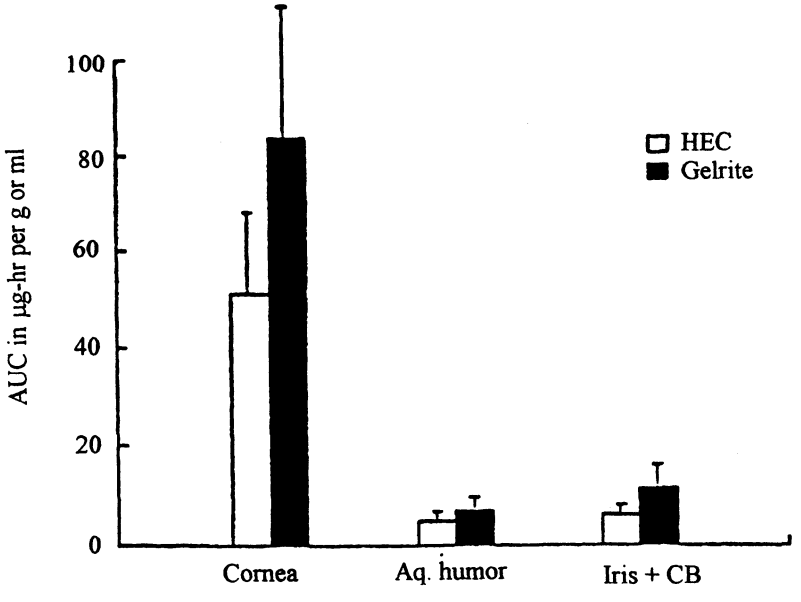

Fig. 3. AUC of timolol concentration versus time plot after instillation of 0.25% in Gelrite or hydroxyethylcellulose.[168]

gelling upon warming up from ambient to body temperature.[175] Interestingly, the temperature of transition of poloxamers can be modulated by adding solutes or polymers such as poly(ethylene glycols)[176] or cellulosic derivatives such as MC or HPMC[177] to the formulation. Poloxamers commercially available as Pluronic® (BASF-Wyandotte, USA) are the most commonly used thermal setting polymers in ophthalmology owing to their low toxicity, mucomimetic properties and optical clarity. However, the disadvantage of poloxamers as compared to Gelrite® lies in their mechanism of gellation. In fact, since sol-gel transition takes place as the temperature increases, accidental gellation during conservation may occur.

A new attractive thermal sensitive hydrogel, Smart Hydrogel™ composed of a polymeric network of poly(acrylic acid) and poloxamer, has been described by Gilchrist et al.[178] After administration, rabbits did not show irritation symptoms. Furthermore, gamma scintigraphic assessment demonstrated a significant increase of the precorneal residence time and the half-life time of elimination was multiplied by 25 when compared with a saline solution.

IV.A.2.c. In situ forming gels influenced by pH Pseudo-latexes have been defined by El-Aasser[179] as artificial latices obtained by the dispersion of a pre-existing polymer in an aqueous medium. Such systems correspond to low viscosity aqueous dispersions, which can undergo spontaneous coagulation in the conjunctival cul-de-sac owing to an increase of the local pH. The massive swelling of the particles is due to the neutralization of the acid groups contained in the polymer chain. The increase in viscosity is by several orders of magnitude.[158,180,181] In situ gelling pseudo-latexes can be prepared by two manufacturing processes; the solvent evaporation process[181–183] and the salting out process.[183–185] Independent of the chosen fabrication process, the final product obtained is a lyophilized and easily redispersible powder. Such a dry form represents a great advantage as a tool to carry drugs sensitive to aqueous media, which could undergo premature degradation during storage if in solution. Studies on the use of pH-sensitive nanoparticulate systems in ophthalmology began in the 1980s and have since been extensively studied by Boye[180] and Ibrahim,[186] who advocated the use of cellulose acetate phthalate (CAP) as the polymeric component for reason of stability and compatibility. The superiority of CAP pseudo-latexes over simple saline has been clearly demonstrated by several authors by evaluating either pharmacological drugs effects[158,184,186] or mean residence time on the corneal surface.[158,186]

IV.A.3. Comments on the formulation of hydrogels as drug delivery systems

It is generally accepted that the instillation of a viscous solution should influence as little as possible the tears' normal flow behavior. Tears show a pseudoplastic and viscoelastic behavior, thus similar rheological properties should be inherent within the delivery system.[193,194] Non-newtonian vehicles such as gellan gum, hyaluronic acid and carbomer are more effective than newtonian formulations containing PVA or celluloses in a similar viscosity range. Furthermore, it is important to note that the viscosity and the rheological behavior are not the only factors which must be considered. Mucoadhesive and wetting properties are also critical parameters to take into

consideration when formulating a hydrogel ocular delivery system.[157,195,196] In fact, the conjunctival tissue surface carries a negative charge due to the presence of sialic acid residues and contains functional groups which can participate in adhesive interactions with polymers such as carbopol. Mucoadhesive polymers contain numerous hydrophilic functional groups providing for electrostatic and hydrophobic interactions, as well as hydrogen bonding with the underlying corneal surface. It is important to note that the mucin layer turnover (15–20 h) is an important limiting factor to the use of hydrogels based on mucoadhesive polymers.[197]

Semi-solid hydrogels are widely accepted as tear substitutes in humans, because of their mucomimetic properties and their prolonged residence time on the eye surface.[198] Tear substitutes developed for human use may therefore be used to advantage in most cases for the treatment of common KCS in animals.

Aqueous gels are only of limited interest for sustained drug delivery. In fact, these vehicles only show a moderate improvement in the release characteristics of at most a few hours compared to several days obtained with inserts (see Section IV.B.). Furthermore, their administration to humans often results in blurred vision, crusting of eyelids and sometimes lachrymation. As a consequence, patients generally prefer to use eyedrops even though a more frequent administration is required. However, these formulations could be advantageously used in the veterinary field, by reducing the number of administrations and by facilitating the administration by the owner of the animal, compared to inserts, which have to be placed most often by a veterinarian. It is important to point out that most ophthalmic formulations have been evaluated in rabbits and in humans, and that the resulting data do not always match. Similar discrepancies would to be expected between other animal species, because of physiological and anatomical differences, such as blinking rate, tear basal turn over or cation concentrations in tears.

IV.B. Inserts

This section is devoted to solid devices delivering drugs to the anterior segment of the eye that are denoted by the general name *insert*, originating from the Latin *inserere*, to introduce. Historically, the first solid medication precursors of the present insoluble inserts, were described in the 19th century. They consisted of squares of dry filter paper, previously impregnated with drug solutions (e.g. atropine sulfate, pilocarpine hydrochloride);[199] small sections were cut and applied under the eyelid. *Lamellae*, the precursors of the present soluble inserts, consisted of glycerinated gelatin containing different ophthalmic drugs.[199] However, the use of lamellae ended when more stringent requirements for sterility of ophthalmic preparations were enforced. Nowadays, ophthalmic inserts are again provoking great interest, as evidenced by the increasing number of publications in the field in recent years.

The uses of ocular inserts have been extensively reviewed by Felt et al.,[200] Bawa,[201] Saettone et al.,[199,202] Khan et al.[203] and Shell.[204,205] Gurtler and Gurny[206] have reviewed ophthalmic inserts in the patent literature while Baeyens et al.[207] have described inserts as potential ocular drug delivery devices in veterinary medicine. The aim of

this section is to give an overview of the different drugs to which insert therapy has been applied in the veterinary field.

Ophthalmic inserts are defined as preparations with a solid or semisolid consistency, whose size and shape are especially designed for ophthalmic application (i.e. rods or shields).[206] These inserts are placed in the lower fornix and, less frequently, in the upper fornix, or on the cornea. They are usually composed of a polymeric vehicle containing the drug and are mainly used for topical therapy.

The advantages of inserts include all those discussed above for hydrogel systems plus accurate dosing, absence of preservatives and increased shelf live, due to the absence of water.[207] However, inserts have one significant disadvantage, their solid consistency, which means that they are perceived by patients and animals as a foreign body in the eye.[202] Besides the initial discomfort upon administration, other potential disadvantages arising from their solid state are, possible movement around the eye, occasional inadvertent loss during sleep or while rubbing the eyes, interference with vision and difficult placement (and removal for insoluble devices).[202] Most of the ongoing research is therefore dedicated to improving ocular retention and to ensure an easy placement, while reducing the foreign body sensation in the eye.

Ophthalmic inserts are generally classified according to their solubility behavior and their possible bioerodibility.[206]

IV.B.1. Soluble inserts

Soluble inserts are the most frequently investigated class of ophthalmic inserts in veterinary applications (Table 5). Their main advantage relies on their complete solubility compared with their insoluble counterparts, so that they do not need to be removed from the eye after therapy. They are usually divided into two categories, according to their polymer composition. The first type is based on natural polymers whereas the second is derived from synthetic or semisynthetic polymers. Drug release from soluble inserts is generally characterized as a diffusion process occurring in two steps.[206,208] The first corresponds to the penetration of tear fluid into the insert, which induces a rapid diffusion of the drug and forms a gel layer around the core of the insert. This external gelification induces the second phase corresponding to a decreased release rate, again controlled by diffusion. The major problems of these soluble inserts are the rapid penetration of the lacrimal fluid into the device, the blurred vision caused by the solubilization of insert components and the glassy constitution of the insert increasing the risk of expulsion.

IV.B.1.a. Natural polymers Natural polymers include collagen, which was the first ophthalmic insert excipient described in the literature. Inserts containing collagen were first developed by Fyodorov[201,230] as corneal bandages following surgical operations and eye disease. Later, collagen shields as drug carriers were suggested by Bloomfield et al.[220] As described for contact lenses, the therapeutic agents are generally absorbed by soaking the collagen shield in a solution containing the drug and, once placed in the eye, the drug is gradually released from the interstices between the collagen molecules, as the collagen dissolves. Accordingly, the residence time of drugs[231] such as antibacterials,[232,233] anti-inflammatory agents,[234,235] antivirals[236,237] or

Table 5
Currently investigated soluble inserts for ocular delivery of drugs

Drugs	Carrier	Remarks	Species[a]	Reference
Pilocarpine	Hydroxypropylcellulose/D-lactose/ glyceryl palmito-stearate/Eudragit® RS	Increased lipophilic character and coating result in an increased pilocarpine bioavailability	R	209
	Polyvinyl alcohol/Xanthen gum/ Hydroxypropylmethylcellulose/ hyaluronic acid/ glyceryl behenate/Eudragit® RS 30 D	Coated inserts show a sustained release of 9–10 h duration, shift to the peak time to 120–240 min, over 3-fold increased AUC over the standard aqueous solution	R	210
	polyvinyl pyrrolidioe	2.5-fold increased AUC over the aqueous solution	R	211
	alginate; methylcellulose	Prolongation of the duration of the miotic activity to a greater degree than solutions of the hydrochloride in the presence of methyl cellulose	R	212
	Hydroxypropylcellulose	Delayed and decreased peak concentration of pilocarpine in general circulation compared to aqueous solution	R	213
	Hyaluronic acid; ethyl ester, hyaluronic acid	Increased bioavailability of pilocarpine compared to the standard aqueous vehicle	R	214
	Polyvinyl alcohol (NODS®)	Increased bioavailability of pilocarpine with respect to standard eyedrop formulations	R	215
Timolol	Hydroxypropylcellulose/Eudragit® RS	Coated inserts antagonized isoproterenol-induced ocular hypertension significantly more than timolol eyedrops and uncoated inserts; sustained release of timolol in tear fluid and decreased systemic peak concentration with coated and uncoated inserts compared to the aqueous solution	R	216
	Hydroxypropylcellulose; polyvinyl alcohol; PVA/ Carbopol® 940	Drug release from the insert decrease in the order polyvinyl alcohol > hydroxypropylcellulose > polyvinyl alcohol/ Carbopol® 940; reduction in peak plasma timolol concentration 2–5 times compared to the aqueous solution	R	217
Timolol and prodrugs of Timolol	Polyvinyl alcohol; hydroxypropylcellulose; hydroxypropylcellulose/Carbopol® 940	Timolol release from the insert decreased in the order polyvinyl alcohol > hydroxypropylcellulose > polyvinyl alcohol/ Carbopol® 940; release rate much slower for timolol prodrugs compared to the timolol-containing inserts	R	218
Tilisolol	Poly(2-hydroxypropyl methacrylate)	Release of tilisolol follows a non-fickian mechanism	R	219
Chloramphenicol	Polyvinyl alcohol (NODS®)	Increased bioavailability of chloramphenicol with respect to standard eyedrop formulations	R	215

Gentamicin	Collagen	After 3 h, collagen insert gives the highest tear film and tissue concentration of gentamicin compared to ointment, aqueous solution and subconjunctival route	R	220
	Collagen	Concentrations in the precorneal tear film approximate the minimum inhibitory concentration for gentamicin during the 24 h after insertion	B	117
	Hydroxypropylcellulose /ethylcellulose/ Carbopol® 934P	Concentrations in the precorneal tear film approximate the minimum inhibitory concentration for gentamicin during the 72 h after insertion	R/D	221
Gentamicin + dexamethasone	Collagen	Collagen shields impregnated with gentamicin-dexamethasone are comparable to the subconjunctival delivery of these drugs over a 10-h period	R	222
	Hydroxypropylcellulose/ethylcellulose/Carbopol® 934P	Concomitant release of dexamethasone and gentamicin for 24 and 48 h, respectively; increased lacrymal availability for both drugs when compared to commercial solution	R,D	223–225
Tobramicyn	Collagen	No significant difference between collagen and aqueous solution in the treatment with *Pseudomonas Aeruginosa*-induced keratitis	R	226
Erythromycin + erythromicin estolate	Copolymers of n-vinylpyrrolidone	Complete suppression of a *Chlamydia Trachomatis* infection; inserts remain 7 days in the eye	M	227,228
Dexamethasone	Polyvinyl alcohol; hydroxypropylcellulose; ethylcellulose; cellulose acetate phthalate; Eudragit®	Increased dexamethasone concentration in eye tissues compared to suspension	R	229

[a] B: bovine; D: dog; M: monkey; R: rabbit.

combination drugs[222] was increased when compared to traditional eye drops. For example, Bloomfield et al.[220] compared the levels of gentamicin in tears, cornea and sclera of the rabbit eye after application of a collagen insert, drops, an ointment or following subconjunctival administration. After 3 h, they found that the collagen insert gave the highest concentration of gentamicin in the tear film and in the tissue. However, as observed for contact lenses, most drugs are released quite rapidly by a diffusion process, whereas dissolution requires a much longer time. The corneal shields, currently available for clinical use, do not contain drugs, but are designed as disposable therapeutic corneal bandages.[202] For example, Bio-Cor® (developed by Bausch and Lomb, Clearwater, Florida) is made of porcine scleral collagen, while Medilens® (developed by Chiron Ophthalmics, Irvine, California) and ProShield® (developed by Alcon Surgical, Fort Worth, Texas) are prepared from bovine corium tissue.[237–239] The main advantages of collagen shields over contact lenses is their solubility and, that they do not need to be removed. However, collagen may cause an inflammatory response in the ocular tissues. Also, if shields are not used in association with antibacterials, a secondary infection may occur.[236] Nowadays, these devices have the further disadvantage of not being well accepted by the authorities, because of possible prion-based infection.

IV.B.1.b. Synthetic and semisynthetic polymers Ophthalmic inserts containing synthetic, i.e. PVA,[210,229] and semisynthetic, i.e. cellulose based[209,210,212,213,216,221,229] polymers are extensively described in the literature. This stems in part from their advantage of being based in products well adapted for ophthalmic use and their ease of manufacture by conventional methods, including extrusion,[221] compression[209] and compression molding.[240] Ethylcellulose, a hydrophobic polymer, can be incorporated in the formulation to decrease insert deformation, and therefore prevent blurred vision.[221,224,229] Regarding the risk of expulsion, several authors[217,218,221,224,225] have incorporated carbomer, which is, at low concentrations, a strong, but well-tolerated bioadhesive polymer. Recently, Baeyens et al.[223–225] and Gurtler et al.[221,241] have used CAP in combination with gentamicin sulfate to decrease drug solubility rate. Prolonged release of the drug above the minimum inhibitory concentration (MIC) was obtained for more than 50 h, while gentamicin incorporated without CAP was released for less than 24 h. Subsequently, a new insert, providing release of gentamicin and dexamethasone at different rates was developed. The prolonged release of gentamicin – an antibacterial agent – is combined with the immediate release of dexamethasone – an anti-inflammatory agent – against structural damage that can be caused by the infection (Figure 4). A recent study (unpublished results) demonstrated that the deposition of one insert (Figure 5) was as effective as a reference eyedrop treatment of one week (3 instillations per day during 7 days) to cure KCS, conjunctivitis and superficial corneal ulcer in dogs. These encouraging results demonstrate that the reduced number of manipulations with the insert therapy (one deposition) when compared with the classical eyedrop treatment (21 instillations) makes inserts interesting candidates for improvement of compliance.

The release rate from ocular inserts can also be decreased by using Eudragit® as an insert coating agent.[209,210,216] This polymer is normally used for enteric coating, and

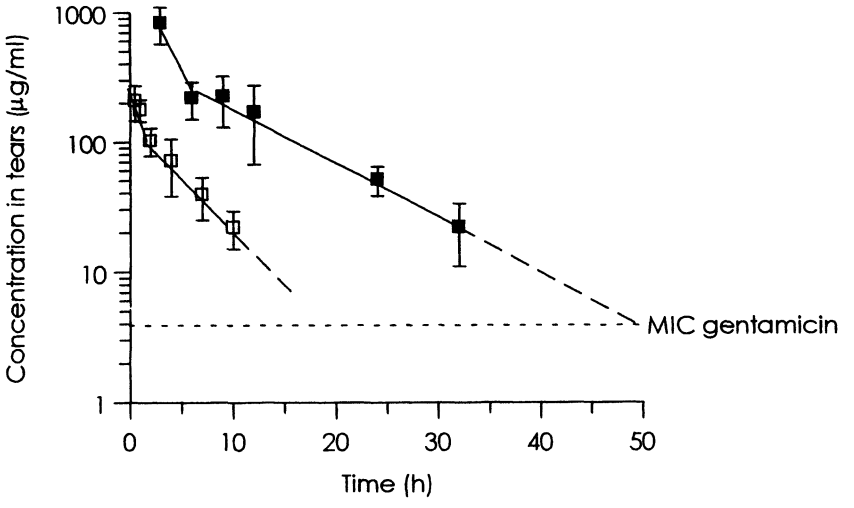

Fig. 4. Concomitant release of gentamicin sulfate (■) and dexamethasone phosphate (□) from a single insert containing 5.0 mg of gentamicin sulfate (25.0% w/w) and 1.0 mg of dexamethasone phosphate (5.0% w/w) (tested in rabbit, mean ± SEM, $n = 6$).[224]

Saettone et al.[209] have observed in rabbits that Eudragit® coated inserts containing pilocarpine induced a miotic effect of longer duration, compared to the corresponding uncoated products.

Lacrisert® is a soluble insert that has been commercialized by Merck Sharp and Dohme in 1981.[199] The device weighs 5 mg, measures 1.27 mm in diameter with a length of 3.5 mm and is composed of HPC and is useful in the treatment of dry eye syndrome. The device is placed in the lower fornix where it slowly dissolves over 6–8 h to stabilize and thicken the tear film.[242]

New Ophthalmic Delivery System (NODS®), originally patented by Smith and Nephew Pharmaceuticals Ltd in 1985, consists of a medicated flag (4 × 6 mm, thickness 20 µm, weight 0.5 g) which is attached to a paper-covered handle by means of a short (0.7 mm) and thin (3–4 µm) membrane (Figure 6).[215] All components (flag, membrane and handle) are made of the same grade water-soluble PVA. For use, the flag is touched onto the surface of the lower conjunctival sac. The membrane dissolves rapidly releasing the flag which swells and dissolves in the lacrimal fluid, delivering the drug. When evaluated in vivo, NODS produced an increase in bioavailability for pilocarpine and chloramphenicol with respect to standard eyedrop formulations.[215]

IV.B.2. Insoluble inserts

Insoluble inserts can be classified into two categories:[225] reservoir and matrix systems.

IV.B.2.a. Reservoir inserts Reservoir inserts consist of a central reservoir of drug enclosed in a specially designed semipermeable or microporous membranes which

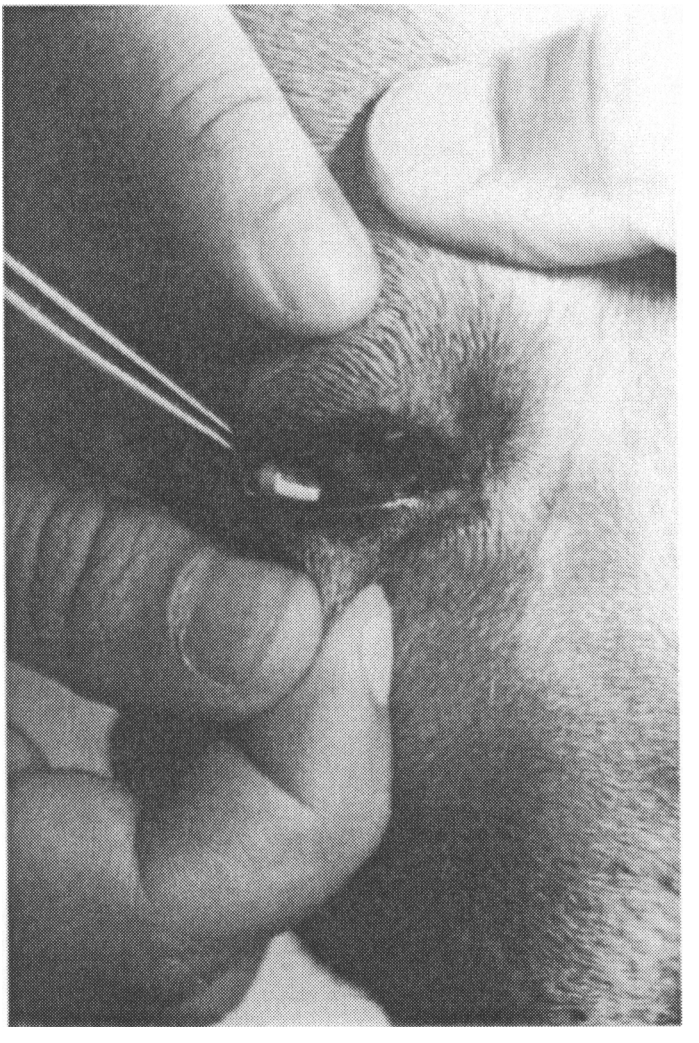

Fig. 5. Bioadhesive ophthalmic drug insert (2 mm diameter, 5 mm length) after deposition in the inferior conjunctival sulcus of a beagle dog.

allow the drug to diffuse from the reservoir at a precisely determined rate in a zero order release fashion.

Ocusert® (developed by Alza Corporation, Palo Alto, California) is undoubtedly the most extensively described insoluble insert in the literature (Figure 7).[202,205,243–249] It is flat, flexible elliptical device which consists of a pilocarpine reservoir comprising alginic acid, which is surrounded on both sides by a membrane of ethylene-vinyl acetate copolymer. The device is encircled by a retaining ring impregnated with titanium dioxide. The dimensions of the elliptical device are: major axis, 13.4 mm; minor axis, 5.7 mm; thickness, 0.3 mm. Two types of Ocusert® are available: the Pilo-

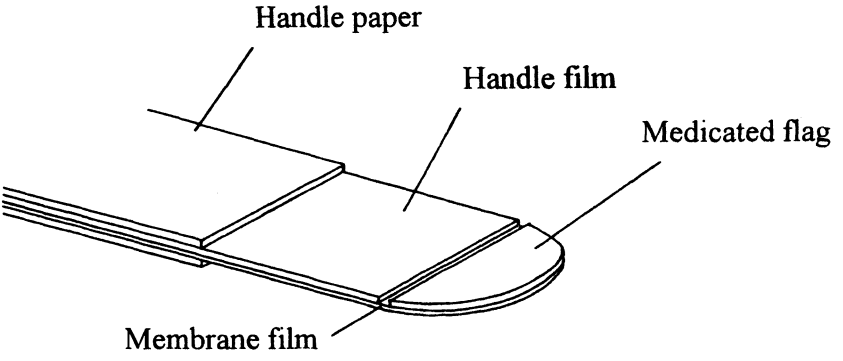

Fig. 6. Schematic representation of the NODS® system (adapted from Ref. 215).

20® and Pilo-40®, providing two different release rates for pilocarpine (20 and 40 μg/h, respectively) over a period of 7 days.[243] In rabbits, Sendelbeck et al.[243] have compared the distribution of pilocarpine in ocular tissues after administration by eyedrop or by the Ocusert® system. After the administration of eyedrops, pilocarpine levels in ocular tissues rose and fell within each 6-h intervals between eyedrops. On the other hand, pilocarpine levels remained constant over a two- to eight-day period with the delivery system.

Reservoir inserts based on an osmotic release mechanism of the drug are mostly described in the patent literature, however in vivo tests on such technologies are rarely reported.[206] These types of ocular delivery systems are generally made up of a unique central reservoir surrounded by a peripheral component.[250,251] The peripheral part of these osmotic inserts comprises in all cases of a covering film made of an insoluble semipermeable polymer. However, the central part can comprise a single reservoir or two distinct compartments (Figure 8).

The single reservoir design contains the drug, with or without an additional osmotic solute, dispersed throughout a polymeric matrix, so that the drug is surrounded by the polymer as discrete small deposits. If the central part is made up of two compartments,

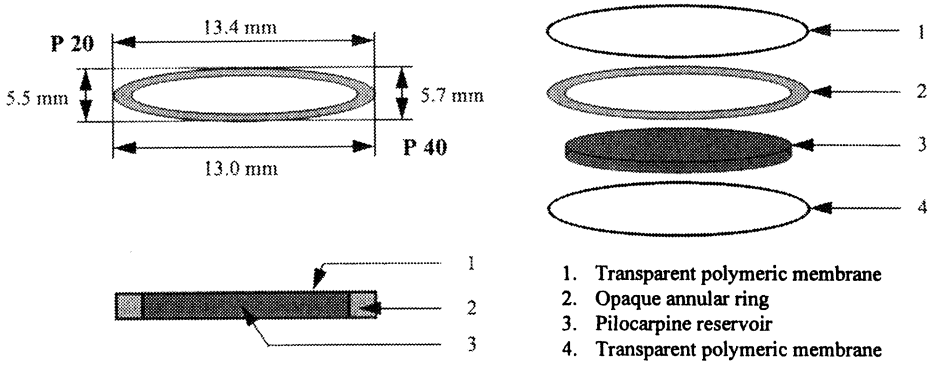

Fig. 7. Schematic representation of the Ocusert® system (adapted from Ref. 201).

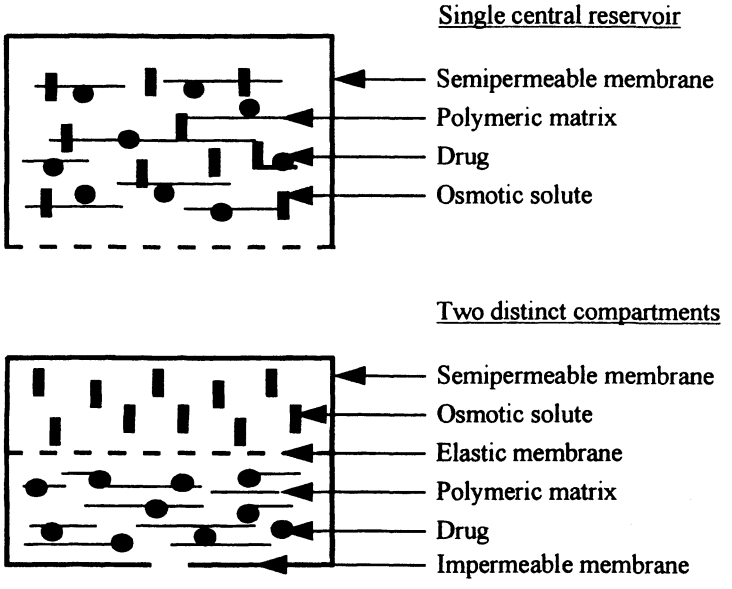

Fig. 8. Schematic representation of the two types of osmotic inserts.

the drug and the osmotic solute are placed in separate chambers, the drug reservoir being surrounded by an elastic impermeable membrane, and the osmotic solute reservoir by a semipermeable membrane. These systems are characterized by two distinct compartments and a single aperture having a very small diameter.

The release of drug from osmotic inserts can occur via two distinct release mechanisms: osmotic or diffusional.[206] When the insert is placed in the ocular environment, this starts the osmotic release. In this case tear fluid diffuses into the peripheral deposits through the semipermeable polymeric membrane, wets them and induces their dissolution. The solubilized deposits generate a hydrostatic pressure against the polymer matrix triggering its rupture in the form of pores releasing the drug. This osmotic part of the release is characterized by a zero order release profile. Ideally, the drug is continuously released from the osmotic insert by the increasing formation of apertures in the device forming a lattice of composition dispensing paths in the polymer on all sides of the inserts. In fact, these paths are interconnected, forming tortuous microchannels of irregular shapes and size causing a second drug release corresponding to a diffusional non-constant release.

The release of drug from systems having a central reservoir subdivided into two compartments[252,253] starts as soon as the device is placed in the eye. Tears diffuse into the osmotic compartment, inducing osmotic pressure that stretches the elastic membrane and contracts the compartment containing the drug, so that the active compound is forced through the single drug release aperture.

A constant zero order release can also by achieved by using reservoir devices based on silicone polymers. The prolonged release of drugs can be controlled either by a diffusion process[254,255] or by an osmotic mechanism.[256,257] Recently, Chetoni and co-

workers[257] manufactured bioadhesive polydimethylsiloxane rod-shaped inserts maintaining lacrymal concentrations of oxytetracycline above the MIC for at least 3 days.

IV.B.2.b. Matrix inserts The matrix insoluble inserts are typically represented by the contact lenses. The initial use of contact lenses was for vision correction. Its use has been extended to drug delivery devices by presoaking them in drug solutions. The main advantage of this system is the possibility of correcting vision and releasing drug simultaneously. Contact lenses are composed of a hydrophilic or hydrophobic polymer which swells by absorbing water. The swelling, caused by the osmotic pressure of the polymer segments, is opposed by the elastic retroactive forces arising along the chains as cross links are stretched until a final swelling (equilibrium) is reached.

Refojo[258] has proposed classifying contact lenses according to five groups, namely rigid, semi-rigid, elastomeric, soft hydrophilic and biopolymeric. Rigid contact lenses have the disadvantage of being composed of polymers e.g. poly[methylvinylether/malicanhydride] (PVMMA) which are hardly permeable to moisture and oxygen. These systems are not suitable for prolonged delivery of drug to the eye and their rigidity makes them very uncomfortable to wear. The permeability problem can be resolved by using gas permeable polymers such as cellulose acetate butyrate. However, the discomfort associated with the foreign object and long adaptation period remain the short-comings. For this reason, soft hydrophilic contact lenses were developed for prolonged release of drugs such as pilocarpine,[259] chloramphenicol and tetracycline,[260] and prednisolone sodium phosphate.[261] The most commonly used polymer in the composition of these types of lenses is hydroxy methyl metacrylic acid (HEMMA) copolymerized with polyvinyl pyrrolidone (PVP) or ethylene glycol dimethacrylic acid (EGDM). PVP is used for increasing water of hydration, while EGDM is used to decrease the water of hydration.

Shell and Baker[208] have shown that drug release from presoaked contact lenses was extremely rapid, with an in vivo residence time in general not longer than 24 h.[203] In addition, preservatives, such as benzalkonium chloride, cannot be avoided and they have greater affinity with the hydrophilic contact lens material than for the aqueous drug solution.[201] Bawa has described other approaches to decrease drug release rate from contact lenses.[262,263] These include the introduction of the drug into the monomer mixture followed by polymerization of the monomers in the presence of the drug. This procedure removes the need for preservatives and consequent eye sensitization, since the drug is added in the matrix as a solid.[262,263] However, the main problem associated with all contact lenses is their high cost of manufacture, and the difficulty of incorporating a precise amount of drug into the matrix. Disposable contact lenses have been commercially available for many years, and the continued progress made in polymer chemistry should facilitate the development of this type of ocular insert.

IV.B.3. Bioerodible inserts

These inserts are formed by bioerodible polymers, i.e. materials that undergo hydrolysis of chemical bonds and hence dissolution (crosslinked gelatin derivatives, polyester derivatives) (Table 6).[213,229,243,264,265] The great advantage of these inserts is the

Table 6
Currently investigated bioerodible inserts for ocular delivery of drugs

Drugs	Carrier	Effects	Species[a]	Reference
Pilocarpine	Poly(methyl vinyl ether/ maleic anhydride)	Delayed and decreased peak concentration of pilocarpine in general circulation compared to aqueous solution	R	213
	Gelatin	2-fold increase in duration of effect as compared to pilocarpine administered as a solution of 1.0 and 60 cps viscosity	R	267
	Gelatin (Gelfoam®)	Gelfoam® device is 2–3 times more effective than conventional pilocarpine dosage forms in prolonging the duration of the pilocarpine	R	266
Timolol	Poly(methyl vinyl ether/ maleic anhydride)	Reduction in peak plasma timolol concentration 2–5 times compared to the aqueous solution	R	217
Timolol and prodrugs of timolol	Poly(methyl vinyl ether/ maleic anhydride)	Release rate much slower for timolol prodrugs compared to the timolol-containing inserts	R	218
Gentamicin	Gelatin	Ocular irritation	B	265
Dexamethasone	Gelatin	Increased dexamethasone concentration in eye tissues compared to suspension	R	229
Idoxuridine	Polypeptide	Better results than drops and ointment in the treatment of Herpes Simplex Keratitis	R	264

[a] B: bovine; R: rabbit.

possibility of modulating their erosion rate by modifying their final structure during synthesis, and by addition of anionic or cationic surfactants. A crosslinked gelatin insert was used by Attia et al.[229] in order to increase bioavailability of dexamethasone in the rabbit eye. The dexamethasone levels in the aqueous humor were found to be 4-fold greater compared to a dexamethasone suspension. More recently, Gelfoam® devices containing pilocarpine were tested in rabbits.[266] These devices are made of absorbable gelatin sponges of $2.5 \times 2.5 \times 1.0$ mm sorbed with pilocarpine to form a matrix. The application of the device in the lower conjunctival sac produces a 2–3-fold increase in the duration of effect of pilocarpine over conventional eyedrops and gels.

The disadvantage of erodible systems is that they can have significantly variable erosion rates based on individual patient physiology and lachrymation patterns. In addition, degradation products and residual solvents used during the polymer preparation can cause inflammatory reactions.

IV.B.4. Concluding comments on ocular inserts as drug delivery systems

In conclusion, the majority of therapeutic agents used in the eye can be delivered using

ocular inserts. Such delivery systems are a promising alternative to classical dosage forms because of their various advantages. However, only a few insert preparations have been commercialized. This can be attributed to the reluctance of ophthalmologists, patients and owners of animals to replace the traditional ophthalmic solutions as well as the cost and the need to train both the prescribers, patients and owners of animals to place the inserts correctly in the eyes.

In the future, the use of ophthalmic inserts will certainly increase because of the development of new polymers, the emergence of new drugs having short biological half-lives or systemic side effects and the need to improve the efficacy of ophthalmic treatments by ensuring an effective drug concentration in the eye for several days.

V. Iontophoresis: a novel approach for drug delivery to the eye

Iontophoresis is a process that increases the penetration of ionized substances into or through a tissue by application of an electrical field (Figure 9). When iontophoresis is used for drug delivery, the best candidate drug is a low molecular weight charged molecule. According to electrical principle, if the molecule is positively charged it is driven from the anode and if it is negatively charged it is driven from the cathode. Although iontophoretic delivery should be inversely dependent upon the molecular weight of the penetrant,[268] it has been shown that neutral molecules and high molecular weight molecules such as proteins could be delivered across tissues by means of iontophoresis.[269,270] Interestingly, although the size of the molecule is an important factor that influences iontophoretic delivery, it has been demonstrated, using oligo-

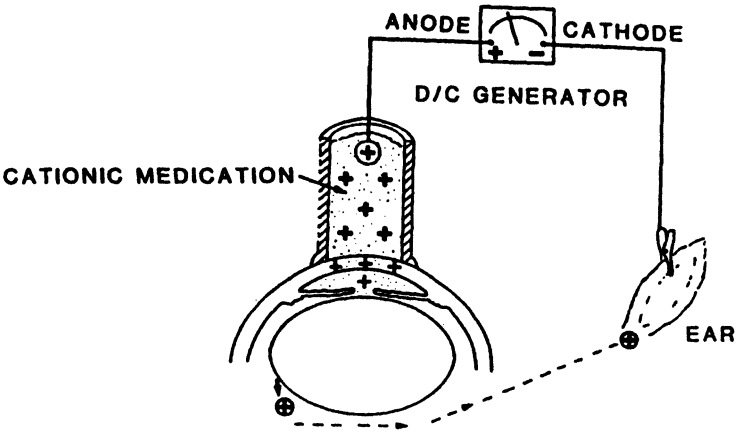

Fig. 9. Schematic representation of iontophoresis in the rabbit. The drug is placed in a cylindrical eye cup. The inner circumference of the eye cup fits within the corneoscleral limbus. The current is controlled by a rheostat on the direct current transformer. In the case illustrated here, the drug molecules (cations) have a positive charge. Therefore, the platinum electrode connected to the anode (the positively charged pole) is placed in contact with the solution. The other electrode (cathode) is connected to the ear of the rabbit to complete the circuit. The positively charged anode drives the positively drug molecules from the solution into the eye at a greater rate than would be observed with simple diffusion (adapted from Ref. 272).

nucleotide penetration through the skin, that not only the base composition, but also the sequence, affects steady-state transport across biological membrane. Therefore, the molecular structure could influence iontophoretic transport as well.[271]

The physical and biological principles of drug penetration by iontophoresis remain unclear. Most of the investigations that have been conducted to explore the mechanisms implicated in drug penetration have been done on skin explant ex vivo, in a different arrangement from that of therapeutic iontophoresis. Several theories are hypothesized, including the Shunt pathway, the flip–flop gating mechanism and electroosmosis.

The shunt pathway suggests that drugs cross the stratum cornea barrier of the skin via glands, follicules and imperfections in the skin.[273–275] Recently, iontophoretic pathways have been identified and quantified using confocal microscopy, within hairless mouse skin. This confirms that follicular transport enhances the delivery to a significant depth into the barrier and that the efficient follicular pathway could be considerable when the surface area is taken into account.[276]

The 'flip–flop' gating mechanism hypothesizes that the permeability of the skin could be altered by the current applied.[277,278] The polypeptides of the cell membranes could follow a parallel arrangement, that allow the formation of voltage-dependent pores.[270,279] During the non-conducting state, the alpha helices of the polypeptides arrange themselves in an antiparallel manner within the bilipidic layer, that could switch ('flip–flop') to parallel fashion when an electric potential is applied. However, the existence of such voltage-dependent pores has not been demonstrated. More recently Monteire-Riviere et al.[280] used transdermal iontophoresis of mercuric chloride on pig skin and demonstrated by ultrastructural microscopy that an intercellular and intracellular penetration was observed. Mercury precipitate were localized in the outer membrane of the mitochondria in epidermal cells, dermal fibroblasts and capillaries, demonstrating a transdermal delivery and a systemic exposure to mercury.

Electroosmosis could be one of the mechanisms implicated in the transport of molecules through the skin. Under the influence of a direct current, the passage of a solvent can carry with it other dissolved substances. This effect is dependent upon the pH. At physiological pH, the skin carries a negative charge, which enhances the migration of cations at the anode. This migration could drag the solvent through the skin, with any dissolved substances with it.[281–284] Conventional electroosmosis could be modulated by lipophilic, cationic substances, modifying the permselectivity of the skin.[285]

For ocular application, transconjunctival, transcorneal and transscleral iontophoresis (see Sections V.A.–V.C., respectively) have been used under variable conditions. The mechanisms of drug penetration that have been previously described to occur through the skin can hardly be extrapolated to ocular iontophoresis, each ocular tissue possessing its own characteristics. Moreover, the distribution of the drug into the eyeball, following iontophoresis is difficult to anticipate. To date, no systematic study has been conducted to elucidate the mechanisms implicated in ocular drug penetration using iontophoresis.

Since the earliest description of zinc salt transcorneal iontophoresis by Wirtz in 1908, described by Duke-Elder,[286] and in spite of a century of publications, clinical

use of iontophoresis has not been widespread. In fact, the absence of scientific rationale for drug penetration into or through ocular tissues, the lack of systematic pharmacokinetic studies, and the effect of pathology on the drug concentration time course; and the description of tissular lesions induced by iontophoresis using high current densities, have hindered clinical development of iontophoresis. However, iontophoresis could have potential interest in many therapeutic fields in ophthalmology particularly to treat posterior segment inflammations and infections and to deliver new potential antiangiogenic or trophic agents to the retina and/or the choroid. The innovative application of modern electronics and materials science as well as further research in ocular toxicology could help to place iontophoresis among the efficient means of treatment of the posterior segment of the eye.

V.A. Transconjunctival iontophoresis

V.A.1. Transconjunctival iontophoresis of antimitotics

Transconjunctival iontophoresis of 5-fluorouracil (5-FU), was investigated in the rabbit to inhibit sub-conjunctival and scleral fibroblast proliferation. Using 0.32 mA/cm^2 current density for 30 s, the acute 5-FU concentration in the conjunctiva was 480 and 168 mg/ml in the sclera, at 10 h it was 0.6 and 1.2 mg/ml, respectively, still above ID50 levels for cultured conjunctival fibroblasts. The amount of 5-FU introduced by iontophoresis was about 0.1% of the dose given to patients by subconjunctival injections.[287]

V.A.2. Transconjunctival iontophoresis of anesthetics

Sisler[288] reported iontophoresis of lidocaine to tarsal conjunctiva from a cotton pad prior to surgical excision of intra- and sub-conjunctival lesions in 27 patients. The excision was painless for 24 patients, and three others required classical injection of local anesthesia.

V.B. Transcorneal iontophoresis

Transcorneal iontophoresis has been used to deliver fluorescein, antibiotics and antiviral drugs into the cornea and in the aqueous humor. Transcorneal iontophoresis results in high and sustained drug concentrations in the cornea and the aqueous humor, but in low drug concentrations in the posterior segment of phakic eyes.

V.B.1. Transcorneal iontophoresis of fluorescein for aqueous humor dynamic studies

In 1966, Jones and Maurice used iontophoresis of fluorescein and a slit lamp fluorophotometer to measure the rate of flow of aqueous humor in the patient. Iontophoresis was performed with a 10% fluorescein solution, 2% agar, and 0.1 solution of methylhydroxybenzoate as a preservative.[289] No corneal lesions were observed on numerous patients who received iontophoresis with a 0.2 mA current intensity for 10–15 s. The same results were obtained by Starr et al.[290] with a 1 min treatment. Tonjum and Green[291] assayed the effect of current intensity and duration of treatment on rabbit eyes in vitro and demonstrated that fluorescein penetration in aqueous humor was already optimal with a 0.5 mA current intensity for 10 s. In 1982, Brubaker used

iontophoresis of fluorescein with a central 5 mm gel containing 2% agar and 10% fluorescein, with an intensity of 0.2 mA for 5–7 min in more than 1000 patients without any lesions except some epithelial defects.[292] This abrasion was apparently caused by part of the apparatus that contained the agar and did not result as a direct consequence of iontophoresis. These studies demonstrated that under specific conditions, iontophoresis is a safe procedure on patients.

V.B.2. Transcorneal iontophoresis of antibiotics

The efficacy of transcorneal iontophoresis of antibiotics has been assayed both in pharmacokinetic studies and on corneal abcess models. Table 7 gives a summary of the main studies using transcorneal iontophoresis.

Hughes and Maurice[293] reported iontophoresis of gentamicin to uninfected rabbit eyes and showed rapid and sustained efficient concentrations of drug in the cornea and the aqueous humor Fishman et al.[294] iontophoresed gentamicin to aphakic rabbit eyes. The peak corneal and aqueous humor concentrations were obtained 30 min after iontophoresis (Table 7), while the peak vitreous concentration was obtained (10.4 mg/ml) at 16 h after treatment, demonstrating that transcorneal iontophoresis could potentially deliver therapeutic antibiotic concentrations for endophthalmitis in aphakic eyes. Grossman et al.[295] demonstrated that the concentration of gentamicin after iontophoresis resulted in higher and longer-lasting gentamicin levels compared to subconjunctival injections. In addition, the inclusion of 2% agar solution to the 10% gentamicin led to very high drug concentrations in the cornea and the aqueous humor. Iontophoresis of tobramycin has been demonstrated to be efficient in the treatment of experimental *Pseudomonas aeruginosa* keratitis in the rabbit.[296] Transcorneal iontophoresis performed 22 h and 27 h after inoculation resulted in 'sterile' corneas over half of the animals 1 h after the treatment. Tobramycin iontophoresis allowed on average a 6-log reduction in colony-forming units in the cornea relative to untreated corneas. Safety of tobramycin iontophoresis was demonstrated by Rootman et al.[297] Iontophoresis of tobramycin delivered high concentrations both to uninfected and pseudomonas-infected corneas; 20 times more than application of fortified tobramycin (1.36%) drops.[298] Moreover, iontophoresis of 2.5% tobramycin resulted in a 3-log reduction in the number of a tobramycin-resistant strain of Pseudomonas, demonstrating the power of this method to deliver very high concentrations of drug.[299] Quinolone iontophoresis was also an efficient way to treat Pseudomonas keratitis.[300] Interestingly, transcorneal iontophoresis of vancomycin was as efficient as subconjunctival injection. The peak concentration was 122.4 mg/ml at 2 h after iontophoresis and 14.7 mg/ml 4 h after 2.5 mg subconjunctival injection. This was the first report that demonstrated that a high molecular weight glycopeptide (1448 Da) could be delivered by iontophoresis, into the cornea and the aqueous humor.[301]

In conclusion, transcorneal iontophoresis has been shown to be an efficient method that enhances aqueous and corneal antibiotic concentrations by a factor of 25–100 compared to topical applications. Except in aphakic rabbits, transcorneal iontophoresis did not result in high drug concentrations in the posterior segment of the eye.

Table 7
Transcorneal iontophoresis of antibiotics

Drug; concentration	Current density (mA/cm^2)	Duration treatment (min)	Tissues measured	Time (h)	Concentration ($\mu g/ml$)	Animal model	Reference
Tobramycin sulfate; 25 mg/ml	0.22	10	Cornea	5	230	Rabbit pyocyanic corneal abcess	296
			Aqueous humor	6	163		
Tobramycin sulfate; 25 mg/ml	0.2	10	Cornea	1	610	Rabbit pyocyanic corneal abcess	298
Gentamycin sulfate; 50 mg/ml	0.95	10	Cornea	0.5	72	Aphakic rabbit	294
			Aqueous humor	0.5	77.8		
Gentamycin sulfate; 100 mg/ml	0.66	1	Aqueous humor	2	8	Rabbit	293
Gentamycin sulfate; 100 mg/ml	8	10	Cornea	2	376		
			AH	2	54.8	Rabbit	295
			AH	16	23.2		
Ciprofloxacin; 10 mg/ml	0.2	10	Cornea	n.a.	n.a.	Rabbit pyocyanic corneal abcess	300
Vancomycin; 50–100 $\mu g/ml$	n.a.	10	Cornea	0.5	11	Rabbit	301
			Aqueous humor	2	12		
Ketoconazole; 100 mg/ml	14.8	15	n.a.[a]	n.a.	n.a.	Rabbit	302

[a] n.a.: non available.

V.B.3. Transcorneal iontophoresis of antiviral drugs

Antiviral drugs have been delivered into the eye using transcorneal iontophoresis for the treatment of herpetic keratitis and uveitis. Hill et al.[303] demonstrated that iododesoxyuridine (IDU), phosphoacetic acid (PAA) and vidarabine monophosphate could be iontophoresed into mouse cornea and studied the pharmacokinetics of radiolabeled vidarabine monophosphate (Ara-AMP) after transcorneal cathodal iontophoresis (0.5 mA, 4 min) in rabbits.[304] When compared to topical applications, the amount of radioactivity measured in the cornea, the iris and the aqueous humor was 3–12 times higher. Moreover, such a treatment did not induce corneal changes at the ultrastructural level of observation. Transcorneal iontophoresis of Ara-AMP was efficient in treating a herpetic keratitis model on the rabbit and required a lower treatment frequency and lower total applied dose than topical treatment.[305] On a stromal herpetic lesion induced on the rabbit cornea, iontophoresis of 3.4% Ara-AMP (0.5 mA, 4 min) or 5% acyclovir (ACV) (0.5 mA for 4 min) was compared to 50 mg/kg intravenous ACV. Iontophoresis was performed daily for five consecutive days, and intravenous injections twice daily for eight consecutive days. The efficacy of treatments was evaluated clinically by slit-lamp examination. Iontophoresis of either Ara-AMP or ACV was as efficient as intravenous treatment but significantly reduced the total administrated dose of drug. This study suggested that iontophoresis could be of use for the treatment of profound corneal herpetic lesions, either alone or combined with systemic therapy.[306]

V.B.4. Other drugs for transcorneal iontophoresis

Other drugs have been transferred into the anterior segment of the eye using transcorneal iontophoresis such as adrenergic agents to create models of recurrent herpes keratitis. Kwon et al.[307] gave the earliest demonstration that iontophoresis of 0.01% epinephrine at 0.8 mA for 8 min for three consecutive days could induce herpes simplex virus-1 (HSV-1) shedding in rabbits harboring latent HSV-1. Since these initial results, many studies[308–311] have contributed to establish highly reliable animal models for the study of herpes reactivation, using corneal iontophoresis either on rabbits or on mice.

More recently, transcorneal iontophoresis of an analogue of arginine (L-NAME) was used to inhibit the inducible nitric oxide synthase activity in endotoxin-induced uveitis in rats. This study demonstrated that under well defined experimental conditions, iontophoresis of L-NAME could reduce nitric oxide production in aqueous humor and reduce the corneal edema that is observed during this inflammation. Therefore, iontophoresis could be an interesting way to assay novel anti-inflammatory drugs, avoiding undesirable systemic side effects.[312]

V.B.5. Is transcorneal iontophoresis safe?

Safety of corneal iontophoresis is dependent on the density of current applied. According to Maurice,[293] current densities up to 20 mA/cm^2 for 5 min have been found to be well tolerated. Table 8 gives a summary of lesions that have been observed after transcorneal iontophoresis in different studies. It seems that current densities up 2 mA/cm^2 for 10 min could allow for both efficacy and safety. However, because topical

Table 8
Corneal lesions induced by transcorneal iontophoresis on the rabbit

Drug	Current density (mA/cm^2)	Duration (min)	Method of analysis	Lesion observed	Reference
Vidarabine	0.6	4	TEM[a]	Epithelial defect	305
Fluoresceine	25	1–5	TEM	Stromal edema	293
Tobramycine	0.8	10	TEM	Epitheial defect	297
NaCl 0.9%	7	5	TEM	5% of endothelial cell loss	301
	3	10	TEM	Endothelial cell loss	295
Ketoconazole	21	15	Clinical	Corneal opacities	302

[a] TEM: transition electron microscopy.

treatment is efficient in the treatment of most of the anterior segment pathologies, iontophoresis could be mainly of interest in clinical practice for drugs that can not diffuse across the cornea or when high stromal concentrations are needed with an intact epithelial barrier (i.e. stromal herpetic keratitis).

V.C. Transscleral iontophoresis

Transscleral iontophoresis has been used to achieve high drug concentrations of antibiotics, antiviral drugs, corticosteroids and fluorescein in the posterior segment of the eye. Most of the probes designed to date have been tubular with a reduced area of contact with the sclera over the pars plana, leading to a very high current density. Small burns over the area where the current was applied have been commonly described. Under these conditions, high drug concentrations in the vitreous were observed. However, the mechanism of penetration could be attributed, at least in part, to facilitated diffusion of the drug through ruptured tissue barriers. Very few studies reported complete drug pharmacokinetics after iontophoresis in all ocular tissues, which could contribute to understanding this method of administration.

V.C.1. Transscleral iontophoresis of antibiotics

Table 9 summarizes the principal studies on transscleral delivery of antibiotics. Barza et al.[313] used a very small probe (1 mm in diameter), placed over the pars palna to deliver gentamicin, ticarcillin and cephazolin in the rabbit vitreous. High concentrations of those drugs were measured in the vitreous after iontophoresis of uninfected rabbits, but using high current densities, burns were observed at the site of iontophoresis. Therefore, the penetration of the drug directly to the vitreous could result, at least in part, from direct penetration through disrupted tissues. Transscleral iontophoresis of gentamicin was clinically a useful supplement to intravitreal injection in an experimental endophthalmitis due to *Pseudomonas aeruginosa* in the rabbit. Higher rates of sterilization were observed in eyes that received both transscleral iontophoresis of gentamicin and intravitreal injections of gentamicin compared to intravitreal injections alone.[314] In the monkey, therapeutic levels have been obtained in the vitreous with transscleral iontophoresis of gentamicin. Electroretinograms were normal in all eyes after iontophoresis and indirect ophthalmoloscopy showed localized area of retinal burns in the area of pars plana where the electrode had been placed.[315] Other studies reported lower antibiotic concentrations in the vitreous with much lower current densities.[316] However, tissue concentrations were not measured. It has been suggested that high and long lasting concentrations of gentamicin could be obtained in the vitreous without any retinal lesion, by using 2% agar in the 10% gentamicin solution, and performing a transscleral iontophoresis with a 2 mm in diameter probe, 2 mA and 10 min treatment.[295] Vancomycin, a high molecular weight glycopeptide, was iontophoresed with a 5% drug formulation in contact with 25–30 mm^2 of the temporal sclera overlying the pars plana with a 3.5 mA current intensity for 10 min. Bactericidal concentrations in the vitreous were observed for about 12 h after a single treatment. This was the first demonstration that a high molecular weight mole-

Table 9
Transscleral iontophoresis of antibiotics

Drug; concentration	Probe diameter (mm)	Current density (mA/cm^2)	Duration (min)	Tissue	Time	Concentration (μg/ml)	Animal	Reference
Gentamycin sulfate; 100 mg/ml	2.5	10.7	3	VB[a]	24	8.9	Rabbit	316
Gentamycin sulfate; 25–50 mg/ml	1	3.33	10	VB	3	<2	Rabbit	313
Gentamycin sulfate; 25–50 mg/ml	0.5	200	1	VB	24	28	Monkey	315
			2	VB	24	11–44 (burn)		
Cefazolin sodium	1	27	10	VB	3	35	Rabbit	313
		67	10	VB	3	119 (burn)		
Ticarcillin	1	27	10	VB	3	34	Rabbit	313
		67	10	VB	3	94 (burn)		
Ketoconazol; 100 mg/ml	3	14.8	15	VB	1	10.2 <MIC	Rabbit	302
Vancomycin	3	12	10	VB	2	13.4	Rabbit	301
					16	3		

[a] VB: vitreous body.

cule could be delivered in the posterior segment of the eye by means of transscleral iontophoresis.[302]

V.C.2. Transscleral iontophoresis of antiviral drugs

Antiviral drugs against cytomegalovirus (CMV), such as gancyclovir and foscarnet have been administered by iontophoresis in order to replace intravitreal injections. A 20% gancyclovir solution was used for a transscleral iontophoresis with a 263 mA/cm^2 current density for 15 min. Therapeutic levels were obtained until 24 h after a single iontophoresis of gancyclovir in the rabbit.[317] However, the pH of such a solution was above 11, and this is an obvious difficulty when applying such a solution in direct contact with an eye. Forscarnet was administered by a 0.19 mm diameter electrode probe, and the iontophoresis was performed with a current intensity of 1 mA for 10 min. Under these conditions, efficient vitreal concentrations were still measured 60 h after a single transscleral iontophoresis of foscarnet.[318] However, with a calculated current density of 526 mA/cm^2 for 10 min, small burns were observed in the retina and the choroid adjacent to the application of the probe. No electroretinographic changes and no histological (light and electron microscopy) lesions were observed elsewhere than at the application site. After 21 consecutive days of the same treatment, the site of burn was not increased compared to a single iontophoresis procedure.[319] Iontophoresis could thus be an interesting alternative to intravitreal injections for the treatment of CMV retinitis, since patients require repeated and continuous invasive treatments.

V.C.3. Transscleral iontophoresis of corticosteroids

Corticosteroids are widely used in posterior ocular inflammation. In 1965, Lachaud demonstrated in a non controlled trial that iontophoresis of hydrocortisone acetate was beneficial for uveitic patients. More than 20 years later, Lam et al.[320] showed that transscleral iontophoresis of 30% dexamethasone, with a current density of 421 mA/cm^2 and a 25 min treatment induce a peak concentration in the vitreous of 140 mg/ml compared to 0.2 mg/ml after subconjunctival injection. Chorioretinal and vitreal concentrations of dexamethasone were higher and lasted longer than sub-conjunctival and retrobulbar injections. Efficiency of iontophoresis of dexamethasone was compared to systemic administration in an ocular model of panuveitis in the rat. Using a 2.6 mA/cm^2 current density, a 400 mA current intensity and 4 min treatment duration, iontophoresis was as efficient as intraperitoneal administration of dexamethasone to treat both the anterior and the posterior segment of the eye, with no effect on systemic production of cytokines. Iontophoresis of dexamethasone resulted in a reduced systemic effect of corticotherapy with a strong ocular effect.[321]

V.C.4. Is transscleral iontophoresis safe?

Table 10 summarizes the lesions observed after transscleral iontophoresis. Lesions that were observed were well circumscribed over the site of current application. However, the size of the lesion was correlated to the time of treatment.[322] According to Yoshizumi et al.[319] repeated treatment did not increase the size and the importance of focal retinal and choroidal burns.

Table 10
Lesions induced by transscleral iontophoresis

Drug	Current density (mA/cm^2)	Duration (min)	Animal	Lesions observed	Reference
Gentamycin	255	5	Rabbit	Retinal and choroid necrosis	313
Gentamycin	764	10	Monkey	Retinal necrosis	314
Phosphate buffer solution	350 535	Lesion if time $>$ 1 min	Rabbit	Choriocapillaris occlusions, cell infiltrate, necrosis of retinal pigmented epithelium and retinal cells	322
NaCl 0.9%	531	Lesion if time $>$ 25 min	Rabbit	Retinal necrosis	322
Foscarnet	530	10	Rabbit	Retinal necrosis. Localized area of choroid, retinal pigmented epithelium and retina over current application	319

The mechanisms of injury could be related to direct effect of high current density (inducing cell membrane damage), heat insult, chemical burns due to hydrolysis and modification of the pH at the surface of the eye. However, it seems that efficient tissue concentrations of drugs can be achieved without any induced lesions when the current density is controlled, and remains inferior to 100 mA/cm^2 for 5 min. When focal lesions are induced by iontophoresis, the drug directly penetrates into the vitreous through disorganized tissues, following the kinetics of an intravitreal injection. Without any observable lesion, the drug penetration should follow other mechanisms, which could be better understood by systematic pharmacokinetic studies in all ocular tissues.

V.D. Concluding comments regarding iontophoresis as drug delivery systems

Transcorneal iontophoresis could have a potential use in the treatment of deep corneal infections or for the administration of drugs that do not cross the corneal barrier. Transscleral iontophoresis could be of interest to achieve therapeutic concentrations of drugs in the posterior segment of the eye. To date, both intravenous and intravitreal injections (see Section VI) are commonly used. Table 11 compares advantages of iontophoresis over intravitreal injections. Intravenous injections of antibiotics or corticosteroids are effective to treat the posterior segment of the eye but expose the patient to high levels of drugs and systemic side effects. Iontophoresis has been demonstrated to allow the delivery of therapeutic concentrations of drugs to the eye and particularly to the posterior segment with a reduction of the total amount of drug delivered to the organism. A better control of electrical parameters as well as a respect of safety limits could increase the benefits of this method of drug delivery, while reducing side effects.

Table 11
Comparison between transscleral iontophoresis and intravitreal injections

Observation	Route of administration	
	Iontophoresis	Intravitreal injections
Pain	No	In 10% of cases
Retinal detachment	No	In 11–23% of cases
Intravitreal hemorrhage	No	In 8–25% of cases
Infection	No	3%
Focal burns	Yes[a]	No
Daily treatment	Yes	No
Depot effect	Yes[b]	No
High molecular weight molecules	Non-available	Yes
Reduced systemic effects	Yes	Yes

[a] With high current densities.
[b] Depending on treatment parameters.

VI. Intravitreal injections for the treatment of ocular diseases

Because many drugs penetrate the eye poorly when administered systemically or even when injected subconjunctivally or retrobulbarly, intraocular injection and implantation of sustained-release drug delivery systems have been tested. In veterinary practice, intravitreal injections are sometimes used for chemical ablation of the ciliary epithelium in glaucomatous eyes and in the treatment of infectious endophthalmitis to achieve therapeutic concentrations of antibiotics or antifungals.[323] The vitreous humor acts as a gel through which drugs diffuse readily before leaving via the anterior or posterior route. The drugs that leave the vitreous humor via the anterior chamber, such as the aminoglycoside antibiotics, exhibit a long vitreal half-life (about 20–30 h). In contrast, posterior-route drugs, such as cephalosporins, are transported actively across the retina and have a short half-life that ranges from 2–10 h.[324] As clinical applications of intravitreal drug therapy may be limited by the relatively short time that it takes for drugs to leave the vitreous cavity, sustained-release devices have been developed to achieve an effective drug concentration for an extended period of time. Liposomes have been tested as drug carriers and it was found that cholesterol-containing, large unilamellar vesicles are a superior formulation for vitreous injection.[325] Liposomes have been evaluated experimentally and clinically for intraocular delivery of different drugs including antifungal (amphotericin B), antiviral (gancyclovir, trifluorothymidine) and antimetabolite (5-FU, cytosine arabinosine) agents.[326] They appear as potential site-specific and sustained-release vehicles for therapy of intraocular diseases. Polymers of lactic or glycolic acid (or a combination of the two) have also been reported as being suitable for extended delivery of antimetabolites into the eye[327] and a recent experiment presents some evidence that an implant consisting of a polyvinyl alcohol/ethylene vinyl acetate-coated cyclosporine pellet is well tolerated when injected intravitreally into the equine eye and could be a promising treatment for recurrent uveitis.[328]

VII. Conclusion

The compilation in this chapter shows some possible new ways that can be exploited for controlled and targeted release of bioactive substances such as specially designed inserts, or iontophoresis for ocular treatment.

The understanding of ocular physiopathology in human and animal health has made big progress over the last decade and many new chemical entities have been introduced in the market. The total market share for ocular veterinary products however only represents 1%. The products with labels for veterinary use (like antiglaucoma, antifungal and antivival preparations) are very limited. At the end of the 20th century still very little attention is given to the fact that the compliance of the owner to treat companion animals is extremely poor. Large efforts need to be made in the very near future to abolish the frequent use of products labeled for human use for the treatment of ocular diseases in animals. This habit is primarily based on the experience gained during preclinical testing of the substances and that many problems affecting the eye

of animals are in some cases quite similar to conditions known in man. Therefore the following statement can be made: 'Better compliance equals better clinical results'. In order to reach this target we need better formulations specifically designed for animal use. This will be the challenge for the next years for the veterinary industry.

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Rathbone and Gurny (eds.), Controlled Release Veterinary Drug Delivery: Biological and Pharmaceutical Considerations

CHAPTER 6

Design and development of controlled release intravaginal veterinary drug delivery systems

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Overview

I. Introduction

Intravaginal veterinary drug delivery has a long history dating back to the early 1960s

when polyurethane sponges were investigated as a means to deliver synthetic progestagens to sheep[1–8] and goats.[9] Extensive field trials in both Australia[1–3] and confirmatory experiments in the UK[4–6] resulted in an intravaginal delivery system used to control the estrous cycle of sheep. A commercially available product resulting from this research is still marketed (Figure 1). In the mid 1970s silicone was investigated as a platform for the release of progesterone, again to control the estrous cycle.[10–19] In the 1980s further work with silicone was performed in sheep and goats[20–23] and later in cattle[24–39] for the same clinical application. Three commercially available intravaginal drug delivery systems arose from this research; PRID, CIDR-G and CIDR-B. In the 1990s, microchip technology was investigated as a means of delivering multiple drugs at different times and rates for the precise control of estrous synchrony and fertility.

Historically, the need to use the intravaginal route for drug delivery arose out of the inabilities of other routes to achieve the clinical requirements of animal scientists. For example, research to deliver progestagens to control the estrous cycle using subcutaneous implants began in the mid 1960s in sheep[40–42] and in the 1970s in cattle.[42–48] However, it was realized that this route of administration could sometimes be impractical because of the size of the implants, high drug loading and the difficulties in administering the delivery system in the commercial setting.[14] Despite this observation, some subcutaneous approaches were successful. A small implant (2 × 18 mm) containing norgestomet (5% w/w) dispersed throughout Hydron was successfully developed during the 1970s for administration into the ear of cattle.[46,49–62]

Scientists with animal, veterinary or plastic engineering backgrounds appear to have taken the initiative to design delivery systems for intravaginal administration

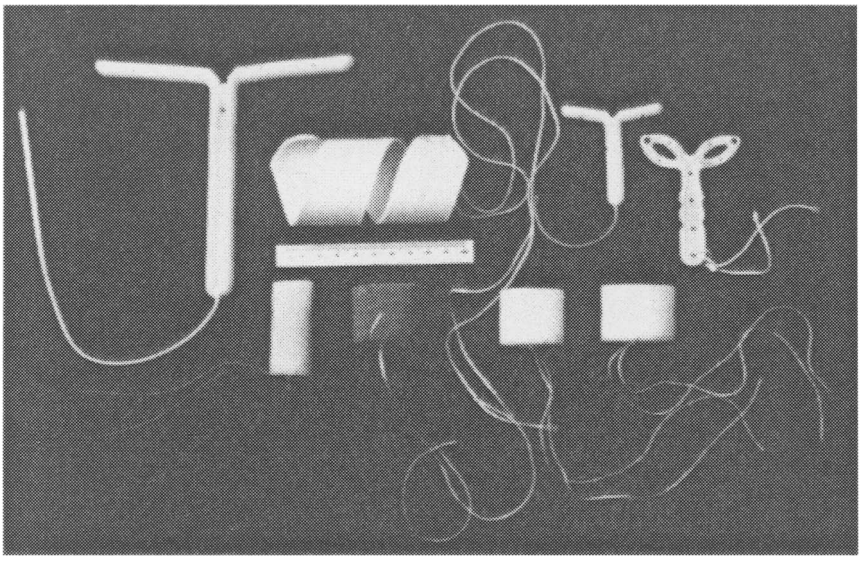

Fig. 1. Photograph of various commercially available intravaginal veterinary drug delivery systems used in farmed animals. Top row (left to right): CIDR-B, PRID, CIDR-G and CIDR-S; bottom row: various intravaginal sponges.

to animals. Indeed, a notable feature of commercially available intravaginal drug delivery systems is the lack of input into their design by formulation scientists. As a result, all the current intravaginal drug delivery systems used in animals have inherent pharmaceutical problems (which would severely limit their use in humans) such as high residual drug loads, induction of a mucopurrelant discharge, etc. However, these limitations have not appeared to have affected their acceptability with the end-user (where cost per treatment and effectiveness is a more important consideration).

A review of the literature highlights the avenues of research taken by these innovative scientists with animal or plastic engineering backgrounds. It is the purpose of this chapter to critically review the literature in order to glean the available information relating to the design and development of intravaginal veterinary drug delivery systems. This information will then provide the basis upon which to design and assess future intravaginal veterinary drug delivery systems.

II. Historical perspective

Around the 1960s the pioneering work on intravaginal veterinary drug delivery was performed by Robinson.[1,2] Since that time, several conceptual[63–69] and commercially available intravaginal veterinary drug delivery systems (PRID, CIDR-G, CIDR-S and CIDR-B) have been described in the literature (Figure 1).

Notable features of currently available intravaginal drug delivery systems include: the lack of input into their design by formulation scientists – most intravaginal drug delivery systems were developed by animal scientists or plastic engineers; at the time of development, sensitive and specific drug plasma assays were not available for the drugs that were being delivered and consequently the effectiveness of the device was based on clinical observations; currently marketed intravaginal drug delivery systems all have problems associated with them, e.g. sponges and the PRID device induce a mucopurrelant discharge during insertion;[1,2,5,8,70–76] relative to the CIDR-B and CIDR-G, the PRID is more difficult and more time-consuming to insert[74] and the PRID, sponges and CIDR-B all have high initial drug loads, all exhibit poor drug utilization, and consequently have high residual contents following removal.[13,37,38,77–86]. In some cases this has encouraged re-insertion (although this is not recommended by the manufacturers).[77,78,87–92] Each device appears to have undergone rapid development and has reached the market within only a few years of conception. Attempts to design a universal intravaginal drug delivery system capable of being used across all species has yet to be achieved.

These historical features have significantly affected our current knowledge on how to design and develop an intravaginal veterinary drug delivery system. The background of the scientists involved in these pursuits has resulted in little pharmaceutical information appearing in the scientific literature regarding the design and development of an intravaginal veterinary drug delivery system. Most of the publications in this area have focused on clinical results and are published in the animal science literature. However, more recently, structured and pharmaceutically based research approaches

to intravaginal veterinary drug delivery have been performed.[67–69,93–96] A combination of the early and more recent work has provided an insight into the pharmaceutical processes for designing, developing, optimizing and assessing an intravaginal veterinary drug delivery system.

III. Drug, polymer and animal candidates

To date, drugs successfully utilized for systemic delivery via the vaginal cavity of animals have been restricted to synthetic and natural hormones used to control the estrous cycle. Specifically, these compounds include progesterone, methyl acetoxy progesterone, fluorogestone acetate and estradiol benzoate.[1–39] Other drugs, e.g. melatonin, prostaglandin $F_{2\alpha}$ and a variety of other synthetic progestagens have been shown to be systemically absorbed from the vagina of farmed animals.[3,97–103] The short list of suitable drug candidates is a limitation for this route of veterinary drug delivery. Drugs must exhibit certain inherent physico-chemical characteristics in order to be useful for this route. These include rapid absorption characteristics, be effective in relatively small doses, have a low minimum effective concentration, exhibit some degree of solubility in the vaginal fluids, be sufficiently stable under the conditions of manufacturing and upon storage, have a wide margin of safety, be non-toxic to the end-user, be non-irritant to the vaginal mucosa and be compatible with the polymer selected for the delivery system.

To date, polymers utilized for systemic delivery of drugs via the vaginal cavity of animals in commercially available products have been limited to polyurethane and silicone.[104] Sponges are manufactured from polyurethane, whereas the PRID, CIDR-B and CIDR-G devices and many conceptual delivery systems are manufactured from silicone. Factors which influence the choice and use of a polymer in an intravaginal veterinary drug delivery system includes convenience, cost, availability, ease of fabrication, low cost fabrication methods and manufacturing processes, biocompatibility, international registration status, physical and biological stability, inertness, drug compatibility and release characteristics.

It would be imagined that any female animal could be a candidate for an intravaginal veterinary drug delivery system. However, some animals are more suitable candidates, such as dairy cows, sheep or goats, because they are used to being handled and accept the end-user working around their rear end. In addition they have relatively large vaginal dimensions.[105] In contrast, some animals, e.g. cats and dogs, may require restraint or even sedation to administer an intravaginal delivery system and would exhibit nervousness if they were approached from behind. In addition, animal production and health needs, and the availability of alternate and more suitable routes for treatment, would also influence the animal candidate list.

It should be remembered that a delivery system designed and optimized for one species may not be as effective in a second species. This may be for several reasons including differences in vaginal size and structure as well as different dosage requirements. However, the basic design concept or technology used to manufacture an

intravaginal veterinary drug delivery system for one particular animal may be able to be applied cross species, but that technology will have to be specifically tailored to each individual animal species. An example of this is the CIDR technology (high temperature injection molding of progesterone loaded silicone) where two different sized devices with different drug loads needed to be designed for sheep (CIDR-G) and cattle (CIDR-B). A second example is the different sized and loaded polyurethane sponges manufactured for sheep and goats. However, there are exceptions to this general rule, e.g. the CIDR-G device can be used in sheep and goats with no modification to its shape, size or drug load.

IV. Factors affecting design

Several factors affect the design and development of an intravaginal veterinary drug delivery system. These are listed in Table 1. Each of these factors is considered in detail below.

IV.A. Applicator

Because intravaginal veterinary drug delivery systems can be easily inserted by hand into farmed livestock and potentially other animals, the design of the applicator, its dimensions and shape, are often considered late in the development of an intravaginal veterinary drug delivery system. This may not be the optimum approach, since our experience has shown that it is easier to design a delivery system around an applicator than vise versa. Applicators are more simple in terms of the requirements expected of them than delivery systems, and delivery systems are more versatile in their inherent capabilities than applicators; release profiles from delivery systems can be modified by many means and several geometries can achieve the desired release profile and retention characteristics. However applicators must fulfill certain specific criteria and the means to achieve those criteria is limited by vaginal anatomy as well as design constraints (detailed below).

Any intravaginal applicator must fulfill certain design constraints. They must be: simple in design; simple and easy to operate; preferably allow one-handed application leaving the other hand free to lift an animals tail, wipe the vulva clean, apply antiseptic to the vulva, assist in the insertion process, etc. preferably be reusable, be strong and sturdy, allow easy and speedy loading of the device, allow easy and speedy expulsion of the device, painless to the animal during insertion into the vagina, produce no damage or irritation to the animal, easily lubricated with the minimum of lubricating fluid, minimize the chance of injury to animal or user, easily washed before re-use, able to be sterilized between herds/flocks, unlikely to transmit vaginal infections and must be of a minimal cost to reduce the overall cost of the treatment.

To date all applicators used to insert a delivery system into the vagina of animals are of a 'single-shot' design, i.e. allow only one device to be administered per vaginum before needing to be re-loaded. Given the current shape and sizes of intravaginal drug

Table 1
Factors affecting design of an intravaginal veterinary drug delivery system

Factor affecting design	Factor influences	Factor influenced by
Applicator design	Dimensions and geometry of delivery system. Ease of use. Animal comfort. End-user acceptance	Size and shape of vaginal cavity. Dimensions and geometry of delivery system
Retention rate	Overall efficacy of delivery system	Retention mechanism. Dimensions and geometry of delivery system
Retention mechanism	Ease of use. Retention rate	Dimensions and geometry of delivery system
Dimensions	Release rate. Ease of use. Animal comfort. End-user acceptance	Size and shape of vaginal cavity. Applicator design. Desired release rate
Geometry	Release rate. Ease of use. Animal comfort. End-user acceptance	Size and shape of vaginal cavity. Applicator design. Desired release rate
Irritation (mucopurrelant discharge production)	End-user acceptance. Release rate	Dimensions and geometry of delivery system. Polymer used to manufacture delivery system. Drug incorporated into delivery system. Presence or absence of additives
Removal	Ease of use. End-user acceptance. Animal comfort	Retention mechanism. Dimensions and geometry of delivery system
Damage to vaginal mucosa and cervix	Applicator design. Removal mechanism. Retention mechanism	Dimensions and geometry of delivery system
Environmental considerations	End-user acceptance. Environment. Regulatory acceptance. Optimal drug load	Polymer. Method of disposal. Drug load. Residue
Release characteristics	Efficacy of delivery system	Polymer used to manufacture delivery system. Drug incorporated into delivery system. Presence or absence of additives. Manufacturing process

delivery systems, multiple dosing units appear impractical and of complex design. Consequently this situation is not likely to change in the near future.

Two basic designs of applicators are currently on the market (Figure 2). Two-handed applicators comprise a tube or speculum appropriately shaped (tapered) at one end to aid insertion and a plunger that runs smoothly along the inside of the tube to expel the delivery system. This type of applicator is used to insert sponges in sheep and goats and the PRID device in cattle (Figure 2A). A single-handed operation applicator has been designed for the administration of CIDR-G into sheep and goats (Figure 2B) and a modified (larger) version to administer the CIDR-B into cattle (Figure 2C). Dimensions (lengths, diameters, etc.) of each of these applicators were specifically tailored to administer the given delivery system for a defined species, or range of species exhibiting similar vaginal characteristics, e.g. goats, sheep and deer.

IV.B. Retention mechanisms and rates

Retention is an important consideration in the design of an intravaginal veterinary

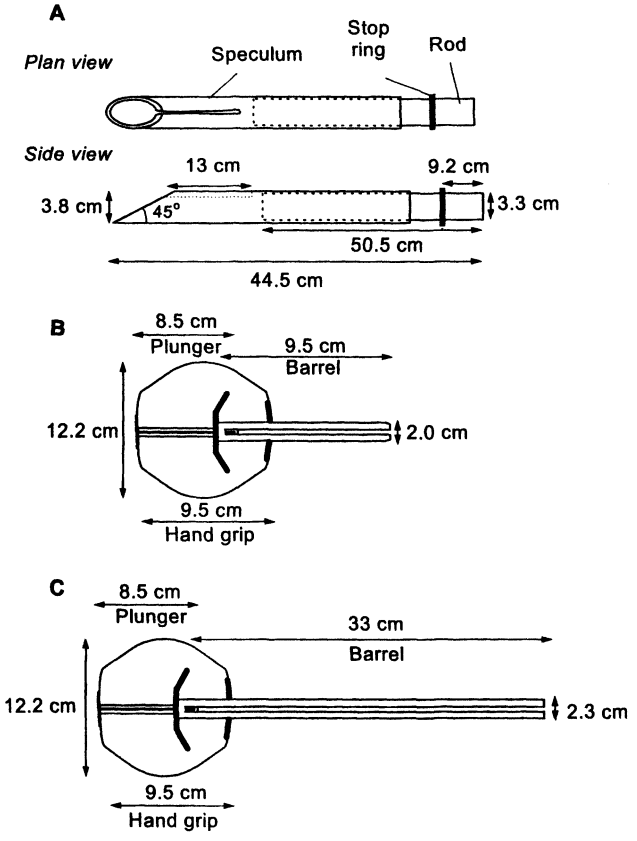

Fig. 2. Schematic diagram and dimensions of some commercially available applicators for the insertion of intravaginal drug delivery systems to farmed animals. A, PRID applicator; B, CIDR-G applicator; C, CIDR-B applicator.

drug delivery system. A device will only be effective if it remains in the vagina for the duration of the treatment period. Poor retention rates would reduce the overall efficacy of a treatment. As a rule of thumb if an intravaginal veterinary drug delivery system is to be of practical use it must possess a retention of at least 95%.

Current intravaginal veterinary drug delivery systems utilize some form of an 'expansion mechanism' to retain the device in the vagina for the duration of treatment. Following insertion, either the entire device expands outwards (e.g. sponges or PRID), or only a part of the device expands outwards (e.g. the 'wings' of the CIDR-B or CIDR-G). In both cases, the expanded device exerts gentle pressure on the vaginal walls resulting in retention. Conceptual delivery systems reported in the literature do not appear to utilize any further mechanisms to aid retention. Consequently, shapes of intravaginal veterinary drug delivery systems are either cylindrical (e.g. sponges) coil (e.g. PRID) or 'T' shaped (e.g. CIDR-B and CIDR-G).

The inherent characteristics of the sponge, e.g. density, flexibility, size, etc. ensure

retention following insertion. In contrast, in order to retain silicone-based delivery systems, the design of the delivery system incorporates a second material, suitably chosen, which impels the retention characteristics to the device. Metal (e.g. stainless steel; PRID) or rigid polymers (e.g. nylon; CIDR-B and CIDR-G) have been used. The grade of stainless steel appeared to be an important consideration in the development of the PRID.[14] Early studies suggested that too stiff a grade caused excessive irritation to the vaginal mucosa.[14] Correct design of the hinged regions of the CIDR-G and CIDR-B (where the wings meet the body) were the key to the success of this approach. Other polymers that are potential candidates for this purpose include polypropylene (e.g. INVAS).[106]

IV.B.1. Cattle devices

The retention rates for CIDR-B are excellent (in excess of 97% in both cows and heifers, Table 2). This suggests that the wing-type retention mechanism is an effective approach. As alluded to earlier, the design of the hinges is crucial to ensure a balance between high retention rates and damage or irritation to the vaginal mucosa.

The coil mechanism for retention was investigated using coils 5.5 and 7.0 cm in diameter.[17] Coil diameter had an affect on retention rate of PRID devices. Trials indicated that the diameter of the coil was inversely related to the retention rate over a 12 day insertion period.[17] The author suggested that a larger coil diameter was more likely to stimulate and irritate the vaginal musculature resulting in expulsion of the coil.[17] This author also cited studies in progress (but not reported) which indicated that reducing the diameter of the coil to 4.6 cm could improve retention

Table 2
Retention of CIDR-B in cows and heifers

Animal details	Insertion period (days)	Number of animals treated	Number of CIDR-B removed	Retention rate (%)	Reference
Cows	4	135	134	99.3	30
	5	136	131	96.3	30
	6	122	120	98.4	30
	7	121	121	100	30
	7	64	62	96.9	33
	10	497	474	98.9	107
	10	72	72	100	74
	12	265	248	93.5	24
	7, 12	1091	1065	97.6	108
	NR	1559	1503	96.4	109
Average				97.7	
Heifers	6	412	406	98.5	110
	7	465	464	99.8	26
	7	133	132	99.25	74
	10	154	150	97.4	111
	12	262	260	99.2	26
	15	81	81	100	32
	15	90	90	100	34
	7, 10, 12	1227	1220	99.4	112
Average				99.2	

Table 3
The retention rate of silastic coils impregnated with progesterone following a 12 day insertion period[109]

Year of trials	Diameter of coils (cm)	Type of animal	Number of coils inserted	Number of coils removed	Retention rate (%)
At pasture					
1974	5.5	Cow	784	724	92
1974	5.5	Heifer	499	480	96
1975	5.5	Cow	1257	1155	92
1975	7.0	Cow	1142	984	86
1975	5.0	Heifer	680	649	95
Housed					
1974	> 5.0	Cow	309	271	88
1974	> 5.0	Heifer	128	117	91
1975	5.2	Cow	368	360	98
1975	5.2	Heifer	301	295	98

rates to 95% in cows over a 12 day insertion period. O'Farrell also used PRID of two different diameters (5 and 7 cm) in both cows and heifers during a 12 day insertion period.[113] The retention rate was low for the 7 cm diameter coil in both cows (approximately 86%) and heifers (approximately 90%). The author suggested that this was a result of the larger diameter coil causing greater irritation to the vaginal walls, therefore causing increased straining to expel the coil.[113]

Retention rates of PRIDs were compiled and reported by Roche in a review in 1976 (Table 3).[114] The data shows that PRID devices have a retention rate in excess of 95% in both cows and heifers under a variety of farm management conditions. These early observations are supported by our own compilation of available data (Table 4).

A comparison of the retention rates between the PRID and CIDR-B (Tables 2–4) suggests that the design of the device is an important factor determining retention rates. In general, PRID coils are considered to be expelled more often from the vagina than the CIDR-B devices.[115] However, retention data should be evaluated bearing in mind that the design of the device is not the only factor which influences the retention rate of intravaginal veterinary drug delivery systems. Other factors include: recipient parity (size of cow vagina, vaginal muscle tone,[115] nutritional stress, health problems, abnormalities of the vaginal tract) operator skill (whether or not the devices are inserted by experienced veterinarians),[74] differences in breeds and sizes of cattle used,[76] differences in age and type of cow treated (e.g. heifers have a higher retention rate than parous cattle[17] although Smith et al. remarked that in their experience the rate of PRID loss did not appear to be affected by cow age or parity[116]), investigation and removal of a protruding tail by a herd mate, and the presence of a vaginitis due to omission of antibiotics from the treatment regime.[76]

A final consideration to bear in mind when evaluating retention rates from the literature, is that retention rates are sometimes reported from experimental studies involving small animal numbers. False conclusions can be drawn from studies of this type, and the tables above have been compiled from studies which used in excess of 100 animals. In general, when designing an intravaginal veterinary drug delivery

Table 4
Retention rate of PRID in heifers and cows

Animal details	Insertion period (days)	Number of animals treated	Number of PRID removed	Retention rate (%)	Reference
At pasture	12	670	620	92.5	14
Dairy cows	18	70	67	95.7	14
Suckling cows	12	114	104	91.2	14
Heifers	7	88	86	97.7	14
	18	60	58	96.6	14
	12	499	480	96.2	14
	7	37	34	92.0	14
Housed	12	309	271	87.7	14
Suckling cows	12	167	160	95.8	14
Heifers	7	65	64	98.5	14
	12	128	117	91.4	14
Heifers	7	19	19	100	14
Heifers	12	156	153	98	117
Dairy cows	12	412	378	91.7	117
Heifers	12	56	53	94.6	118
Heifers	12	159	150	94.3	16
Suckler cows	12	143	138	96.5	16
Heifers	12	240	232	96.7	19
Dairy cows	12	367	340	92.6	11
Suckler cows	12	344	328	95.3	17
Dairy heifers	12	350	336	96	17
Suckler cows	12	466	413	88.6	17
Heifers and cows	14	107	103	96.6	119
Heifers and cows	12	200	194	97	120
	14 and 16	300	279	93	120
	14 and 16	500	470	94	120
	12	198	198	100	120
Dairy cows	12	47	42	89.4	115
Cows	8	44	36	81.8	121
	8	43	36	83.7	121
Beef cows	12	200	188	94	122
Heifers	7	130	116	89.2	74
Heifers	7	93	90	96.8	74
Diary cows	12	76	71	93.4	123
Beef and dairy cows	12–16	200	195	97.5	124
Japanese Black cows	12	12	9	75	76
Heifers and suckling cows	9	109	102	93.6	125
Cows	12	343	307	89.5	113
Heifers	12	297	277	93.2	113
Heifers and cows	10	47	47	100	126
Cows	9	247	245	99.2	127

system, retention rates must be determined and involve large animal numbers (in excess of 100), utilize commercial conditions and involve a variety of operators if a true estimate of the retention rate is to be determined.

IV.B.2. Sheep, goats and deer devices

Reported retention rates of CIDR-G in sheep, goats and deer are compiled in Table 5. To ensure the high retention rates observed with the CIDR-G it is important to insert the device into the anterior region of the vagina. Some investigators have experienced that the CIDR-G discharges incompletely from the applicator and is pulled posteriorly as the applicator is withdrawn.[137]

Retention of sponges in sheep has been investigated by numerous authors and generally exceeds 98% (Table 6). Robinson reported that size influenced retention rates of sponges.[1,2] Sponges 2.5 cm thick and either 3.2, 3.5 or 3.8 cm in diameter were investigated and the larger sponges were retained better than the smaller ones. Texture of the sponges also appeared important with denser sponges being preferred.[1,2]

The type of drugs incorporated into sponges may also affect retention. Gordon suggested that incorporation of estradiol into sponges affected retention rates.[142] Retention rates after inclusion of 800 μg estradiol per device was significantly lower 91.8% (124/135) than in controls 97.7 (129/132). The authors suggested that estradiol may have influenced the irritability of the vaginal musculature and that this led to the higher rate of ejection.[142] The same authors gathered little evidence to suggest that the incorporation of stilbestrol into sponges affected retention rate.[158] Conversely, progesterone impregnated sponges appeared to be retained at a lower rate (85%) than fluorogestone acetate-containing ones (98%) over a 17 day insertion period.[2] Later work by the same authors also confirmed these observations (progesterone 500 mg sponges: 2213/2852 = 77.6% retention rate; fluorogestone acetate 30 mg: 503/534 = 94.2% retention rate).[138] The authors suggested that this was due to differences in the density and flexibility of the sponge as a result of the mass of

Table 5
Retention rates of CIDR-G in sheep, goats and deer

Insertion period (days)	Number of CIDR inserted	Number of CIDR removed	Retention rate (%)	Reference
Sheep				
12	150	149	99.3	128
12	50	50	100	129
11	27	27	100	130
12	62	62	100	131
12	25	24	96	131
12	428	424	99	131
Goats				
18	54	49	90.7	132
Deer				
14[a]	10	10	100	133
12[a]	215	205	95.3	134
12[b]	18	17	94.4	135
14[b]	Not reported	Not reported	> 99	136

[a] Red.
[b] Fallow.

Table 6
Retention rates of various sponges in sheep

Sponge details	Drug	Insertion period (days)	Number inserted	Number retained for insertion period	Retention rate (%)	Reference
Hand made	Fluorogestone acetate	17	52	51	98	2
Hand made	Progesterone	17	54	46	85.2	2
Hand made	Fluorogestone acetate	14	100	100	100	8
Hand made	Progesterone 500 mg	12	62	62	100	131
2.5 cm in depth, 3.2 and 3.5 cm in diameter grade 300	Progesterone 500 mg Fluorogestone acetate 30 mg	15–18	2852 534	2213 503	77.6 94.2	138
2.5 cm in depth, and 3.5 cm in diameter grade 800	10, 20 or 30 mg	19	90	90	100	139
Prototype sponges made by Abbott laboratories	Fluorogestone acetate 10, 20 or 30 mg	16–19	9552	9517	99.6	140
Syncro-Mate	Fluorogestone acetate	12	40	40	100	4
Syncro-Mate	Fluorogestone acetate 20/30 mg	16–17	2797	2836	98.6	5
Syncro-Mate	Fluorogestone acetate 30 mg	16	238	236	99.2	5
Syncro-Mate	Fluorogestone acetate 30 mg	16	248	244	98.4	5
Syncro-Mate	Fluorogestone acetate 20 mg	16	735	734	99.9	5
Syncro-Mate	Fluorogestone acetate 30 mg	12	272	264	97.1	141
Syncro-Mate	Mixture of fluorogestone acetate 30 mg and fluorogestone acetate 30 mg/progesterone 400 mg	12	132	129	97.7	142
Syncro-Mate	Fluorogestone acetate 30 mg alone and fluorogestone acetate 30 mg/progesterone 400 mg	14	53	53	100	143
Syncro-Mate	Fluorogestone acetate 30 mg	15	208	207	99.5	73
Chrono-gest	Fluorogestone acetate 30 mg	12	50	50	100	129
Chrono-gest	Fluorogestone acetate 30 mg	12	61	61	100	131
Veterdif-cycle	Methyl acetoxy progesterone 30 mg	14	84	82	97.8	144
Veterdif-cycle	Methyl acetoxy progesterone 50 mg	14	84	83	98	144
Veterdif-cycle	Methyl acetoxy progesterone 50 mg	14	406	406	100	145
Spogosan	Methyl acetoxy progesterone 50 mg	14	406	406	100	145
Veterdif-cycle	Methyl acetoxy progesterone 50 mg	14	355	350	98.6	146
Spogosan	Methyl acetoxy progesterone 50 mg	14	361	354	98.1	146
Veterdif-cycle	Methyl acetoxy progesterone 50 mg	14	438	434	99.1	147
Spogosan	Methyl acetoxy progesterone 50 mg	14	478	471	98.6	147
Veterdif-cycle	Methyl acetoxy progesterone 50 mg	14	577	571	99	148
Spogosan	Methyl acetoxy progesterone 50 mg	14	574	562	98	148

Table 6 (*continued*)

Sponge details	Drug	Insertion period (days)	Number inserted	Number retained for insertion period	Retention rate (%)	Reference
Repromap	Methyl acetoxy progesterone 40 mg	13	49	41	83.7	149
Repromap	Methyl acetoxy progesterone 40 mg	13	88	86	97.7	150
Veramix	Methyl acetoxy progesterone 60 mg	13	264	241	91.3	151
Same study	Methyl acetoxy progesterone 60 mg	14	200	171	85.5	152
	Fluorogestone acetate 40 mg	14	200	199	99.5	
Same study	Methyl acetoxy progesterone 60 mg	14	304	250	82.2	153
	Fluorogestone acetate 40 mg	14	314	313	99	
Repromap	Methyl acetoxy progesterone 60 mg	14	30	28	93.3	71
Repromap	Methyl acetoxy progesterone 60 mg	13	188	186	98.9	71
Repromap	Methyl acetoxy progesterone 60 mg	12	1222	1188	97.2	154
Repromap	Methyl acetoxy progesterone 70 mg	12	300	297	99.0	155
Repromap	Methyl acetoxy progesterone 60 mg	12	62	61	98.4	131
Polyurethane sponges measuring 3 cm in diameter × 2.5 cm in height	Methyl acetoxy progesterone 60 mg	14	100	96	96.0	156
5.08 cm long × 1.8 cm in diameter	Methyl acetoxy progesterone 60 or 80 mg	13, 15 or 17 days	504	494	98.0	157

progesterone incorporated into it.[138] In contrast to these observations, a study comparing sponges impregnated with 40 mg fluorogestone acetate and 400 mg progesterone suggested that the two sponges were retained equally well (96.9% versus 94.7% retention). Moore and Holst reported similar results.[159]

From Table 6, intravaginal sponges impregnated with methyl acetoxy progesterone appear to be lost more readily than sponges impregnated with fluorogestone. This observation is supported by a study by Ainsworth in which retention rates of methyl acetoxy progesterone-impregnated sponges were directly compared to fluorogestone acetate-impregnated sponges in the same study under the same conditions.[153] Lower retention rates (250/304) were observed for the methyl acetoxy progesterone-impregnated sponges than the fluorogestone acetate-impregnated ones (311/314).[153] Other studies which support these differences have been reported[149,152] but not all workers have observed such differences.[160–163] When Ch'ang et al.[164] observed poor retention rates (71/96; 73.9%) during a 14 day insertion for methyl acetoxy progesterone, they suggested that failure to lodge the sponge in the anterior region of the vagina was the likely cause of sponge losses.[164] It would be easy to suggest that this was the reason in other studies and is therefore the general explanation for the results. However, although correct insertion technique is an important factor to consider when interpreting these observations, Ainsworth et al. demonstrated that when sponges were inserted correctly, the time of loss of intravaginal sponges containing fluorogestone acetate (40 mg) and methyl acetoxy progesterone (60 mg) was random and therefore not affected by drug content.[153] Differences exist between the physical properties of the two sponges in which the methyl acetoxy progesterone and fluorogestone acetate is incorporated including shape, length, diameter, consistency, and surface texture (smoothness).[152] These factors must also be considered when interpreting results.

IV.C. Dimensions

The dimensions of an intravaginal drug delivery system are likely to be more complex than first imagined. In nearly all cases, the intravaginal drug delivery system will have a different shape during insertion than after insertion, as the change in shape to a more expanded system is often used to retain the device in the vaginal cavity. The dimensions of the delivery system (length during and after insertion, diameter, width during and after insertion) are dictated not only by the anatomical constraints and resilience of the vagina but also by the applicator design. This interrelationship between delivery system design and applicator design has been discussed earlier. As pointed out, this dependence of the size and shape of the delivery system upon the applicator design is often not fully appreciated until development work governing the size and shape of the delivery system itself has been performed – which is generally based purely on the size and shape of the vagina. A result of this approach is the potentially difficult task of designing an applicator to administer the delivery system. The most practical approach would be to have some insight into the delivery system, design an appropriate applicator around this tentative design and the vaginal anatomy, and then develop the delivery system around the constraints of both the vagina and applicator.

IV.D. Geometry

The shape of the delivery system is based on the shape and dimensions (length, width, etc.) of the vagina, the choice of retention mechanism which will be used to retain the device in the vagina following insertion and the design of the applicator. The vagina will influence the overall maximum dimensions that the device can be, while the latter two parameters will govern the shape of the device during and after insertion and consequently its overall geometry.

IV.E. Irritation (mucopurrelant discharge production)

The vagina reacts to the presence of any intravaginal drug delivery system. This reaction generally manifests itself in the form of a mucopurrelant discharge. However, if a delivery system is poorly designed an infection, ulceration, perforation or lesion may occur. The production of a discharge has become accepted by workers in the field as a part of the profile of an intravaginal veterinary drug delivery system. However, the formation of ulcers, perforation of the vaginal mucosa or lesion formation would be unacceptable properties. The amount of mucopurrelant discharge varies between delivery systems with the sponge[1,2,5,8,71,72,165] and PRID device exhibiting a high degree of discharge[73,74] and the CIDR-B[73,74] and CIDR-G[23,71,129,166] exhibiting a low degree of discharge. This results in the CIDR-G being the preferred device over sponges[23,71] and the CIDR-B the preferred device over the PRID[74] in certain countries or by certain investigators.

Other reactions to specific delivery systems have been reported in the literature. Polyurethane sponges impregnated with fluorogestone acetate were found to adhere to the vaginal wall in some maiden sheep.[8] Wishart observed that insertion of an intravaginal sponge into maiden sheep necessitated rupturing of the hymen and that over the treatment period, adhesions formed between the vagina and the sponge making removal extremely difficult and causing pain and trauma to the ewe.[5] van der Westhuysen also surmised that physical trauma to the hymenal region during insertion in maiden ewes resulted in cicatrix formation with partial occlusion of the vagina and considered that both insertion and removal of the sponges must be a painful experience.[72] Other workers have also reported that in a small proportion of treated ewes the polyurethane sponge adhered to the vaginal wall.[71,165]

The physical presence of the sponge produces an accumulation of a foul-smelling mucopurrelant discharge.[1,2,5,8,71,72,165] This does not appear to affect their efficacy, animal health or the acceptability of the product by the end-user. The mucus has been shown to be a good medium for bacterial growth but rarely results in infection of the vagina. In his original publication, Robinson reported that there was no obvious or serious infection on removal[2] and such observations have been confirmed in later studies.[72] Over longer insertion periods (up to 4 weeks) most animals develop a mild vaginitis[167] which does not, however, result in more generalized infections and clears rapidly after removal of the sponges.[167] In goats, a bacteriological examination on the mucus found that the results were negative from seven sponges, *E coli* was isolated from one and non-heamolytic staphylococcus from a second.[9] Vaginal smears from

sheep, which had intravaginal sponges inserted and removed, did not contain large numbers of leucocytes, but rather mucus, a few desquamated cells, some cellular debris and a few leucocytes.[1,2] A large number of bacteria were present in every sponge examined. However, the flora was non-specific and no pathogens were detected.[1,2] *P aeruginosa* was isolated from 12 sponges and two appeared to represent severe infections; however, 2–3 days later at insemination the vagina of each ewe appeared clinically normal.[1,2] In a further study results from vaginal swabs taken following insertion and removal of intravaginal sponges in sheep showed that there was a preponderance of non-hemolytic coliforms suggesting that contamination had occurred during device insertion.[5]

The presence of this discharge has prompted the practice of applying an antibiotic dust, cream or solution onto the surface of the sponge immediately prior to insertion. A review of the literature shows that this practice has little rationale. van der Westhuysen et al.[72] studied the effect of changes in the vaginal environment as a result of insertion of a fluorogestone acetate-impregnated sponge co-administered with and without antibiotic. Vaginal swabs were taken before insertion, immediately following removal and 2 days after sponge removal. Although no bacterial counts were made, the types of bacteria which were isolated from both aerobically and anaerobically cultured swabs showed only random variation between treatment groups and between the three sampling periods. Non-specific, non-pathogenic coliform bacteria were found in all cultures as were *Corynebacterium* species. None of the vaginas appeared infected and were clinically normal 2 days after sponge removal.[72] The authors concluded that the use of antibiotics had no effect upon vaginal flora after a 15 day insertion of the sponges.[72] Similar results were observed by Moore et al. who inserted sponges into the vagina of sheep which were pretreated with no antibiotic, Duocillin powder (penicillin), Zephiran solution (1:1000 aqueous solution Zephiran which was equivalent to a 10% aqueous solution of alkyldimethylbenzyl ammonium chlorides) or Savlon cream (cetrimide BP 0.5%, Hibitane 0.1%).[168] Bacteriological examination on removed sponges revealed that only gram positive bacteria were significantly affected by the bacteriocide, with their incidence being lowest with Zephiran and Savlon and highest with no application.[168]

A mucopurrelant discharge is also seen in cattle following insertion of PRID or CIDR-B devices.[73,74] The amount of discharge is less with the CIDR-B.[73,74] Indeed, only one early study reports any significant vaginal discharge when CIDR-B were removed.[87]

Uehlinger et al.[73] investigated the use of PRID and CIDR-B in seven ovariectomized cows. Cows were slaughtered 20–22 h after removal of the devices. Tissue samples of vagina, uterus and cervix were histologically investigated. Table 7 summarizes the clinical and histopathological results obtained in this trial.[73]

Because of the large surface area of PRID in contact with the vaginal mucosa a diffuse inflammation appears to be caused while hardly any locations of specially intensive irritation were observed. In contrast, the CIDR-B caused less diffuse inflammation because of restricted localization on the vaginal wall and its thinner shape. However, after removal a localized reddening was observed on the vaginal wall where the CIDR-B wings had been in contact with it.[73] The main difference between the two

Table 7
Clinical and histological comparison of PRID and CIDR-B devices[73]

Inspection[a]					Histology[a]			
Cow	PRID		CIDR		Device	Vagina	Cervix	Uterus
	Reddening	Pus	Reddening	Pus				
1	+ + +	+	+ + +	+	PRID	+ +	−	−
2	+ + +	+ + +	+ +	+	PRID	+	−	−
3	+ + +	+ + +	+ + +	+ +	PRID	+ +	−	−
4	+ +	+	−	−	CIDR	−	−	−
5	+ + +	+ + +	+ +	+	CIDR	+ +	−	−
6	+ + +	+ +	+	−	CIDR	+ +	−	−
7	+ + +	+ +	+ + +	+ +	CIDR	+ + +	−	−

[a] + , low level; + + , medium level; + + + , strong level.

devices was reported to be in the localization of the vaginal reactions; with the PRID a more generalized vaginitis was observed.[73]

Upon removal of the PRID device volumes of a mucopurrelant discharge are experienced.[75] The discharge is not bacterial in origin and does not affect insemination procedures, or fertility.[34] In addition the discharge disappears within a few days of treatment cessation.[75,76] Bulman et al. investigated the bacterial content of the discharge of cows in which PRID had been inserted for 14 days.[75] Vaginal swabs from nine out of ten cows examined showed a mild infection in the anterior vagina. The infections were observed to resolve spontaneously following removal of the device. The authors identified numerous organisms in the swabs, the majority of which were among those characteristically present on the skin and in the feces of cattle. The authors presumed that they were introduced into the vagina when the PRID was inserted and once in the vaginal cavity the presence of the PRID resulted in the accumulation of secretions in which the organisms could multiply.

IV.F. Damage to vaginal mucosa and cervix

A successful intravaginal veterinary drug delivery system must not cause damage to the vagina of the species for which it was designed. Problems can arise when a delivery system designed for an older animal is used with a younger animal. This may not be due to the difference in dimensions of the vagina between an adolescent and mature animal (which are often quite similar), rather it may be due to the presence of an anatomical feature which is present in the younger animal, but not in the older one, e.g. hymen. The problems arising from this have been discussed earlier.

Intravaginal drug delivery systems which utilize point loading for retention (e.g. CIDR-G and CIDR-B) as opposed to whole-device loading (e.g. sponges and PRID) do have the potential to cause more damage to the vaginal mucosa, however, there is very little evidence in the literature to suggest that such systems cause any damage to the mucosa. It is not uncommon to detect small ridges in the vaginal wall where the tips of such devices were localized during the insertion period.

IV.G. Removal

The majority of delivery systems administered intravaginally to animals are not biodegradable and therefore require some means of removal from the vagina to end the treatment. Removal must be achieved quickly, safely and without damage to the vaginal mucosa. Typically removal of devices is aided by the addition of a tail into the design of the device. The tail must be firmly attached to the device and must not detach itself from the device either during insertion or during the removal operation. It must also be long enough to protrude externally from the lips of the vulva and be of sufficient length to permit a firm hold by the remover. It must not cause irritation to the vulval lips, it should hang downwards and it should be unobtrusive during the insertion period so as to not attract interest from fellow herd or flock mates.

An early problem with the sponge was 'pull-through'. Tissue infiltration followed by some degeneration of the polyurethane occurred during the insertion period of polyurethane sponges manifesting itself in the strings being pulled out on removal.[1,2] This problem was a result of the distance between the string strands passing through the sponge matrix. By having the strands of the withdrawal string widely spaced, pull-throughs were eliminated.[5] Other factors which were identified as affecting string pull-through included tensile strength of the polyurethane, duration of intravaginal insertion period and presence or absence of adhesions.[5]

Tails have been made from plastic, monofilament nylon cord and multifilament cord. A monofilament cord is preferable since multifilament cords act as wicks allowing bacteria to travel up and into the vagina causing infections. Monofilament nylon cords are very flexible and consequently hang downwards and are unobtrusive. They generally do not attract the attention and interest of flock or herdmates. Their flexibility can also be a disadvantage; they can be drawn up into the vagina making it appear that the device has fallen out and make removal more difficult. Plastic cords offer the advantage that they can be colored and therefore be useful for identification purposes in developmental experiments, animal identification, etc. If they are designed too rigidly they can protrude directly outwards from the vagina attracting interest from fellow herd or flock mates. Rigid tails may also be uncomfortable to, and irritate, the animal.

IV.H. Release characteristics

The release characteristics of the device are tailored to the desired plasma drug concentrations required for clinical efficacy. Tailoring of release profiles can be achieved from in vitro and in vivo studies as described below. Tailoring involves the basic principles of pharmaceutics; identification of the mechanism of release, examination of release equations and investigations involving the manipulation of the parameters of the release equations until the desired release rate is achieved (e.g. by the addition of excipients to affect drug solubilities in the polymer, diffusion coefficients, etc. alteration of surface area, alteration of the thickness of the rate-limiting membrane to modify diffusional pathlengths, etc.). Tailoring of release profiles is often the last step in the development process. An examination of the current

literature would suggest that this stage of development was rarely undertaken with those products currently on the market. The reason is probably 2-fold: (i) formulation scientists were not involved in the early development work of currently marketed products and (ii) the inherent release characteristics of the synthetic or natural progestagens from polyurethane or silicone, respectively, were ideal for the required clinical purpose. However, although drug release was ideal, the initial loads, poor drug utilization and high drug residuals remaining in these devices following removal were issues that were not addressed. Interestingly, this stage has, or is, now being addressed with the sponge[67–69] CIDR-B and CIDR-G[93–95] where, in the case of the latter two, the currently marketed products are being optimized. The one exception to this rule was the excellent work of Chien[67–69] who initially set out to optimize the sponge, but resulted in rationally developing a series of intravaginal drug delivery systems manufactured from silicone, which utilized the sponge as a means of retention.[67–69]

V. Assessment of intravaginal drug delivery systems

The rational design and development of an intravaginal drug delivery system necessitates in vitro and in vivo release studies, stability studies and retention studies to be performed on the device.

V.A. In vitro release

The advantage of developing an in vitro test is the rapidity with which results can be generated. It can be an excellent screening tool for formulations. It is important, however, that the test be predictive of what happens in vivo. Often in vitro tests are designed to be representative of the in vivo conditions (e.g. same pH, ionic strength, agitation conditions, etc.), however, representative may not necessarily mean predictive. In our experience it is worth spending some time developing a predictive in vitro test and this may involve moving away from conventional methods (e.g. modification of the dissolution tests found in Pharmacopoeias). Ultimately, an in vitro release assessment test will need to be developed for regulatory purposes. This should be considered a separate issue and will need to follow guidelines reported in Pharmacopoeias.

Examples of release assessment of intravaginal veterinary drug delivery systems have appeared in the literature.[67,94] The actual PRID device has never been assessed in vitro (at least there are no reports in the literature). One report[13] used progesterone-impregnated silicone discs loaded with the same drug concentration to mimic a PRID, however, it was not subjected to the exact manufacturing process as that device, which may have affected release profiles. It would not seem unreasonable to suggest that an expansion of this approach could be used to test devices of the type the PRID represents (uniform layer of polymer impregnated with a uniform dispersion of drug). A representative sample of the device could be taken from it and mounted so that only one side was accessible to a release media which impelled sink conditions. Such an approach seems feasible, provided it can be shown that drug is uniformly and consis-

tently distributed throughout the delivery system such that the sample taken from the device is representative of the entire device.

Chien developed an in vitro release apparatus which enabled analysis of the in vitro release characteristics of fluorogestone acetate from polyurethane sponges.[67] The apparatus was adapted to also allow determination of the absorption characteristics of the drug after release from the sponge.[67]

Recently Bunt et al. described an in vitro release assessment test for the CIDR-B which was based on the USP dissolution test.[94]

V.B. In vivo release

Despite the convenience, speed and cost advantages of in vitro release methods, such methods are only useful in product development if the results generated from such studies are predictive of what happens in vivo. At some point in the development process the in vivo release profile from the intravaginal veterinary drug delivery system may need to be determined. This is a reasonably easy process which can be achieved by removing inserted devices after given time periods and assaying for remaining drug content in the device. Knowledge of the initial amount incorporated into the device allows calculation of the amount released over a specified time period and construction of an in vivo release profile. The limitation of this approach is that without confirmatory pharmacokinetic studies, the amount of drug released from the device may not be representative of the amount of drug that actually reaches the systemic circulation. Such a methodology has been used by several investigators to determine in vivo release profiles from intravaginal veterinary drug delivery systems.[13,65,83,85] Robinson determined the in vivo release profile of fluorogestone acetate from polyurethane sponges[83,85,86] and Winkler has used this approach to determine the in vivo release profile of progesterone from PRID devices.[13]

V.C. Stability

Final product chemical stability is a requirement if any device is to be registered. In general, a 6 month accelerated study is acceptable to most regulatory bodies in lieu of real-time data subsequent to the initiation of the registration process. The stability study, which may have to undergo regulatory authority approval, will need to be designed such that it demonstrates product stability and it will require the development of a fully validated stability-indicating assay. If the product is to be registered, the stability study must be performed on the final product which is to be marketed. Such a study can therefore only be initiated when the design and development processes have been completed and scale-up has been achieved. At this stage in the development process, it would be rather embarrassing to discover that the drug in the delivery system was unstable. Hence, it is advisable to initiate preliminary chemical stability trials early on in the development of an intravaginal veterinary drug delivery system using prototype devices. This procedure could identify problems well in advance.

V.D. Retention

Retention studies should be performed on an adequate number of animals. Conclusions drawn from trials performed with small animal numbers can be misleading. High retention rates may be encouraging in initial trials. Always remember that initial studies may have been performed on experimental animals which are used to being handled, devices are inserted by experienced operators and under experimental conditions which may not reflect those found in the commercial setting. Continual observation of high retention rates in early development studies would be encouraging, and suggest that only minor modification to the geometry or retention mechanism of the device would be needed if devices used under commercial conditions exhibited less than ideal retention rates. On the other hand, continual observation of low retention rates in early development studies would indicate that work should be performed on the design of the device at an early stage in the development process.

VI. Future issues

A variety of intravaginal vaginal drug delivery systems are available. These are used to deliver progestagens and potent analogues of non-steroidal compounds to livestock in order to control their estrous cycles. Such delivery systems are used as part of an overall synchrony program designed to impel precise synchrony and normal fertility. Many synchrony programs require the administration of several drugs independently at different times throughout the program. Developments in this area will continue in close association with reproductive physiologists and veterinarians. The current aim of formulation scientists in this area is to deliver multiple drugs in a controlled or pulsed fashion, at different rates and times from a single device. Such aims are demanding, but recent research has suggested that it may be achievable using microchip-controlled delivery systems. A second issue facing scientists in this area is the search for suitable drug candidates which possess ideal physicochemical properties and for which there is an animal health or production demand. The use of penetration enhancers to improve membrane permeability, the search for suitable polymers as platforms for intravaginal drug delivery, and the design of retention mechanisms which permit longer insertion periods with minimal damage to vaginal mucosa are other possible future issues.

VII. Conclusions

The intravaginal route in animals offers unique advantages and challenges to the formulation scientist. A review of the literature reveals that it remains an underexploited area of pharmaceutical research. Much fundamental and applied work has still to be performed before we will fully appreciate the complexities and full potential of the intravaginal route for drug delivery in animals.

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CHAPTER 7

Controlled release drug delivery systems for estrous control of domesticated livestock

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Overview

I. Introduction

The need for controlled release drug delivery systems in the field of estrous control of domesticated livestock arises due to the physicochemical and pharmacokinetic char-

acteristics of the drugs (e.g. progesterone) which control the estrous cycle of domesticated livestock.[1,2] In general such drugs have low aqueous solubilities, are rapidly metabolized by the body, are extensively degraded in the chemically harsh environment of the rumen (of ruminant animals) or stomach (of monogastric animals) and require a rapid decline in their plasma concentrations to occur at the end of treatment in order to initiate estrus.[1,2] Some of these characteristics necessitate the need to utilize routes of drug delivery other than the oral route, some dictate the need to formulate the active compound into a controlled release drug delivery system, and some result in the need to be able to remove the delivery system (or for the formulation to exhaust itself of drug) at the end of the treatment period to abruptly terminate delivery of the active compound.

The first use of controlled release drug delivery systems to control the estrous cycle of sheep and cattle dates back to the mid 1960s and mid 1970s, respectively.[1–3] In contrast, controlled release drug delivery systems for estrus control of horses and pigs has evolved more recently,[4–7] despite the fact that the need and desire for such products for these animals has been ongoing since the 1950s. In spite of the long history associated with this area of drug delivery, the opportunity to develop and commercialize controlled release drug delivery systems to control the estrous cycle of domesticated species has never been greater.[8] This is because of the rise in on-farm use of procedures such as embryo transfer, new assisted reproductive technologies such as super ovulation, in vitro fertilization, intracytoplasmic sperm injection and cloning, all of which increase the need to synchronise domesticated livestock. In addition, recent exhaustive efforts by animal scientists have resulted in a clearer understanding of the endocrinology and physiology of follicular development and ovulation in cattle, sheep, horses and pigs.[1–3] Information which can be exploited by the formulation scientist when developing a controlled release estrous control product for domesticated livestock.

This chapter will describe the controlled release drug delivery systems which are currently available or in development for the control of the estrous cycle in sheep, cattle, pigs and horses. It will describe some of the formulation considerations for such products and highlight recent advances in the field of estrous control in horses and pigs.

II. The need for control of estrus and ovulation in domesticated livestock

There are several reasons why a modern day farmer would want to control estrus and ovulation in domesticated livestock (Table 1).[8] Likewise there are several reasons why a formulation scientist would focus on formulating the drugs used to control the estrous cycle of domesticated animals into a controlled release drug delivery system (Table 2).

Table 1
Reasons for farmers needing to control estrus and ovulation in domesticated livestock (adapted from Ref. 8)

Reason
To reduce the time and labor which is currently devoted to estrous detection
To allow for more cost effective implementation of timed insemination programs
To increase the use of artificial insemination with fresh, transported or frozen semen in order to utilize desirable genes
To reduce natural service in order to decrease the transmission of communicable diseases
To synchronize donor and recipients for embryo transfer allowing the use of new assisted reproductive technologies such as super ovulation, in vitro fertilization, intracytoplasmic sperm injection and cloning
To allow seasonal breeders to be bred early in the season or even out of season when commercially important
To maximize reproductive efficiency with ovulation control programs to provide producers with substantial opportunities to reduce production cost and enhance profitability

III. Routes for controlled drug delivery for estrous control

Both the subcutaneous and intravaginal routes have been commercially exploited for the controlled delivery of drugs to control the estrous cycle of cattle and sheep.[1–3] In pigs, historically, both of these routes have been examined for controlled drug delivery, but with limited success.[4,9] However, more successful attempts have been made recently.[5,7] The intravaginal route has been conceptually examined in horses using delivery systems designed principally for cattle[10–17] which has provided some indication of the possibilities of this route, however, the commercially successful routes for drug delivery in horses are the subcutaneous and intramuscular routes.[18–33]

IV. Drugs, polymers, formulations and manufacturing techniques for controlled release drug delivery systems

Because of the low commercial value of sheep, cattle and pigs and the fact that farmers control large herds or flocks of these animals, the drugs, polymers and any other excipients used in controlled release drug delivery systems for cattle, sheep and pigs need to be cheap to enable low cost products to be manufactured and retailed.

Table 2
Reasons why controlled release products for estrous control are advantageous in domesticated livestock

Reason
To reduce labor and the associated handling stress to the animals and producers
To integrate treatment with farmed management practices
Controlled release delivery systems are the ideal technology to deliver the type of drugs used to control the estrous cycle of domesticated livestock (see Section I)
Specific controlled release drug delivery systems can be tailored to maximize reproductive efficiency based on the unique reproductive and economic characteristics for a given species
Controlled release delivery systems are ideal to enable the formulation scientist to utilize routes of drug delivery which are not associated with residue issues (e.g. ear in cattle and sheep; vagina in all domesticated species)

Delivery systems for sheep, cattle and pigs have revolved around injection moldable biocompatible polymers such as silicone and Hydron (poly ethylene methacrylate).[1–3] In addition for sheep, polyurethane in the form of a sponge has been utilized as a controlled release drug delivery system.[1–3] Horses tend to possess an inherently higher value and therefore there is more scope for the delivery system to comprise a more complex formulation, incorporate more expensive bioactive materials and/or be manufactured by a more complex manufacturing process. Consequently, poly (DL-lactide) copolymer has been used in the development of a microencapsulated product for use in horses.[18–24]

V. General considerations for developing a controlled release drug delivery system for the control of the estrous cycle of domesticated livestock

When developing a controlled release drug delivery system for estrous control several factors should be taken into account. These are presented in Table 3 and briefly discussed below.[8]

Interactions between the drug and the formulation ingredients both during and after the manufacturing process should be identified and avoided.

Sterility issues must be addressed early in product development. If the product is an injection such issues will be of paramount importance and methods of sterilization together with the effect that those methods have on the release properties of the active must be investigated. Intravaginal delivery systems do not need to be sterilized following manufacture, and to date none of the commercially available ear implants are sterilized following manufacture.

Table 3
Formulation development issues. Adapted from Ref. 8

Issue
Intellectual property issues relating to actives, use and process patents
Physiochemical properties of the drug
Interactions between the drug and the formulation ingredients
Duration of release
Formulation processing conditions and facilities with respect to both initial development and scale-up
Sterility issues
Tissue residue issues in food producing animals
Ability to fit within farm management practices
Packaging requirements
Method of administration
Dose utilization
Cost of raw materials
Size of the delivery system
Successful formulations must offer improvements in control of the estrous cycle
Cost effectiveness
Safety to the end-user
In vivo stability
Retention of the delivery system

Formulation processing conditions and facilities are important factors that must be considered during development and scale-up. These include the need for dedicated facilities when working with steroids such as estradiol. Also, solvents used in many polymer based microencapsulation processes may require explosion proof processing areas and special waste handling systems.

In food producing animals tissue residue issues must be addressed both for the active and the biodegradable matrix because the animal enters the food chain. The delivery system should not induce local reaction (irritation or swelling) and should cause little or no damage to the carcass. Better yet, the delivery system should be designed to be administered and retained in non-edible tissue of the animal (e.g. ear in cattle and sheep; vagina in all domesticated species).

Drug properties such as molecular weight, aqueous solubility at different pHs, lipophilicity, pK_a, etc. should be considered during the pre-formulation stage since these will have an effect upon both the biological activity of the drug and the release characteristics of the drug from the delivery system. The active compound should also be chemically stable during process and manufacturing and upon storage in the finished product.

The size of the final dosage form will be dictated by the dose size, the amount of excipents needed to formulate the delivery system and the type of delivery system being formulated (implant/injection/intravaginal insert). Typically, the size of a commercially feasible subcutaneous implant delivery systems must be small to aid easy administration (maximum dimensions of say 15 mm in length, 3 mm in diameter). Aqueous or oily injections should be of small volume, e.g. <10 ml, to reduce pain and distress to the animal upon administration. The size of an intravaginal delivery system varies between animal species but would be tailored to fit the internal dimensions of the vagina of the target species. The size of intravaginal drug delivery systems are also influenced by the need for ease of administration, mechanism of retention, ease of removal and desired drug delivery profile.

The duration of release varies from drug to drug and species to species. For example, the delivery of progesterone to cattle is at least 5 days for intravaginal products and up to 12 days for subcutaneous implants. In horses and pigs 12–14 day administration periods are used for progesterone. In sheep periods of 14 days are not uncommon for the delivery of synthetic progestagens.

Administration must be able to be integrated into different management practices. The administration procedure must also be relatively free from discomfort for the animal. Parenteral solutions are easier to administer compared to implants which may require specially designed implanters with large bore needles. Intravaginal administration requires the use of specially designed applicators which are unique in size and shape and tailored to the delivery system.

The price of the controlled release delivery system must be cost-effective for sheep, cattle and pigs to realize commercial potential. As indicated above, there is more latitude in cost when formulating an estrous control product for horses.

The delivery system must be designed to efficiently utilize the incorporated dose. To date this has not been a characteristic of commercially available subcutaneous or

intravaginal drug delivery systems. However, it is more characteristic of recently developed systems.

The delivery system must be reasonably aesthetic to appeal to the end-user. It must also be easy to administer, offer improvements in control of the estrous cycle, adapt to farm management practices, be safe to the end-user and be cost effective.

After administration the formulation will be exposed to water from the surrounding interstitial spaces (subcutaneous or intramuscular implants) or vaginal fluids (intravaginal delivery systems). This will result in the drug existing at high concentrations in an aqueous environment which is elevated to temperatures of 37–39°C. Thus the stability of the drug, the polymeric matrix and any excipients must be investigated during the development of a controlled release delivery system.

The retention of the delivery system for the duration of release is a high priority since the overall effectiveness of the treatment program relies on the delivery system remaining in situ. An intravaginal drug delivery system would require a retention rate in excess of 95% (more appropriately 98%), while a subcutaneous implant would be expected to be retained in >99% of cases.

VI. Controlled release products for horses, cattle, pigs and sheep

VI.A. Horses

VI.A.1. Intravaginal estrous control products for horses

Although no specific intravaginal product has been formulated for use in horses, both the commercially available PRID and CIDR-B products have been successfully used to control the estrous cycle of horses[1,3,10–15] and sponges have been investigated conceptually.[1,3.16,17]

VI.A.2. Intramuscular estrous control products for horses

VI.A.2.a. Biodegradable polymeric microspheres Intramuscularly administered products for estrous control of horses have revolved around the formulation of biodegradable polymers. The potential of biodegradable polymers in estrus control formulations was first shown by Beck et al. in 1979[34] who formulated injectable microsphere systems containing contraceptive steroids. This was followed by the elegant work of Vickery et al.[35] who formulated a biodgradable preparation incorporating a GnRH agonist for long-term ovulation inhibition. The promise of such research in laboratory species studies eventually led to the development of biodegradable microspheres for controlling estrus and ovulation in mares.[18–24] The product, P+ (originally named Lutamate Plus), is for use in cyclic and late transitional phase non-cyclic mares. It comprises a single intramuscular injection of estradiol and progesterone formulated into biodegradable microspheres (Figure 1) administered as a sterile aqueous injection. P+ was specifically formulated to release sufficient progesterone and estradiol to block estrus for the duration of approximately one luteal phase (14–18 days) to allow for a normal ovulation to occur about 1 week later.

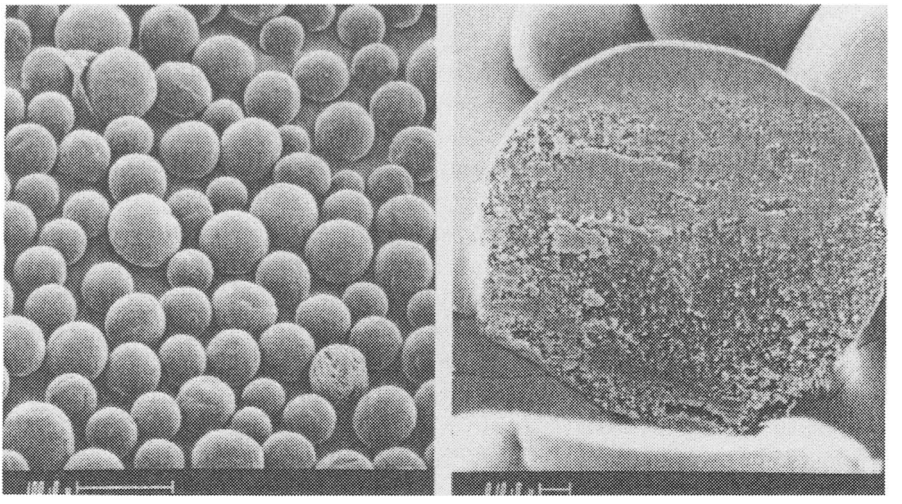

Fig. 1. Biodegradable poly (DL-lactide) microspheres containing 100 mg estradiol and 1.25 g progesterone.

The microspheres were prepared using a solvent extraction process where a mixture of poly(DL-lactide), drug and solvent were emulsified in water with subsequent removal of the solvent to afford discrete particles (Figure 2).[36] The microspheres

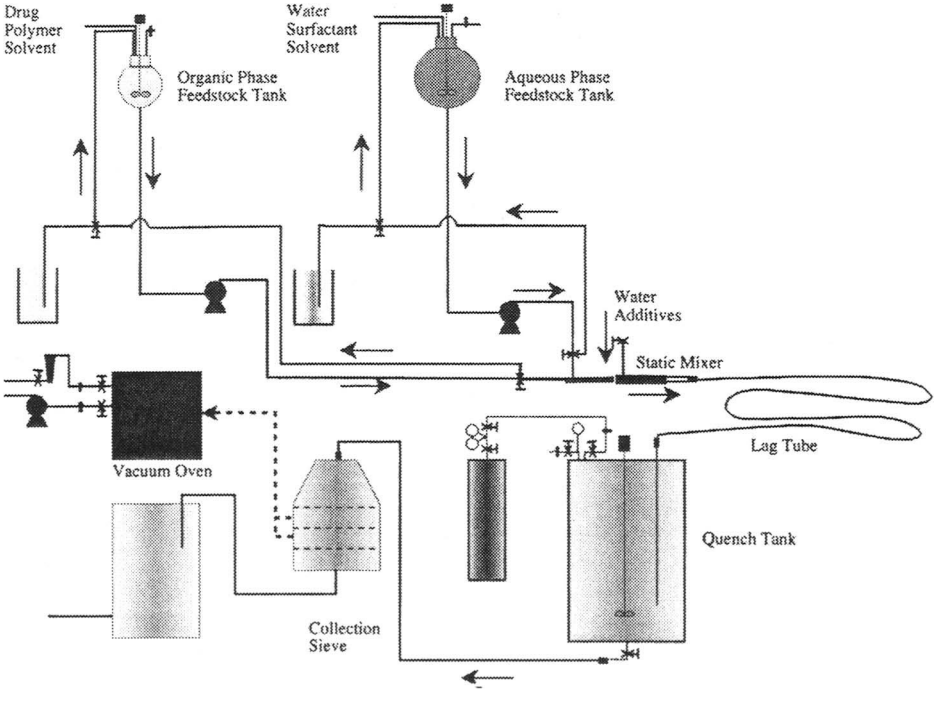

Fig. 2. Diagram of continuous microencapsulation process used to manufacture poly (DL-lactide) microspheres.

were then collected by filtration, washed extensively with purified water and dried under vacuum.[7] The microspheres resulting from this preparation method have been characterized by determining their core loading, SEM surface morphology and particle-size analysis after sterilization by exposure to gamma radiation.[7]

Following administration of P+ containing 100 mg estradiol and 1.25 g progesterone, plasma concentrations of these compounds were observed to be elevated and sustained (Figure 3).[36]

The clinical efficacy of P+ to control estrus and ovulation in late transitional noncyclic mares, as well as in cyclic mares, has been examined in a controlled multicentered clinical trial using a total of 135 mares.[20] Combined results from all sites demonstrated that P+ treatment increased the proportion of mares displaying a normal estrus following treatment (87% in treated mares versus 63% in control mares). Treatment was effective in controlling estrus and ovulation as indicated by significant reductions in variation for days to estrus, days to ovulation and length of estrus following treatment. Conception rates for the estrus following treatment were similar and averaged 63% in treated mares and 52% in control mares. Conception rates for the entire breeding season also were not different and averaged 89% in treated mares and 84% in control mares.

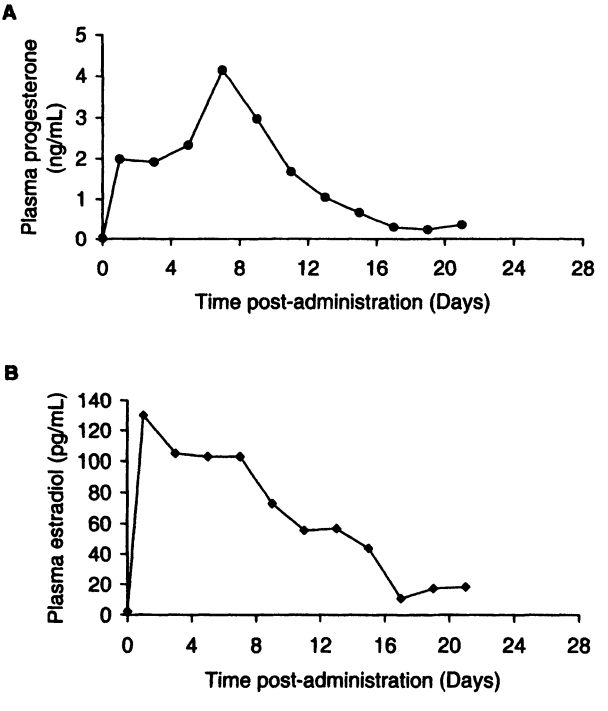

Fig. 3. Mean plasma concentrations of progesterone (A) and estradiol (B) in anovulatory mares following a single intramuscular injection of P+, an intramuscular injection formulation comprising biodegradable poly (DL-lactide) microspheres containing 1.25 g progesterone, 100 mg estradiol manufactured by continuous encapsulation process. Redrawn from Ref. 36.

Although these and other extensive studies[3,18–24] demonstrated the effectiveness of the biodegradable steroid microsphere formulation to control estrus and ovulation in mares, scaling up to commercial microsphere production to meet the demands of the domesticated livestock market proved to be a very complex process requiring costly specialized facilities, water systems and equipment.

VI.A.2.b. SABER delivery system The SABER delivery system has recently been investigated as a more cost effective platform than microspheres to deliver several important reproductive hormones including the potent GnRH analog Deslorelin for a period of hours for induction of ovulation in estrus mares with preovulatory follicles,[37–39] and estradiol to mares for a period of several weeks.[40,41]

The SABER delivery system comprises a unique high viscosity base compound (sucrose acetate isobutyrate, SAIB; Figure 4) which, when mixed with a small amount of solvent, converts to an easily injectable liquid.[39–41] However, following administration, it solidifies to form a semi-solid, biodegradable implant which acts as a platform for the delivery of the drug or antigen it has been formulated with. SABER formulations can be administered by multiple routes including intramuscular, subcutaneous, intranasal, intravaginal or intrauterine. Moreover, unlike the very complex and costly processes associated with microsphere formulations, the production of SABER formulations involves a simple mix and fill operation (Figure 5).

SAIB has attained regulatory status in several countries world wide. It is approved as a Direct Food Additive in parts of Europe, Asia, the Middle East and South America, and it was granted US approval as a Direct Food Additive in June 1999. SAIB has several properties which combine to make it a unique platform for drug delivery (Table 4).

Several experimental SABER formulations containing various amounts of estradiol were manufactured by the process shown in Figure 5. Based on in vitro evaluation, two were selected for in vivo investigation.[41] The first test formulation contained a SAIB:ethanol ratio of 85:15 and 5% estradiol in a 2 ml dose. The second contained a SAIB:ethanol ratio of 85:15 and 10% estradiol in a 1 ml dose. Twelve light horse geldings between 6 and 12 years of age, weighing between 490 and 560 kg were used in the trial. Three geldings received 2 ml of physiologic saline intramuscularaly and

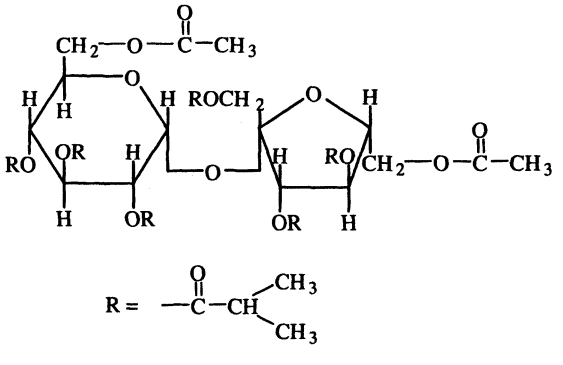

Fig. 4. Chemical structure of sucrose acetate isobutyrate (SAIB) molecule.

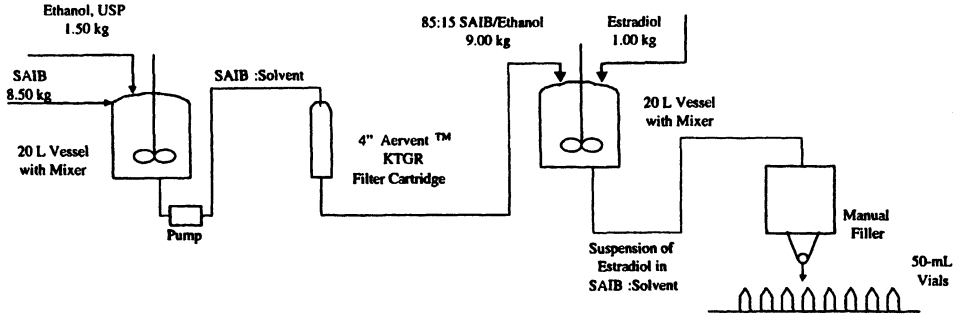

Fig. 5. Process flow diagram for the manufacture of a SABER formulation.

served as negative controls. In the treatment groups, three geldings received the positive control containing 100 mg of estradiol microspheres suspended in 2 ml of injection vehicle injected intramuscularaly and three geldings per group received the SABER formulations as a 5% (2 ml) or 10% (1 ml) suspension injected intramuscularaly. The estradiol plasma levels obtained following administration of these formulations is shown in Figure 6.[41]

Another product SABER Mate E (Equine) has also been developed using the SABER delivery system (Figure 7).[37–39] SABER Mate E is a short acting controlled release preparation comprising deslorelin acetate formulated with SAIB. It is indicated for stimulation of ovulation within 48 h in estrus mares with a follicle between 30 and 40 mm. SABER Mate E has been shown to be clinically effective 90% (9/10), 90% (18/20) and 100% (36/36) of the time in stimulating ovulation within 48 h in research trials in Brazil, Colorado and Colorado, respectively.[38] Recently it has been successfully scaled up to a 10 000 dose batch size.

VI.A.3. Subcutaneous estrous control products for horses

Controlled drug delivery systems for GnRH agonists have been evaluated in mares for both induction of ovulation during seasonal anestrus (using biodegradable subcutaneous implants[42]) and induction of ovulation in estrus mares with preovulatory follicles (using bioabsorbable implants).[43]

VI.A.3.a. Ovulplant™ Ovulplant™ (Figure 8) has been available in Australia for several years and was approved for use in the US in 1998. In both countries it is

Table 4
Properties of SAIB (sucrose acetate isobutyrate)

Properties
High viscosity liquid
Hydrophobic
Biocompatable
Biodegradable
Adhesive

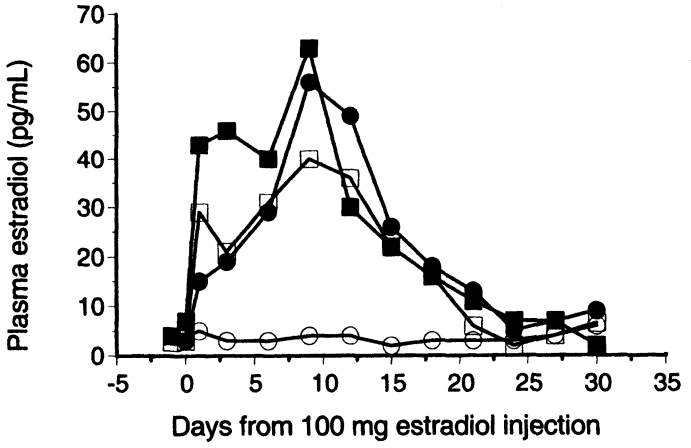

Fig. 6. Estradiol plasma levels following administration of a SABER formulation containing estradiol. Saline (○), poly (DL-lactide) microsphere preparation (□), 5% SABER formulation (■) and 10% SABER formulation (●). From Ref. 41.

used for stimulation of ovulation within 48 h in estrus mares with a follicle between 30 and 40 mm. Ovuplant is a biocompatable subcutaneous implant (2.3 mm in diameter × 3.6 mm in length) containing 2.1 mg Deslorelin (GnRH analog).[3,12–14,25–33] It has been shown to be clinically effective 86.9% (73/84) and 78.1% (32/41) of the time in stimulating ovulation within 48 h in six site and three site multi-site clinical trials, respectively.[44]

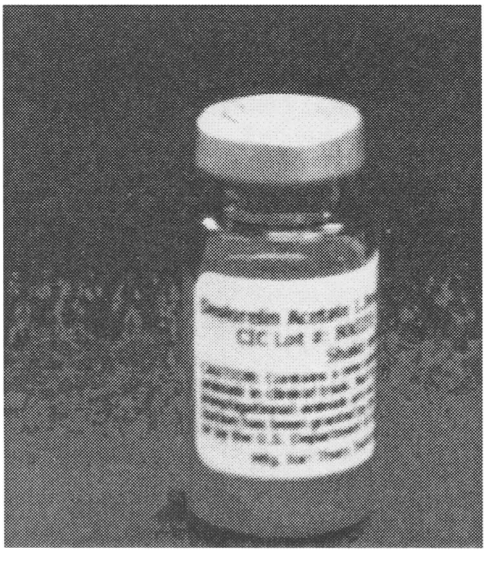

Fig. 7. SABER Mate E product.

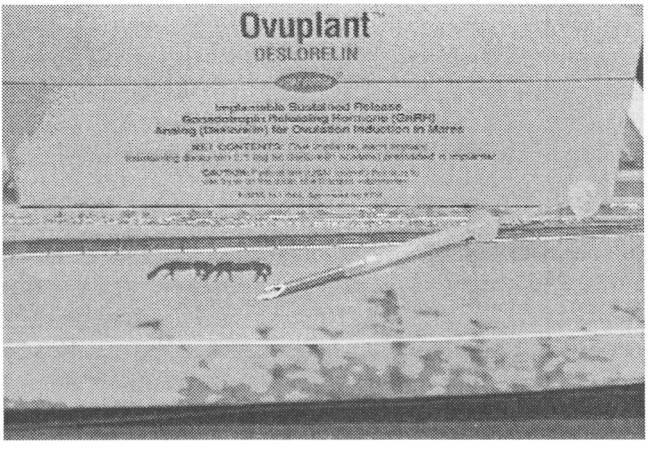

Fig. 8. Commercially available Ovuplant product.

VI.B. Cattle

VI.B.1. Intravaginal estrous control products for cattle

Several intravaginal products are commercially available or have been conceptually investigated for the control of the estrous cycle of cattle.[1–3]

The PRID comprises a stainless steel strip covered on both sides with a matrix comprising silicone and progesterone and rolled to produce a coil approximately 4 cm in diameter and approximately 12 cm in length.[1–3,45] The coil is supplied with a hard gelatin capsule containing 10 mg estradiol benzoate glued to its inner surface. A length of string is tied to one end of the device to aid removal. The PRID has been extensively studied in the literature (see Refs. 1–3 for review of articles).

The CIDR-B also comprises progesterone homogeneously distributed throughout a silicone rubber matrix.[1–3,46] However, it has a different shape and size compared to the PRID and is manufactured using a different injection molding process. The CIDR-B is T-shaped and manufactured by an injection molding process in which the progesterone/silicone mixture is cured over a pre-molded annealed nylon spine at high temperatures (approximately 190°C). Following curing a plastic tail is attached to the lower end of the body of the CIDR-B. The tail protrudes from the vulva following insertion and aids in removal of the device from the vagina. The CIDR-B has been extensively studied in the literature [see Refs. 1–3 for review of articles]. The CIDR-B was first marketed in New Zealand in 1987 and originally contained 1.9 g progesterone and was designed for a 12 day insertion period. Recent advances in our understanding of animal physiology and endocrinology of the cattle estrous cycle has resulted in the treatment period for this product reducing to 7–10 days. A consequence of this is that the CIDR-B contains a high residual load following its removal after 7 days (1.32 g). Recently it has been re-engineered to account for the newer treatment insertion periods.[47] Extensive in vitro and in vivo characterization[47–51] resulted in the design parameters for a new product to replace the CIDR-B (Figure 9) whose initial load is 1.38 g,

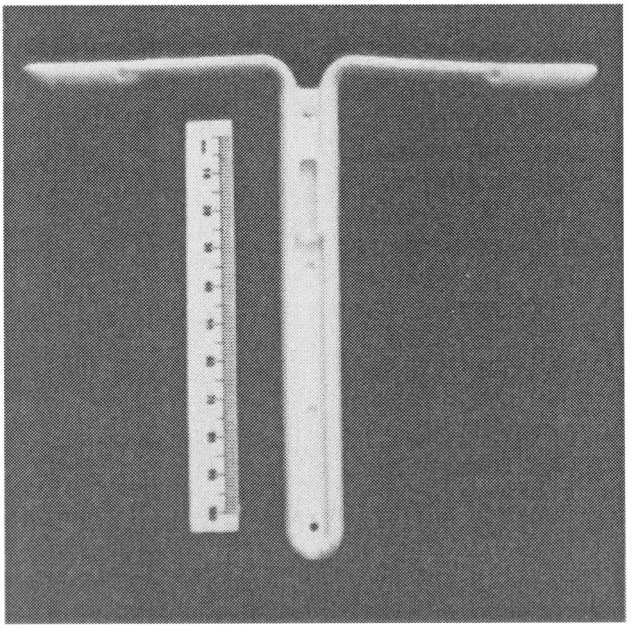

Fig. 9. Commercially available CIDR-B intravaginal insert.

and which exhibits a residual of approximately 650 mg following its removal after a 7 day insertion period.[47]

Another delivery system, similar in shape to the CIDR-B, named the intravaginal application system or INVAS, has been described in the patent literature.[1,2,52,53] The T-shaped device comprises a polypropylene spine and is produced using a manufacturing technology which is different from the CIDR-B.[1,2,52,53]

Rajamehendran et al. fabricated an intravaginal drug delivery system from silicone rubber tubing.[54–56] The device contained progesterone within the tubing lumen and estradiol 17β contained in coated tips which covered both ends of each tube.

Polyurethane sponges impregnated with varying amounts of progesterone and of various lengths, diameters and densities have been inserted in cattle.[57–66] The sponges exhibited variable retention characteristics which was explained by factors such as diameter, length, presence or absence of antibiotic, type of antibiotic applied, age of the animal, size of the vagina, hormone type, rectal palpation, tail characteristics and sponge density.[57,59,63–65]

The most recent and technologically advanced controlled release drug delivery system was developed in New Zealand and was named the intelligent breeding device (IBD) and was recently described by Rathbone et al.[1–3] The IBD was designed to administer progesterone, estradiol and prostaglandin at the required amounts, rates and times to precisely control the estrous cycle of cattle. The device comprises four drug reservoirs (a large one at the base of the device and three smaller ones at the head of the device), a circuit board and batteries. In addition a spoke-type retention mechan-

ism is located at the head of the device and a tail is attached to its base to aid its removal from the animal. The large drug reservoir contains an organic-based solution of progesterone which is released from the device via a small orifice. The amount and rate of progesterone release is controlled by the opening and closing of the orifice. Two of the three small drug reservoirs contain solutions of prostaglandin and estradiol. The third is currently not utilized. The release of these latter actives is controlled via the electronic circuit board. The IBD is programmed to continuously deliver progesterone over a 10 day period, and deliver a pulsed administration of estradiol 1 h after administration and a pulsed administration of prostaglandin 6 days after administration.

Recently poly (ε-caprolactone), a biodegradable polyester, has been shown to be suitable for the manufacture of an intravaginal drug delivery system for the delivery of progesterone to control the estrous cycle in cattle.[67,68] Two groups of six ovariectomized cattle were treated for 7 days with either a poly (ε-caprolactone) insert containing 10%w/w progesterone or a CIDR-B (containing 10%w/w progesterone).[67] The poly (ε-caprolactone) intravaginal insert exhibited slightly lower average plasma progesterone concentrations compared to the CIDR-B (Figure 10), and released slightly less progesterone (0.68 g) compared to the CIDR-B (0.72 g) over the 7 day insertion period. The trial demonstrated that plasma progesterone levels could be elevated and sustained thus demonstrating the feasibility of using poly (ε-caprolactone) as a platform for the intravaginal delivery of progesterone to cattle. Further trials demonstrated how progesterone release from the poly (ε-caprolactone) intravaginal inserts could be modified by the addition of various excipients to the insert to elevate plasma progesterone levels.[68]

VI.B.2. Intramuscular estrous control products for cattle

VI.B.2.a. Biodegradable microspheres The promising research conducted by Burns et al. into the development of biodegradable microspheres for controlling estrus and ovulation in mares (P+)[18–24] led to the evaluation of a similar product in cattle.[69]

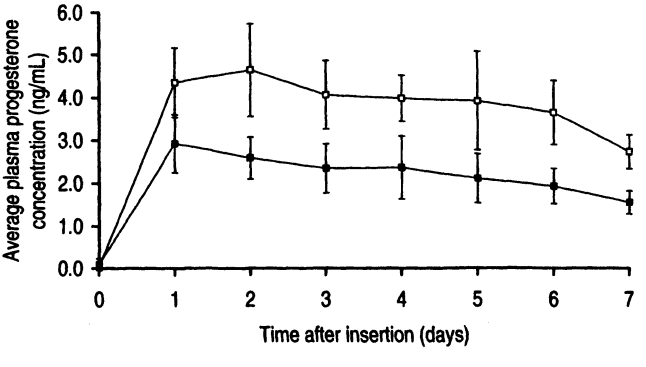

Fig. 10. Plasma progesterone levels in treated cows following administration of CIDR-B (□) and poly (ε-caprolactone) intravaginal inserts (■). From Ref. 67.

P+ for cows is a cycle controlling product for use in cyclic and peri-pubertal heifers and involves a single intramuscular injection of biodegradable progesterone microspheres. Plasma levels of progesterone following administration of two different microsphere formulations were shown to be elevated following administration and declined quickly when the microspheres became depleted of drug (Figure 11).[69]

VI.B.2.b. SABER delivery system SABER Mate B (Bovine) is a recently developed short acting controlled release deslorelin acetate formulation for use in the newly developed OVSYNCH protocol which synchronizes follicular development, luteal regression and time of ovulation, thereby allowing for timed insemination 12–24 h after the completion of the OVSYNCH treatment protocol (GnRH/$PGF_{2\alpha}$/GnRH).[70]

VI.B.3. Subcutaneous estrous control products for cattle

VI.B.3.a. Subcutaneous implants (body) Both natural (progesterone)[71] and synthetic (melengestrol acetate;[72] or norethandrolone[73–76]) progestogens have been delivered from subcutaneous implants in an attempt to control the estrus cycle of cattle. Such implants were manufactured using either silicone[71–73] or Hydron.[74–76] The quantity of progesterone needed for effective control resulted in implants of large size. This factor, together with the need to restrain the animal to administer the product, the requirement for aseptic techniques during administration and the need for minor

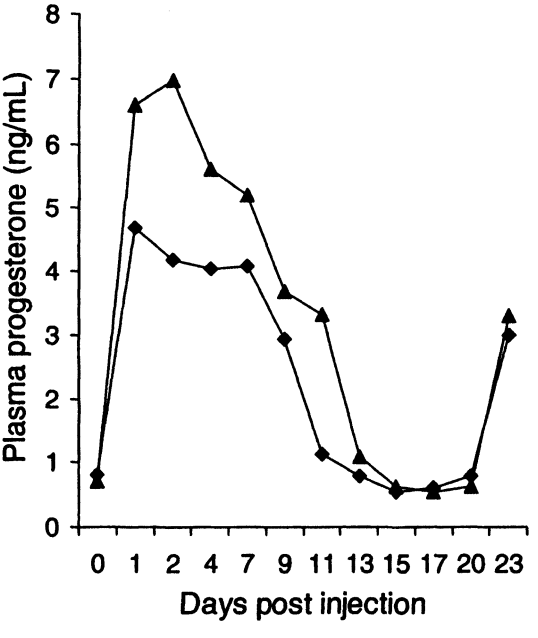

Fig. 11. Plasma progesterone levels in treated cows following administration of two different biodegradable microsphere formulations containing progesterone.

surgery to remove the implants to produce a precipitous decline in progestagen levels, has resulted in this approach having limited practical application.

VI.B.3.b. Subcutaneous implants (ear) A small ear implant (SYNCRO-MATE-B ear implant; Figure 12) has been commercialized for use in cattle and numerous authors have investigated the norgestomet impregnated Hydron polymer ear implant in cattle (see Refs. 1–3 for extensive lists of references). The SYNCRO-MATE-B ear implant contains 6 mg norgestomet homogeneously distributed throughout a polymer called Hydron. The implant measures approximately 3 × 18 mm and weighs approximately 0.125 g. They come encased within an open ended protective plastic sheath and are supplied in sealed foil packaging. The ear implant is part of a treatment program which includes an intramuscular injection comprising 2.0 ml sesame oil containing 10% benzyl alcohol, 5 mg estradiol valerate and 3 mg norgestomet.

The release of norgestomet from the SYNCRO-MATE-B ear implant follows a declining release profile (i.e. square-root-of-time dependence).[77,78] Chien developed an ear implant which utilized the microsealed drug delivery (MDD) technology which released drug according to a zero-order release pattern.[79] The ear implant was manufactured by dispersing crystalline norgestomet in microreservoirs of aqueous PEG 400 throughout a matrix of polymerized silicone. The resulting solid was then cut into cylindrical rods.[80] A variety of implants were manufactured which were designed to release norgestomet at different amounts per day. The norgestomet-containing MDD implants were shown to release in vivo at a constant rate for up to 20 days in both heifers and cattle.[79] Chien demonstrated that the biological effectiveness of a MDD implant which delivered 176.8 μg/day compared favorably with the commercially available SYNCRO-MATE-B product.[79]

The Crestar ear implant (Figure 13) is another commercially available ear implant. It contains 3 mg norgestomet dispersed throughout a silicone matrix. It is marketed with a 2 ml injection comprising 3 mg norgestomet and 5 mg estradiol valerate.[81–87]

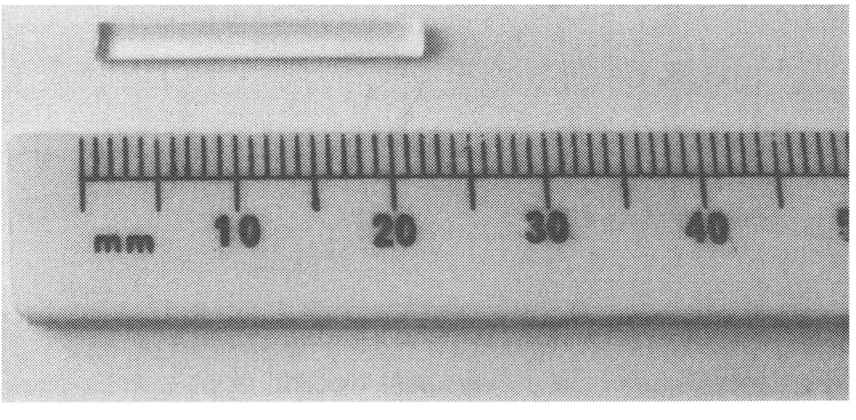

Fig. 12. Commercially available SYNCRO-MATE-B ear implant.

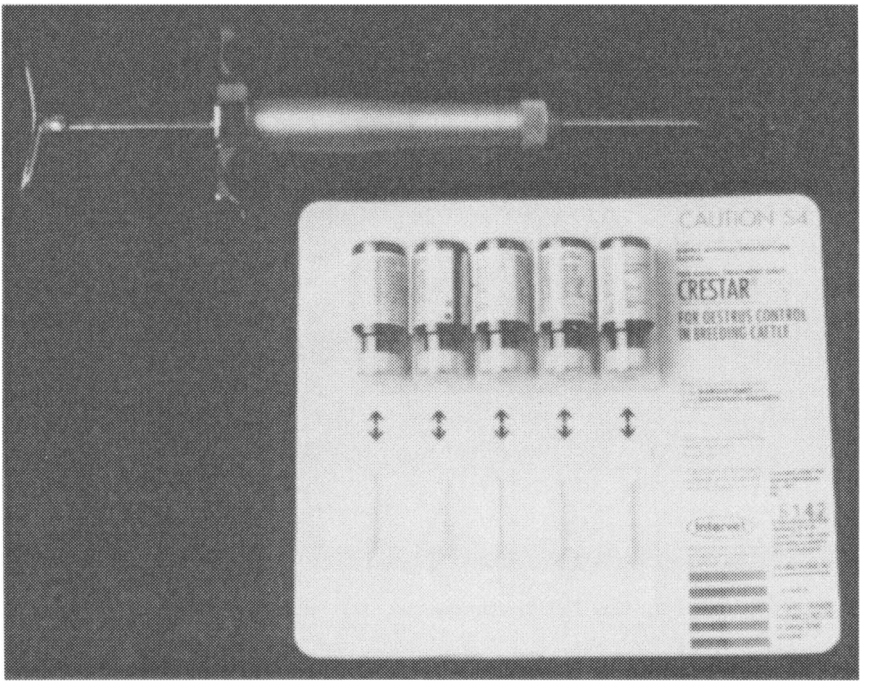

Fig. 13. Commercially available Crestar ear implant showing silicone implants, supplementary estradiol injection and implanter.

VI.C. Pigs

VI.C.1. Intravaginal estrous control products for pigs

A silicone/progesterone based intravaginal product for use in gilts and sows has been described.[4] The device contained 0.9 or 1.4 g progesterone dispersed throughout a silicone rubber matrix molded over a nylon spine. The device was similar in shape to a CIDR-B, i.e. its basic shape was in the form of a T (Figure 14); however, its body was more elongated and its terminal end was fabricated into a collapsible diamond shape to aid retention. Initial promising results were reported,[4] however further development of the intravaginal device for cycle control in pigs has not been forthcoming.

Recently an improved silicone/progesterone intravaginal insert has been developed to control estrus and ovulation in gilts.[5,6] The insert (Figure 15) was trialed in intact gilts. Retention rate for this product was 96% over the 14 day insertion period. None of the treated gilts showed signs of cystic follicles, indeed, the animals showed a significant increase in the number of corpora lutea and a trend toward increased numbers of live embryos compared to control animals.[5,6] Ninety-five percent of the gilts showed estrus 3–6 days after insert removal. The product has also been inserted into ovariectomized gilts (Figure 16).

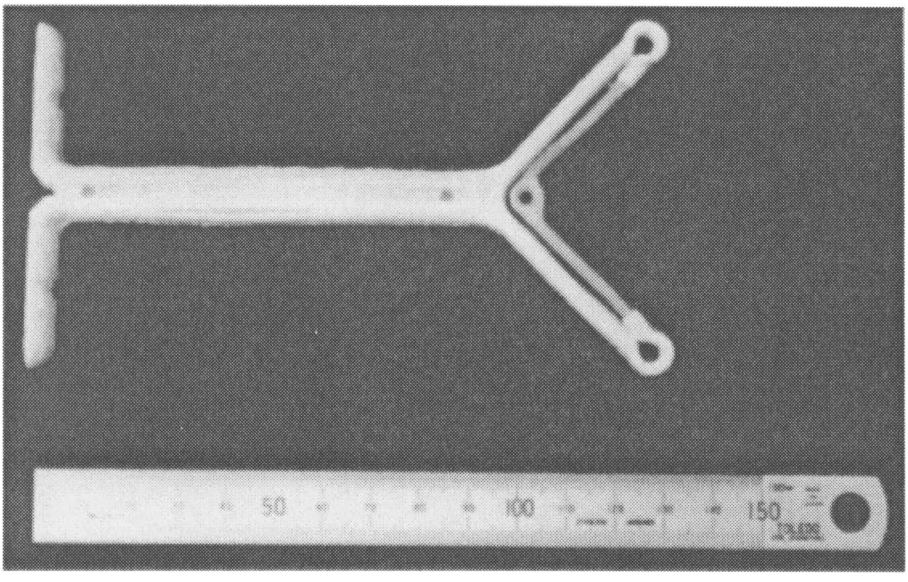

Fig. 14. CIDR-P silicone/progesterone intravaginal insert for gilts.

VI.C.2. Intramuscular estrous control products for pigs

VI.C.2.a. SRE2 SRE2 is a cycle controlling product for use in cyclic gilts which involves a single intramuscular injection of estradiol formulated into biodegradable microspheres to induce pseudopregnancy followed by $PGF_{2\alpha}$.[7] The rationale for the SRE2 treatment regime is depicted in Figure 17.

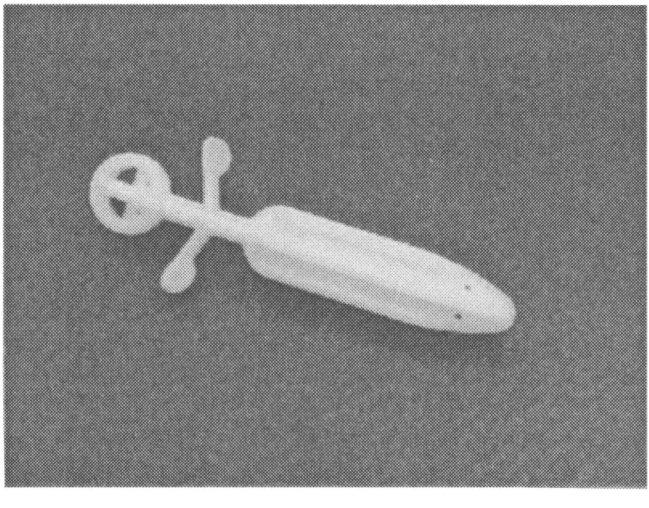

Fig. 15. Silicone/progesterone intravaginal insert for gilts.

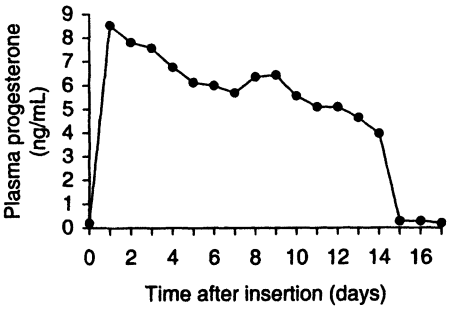

Fig. 16. Plasma progesterone levels in treated ovariectomized gilts following administration of a silicone/progesterone intravaginal insert. From Ref. 6

Initial results were promising. Plasma concentrations of estradiol became elevated following administration of the microsphere product, and peak heights were shown to be dependent upon the initial dose of estradiol (Figure 18).[7] In addition, 86% of PG 600 induced diestrous gilts given SRE2 at doses between 25 and 100 mg were pseudopregnant on day 59 (Figure 19) and 84% of those pseudopregnant gilts responded to $PGF_{2\alpha}$ (Figure 20).[7]

VI.C.2.b. SABER delivery system Recently a SABER Mate P (Porcine) has been developed.[88] It is a short acting controlled release deslorelin acetate/SAIB formulation for stimulation of ovulation within 40 h in estrus gilts and sows.[88]

VI.C.3. Subcutaneous estrous control products for pigs

Osmotic pumps (ALZET 2 ml osmotic mini pumps) have been investigated in a small number of cycling gilts weighing approximately 130 kg.[9] Six osmotic pumps were subcutaneously implanted for 7 days in gilts in the late luteal phase of their estrous cycle. The combined delivery of progesterone from the pumps was estimated to be 115 mg/day. Plasma progesterone levels greater than 10 ng/ml were observed over the insertion period. During insertion follicular growth was suppressed which in turn

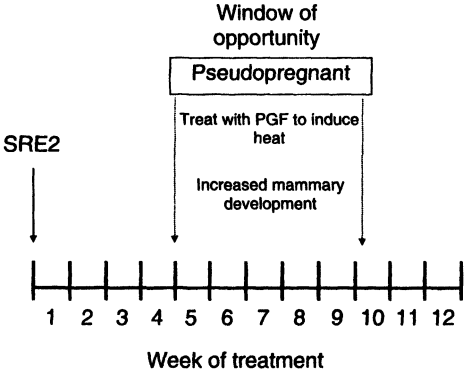

Fig. 17. SRE2 treatment regime time scale picture showing window of opportunity.

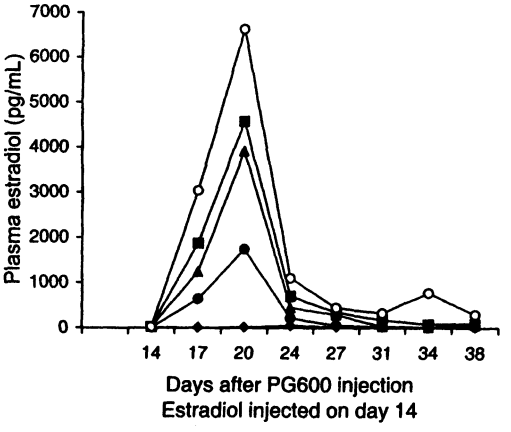

Fig. 18. Plasma estradiol profiles following administration of microsphere preparations containing 0 (◆), 12.5 (●), 25 (▲), 50 (■) and 100 mg (○) of estradiol. From Ref. 7

prevented the gilts from exhibiting estrus, i.e. effectively the implants extended the estrous cycle for 7 days. Removal of the pumps resulted in normal preovulatory endocrine changes. Follicular cysts were observed in 1/5 gilts.[9]

VI.D. Sheep

VI.D.1. Intravaginal estrous control products for sheep

Intravaginal sponges containing various amounts of fluorogestone acetate have been extensively investigated in sheep (see Refs. 1,2 for an extensive list of published

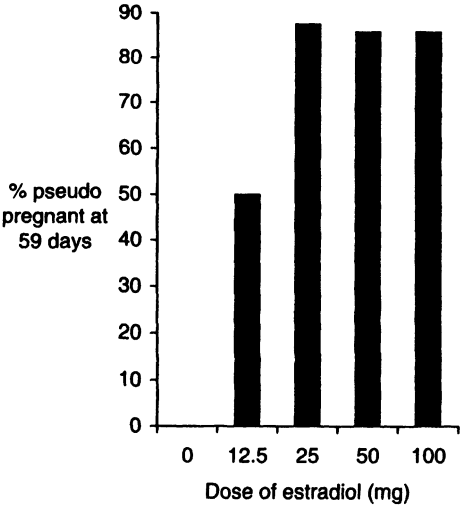

Fig. 19. Percentage of gilts pseudopregnant at day 59 following administration of microsphere preparations containing 0, 12.5, 25, 50 and 100 mg of estradiol. Data from Ref. 7

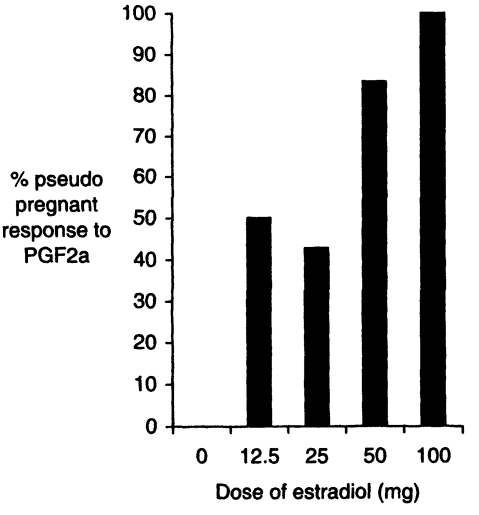

Fig. 20. Percentage of pseudopregnant gilts responding to PGF2α at day 59. Data from Ref. 7

work). In the early 1960s Robinson and colleagues pioneered the use of intravaginal sponges impregnated with a progestagen for synchronization of estrus of sheep.[89–91] In the 1980s it was realized that the amount of drug being released from the sponge was in excess of that being absorbed, and Chien and co-workers investigated this problem. Chien demonstrated that the release of fluorogestone acetate from sponges followed a square-root-of-time relationship and a new sponge was developed which released drug with a zero-order release profile. The new sponge design utilized an unloaded sponge as a carrier for a silicone based drug reservoir.[92–94] Chien characterized both the in vitro and in vivo pharmaceutical properties of various designs and identified the formulation factors that affected release. These factors were then manipulated to develop a delivery system which delivered sufficient fluorogestone acetate to control the estrous cycle in sheep.

The CIDR-S was rabbit eared in shape and contained progesterone homogeneously dispersed throughout a silicone rubber skin which was cured over a nylon spine.[1,2,95] The CIDR-S was superseded by the CIDR-G which is essentially a smaller version of the CIDR-B, i.e. a T-shaped intravaginal device comprising of a pre-formed annealed nylon spine which is coated with silicone impregnated with progesterone.[1,2,96] The device has a filament of flexible nylon pre-formed onto the spine which aids its removal from the animal. The CIDR-G has a slimmer profile than the CIDR-S and was therefore much easier to insert and permitted its use in lambs, goats and ewes, all of which have smaller vaginas.

A C-shaped plasthyd device for use in sheep and containing 210 mg of progesterone dispersed throughout a solid polymer referred to as 'plasthyd' was recently described by Mandiki et al.[97] The device was 'C'-shaped and specifically designed to reduce the excessive vaginal secretions often observed following insertion of the commercially available progestagen sponges.[97]

Recently poly (ε-caprolactone) was investigated as a biodegradable polymer suitable for fabrication into an intravaginal insert containing progesterone for control of the estrous cycle in sheep.[98] Poly (ε-caprolactone) inserts were manufactured and inserted into the vagina of anestrus sheep for 14 days. The plasma profiles obtained following insertion of the poly (ε-caprolactone) inserts (Figure 21) mimicked those observed following insertion of the commercially available silicone product used in sheep, the CIDR-G.

The retention rate of the poly (ε-caprolactone) inserts was excellent over the 14 day treatment period, no vaginal damage was observed due to the presence of any inserts over the course of treatment and discharge was minimal. Further studies showed that plasma levels were affected by both surface area and drug load.[98]

VI.D.2. Subcutaneous estrous control products for sheep

VI.D.2.a. Sil-Estrus A subcutaneous implant measuring 0.9 cm in diameter and 5.0 cm in length comprising a solid silicone rod containing 375 mg of progesterone (11.0% w/w initial load) has been reported in the literature.[99–106] The implant was named Sil-Estrus and was manufactured by Abbott Laboratories, IL, USA (Figure 22). The literature is sparse regarding information on the method of manufacture of the implant and there is little documented information on the formulation of the delivery system. Administration of the implant was reported to result in precise synchronization of estrus.[99,101] However, fertility of the first estrus following treatment was observed to be depressed compared with untreated ewes.[99] In addition, the implant was generally awkward to use,[101] the implantation process was slow since it required the sheep to be handled with much more effort, compared to the sponge the need for aseptic precautions was greater and field tests revealed resistance by farmers to the minor surgery required for implantation and removal.[105]

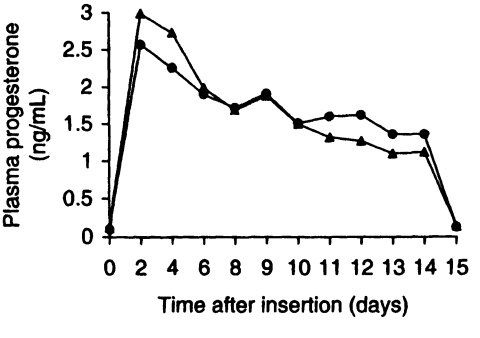

Fig. 21. Plasma progesterone levels in treated sheep following administration of CIDR-G containing 9% w/w progesterone (●) and poly (ε-caprolactone) intravaginal inserts containing 9% w/w progesterone (▲). From Ref. 98.

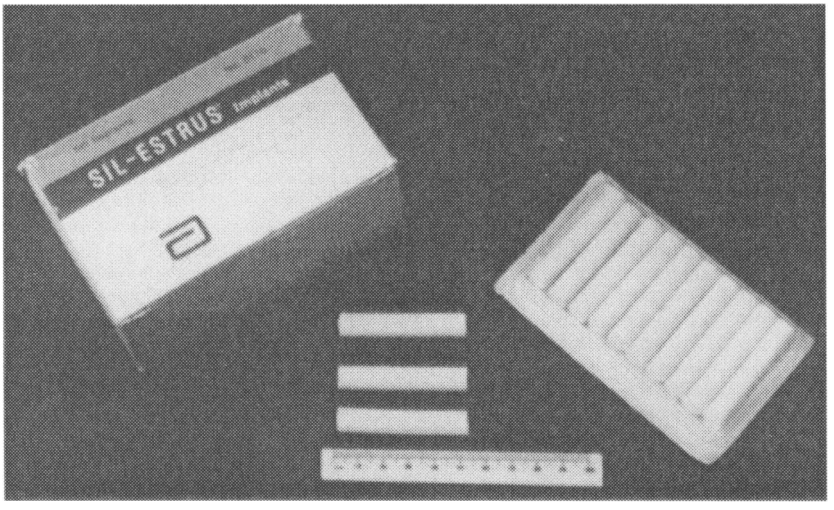

Fig. 22. Sil-Estrus subcutaneous implant used in sheep.

VII. Conclusions

This chapter has demonstrated that the opportunity to develop new, efficient and cost effective methods for the control of estrus and ovulation in domesticated livestock now exists with advanced controlled release systems which utilize the latest polymers, manufacturing processes and controlled release technologies. It is clear that biodegradable drug delivery systems have begun to make their presence known in the area of estrus and ovulation control. However, concerns over cost will limit success to products that can offer the end user reduced production cost and enhanced profitability.

In nature reproduction has evolved so that in general animals breed annually and that parturition occurs in the spring when environmental conditions such as temperature and rainfall are favorable for survival of the young and food for the mother is abundant so that adequate lactation is ensured.[8] However, effective ovulation control in the future will be strongly influenced by the availability of controlled release drug delivery technologies which are now available or currently in development.

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CHAPTER 8

Controlled release products for control of ectoparasites of livestock☆

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Overview

I. Introduction

Within the larger domain of controlled release drug delivery systems, applications in veterinary medicine are a relatively small portion. Smaller yet is the part of controlled release delivery systems in veterinary medicine devoted to the products for the control of ectoparasites of livestock. If controlled release products in the field of veterinary medicine can be described as growing, then those products specifically aimed at control of ectoparasites would best be described as emerging. It is an area often ignored by even those interested in veterinary applications probably because of the perceived lack of market or profit potential.

A variety of insects, ticks, and mites affect the well-being and productivity of livestock. These pests can cause loss of blood, increase susceptibility to disease and infection, transmit diseases, and by their general annoyance and irritation can interfere with the feeding and resting patterns of the host. Direct results of this parasitism are reduced efficiency of feed conversion, reduced weight gains, decreased milk produc-

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tion and decreased wool and mohair production. In some cases, damage inflicted by ectoparasites decreases the value of hides and carcasses. While loss estimates on a world-wide scale are not available, Cardinal and Witchey-Lakshmanan[1] reported that ectoparasiticides used for control of these pests represented US$416 million of a total US$6.6 billion world animal health product market in 1986. Therefore it is safe to assume that the economic damage far exceeds that expended for control. In the US, economic damage to the livestock producer is estimated in excess of US$3 billion annually. The largest portion, over US$2 billion annually, is suffered by the beef cattle industry. Kunz et al.[2] presented a list of the more important pests and the estimated losses associated with each (Table 1). These include horn flies, stable flies, horse flies, mosquitoes, face flies, cattle grubs, lice, scabies and ticks. The major pests responsible for these losses are biting flies, primarily the horn fly, *Haematobia irritans* (L.) and stable fly, *Stomoxys calcitrans* (L.). Estimates of losses due to the horn fly exceed US$875 million a year while stable flies cause losses estimated at US$430 million annually. While losses individually attributed to the other pests are considerably less than these two major pests, they are nevertheless significant at an estimated combined cost to the producer of US$880 million annually. Insecticides, labor, and equipment to control these parasites represent a major cost to the producer. An estimated 4.5–5.5 million kg of insecticides are applied annually to livestock at a cost of US$60 million for the pesticides alone. Although figures are not available on the cost of labor, equipment and debilitation to animals, these probably exceed the cost of the pesticide.

Drummond et al.[3] and Kwan[4] presented comprehensive reviews of the history and current conventional methods of control of arthropod pests of livestock. The control of these livestock ectoparasites is almost solely dependent on the use of conventional pesticides from the organophosphorous, pyrethroid, carbamate and avermectin groups.

Table 1
Potential losses due to insect and tick pests of cattle in the US[a]

Pest type	Cattle	Loss (US$ million)	Total loss (US$ million)
Horn fly	Calves	264	
	Stockers	612	876
Stable fly	Feedlot	155	
	Dairy	277	432
Horse fly	Stockers	79	
	Calves	63	
	Dairy	48	190
Mosquitoes	Stockers	19	
	Calves	31	50
Face fly	Stockers	69	
	Calves	54	123
Cattle grubs	Slaughter	56	56
Lice	Stockers and calves	38	38
Scabies	Feedlot	259	259
Gulf coast ticks	Cattle/calves	75	
Lone star ticks	Calves	16	
	Stockers	13	104
Total estimated losses	All cattle		2128

[a] Adapted from Ref. 2

These are applied primarily as sprays, dust, pour-ons, or as insecticidal ear tags. Adequate insecticides have been available for ectoparasite control for over 40 years. However, when applied as sprays, dusts or pour-ons, the chemicals are effective for short periods and must be frequently applied to achieve adequate control.

Despite the inherent environmental problems of chemical control and the inevitable problem of resistance, pesticides will remain a significant part of the producer's defense against these pests either alone or as part of an integrated pest management strategy. Therefore we are motivated to continue to develop means of using these pesticides in a judicious and safe manner. Novel and improved delivery systems provide a means of minimizing the quantity of chemical needed for control of the pests, reducing the labor involved in gathering and treating cattle at frequent intervals, and reducing the environmental hazards associated with chemical control.

The purpose of this chapter is to review various controlled release delivery systems that have been developed or are under development for the control of livestock ectoparasites. No attempt will be made to review the innovative developments of recent years for controlled release delivery of anthelmintics except where those devices have application for ectoparasite control as well as for control of endoparasites. This omission is dictated by the authors lack of expertise in the area of control of internal parasites and by the availability of excellent reviews by Cardinal[5] and Cardinal and Witchey-Lakshmanan.[1] Moreover, the focus of this discussion will be on the products, their use and their limitations with only limited detail as to how the various controlled delivery systems work. Such descriptions are adequately covered in the literature that is referenced herein.

II. Back rubbers, insecticidal dust bags, eartags, neckbands and tailtags

It could be argued that cattle back rubbers and insecticidal dust bags represent the first controlled release applications for livestock pests. Back rubbers are fabricated from absorptive materials either stuffed into a fabric sleeve or wrapped with burlap. They are treated periodically with an oily solution of pesticide and hung in a location and in such a manner that cattle can walk beneath the device and treat themselves as it rubs against the back and sides of the animal. Similarly, when insecticidal dust is placed into porous bags, cattle are dusted as they walk beneath the suspended bags which rub against the head and backline. In each of these cases, a small quantity of pesticide was delivered at frequent intervals. Moreover, the cattle learned to use the devices in response to the irritation caused by the pests.

Notwithstanding the above argument, the introduction of the cattle insecticidal ear tag represents a major innovation in the control of ectoparasites of livestock. In their pioneering work in late 1960s, Harvey and Brethour[6] attached dichlorvos-impregnated polyvinyl chloride strips to collars or ear tags of cattle. They found that sustained-release of the pesticide from the polymer controlled horn flies on cattle for 1 week to 1 month. Although of rather limited success, this research led others to explore the potential of various external attachments. A series of studies was conducted to deter-

mine which type of attachments might work best on cattle and to explore more effective pesticides. To determine which areas of the cow's body might be treated and the extent of material transfer as a result of various types of attachments, a rather simple study was conducted.[7] Felt pads containing a dye were attached to halters, ear tags, neckbands, leg bands and tail tags which were applied to white cows. Each device provided a unique pattern of distribution of the dye to the animals' hair coat as a result of the behavior of the cattle (Figure 1). Although tail tags provided the greatest coverage with a distribution of material over the rear half of the animal, tail tags were more difficult to attach and retain on the tail switch. Ear tags were judged more acceptable because of producer familiarity with the numbered identification tags.

In the mid-1970s, commercially produced insecticidal ear tags containing stirofos, an organophosphorous compound, were introduced to the market (Figure 2). These were originally developed for control of the Gulf Coast tick, *Amblyomma maculatum.*[8] The tag ultimately found its greatest use in the control of the horn fly, a much more serious pest of cattle. The application of a 10 g tag containing 1 g stirofos in each ear controlled horn flies on cattle for 12–14 weeks.[9] Subsequently, ear tags were developed to deliver pyrethroids such as permethrin, fenvalerate, cyhalothrin, cyfluthrin and cypermethrin. These tags were generally capable of controlling susceptible horn flies on cattle for up to 5 months. With the exception of the Gulf Coast and in South Texas regions, producers could apply the pyrethroid ear tags once in the spring and obtain season-long control. Therefore the insecticidal ear tag provided a convenient and economical tool for control. Since that time, several tags containing organophosphorous compounds such as diazinon, pirimophos methyl, fenthion and ethion have been marketed in an effort to combat the resistance problem encountered with pyrethroids. In addition, a few tags with a combination of a pyrethroid and an orgaonophosphorous have been developed. There are approximately half a dozen major manufacturers of

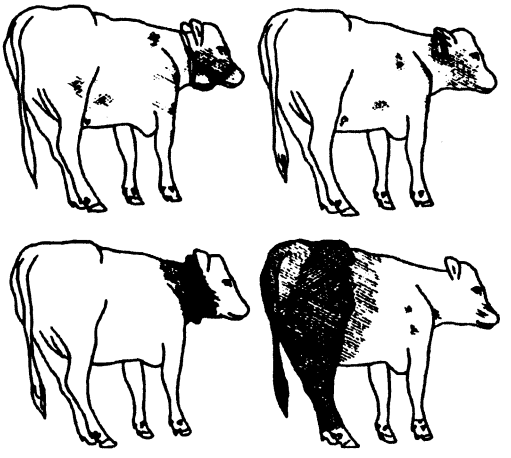

Fig. 1. Pattern of dye transfer to the hair coat of cattle (clockwise from upper left) resulting from halter, eartag, tailtag, or neckband delivery system.

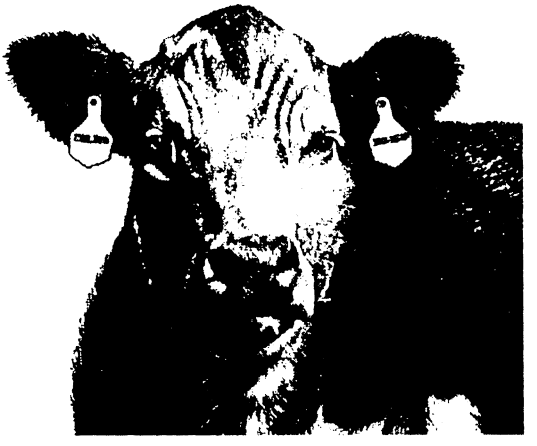

Fig. 2. Cattle can be fitted with insecticidal ear tags for control of horn flies, face flies and ear ticks.

insecticidal tags (Table 2). Each manufacturer has its own tag shape and color for easy identification. Shapes range from triangles, bells, rectangles and hexagons and colors across the spectra from purple to red. However, the tags were basically alike in that the pesticide was incorporated into a plasticized polyvinyl chloride matrix and usually comprised 8-40% active ingredient.

The pesticide diffuses to the surface of the tag where it is rubbed onto the hair coat of the animal and contacted by the parasite. In the case of the horn fly, it is not necessary that the pesticide be uniformly distributed over the body of the cow. The fly moves over various areas of the animal in response to sun, shade, temperature and animal defensive behaviors and therefore comes in contact with the treated area. Indeed, insecticidal ear tags work well for horn flies because of the flies movement pattern on the cattle. The ear tag works well for the Gulf Coast tick because their preferred feeding site is the ear. However, ear tags do not control other tick species such as lone star ticks, *Amblyomma americanum* (L.). This is because these ticks tend to move to and attach to the neck and escutheon areas. In such cases, part of the control strategy should be to match the behavior of the pest with the unique distribution of the attachment so that the pesticide will be applied to areas which are sure to be contacted by the pest.

The release rate of these monolithic tags containing pyrethroids have been shown to conform to the Baker and Lonsdale model based on Fick's law of diffusion.[10] Figure 3 shows the release rate for a 10% permethrin tag and an 8% fenvalerate tag under normal use on cattle. The minimum effective delivery rate for control of horn flies on cattle was determined to be 1.9 mg/day for the permethrin tag and 1.0 mg/day for the fenvalerate tag.[11] The tags tend to lose their effectiveness when 50% of the insecticide has been released.

Several other controlled release systems have been explored for use as insecticidal ear tags. A membrane system developed by Bend Research and Conseps Membranes has been applied to ear tags.[12] Essentially, an insecticide reservoir, one side of which is

Table 2
Available insecticidal ear tags

Class of insecticide	Active ingredient	Trade name (manufacturer)
Pyrethroids	Permethrin	Atroban (Mallinckrodt Vet), Atroban Extra (Mallinckrodt Vet), Deckem (Fearing), Ear Froce (Anchor), Expar (Mallinckrodt Vet), Gard Star Plus (Y-Tex), Perma-Tect (Farnam), Z Permethrin (Farnam)
	Fenvalerate	Ectrin (Boehringer Ingelheim), Super Deckem (Fearing)
	Cyfluthrin	Cutter Gold (Bayer)
	L-Cyhalothrin	Excalibur (Mallinckrodt Vet), Saber Extra (Mallinckrodt Vet), Saber (Mallinckrodt Vet)
	Z-Cypermethrin	Python (Y-Tex), Zeta Gard (Y-Tex)
Organophosphate	Diazinon	Terminator (Anchor), X-Terminator (Fearing), Patriot (Boehringer Ingelheim), Optimizer (Y-Tex), Optimizer Calf (Y-Tex), Bovagard (Y-Tex), Cutter 1 (Bayer), Z Diazinon (Farnam)
	Ethion	Commando (Boehringer Ingelheim)
	Fenthion	Cutter Blue (Bayer)
	Pirimiphos-methyl	Dominator (Mallinckrodt Vet), Rotator (Mallinckrodt Vet), Tomahawk (Mallinckrodt Vet)
	Stirofos	Rabon (Boehringer Ingelheim)
Combination	Permethrin + chlorpyrifos	Ear Force Ranger (Anchor)
	Cypermethrin + chlorpyrifos	Max-Con (Y-Tex)
	L-Cyhalothrin + pirimiphos-methyl	Double Barrell (Mallinckrodt Vet)
	Diazinon + chlorpyrifos	Diaphos Rx (Y-Tex), Warrior (Y-Tex)

a rate controlling membrane, is formed on the surface of an ear tag. The release rate is controlled by the permeability, surface area and thickness of the membrane. Unlike the monolithic tags, this configuration gives near constant release of active.

The research group at Knipling-Bushland US Livestock Insects Research Laboratory studied a trilaminate system in which an insecticide impregnated core was

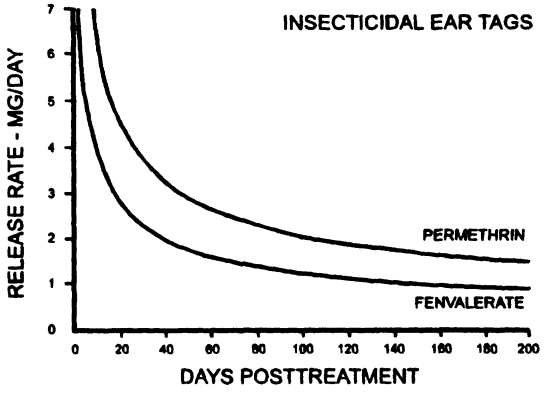

Fig. 3. Release rate from permethrin and fenvalerate ear tags on cattle held on pasture.

sandwiched between two rate controlling membranes. Such a configuration was shown in field trials on cattle to provide near zero-order release until approximately 70% of the load is dispensed (unpublished data). Such systems loaded with pyrethroids have been successfully field tested on cattle against horn flies.

Both monolithic and reservoir neckband systems have been used for controlled delivery of insecticides to cattle. These are similar to the tick and flea collars marketed for pets. One of the important advantages of a neckband is the ability to carry greater loads of active agent. The ear tag is limited to an approximate total weight of 17 g; if it weighs greater than 17 g then it enlarges the hole in the ear and is eventually lost. In contrast, a neckband can be as heavy as 100–200 g without difficulty. Neckbands containing amitraz, a potent acaricide, in an ethyl vinyl acetate polymer have been shown to be effective against ticks on range cattle.[13] Using thin-walled PVC tubing as a reservoir, a neckband for cattle can deliver organophosphates at rates of 20–25 mg/day.[14] The variety of materials available for fabrication of reservoirs means that release can be tailored to practically any need.

Tail tags as a delivery system have not received much attention but appear to have potential. Tail tags have been used effectively for both control of horn flies and lone star ticks on cattle.[13] Cattle switch their tails as part of their defense against the horn fly and therefore the tail is a logical insecticide applicator. One of the main difficulties is the development of a system for retention of the tag in the tail switch. The combination of a neckband and a tail tag have been used successfully for the control of lone star ticks on cattle.[13] With this particular species, 80% of the ticks attach in the area of the neck, dewlap and brisket area and in the escutcheon area from the tail head to the udder of cattle. Therefore controlled release devices that impact those areas can provide good tick control.

The Morgan Recharger is an interesting device that can be used either as an ear tag or a tail tag.[15] The one piece, hinged unit is molded of polypropylene and contains a reservoir for repeated loading with pesticide. The reservoir, filled with cotton packing, has a capacity for 7 ml of liquid pesticide. Small diameter nylon and rayon cords protrude from one end of the loading chamber for wicking pesticide from the chamber. It is interesting to note that when fully loaded, the device has a weight of approximately 20 g which exceeds the generally acceptable limit for ear tag weight. The device provided up to 8 weeks control of horn flies when applied as two ear tags loaded with 5 ml of 20% diazinon. When used as a tail tag, the device was less effective and difficulties were encountered with its retention on the tail switch of cattle. These devices have been less effective in control and less efficient in pesticide usage than monolithic PVC tags.

III. Controlled release oral dosage forms

Drenches, gels, pastes, tablets, capsules, boluses, medicated feeds or water have all been used for oral delivery of therapeutics to livestock. Anthelmintics, antibiotics, minerals and trace elements are among the most commonly delivered actives by these oral dosage forms. Because some ectoparasites such as the horn fly and the face fly

breed in the manure of cattle, researchers have considered oral treatment as a practical means of control of the immature stages. Miller and Miller[16] presented a thorough review of the history of this approach dating back to the 1920s. Because of the need for larger dosages of earlier compounds, most of the effort was on developing medicated feed, water and mineral products. For example, in 1967, only three compounds, phenothiazine, ronnel and coumaphos were registered for use as feed-through chemicals for fly control in cattle. These larvicides, and the addition of stirofos a few years later, were effective against horn flies and face files in manure when given to cattle at 1–6 mg/kg body weight per day. It was not until the mid-1970s that a new group of compounds known as insect growth regulators (IGR) became available.[17,18] The IGRs such as methoprene and diflubenzuron were effective in inhibiting development of the immature stages of horn flies and face flies in manure of treated cattle at much lower dosages. Moreover, they were capable of passing through the digestive tract and reaching the manure in efficacious quantities. In addition, they are relatively safe because of the lack of residues in meat and milk. These developments and the subsequent availability of other highly effective control agents provided the opportunity for the development of controlled release oral dosage forms.

The unique digestive system of ruminants (e.g. cattle, sheep and goats) provides interesting possibilities for the use of delivery systems. The stomach of ruminants consist of four compartments. Each of these compartments, the rumen, the reticulum, the omasum and the abomasum, has its own unique physical, chemical and microbial characteristics designed to enable digestion of cellulosic foodstuff. The rumen is the largest compartment and, in cattle, can have a volume of 50–60 l. In these compartments the food is digested by microorganisms before continuing through the digestive tract. Because microbial degradation is most efficient when the particle size is small, an interesting system to reduce particle size has evolved in ruminants whereby foodstuff is chewed, swallowed, regurgitated and chewed again. These features and processes offer unique challenges to the formulation scientist.

The large rumino-reticular compartment provides a unique repository for controlled release devices. However, a primary problem is that of retention of a device in the desired compartment. Retention of a device in the desired compartment can be accomplished either by density or by shape. Research has shown that a specific gravity of at least 1.6 is required for retention in the rumen and 2.0 for retention in the reticulum.[19] Using shape as a method of retention means that the minimum diameter of the device in the rumino-reticular compartment must be significantly greater than the diameter of the reticular-omasal orifice or the esophagus.

One of the simplest approaches is to develop a high density composition that slowly erodes in the rumen or reticulum. This approach may have originated with the development of the so called 'cobalt bullet'[20] to provide a trace element supplement to livestock in cobalt deficient areas. The cobalt-oxide pellet, placed in the reticulum, has had a significant economic impact on the grazing industry in Australia. Similar devices were subsequently made available for delivery of other trace elements such as selenium, copper, zinc and magnesium. Of course the density of these heavy metal boluses made retention a minor problem.

A similar concept was developed to deliver IGRs to cattle for control of dung

breeding flies such as the horn fly and the face fly. Boluses were fabricated using a blend of monostearin, carnuba wax, and the IGR with barium sulfate as the weighting agent to increase the specific gravity to greater than 2.0.[21] These boluses lodge in the reticulum where they slowly erode, releasing the active agent into the digestive tract and ultimately into the manure. The presence of the IGR in the manure dropping of cattle prevents the development of the immature stages of horn flies and face flies. Two erodible boluses using this concept have been marketed, the Vigilante® by American Cyanamid and the Inhibitor® by Zoecon (Figure 4). The Vigilante bolus contains a chitin inhibitor, diflubenzuron, and the Inhibitor contains a juvenile hormone mimic, methoprene. These boluses provide 10–16 weeks control of horn fly and face fly development in the manure of treated cattle.[22,23] Formulations can be developed that are active for 24–30 weeks. As might be expected, release from these systems is not zero-order or uniform. As the bolus erodes, surface area decreases resulting in a decline in the rate of delivery over time. For this particular application, maintaining a low cost of treatment is more important than uniformity of delivery.

Similar erodible boluses have been used for delivery of famphur for systemic control of Gulf Coast ticks and lone star ticks feeding on cattle.[24] In addition, the concept has been tested for delivery of stirofos to cattle for control of face flies.[25] However, in both of these studies, the large dosage required for effectiveness proved a limiting factor.

Zero-order or near zero-order delivery can be achieved with a bolus through the use of devices such as the Paratect® bolus,[26] the Paratect Flex® bolus[27] and the Rumisert® bolus (push-melt osmotic pump).[28] Although the Paratect bolus and the Rumisert bolus offer the advantage of zero-order delivery, they have the disadvantage of being considerably more expensive and the spent devices remain permanently in the rumen. Such remnants can pose a serious problem to equipment in conventional slaughter operations where rumen content is often processed through grinding equip-

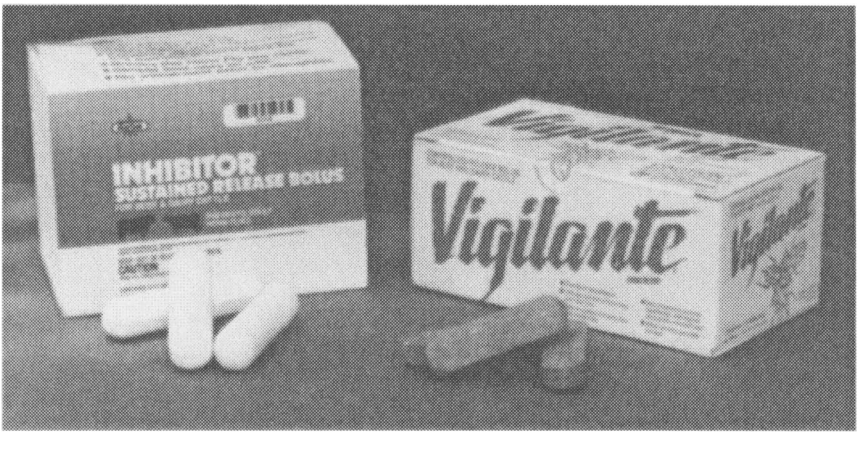

Fig. 4. Erodible boluses for delivery of insect growth regulators, methoprene (Inhibitor) and diflubenzuron (Vigilante) to the rumen of cattle.

ment. Although any of these devices could be used to deliver agents for the control of ectoparasites in livestock, only the Rumisert bolus has been used for this purpose.

The Rumisert bolus technology was developed by Alza Corporation and Merck Animal Health and is currently marketed as the IVOMEC® SR Bolus for delivery of ivermectin to cattle for control of both endo- and ectoparasites (Figure 5). The bolus uses the push-melt osmotic pump concept and consists of four basic components. An osmotic driving tablet composed of a salt and a swelling hydrogel is located at the base of the bolus. The drug containing vehicle is in the center of the bolus. To increase the density, a metal element with an exit orifice is placed at the top end. These three elements are encased in a semipermeable, rigid capsule. In the rumen, water permeates the capsule wall causing the salt-hydrogel osmotic element to swell and force the drug and vehicle through the exit orifice and into the rumeno-reticular compartment.

A prototype of this osmotic bolus was shown to deliver 6–9 mg/day for 110–120 days in both in vitro and in vivo trials.[28] Several studies have demonstrated the efficacy of the bolus against various tick species, including *Ixodes* spp., *Boophilus* spp., *Hyalomma* spp., *Rhipicephalus* spp., and *Amblyomma* spp.[29,30] In a recent trial in South Texas, a single IVOMEC® SR Bolus provided <30% control of the cattle tick, *Boophilus annulatus*, on pastured heifers weighing approximately 200 kg. Two boluses per heifer were required to provide complete control of the tick (Miller, unpublished data). A single bolus, which contains 1.72 g of ivermectin, produced levels of 6–8 ppb in the serum of the treated heifers for approximately 16 weeks (Figure 6). Two boluses per heifer resulted in 16–20 ppb ivermectin in the serum. The levels declined after 16 weeks to <2 ppb at 20–21 weeks post-administration.

The use of shape for retention in the rumen is an interesting concept being pursued

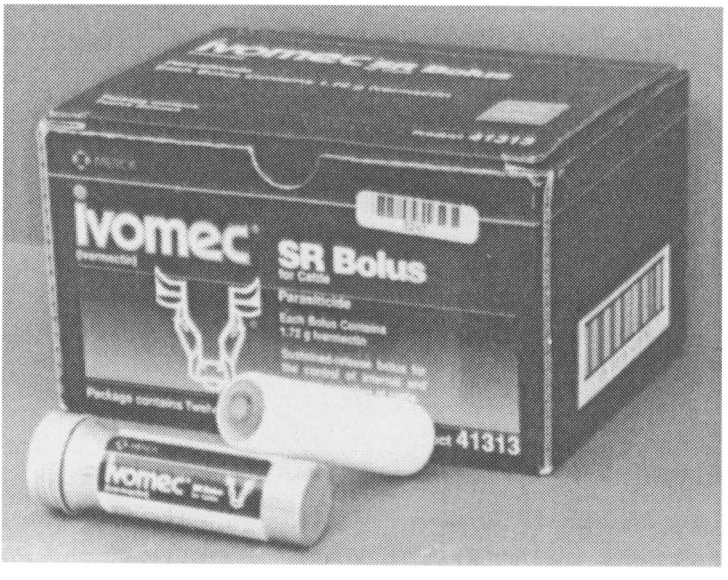

Fig. 5. Ivermectin is delivered to the rumen of cattle using the IVOMEC® SR bolus, a push-melt osmotic pump.

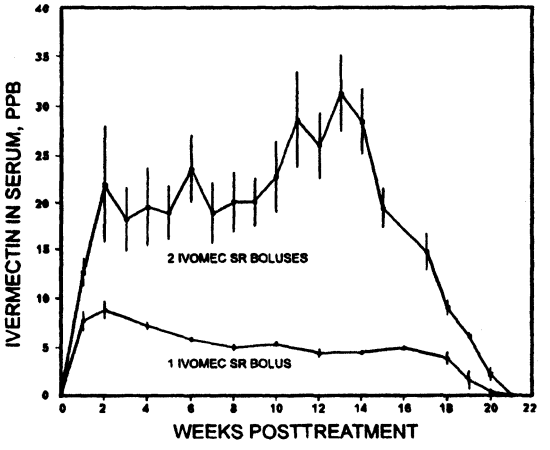

Fig. 6. Concentration of ivermectin in serum of cattle treated either with one or two IVOMEC® SR boluses. Cattle were held on pasture in South Texas.

both in the US and Australia[31,32] and would certainly have application for delivery of larvicides and IGRs.

Trilaminate systems,[33] developed several years ago for zero-order delivery of drugs including pesticides and pheromones in residential, forest and crop applications, are finding application in the veterinary area. The trilaminate has a polymer core containing the drug sandwiched between two rate controlling films. In one configuration, the rate controlling films are permeable allowing the drug to move from the core to the surface. The mechanism of delivery is very much like that of a reservoir system. A second configuration uses impermeable barriers on either side of the drug loaded core. Holes through all three layers provide the release path. Rate of release can be controlled by the number and diameter of holes in the system and the solubility of the active agent.[27] To administer the product the trilaminate is tightly rolled and held in a cylindrical shape by water soluble tape. Upon arrival in the rumen, it opens to a slab configuration and is retained by virtue of its size and shape. While this technology is currently being used for delivery of an anthelmintic in the form of the Paratect® Flex bolus, it has not yet been applied to the delivery of IGRs, larvicides or systemically active pesticides.

IV. Implants and injectables

Controlled release implants and injectables are popular dosage forms for delivery of drugs in veterinary medicine. They have long been available for delivery of antibiotics and growth promotants. Synovex®, Ralgro®, and Compudose® are familiar trade names in the industry and have been shown to increase feeding efficiency and rates of gain in cattle.[34]

One of the requirements of the drugs delivered by controlled release implants or injections is that they be active at very low dosages usually in the μg/kg body weight range. Generally speaking, chemicals used for the control of ectoparasites require higher dosages, typically in the mg/kg body weight range, to provide efficacy. The practical limitations of implant or injectable size or volume required by such dosages have hitherfore prohibited development of this means of delivery for ectoparasite control.

Systemically active IGRs were formulated into implantable pellets and microspheres of either poly(D,L-lactic acid) or copolymers of lactide/glycolide(PLA/PGA) for trials against cattle grubs.[35] When injected subcutaneously in the ears of infested cattle, those formulations containing methoprene, a juvenile hormone mimic, prevented the emergence of adult cattle grubs. In a second series of studies, methoprene either formulated into the implantable pellets or loaded into microporous polycaprolactone reservoirs also provided control of the cattle grubs in infested cattle.[36] The implantable polycaprolactone reservoirs were also used to successfully deliver insect steroid analogues against ticks.[37]

In a rather simplistic approach, a sustained release implant was fabricated to deliver ivermectin to cattle. The implant containing 20% ivermectin was formulated by dissolving the drug into a high molecular weight polyethylene glycol.[38,39] When these implants were injected subcutaneously into the ears of Hereford steers at a dosage of 200 μg/kg body weight, sufficient drug was released to provide >70% control of adult *Amblyomma cajennense* (F.) and >85% control of *Amblyomma americanum* feeding on steers for over 7 weeks. By comparison, when steers were treated with a single subcutaneous injection of the commercially formulated injectable at an equal dosage, effects on these ticks could only be seen for 1 week post-treatment. When the loading in this polyethylene glycol implant was increased to 30% active ingredient (AI) and cattle were treated at 400 μg/kg body weight, ticks were controlled for up to 11 weeks.

In more recent studies, a bioabsorbable, injectable microsphere (IMS) formulation containing ivermectin in poly(lactide-co-glycolide) copolymer (PLA/PGA) was developed to provide long-lasting delivery of the drug for control of livestock pests.[40] The solvent evaporation technique of Tice and Gilley[41] was used to produce the spherical beads containing approximately 30% ivermectin and ranging in size from 25 to 250 μm (Figure 7). The pattern of delivery of the drug into the blood stream of Spanish goats was characterized for a 50:50 PLA/PGA, a 90:10 PLA/PGA copolymer formulation, and a PLA monomer formulation. All three formulations produced an initial peak in the serum concentration of ivermectin between 1 and 4 days post-treatment (Figure 8). The 50:50 and the 90:10 copolymer formulations produced similar release patterns with initial peaks of about 20 ppb occurring less than 1 week post-treatment and subsequent peaks of 15 and 20 ppb at 6 week post-treatment, respectively. Minimum concentrations of 3–5 ppb were detected 2–3 weeks after treatment, and no ivermectin was detected after 12 weeks. Likewise, the PLA monomer formulation produced an initial peak of 10 ppb within the first week post-treatment, but the second peak of 12 ppb occurred at 17 weeks post-treatment and no ivermectin was detectable between 3 and 10 weeks or after 25 weeks.

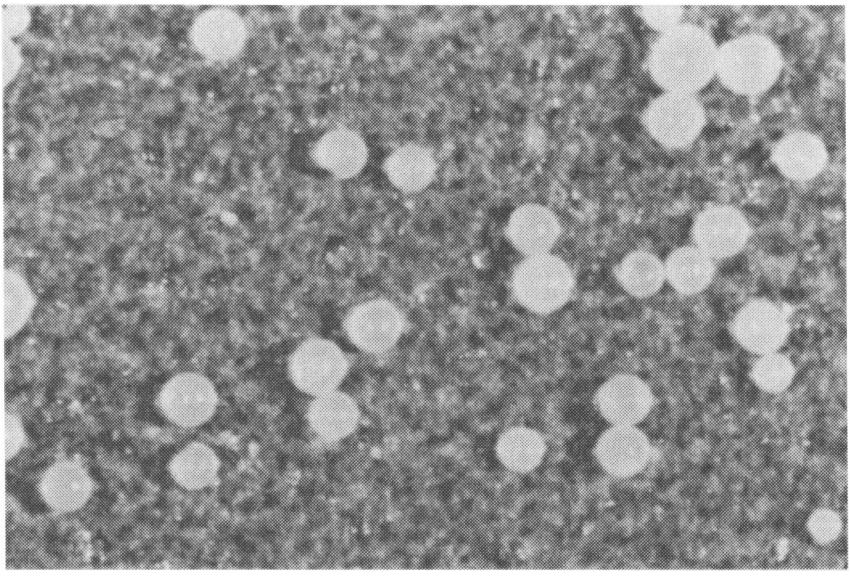

Fig. 7. Bioabsorbable, injectable, PLA/PGA microspheres were used to deliver ivermectin to cattle. Mean diameter of the solid beads range from 25 to 250 μm.

When the 50:50 PLA/PGA formulation was used in cattle at the rate of 2 mg(AI)/kg body weight, two peaks of 45–50 ppb ivermectin in serum were observed. The results of the blood serum analysis indicated that the profile of ivermectin concentration over time was similar for each of the treated animals (Figure 9). The first peak was at about 1 week post-treatment, and the second peak, which was broader than the first, occurred at about 6–7 weeks post-treatment. The concentration in the valley between the 2 peaks was approximately 10 ppb. The level of ivermectin in the blood dropped to <2 ppb (limit of quantification) for all three treated animals between 9 and 12 weeks post-treatment.

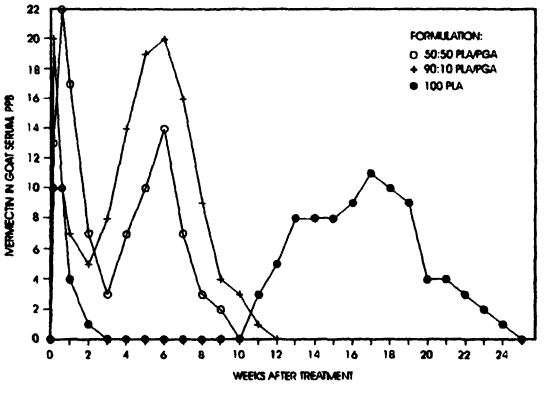

Fig. 8. Concentration of ivermectin in serum of Spanish goats resulting from 50:50, 90:10 and 100:0 PLA/PGA microspheres injected subcutaneously at a dose of 2 mg (AI)/kg body weight.

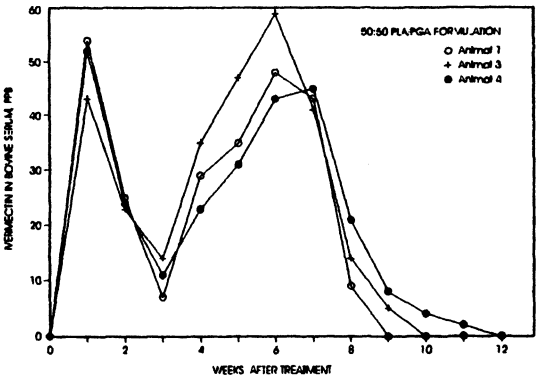

Fig. 9. Concentration of ivermectin in serum of cattle treated (SC) with 50:50 PLA/PGA copolymer formulation of the injectable microspheres at a dosage of 2 mg (AI)/kg body weight.

Table 3 shows percentage inhibition of estimated larvae (EL) for the lone star ticks placed on both the untreated control animals and those treated with the IMS formulation for 12 weeks post-treatment. Since no engorged ticks were collected from any of the treated animals for 8 weeks post-treatment, the treatment produced 100% inhibition of EL for that period. In the ninth week, only one of the three treated animals produced engorged ticks. Percentage inhibition of EL for the ticks placed on treated animals during week 9, 10, 11 and 12 was 75, 57, 46 and 44%, respectively.

In addition, the treatment provided 98–100% inhibition of larval horn flies in the manure of the treated animals for 10 weeks (Table 4). The efficacy of the treatment declined thereafter but in one animal, the treatment continued to provide 100% control of horn flies in the manure through the 14th week post-treatment when sampling was terminated. Both the larval horn fly and the tick bioassays are in agreement with the pharmacokinetics of the drug from the IMS formulation.

Table 3
Control of lone star ticks on cattle treated with the injectable microsphere formulation (50:50 PLA/PGA) containing ivermectin

	Animal number	Estimated larvae and % control at indicated weeks post-injection					
		0–7[a]	8	9	10	11	12
Controls	2	36 877	41 563	43 524	28 652	45 821	49 564
	5	59 450	59 593	63 449	35 539	69 674	92 207
	6	61 386	36 575	59 607	86 236	80 539	61 643
	Totals	157 713	137 731	166 580	150 427	196 034	203 414
Treated	1	0	0	41 314	37 899	56 027	61 211
	3	0	0	0	15 841	16 273	25 176
	4	0	0	0	11 599	33 456	28 283
	Totals	0	0	41 314	65 339	105 756	114 670
	% Controls	100	100	75	57	46	44

[a] Mean value for weeks 0–7 post-injection.

Table 4
Mortality of immature horn flies in the manure of cattle treated with the injectable microspheres formulation (50:50 PLA/PGA) containing ivermectin

Animal	% Control at indicated weeks post-injection						
Number	0–8[a]	9	10	11	12	13	14
1	100	100	98	7	42	22	0
3	100	100	98	95	33	26	0
4	100	100	100	100	100	100	100

[a] Mean value for weeks 0-8 post-injection.

Although injection sites were not examined for lesions or residues, we did observe that even at 24 h post-injection, we could not see or feel the site of injection and no obvious reactions were detected (Miller, unpublished data).

The efficacy of the injectable microspheres containing ivermectin for control of the cattle tick was tested on pastured cattle in South Texas.[42] Cattle in one pasture were injected subcutaneously in the neck with a controlled-release microsphere formulation of ivermectin at the rate of 2.4 mg(AI)/kg body weight. For this trial, the injection consisted of a blend of microspheres half of which were fabricated of the 50:50 PLA/PGA copolymer and half of which were 65:35 PLA/PGA containing 30% ivermectin. A second group of cattle in another pasture were controls injected with carrier only.

The mean level of ivermectin in the serum of the treated cattle over the 16 week study resulting from the IMS formulation showed three peaks in concentration as a result of the release of the drug over time (Figure 10). The first peak of approximately 35 ppb observed at 1 week post-injection is the result of the free ivermectin on the surface of the beads at the time of injection and possibly a small amount of diffusion from the polymeric microspheres. The second peak of approximately 65 ppb occurred at 4 weeks post-injection and is the result of the release during the hydrolysis of the 50:50 copolymer component of the injection. As the 65:35 copolymer began to

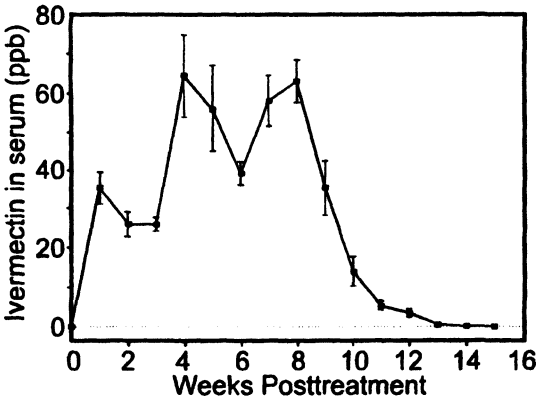

Fig. 10. Serum concentration of ivermectin in cattle treated (SC) with half 50:50 and half 65:35 copolymer formulation of microspheres at a dosage of 2.4 mg (AI)/kg body weight.

degrade and release its drug load, a third peak of approximately 63 ppb was detected at about 8 weeks post-injection.

The number of engorging females (≥5.5 mm diameter) was counted each week on both the treated and untreated heifers throughout the study (Figure 11). At the time of treatment, both groups had an average of about 60 such ticks. However, after treatment the average number of engorging females on the treated cattle began to decline. No engorged ticks were found on any of the treated cattle beginning 4 weeks after treatment and throughout the reminder of the study. When a pair of untreated, tick-free heifers was put in the control pastures during weeks 11 and 12, and again during weeks 14 and 15, approximately 20 500 and 17 500 engorged females were collected from these sentinels, respectively. In contrast, no ticks were recovered from those heifers exposed with the treated group at either of these same time periods. The PLA/PGA injectable microspheres delivered sufficient quantity of ivermectin not only to eliminate *B. annulatus* from the treated cattle, but also eliminated the tick population in the pasture where the cattle were held.

The injectable microsphere delivery system may also prove useful for control of parasites in a variety of other animals, such as heartworm in dogs, mites and ticks in exotic game animals such as such as bighorn sheep[43] and meningeal worm in llamas[44] and other zoo animals.

V. Controlled release technology and the resistance problem

Conventional control technology for horn flies requires the repeated application of pesticide at 3–5 week intervals. This approach is expensive in terms of labor of gathering, debilitation of cattle and quantity of pesticide. In the mid-1970s, controlled release technology provided the producer with an alternative that appeared to be the ultimate solution. The insecticidal ear tag provided convenient, easy to apply, relative low cost season long control. Moreover, a tag containing only 1 g of a pyrethroid

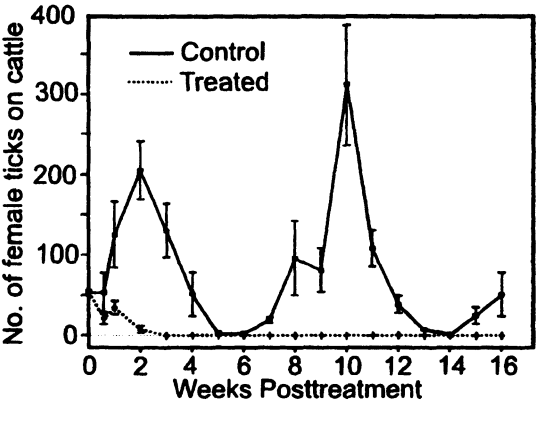

Fig. 11. Number of female *Boophilus annulatus* (Say) on cattle treated with the formulation described in Figure 10 as compared to the number of ticks on untreated controls.

provided control with a >98% reduction of pesticide compared to conventional treatments.

As a result of the rapid acceptance and wide use of the insecticidal ear tag, in the US, horn fly populations were subjected to greater selection pressure by a single class of pesticide than ever before.[45] As many as 13–14 generations of horn flies were continuously exposed in a single season. Consequently, this heavy selection pressure exerted by the insecticidal ear tag led to the development of significant resistance in some areas in as little as 2–3 years of treatment.[46–48] Soon resistance was being reported in every state in the US.

Researchers are actively seeking solutions to the problem through resistance management strategies. Both the problem and the solutions closely parallel that of bacterial resistance to antibiotics in human medicine. The strategies to prolong the usefulness of both the pesticide and the delivery system range from a periodic change in classes of control agents in the delivery system to completely eliminating the use of controlled release delivery and reversion to the conventional technologies of earlier days. Unfortunately, the insecticidal ear tag, one of the first and most innovative technologies, is now being credited with the problem rather than the solution to horn fly control.

VI. The future of controlled release technology for control of ectoparasites of livestock

The needs of the producer/rancher for effective, inexpensive ectoparasite control remain, and present, both a unique challenge and opportunity. The key issues of cost/benefit ratio, ease/speed of administration, frequency of dosing, tissue residue, side effects, environmental/handler safety and compatibility with slaughter procedures listed by Cardinal and Witchey-Lakshmanan[1] for veterinary medicine are also central to acceptability in ectoparasite control. In ectoparasite control, the issue of cost/benefit ratio is even more important than in other applications in veterinary medicine. In most cases the survival of the animal is not in jeopardy because of the pests, and the economic impact may not be as well defined.

If history is any indication, the introduction of new, innovative controlled drug delivery systems for livestock pest control will be slow. Progress in the past has been mainly through the development and availability of new chemicals. However, both the discovery of new pesticide entities and the development of novel delivery systems are important to improvements in control technology and management of livestock ectoparasites. The development of novel delivery systems can improve efficacy of the treatment, efficiency of pesticide usage, and safety to the animal, the applicator and the environment. Ideally, a synergy would exist between the control agent, the delivery system, animal behavior, and an understanding of the population dynamics of the pest to optimize management practices.

Because of the perceived lack of market or profit potential and the limited research in the area, it appears likely that future progress will focus on the use of delivery systems used in other areas of veterinary medicine.[49] With the availability of control

agents which are active at lower dosages and present less tissue residue concerns,[50] the potential of using implants, injectable microspheres or microcapsules, and intraruminal devices for delivery become more practical. Pulsed release in the rumen or as injectable microspheres as earlier described appears to be a practical and achievable goal worthy of development for systemic control of ticks. It will be a continuing challenge to control the higher cost usually associated with more sophisticated delivery systems.

In addition to the challenge of developing practical control release delivery systems, the producer/rancher will be challenged to use these systems in an effective, strategic management approach. Researchers, marketers, and users will need to be realistic in assessment of new tools; a single chemical entity or delivery system will not be the answer to all our ectoparasite control needs. Wise, strategic management using a broad arsenal of technologies will be needed to prolong the utility of any control agent and delivery system by avoidance of the resistance problem.

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CHAPTER 9

Controlled drug delivery and the companion animal

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Overview

I. Introduction

The purpose of this chapter is to (a) provide a perspective on controlled release drug delivery systems for companion animals, (b) highlight some of the reasons why controlled release drug delivery systems are developed for companion animals and (c) describe relevant issues pertaining to the design and development of controlled release products for various routes/clinical conditions.

Companion animals are those which are considered as pets, primarily dogs, cats and horses. Animals such as birds, lizards, rabbits, etc. can also be considered as companion animals; however, these are more properly classified as 'exotic' animals. Since

the largest body of controlled release literature focuses on dogs and cats as companion animals, these two species will be the primary focus of this review. It should be realized, however, that the other species mentioned above also present unique and interesting opportunities for controlled delivery of medicinals.

The companion animal market is quite different from the food animal market since the factors supporting each are divergent. For example, in the food animal industry the main factors that drive the product development are the costs associated with the handling and dosing of the animals. In that industry, often the profit margins of the farmers are quite low. Therefore, the formulation must be inexpensive and yet be able to release the active ingredient over the course of a long period of time to minimize the frequency of dosing and handling of the animals. In contrast, companion animals are often considered as part of the family. As a result the market is better able to support the expense of dosage forms, similar to human medicine.

II. Why controlled release delivery for companion animals?

Physiologically, most companion animals, such as dogs and cats, are monogastrics. Therefore, the oral delivery challenges facing the formulation scientist are similar to those encountered when developing a formulation for use in man. Indeed, at times human medicines are actually used for companion animal applications. In such cases, typically pediatric medicines are administered, since the difficulties experienced in dosing a dog or cat are comparable to those of dosing a child. Likewise, the issues of taste, frequency of dosing and size are often similar as well. However, children do not hide under the couch if they do not like the medicine! Hence, the development of a controlled release medication for a companion animal can provide an opportunity to improve patient compliance, and therefore, improve the overall therapeutic index of the drug.

For injectable formulations, it is desirable to have dosage forms that release from as short as a full day, to a week, to as long as 6 months. For example, for some antibiotic therapies the most desirable product might be an injection dosed once that would then provide efficacious levels of drug for no more than 24 h, followed by oral therapy of the same active agent. This dosing pattern would allow the veterinarian to not only attack the infection immediately, but also give her/him the opportunity to change the drug during the oral dosing phase if the infectious organism failed to respond to the therapy. In contrast, in the instance in which the infection was known to be susceptible to a particular active agent, a product that provides efficacious drug levels over an entire week would be preferred. In this case, one injection releasing the active agent over seven days would improve patient compliance and client convenience over the entire course of therapy. In comparison, a much longer duration would be useful for therapies against ticks and fleas. Thus, a single injection or implant that releases the active over six months would allow for treatment over the course of a whole season.

Many other therapies offer similar sets of needs and issues for similar challenges. This paper reviews sterile systems, such as depot, particulate and implant technologies as well as ophthalmic drug delivery and wound dressings. It continues with a discus-

sion of non-sterile applications, focussing on parasiticides and osmotics. Finally, recent initiatives in protein/peptide, vaccine and gene therapies are explored. As mentioned, products which are available for dogs and cats will be primarily reviewed, with the understanding that similar technologies and in some cases the exact same products are adapted for use in more exotic species.

It should be noted that the regulations that govern companion animals and other areas of veterinary medicine are identical to those that govern human medicine. Therefore, all the same regulatory requirements, such as stability, choice of excipients, quality of manufacture, and product/process reproducibility, are to be met in the development of a new delivery system.

III. Companion animal controlled drug delivery

III.A. Sterile delivery systems

As discussed in the previous section, in companion animal medicine it can be most beneficial to formulate a therapeutic ingredient into a sustained release injectable dosage form. This would permit a veterinarian to administer the drug at the clinic and obviate the need for the pet owner to have to administer tablets. In fact, it is very challenging for the owner to administer tablets to a pet, especially to cats. Animals often spit the tablet or liquid out if the flavor is not right, or may hide under the furniture or behind a cabinet at the next dosing time. Thus, a long-acting injection can improve 'patient compliance' and reduce stress to the administrator.

III.A.1. Depot systems

Depot injections are defined as those suspensions or solutions, which upon injection remain at the site as a precipitate or a crystal form. This solid drug substance remains as long as it takes for the drug to solubilize and to become systemically available.

One classic depot injection in companion animal medicine is insulin used for the treatment of diabetes in animals. Most insulin products used in companion animals are those developed for humans. Historically, the most typically available products have been the bovine and porcine insulins. Since dog insulin is identical to porcine insulin, and cat insulin is nearly identical to bovine insulin differing by only one amino acid group,[1] these human products are regularly used to treat diabetes in cats and dogs. Now that recombinant human insulin is available, companion animals can be treated with that as well.

Short, intermediate, and long acting insulins are all currently available for animals. For the immediate treatment of high blood glucose levels in animals, short acting insulin is usually utilized. Intermediate, or lente, insulin is typically given to an animal twice per day, with feedings scheduled according to the injection time. Long acting, or ultralente, insulin can be used for once a day delivery. In addition, there are several combinations of insulin products, which have been specifically formulated to provide certain release profiles. Greco et al.[1] have compiled an excellent table of all types of insulin products which are available commercially and can be used in the companion animal.

In general, pharmacokinetics of an insulin product is determined by the crystal size of the insulin itself. For example, solution formulations of insulin allow the drug to be available immediately upon injection. Lente insulin contains small zinc-insulin crystals, which release the insulin molecules slowly as compared to a solution type of insulin. In contrast, ultralente insulin is composed of large zinc crystals that dissolve even more slowly, releasing the insulin over an even longer period of time.

Many other injectable suspensions are commercially available for companion animals; however, one of the most interesting is the new Program® injectable suspension from Novartis. This product is indicated for flea control and is given once every six months, covering the entire season. Like insulin, the drug substance is nearly insoluble, and remains at the site after use. However, the rate of dissolution is very slow; therefore, the ectoparasites are controlled over several months. The injection is indicated only for cats, since injection site irritation is observed in dogs.

III.A.2. Particulate systems

In companion animals, the use of particulate drug delivery approaches, such as beads, microspheres, liposomes, etc. are still primarily experimental. Beads are characterized as round solid polymeric matrices, several millimeters in diameter, into which the active agent is dispersed. Beads are typically on a wire 'string' and surgically implanted at the location of interest. Once the course of therapy is completed, the beads and wire are then surgically removed. Since the 1970s, polymethylmethacrylate beads containing gentamicin or other antibiotics have been used for the treatment of bacterial infections. Clinical applications of this technology can be found in companion animals for the treatment of osteomyelitis and associated conditions.[2]

Since beads require surgical removal, an obvious improvement would be the development of a biodegradable delivery system. Examples of such work can be found in the studies from Garvin et al. [3] and Gupta et al,[4] in which polylactide-co-glycolide (PLGA) type polymers were prepared as microspheres or compressed rods and administered to dogs. These systems were actually being developed for eventual use in humans; however, they demonstrate the feasibility of such drug delivery approaches in canine medicine.

Liposomes are complex emulsion-like systems consisting of bilayer structures of mainly phospholipids. An active agent can be entrapped in either the aqueous or lipid layer depending on the physical-chemical properties of the drug. In companion animals, liposomes have been used for the treatment of a variety of infections and cancers.[5–8] Gentamicin[5] and many other biologically active agents[6,7] have been encapsulated into liposomes for injectable dosage forms. Long-circulation liposomal drug delivery systems were achieved by inhibition of the rapid uptake of liposomes by the macrophage–monocyte system and reduction of the rate of drug leakage. Liposome localization in tumors appears to be the result of enhanced rate of extravasation through abnormally permeable microvasculature coupled with impaired lymphatic drainage.[7]

III.A.3. Implants

Parenteral implants can have the active agent incorporated into a polymeric matrix or

into a vehicle held within an enclosed reservoir. They have been shown to provide either site specific or systemic sustained release of the active. In companion animals, implant technology has been widely used in the areas of estrous control and parasite control. Estrous control is important for controlling free-ranging canine and feline populations as well as for synchronizing the estrous in a predictable manner for breeding. Currently, several products are available; however, most of them are not long acting.

The potential use of testosterone implants has recently been examined in a few studies for the inhibition of estrous in dogs.[9,10] For example, Vincent et al.[10] examined the plasma concentration of testosterone in castrated dogs following the implantation of polydimethylsiloxane capsules, which were filled with various amounts of testosterone. Other researchers have explored the use of a medroxyprogesterone acetate implant for canine population control. The product, known as Perlutex Leo® in Norway, has become popular in replacing surgery and has been used effectively to reduce the number of free-ranging dogs in various locations.[11] However, the utilization of this product may be associated with the formation of uterine lesions and canine mammary tumors.[11,12]

Intravaginal inserts, such as the progesterone releasing intravaginal device (PRID), the controlled internal drug release (CIDR) device and sponges, have been shown to be effective in controlling estrous of horses.[13] The CIDR intravaginal devices utilize technology that involves a high temperature injection molding process to cure a silicone matrix impregnated with a homogenous dispersion of progesterone over a nylon spine. Retention of CIDR in horses has been reported to be excellent with a 15-day insertion period.

The use of injectable gel technology to control estrous in horses has also been explored.[14,15] This system consists of an easily injectable solution of the active agent with sucrose acetate iso-butyrate (SAIB). Upon injection, the sucrose derivative gels in the presence of physiological fluids. The active agent then slowly releases from the hardened mass. Over time the sucrose acetate iso-butyrate completely degrades into innocuous esters. In these recent studies, it was shown that the estrous of mares is successfully controlled using this technology.

Long releasing implants have also been explored in the area of parasite control. Specifically, poly(ortho ester) (POE) polymer has been reported to be used in matrix systems to release ivermectin for the control of *D. immitis* heartworm infestation in dogs.[16] Ivermectin was covalently bonded to the poly(orthoester) monomers and slowly reacted with them to form the device. Once the device was shaped into a rod, it was injected subcutaneously, releasing the ivermectin for as long as 6–12 months. More than one implant may be used if needed. The implant was completely biodegradable, ensuring that the accumulation of polymeric material was minimized with repeated dosing. An acid or base is typically incorporated in the polymer matrix to control the rate of the erosion process, since orthoester bonds are unstable under acidic conditions but relatively stable at a neutral or basic pH.[17,18] The release mechanism of ivermectin is primarily due to the surface and bulk erosion of the crosslinked poly(ortho ester) matrix. As the crosslink density of the matrix decreases with time, the diffusion mechanism becomes more important. This occurs because an increasing

fraction of the ivermectin is freed from the matrix itself. In fact, a peak in the plasma profiles was often observed after 16 weeks drug delivery (Figure 1), which may be attributed to complete matrix erosion, thereby leaving the drug to be absorbed freely.[16]

It should be noted that this approach could also be applied to many other drugs as long as the drug molecules possess two or more hydroxyl groups. These hydroxyl groups allow the drug molecules to be chemically bound as part of the POE backbone through a condensation reaction. Similar to polylactide-co-glycolide systems, POE delivery systems can also be prepared without covalently binding the active agent to the polymer backbone.

Various implants have also been investigated to control insulin release under circumstances more naturally modulated than the injections discussed earlier in this chapter.[19–22] For example, islets have been encapsulated into hydrophilic polymers, which support the viability of the cells. When the glucose level in the body is high, the glucose molecules migrate through the hydro-polymer to the cells. The cells respond by producing insulin, which then migrates back into the body and becomes available systemically. Insulin has also been formulated into an implantable reservoir system that contains a hydrogel material to provide similar effects. In this device, various types of glucose sensitive moieties were placed onto the polymer that permitted the polymeric material to expand in the presence of glucose. When the polymer network expanded, insulin molecules could diffuse out easily. When the glucose level decreased, the moieties responded by causing the contraction of the polymeric material, which inhibited the diffusion of the remaining insulin. Both of these approaches are still in the experimental stage.

Researchers have also been exploring the use of collagen implants in the canine. These implants are designed to slowly release chemotherapeutic agents for the control and elimination of tumors. The implants are typically injected into the tumor directly in a cross-hatched array. The therapeutic agent is then slowly released over a period of time based on the formula developed.[8]

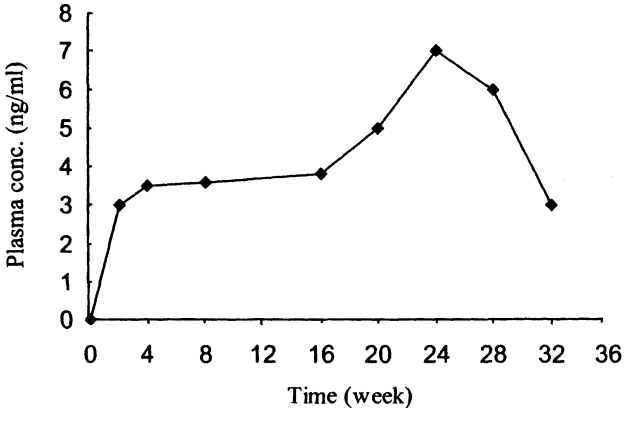

Fig. 1. A typical plasma ivermectin concentration profile.

III.A.4. Ophthalmic drug delivery systems

In general, veterinary ophthalmic drug delivery systems are similar to those for humans, except the eye anatomy and physiology of the animals need to be considered.[23] Usually the cost and convenience of the application determine the choice of medication. Historically, simple solution eye drops have been the dosage form of choice. The drops can be cleared from the eye quite quickly, and as a result the medicine must be administered several times a day. Frequent application of eye drops to an animal is often a challenge and is inconvenient because one has to hold the animal's head still. Therefore, a long acting ophthalmic dosage form is preferred.

Baeyens et al. reviewed the ophthalmic diseases and the agents used to treat them in the field of veterinary medicine.[23] The available products currently on the market and a disclosure of the technologies being explored to prolong the release of the active agents were also presented.[23] An oil-based system is usually the first line of formulation used to prolong drug delivery. This was the approach taken for the canine product Optimmune (Schering-Plough). Alternative approaches include the addition of various excipients, such as polymers, which help the active agent adhere to the local conjunctiva for a longer period of time. Summarized mechanisms of these approaches and common polymer excipients used in ophthalmic drug delivery are listed in Table 1.

As indicated in the table, the typical excipients added to prolong the release of an ophthalmic product are mainly polymers, such as water-soluble celluloses (microcrystalline cellulose (MCC), hydroxypropylmethylcellulose (HPMC), hydroxypropylcellulose (HPC), etc.), polyvinyl alcohol (PVA), hyaluronic acid, carbomer (polyacrylic acid), cellulose acetate phthalate (CAP) latex, polysaccharides, poloxamers (polyoxyethylene–polyoxypropylene copolymer), etc. Celluloses and polyvinyl alcohol can increase the viscosity of the ophthalmic solutions significantly and, therefore, improve the retention of the solution in the conjunctival sac. Polysaccharides, such as gellan or carrageenan, crosslink in the presence of the divalent cations in the tear fluid. This crosslinking increases the viscosity of the formulation and also acts as a matrix for the drug molecules, thereby extending the residence time of the product in the eye. Carbomer and CAP preparations are usually compounded at pH values around 3–6 to ensure that they can be dripped into the eye. They then gel in situ when the local pH increases to physiological pH. Poloxamers can be utilized to formulate a liquid dosage

Table 1
Mechanisms of oil-based or polymer-based products used in ophthalmic drug delivery

Type	Mechanism
Oil-based	Lack of solubility
Water-soluble celluloses	Increased viscosity of the preparation
Polyvinyl alcohol	Increased viscosity
Hyaluronic acid	Increased viscosity
Carbomer	pH-dependent gellation
Cellulose acetatephthalate latex	pH-dependent gellation
Carrageenan/gellan	Ca^{2+}- or K^{+}-dependent gellation
Poloxamers	Reverse thermal gellation

form at room temperature, which will gel at body temperature. All of these gelling formulations typically provide a moderate improvement of 12–24 h;[24] however, their side effects may include blurred vision, crusting of eyelids and lacrimation, as well as a 'foreign body' sensation.

Ophthalmic inserts and implants have also been investigated for companion animal use. Usually ophthalmic inserts are classified as soluble, insoluble or bioerodible. Soluble inserts are most desirable since they do not require surgical removal after use. However, some of these inserts have been shown to be irritating to the eye.[23] Gurtler et al. [24] discussed the development of a veterinary insert containing gentamicin for the treatment of ocular infections. In this device, a combination of hydroxypropyl cellulose, ethylcellulose and carbomer (polyacrylic acid) were utilized and the device was shown to provide efficacious drug levels for as long as 72 h. A silicone implant has also been studied experimentally for the treatment of glaucoma in dogs.[25] However, these implants need to be removed surgically once the therapy is complete.

Other approaches, such as prodrugs, intravitreal injections (liposome, biodegradable microspheres and plugs) and iontophoresis, may also have the potential to be used for the ocular drug delivery in companion animals.[23]

III.A.5. Wound dressings

The primary purpose of wound dressings is to accelerate the healing process, retard infection and minimize pain. Wound dressings can be found in many different forms, including the recently introduced film dressings and gel dressings. Several general classifications of dressing forms can be made, as outlined in Table 2. These classifications include bandages, petrolium-based gauzes, alginate dressings, polyurethane films, hydrocolloid dressings, hydrogel dressings, hydrophilic dressings, foam dressings and biological dressings.[26] These dressing materials can provide an excellent matrix for controlled release of biologically active agents, such as growth factors, antibiotics, antiseptics, etc. For example, transforming growth factor-beta1 (TGF-β1) was incorporated into a poloxamer gel that provided a sustained release of TGF-β1

Table 2
Typical wound dressings and their common application forms

Wound dressings	Common application forms
Adherent	
Bandages	A wide mesh gauze
Non-adherent	
Petroleum-based	Petroleum-impregnated gauze or a wide-mesh gauze coated with petroleum-base antibiotic ointment
Calcium alginate dressings	Flat, non-woven pads, either calcium-sodium alginate fiber or pure calcium alginate fiber. Form a gel when absorb wound exudate.
Polyurethane films	Polymer films
Hydrocolloid dressings	Suspensions of starch polymers in an adhesive matrix
Hydrogel dressings	Insoluble hydrophilic polymers paste or adhere to a mesh or fiber
Hydrophilic dressings	Polymer beads, flakes, powders and pastes
Foam dressings	Polyurethane and a semipermeable film
Biologic dressings	Tissue culture-derived membranes, collagen-synthetic bilaminates

and a significant enhancement in wound healing.[27,28] Gentamicin or amikacin has also been placed between a collagen membrane and a collagen sponge wound dressing, allowing for the slow release of drug over 3 days.[29] A novel controlled release wound dressing with an antibiotic delivery system stimulated by microbial infection has also been developed. In this system gentamicin is incorporated into the dressing by binding it to polyvinylalcohol hydrogels. The drug can only be cleaved by the proteinase that exists in an infected wound.[30–32] Once cleaved, the drug is free to migrate to the infected area.

The most widely used film dressing materials are made of semi-permeable synthetic polymers, such as polyurethane films, while hydrocolloid dressings are suspensions of starch polymers in an adhesive matrix. When in contact with a moist wound, they form non-adherent gels, which are permeable to water vapor, oxygen and carbon dioxide, but are impermeable to organisms, such as bacteria.

Hydrogel dressings have been studied most extensively. Hydrogels are made of insoluble hydrophilic polymers that usually can absorb substantial amounts of fluid from wounds and provide a moist wound environment.[33] Silicone rubber-hydrogel[34], acemannan hydrogel (extracted from aloe plant)[35,36] and polyacrylamide hydrogel[37,38] fall in this category. Hydrophilic dressings can be found in many forms, such as beads, flakes, powders and pastes. Each of these forms is able to absorb wound exudate.[39] The beads can be used to separate substances in wounds by capillary action. Large particles such as microorganisms, plasma proteins, fibrinogen, and wound debris move to the spaces between beads. Smaller molecules such as moisture, electrolytes, prostaglandins and hormones absorb into the beads, causing the beads to form a gel.[40] Foam dressings are typically made from polyurethane and a semipermeable film. Tissue culture-derived membranes[41] and collagen-synthetic bilaminates are biologic dressings used for temporary coverage of open wounds.

There are many other wound dressing materials, such as live yeast cell derivatives, aloe vera extract gel,[39] polyethylene oxide-aloe vera gel matrix,[42] chitosan,[43] gelfoam, etc. With more complete understanding of wound healing process and advances in polymers and breathable adhesive technology, more types of controlled release wound dressing products will be developed, providing additional significant technical and clinical benefits.

III.B. Non-sterile dosage forms

III.B.1. Parasiticides

Endoparasite and ectoparasite control and elimination represent one of the largest markets in companion animal medicine. The most popular products in endoparasite control are Interceptor (Novartis) and Heartguard (Merial), in which milbemycin and ivermectin, respectively, are the active ingredients. These compounds are quite potent and have long biological half-lives. These properties allow small dosages to be formulated into meat-flavored tablets that are easily given to companion animals, in particular dogs, at monthly intervals.

The ubiquitous flea collar is the most traditional ectoparasite control product. A history of ectoparasiticide collars and the development of collar technologies have

been recently presented.[44] Incorporation of an active ingredient into a plastic matrix is the main basis of this technology. These collars were first developed in the late 1960s using vinyl resins. Subsequent improvements focused on prolonging the release rate and increasing the fraction of active agent delivered over the life of the product. These changes were accomplished by modifying the matrices by using a variety of polymers and processing aids. The basic mechanism of the design is to use the incompatibility between the plastic matrix and the liquid/solid active agent to force the active to migrate onto the surface of the collar. The drug then comes in contact with the fur and skin of the animal, thereby being available to eliminate the parasites. Many other technologies have also been investigated, such as reservoir systems in which the active agent is incorporated into a depot that is surrounded by a rate controlling membrane. Mechanical approaches, such as pumps that push the active agent slowly out of a device over a long period of time, have been studied as well.

Over the last decade, the most significant improvements in ectoparasite control have been made through the advent of highly potent compounds that possess long systemic and local activity. Similar to the endoparasite products already described, oral ectoparasite products, such as Program® and Sentinal® (Novartis) also provide a month long protection against flea infestations. However, these compounds are not effective against adult fleas.

Compounds have also been formulated into once-a-month spot-on products. Only a few milliliters of the spot-on liquid need to be applied along the back of the animal or at the base of the neck. These products include Frontline® TopSpot (Merial), Advantage® (Bayer) and Ex-Spot® (Schering-Plough).[45] The retention power of these medications is determined by the unique properties of the active agents and excipients with which they are compounded. For example, in Frontline®, the active ingredient sequesters into the sebaceous oils of the animal and is released slowly from there, unaffected by bathing or rain.

III.B.2. Oral osmotic systems

The oral osmotic controlled release dosage form was developed to reduce the dosing frequency and plasma fluctuation by using osmosis as a constant driving force to control the delivery rate.[46] Historically, this dosage form is composed simply of a tablet core, a semipermeable membrane coating and a laser-drilled orifice. As illustrated schematically in Figure 2, the tablet core is comprised of active agent intimately mixed with the osmotic engine material. The osmotic engine material is typically a hydrogel polymer containing sodium chloride to provide the osmotic driving force. Upon dosing, water migrates across the membrane via osmosis to dilute the concentration of salt within the tablet, hence, the term 'osmotic' system. This water then swells the hydrogel, pushing both gel and drug out through the orifice. This system was first developed for water-soluble drugs,[46] or for solubilized poorly water-soluble drugs.[47]

A similar 'push–pull' osmotic pump design, as shown in Figure 3, can also be used for poorly water-soluble compounds. In this design, the drug and the osmotic agent are compressed into a bilayer tablet, then coated with a semipermeable membrane. An orifice is then drilled on the drug side of the membrane. Water molecules migrate

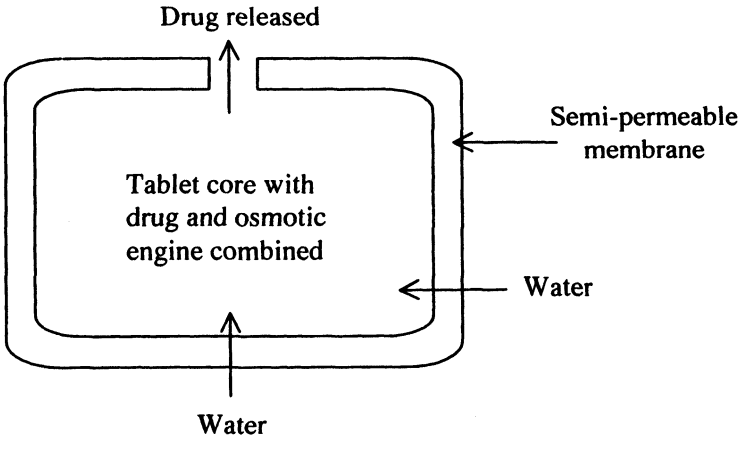

Fig. 2. An osmotic pump for controlled delivery of water-soluble drugs.

through the membrane and are absorbed into the polymer half of the tablet via osmosis. A drug suspension is formed when the water enters the device, and the suspension is pushed out through the orifice by the expanding osmotic engine. This mechanism of operation allows the delivery of insoluble drugs in a finely divided form ready for dissolution and absorption.[48] The delivery rate obtained from either design is determined by osmotic properties of the drug, drug loading, permeability of the membrane, membrane surface area and thickness. Once these parameters are determined, the release rate should be nearly constant, unaffected by environmental pH and gastrointestinal motility.

Several osmotic drug delivery systems have been successfully developed for human use. In the course of that work, many in vivo pharmacokinetic or pharmacodynamic studies have been conducted on dogs using such osmotic systems. These studies include systems containing the β-adrenoceptor antagonists, metoprolol and oxprenolol Oros® system[49]; EOP-indomethacin[46]; nifedipine, a calcium channel blocker used for the treatment of angina pectoris[48]; tromethamine salt, a potent HMG-CoA reduc-

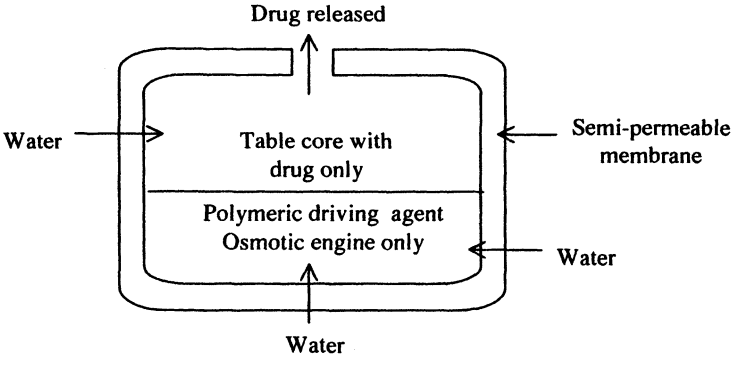

Fig. 3. A push-pull osmotic pump for controlled delivery of insoluble drugs in suspension.

tase inhibitor and cholesterol lowering agent[50]; diltiazem hydrochloride, a potent calcium-channel blocker[51,52], etc. These studies showed reasonably good agreement between the in vivo performance and the in vitro dissolution results. Such experiments demonstrated the feasibility of using this technology in animals; however, it is believed that the overall GI transit time in companion animals may be too short to utilize the specific oral osmotic technologies investigated in these studies. Thus, for the companion animal market, osmotic technology must be exploited in a different manner if it is to be applied to animal health indications.

IV. Recent initiatives

IV.A. Protein and peptide delivery

At present, not many proteins or peptides have been studied for veterinary use due to their high cost and a lack of availability. As biotechnology becomes more prevalent, the application of new entities will become more available. Somatotropins (bovine or porcine) or somatotropin releasing hormone, which are used to increase milk production, accelerate growth, and reduce fat deposition, etc., are currently the proteins which have been studied the most in farmed animals.[53] In the market of companion animals, luteinizing hormone releasing hormone or luteinizing hormone releasing hormone agonists (e.g. nafarelin acetate and leuprolide acetate) may be applied to control of fertility, stimulation of spermatogenesis and ovulation and estrous suppression.

Formulations designed to prolong protein and peptide release include implants,[54–60] hydrogels,[61–64] microparticles,[65,66] liposomes,[67,68] aqueous solutions gelled with polymers[69] and emulsions.[70,71] Several factors must be considered carefully for a successful formulation. Specifically, the stability of the entity must be taken into account to prevent aggregation, deamination or oxidation.[72] Additionally, the bioactivity of the substance must be maintained at the proper potency level. Physiochemical properties of the protein, such as isoelectric point, molecular weight, amino acid composition, aqueous solubility at various pHs and salt concentrations and organic solubilities, will also be useful in establishing proper conditions for maintaining stability and bioactivity.[72]

One example of proteins/peptides that might have market potential in companion animals is bone morphogenic proteins (BMPs). Their presence, activity and sequences have become known only over the last decade. The function of these proteins is to differentiate mesenchymal cells into chondroblasts and osteoblasts, inducing the formation of bone. Interestingly, these factors can be included into implants, which can be placed into large fractures and promote healing where proper healing would not normally occur. One can imagine that such a device might be most lucrative in the equine industry.

The development of delivery devices containing bone morphogenic proteins and the associated patent literature has been reviewed.[73] The delivery device primarily employed to date has been a matrix of bone-derived materials, such as demineralized bone matrix (DBM) and inactive collagenous bone matrix (ICBM), and/or synthetic polymers. The most common biodegradable synthetic polymers cited in the literature

for the delivery of BMPs have been polylactide-co-glycolide and poly(orthoesters). The mechanisms of releasing the active proteins are typically a combination of diffusion through the porous matrix and the degradation of the device. A veterinary perspective of BMP technology has also been presented.[74] In this paper, Kirker-Head discussed the potential uses of BMPs and possible delivery devices for veterinary medicine such as bone graft replacement and bone/soft tissue joining. At present, the cost and reproducibility of manufacture of the bone morphogenic proteins continue to be the main issues in developing BMP drug delivery systems.

IV.B. Vaccine delivery

While conventional vaccine formulations are indisputably helpful and necessary in preventative therapy, many possess potential safety issues as well as limited efficacy. For example, the delivery of antigens from oil-based adjuvants[75] has resulted in the reduction of the number of doses needed. However, some of the oil-based adjuvants possess inherent toxicity. As a result, researchers continue to focus on the development of more suitable adjuvants in vaccine formulation.

One approach has been the delivery of antigens via particulate delivery systems, rather than in solution. This approach facilitates recognition by the phagocytic systems and results in presentation of the antigen to the lymphocyte.[76] Moreover, these vaccine delivery systems can theoretically provide the ability to deliver antigens in a continuous manner, resulting in an improved control over the production of antibodies.[77] Particulate systems that have been studied as vaccine delivery vehicles include emulsions, liposomes, microparticles, etc. These approaches are discussed individually below.

Oil-based emulsions have proven to be effective vaccine delivery vehicles for a wide range of veterinary species.[78] The vaccine adjuvant and antigen are usually included during the emulsification stage and become incorporated within the droplets, which act as a depot either at the site of injection or in antigen presenting cells.[78] The product performance characteristics of an emulsion are dependent upon certain properties of the emulsion, such as the oil/water phase ratio, the emulsion droplet size and the viscosity of the oil phase. For example, high oil content can cause unnecessary injection site irritation. Inappropriate droplet size can result in a physically unstable product, thereby reducing product shelf life.

An example of using emulsion formulations for incorporation of a new adjuvant is the preparation of gliding bacterial adjuvant (GBA). The results showed that GBA appeared to be a potent stimulator of feline T-cell proliferation and could induce both interferon (IFN) and interleukin (IL-2) production in cats.[79] GBA has relatively low local and systemic toxicity and exhibits potent activity, both in phosphate buffer solution or within oil vehicles. Hence, GBA may be an interesting new adjuvant for feline vaccines.

Antigens have also been entrapped in liposomes, which serve as carriers and as depots for the controlled release of antigens, as well as targeting agents for the delivery of novel antigens to antigen presenting cells. Liposomes can induce both a humoral and cell-induced immunity, which is clearly an advantage when formulating

a vaccine.[80] There are currently no commercial animal vaccines based on liposomes. Lack of stability and the utilization of organic solvents in process, which may adversely affect antigens, are included in the limiting factors.[78]

In contrast to liposomes, microspheres or nanospheres are prepared from synthetic polymers, such as polylactide and glycolide copolymers. These polymers are biodegradable, biocompatible and approved by the FDA for use in therapeutic products.[81] Use of microsphere systems for delivering vaccines allows one to employ multiple mechanisms to control release. These mechanisms include polymer type, polymer molecular weight, and particle size and distribution. For example, glycolide polymers degrade more rapidly than lactides, and the ratio of glycolide to lactide can dictate the rate of erosion. In addition, low molecular weight polymers degrade completely more quickly than high molecular weight polymers. Finally, use of particles of mixed sizes, especially larger particles that avoid uptake by macrophages, can also sustain the release of antigens.[78] The small particles will release the antigen more quickly than the larger particle, primarily due to their more rapid degradation. However, the larger particles allow for the diffusion of the antigen through pores, as well as erosion, to help sustain the release profile. Regardless of the system used, the effectiveness of the vaccine delivery may also be affected by the stability of the antigens during the manufacturing process as well as in the pH of the microenvironment upon administration, which may change due to the hydrolysis of polymers.

Though veterinary applications of microparticulate vaccines are limited, the number of investigations in this area continues to rise. An example is a bait delivery system for rabies immunization of dogs in Tunisia.[82] The system was prepared by coating a freeze-dried core unit containing a biomarker with a paraffin matrix. The bait systems were placed on transect lines, which made them available to free ranging dogs which were not accessible via a dog owner. Unfortunately, this method was found to be less safe, not species specific, costly and, therefore, not readily accepted by the human population.

IV.C. Genetically engineered vaccines/gene therapy

At present, marketed vaccine technology often provides only temporary immunity based on weak vaccines. The weaker the vaccine, the larger the dose needed to stimulate the immune response and, therefore, the greater the risk of over-activating the immune system resulting in shock and death. Through genetic engineering, new vaccine technology is now providing to us with the ability to devise genetic material designed for inducing immunity against specific diseases.[83] These genetically engineered vaccines theoretically do not over-stimulate the immune response, since only those proteins or subunits that are most critical in inducing protection are administered. These most critical proteins may include not only the highly purified subunit, synthetic peptide or live recombinant vaccines, but also live attenuated organisms in which deletions have been made in specific genes involved in replication. These attenuated organisms are further engineered to ensure that the organism has a low probability of back mutation and restoration to virulence.

Since the antigens are very specific, genetically engineered vaccines offer the

possibility that multiple antigens from a variety of pathogens could be combined. One might hope that the specificity of the antigens might also extend to each animal species. These antigens might, therefore, provide the opportunity for the development of one product that could be used on various species. Theoretically, the agent could be designed to induce various types of immune responses, depending on the animals being immunized.

It should be noted, however, when the first genetically engineered hepatitis-B vaccine was developed, it cost US$150 for three injections. Although the vaccine is safe and effective, few pet owners would be willing to pay so costly a price for such a series of vaccines. Consequently, similar to other technologies, genetically engineered vaccines may not be marketable in veterinary medicine until the technology further matures.

As discussed, genetically engineered vaccines allow for the stimulation of the immune system through the choice of specifically designed antigens. In contrast, gene therapy replaces the entire gene whose function/expression has been lost in a cell. The DNA/vector combination delivered in such therapies is regulated as the drug substance. Thus, issues similar to those encountered in the development of other drugs and proteins such as bioavailability, toxicity, activity, and cost/risk benefit must be evaluated during their development.[84] Ideally the system delivering the gene should be formulatable, non-immunogenic, non-toxic and have a low cost/risk ratio. It must reach target cells, and promote the movement of the DNA to the nucleus where gene expression takes place. Then the gene must be expressed in a predictable and controlled fashion.

The development of gene delivery systems has included both viral and non-viral vector systems to deliver the gene to the target cells. Viral vectors are constructed by extensively modifying the viral core DNA with the therapeutic gene without compromising the ability of the virus to infect the desired cells. These systems are most typically delivered locally to maximize the exposure of the system to the site of interest, and to minimize the immune response of the body to the viral invasion. Nonetheless, research continues in the area of developing viral vectors appropriate for systemic delivery.

Non-viral vectors consist of complexes made with the naked DNA gene structure and other materials such as lipids or surfactants. These are engineered to transfect the cells without the aid of a natural system, such as a virus. As one might expect, transfection by non-viral systems is less efficient than the transduction of viral vectors. However, researchers realize the exploration of non-viral vectors is merited due to the risk of exaggerated immune response possible with viral delivery systems.

Therapeutic applications of gene therapy in animals are currently limited. However, the most active areas have been applications of nucleic acid probes or recombinant DNA products for clinical or diagnostic use in veterinary medicine.[85,86] As the knowledge of the human genome has grown, hormones, growth-stimulating factors, enzymes, cytokines and immunomodulating proteins have all been cloned from the human genome for use in veterinary medicine.

V. Summary

Controlled release drug delivery in companion animals covers not only all the different types of dosage forms that exist in the overall body of medicine, but also unique delivery systems in the area of estrous and parasite control technologies and topical antiparasiticides. Differences in anatomical and physiological characteristics of the individual animal species have to be considered in the dosage form design, in addition to product stability, reproducibility in processing, and other stringent regulatory requirements. Many technologies have already been developed to overcome the challenges associated with the delivery of drugs to companion animals and numerous examples of innovative controlled release drug delivery systems exist in the literature with more available for future exploration.

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CHAPTER 10

Controlled release vaccines in veterinary medicine

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Overview

Introduction

Infectious diseases cost livestock producers billions of dollars in losses annually. Prevention of infectious diseases is also a primary source of veterinary visits for companion (dogs, cats, horses) animals. Without a good vaccination program, companion animals would suffer many serious infections. Successful vaccination depends on the vaccine not causing disease or negative side effects in the host while engendering

an immune response capable of protection against the natural pathogen. This can be achieved by using an attenuated or modified live form of the pathogenic organism. Inoculation of a modified live organism is intended to induce an asymptomatic infection with complete recovery. This in turn results in a long-lived, protective immune response. However, in many cases attenuated forms of the pathogen have not been obtained, or may be unstable and revert to the virulent form. This results in some unacceptable side effects of infection.[1–5] Consequently, for diseases where an attenuated vaccine is not available, many strategies have been employed that use killed organisms or parts thereof as components of protective vaccines.

The major advantage to an inactivated vaccine strategy is safety, in other words, zero risk of infectious disease being induced. This strategy has been the basis for many traditional vaccines that have been successful for many, but not all, infectious diseases. Killed organism or subunit vaccines have their own unique set of difficulties that need to be addressed if they are to be successful. Their greatest disadvantage is that such vaccines do not replicate in the host and as such are not as immunogenic as their attenuated counterparts. With the ever-growing advantages of biotechnology, the cloning of genes, and the increased ease of identifying protective immunogens, the basis for extremely safe, highly specific vaccines is now possible. Unfortunately, these new antigens are not always highly immunogenic, therefore, there is a growing need for better adjuvants as well as better methods of delivery that enhance the effectiveness of these new generation vaccines. Consequently, delivery techniques and formulations that overcome these difficulties have become an increasingly important topic of investigation.

The biotechnological revolution in vaccines has resulted in many novel methods of delivering vaccines using recombinant vectors that can colonize a host, and remain viable while expressing the subunit vaccine in vivo. Alternative approaches that produce pathogens genetically altered so as to render them non-pathologic have also been used as vaccines that generate protective immunity in the host without causing disease.[6–8] There are also particulate representations of bacteria (ghosts) and virus like particles in which soluble subunit antigens can be delivered in a manner that avoids the risks associated with a viable vaccine. At the same time they present the antigen in a manner that is more similar to the way in which it would be normally encountered by the host immune system.[9–10] While many of these molecular techniques of vaccination can be considered unique vaccine delivery systems, they have been reviewed elsewhere[11] and will not be discussed in this review. Rather, this review will address the delivery systems based on polymeric encapsulation or co-administration that assures sustained release of antigen, and the induction of a protective immune response.

I.A. The need for delivery systems for veterinary vaccines

As our knowledge of the immune system and its interaction with pathogens has increased, it has become possible to increase the efficacy of non-replicating vaccines. This has been accomplished by tailoring adjuvant and delivery formulations to specifically induce the specific mechanisms of immunity (mucosal, cell mediated, opson-

sizing or neutralizing antibody responses) necessary to engender protection. In addition, all new delivery systems or adjuvant formulations applied to veterinary medicine must address safety and practicality issues that pertain to food and/or companion animals. These issues include the need to vaccinate large or free ranging herds of animals, especially cattle, before they are exposed to multiple pathogens, in an economical manner that does not require individual handling of each animal. There is also a need to vaccinate young animals in a manner that overcomes vaccine interference due to high levels of maternal immunity (maternal interference). Such a method would avoid the need for multiple vaccinations over an extended period of time in order to assure that the animals are vaccinated and active immunity is induced at the exact time passively acquired immunity wanes. There is a growing desire to decrease adverse side effects of vaccines at the site of injection such as fibrous reactions that degrade prime cuts of meat, or the vaccine associated sarcomas in cats.[12] There is also a concern that repeated vaccinations may induce adverse immune diseases in companion animals that may have high susceptibilities to allergic reactions. There are diseases (rabies and lyme disease) in wildlife that public health officials would like to be better controlled, and that will most likely require novel methods of delivery. There is a growing need to have vaccines that control allergies, perhaps by inducing immune tolerance, and also vaccines for sterility/fecundity. Finally, the veterinary profession should take advantage of therapeutic cancer vaccination strategies that are currently under development for humans, and determine if they may be useful in (aging) companion animals.[13–15]

The successful application of vaccines to address these issues will depend greatly on the method of delivery as well as the antigen preparation. One of the areas where this is especially important is in ease of administration, especially to a large number of animals. How can large herds of cattle or swine not only be vaccinated, but boosted in a way that is timely and does not interfere with the overall production of the animals? Many vaccines are effective if given by injection at least two to three times. Unfortunately, management of livestock in large confinement operations does not make it easy to handle animals to accomplish this. Vaccines that are long acting, pulsatile, have timed release, or that can be administered in a way that requires minimal handling, are desired.

There are many exciting novel technological strategies for the delivery of vaccines. These include the use of virus like particles, bacterial ghosts, naked nucleic acids, viral and bacterial vectors to deliver vaccine antigens. Live vectors/organisms can overcome the disadvantage of live vaccines while retaining the positive aspects. These methods have been reviewed previously.[11] This review chapter will address advances in delivery systems that have the greatest potential for improving the efficacy of veterinary vaccines. These vaccines address issues such as ease of delivery (sustained, pulsatile release of antigen), specificity of desired protective immune response (humoral versus cell mediated, or Th 1 versus Th 2 responses), safety, and a desire to induce mucosal immunity.

I.B. The need for delivery of vaccines to alternative (mucosal) sites

Oral administration of vaccines is obviously one method of vaccination that reduces handling by administering vaccines through the feed or water. This method of delivery is not only labor efficient, and less stressful to the animals, but has another advantage required of new generation vaccines: the ability to induce mucosal or local immunity to infectious organisms. Although most infections begin at mucosal sites, most vaccines have been administered parenterally to induce circulating antibodies that do not necessarily cross to all mucosal sites. Induction of immunity at mucosal sites can prevent invasion of the host, the serious pathology associated with infections due to the release of inflammatory cytokines, and the life threatening effects that microbial endotoxins and exotoxins may have on the host's organ systems.

All pathogens must first gain access to their preferred site of colonization in order to establish infection and cause disease in the host. Most pathogens can be prevented from causing disease in the host by a potent mucosal immune response that prevents the pathogen from either penetrating or colonizing mucosal tissue.[16] However, despite the desirability to induce protective mucosal immune responses against many pathogens, the practical difficulties of mucosal vaccination have minimized the success of mucosal immunization. The major barrier to inducing mucosal immunity is delivery of safe vaccines to the mucosal site. The need for good restraint and individual administration to a large number of animals often makes such delivery impractical. Fortunately, all mucosal sites are interconnected by a common mucosal immune system (CMIS). In this system, administration of antigen to an inductive lymphoid tissue site, e.g. the Peyer's patches in the intestinal tract, bronchus or nasal associated lymphoid tissues, stimulates the production of antigen specific lymphocytes. These lymphocytes enter the blood, circulate through the body, homing back to the site where they were induced. However, a significant number of these lymphocytes also migrate to other mucosal sites in the body. These lymphocytes can then respond to infectious disease organisms at mucosal sites distant from where the immune response was induced. This means that the oral, intranasal, or aerosolization of vaccines can result in an immune response at other mucosal sites in the body.[17–20]

Although mucosal administration of antigens is beneficial to the host, there are obstacles to be overcome to induce an effective response. Mucosal surfaces are coated with sIgA to exclude pathogens or potentially damaging antigens or toxins. Nonspecific factors (mucus, lysozymes, ingesta that can bind or degrade peptides, gut motility, intestinal microflora) can degrade antigens, or prevent uptake and processing of antigens. Orally administered vaccines must be protected from the low pH and digestive enzymes of the stomach enroute to the Peyer's patches in the small intestines. These factors must be overcome to successfully deliver antigen to the inductive lymphoid tissue and antigen presenting cells where the immune response is initiated. Delivery mechanisms are needed to address this issue.

One alternative for mucosal vaccination with inactivated antigens is to encapsulate the vaccine in microparticles (MP, described below). Orally administered MP are taken up by Peyer's patches and the CMIS stimulated to induce an immune response at distant mucosal sites.[20–21] Intranasal administration of antigens in MP, or formulated

with mucosal adjuvants induce very good immune responses at distant mucosal sites in a manner not yet fully understood. Antigen encapsulated within MP (polylactide-co-glycolide (PLG), alginate, ISCOMs, or liposomes) has been administered orally and successfully induced mucosal immune responses.[22–25] Many studies have been described using PLG MP, primarily because of the reasons discussed below which detail the advantages of using aliphatic esters as vehicles for parenteral immunization (discussed later). Many vaccines of human interest have been tested with these MP following oral administration in laboratory animals. However, limited studies have been carried out using antigens of commercial veterinary interest for oral administration. Many of the ones that have been studied are discussed below.

I.C. Adjuvant-like quality of delivery systems

One of the most critical components of a parenterally administered vaccine that can affect its efficacy and method of delivery is the adjuvant. It is not the intent of this chapter to provide an extensive review on the field of adjuvants (this has been recently reviewed[26,27]). Instead, this chapter will attempt to address advances in the field that will allow inactivated vaccines to be successfully used to the greatest advantage of veterinary medicine. Several distinct classes of adjuvants have been used in the field of veterinary vaccines. Adjuvants are considered to exert their effects through one or both of two basic mechanisms of action.

In the first case, it is necessary that adjuvants physically present the vaccine to the immune system. As such, adjuvants need to associate with the vaccine, physically retaining it in high concentrations. This ensures that the components of the immune system are exposed to the vaccine in sufficiently high levels and for a sufficient period of time that a significant immune response is induced and allowed to mature. This property, usually referred to as a 'depot', is achieved by the adjuvant adsorbing, entrapping or aggregating the vaccine antigen so that it is particulate, and may be exposed to the immune system over an extended time period. An important advance in the concept of vaccine depots that will be discussed in more detail is the use of adjuvant formulations that target vaccines to specific areas within the body so they induce the appropriate aspects of the immune response that mediate protection against the intended pathogen.

The second aspect of the adjuvant mechanism of action is the ability to quantitatively and qualitatively direct the immune response that is engendered to the incorporated vaccines. As such, many adjuvants contain immunostimulatory molecules that are obtained from plants or pathogenic classes of organisms. Some of the cellular components of the immune system respond to the immunostimulatory components of the adjuvant by elaborating various cytokines and chemokines that change the overall quality of the immune response such that some aspects are enhanced while others are down-modulated. As our knowledge of the host's interaction with particular pathogens has increased, so has our ability to qualitatively influence aspects of the vaccine induced immune response that will enhance its protective capacity. Some of these immunomodulatory alterations pertaining to specific usage with delivery systems will be discussed in more detail later in this review.

II. Vaccine delivery methods

What makes a good vaccine delivery system? In the context of this chapter, any chemical structural formulation that enhances the immune response by one of two basic mechanisms would qualify. The first mechanism is to enhance the long-term release of antigen to eliminate or reduce the need for subsequent inoculations. The second and not necessarily exclusive mechanism, is to provide a unique method of administering vaccines so that they stimulate an aspect of the immune system that is very effective at protecting against the intended disease. Of paramount interest here is the use of polymer encapsulated antigens for the controlled delivery of antigens. Since the realization that adjuvants enhance immune responses to vaccines, there has been interest in ways to reduce the number of vaccinations needed to induce protection without the undesired side effects of injection site irritation (for example the severe granulomas and abscesses induced by Freund's complete adjuvant). Polymers have been studied for nearly 20 years as a means to deliver antigens in a controlled manner. A variety of different polymer formulations have been investigated as solid implants, microparticles, or soluble mixtures with antigens, some of which form a gel when injected into the body. Each of these methods of polymer delivery systems will be discussed in this review with respect to their application to sustained release vaccines. It will also address the need for alternative sites to subcutaneous injection for the delivery of vaccines.

II.A. Implants

II.A.1. History and overview

One of the earliest described polymeric systems for sustained antigen release was a non-degradable ethylene vinyl acetate copolymer implant that contained antigen.[28] Antigen was encapsulated in a solid phase polymer that was implanted under the skin by surgical implantation, or by injection through a large bore needle. A single administration of antigen encapsulated in such an implant induced an immune response comparable to that of antigen adjuvanted with Freund's complete aduvant (FCA). Several model antigens were encapsulated in ethylene vinyl acetate implants including bovine serum albumin (BSA), gamma globulin, and ribonuclease (proteins of different molecular weights from 14 to 158 kDa) each successfully inducing an immune response in mice. This study was important because it demonstrated the feasibility of implants to deliver vaccines in one dose. However, it also showed the limitations of such a system – being non-degradable the implant would have to be removed at some point. Long-term safety and host response to the implant were concerns along with the issue of maintaining sterility of the implant (both during its preparation, as well as while in the body of the host). Since that time, many other formulations have been investigated. In the text below we have attempted to discuss those with veterinary relevance as shown by research for veterinary pathogens or antigens of interest, as well as some that have shown good potential in human studies and which could be applied to veterinary related antigens.

Another advantage of vaccine implants is that they can be designed so that they do

not require booster immunizations (self-boostering vaccines). Although vaccines are advantageous to producers because they reduce the need for antibiotics to control infectious diseases, repeated immunizations are required to re-expose the immune system to an antigen to further enhance the magnitude and avidity of an immune response. This boostering leads to a faster acting and more effective immune response when the host is exposed to an infectious agent or the inciting antigen (in the case of sterility vaccines, or an allergen). Parenteral administration remains the most commonly used route of administration for both companion and food animal species. In most instances repeated immunizations are necessary not only to induce the appropriate levels of protective immunity but also to overcome the negative interference of maternal immunity as well.[29] However, in many cases it is logistically impractical to individually handle animals for repeated immunization (for example, beef and swine operations in the USA, or range cattle and sheep in Australia). It is laborious and therefore expensive to handle large numbers of animals repeatedly. The handling can also stress animals, increasing their susceptibility to diseases, and decreasing their performance.[30] Therefore, a single injection technique with controlled or sustained release of antigen so that it is the equivalent of primary and booster vaccinations is desirable.

Although the prospect of a vaccine that would slowly release antigen over time to induce and maintain an immune response is an exciting innovation, it is questionable as to whether it is a reasonable goal. Simply exposing the immune system to an antigen is not guaranteed to induce an immune response. In fact, if the antigen is soluble and no adjuvant is present, the result may be induction of specific immune tolerance.[31] Animals exhibiting antigen-induced tolerance do not mount an effective immune response to the antigen usually due to anergy within the T cells. Without the appropriate T helper cells, antigen specific B cells will not produce antibodies (see Ref. 32 for a review of immune tolerance). Consequently, single shot vaccine delivery vehicles that are designed to deliver long continuous infusions of a soluble vaccine (for example a single recombinant protein antigen of a virus) primarily by diffusion from a polymer matrix may induce a tolerant state within the animal. In contrast, particulate antigens are much less likely to induce immune tolerance. Thus one advantage of combining an adjuvant with an antigen is that it will aggregate the included antigens thereby ensuring that they are more particulate in nature.

There are contradictory experiments regarding this phenomenon of immune tolerance by soluble antigens. The early work of Dresser,[33] reported that repeated administration of small doses of soluble bovine gamma globulin induced immunological tolerance. These findings were extended in studies in which antigen was administered by an osmotic pump to continuously deliver TNP-KLH over a period of 7 days.[34] In this case the animals had significant suppression of the IgG, but not the IgM component of the immune response. This suggested there was a lack of specific T cell help. However, more recent work with the sustained release of antigens from polymer matrices, cholesterol implants or osmotic pumps have demonstrated potent induction of IgG immune responses to soluble antigens such as BSA.[28,35,36] This suggests that sustained delivery systems not only have a place in vaccine technology, but their

application as a single immunization vehicle offers significant advantages over classical adjuvant formulations. Examples of veterinary significance are discussed below.

II.A.2. Cholesterol: lecithin implants

Recently a cholesterol:lecithin implant system has been tested in sheep using recombinant pilus antigens from the etiologic agent (*Dichelobacter nodosus*) for footrot in sheep.[37] Sheep vaccinated with these implants had increased serum antibody titers. The implants were made by compressing antigen mixed with the cholesterol and lecithin along with an adjuvant, in this case, Quil A. Methodology such as this is advantageous in that the technology is straightforward. It does not depend on chemical linking of components but rather on a mixture; no harsh chemicals are used that could affect the immunogenicity of the antigens. In addition most adjuvants could be incorporated in the implant along with the antigen. It is thought that the antigen is released by diffusion from the implant. In regard to how the immune system would process such a soluble antigen, it is interesting to note that there was a better immune response to implants that contained both antigen and an adjuvant. This methodology shows promise although longer retention of antigen within the implant and slower release would be desirable in order to induce long lasting immunity. Further refinement of the formulation is likely to make this possible.

II.A.3. Atrigel™ formulations

Biodegradable implants composed of copolymers of aliphatic polyesters, such as PLG have been frequently described in the literature. This is because there is a wide variety of biocompatible aliphatic esters whose controllable molecular weights lead to predictable degradation rates in aqueous environments. The proprietary Atrigel™ formulations of Atrix Inc. (Fort Collins, CO) are aliphatic esters dissolved in biocompatible solvents that can be mixed with antigen. In this state they are stable, liquid gels, that can be administered to the host as syringable vaccines. When such vaccine formulations are injected into the aqueous environment of the host tissues, the solvent dissipates and the aliphatic ester precipitates. The incorporated vaccine included in the formulation becomes entrapped in the precipitated polymer and is slowly released as the polymer degrades.[38] As described previously, candidate polymers for the Atrigel™ delivery system include the polylactides, polyglycolides, and their co-polymers. These water-insoluble aliphatic esters contain functional groups within their chemical backbone that are hydrolyzable and thus facilitate a decrease in polymer chain length until it eventually becomes water-soluble. Consequently, the biodegradation times and therefore the release rates of incorporated vaccine are defined by varying the chain length of the constituent polymers.

We have previously described our pilot experiments with Atrigel™ polymers incorporating ovalbumin (OVA) as the antigen that were performed in mice and swine.[11] These studies showed that single injections of Atrigel™ polymers based upon polylactides or polyglycolides could effectively immunize swine to produce IgG responses against small amounts of incorporated OVA. Furthermore, the profile of the immune response was greater and more sustained than that seen with classical adjuvants (Fig. 1), peaking quickly and then maintaining higher titers than an adjuvant used in

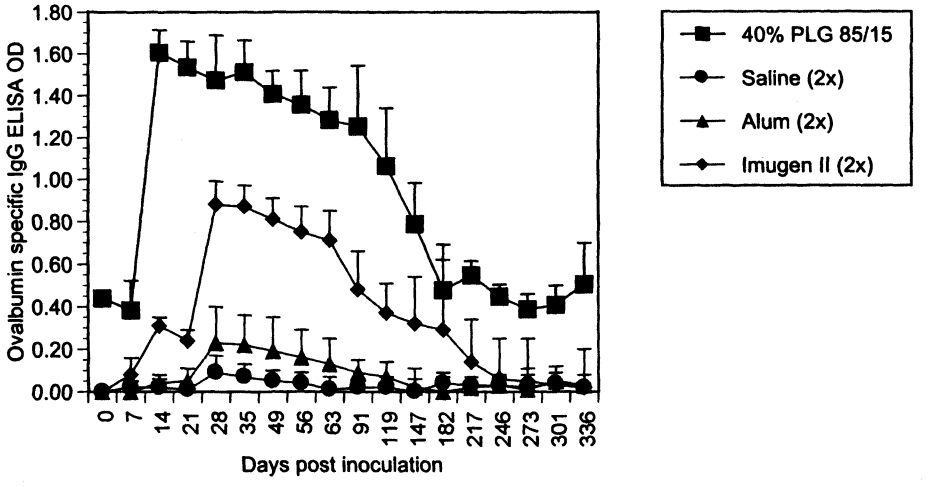

Fig. 1. Serum IgG to OVA in swine immunized with different adjuvant formulations. Groups of four pigs were vaccinated once with an Atrigel™ formulation (40% PLG 85/15) at day 0, or twice with conventional adjuvant formulations (day 0 and 14) containing 1mg of OVA. Animals were bled at frequent intervals thereafter and the separated serum stored at − 20°C. After the study had been completed (336 days) all of the serum samples were analyzed in an ovalbumin specific ELISA (at a 1:25 dilution of serum) for the presence of IgG antibodies. The data is shown as the mean of four individual animals together with the standard deviation.

commercial swine vaccines (Imugen™ II, or Alum). Subsequent experiments have demonstrated that Atrigel™ polymers can deliver complex vaccine antigens such as inactivated pseudorabies (Aujeszky's disease virus) virus (PRV) vaccines and canine parvovirus.[11] We have not investigated the mechanism of action of the Atrigel™ polymers any further but our data indicates that a substantial portion of the entrapped antigen is released immediately after implant formation while the remainder is slowly released for the duration of the implant's existence. Experiments to date would suggest that Atrigel™ formulations have considerable promise as single administration vehicles for soluble antigens and inactivated virus vaccines in food animals. However, the current use of water miscible solvents precludes their use for more labile antigens such as gram negative bacterins. Further investigation into more compatible solvents is likely to overcome this problem.

Similar release profiles have been described for other successful implanted vaccine delivery devices.[28,35] Overall, these data suggest that devices capable of the controlled release of inactivated vaccines cannot only compete with the classical adjuvant formulations, but may also offer significant advantage if used as single administration, biocompatible implants. As our knowledge of the effects that different antigen delivery profiles have upon the quality of an induced immune response increases[36] it will become possible to transfer the wealth of pharmacological knowledge available for controlled drug delivery to more effective as well as convenient vaccine formulations.

II.A.4. Needleless implants

A novel implant delivery system for sustained release vaccines is comprised of a

compressed tablet containing the antigen and sustained release materials encased within a biodegradable and biocompatible food additive shell.[39] What makes this system unique is that it is designed for needleless administration. Animals can be vaccinated using compressed air to propel the implant into the animal from a distance of 3–6 m. This would permit vaccination of a large number of animals without the need for individual handling by forcing them through a chute where they would be stressed. The implant itself would then deliver a vaccine that would not need to be boostered. The system has been evaluated and shown to induce minimal tissue damage, and the implant wound quickly heals. This system additionally has the potential to reduce injection site reactions, a serious problem with injectable vaccines. Studies have shown promise for this system in administering a contraceptive vaccine to horses.[40] The needleless implants represent an improvement over the use of the tableted vaccine described previously[41] using rinderpest virus antigen and administration by way of a device designed to administer hormonal implants in the subcutis (see Section II.A.5.).

II.A.5. Tablets

An earlier implant method developed for the administration of modified live Rinderpest virus to cattle has been described.[41] Rinderpest is more stable at room temperature and atmospheric conditions when dry. Thus, a tablet would be a good way to store the vaccine. The virus was stable and viable in the tablets for at least 70 months post-preparation. The tablets were inoculated into cattle by subcutaneous implantation using a tool made for hormone tablet implantation. Seven of eight cattle responded to the vaccine with demonstrable protection from challenge by 15 days following vaccination. Further studies were not done to determine how long immunity from administration of one tablet would last in cattle. Since in this case a live virus was the antigen, and assuming the virus was viable upon administration, it is likely that immunity would have been long-lived.

II.B. Microparticle delivery systems

Microparticles have been used to deliver pharmaceutical products either in a time release fashion or as a sustained release formulation.[42,43] It is a logical extension that they be used to deliver vaccines. The primary goals for using microparticles have been to reduce the number of injections or administrations needed to induce protective immunity, but when engineered appropriately they can be used as oral delivery vehicles. Microparticles can be made to both release the encapsulated material in a sustained manner, but also can incorporate materials that enhance the immune response. Many materials used to make microparticles are either biodegradable or biocompatible, and ideally, both.

II.B.1. Lipid based microparticles

II.B.1.a. ISCOMs Immunostimulatory complexes (ISCOMs) are small, stable cage-like particles (30–40 nm in diameter) made of cholesterol, phospholipids, and immu-

nomodulatory extracts of the Quillaja saponins (Quil A).[44–45] A vaccine antigen can be added in the production of these particles without an undue effect upon ISCOM size or function. ISCOMs induce a much more expansive immune response than vaccines made with depot (oil or aluminum precipitation based) adjuvants. ISCOMs are reported to have several advantages over conventional vaccine formulations, both as a delivery system and an adjuvant formulation. For example, a 1 μm dose of antigen induces a 10-fold greater antibody response than other adjuvanted vaccines.[44–45] Also, ISCOM vaccines have been shown to induce immunity that lasts for longer than 1 year.[46]

Although ISCOMs are not frequently used as an adjuvant for veterinary vaccines, they have undergone extensive investigation for veterinary applications (see Ref. 11, and Table 1). ISCOMs are currently used in a commercial equine influenza vaccine that has proven to be effective.[47] Ponies vaccinated with inactivated equine influenza virus or purified haemagglutinin (Equip™) encapsulated in ISCOMs, had fewer days of fever, less coughing, greater titers of serum antibodies, and shed less virus than non-vaccinated ponies.[47,48] In addition, antiviral immune responses were maintained for 15 months, a much longer duration than that for responses induced by conventionally adjuvanted whole virus vaccines that required booster vaccinations as frequently as every 3 months. This is a very demonstrable advantage of this vaccine technology, avoiding multiple vaccinations in a host that can be difficult to handle and distrustful of needles. More recently, extracts of bovine viral diarrhea virus (BVDV) have been incorporated into ISCOMs to produce a parenteral bovine vaccine, and for which, field trials have been reported to be performing well.[49] Like influenza in horses this is a disease for which a safe, effective vaccine is needed. The BVDV ISCOM vaccine is a subunit preparation and therefore safety, particularly in gestating animals is inherent. This is an important issue as modified live BVDV vaccines can cause abortions, fetal abnormalities, or tolerance in calves making them susceptible to a virulent different strain of virus which is more likely to cause severe disease in susceptible animals.

II.B.1.b. ISCOMs and oral delivery ISCOMs address another issue in vaccination protocols, and that is ease of administration. Many reports in the literature have demonstrated that ISCOMs are effective when administered orally. Not only does this avoid injection and associated side effects (pain, swelling, possible abscessation or fibrosis), but induces mucosal immunity as well. Orally administered antigens encapsulated in ISCOMs prime CD4 T cells that release their corollary cytokines and support induction of IgG1 and IgG2a antibody responses similar to those detected when ISCOM vaccines are administered parenterally. Mice fed OVA ISCOMs had detectable cytotoxic T cells specific to OVA in their spleen, and increased OVA specific intestinal secretory IgA and serum IgG antibodies. None of which were found in mice fed unencapsulated OVA.[50] The priming of Th1 and Th2 responses may be indicative of the excellent adjuvant activity of ISCOMs.[51] Chickens developed serum antibodies to Newcastle virus encapsulated in ISCOMs and administered orally.[52] Glycoprotein or nucleoprotein subunit vaccines to influenza virus induced very good protective immune responses in mice and chickens following oral administration of the subunit vaccine in ISCOMs.[53,54] ISCOMs are

Table 1
Delivery systems and antigens delivered for use in target species

Organism	Target host	Delivery				
		ISCOM	Liposome	Microparticle	Macroparticles	Implant
Viruses						
Newcastle virus	Chickens	148	182			
Rabies virus		149	183,184	202		
Equine influenza virus	Horses	47,48				
Avian influenza virus	Poultry	150,151[a]	185	203–205[b]		
Equine herpes virus	Horse	152,153				
Pseudorabies virus	Swine	154,155				11
Infectious bovine rhinotracheitis virus	Cattle	156	65,154,186	112		
Feline leukemia virus	Cats	157				
Bovine leukemia virus	Cattle	158	187			
Canine distemper virus	Seals (phocid), dogs	159,160				
Bovine viral diarrhea virus	Cattle	161–163				
Bovine adenovirus	Cattle	164				
Feline immunodeficiency virus	Cats	165,166				
Foot and mouth virus	Ungulates		188–190			
Reovirus	Many			206[a]		
Rotavirus	Swine, cattle, horses	167		207–209[a]		
Venezuelan equine encephalitis virus	Horses			210		
Parainfluenza-3 virus	Cattle			82		
Bovine respiratory syncitial virus	Cattle		191			
Bacteria						
Mycoplasma gallisepticum	Chickens	56,168	192–195			
M. hyopneumoniae	Swine				137	
M. mycoides	Cattle	169				
Rhodococcus equi	Horses	170				
Francisella tularensis	Cats	171,172				
Clostridium tetani	Horses	48		211–213[a]		
Salmonella	Poultry, cattle, swine	173	196,197	95,96	24	

Table 1 (*continued*)

Organism	Target host	Delivery				
		ISCOM	Liposome	Microparticle	Macroparticles	Implant
Staphylococcus spp.	Cattle		198			
E. coli	Cattle, swine		57			
Pasteurella haemolytica	Cattle				100	
Pasteurella multocida	Rabbits			121		
Dichelobacter nodosus						37
Actinobacillus pleuropneumoniae	Swine	69				
Vibrio anguillarum	Fish				216	
Renibacterium salmonaium	Salmonids			139		
Protozoa, worms, etc.						
Neospora caninum	Dogs	174				
Trichinella spiralis	Swine	175				
Echinococcus granulosis	Dogs, sheep	176,177				
Boophilus microplus	Cattle	178				
Toxoplasma gondii	Sheep, goats	179,180	199			
Ascaris suum			62			
Nippstrongylus brasiliensis			63			
Fasciola giganticum	Sheep			97		
Fasciola hepatica	Cattle			214		
Theileria annulata	Cattle	181				41
Contraception						
LHRH	Dogs			93,94		
Zona pellucida	Horses					40
Nucleic acids						
	Mice		67,200,201	118,209,215		

[a] Could be applied to animals although intent of studies is not directly for veterinary usage.
[b] Could also be applied to other animal species – horses and swine.

also described as being excellent for the induction of immunity to parasitic diseases. An orally administered subunit vaccine for *Eimeria falciformis* reduced oocyst excretion, weight loss, and incidence of death in mice.[55]

ISCOMs have also been administered by other mucosal routes. Chickens have been vaccinated intranasally (IN) and ocularly with ISCOMs containing subunit antigen of *Mycoplasma gallisepticum*.[56] This approach may be useful to consider for the prevention of reproductive diseases in cattle, swine, and companion animals. ISCOMs containing cell wall components of a K88 piliated, heat labile toxin subunit B, toxigenic *E. coli* from pigs were shown to induce antigen specific serum antibodies when administered orally to pigs.[57] *Enteric colibacillosis* is an economically important disease in cattle and swine. An effective vaccine to reduce the incidence of this disease would have a major impact on food animal production.

ISCOMs represent a major improvement in the formulation of veterinary vaccines, as they overcome many of the deficiencies described for current vaccines. These include the lack of induction of cell-mediated immune responses, poor local immunity, poor adjuvant activity and the need for frequent booster immunizations. Consequently, as shown in Table 1, antigens from a variety of animal pathogens including viruses, facultative and obligate intracellular bacteria, arthropods vectors, protozoa, helminths and trematodes have been incorporated into ISCOMs, and tested experimentally in vivo in the target host or laboratory animals.[11]

Although vaccines using ISCOMs have yielded very encouraging results, increased commercial application will require that several issues be addressed. These include the cost of production, injection site reactions (although no adverse reactions were seen in horse studies[46–48]) and, although ISCOMs are thermodynamically stable, they are chemically unstable (the saponin component breaks down with time). In addition, some saponin preparations are toxic for the host. The toxicity of the Quil A can be reduced by oral administration compared to administration by other routes. The QS21 fraction of Quil A has been investigated as a less toxic derivative of Quil A.[58] Hydrophobic antigens can be readily incorporated into the lipophilic ISCOMs. However, hydrophilic antigens must be chemically modified to expose hydrophobic groups or hydrophobic groups must be linked to the proteins. This has been accomplished but does not always result in successful induction of a protective immune response, perhaps due to modification of immunogenic epitopes of the antigens.[58,59] As these problems are resolved, ISCOMs have great potential as an adjuvant and delivery vehicle for the next generation of subunit or inactivated veterinary vaccines.

II.B.1.c. Liposomes Liposomes are bilayered phopholipid particles consisting of phospholipids or sterols surrounding an aqueous solution. Liposomes can vary in diameter from 0.01 to 150 μm. Liposomes can be used to encapsulate either water-soluble material (in the aqueous center) as well as hydrophobic agents in the outer lipid layer. This permits the encapsulation of almost any type of antigen. It is thought that liposomes act as a depot of antigen, rendering soluble antigens particulate, and targeting delivery to antigen presenting cells in a manner similar to that for ISCOMs.[23] Liposomes readily adsorb to most mammalian cells where they are readily taken up into lysosomes leading to the release of antigen. They are also phagocytosed by

antigen presenting cells (APC) where they release their antigen.[60] Liposomes can interact with macrophages to induce antigen specific cytotoxic T lymphocytes (Tc) as well as responses dependent upon exogenous presentation of antigen, thereby enhancing the repertoire of cell mediated immune responses. There are many reports in the literature of liposomes used to encapsulate antigens of interest to both human[61] and veterinary diseases (Table 1).

The greater protective response seen with vaccines in which the antigen was incorporated into positively charged liposomes might be attributed to the preferential uptake of liposomes by APC. Not only charge, but size, composition and method of preparation can affect the delivery of antigens by liposomes. The increased uptake of liposomal vaccines by APCs enhances the delivery of antigen to draining lymph nodes thus improving the priming of T lymphocytes. Liposomes whose stability is sensitive to pH can fuse with endosomal membranes, release their antigen into the cytosol where it is processed to induce an class I MHC restricted Tc response. Liposomes can also be used to encapsulate multiple antigens thereby maximizing efficacy of immunization of both T and B cell epitopes. Their flexibility for encapsulating a variety of antigens, coupled with the safety, biocompatibility, biodegradability, and utility as both parenteral and oral vaccines make liposomes an excellent candidate to replace standard adjuvants/delivery formulations.

There are many examples of veterinary applications using liposomal vaccines for encapsulation of viral, bacterial, and parasitic antigens. In the examples described briefly here, it is interesting to note that organisms for which cell mediated immune responses are required (viruses, parasitic worms, and fungi) are good candidates for encapsulation in liposomal vaccines. Pigs injected with crude extracts of *Ascaris suum* larvae encapsulated in liposomes were partially protected from migrating larvae.[62] Mice receiving solubilized surface antigens from adult *Nippostrongylus brasiliensis* encapsulated in liposomes had fewer adult intestinal worms after challenge.[63] Mice inoculated with a soluble antigen of the fungus *Cryptococcus neoformans* (the cause of neural infections in humans and animals) encapsulated in liposomes had increased cell mediated immunity as detected by an increased DTH response.[64] Liposomal vaccinated mice were also better able to clear organisms from tissues than were control mice.

One advantage of delivery systems for vaccines is the capability to coadminister immunomodulators with the vaccine antigen. Liposomes have been used to co-encapsulate cytokines with antigens. Interleukin-12 (IL-12) encapsulated in liposomes along with a glycoprotein of bovine herpes virus (BHV-1) stimulated increased antigen specific IgG2a antibodies and IFN-gamma secreting cells when administered to cattle.[65] Alternatively, the co-encapsulation of IL-4 with the glycoprotein increased antigen specific IL-4 secreting cells. This study showed that the desired final immune response could be manipulated by co-encapsulated cytokines to induce either a TH1 or TH2 biased response.

Liposomes with a positive surface charge can be targeted to antigen presenting cells leading to a greater immune response. Positively charged liposomes are taken up by cells to a greater degree than neutral or negatively charged liposomes. Such liposomes have been found to induce better inhibition of tumor cell growth than other liposomal

preparations.[66] Positively charged liposomes have also been used to transfect cells with naked DNA for vaccination purposes.[67] The better transfection of cells results in greater expression of the foreign protein leading to an enhanced immune response.

One disadvantage of liposomes is that although they bind to and are efficiently taken up by phagocytes, they often remain in phagosomes and are degraded by lysosomes before the antigen load can be released to the cell. Acid sensitive liposomes containing the listeriolysin of the bacterium *Listeria monocytogenes* are taken up by phagosomes. As the liposomes break down, the listeriolysin causes the lysosome to become leaky releasing the antigen load. This results in ready access of antigen to the cytosol of the cell where it can enter the endocytic processing pathway.[68] This unique delivery system further enhances the potential liposomes for subunit protein vaccines that induce a Tc response.

II.B.1.d Liposome–ISCOM hybrid Liposomes and ISCOMs have also been combined to produce a hybrid delivery formulation. The advantage of such a formulation is that while hydrophobic antigens are readily encapsulated into ISCOMs, it is easier to encapsulate hydrophilic antigens into liposomes. The liposomes in turn can be encapsulated easily into the ISCOMs. This system is applicable for multiple antigen formulations such as subunit bacterial vaccines that often require more than one component to induce protective immunity. Chin et al.[69] used liposome–ISCOM hybrids to encapsulate the outer membrane proteins and LPS of the bacterium *Actinobacillus pleuropneumoniae* a pathogen that causes a bacterial pneumonia that inflicts serious economic losses in the swine industry. Mice vaccinated intradermally with the hybrid formulation had reduced clinical disease, and more active bone marrow haematopoiesis indicating a better capacity to fend off infection, than did antigen vaccinated mice. In addition the hybrid formulation induced greater serum IgG, M, and A antibody titers to the antigens.[69] The intradermal administration of antigen using this delivery system also resulted in a balanced Th1/Th2 immune response. Vaccination with unencapsulated antigen induced primarily a Th2 response that included recruitment of mast cells to the lung, a situation that could potentially induce more pulmonary pathology. This hybrid technology is a novel approach that takes advantage of the complementary strengths of the two different delivery systems.

Although encouraging in many respects, especially in regard to inducing protective immunity to helminths whose subunit antigens are difficult to produce, to date there are currently no commercial animal vaccines based on liposomes. The usefulness of liposomes for vaccine delivery is limited by their lack of stability and the use of organic solvents in their preparation that can adversely affect antigens. Once these factors are addressed it is likely that liposome formulations will find application in the veterinary infectious disease arena.

II.B.2. Polymeric microparticles

Currently there are no commercially available veterinary vaccines based upon microparticles, however, there are many reports in the literature documenting the great potential of this delivery method for the prophylactic control of veterinary

pathogens. The term microparticles (used interchangably here with microspheres, MS) is used here to describe an array of vehicles that include small particles (1–200 μm in diameter) consisting of the vaccine antigen and a carrier formulation. The distinguishing feature that separates this vehicle from ISCOMs is size. As mentioned above ISCOMs have an approximate size of 40 μm, whereas the microparticles described below are almost an order of magnitude bigger with sizes tending to be 1 μm and above. The various approaches to using microparticles as vaccine delivery vehicles share the goal of entrapping viable antigen in a small particle in a stable manner that can be safely delivered to the appropriate site of the host. The major premise for the pursuit of microparticles to deliver vaccines is the observation that antigens encapsulated in microparticles induce a much greater immune response than unencapsulated antigens. This is especially true for subunit antigens.[70,71] The immune response to antigens encapsulated in microparticles is equal to or in some cases, better than that seen using standard adjuvants such as alum precipitation or Freund's adjuvants.[72] The materials and techniques used to produce microparticles differ greatly from each other. Some of the more frequent approaches to vaccine delivery that have either been tested using antigens of relevance to veterinary medical needs, or have potential for use in veterinary medicine are described below.

II.B.2.a. Aliphatic polyester microparticles Copolymers of the polylactide and polyglycolide esters, as well as poly (ε-caprolactone) and their copolymers have been used to produce small biodegradable microspheres that act as depots for the delivery of vaccine antigens.[73] The polylactide and polyglycolide polymers have proven safety, biocompatibility, and biodegradation; as such their use has been approved by the FDA in humans for absorbable sutures, and as drug delivery vehicles.[74,75] Another important feature that makes them attractive as a sustained release vaccine delivery, is their well characterized hydrolysis rates which allow sustained antigen release over periods up to 1 year or greater.[76]

Particles are typically made by spray drying, coacervation, or an emulsion technique. Once made, the particles containing antigen can be lyophilized and are very stable. Antigen is dispersed homogeneously throughout the polylactide-co-glycolide (PLG) microparticles. This is potentially a major advantage of PLG microparticles since they could be used in situations where refrigeration is not readily available such as in developing countries or for vaccination of large groups of animals (on the range).

The production process is optimized to generate particles of particular sizes. This allows particles sizes in the 1–10 μm range, a size that is optimal for phagocytosis by macrophages and antigen processing cells. It also allows production of larger particles up to 125 μm in diameter that slowly breakdown in vivo, decreasing in size until they can be phagocytosed. This is important for the sustained release of antigen and the prolonged immunostimulation necessary for a single shot vaccine to induce long-lived immunity. Using PLG particles of different sizes can induce long lasting immunity. Phagocytes readily take up smaller particles resulting in a rapid release of antigen and an immediate immune response. Larger particles (20–125 μm) are less likely to be taken up immediately by macrophages and eventually break down or erode into

smaller particles that are then taken up and processed releasing antigen in macrophages. When these principles are used together, a short onset, self-boosting, long lasting immunity can result.[74,77–79] These principles are true regardless of the target host species. Monocytes and polymorphonuclear neutrophils of cattle and alveolar macrophages of swine have been tested for phagocytosis of PLG microspheres.[80,81] Microspheres with a diameter less than 10 nm in diameter were taken by these antigen-presenting cells of both species. It is important that in vitro tests confirm this activity as it is expected that uptake of particles is the first step in successful antigen delivery and in inducing an immune response. There is one report of dual pulse PLG microparticles being used to induce an antibody response in cattle that was greater than that induced by a conventionally prepared vaccine.[82]

Microparticles are commonly made with either polylactide, polyglycolide, or a combination of these esters (PLG) in different ratios. Furthermore, each ester is available in a range of different molecular weights. These parameters can be used to produce MP that hydrolyze at different rates. Long lasting immunity can be induced by the (parenteral) administration of MP made with different ratios of each ester, or with different molecular weights that hydrolyze (and hence deliver antigen) over a period of time. In general, the greater the ratio of lactide:glycolide, the slower the release of antigen, and the later the onset of an immune response. Typically, particles consisting of one polymer, either glycolide or lactide, hydrolyze more slowly than particles made of a combination of polymers. Poly(L-lactide) hydrolyzes at the slowest rate with complete hydrolysis by 18–24 months, polyglycolide by 12–16 months, 85:15 ratio of poly(L-lactide) to glycolide by 5 months, and 50:50 combination by 2 months. In one study it was found that particles with ratios of lactide:glycolide of 50:50, 85:15, and 100:0 had an increasingly later onset of immunity from day 50, 130, 230, respectively.[83] The onset of the immune response is directly related to the rate of biodegradation of MS made from these polymers. Since breakdown of these esters is non-enzymatic, the greater the molecular weight of the polymer, the slower the hydrolysis of the polymers as well.

Other methods have also been used to alter the release of antigens from PLG MS. The concentration of antigen affects the rate of antigen release and subsequent induction of an immune response. The greater the antigen load, the faster the rate of antigen release due to the presence of more antigen nearer the surface of the MP.[71,82] The controlled release of antigen from PLG MS depends on many variables (ratio of polymer, particle size, loading of antigen, hydrophobicity, etc.). These are interrelated so that it is possible to generate many combinations of formulations of MP to achieve a long lasting immune stimulation. In this manner one injection of a vaccine can result in long lasting immunity obviating the need to booster animals. This is important for vaccination of livestock where the repeated administration of vaccine to a large number of animals is logistically challenging, labor intensive, stressful, and therefore counter productive for the efficient production of animals.

An important factor in developing a sustained release vaccine is the concept that antigens presented to the host in a manner that mimic natural infection will stimulate the most effective immune response.[85] The optimal method of antigen release has not been determined, that is, whether it is more desirable to have a pulsatile antigen

release or a continuous release of antigen over time to induce a long lasting immune response. In general, this relates to a large exposure to an antigen followed by a decreasing level over time often with subsequent larger exposures at a later time. The general assumption is that a pulsatile administration of antigen is optimal to mimic natural infection, and to avoid induction of immune tolerance by the continual administration of antigen at a low level. However, some studies have shown long lasting high titers with continuous antigen release, apparently avoiding induction of immune tolerance.[86] One approach to produce a timed burst release was achieved by engineering PLG MS to release at different times. This not only achieved a pulsatile antigen delivery, but a more efficient delivery of antigen to antigen presenting cells.[87] This approach addresses not only how the antigen is received by the immune system, but also the practical implication of delivering most of the antigen to the site of induction of an immune response. More work is needed to resolve the importance of pulsatile release to the induction of a long-term immune response. The current methodologies for manufacturing MS formulations have the capability of inducing much longer lasting immunity than most vaccine formulations available to date.

One important aspect of using microspheres for vaccination is that they can be phagocytosed by antigen presenting cells such as macrophages of the veterinary species of interest. Phagocytosis by bovine cells in vitro was demonstrated using PLG microspheres of different diameters.[80] This study confirmed earlier work by Eldridge[70] that murine macrophages were better able to phagocytose particles less than 10 μm, than larger micospheres. This study also showed that antigen release could be modulated using microspheres of different sizes, with the small MS releasing antigen faster than the larger MS. A similar study was performed using alveolar macrophages from swine.[81] In this study alveolar macrophages were used to evaluate uptake of MS by cells present at a mucosal site. MS less than 10 μm were taken up equally well regardless of whether they were less than 5 μm or between 5 and 10 μm in diameter. These two studies are among the very few to evaluate uptake of a polymeric MS by cells from a target species of animals for which vaccines could be developed.

Many vaccine studies using PLG microspheres have been done in vitro and in vivo with microbial antigens of interest to prevent human infectious diseases, e.g. HIV, *E. coli*, and tetanus.[79,84,88,89] PLG microspheres have been administered in a variety of ways. Parenteral, oral, intratracheal, or intranasal administration of antigen encapsulated in PLG microspheres induces systemic or mucosal immunity in rodents as well as non-human primates.[22,89–91] Although no vaccines are currently on the market for use in animals or humans, this technology has been thoroughly evaluated for many veterinary applications, including the control of reproduction (via sterility) as well as to prevent diseases caused by bacteria, viruses, protozoa, and parasitic worms (Table 1). For example, the incorporation of luteinizing hormone releasing hormone (LHRH) conjugated to diphtheria toxoid has been described as a contraceptive vaccine for use in companion animals, with one injection inducing sterility that lasted 8–9 months.[92,93] This would allow owners to control estrus in pets and still have the option of breeding in contrast to the finality of a neutering surgery. It would also be beneficial to livestock producers because current methods of feeding animals of mixed sexes result in reduced efficiency of weight gain. Females in estrus have a negative impact on weight

gain and production of meat animals when sexes are mixed in a feeding regimen. This would eliminate the need for ovariohysterectomies for feedlot cattle or segregation of sexes of swine to optimize feeding regimens.

Enteric bacterial diseases not only cause disease in livestock but can also be transferred in meats, eggs, or milk to cause disease to humans. Some of these zoonotic diseases are caused by bacteria that are either resistant to antibiotics, or if treated with antibiotics, can cause more serious disease in humans. There is a growing need to control these organisms by vaccination of animals. One of the most common zoonotic foodborne pathogens is *Salmonella enteritidis*. This organism is found in chicken eggs, is transmitted to young birds through the eggs, and is found in broilers and chickens raised for meat. The search for an effective vaccine in poultry has included the use of PLG encapsulated antigens. *S. enteritidis* bacterin was encapsulated in PLG MS and administered to hens in an attempt to induce passive immunity in the yolk, consequently preventing infection in chicks. A preparation of microparticles comprising a ratio of 50:50 PLG and poly lactic acid (PLA) was effective in stimulating the immune system for 9 months.[94] This vaccine could be well received since food borne disease due to *S. enteritidis* is a growing worldwide problem. More recently antigens of *S. typhimurium*, another foodborne pathogen, as well as a cause of enteric disease in swine, cattle, and horses was encapsulated in PLG MS and administered orally to mice.[95] These mice had good intestinal sIgA titers and had the best protection from intestinal challenge by *S. typhimurium* when compared to other vaccines. Non-typhoidal *Salmonellae* are the most frequently isolated bacteria in foodborne disease, potentially making this type of vaccine extremely important if it prevented foodborne disease by decreasing colonization and shedding by food animals at slaughter.

There are many viral infections of animals that require vaccines not only to induce antibodies, but in many cases, cell mediated immunity is also important in prevention of disease. Formalin inactivated Venezuelan encephalitis virus (VEE) has been encapsulated in PLG MS as well. Mice vaccinated with encapsulated formalin inactivated VEE were better protected than mice vaccinated with unencapsulated virus.[96] Mucosal vaccination was as effective or more so than that induced by systemic administration of this vaccine. Such a vaccine may be useful to prevent encephalitis for horses as well as humans. Bovine parainfluenza 3 virus (PI3) has been encapsulated in PLG microspheres and administered as a subcutaneous (SC) injectable vaccine for cattle. In this particular study, one inoculation conferred high antibody titers for up to 70 days following SC inoculation, making this a more effective method of inoculation than intramuscular (IM) inoculation for inducing high antibody titers.[93] This is an important consideration as any adverse reactions to the SC vaccine could be easily trimmed at slaughter whereas abscesses or fibrosis in deep muscle lowers the value of the carcass. This virus is part of the respiratory disease complex, the greatest cause of economic losses in beef cattle in North America. Such a vaccine, if effective, would offer a great benefit to the health of cattle.

In many ways parasitic worms and protozoa offer the greatest challenges for vaccination. These organisms are difficult to impossible to cultivate in vitro making their study, as well as the preparation of modified live vaccines, extremely difficult. Subunit antigens have been cloned, but the complexity of the organisms makes the possibility

that one to two subunits can induce protective immunity unlikely. This sets a challenge for the vaccine formulation to deliver the antigens in a way that makes them as antigenic as possible, and in a way that optimizes the desired immune response (e.g. cell mediated or local immunity). One worm that has an economic impact on cattle and sheep industry is the liver fluke. Damage to the liver can threaten the life of the animal, but more importantly, it causes loss of the liver at slaughter – an important source of income to packers. The encapsulation of *Fasciola gigantica* subunit antigens into PLG MS demonstrated the feasibility of PLG MS for delivering subunit antigens from intestinal/systemic parasites of veterinary importance.[97]

The above examples are some of a growing number that demonstrate the efficacy of PLG microparticles in target animal species. Parenteral application is expected to be similar in efficacy to that demonstrated in rodent models with human antigens, for example, influenza virus, tetanus toxoid, as well as *Bordetella* and streptococcal antigens. Some of the infectious agent antigens successfully encapsulated in PLG MP that have potential for use in veterinary medicine are shown in Table 1.

Although there have been many studies evaluating the efficacy of mucosally administered infectious disease antigens encapsulated in PLG microspheres in rodents, there is much less information regarding delivery to host species of veterinary interest. The administration and uptake of microparticles is likely to vary between species due to differences in retention time in the stomach, intestinal motility, and total surface of area of inductive sites (Peyer's patches) versus total volume of the small intestines. A study in which the immune response to pilus antigens of *E. coli* was evaluated following oral or parenteral administration in swine was recently performed.[98] The pigs vaccinated by subcutaneous injection mounted an immune response to the antigen confirming that the antigen was released in a form to be immunogenic in the pig. However, uptake of particles was low even though 90% of the particles were less than 10 μm in diameter. This raises the question 'in smaller species such as mice, how well does the number of particles needed to induce an immune response following oral administration relate to other species where the relative number of Peyer's patches per area of intestine may be much smaller?' Furthermore, the number of M cells per Peyer's patch varies between species with rabbits and pigs having the greatest (50% of cells) ratio of M cells per Peyer's patch. This factor could dramatically affect uptake of particles and the number needed to induce an immune response. In addition, other factors must be addressed with oral administration in order to optimize the usefulness of PLG as a mucosal delivery system. This includes the number of administered particles, the role of size of particles (presumably the smaller the better to induce a mucosal immune response), as well as the dose of antigen and the optimum timing of the administration. The way in which mucosal vaccines are evaluated in inducing an immune response must also be re-examined. Strictly measuring (serum) antibody responses may not be sufficient to detect a meaningful protective immune response. Studies have shown that protective immunity can be induced without demonstrable antibody titers to pathogens.[99,100]

The wealth of information regarding model antigens as well as antigens of interest in human diseases, suggests that PLG microspheres have great potential for wide use

in veterinary medicine. Technical issues that need to be addressed include the stability of the antigens during the manufacturing processes, since antigens may be exposed to organic solvents or lyophilization during the encapsulation process and this can alter antigen conformation and stability. There is also concern about the stability of antigen within the microparticles as they are stored under hydrolyzing conditions. In addition, as the polymers break down in vivo they create an acidic microenvironment that can inactivate antigenic epitopes, making a vaccine ineffective.[101] Since PLG is hydrophobic in nature, encapsulation with hydrophilic antigens may result in some incompatibilities in the loading and stability of encapsulated antigens.[102] Other factors to consider in PLG MS based vaccines include the stability of the polymers at room temperature (for optimal usage in isolated regions), MS flow properties as a function of storage temperature, stability of the protein within the MS (especially if lyophilized), and antigen release characteristics from MS (changes in polymer properties with aging may affect epitopes of antigens). Long-term storage may lead to aggregation of particles, oxidation, deamidation, or proteolysis of antigens.[103] Assurance of sterility of the final product is also a challenge – irradiation reduces the stability of PLG MS making other sterilization methods necessary. Many of these concerns apply to other MS systems as well. The known hydrolytic properties, relative ease of production (although scale-up may be a problem), wide utility for administration by a variety of routes, biodegradability and biocompatibility make this a leading candidate for vaccine development for most animal species.

II.B.2.b. Poly ε-caprolactone There are other polyesters that can be used to encapsulate antigens. The second most common one after PLG is poly ε-caprolactone. The major reason for the use of these polymers is that they do not breakdown into acid subunits. Another potential advantage of the poly ε-caprolactones is that they can be made in a variety of ways. Regardless of how they are made, they release antigen at a constant rate. There is no large burst effect of release of a large amount of antigen contained on the surface of the microparticles. There is delayed breakdown of poly ε-caprolactones thereby resulting in the potential for sustained release of antigen over a period of time. The slower release is related to the method of production. The major drawback to the use of poly ε-caprolactones is that they use heat or solvents in their preparation. This could affect the structure and immunogenicity of some antigens. One other drawback is that the release rate may be too slow to be of benefit to the immune system. This will need to be investigated further if microparticles of poly ε-caprolactone are to be useful in vaccines.[104]

II.B.3. Ionic polymeric microparticles

II.B.3.a. Polyphosphazene hydrogels Polyphosphazene molecules are bioactive, biocompatible, biodegradable polymers of alternating nitrogen and phosphorus atoms with two side groups attached to each nitrogen. The most common polyphosphazene is poly[di(carboxylatophenoxy)phosphazene] (PCPP). Polyphosphazenes are ionic polymers that have adjuvant qualities when administered with an antigen. In fact, soluble PCPP as well as PCPP MP containing antigen are more effective than

Freund's complete adjuvant (FCA) at inducing high serum antibody responses to influenza in mice.[105] Soluble PCPP formulations have also been used as a controlled release formulation, inducing an immune response that began at 25 weeks post-administration. The remaining discussion of polyphosphazene vaccine preparations will focus on the use of MP for delivery since they can be used to modulate the release of antigen and generate long lasting immunity after a single inoculation.

When polyorganophosphazene polyelectrolytes are mixed with divalent cations at room temperature, they gel to form solid particles or microparticles. This is a major advantage of PCPP MP over PLG MP preparations. There are no harsh chemicals or extreme temperatures needed for polymerization. PCPP also offers many other advantages over other polymeric delivery systems. PCPP MP are stable when lypophilized with no change in size or shape; they are non-toxic to cells and encapsulated proteins. PCPP MP have a hydrophobic, charged surface making them more likely to be taken up by antigen presenting cells when administered at a mucosal site.[106] PCPP have a lot of similarities to natural polymers such as alginates (described below), but because they are synthetic, have much greater consistency in molecular weight and purity making for a more consistent product. PCPP MP have adjuvant-like properties, requiring smaller amounts of antigen to induce an immune response than other methods of delivery.

PCPP MP are formed by creating small droplets of PCPP, usually by use of an ultrasonic spray nozzle, which are then collected in a calcium bath where the droplets gel and solidify on contact. The PCPP MP are stable under most conditions but will disintegrate in basic or high ionic strength solutions. Crosslinking the PCPP MP with positively charged electrolytes like poly-L-lysine (PLL) results in MP that are more stable in physiological saline. The PLL can also be used to alter the hydrophobicity of the surface of the PCPP particles.

PCPP particles can be used for controlled release of encapsulated materials including antigens. Several methods can be used to generate polyphosphazene MP that release antigen over time. The concentration of PCPP or its salt used to form a coacervate solution to generate microdroplets of polymer affects the eventual release of proteins from MP.[106] As the concentration of each material, the time of coacervation, or the molecular weight of the polyphosphazene is increased, the rate of release of an encapsulated protein decreases, and the size of the particles increases. Crosslinkage with PLL can further modulate the release of encapsulated protein. In general, the greater the concentration of PLL, the longer it is allowed to react with the PCPP, and the lower the molecular weight of PLL used, the greater the retention of proteins. However, when greater concentrations of, or higher molecular weight PLL is used, the release becomes biphasic. There is an initial burst of release of material with a slower release of the remaining material. This methodology lends itself well to production of controlled release vaccines. A further decrease in permeability can be accomplished by coating the PLL-coated PCPP microparticles with another coat of PCPP. This also can alter the hydrophobicity of the MP. Faster erosion (and release of proteins) of the PCPP MP can be accomplished by incorporation of chlorine atoms or amino groups (such as glycinato groups) into the polymer chain.[107] Incorporation of other side groups can also affect the rate of release of antigens from PCPP MP.

PCPP MP containing formalin inactivated influenza virus induced a strong antibody response in mice that was of similar magnitude to that induced by FCA.[105,108] The immune response to influenza included not only IgG1 but IgG3 antibodies as well as haemagglutination inhibition activity that were greater than that induced by FCA. The immune response to tetanus toxoid was also greater than that seen with alum and FCA as an adjuvant. Antibody titers increased faster and lasted longer in mice vaccinated with antigen encapsulated in PCPP MP.[105]

Not only can PCPP MP containing antigen induce an immune response when administered by parenteral injection, but also when administered directly to a mucosal surface. Tetanus toxoid encapsulated in alginate/PCPP MP administered in one dose intranasally to mice induced a longer lasting, higher titer response than when administered unencapsulated.[105] Other antigens successfully encapsulated in PCPP microparticles include hepatitis B surface antigen, herpes simplex virus glycoprotein gD2, and the capsular polysaccharide from *Haemophilus influenzae* type b. Although there is no information currently available regarding PCPP vaccines for veterinary applications, most of the antigens described above could be developed for use in animals (such as influenza and tetanus toxoid) or have closely related antigens in pathogens of veterinary significance (herpesviruses in cats, horses, cattle, etc. or *H. somnus* or *Pasteurella* spp.). Liposomes containing antigen have also been encapsulated in PCPP showing the broad ranging possibilities of encapsulating other highly hydrophobic as well as water soluble antigens.[109] These studies demonstrate the feasibility of using PCPP MP as a controlled release delivery system for administration of vaccines to animals as well as humans.

II.B.3.b. Polyanhydride copolymers Besides PLGA there is only one other biodegradable polymer approved for delivery of drugs in humans and that is polyanhydride. Polyanhydrides are hydrophobic in nature, and therefore, not easily hydrolyzed. This makes them ideal for sustained delivery since they degrade in a manner that mimics natural infection, that is a burst of release followed by a slower sustained release of antigen. Microparticles made of polyanhydrides have incorporated a tyrosine polymer as well. The advantage of being able to copolymerize with tyrosine is that it is a natural adjuvant enhancing the immune response to co-administered antigens. Another advantage of using tyrosine is that the tyrosine breaks down at a rate similar to the rate of release of the antigen thereby simultaneously providing an adjuvant. Polyanhydrides can be bioadhesive as well, increasing the chance that microparticles will come into contact with the mucosa and Peyer's patches.[110]

The release rate of antigen can be altered by the make-up of the polyanhydride monomers. If a larger amount of polyanhydride is used for example, the particles degrade and release at a much slower rate. The polyanhydrides degrade more rapidly in a basic pH than acid. This is advantageous as it makes them stable in the stomach acid where they can protect the antigen while hydrolyzing in the higher pH of the lower intestine or within antigen presenting cells. The degradation rate of polyanhydrides can be modified to take advantage of the fact that the MP degrade from the surface only, allowing for a continued release of antigen over time without a sudden

dumping of load. However, as they erode the encapsulated proteins tend to aggregate in the core. Adding sucrose to the formulation can modulate the aggregation. This type of formulation has demonstrated that recombinant bovine somatotropin can be encapsulated and retain its biological activity. This is quite an achievement since this peptide tends to aggregate in vitro. Animal testing is in progress for this delivery system. The major challenge facing this delivery technology is contamination with solvent residues used to prepare the microparticles, and the stability of the protein both during encapsulation and within the core as the MP erode. In vivo studies in target animal species are needed to validate the advantages shown in vitro.

II.B.3.c. Alginate microparticles Sodium alginate is a naturally occurring polysaccharide made from brown seaweed species such as *Macrocystis pyrifera*, and *Ascophyllum nodosum*. Alginic acid is an unbranched glycuronan composed of mannuronic and guluronic acid, a viscous liquid that polymerizes into a solid matrix when mixed with divalent cations.[111] The mild conditions needed to generate solid particles make alginate ideal for encapsulation of a wide range of antigens as well as nucleic acids. The efficiency of encapsulation of different virus antigens varies within spermine-alginate MP and within other polysaccharides (chondroitin, carboxymethylcellulose). However, the immune response induced in mice is less dependent on the antigen load than on the polymer contained in the MP. Part of the reason for this observation may be the processing of alginate microspheres within the antigen presenting cells. Spermine-alginate MP are preferentially taken up by dendritic cells and these are extremely effective antigen presenting cells. One possibility is that alginate may enhance delivery of antigens into professional antigen presenting cells such as dendritic cells.[112] Alginate may also affect the immune response by activating macrophages and inducing cytokine production.[113,114] Alginate, especially the mannuronic acid block component, stimulates pro-inflammatory cytokines such as interleukin 1 and 6 (IL-1, IL-6) and tumour necrosis factor (TNF) production, which could modulate the immune response. This could also affect the immune response to antigens contained within alginate MP. Another alternative is that these MP may release antigen in a manner that maintains stimulation of the immune response for a longer period of time. The overall conclusion of this data is that alginates have intrinsic adjuvant activity that probably contributes to their success as MP based vaccines. In studies in our laboratory, mice vaccinated either orally or by subcutaneous (SC) inoculation of porcine serum albumin (PSA) encapsulated in alginate MP had an immune response similar to that seen in mice inoculated by subcutaneous injection with PSA adjuvanted with a commercial adjuvant Imugen™ (Fig. 2). Mice inoculated once with alginate MS SC had an immune response that was not only equal in titer, but also in duration to that induced by antigen adjuvanted with Imugen™. Mice vaccinated orally received three doses of antigen. It is interesting to note how long the immune response was sustained since the titer was comparable to that induced by the SC administered antigens.

The potential use of alginate MP has been studied using several model antigens as well as subunit antigens of microorganisms. Ovalbumin (OVA) encapsulated in alginate microspheres induced an immune response similar to that induced by antigen

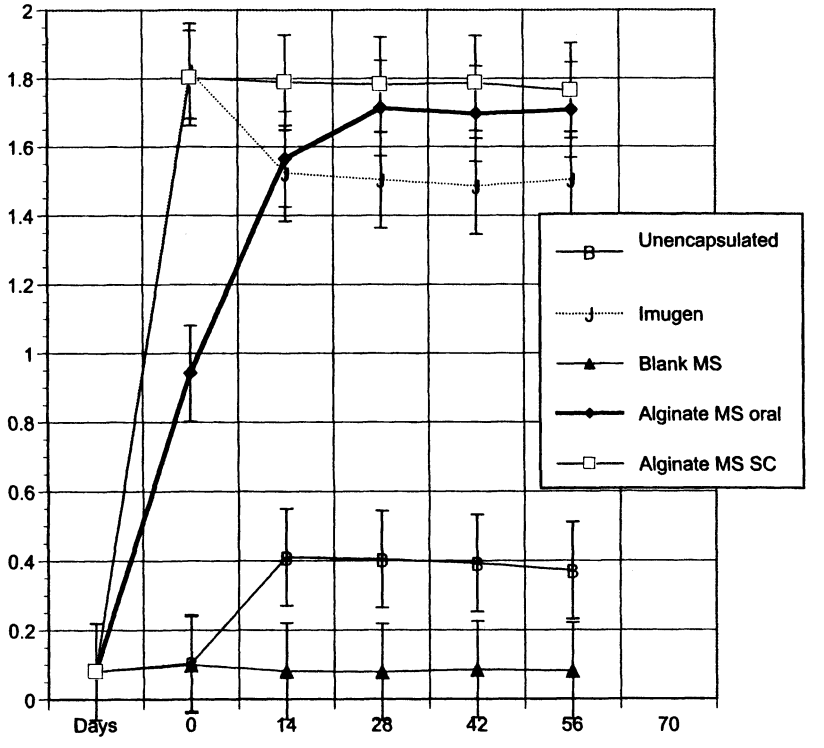

Fig. 2. Mean serum IgG response of mice vaccinated either orally or by subcutaneous injection with antigen porcine serum albumin (PSA) encapsulated in alginate microspheres. Each group has five mice. Error bars represent one standard deviation. Unencapsulated, porcine serum albumin administered orally in saline; Imugen™, vaccine administered by SC injection using the commercial adjuvant Imugen™; Blank MS, alginate microspheres without antigen; Alginate MS, alginate microspheres containing porcine serum albumin; SC, subcutaneous administration.

adjuvanted to aluminum hydroxide or incomplete Freund's adjuvant in mice following parenteral inoculation.[24] Inactivated rotavirus encapsulated within spermine-alginate MP induced a greater immune response in mice when administered by intramuscular injection than did unencapsulated virus.[115] Spermine-alginate MP containing bovine herpes virus gD induced greater and longer lasting titers than MP made with other polymers, despite have less antigen than the comparator MP.[112] A commercial bacterial vaccine for *Pasteurella haemolytica* for cattle and a subunit *P. multocida* vaccine have been successfully encapsulated in alginate microspheres.[116] In addition, *P. multocida* antigens encapsulated in alginate MP were very effective in stimulating mucosal immunity in rabbits when administered orally. Bovine respiratory virus (unpublished data), influenza virus.[113] and nucleic acids[117] have also been encapsulated with variable success. The use of MP such as alginate is an interesting method of getting nucleic acids taken up into the body without the need for injection. Recently it was shown that bacterial beta galactosidase gene co-encapsulated with a bovine adenovirus administered orally to mice resulted in expression of the gene in the intestine,

liver, and spleen.[118] The co-encapsulation of the adenovirus enhanced the expression of the gene in the mouse. This shows the usefulness of alginate microspheres to deliver nucleic acids orally for vaccines as well as for gene therapy.

One effective method to deliver antigens to mucosal sites is to use attenuated live pathogens as vaccines. Although the mucosal administration of modified live pathogens is effective in inducing local immunity to infectious agents, as with parenteral administration, this method is not without risks. Too high a dose, administration to stressed or immunocompromised individuals, or reversion to virulence, can induce full blown disease in the host. The side effects of live vaccines (for example, decreased appetite, nasal discharge) can adversely affect the economics of production in livestock by reducing weight gain or possibly inducing severe disease.[4,119] Therefore, a safer alternative method of delivery of vaccines to induce mucosal immunity is desired.

We have examined the efficacy of alginate MP for oral administration of vaccines to animals. Oral administration of ovalbumin encapsulated in alginate microspheres to cattle induced an antigen specific pulmonary IgA response after one administration of MP.[120] Oral administration of antigen induced only an IgA response in these calves. Administration of *Salmonella enteritidis* antigens to chickens induced a delayed type hypersensitivity reaction. This suggests that a systemic cell mediated immune response as well as a humoral immune response can be induced by the oral administration of antigen encapsulated in alginate microspheres.[24]

The best indication of the efficacy of an oral vaccine is protection of vaccinated animals against challenge with an infectious agent. Rabbits that received bacterial outer membrane proteins and exotoxin encapsulated in alginate microspheres by either oral or intranasal (IN) administration had increased IgA in nasal washes, and reduced pulmonary lesions compared to non-vaccinated controls following intranasal challenge with *P. multocida*.[121]

We recently expanded our studies with alginate MP to investigate the efficacy of alginate MP prepared with an emulsion method to stimulate a serum IgG and IgA response. The immune responses were compared in mice that were vaccinated by oral or subcutaneous inoculation of porcine serum albumin (PSA) encapsulated in alginate microspheres, SC inoculation with PSA adjuvanted with a commercial adjuvant (Imugen™), oral inoculation with unencapsulated PSA, and oral inoculation with blank MP. Mice vaccinated by SC inoculation with PSA encapsulated in alginate MP, or adjuvanted with the Imugen™ had similar immune responses, which in turn were similar to mice that received oral inoculations of PSA encapsulated in alginate MP (Fig. 2). These data suggest that alginate MP have adjuvant like qualities in stimulating a long lasting immunity when administered by a parenteral route. The adjuvant effect also appears to be effective following oral administration, as mice that received oral MP containing PSA maintained an immune response 4 weeks after the last oral inoculation.

Alginate MP can also be used to overcome maternal blocking antibodies in newborns. One important advantage of oral vaccines using MP delivery is the capacity to avoid neutralization of antigens by passively acquired (maternal) antibodies. MP protect the antigen from these antibodies, while facilitating uptake by antigen present-

ing cells where they can then induce an active immune response in neonatal animals. Orally administered alginate MP containing reovirus were administered to mice that had suckled dams which had been hyperimmunized to reovirus. The oral vaccine induced an active immune response in mouse pups despite the presence of high titer passively acquired antibodies.[122]

The mild conditions used for encapsulation make alginate MP a very useful delivery system. The successful stimulation of immune responses will depend on successful encapsulation of microbial or parasitic antigens against diseases of economic importance. Studies with various viruses have indicated that it is not necessarily a universal process to encapsulate all antigens. More information is needed to better understand why the encapsulation process for certain antigens (like influenza virus) is not as good as others. At this time there is nearly as much animal information regarding oral administration and parenteral administration of alginate MP vaccines. An understanding of the consistency of uptake and induction of protective immune responses in challenge models is needed to understand how well this delivery system can be applied to a variety of host species and antigens. The hydrophilic nature of alginate will be a major concern for the oral administration of MP vaccines.

II.B.4. Other polymeric delivery systems for vaccines

There is a wide range of natural and synthetic polymers that have been used to encapsulate pharmaceuticals, and in some instances vaccines. However, there is not sufficient space to cover these in depth in this review, and the reader is referred to other references for detailed information.[123,124] Little information regarding studies with antigens of interest to veterinary medicine is available at this time, although this is likely to change in the near future. Natural polymers including collagen, chitin, cellulose, gelatin, dextran, starch and hyaluronic acid all have potential to act as polymer candidates for MP synthesis. As natural polymers they have the advantage of being biocompatible and/or biodegradable. However, many also suffer from being antigenic themselves and that will limit their usefulness in vivo. A few applications of these are mentioned in brief below.

II.B.4.a. Polyacryl starch Starch is cheap, inert, readily available, has proven safety, and a history of use in tablets. It is naturally hydrophilic, yet can be grafted with a hydrophobic material such as polydimethylsiloxane (PDMS) to render the surface of microparticles hydrophobic. Starch copolymerized with silicone produces unique microparticles that are effective when administered orally or intranasally. Oral administration has stimulated primarily a Th2 response that may be advantageous for the induction of oral tolerance and thus reduction of food allergies.[125,126] So far only model antigens such as human serum albumin have been tested in starch microspheres.[127] In one study, encapsulated human serum albumin stimulated an equivalent immune response to antigen formulated with FCA.

II.B.4.b. Chitosan Chitosan is a biocompatible, biodegradable, polycationic, naturally occurring polysaccharide material. The positively charged chitosan can have strong electrostatic interaction with mucous, or the negative charge of

mucosal surfaces, giving it mucoadhesive properties. Microspheres made of chitosan retain this mucoadhesive property.[128] Chitosan also has the advantage of possessing adjuvant like properties that can enhance the immune response when simply mixed with antigen.[129,130] These properties make chitosan a good candidate for mucosal delivery of vaccines. Microparticles made of chitosan and coated with paraffin or polylactic acid to stabilize them, retained diptheria toxoid for 6 months and could maintain an immune response in rats for at least 5 months.[131] Chitosan nanoparticles coated with ethylene oxide-propylene oxide co-block polymer retained tetanus toxin for at least 15 days in vitro, releasing antigen at a constant rate.[132] Chitosan microparticles have also been used to deliver DNA to mucosal surfaces. Oral administration in mice of peanut antigen DNA incorporated into chitosan nanoparticles induced an IgA and IgG2a response that blocked anaphylaxis to peanut allergens. This could lead to a strategy capable of ameliorating food allergy.[133] Although most of these applications to date are not directly usable in veterinary medicine, the potential is certainly obvious, especially since oral applications have great relevance to many vaccine situations.

II.C. Oral administration delivery systems

II.C.1. Enteric coatings for vaccines

II.C.1.a. Eudragit™ Delivery systems using enteric coating methodologies have also been developed to protect antigens from the acid pH of the stomach, allowing later release of antigens in the intestine. Methacrylic acid co-polymerized with acrylic esters (Eudragit™) has been used to coat non-pareil seeds (NPS, made of sugars for example) that have been coated with antigen. The Eudragit™ protects the antigen from low pH but dissolves readily at basic pH. A vaccine composed of inactivated *Vibrio anguillarum* was coated onto NPS and fed to salmonid fish, engendering an active immune response that resulted in protection in fish challenged with the organism.[134] Eudragit™ has also been used to coat granules made of antigen and inert additives such as cellulose, starch, sucrose or gelatin. Granules made with rotavirus have been used to vaccinate animals orally using live rotavirus as the antigen.[135] Rotavirus is a pathogen of most mammalian species in neonates or young animals. Not only is the antigen of veterinary interest, but this method lends itself to oral vaccination of food producing animals since many feeds and feed additives are currently administered as granules or pellets.

II.C.1.b. Cellulose acetate phthalate Cellulose acetate phthalate (CAP) is an acrylic resin used to coat tablets or granules to protect their drug load from the enzymes and low pH of the stomach. CAP can also been used as a component of oral microparticles, with a solvent evaporation method used to encapsulate antigen. Antigen is usually lyophilized, mixed with a sugar, and stirred with a waxy material like paraffin, a surfactant, and a solvent, in order to form capsules. After drying the capsules are ready for administration.

There are several examples of successful oral immunizations using CAP encapsu-

lated vaccines. Inactivated influenza virus coated with CAP was effective as an oral vaccine in humans.[136] An oral vaccine containing inactivated *Mycoplasma hyopneumoniae* was effective in preventing enzootic pneumonia in swine.[137] In this latter study the vaccine was administered orally following an intramuscular priming dose. After challenge the CAP vaccinates had dramatically reduced lung lesion scores, and increased antigen specific antibody titers, compared to non-vaccinated controls. The particles administered to swine ranged in size from 0.3 to 1.5 mm[138] thus demonstrating that an oral vaccine for swine does not have to be made of particles less than 10 μm in size in order to induce an effective immune response. It appears that this delivery system depended on protecting the antigen from the low pH and enzymes of the stomach. Enteric coatings like CAP breakdown in a basic pH, thus releasing their load. In this case the antigen is presumably released in the lumen of the small intestine and taken up by Peyer's patches or other antigen presenting cells in follicular associated lymphoid tissue of the small intestines.

In another example, a heat inactivated whole cell *Renibacterium salmoninarum* vaccine was incorporated into enteric-coated microspheres and administered to coho salmon. After challenge these fish had lower bacterial counts in their kidneys compared to non-vaccinated controls. This again shows that this method can be used to produce successful vaccines for exotic species.[139]

Other enteric-coated vaccines have been tested primarily for pathogens of human concern. However, many of these could be adapted to similar pathogens in veterinary medicine. Rabies and *Salmonella* spp. are examples of pathogens of great importance to veterinary medicine in which enteric coating of vaccines has been tested. In order for enteric coating of antigens to be useful, there must be a good antigen and it must be stable under the conditions used to prepare and coat the vaccine. The technology for preparation of CAP formulations is well developed. However, the technology is most successful for delivery of small molecules that are well absorbed by the intestine. Larger proteins are used for vaccines, and these are not well absorbed, nor necessarily stable in the intestine. Targeting to the Peyer's patches or sites of antigen uptake may also be problematic.

II.C.2. Polymethacrylic acid (PMA) and its derivatives

In general the more hydrophobic the polymer used to make microparticles, the better the adsorption to and uptake by the Peyer's patches.[140] One of the more hydrophobic polymers investigated for uptake in this regard have been PMA and polymethylmethacrylic acid (PMMA). Nanoparticles made of PMMA are good adjuvants, increasing the immune response to antigens administered parenterally more potently than aluminumn hydroxide.[141] An additional benefit is that both PMA and PMMA are bioadhesive.[142] This is advantageous as it increases the chance of particles coming in contact with the mucosa, an important consideration in the uptake and delivery to antigen presenting cells. The adjuvanticity of vaccines made with either PMA or PMMA may not be restricted to parenteral administration, nor to nanoparticle size distributions. Culture supernatants of *Pasteurella haemolytica*, containing primarily leukotoxin, along with other cell associated proteins, were absorbed into 3 × 5 mm discs of PMA. These hydrogels were used to vaccinate cattle by oral administration for

5 consecutive days. The cattle were then challenged with a transtracheal dose of virulent *P. haemolytica* 3 weeks later.[143] The orally vaccinated cattle lived longer and had less pulmonary damage following challenge than non-vaccinated cattle. The mechanism of antigen delivery was not clear. It is possible that the hydrogels of PMA eroded in the reticulum of the cattle and these smaller particles were taken up by Peyer's patches. It is also possible that the antigen eluted from the hydrogels and eroded hydrogel particles as they traversed the intestinal tract and it was the antigen itself that was released and taken up by Peyer's patches. Similar to the study by Weng et al.,[137] this study with PMA discs demonstrated that orally administered, non-microparticle, polymeric based delivery systems can effectively deliver vaccines to livestock, and induce immunity at a distant mucosal site.

II.C.3. Baits

Development of practical methods for delivering inactivated vaccines to feral animals presents a significant challenge. There have been some remarkable successes for delivery systems in this area. One good example is the use of the oral bait rabies vaccines to control the spread of rabies in the feral animal population. Infected feral animals are a common source of exposure to rabies virus for humans and domestic animals. Since there is no effective therapeutic agent to eliminate the disease in humans or animals, the best means of control is by vaccination. Effective vaccines have been developed. However, since the primary reservoir is feral animals, vaccination of individual animals by injection is not practical. So once an effective vaccine was developed the challenge became how to deliver the vaccine to a population of feral animals.

The oral rabies vaccine program (ORVP) was developed as a means of vaccinating feral animal populations over fairly wide geographic areas. Baits containing rabies antigen, usually as either a recombinant vaccinia or adenovirus expressing the immunogenic glycoprotein of rabies, or a modified live rabies virus were developed.[144–146] The antigen component is then packaged in a soft plastic sachet often containing an attractant for the targeted host. The vaccine/bait can be dispersed over a wide area using an airplane. The recombinant vectors are stable in the bait formulation, and persist in the environment for a month. Furthermore, they do not induce any adverse effects in non-targeted hosts. When the targeted host bites into the bait, the recombinant poxvirus colonizes the oral pharnyx, expresses the rabies antigen which in turn induces a rabies specific immune response the animal. The ORVP has been very effective in dramatically reducing the incidence of rabies in raccoons, foxes, skunks, mongeese, and coyotes worldwide in an economical manner. Similar applications are under consideration for population control (sterility vaccines) of certain feral animal species (rabbits or deer), and could also be used to control other infectious diseases with feral animal reservoirs such as Lyme disease.[147]

III. Future considerations

There continues to be many exciting advances in biotechnology that will make devel-

opment of more effective vaccines likely in the future. Genomic sequencing of DNA and RNA, both from mammals (including human) as well as pathogenic microbes is enhancing our understanding of the pathogenesis of disease and mechanisms to control pathogens. Identifying receptors in the host that microbes attach to, possible cross reactive (mimicry) proteins found in host and microbe, as well as epitopes of key antigens will further aid the design of vaccines that are less likely to induce adverse side effects. Advances in our understanding of how the immune system functions will also allow more rational approaches to vaccine design. This will permit the design of vaccines that specifically activate the parts of the immune system necessary for the successful control of a pathogen, whether it be a virus, bacterium, fungus or protozoa. Development of new and better adjuvants increases the likelihood of success for subunit vaccines, again increasing the ability to enhance the appropriate immune response, for example, either a mucosal or systemic response. As we gain a better understanding of the physiology involved in mucosal sites and how materials used in delivery systems, such as microparticles interact with the gastrointestinal system, we will enhance the utility of vaccine delivery systems. Constant improvements in the design of both polymeric and non-polymeric delivery systems will also positively affect the way in which vaccines are administered. This will result in increased availability of vaccines to more patients, safer vaccines with less side effects and better compliance by patients in taking vaccines, all leading to increased herd immunity and, hopefully, reduction in infectious and other diseases. The need for controlled release delivery of vaccines will be an important aspect of new vaccines. As improvements in biotechnology and the understanding of infectious, immune mediated, and cancer associated diseases continue there are specific needs that will require special attention. The growing interest in mucosally active vaccines will increase the need for delivery systems especially for orally administered vaccines. The desire to reduce the number of injections needed to avoid adverse reactions and increase safety will also demand better methods of delivery. An example of novel, anticipated needs for oral vaccine will be for control of allergies by inducing oral tolerance. Other special needs for specific types of vaccination will also increase the need for delivery. The growth of aquaculture will increase the need to control infectious diseases. The need for an efficient method to deliver vaccines to fish will be important to this industry as for traditional food animals. The continued pressure to reduce antibiotic usage in food animals and the environment along with the need to vaccinate a large number of animals that are not easy to handle individually further increases the demand for better delivery methods for vaccine administration.

IV. Summary

This review has attempted to address the major polymeric and microparticle based delivery systems that are or could soon be used to vaccinate animals. We have discussed systems used both in commercial vaccines and used experimentally that are most likely become common in the near future. As our understanding of the pathogenesis and the molecular basis of disease, as well as the role of the immune

system has increased, more rational approaches to vaccine design including controlled release delivery have become possible. The increasing number of adjuvants that not only enhance the immune response, but also direct the type of immune response engendered, has further aided the efficacy of vaccines for animal production and health. This trend is expanding rapidly and will no doubt greatly increase the value of vaccines. The greater need for easily administered, safe vaccines has also increased the need for better delivery systems. These delivery systems are necessary for long acting, mucosally active, minimally reactive vaccines. The desire for alternative methods to prevent infectious disease, as opposed to treatment with antibiotics, continues to be a growing focus of those involved in animal health and well being. This review is meant to demonstrate the possibilities as well as the wide range of methods and approaches to meet the challenge of improving the health of companion animals, livestock, and protecting the health of humans, by development of more effective vaccines.

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Rathbone and Gurny (eds.), Controlled Release Veterinary Drug Delivery: Biological and Pharmaceutical Considerations

CHAPTER 11

In vitro drug release testing of controlled release veterinary drug products

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Overview

I. Introduction

A controlled release veterinary pharmaceutical is specifically designed to release its contents over an extended period at a predefined rate. The release characteristics of the product are dependent upon its formulation (e.g. types of polymers and inert excipients used) and the process conditions used during its manufacture. Once a formulation and manufacturing process have been defined and the product is being produced on a commercial scale the release characteristics of the product may vary from batch to batch if the raw materials are of an inferior quality, or the manufacturing process is not sufficiently controlled. Consequently, manufacturers need some method that allows

them to determine the effect of formulation and manufacturing variables on release rate. This is especially important during development in order to assess batch-to-batch reproducibility of release rate. The use of animals to assess drug release characteristics is expensive and time-consuming. In addition, in vivo testing requires sensitive assays and is subject to marked inter- and intra-subject variation.[1] Therefore it is desirable to develop an inexpensive, rapid, reproducible test that assesses the drug release characteristics of the product in vitro, but which provides assurances to manufacturers and regulatory bodies of the batch-to-batch in vivo performance of the product.

This chapter discusses some aspects of in vitro drug release test method development, optimization and validation. Both compendia and non-compendia methods are discussed, and suggestions to achieve sound, scientific development and validation for such methods are forwarded. The general thoughts presented on this topic in this chapter represent only some of many possible approaches to the development, optimization and validation of an in vitro drug release test. Many other approaches are likely to be equally applicable, or even superior, and case by case development and validation procedures are the order of the day. Indeed, it is important to acknowledge that each controlled release veterinary product is unique and requires considerations specific to that product that may or may not be covered by the ideas presented in this chapter. In addition, the paucity of published articles on this area of research in the veterinary area severely limit the authors' ability to present an exhaustive overview, beyond their personal experiences.

II. Uses of in vitro drug release test

An in vitro drug release test is a necessary requirement for the registration and quality control of a controlled release veterinary pharmaceutical. Indeed, if there is a label claim that the product possesses controlled release characteristics, the manufacturer must include drug release as a quality control tool for assessing batch-to-batch product quality, and include a drug release component in their registration stability trials.[2] Irrespective of the need, or otherwise, to develop an in vitro drug release test for final product testing, it is advisable to develop such a test from the onset of product development since it has several other useful applications beyond those of final product testing quality control. These include: screening of formulations and product candidates during the development phase; a tool for process development, optimization and validation; a tool to monitor ongoing performance of the manufacturing process; a method for detecting possible risks associated with controlled release preparations e.g. dose dumping; a useful tool to determine the effect of scale up; and, if developed to possess a valid in vitro–in vivo correlation, it has the potential to allow the use of in vitro data in lieu of in vivo bioavailability data to justify a change in manufacturing site, raw material suppliers, or minor formulation/process changes.[3–11]

The value of an in vitro drug release test depends upon the extent to which it can predict the in vivo performance of the product.[1] The goal of any in vitro drug release test development should be to establish a test which exhibits a valid in vitro–in vivo

correlation. If this can be achieved, then the test will provide a high degree of confidence to both manufacturers and regulatory bodies that within specification results assure bioequivalence of product from batch to batch. In such cases, the in vitro drug release test can act as a surrogate for extensive animal testing. If the in vitro test does not correlate with the in vivo performance of the product then appropriate testing in animals may be necessary and required.[7] For example, appropriate animal studies may need to be undertaken to demonstrate that a product stored for the stated shelf life exhibits the same in vivo performance,[7] or may need to be performed to demonstrate minor formulation or process changes, equipment changes or site of manufacture have no effect on the in vivo performance of the product.

2.A. Quality control

If a manufacturer makes the claim on the label that the product is controlled release in its nature, it is their responsibility to develop and validate an in vitro drug release test for use as a quality control product release method in order to assure batch-to-batch reproducibility.[2]

The question arises whether a drug release test used as a quality control tool should exactly mimic the in vivo characteristics of the product (i.e. be able to discriminate among formulations that differ in in vivo bioavailability), or simply be developed to be able to discriminate among products that differ in key variables such as excipient amounts and/or processing variables[3] which provide distinction between product which lies outside the acceptable range (i.e. be sensitive enough to ascertain batch acceptability or rejection based on formulation or process variables). Clearly the ideal case would be the former since this would minimize the risk of the occurrence of bioinequivalence among batches.[3,5] However, because an in vitro-in vivo correlation is often very difficult to achieve with veterinary pharmaceuticals which deliver drugs for very extended time periods (weeks or months) this is not always possible. Consequently, regardless of the ability of an in vitro drug release test to predict in vivo performance, the drug release test will remain a regular quality control tool in good manufacturing practice providing, at the very least, a simple and cost effective indicator of a products physical consistency,[4] will remain useful for the purpose of batch acceptance/rejection or as a tool to monitor the consistency of the manufacturing process.

2.B. Research purposes

Regardless of the ability of an in vitro drug release test to relate to in vivo performance, it is a useful tool for screening different formulations during various stages of product development.[4,6] Compared to screening in animals, an in vitro drug release test is a rapid and inexpensive method to evaluate the release characteristics of a controlled release veterinary pharmaceutical. In early product development, in vitro drug release characteristics can be used as the basis for distinguishing between different alternative formulations to select one for further development. In vitro drug release investigations may also be employed to optimize the final formulation or evaluate the effect that changing a process/manufacturing variable has upon the release character-

istics of the product. Thus, given the value of an in vitro drug release test, an investigator would be well advised to develop such a test early in product development and to generate extensive comparative in vitro data during all stages of product development in order to maximize the chances of successful product development.[5,6]

2.C. Stability testing

An in vitro drug release test is an important tool to evaluate the stability characteristics of a controlled release veterinary pharmaceutical.[6] Evaluation of the drug release characteristics of a controlled release veterinary pharmaceutical should be determined in an in vitro drug release test at appropriate times during the stability program since such a test assures that the product will release its contents within acceptable limits for the duration of the stated shelf life. Different release profiles may be observed upon storage if the drug is chemically unstable and degradation products form which have different physicochemical properties such as diffusion rates through the polymer matrix. Alternatively, products may exhibit a physical instability on storage such as conversion into different polymorphs, blooming, or drug particle migration resulting in a non-uniform distribution of drug within the delivery system. Adverse changes which occur on storage and affect the quality of the product can be detected in an appropriately designed in vitro drug release test.

III. Development, optimization, validation and calibration of in vitro drug release tests

3.A. Development of an in vitro drug release test

3.A.1. Development of compendia methods

For veterinary products whose size, shape and drug load is comparable to human controlled release products, the dissolution test apparatus described in official compendia, e.g. USP 24[12] either alone or in combination[4,5] should be able to be used to develop a valuable in vitro drug release test.

The development process starts with the selection of apparatus. For the initial selection, the compendium associated with the country for which the product is to be marketed should be consulted. This will provide a full description of the available apparatus, including specifications of the physical dimensions of the equipment, and definitions of the ranges for the conditions. For example, the current USP 24 describes seven types of dissolution apparatus which can be utilized to develop an in vitro drug release test which fulfills regulatory requirements for the United States.[4,12] Apparatus 1 (rotating basket) and Apparatus 2 (paddle method) are the most widely used within the human pharmaceutical industry.[4] They are simple, robust, standardized, are commercially available worldwide[6] and are supported by the widest experience of experimental use.[6] Therefore, when developing an in vitro drug release test for a veterinary pharmaceutical, it would be appropriate to first evaluate the applicability of the paddle and rotating basket methods. Historically, Apparatus 3–7

were introduced to overcome the unique issues associated with certain new human drug delivery technologies.[4] Apparatus 3 (reciprocating cylinder) is useful for controlled release formulations.[4,13,14] This apparatus replaced the rotating bottle method while still maintaining the free movement of the delivery system through the media.[4,13,14] It is reported to be useful for the analysis of products containing poorly soluble drugs.[4] In addition, unlike Apparatus 1 and 2, the drug release rates with this type of apparatus are reported to be relatively unaffected by the presence of dissolved gases in the media,[4,13–15] and allows for ready changes of media.[13] A drawback of Apparatus 3 from a veterinary viewpoint is that it is only useful for the assessment of small sized delivery systems. Apparatus 4 (flow-through cell) is a versatile apparatus that can be set up to provide either laminar or turbulent flow,[4,16] can be run as an open or a closed system[6,16] and allows for pH changes.[5,16–18] It consists of a reservoir and a pump for the dissolution media, a flow-through cell and a water bath that maintains the temperature of the media.[16] Five types of cells are currently available allowing for the assessment of a variety of formulation types.[4,16,17] Because fresh media continuously flows across the delivery system, the apparatus is particularly suited to products containing poorly aqueous soluble drugs.[4,17,18] The in vitro drug release profile is similar to plasma-time concentration curves and therefore the flow-through method is considered to enhance the possibility of developing of an in vitro–in vivo correlation.[19] The large volume of release media can be an advantage since it can reduce the need for solubility enhancers in the drug release media,[15,19] however, some authors consider that the need for such large volumes of drug release media (approximately 60 l for a typical test) can be a disadvantage.[4,5] Its major disadvantage, however, is that it is not as technically robust or reliable as Apparatus 1, 2 and 3.[4] An approach to developing a method for this apparatus is described by Looney.[17] Apparatus 5, 6, and 7 are used mainly in the human field for transdermals.

For the reasons highlighted above, the initial selection of an apparatus should be focused on the suitability of compendia methods 1 or 2.[4,6] If both methods appear suitable a decision must be made, or experiments must be performed, that identify which of the two apparatus will be more appropriate. If Apparatus 1 or 2 cannot be used, the next step would be to evaluate the suitability of Apparatus 3 or 4. Once a suitable compendia apparatus has been selected, the proponent should familiarize themselves with the procedure for performing a drug release test using that apparatus. For example Section ⟨724⟩ Drug Release[20] (which also refers to Section ⟨711⟩ Dissolution[12]) of the USP 24 documents in detail the variables and their normal ranges associated with the different Apparatus described above. Before use, the apparatus should be checked by the investigator to ensure that it complies with the compendia limits for its physical and mechanical specifications, and whether it will comply to the stringent specifications defined in compendia for alignment, wobble, etc. Lindauer[21] suggests some practical methods by which specifications can be determined on such variables as alignment and positioning of apparatus components, media evaporation and system-dependent variables using analytical instruments which meet recognized reference standards.[21]

Next, the initial conditions for the test are selected. This will initially be done in an

arbitrary fashion[a] but will be based on knowledge of the physical and chemical characteristics of the drug (e.g. aqueous solubility, pK_a) in combination with the factors shown in Table 1.

In essence, the size of the delivery system, the quantity of drug it contains and the physicochemical properties of the drug (e.g. solubility in various solvent systems, pKa and stability[5]) dictate the size of the vessels employed, the choice of solvent system (composition and volume) to maintain sink conditions, and the mechanism by which the release media will be agitated. Once the initial conditions have been selected, preliminary investigations subjecting the drug product to the proposed test conditions will be performed in order to provide an insight into the suitability of the selected Apparatus and initial conditions.

Once the preliminary test conditions have been confirmed as an appropriate starting point, experiments will be performed to further develop the test. Initial experiments would involve the use of an intense sampling procedure to complete drug release in order to identify the appropriate sampling intervals to be used in the development process. This process would identify the most appropriate six to eight sample points with the final sample point at a time at which >75–80% total release occurs. Once the drug release profile has been defined, subsequent developmental experiments would then examine how the drug release profile is affected by experimental parameters. Such investigations would include examination of the effect of agitation rate (rev./min, reciprocating rate and stroke, etc.), the effect of media deaeration, the effect of release media composition (e.g. pH and/or ionic strength and/or surfactants) and volume, and the need for the sample to be filtered (including the effect of pore-size, whether any of the drug is absorbed or adsorbed onto the filter and whether the products excipients clog the filter). It should be noted, however, that there are constraints on the degree to which experimental conditions can be modified. In order that the developed method complies with compendia specifications, the media composition, pH, ionic strength, size of vessels, dimensions of paddles, etc., must fall within the ranges or specifications defined in the compendia in which the method is specified.

In addition to investigations which ultimately define the conditions which will be used in the in vitro drug release test, there are other related decisions or investigations

Table 1
Factors which are used in conjunction with the physiochemical properties of the drug to select the initial conditions in the development of an in vitro drug release test

Factor
Description of the drug product (size, shape, drug load)
Composition of the drug product (excipients, polymers)
Manufacturing process used to fabricate the product
Definition of the purpose of the in vitro drug release test

[a] It would be wise at this stage in the development to initiate investigations that would facilitate the selection of the 'arbitrary' chosen conditions, e.g. experiments which identify the solubility profile of the drug in various solvent systems.

which should be performed at this stage of the development. These would include the type of analytical procedure which will be used, whether automated sampling would be adopted and the stability of the samples following sampling. UV absorbance is the usual analytical procedure of choice[22] since it is rapid and convenient. However, occasionally inherent drug or product characteristics (e.g. a multicomponent drug product) necessitate the need for HPLC or more sophisticated analytical methods. Another consideration related to the analytical method is whether it requires the need for large volumes of samples to be taken thereby necessitating replacement of media. If so, the effect of media replacement and the requirement for such replacement needs to be accounted for in the interpretation of results. If automated sampling is to be used, experiments should be designed and conducted which develop the automated sampling process (e.g. adsorption/absorption onto lines, accuracy of sample times, accuracy of sample volumes, etc.). Finally, the stability of the drug solution between sampling and analysis will require investigation. Such information will dictate whether the sample should be analyzed immediately following removal or will allow definition of the sample analysis window.

III.A.2. Development of non-compendia methods

Within the veterinary pharmaceutical industry there exist several reasons for developing a non-compendia in vitro drug release test i.e. a test which is a modification (either minor or major) of the specified apparatus and/or conditions of a compendia method or which utilizes a unique apparatus and/or uses conditions which are not described and defined in a compendia (Table 2).

For a veterinary drug delivery system which exhibits any or all of the characteristics shown in Table 2, it is often difficult to successfully develop an in vitro drug release test using the apparatus and/or conditions exactly as specified in compendia. In such cases a non-compendia method must be developed, which might exhibit minor or major variations to compendia apparatus or conditions, or (as a last resort) utilize new apparatus and/or conditions. Although flexibility in in vitro drug release test development must be recognized and accepted, it should be acknowledged there is a need to avoid the needless proliferation of methods.[6] It is well accepted within the human pharmaceutical field that the ad hoc development of new methods should be discouraged.[6,7] This applies equally well to the veterinary pharmaceutical field. Therefore, compendia methods should initially be evaluated to exhaustion, and only when they are proven to be inappropriate should a scientifically sound non-compendia method be developed, but within the constraint that it should be based on the underlying principles inherent within the methods described in compendia (i.e. obsessive attention to vessel dimensions, stirrer device wobble and centralization, precise temperature control and closely controlled agitation conditions from one vessel to the next, etc.).

The first step, therefore, in the development of a non compendia method is to thoroughly evaluate the suitability of compendia methods for the veterinary product in question. However, if a compendia method proves not to be feasible (i.e. either the apparatus is inappropriate or the test conditions fall outside the specified ranges), the next step is to attempt to make minor modifications to the most appropriate method. If this is not possible then the proponent should attempt to make major modifications to

Table 2
Reasons for developing a test or for proposing a new apparatus which is a substantial modification to a compendia method

Problem	Issues
Size and shape of the delivery system	Issues relate to accommodating large and/or unusually shaped veterinary drug delivery systems into compendia vessels of defined dimensions, baskets of defined dimensions or utilizing stated paddle heights and sizes
Delivery system composition	Issues relate to: Hydrophilic excipients can deaggregate from the delivery system in a random manner. Waxy or hydrophobic excipients can clog the openings of basket meshes resulting in inhibition of the free flow of release media over the delivery system. Waxy or hydrophilic excipients can accumulate on the surface of filters resulting in inaccurate sampling volumes
Initial drug load in consideration with the drugs inherent aqueous solubility	Issues relate to the difficulty in obtaining and maintaining sink conditions for high amounts of sparingly water soluble compounds using aqueous release media

the most appropriate method. Only if none of the above are feasible, should a unique method be designed for the veterinary product in question. If this sequence of steps is adopted, sufficient data will be generated to clearly justify the necessity of the alternative method over those defined in compendia. Indeed, only when clear-cut evidence can be presented that compendia methods are inadequate for the veterinary drug delivery system, should a non-compendia method be developed. The burden of proof remains with the methods developer,[4] who must demonstrate that the variations are essential to the development of the test method, that the release data is repeatable and (ideally) the method correlates with in vivo performance.

The development of a non compendia method includes considerations of both the apparatus and conditions which, initially, will most likely be simultaneously evaluated in order to develop and define the apparatus. Some points to consider when developing apparatus for a non-compendia apparatus are shown in Table 3.[23]

When the final apparatus design has been defined, conditions will be thoroughly investigated to further develop the method and to provide a thorough understanding of the method for optimization purposes. At this stage, a typical development process would investigate the effect of gross changes of the parameters shown in Table 4 on the release characteristics of the delivery system, and simultaneously investigate the factors listed in Table 5.

Some of the parameters listed in Tables 4 and 5 are considered in detail below.

III.A.2.a. Temperature and pH Historically, in the human field, attempts were made to adjust in vitro release media conditions to make them as close as possible to physiological conditions in order to increase their predictive value. The result was that human drug release tests are usually performed at 37°C ± 0.5°C[4] and the drug release characteristics of the product would be determined over the entire range of physiological pH, which for humans is generally described as being from approximately pH 1–7.5.[4] Consequently, these are the values stated in most

Table 3
Some points to take into consideration when designing apparatus for use in a non-compendia in vitro drug release test[a]

Factor
The apparatus should be simple in design and convenient to operate
The fabrication method, dimensions, and positioning of each component (stirring device, vessel, etc.) should be precisely specified
The apparatus should provide and maintain sink conditions throughout the test period
Agitation conditions should be closely controlled and provide mild, uniform, non-turbulent release media agitation
Flask size, dimensions and shape should be precisely defined and readily fabricated
The apparatus must provide a convenient means for introducing the delivery system into the release media
The apparatus must be able to retain the delivery system in a fixed position
The apparatus must maintain the delivery system completely immersed in the release media throughout the test period
The impact of environmental variables on release must be considered i.e., the apparatus must not impart excessive vibration
The release media vessel must be closed to prevent loss of media due to evaporation
The release media vessel must be thermoregulated at fixed temperature
The release media vessel must preferably be transparent to permit visual observation
The apparatus must lend itself to easy withdrawal of representative fluid samples from the bulk of the release media for analysis, either by manual or automated methods, without interrupting release media agitation
The apparatus should be rugged enough so that results give good interlaboratory agreement
The apparatus should be sensitive enough to reveal process changes, be capable of showing formulation differences and it should yield reproducible results upon repeated testing under identical conditions

[a] Adapted from Ref. 23.

compendia. In the development of an in vitro drug release test for a veterinary pharmaceutical it would not be unreasonable to adopt these values. However, it may be equally appropriate to develop the method using alternate pH or temperature ranges which are justified based on the normal animal physiologic ranges encountered at the sites of absorption, or regions which the drug product may find itself in the animal.

III.A.2.b. Release media composition The release media composition and volume are generally selected based on solubility data and the initial drug load contained in the veterinary drug delivery system such that sink conditions are met. The definition of sink conditions varies between compendia, e.g. the USP defines sink conditions as when the saturated solubility of the drug is equal to or greater than threefold the concentration of a completely dissolved delivery system.[4,5,24]

Table 4
Factors which would be investigated during the development of an in vitro drug release test

Factor
Agitation speed
Agitator position, shape, etc.
Temperature
pH
Media composition
Ionic strength
Deaeration

Table 5
Supplementary factors which would be investigated during the development of an in vitro drug release test

Factors
Sample method (manual or automated)
Sample size (manual: 10–20 ml aliquots, automatic: <3 ml aliquots)
Selection of analytical method – speed, specificity, simplicity, ease of automation, ease of sample preparation
Absorption/adsorption onto sample tubing, glassware, paddle materials, etc.
Stability of the drug in the release media
Filter material and pore size
Collection tube issues (absorption/adsorption)

When deciding on the composition of the release media, it is probably inadvisable to focus on exactly mimicking the in vivo conditions found at the site of delivery.[6] Indeed, as Skoug et al. point out, if an in vitro drug release test can be shown to correlate with the products in vivo performance, and if that correlation can be reasonably validated, then concerns about the physiological relevance of the release media are of secondary importance.[5] The release media composition and volume will be dictated by the interrelationship between volume of release media, the solubility of the drug in the release media and the total amount of drug initially contained in the delivery system (or if necessary, 80% of that value since the final sample will occur around 80% of total release). In practice it is essential to generate (or extract from the literature) data upon the solubility of the drug in a variety of release media. Indeed, since the composition of the release media is a critical variable which can increase the chances of an in vitro-in vivo correlation and improve test ruggedness,[5] it is advisable (particularly if the controlled release drug delivery system contains a sparingly soluble drug) to investigate a range of media comprising various buffers (differing in both pH and buffer components) with or without solubilizing agents at various concentrations to map the solubility characteristics of the drug. Numerous solubilizing agents have been reported in the literature to increase to solubility of sparingly soluble drugs in an in vitro drug release test.[11,19,25–35] Natural solubilizing agents are expensive and are not of practical use for routine drug release studies. In contrast, synthetic solubilizing agents such as sodium lauryl sulfate or polysorbate 80 are more economic for routine use.[26] It is advisable to use the least amount of solubilizing agent in the drug release media since high concentrations of solubilizing agents may not be as effective at differentiating between formulation variables and are likely to cause foaming which can cause problems if the method is automated.[26]

III.A.2.c. Vibration Modern day dissolution apparatus produced by today's leading manufacturers are designed to ensure that vibration arising from the apparatus is insignificant.[15,21,36] Compendia specify that their methods should be free from significant vibration,[21] and this applies equally well to any non compendia apparatus which are designed and built.[21] Indeed, regardless of the source of the apparatus, both internal and external sources of vibration should be identified and minimized during the development of an in vitro drug release test.

III.A.2.d. Sampling considerations The first sampling consideration concerns the mapping of the drug release profile. In the initial stages of the development process the drug release profile of the controlled release veterinary pharmaceutical should be fully characterized with six to eight samples taken at appropriately spaced time points, and collected over the time course spanning complete release of drug from the delivery system. As the development process proceeds and more experience is gained with the delivery system, the number of time points can be reduced to that required to support the anticipated specifications. The specifications for controlled release drug delivery systems characterize the rate and extent of drug release from the controlled release drug delivery system and are normally <20–30% released after 1 or 2 h, time to 50% release and time to >75–80% release. Consequently, appropriate time points should be chosen to coincide with these ranges.

A second consideration is sample volumes. If manual sampling procedures are adopted, then the sampling volumes would usually be between 10 and 20 ml. However, probably the most appropriate sampling method for an in vitro drug release test for a controlled release veterinary product is automated sampling, in which case smaller (<3 ml) sample volumes can be withdrawn.

A third consideration is sample probe size and position. Both of these have been shown to influence the dissolution rate of immediate release tablets and it is possible that they may influence the release rate from a controlled release veterinary product.[3,4,6,37,38] Therefore, the effect of the sample probe position and size should be evaluated during the development stage to determine their influence on agitation conditions and subsequent drug release rate.[6]

III.A.2.e. Agitation conditions The rate of agitation is another variable which should be thoroughly investigated[7] since it can markedly affect the release profile. Once the agitation rate has been established it should be closely controlled within narrow specifications. In general agitation should provide mild, uniform, non-turbulent media agitation. The process for selection of the final agitation conditions raises an interesting point for discussion. Controlled drug release products are specifically designed to release drug in the in vivo environment independently of the conditions it finds itself in, i.e. the rate of release of the delivery system defines the magnitude and duration of the plasma levels. In an in vitro test apparatus agitation conditions may be such that stagnant aqueous boundary layers form adjacent to the surface of the delivery system potentially controlling the release profile in vitro depending upon their magnitude. It would not be unreasonable to suggest that experiments should be conducted that examine the effect of agitation on the release characteristics of the drug product,[7] and then the agitation conditions that are finally selected are based on the rate at which release becomes independent of agitation conditions, yet still providing mild, uniform, non-turbulent conditions. Under such conditions, release profiles will reflect release from the delivery system independent of boundary layer effects.

III.A.2.f. Filter considerations Filtering is usually performed before analysis to remove undissolved drug particles and insoluble excipients that may affect

analytical results. In some cases (e.g. non-disintegrating matrix-type delivery systems) filters may not be necessary. Therefore, experiments should be conducted to determine the need for filters. If filters are necessary, experiments which investigate the potential for absorption/adsorption onto the filter during sampling should be performed to assure that the filter does not significantly alter the concentration of the drug in solution. Experiments are usually designed which investigate the highest and lowest concentration (usually 10 and 110% of label) expected to be analyzed.[5] To be acceptable, the filtered aliquots should provide values which are the same as the concentration of unfiltered media.[5] Studies should be designed to assess the suitability of the filter and to assess the degree of sample contamination from the previous sample due to contaminated filters, thereby defining the volume of filtrate which needs to be discarded before a sample is taken for analysis. In addition, the availability of different filter manufacturers means that different sources of filters should be evaluated.

The use of filters on the sampling probe may affect the agitation characteristics of the release media.[6] Such influences should be characterized during development by demonstrating that results are equivalent with and without the filters.[6] If filters are shown to disturb the flow of the release media they may need to be positioned above the surface of the media until they are needed to take a sample.[39]

III.A.2.g. Physicochemical properties of the drug Knowledge of the physicochemical properties of the drug play an important role in the development of an in vitro drug release test.[5] The solubility of the drug in various solvent systems has already been discussed above and knowledge of this physicochemical property of the drug is required to ensure sink conditions exist throughout the test.

The pKa of the drug defines the degree of ionization of the drug at a given pH. Ionized species are more aqueous soluble than non-ionized species, and therefore knowledge of the pKa is important as it provides information about the solubility of the drug at different pH values.[4]

The stability of the drug in the release media should be determined at the temperature of the release test over the time scale of the test, and at room temperature for time periods which correspond to (at least) those between sampling and the proposed analysis time.[3,4] The latter will define the window between sampling and analysis. The chemical stability of the drug should also be determined as a function of pH at an early stage in the development process since its stability characteristics may limit the pH range of the release media because the chemical stability of a drug may be dependent upon pH.[5] A typical acceptance criteria for stability is $<2\%$ degradation during the course of the drug release test.[5] However, this limit is arbitrary and may not be appropriate in all cases.[5]

III.A.2.h. Agitation methods If a new shaft, stirrer shape or agitation apparatus is devised it should be accompanied by detailed engineering drawings and be manufactured according to such drawings within specified limits and tolerances. Following manufacture the physical dimensions of the shaft or stirrer should be

measured accurately to document that the new shaft or stirrer dimensions fall within the predefined stated specifications.

III.A.2.i. Automated sampling Manual sampling techniques are no longer recommended if more than one aliquot must be taken from a dissolution vessel because when a cannular is inserted into the media the flow of the media is disturbed, which is difficult to control when manual aliquots are taken, making results obtained from all aliquots difficult to reproduce.[39] Automated sampling is therefore highly recommended.[39] Automation of the sampling procedure provides the advantages of small sample volumes and reduced analyst input and error. However, if an automated sampling method is to be used in the final method it should be thoroughly investigated during the development stage. Experiments should be performed to assess whether the drug absorbs into, or adsorbs onto, the sample lines and that there is no carry-over from one sample to the next.[3] In addition, it should be demonstrated that the automated sampling procedure results in the same drug release profile as a manual sampling method under the same experimental conditions.[3]

III.A.2.j. Deaeration Some in vitro drug release tests/delivery systems remain unaffected by dissolved gasses in the release media and require no deaeration procedure prior to commencement of the test. More commonly, however, in vitro drug release methods are sensitive to dissolved gasses. The outcome is a deposit of gas onto the surface of the delivery system itself or in the pores of the basket,[21] resulting in the prevention of release, or a change in the flow characteristics of the release media through the basket, respectively. The outcome is a large variation in release rate and poor test reproducibility.[5,21] Accordingly, the effect of deaeration of the release media on release rate should be evaluated on a case by case basis.[3,6] If the test is shown to be affected by deaeration of the release media, the use of deaerated release media should be specified in the test procedure.[5] It should be noted that there are several methods to deaerate release media, each of which differ in their degree of efficiency.[21,39–41] Therefore several different methods should be evaluated in the development process and only the effective deaeration method or, if appropriate, a combination of methods should be used in the method development.[6,39]

IIIA.2.k. Position of dosage unit The position of a drug delivery system in the release media can affect the rate at which it releases drug. Therefore this aspect should be investigated during the development stage, and, if it proves to be a problem, a solution should be identified and then implemented in the final test method.

III.A.2.l. Analytical methods A validated quantitative analytical method must be developed to analyze drug content in the samples taken during the in vitro drug release test. UV absorbance is the most common analytical method developed for routine use since it is rapid, convenient, sample preparation is simple and it can be easily automated.[5] However, occasionally (e.g. for a multicomponent drug product) the use of HPLC or more sophisticated methods may be necessary.[4] The analytical method must be linear over the expected concentration range (i.e. 10–110% of label)

and recovery of drug should not be significantly affected by filtration or the presence of any formulation excipients.[3,5] There is no requirement for the analytical method to be specific for process impurities and degradation products.[3,5] However, the analytical method must be developed such that it is free from excipient interference.[3] This is an important consideration during the early stages of product development, where an additional excipient may exhibit spectral interference. There is a need, therefore, when using UV to revalidate the analytical method each time the product is reformulated in order to ensure that reformulation has not introduced any spectral interference.[5]

III.A.2.m. Drug product When the method development process is approaching its final stages, it would be advisable (indeed essential) to investigate several batches, to examine product which differs in key manufacturing variables, and (if available) any product which has been used in clinical trials or which has been subjected to storage. This will provide an indication of the potential discriminating power of the method, and provide further insight into the appropriateness of the developed method.

III.B. Optimization of drug release test

At this stage the investigator should have defined the apparatus and its specifications, and have a thorough understanding of the parameters which affect the rate of release of drug from the veterinary pharmaceutical. This would include the effect of system-dependent variables such as media composition (pH, ionic strength, solubilizer concentration, apparatus shape and agitation conditions), as well as the effect of key manufacturing variables on release rate. The next step is to use that knowledge to optimize the developed test. This involves modification of system-dependent variables in order to achieve the maximum discriminating ability between product which exhibits different biological performance or which differ in some key formulation or process variable, but which at the same time exhibits minimum variation between identical formulations.[3,5]

The method can be optimized to exhibit an in vitro–in vivo correlation. Alternatively, the test may be optimized based upon knowledge of the extremes of clinical efficacy; if two formulations can be identified which exhibit different in vivo performance characteristics, the test can be optimized to differentiate between such formulations. Alternatively, optimization might be based upon the ability of the test to differentiate between formulations which differ in some key manufacturing variable (in the absence of any in vivo data). In this case the test is optimized to be sensitive to key variables within the acceptable range of values which would be expected during the manufacturing process. Key manufacturing variables are those materials and/or methods used in the manufacturing processes that, if varied, can significantly affect the release of drug from the product (e.g. coating thickness, excipient concentrations, hardness, etc.). Clearly the different optimization approaches described above differ in both the time, effort, expense and experiments that are needed to reach the optimization goal. However, the approach taken to optimize the method

will dictate the value of the in vitro drug release test. Clearly the most valuable in vitro drug release test would be one that was optimized to be predictive of in vivo behavior since this will provide assurances that the risk of bioinequivalence between batches is minimized. However, the optimization of a method which exhibits an in vitro–in vivo correlation will often require more effort, take a longer time and be more expensive to achieve than if a test was optimized to simply differentiate between products of different composition or which differed in key manufacturing variables.

The optimization process will have several outcomes. The first outcome of the optimization process would be the confirmation of the apparatus used in the development and a detailed definition of its design and specifications. The second outcome would be the identification of the critical parameters of the method, e.g. temperature of test media, agitation rate, volume of release media, composition of release media, sampling depths, etc. Each of these method parameters will be specifically defined, specifications on their limits set and if required, details documented of how and with what frequency and accuracy they should be monitored during the period the test is being performed. The third outcome of the optimization process might be the generation of sufficient data with the final method to allow the setting of quality control final product release specifications. However, this stage may be performed separately, depending upon the available information. The final outcome of the optimization process would be the identification of whether the drug release profile is influenced by the physical behavior of the drug product such as floating, adherence to the walls, etc. If such behavior is observed, it will require critical inspection and observation to be undertaken during the test procedure to explain any 'out-lying' results and it may limit the use of automation for the particular drug product.[6]

III.C. Validation of a drug release test

When the development and optimization processes are completed and all critical variables of the apparatus and release media are defined together with their specifications to ensure complete control over the final method, it should undergo a validation procedure. The aim of this process is to document the precision and ruggeddness of the in vitro drug release test.[42] In addition, if not already performed as a separate validation, the following aspects of the in vitro drug release test should also be validated at this stage: specificity and accuracy of the analytical method, automated sampling procedure and stability of the drug in the release media, stock and working standard solutions.[5]

Leeson [22] presents a perspective of the validation of a dissolution method for an immediate release product and describes in detail a validation approach for the apparatus, analytical method and the developed method. Much of the information contained in that article is relevant to the validation of drug release test.

To validate a drug release test the method is fully defined and documented in detail.[22] This includes a detailed description of the apparatus, together with specifications or limits on dimensions, and a detailed description of the method, again

together with specifications or limits on volumes, temperatures, agitation rates, etc. standard operating procedures for the test method and any associated or related methods should be written and authorized. Product is then assessed using the proposed final method.

Precision expresses the closeness of agreement among individual test results when the same product is subjected to repeated testing and may be considered at three levels: repeatability which is precision under the same operating conditions, same analyst, same instrument, same laboratory over a short interval for repeat determinations of a single homogeneous sample; intermediate precision which is within laboratory variation, e.g. different days, different analysts, different equipment; and reproducibility which is precision between laboratories with the same method. At a minimum, precision determination would involve three repeat runs (performed by the same analyst, on the same equipment, on different days) with each run simultaneously assessing six delivery systems (comprising the same formulation and manufactured under identical conditions). From the results of this data, the average of each run of six on a single day as well as the relative standard deviations should be determined and used to assess the repeatability of the test method. In addition, further studies by the same analyst using different equipment (if available) and by a second analyst (using the same equipment as used by analyst one in the repeatability determinations) is then performed. Such studies would involve a single run which assesses six delivery systems (again, comprising the same formulation and manufactured under identical conditions). From this data, and the repeat runs by analyst one in the repeatability study, the intermediate precision can be determined. Finally, if possible, the same product should be subjected to the test method a further time in a second laboratory in order to assess the reproducibility of the test method.

The ruggedness of the test method is a measure of its capacity to remain unaffected by small but deliberate variations in method parameters and provides an indication of its reliability during normal usage. To assess the ruggedness of the in vitro test method, the same sample (comprising the same formulation and manufactured under identical conditions) is subject to certain key system-dependent variables which have been modified by 5–10% of their stated value. For example, solvent composition, pH, ionic strength, rotation speed, temperature, etc. can all be modified by 5–10% of their stated value and three to six replicates of the same product assessed by the same analyst to determine their effect on the release profile. Different batches may also be selected for examination to assess ruggedness of the method. In addition, if it is deemed appropriate, ruggedness can be assessed further by examining product manufactured by modifying key process variables by 5–10%. The effect of air dissolved in the release media would have been identified in the development phase, however, if the method has been shown to be insensitive to dissolved gasses, this aspect should be assessed in the validation for ruggedness. The average of each run of six on a single day as well as the relative standard deviations, should be determined and the *F*-test for equal variance and two-tailed *t*-test for the comparison of means may be used to compare the day to day results and evaluate ruggedness.

III.D. Calibration of test apparatus

A further issue of interest is qualification and calibration of test apparatus. For veterinary products whose test is based on the USP or BP the current methods for qualifying and calibrating dissolution testing apparatus should be adopted using calibrator products such as USP Prednisone and Salicylic Acid Calibrator Tablets. However, when an in vitro drug release test is developed which deviates in a minor or extensive way from a compendia method, the value of performing the 'standard' qualification and calibration tests with prednisone and salicylic acid are debatable based on their relevance to the new apparatus or conditions. Thus if the test is so different then an in-house calibration test standard should be manufactured, extensively tested and characterized and then used to perform the calibration. In addition, if the test is a major modification of the USP apparatus, but still utilizes key equipment, e.g. vessels, as described in the USP, then in addition to the in-house calibration standard the USP Dissolution Calibrator Tablets should also be used.

Calibration of the final test apparatus should be a routine procedure. Apparatus suitability tests are recommended to be performed not less than twice per year per equipment and after any occasion of equipment change, significant repair or movement.[6,43] In addition, trend analysis helps in the confirmation of the precision of the developed method. The purpose of calibrator products has been discussed by Lindauer.[21] Lindauer concludes that they are not products which allow the investigator to develop correction terms to be applied to the test nor to adjust the apparatus to give correct results – they are qualification probes to reveal system failures, which, once identified, are investigated and resolved.[21]

IV. Specification setting

The ultimate specifications which can be set for an in vitro drug release test are those which provide manufacturers and regulatory authorities with the confidence that, if the tested product meets the specifications, then its in vivo performance will be assured. To set specifications which fulfill this objective extensive knowledge of the in vivo performance of the product is required, and the in vitro drug release test must be developed to have an in vitro–in vivo correlation which takes into consideration the reproducibility of the manufacturing process and the range of 95–105% of stated amount for the average content of drug substance.[6] This is not always possible, and therefore the proponent might opt to set specifications which provide manufacturers with less confidence in a products ability to perform in vivo, but which can accurately monitor the consistency of the manufacturing process. In this case an appreciation of the acceptable range of product quality which occurs both on storage and within the constraints of the manufacturing process will be required. Whatever the case, specifications must be realistically set, tailored to the purpose for which the test is to be used, and the limitations of the specifications be acknowledged and understood by the user.

When setting specifications it is feasible that the specifications set for final product

batch acceptance is wider than the specifications set in the development stage to develop and validate the method. Several methods have be described in the literature to set specifications.[5,6,8,9] Specification setting may involve a series of experiments which encompass in vivo studies which are designed to determine bioequivalence among product which differs in release rate but which are reflective of the quality range of the product. If in the study, any of the product does not demonstrate bioequivalence an opinion on the therapeutic significance of the observations should be sought or further clinical efficacy studies should be performed to justify the use of the in vitro release profile of such products to set specifications.[5] Alternatively, specification setting may involve a series of experiments which determine the release profiles under the conditions of the validated in vitro drug release test method, of product which exhibits acceptable changes which occur during storage, or different product which results from the normal range of manufacturing, or product which varies in key manufacturing or formulation variables. In this case it would be prudent for the investigator to demonstrate that the product used to set specifications was clinically effective. Alternatively, specification setting may involve a series of experiments which encompass bioavailability studies which are designed to determine bioequivalence among several different batches of product which are specifically prepared (differing in critical manufacturing variables) to exhibit altered in vitro release rates.[8,9] Specifications are then proposed utilizing these data to set acceptable target ranges within which future product is assured of providing bioequivalent in vivo plasma profiles if they fall within the limits of the previously investigated manufactured product.

In the ideal case drug release specifications would be based on the drug release profiles of that product which was used in the biostudy, or was used in any pivotal clinical trials or bioavailability studies, taking into account the variability of the drug release test and the manufacturing process, the clinical significance of differences in pharmacokinetics and the specific characteristics of the drug product.[5,6]

For controlled release formulations, drug release specifications should consist of at least three points.[6] The first specification is intended to assess dose dumping[4,7] and therefore should be set after a testing interval of either one to 2 h and should define when less than 20–30% of labeled claim has been released.[6] The second specification point should define the drug release pattern and thus be set at the time taken for 50% release of label claim[6,7] and define the point at which no less than 40%, and no more than 60% of the drug has been released. The final specification point is the time which ensures that (almost) total drug release has occurred, which is generally accepted as $\geq$ 75–80% of label claim.[6,7]

V. Acceptance criteria

Specification setting should include definition of the acceptance criteria, the number of units to be assessed and the procedure for addressing failed results.[6] Currently, many compendia do not give extensive recommendations regarding acceptance criteria and when it is given those recommendations vary considerably between compendia with

regard the number of units to be tested, the number of steps permissible if a failure is observed, and the extent of the failure of individual units. Therefore, it is recommended to follow the acceptance criteria in accordance with the relevant regulatory authority the test is being developed for.

VI. In vitro–in vivo correlation

The ultimate objective of drug release test development and optimization is to obtain a test that can discriminate among formulations that differ in in vivo bioavailability. However, even with highly sophisticated techniques it is often difficult to obtain meaningful in vitro–in vivo comparisons, especially when product which represent the upper and lower specification limits are compared. Various aspects of in vitro–in vivo correlations is a subject of much discussion in the human field.[3,4,6,7,9,10,13,44–57]

The advantage of developing and optimizing an in vitro test which exhibits an in vitro–in vivo correlation is that it enables establishment of a drug release specifications that provide the maximum assurance of batch to batch consistency of in vivo performance.[5] Indeed, since the ultimate goal in the development of a drug release test should be to establish meaningful drug release specifications, it is clear that an in vitro–in vivo correlation is both desirable and important. An additional advantage of developing a test with an in vitro–in vivo correlation is that it may allow the use of in vitro drug release data in lieu of additional bioavailability data to justify a change in manufacturing site, raw material suppliers, or minor formulation or process changes.[5] The achievement of an in vitro–in vivo correlation is therefore a very desirable goal and the extra time, effort and expense taken to achieve this goal is justified on the basis of the increase in value of the in vitro drug release test to the manufacturer.

To establish an in vitro–in vivo correlation, bioavailability data is required. This can come from data obtained during the course of product development (clinical trials, pivotal animal studies, etc.) or alternatively, the in vivo data can be generated for the specific purpose of developing the in vitro–in vivo correlation. Once available, and based on the extensive knowledge obtained during the development stage of the in vitro drug release test, the in vitro drug release test parameters would be modified with the specific objective of establishing an in vitro–in vivo correlation.

VII. Concluding remarks

The development of an in vitro drug release test is probably more challenging and difficult for a veterinary product compared to its human counterparts. There are several reasons for this. These include (but are not limited to): size of the delivery system (compare a controlled release tablet used for human consumption to an intraruminal or intravaginal veterinary drug delivery system); very large quantities of incorporated drug (usually in gram rather than mg quantities); very long delivery periods (weeks and months for veterinary products compared to hours or days for human products); diverse physicochemical properties of the incorporated drug; unique shapes and

geometries and unique release mechanisms. For products which exhibit one or more of the above characteristics, the development of an in vitro drug release test for a controlled release veterinary pharmaceutical is not always a simple matter of following the procedures outlined in the USP or BP for Drug Release.[20] Therefore, the development of a non compendia method must be addressed. However, some constraints must be placed upon the design of the test method since there is no point in the endless proliferation of methods[6] which use specially designed flasks, holders, etc., and which are difficult to reproduce between laboratories. If a compendia method is not appropriate, then minor modifications to the specified USP or BP procedures for Drug Release should be attempted initially, no matter how difficult this may seem, or how attractive the alternative option of developing a unique test method appears. More extensive modifications should only follow if minor modifications do not result in the successful development of the in vitro drug release test. In either case each deviation should be scientifically rationalized and justified. Only when minor or extensive modifications to the procedures specified in the USP or BP are shown to be unacceptable would it be necessary to develop a non-compendia in vitro drug release test. When this occurs, good science should prevail. Indeed, if the developed apparatus and method must stray from those described in the compendia, then both the final apparatus and method must reach the expectations inherent within compendia in terms of scientific merit, geometric configuration and method precision. In addition the demanding specifications associated with the parameters that affect test reproducibility must be identified during development and adequately controlled thereafter.

Development of an in vitro–in vivo correlation is an important objective and should be vigorously and systematically pursued on a product-by-product basis. Such correlations allow one to develop product specifications with bioavailability implications providing maximum assurance and predictability. When this is not possible, discrimination is sought among formulations that differ in key variables such as excipient amounts and/or processing parameters. Optimum discrimination requires both maximum differences in drug release rate among formulations and low variability for each particular formulation. Such a test will have the greatest probability of being related to in vivo bioavailability.

The paucity of literature on the development and validation of in vitro drug release tests for veterinary pharmaceuticals does not reflect the immense challenges and problems that face the proponent when developing such an analytical procedure. Indeed it is the lack of scientific literature on this subject that severely challenges the ingenuity of the proponent when they develop a non compendia in vitro release assessment test method for a veterinary drug delivery system. They have little reference to other investigators problems, and solutions to those problems to build upon.

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CHAPTER 12

Stability testing of veterinary drug products

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Overview

I. Introduction

Each stage of the development process of a veterinary product results in an improvement of its quality, efficacy and safety until the process of optimization is completed resulting in the final product. An outcome of this process is that the original dosage form which was first conceptualized and made on a small scale using bench top equipment may possess different pharmaceutical characteristics to the final product which was commercially produced on large scale manufacturing equipment. As a consequence, the chemical, physical and microbiological properties of the product can potentially change during the development process. Such changes often occur in an unpredictable or unexpected way. It is wise therefore to generate information on the stability of the product throughout the entire development and scale-up process. However, the reasons for performing the stability test, the extent of validation of the methods used and the amount of control over the study may vary depending upon the stage of product development or scale-up.

Three major stages in the development of a drug product can be identified from a stability viewpoint. These are: (i) the initial *development stage* in which active ingredient and bench-made formulations are investigated. In this case the stability studies are designed to provide an insight into potential stability issues relating to the product; (ii) the *pilot batch stage* in which product is made by trained personnel according to current good manufacturing practice (cGMP) using a manufacturing process which is under control. In this case the stability studies confirm that the process can be operated on a large scale to manufacture batches within specification and with uniform properties. Such studies are usually conducted to generate information on the stability characteristics of the drug product (in the container proposed for marketing) which will be used to propose and establish the shelf life of the product; and (iii) the *final product full scale manufacture stage* in which batches of the final product are manufactured post market approval under a manufacturing process which has been validated. In this case the stability studies are designed to provide on-going information which supports the shelf life and provides evidence that the manufacturing process is under control from year to year. Each stage corresponds to a progressive improvement in manufacturing conditions and an increase in the requirement to perform stability tests in accordance with cGMP principles.

Aspects of stability test programs for the assessment of drug product which falls within the latter two stages of product development (pilot batch scale and final product full scale manufacture stage) are discussed in this chapter. The design of such programs should be based on knowledge of the properties of the active ingredient, together with experience gained from stability studies conducted on product made during the development stage and taking into consideration any regulatory requirements. The requirements of a stability testing program can vary between products and regulatory authorities. However, the key to a successful stability testing program is to base all decisions and actions on justifiable scientific reasons.

Given the wide variety of drugs and dosage forms manufactured by the veterinary pharmaceutical industry, the range of potential problems which may be encountered

during a stability testing program are vast. However, when designing a stability program it is advisable that regulatory issues are considered and addressed within the program at the outset. In order to better understand what these issues are, and to appreciate the regulatory complexities that relate to the design and undertaking of stability testing programs, the reader is advised to consult general book chapters and official regulatory authority guidelines.[1–9] Such information can be supplemented by visiting the useful websites listed in Table 1 that provide additional information relevant to stability testing programs. These documents, web site addresses and others in the public domain provide an excellent background on the principles and practices of conducting stability tests on products that fall within the last two stages of product development.

II. Purpose of stability testing

Stability testing is performed to 'provide evidence on how the quality of a drug substance or drug product varies with time under the influence of a variety of environmental factors such as temperature, humidity and light'.[8] Stability testing provides confidence that, provided the drug product is stored under the conditions stated on the label in the proposed containers and closure, it will retain its predefined quality, efficacy and safety up to the end of its expiration date. This implies that during a stability testing program not only should the chemical characteristics of the product, but also all of the physical, and microbial characteristics of the product which affect its quality, efficacy and safety, should fall within predefined specifications until the end of the stated expiration date.

III. Stability studies/tests at various stages in the development of a drug product

III.A. Development stage stability studies

Preliminary stability studies should be performed during the development stage on both active ingredient(s) and on the formulations manufactured on the bench. Both stress testing and accelerated stability studies should be performed.

Stress studies assist in the determination of the intrinsic stability of the drug by establishing degradation pathways in order to identify the likely degradation products.[8] Stress studies should be undertaken on active ingredients, as well as formulations and placebo formulations (in order to assess for potential excipient-related problems). Such studies should be designed to assess the effect of extremes of temperature, humidity, photostability, oxidation and hydrolysis.[3,8,9] Experiments should ideally be designed to determine the degradation kinetics of the active or drug product (i.e. the experiment should involve multiple samples over time). In addition impelled conditions should be mild enough to result in the formation of primary degradants (since these are the most likely compounds to appear under normal

Table 1
Useful organization websites containing information relating to stability testing[a]

Organization	Specific documents of interest	Website address
US Government Printing office	Home page	http://www.access.gpo.gov
	Code of Federal Regulations online	http://www.access.gpo.gov/nara/cfr/index.html
US Food and Drug Administration	Home page	http://www.cvm.fda.gov/
	Electronic reading room	http://www.fda.gov/foi/electrr.htm
	Center for Drug Evaluation and Research (CDER) home page	http://www.fda.gov/cder
	List of, and access to, CDER guidance documents	http://www.fda.gov/cder/guidance/guidance.htm
	FDA Guidance: oral extended (controlled) release dosage forms: in vitro bioequivalence and in vitro dissolution testing	Access via: http://www.fda.gov/cder/guidance/guidance.htm
	FDA Reviewer Guidance: validation of chromatographic methods	Access via: http://www.fda.gov/cder/guidance/guidance.htm
	FDA Guidance for industry: extended release oral dosage forms: development, evaluation and application of in vitro/in vivo correlations	Access via: http://www.fda.gov/cder/guidance/guidance.htm
	FDA Guidance for industry: stability testing of new drug substances and drug products	Access via: http://www.fda.gov/cder/guidance/guidance.htm
	ICH Guideline for industry: Q1A stability testing of new drug substances and products	Access via: http://www.fda.gov/cder/guidance/guidance.htm
	ICH Guidance for industry: Q1B photostability testing of new drug substances and products	Access via: http://www.fda.gov/cder/guidance/guidance.htm
	ICH Guidance for industry: Q1C stability testing for new dosage forms	Access via: http://www.fda.gov/cder/guidance/guidance.htm
	ICH Guideline for industry: Q2A text on validation of analytical procedures	Access via: http://www.fda.gov/cder/guidance/guidance.htm
	Center for Veterinary Medicine (CVM) site map	http://www.cvm.fda.gov/cvm/default.htm
	Access to CVM guidelines and guidance documents	http://www.fda.gov/cvm/fda/TOCs/guideline.html
	CVM Guideline 5. Drug stability guidelines	http://www.fda.gov/cvm/fda/TOCs/guide5part1.html
	VICH GL1 Guidance for industry: validation of analytical procedures: definition and terminology	Access via: http://www.fda.gov/cvm/fda/TOCs/guideline.html
	VICH GL2 Guidance for industry: validation of analytical procedures: methodology	Access via: http://www.fda.gov/cvm/fda/TOCs/guideline.html
	VICH GL3 Guidance for industry: stability testing of new veterinary drug substances and medicinal products	Access via: http://www.fda.gov/cvm/fda/TOCs/guideline.html
	VICH GL4 Guidance for industry: stability testing for new veterinary dosage forms	Access via: http://www.fda.gov/cvm/fda/TOCs/guideline.html
	VICH GL5 Guidance for industry: stability testing photostability testing of new veterinary drug substances and medicinal products	Access via: http://www.fda.gov/cvm/fda/TOCs/guideline.html
	VICH GL10 Guidance for industry: impurities in new veterinary drug substances	Access via: http://www.fda.gov/cvm/fda/TOCs/guideline.html
	VICH GL18 Guidance for industry: impurities: residual solvents	Access via: http://www.fda.gov/cvm/fda/TOCs/guideline.html

Table 1 (*continued*)

Organization	Specific documents of interest	Website address
International Federation of Pharmaceutical Manufacturers Associations	Home page	http://www.ifpma.org/
	Overview of International Conference on Harmonization (ICH) process	http://www.ifpma.org/ich4.html
	Access to ICH topics and guidelines	http://www.ifpma.org/ich5.html
	ICH Harmonized Tripartite Guideline: stability testing of new drug substances and products	Access via: http://www.ifpma.org/ich5.html
	ICH Harmonized Tripartite Guideline: stability testing: Requirements for new dosage forms	Access via: http://www.ifpma.org/ich5.html
	ICH Harmonized Tripartite Guideline: photostability testing of new drug substances and products	Access via: http://www.ifpma.org/ich5.html
	ICH Harmonized Tripartite Guideline: text on validation of analytical procedures	Access via: http://www.ifpma.org/ich5.html
	ICH Harmonized Tripartite Guideline: validation of analytical procedures: methodology	Access via: http://www.ifpma.org/ich5.html
	ICH Harmonized Tripartite Guideline: specifications: test procedures and acceptance criteria for new drug substances and new drug products: Chemical substances	Access via: http://www.ifpma.org/ich5.html
Rainfo	Home page providing a set of links to worldwide regulatory internet sites	http://www.rainfo.com
Regulatory Affairs Professionals Society	Home page providing a set of links to worldwide regulatory internet sites	http://www.raps.org/
Institute of Validation Technology	Home page of the professional organization devoted to validation technology	http://www.ivthome.com
F-D-C Reports	11 publications for pharmaceutical and device industries:	http://www.fdcreports.com
	Pink sheets	http://www.fdcreports.com/pinkout.html
Washington Information Source, Co.	Source for finding FDA documents and other regulatory news on the web	http://www.fdainfo.com
	Inspection monitor	http://www.fdainfo.com/im.htm
	Warning letter bulletin	http://www.fdainfo.com/wl.htm

[a] This is a dynamic table, the information and stages individual guidance documents are at were current at the time of publication. Readers are advised to check the websites to ascertain if more current versions are available.

storage conditions). It is important therefore to perform the studies under conditions that allow for the formation of primary degradants. Some literature methods recommend that the active or drug product be refluxed until 50–60% has disappeared.[3] Under such conditions there is the potential for secondary or tertiary degradants to form, which may have different physicochemical characteristics to the primary degradants. Upon completion of a stress study the resultant solutions can be used to assess the stability indicating power of the analytical method.

Accelerated stability studies should be conducted on the developmental formulation(s) at 40°C/75% RH for up to 6 months. The purpose of such studies would be to compare the effect of formulation components, packaging materials and/or manufacturing processes on product stability. In this respect the aim of such studies would be

to determine the potential for physical and chemical stability issues which may influence the composition of the final product and choice of containers.

The assessment of chemical stability for both the stress and accelerated studies at this stage of the development process would likely use an analytical method which was scientifically sound, but perhaps only partially validated, which had been shown to separate the major degradation products.[10]

III.B. Pilot batch stage stability testing

When the final formulation, container and manufacturing process have been established and developed, a stability testing program should be carried out on pilot batches (using a minimum of three different batches[10–12]) to establish the shelf life and storage conditions of the product for regulatory purposes. Such batches should be manufactured using materials of the same grade and sourced from the same raw materials suppliers as those that will be used to produce the final product.[11] In addition they should be produced by a manufacturing process which represents that which will be used to produce the final product. These conditions apply in order that the stability test assesses a product that appropriately represents the final product.[11]

Both accelerated and long term testing should be initiated simultaneously. The data from the accelerated test would allow the manufacturer to propose a provisional shelf life and the long term data (conducted for the length of the proposed shelf life) would be used to confirm the provisional shelf life. Both accelerated and long term data should cover a minimum of 6 months at time of submission.[8] Since the manufacturer would be required to submit the data generated during its pilot batch stability test in its regulatory submission document, it would be important that such testing was conducted along cGMP principles. This would involve the use of fully validated analytical methods, and necessitate that the pilot batch fulfilled certain criteria for batch size (as defined in appropriate regulatory guidance documents).

As indicated above, the data from the accelerated study can be used to propose a provisional shelf life that may subsequently be approved by the drug regulatory authority. However, in such a case the manufacturer must undertake (by virtue of a signed statement) to continue and complete the required testing to confirm the proposed shelf life and to submit the results to the regulatory authority.

III.C. Final product full scale manufacture stage stability testing

After the product has been approved, regulatory authorities generally require that an appropriately designed long-term testing study is conducted on the first three production batches. International Conference on Harmonization (ICH) Guidance Documents recommend that the first three production batches are subject to the same stability protocol as in the approved drug application.[8] In addition, to ensure ongoing quality, a certain percentage of the total yearly production batches are laid down annually for long term testing. Such stability tests are conducted for the duration of the proposed shelf life of the product in the container in which the product is marketed.[10]

IV. Aspects of the drug product which are monitored during stability testing

A typical stability study performed on a veterinary product would be concerned with the following aspects: (i) how chemical changes are monitored; (ii) how much of the active ingredient is present; (iii) what compounds and how much of them are formed on storage; and (iv) how the physical and microbial characteristics of the product change on storage. The first aspect is dealt with in detail later in this chapter (Section VI). The latter three aspects are discussed below.

IV.A. How much of the active ingredient is present

The amount of active ingredient in a veterinary pharmaceutical may change with time due to chemical decomposition. Active ingredients may be subject to several mechanisms of chemical decomposition. The major ones are hydrolysis, oxidation, photolysis, polymerization and isomerization. Hydrolysis is the most common path of chemical decomposition and, together with oxidation, accounts for most chemical degradation in veterinary pharmaceuticals.

The chemical stability of an active ingredient is relatively predictable based on its chemical structure and can be investigated in stress tests. On the other hand, the degradation of an active ingredient following its incorporation into a veterinary product is more unpredictable, since it is now part of a complex formulation likely to contain a variety of excipients each with its own distinct physical and chemical characteristics. The breakdown of an active ingredient (or the excipients) in such a formulation can involve a number of different pathways and may not be predicted by stress testing. This is the major reason why, although a shelf life can be proposed using accelerated data, stability testing on the final product must be carried out under long term conditions in order to confirm the expiration date and storage conditions.

IV.B. What compounds are formed on storage

An understanding of what compounds are formed on storage is important from several viewpoints. Knowledge of how the active ingredient degrades is an important aspect of developing a stability indicating assay (see Section VI). Identification of the decomposition products of an active ingredient is also important to assess the safety of a product and therefore is of interest to regulatory authorities. In addition, the level of degradation products is a more sensitive indicator of product stability relative to the assay results.

IV.C. Physical changes

Physical changes in a veterinary product can occur upon storage that may not be related to chemical decomposition of the active ingredient. Indeed, a veterinary product may be chemically stable, yet exhibit some form of physical instability that may limit its shelf life. An assessment of the physical changes that a product undergoes on storage is an integral part of a stability testing program. However, unlike

chemical stability where a relationship is expected between long term and accelerated tests, physical stability is not generally predictable from accelerated tests and can generally only be evaluated during long-term testing.

For the two stages being considered in this chapter (pilot scale stage and final product full scale production stage) major physical changes in the final drug product which limit its shelf life would not be expected to manifest themselves since any physical instability of the product would likely have been identified and overcome during the early stages of the development process. However, occasionally, an unexpected physical instability can arise, particularly when product manufacture is scaled up from bench to pilot scale. If a physical instability does manifest itself during the stability testing program it should be documented and the manufacturer would be well advised to perform additional investigations to determine the clinical consequences of such changes. If a valid in vitro–in vivo correlation exists for the drug release test used in the stability test, then it may be sufficient to restrict such investigations to in vitro drug release testing. The aim of such investigations would be to determine if the physical changes significantly affected the drug release characteristics of the product. However, if no in vitro–in vivo correlation existed for the drug release test, or the results did not meet specifications, or they suggested that the physical changes may affect the clinical efficacy of the product, then in vivo studies (e.g. a bioequivalence test) should be performed on stored product to ascertain the effect of the physical instability on the efficacy of the product. Therefore, when performing a stability test, especially on a pilot scale product, it is recommended to store additional product in the unlikely event that an unforeseen physical stability problem occurs and extra product is required for additional in vitro and/or in vivo studies.

In some cases, a degree of physical instability may be considered by a company to be an acceptable characteristic of the final product. In this case the company must demonstrate that the physical instability (e.g. a minor color change over the products shelf life) only affects the cosmetic properties (appearance/elegance) of the product, i.e. it does not diminish the efficacy of the product. In cases where physical changes do influence the efficacy of the product, then the physical stability of the product should play a role in setting its shelf life.

Although some physical changes are routinely considered for certain dosage forms e.g. sedimentation and caking for suspensions,[3,4,13] many changes in the physical characteristics of a veterinary drug product are product dependent and should be evaluated on a case by case basis.

The extent to which the physical stability of a product is reported in a stability program varies considerably between investigators,[4] and can range from a simple visual observation to detailed comparisons of product, to the use of color charts, pH determinations and physical measurements, etc.[4] However, irrespective of the complexity of the test method used in a stability testing program, it should be validated as far as possible.[4] Advice on achieving this aim is limited since the scientific literature is sparse on this topic. However, the key to a successful validation is through the thorough development of a test that is based on sound scientific principles and directed toward achieving a meaningful measurement each time the procedure is performed.

IV.D. Microbial changes

Microbial testing is an important element of a stability program for some veterinary products, e.g. sterile injections.[3] Such products may need to be tested for total count of aerobic microorganisms, the total count of yeasts and molds and the absence of specific bacteria such as *Staphylococcus aureus*, *Escherischia coli*, *Salmonella* and *Pseudomonas aeruginosa*. In special cases, sterility testing or endotoxin testing may be appropriate (e.g. for injectable drug products). For liquids needing an antimicrobial preservative, acceptance criteria for preservative content and effectiveness may need to be studied.[3] With scientific justification it may be appropriate to propose no microbial limit testing for some veterinary dosage forms, e.g. intravaginal products, subcutaneous ear implants.

V. Stability test design

Despite the multitude of veterinary products on the market, each with its own individual, novel features, a stability test conducted on any veterinary product will involve the same essential features. However, the designer of the stability program must be prepared to exhibit a degree of flexibility in order to ensure the design not only fulfils any specific requirements of a particular regulatory authority, but also evaluates any unique or novel characteristic(s) of the product which may influence its stability.

In general, the design of a stability program for a finished drug product will be influenced by knowledge gained from the known physicochemical properties and characteristics of the active ingredient, experience gained from product development studies, characteristics of the chosen dosage form, experience gained from clinical batch formulation studies, the proposed recommendations for use of the product and any published regulatory requirements.

A key to conducting a successful stability testing program is the adoption of the principles of GMP within the testing laboratory. This will ensure that the testing laboratory performs adequately and consistently, achieves the quality objectives inherent within the operations required to perform the stability test and is capable of fully documenting all of the operations. This will involve qualified and well-trained personnel, fully validated and monitored stability chambers, fully qualified and calibrated analytical equipment, standard operating procedures for all test methods, an approved stability protocol,[11] an out of specification results protocol,[14–16] documented data review, trending and reporting processes, and backup and archiving procedures.

Testing should focus on those features likely to change during storage that may influence the quality, safety or efficacy of the product. Analytical test procedures used in the study should be fully validated and the assays should be stability indicating. The number of samples assessed at each time point will depend on the precision of the test procedures.

V.A. Stability testing protocols

A stability testing protocol is a written procedure that details how the stability program will be conducted. It should cover not only chemical stability but, where appropriate, physical and microbial testing. The stability testing protocol should include: information on the number of batches to be tested; the minimum size of those batches; product details such as strength, composition, etc.; closure details such as size, composition, etc.; a statement on the purpose of the study; define the storage conditions and sample time points; detail the tests to be performed including references to the validated methods or specifications which will be used; and define the number of samples to be tested at each sample point.[11] In addition, the protocol should include: directives to assess the chemical stability of active ingredients, excipients, preservatives etc.; to monitor and quantify impurities and degradation products; and, if the product has a label claim for controlled release, to determine the in vitro drug release profile. It should also specify what form of statistical evaluation should be used on the results in order to establish a meaningful shelf life statement.[10]

V.B. Selection of batches

When a stability test is designed to establish the expiration date of the product, the product used in that test is usually manufactured before final product full scale production begins. As a result, pilot scale batches are used in the test. It is important that the product manufactured in these batches should have the same characteristics as production batches and be fully representative of final product production. Some modification to the manufacturing process may occur between the time the stability test is laid down and final product production begins. If such changes are minor there is little or no issue, but major changes may invalidate the conclusions of the stability study performed on the pilot batches. In either case, product from the first three commercially manufactured batches must be placed on accelerated and long term stability testing following market approval using the same stability protocol that was used in the approved drug application.[8]

Use of a single batch alone to propose a shelf life would not permit assessment of batch-to-batch variability, and the use of only two batches is an unreliable means for establishing an expiration dating period.[17] Although it is true that the more batches that are investigated the greater would be the confidence in the results, practical considerations prevent the use of an unlimited number of batches. As a result, it is usual that stability data used to propose a shelf life is based on three batches. In general two of the three batches should be obtained from a manufacturing run equivalent to pilot scale (at least 1/10th final production batch size) but the third may be smaller.

When the on-going annual stability testing is performed post-approval, ideally, the batches selected for such testing should constitute a random sample from a population of the production batches.

V.C. Packaging materials

The choice of container in which the product is marketed would be based on results from stability studies performed on developmental products and influenced by company policy, market requirements and package engineering facilities at the manufacturing site. There are no specific guidelines on the selection of appropriate packaging material for veterinary products and selection is based on the manufacturer's judgment that it is the best for the intended use of the product and provides optimum protection. Once chosen, stability testing should be performed in the container proposed for marketing.

It should be noted that if the product is to be registered in more than one packaging form, stability data would normally be required for each packaging form. This is necessary even if the packaging materials meet suitability tests required by the USP for plastic containers and rubber or plastic closures.

V.D. Storage test conditions

V.D.1. Storage conditions

The length of a stability test and the storage conditions involved should be appropriate to cover the anticipated length of time the product is in storage, shipment and subsequent use.[8] The storage conditions normally used for accelerated testing are 40 ± 2°C/75 ± 5% RH, and for long term testing 25 ± 2°C/60 ± 5% RH,[8,12] although other storage conditions are allowable if scientifically justified.[8] If a significant change (failure to meet the specification[8]) occurs at 40°C/75% RH, then changes under intermediate testing conditions, e.g. 30°C/60% RH should be evaluated.[8,18] When a stability testing program is designed to establish an expiration date for the product, even if preliminary stability data suggest a stable product, it is prudent to include a provision in the stability protocol to lay down an intermediate testing protocol in case the product demonstrates a significant change during accelerated storage.

It should be noted that countries are assigned to different climatic zones which have specific storage conditions. Therefore, to minimize the overall cost of the test and to avoid the discovery of protocol limitations following submission of data based on 6–12 months work, the manufacturer should become familiar with the regulatory requirements of the markets upon which the product will be sold. In addition, regulatory authorities have different rules with respect to the conclusions that can be drawn from stability testing. For example, in Australia, if no trend is observed after 'x' months storage at a temperature of at least 10°C or more greater than the maximum recommended storage temperature of the product, then an interim shelf life of a maximum of $2x$ months may be permitted provided that $2x$ is not greater than 3 years.[5]

V.D.2. Testing frequency

The testing frequency should be sufficient to establish the stability characteristics of the product. ICH Guidelines suggest that testing should be every 3 months over the first year, every 6 months over the second year and then annually.[8,11] However, it is necessary to be familiar with the regulatory requirements of the specific country in

case additional testing time points are required. For drug products predicted to degrade rapidly, more frequent testing would be necessary.

V.D.3. Analysis window

There are no written guidelines on the time that may elapse between sampling and assay of a drug product. Ideally, samples should be tested within 24–36 h but factors such as number and variety of products to be analyzed, analytical equipment capability, assay length, and unforeseen circumstances such as equipment malfunction may prevent assay of the complete set of stability samples within such a short time frame. In such cases, long term freezing before analysis would not be considered an acceptable option since the drug product may be adversely affected by low temperature storage and/or the moisture content of the refrigerator. A more suitable approach would be to assay within 10 working days of sampling, during which time the product should be kept in the laboratory environment under ambient conditions. In this scenario, stability data from long term testing should be used to verify the stability of the product during the period between withdrawal and analysis.

VI. Analytical methods

VI.A. Validation master plan

The cGMP regulations section 210.3(b)(25) define method validation 'as establishing, through documented evidence, a high degree of assurance that an analytical method will consistently yield results that accurately reflect the quality characteristics of the product tested'. In essence, validation is providing documented evidence that something does what it purports to do.[19–21]

The value of producing a Validation Master Plan for Analytical Test Methods prior to initiating the validation of an analytical test method which will be used in stability tests for registration purposes is now well recognized.[22] Such a master plan documents the procedures and protocols necessary to validate an analytical test method.[22–26]

The components of a technically sound validation include regulatory, scientific and technical issues.[22] The need to comply with cGMP regulations should be combined with the need to choose, develop and validate scientifically sound analytical methods that are accurate, sensitive, specific, reproducible and rugged and technically suitable for the function they were developed for.[22] These aims can be achieved through a well thought out Validation Master Plan for Analytical Test Methods.[22,27] Such a plan should include: a definition of the technical objective; definition of the roles and responsibilities of each department involved in the validation procedure; and a statement on the type and purpose of the analytical method.[22,26,27] It should also provide an overview of how to select a suitable analytical technique, provide guidelines on the development procedure, define the protocol for the validation experiments, provide a statement on the performance parameters that the analytical method must meet, provide documented guidance on how to write development and validation reports, and how to cross reference raw data, instrument outputs, etc.

VI.B. Qualification of equipment

Accurate and reliable analytical data can only be obtained using validated equipment, methods and processes.[28] Many regulatory authorities now expect that equipment and laboratory procedures used to generate analytical data for stability testing be qualified and validated. Indeed, validation is fundamental to the demonstration of cGMP compliance.[27] For any laboratory wishing to operate to cGMP, installation qualification (IQ), operational qualification (OQ) and performance qualification (PQ) and requalification protocols should be in place for each piece of equipment used to generate data in a stability program.[28] IQ and OQ is a verification method used to establish documented evidence that the engineering requirements and specifications for the equipment/system have been satisfied, the equipment/system and software have been properly installed and the equipment/system operates per intended use.[29] The IQ and OQ of equipment may be performed by either the user, a representative of the vendor, or a third party.[27,28] Whichever the case, the installation and testing should follow written protocols.[24–26,28–31]

VI.C. Chemical assay considerations

The analytical methods that are used to assess the chemical stability of a product during a stability testing program may differ in either their complexity or degree of validation over the various stages of product development. For example, a fully developed and validated stability indicating assay may not be necessary for developmental studies on early formulations, (but note that such an assay must be scientifically sound, be partially validated and generate reliable data). In contrast, if the data is to be included in a regulatory submission (pilot batch stage and final product full scale manufacture stage), the method should be fully validated.

Pre 1990s, it was common for analysts to apply ultraviolet spectroscopy (UV) to quantitative analysis and thin layer chromatography (TLC) to detect degradation products. However, because UV absorption is not specific[32] and because TLC is only semi-quantitative, nowadays it is recognized that neither technique is useful as a basis for a stability-indicating assay. In addition, since 1990 there has been a tightening of regulations. As a consequence of these two factors, analysts have turned to the use of high performance liquid chromatography (HPLC), which today is the most common analytical technique for developing stability-indicating assays used in stability testing programs.

VI.C.1 Selection of stability-indicating assay

The first step in the selection of an analytical method is to establish what is to be measured and how accurately it should be measured.[33] A literature search should then be performed which, combined with knowledge of the chemistry of the compound, should allow identification of an appropriate method for development. Alternatively, if no method appears to be appropriate for the purpose, a decision would be made that a new method is required.[33] Sources of information on which to base the development of an appropriate assay include compendial methods, supplier of the drug substance

and the scientific literature. If a compendial method exists but a manufacturer chooses to use an alternative method, the two methods should be compared to demonstrate that the in-house method is equivalent or superior to the official procedure.[34] If a compendial method is chosen, then the manufacturer should verify that the method works under the actual conditions of use.

VI.C.2. Development of assay

When developing a stability-indicating HPLC assay for use in a stability program, the practicality of the assay method should be considered.[35] In this respect, repeatability, sample throughput, run time, the relative merits of gradient versus isocratic elution and system suitability parameters should all be considered.

The method must be able to differentiate the drug from any degradation products, dosage form excipients or impurities if it is to be considered stability-indicating. Stress testing should be performed during the development of the method and used to demonstrate the ability of the method to differentiate the drug from its degradation products. Impurities in a drug product can arise from several sources including process-related impurities, synthetic intermediates, starting materials, degradation products, residual solvents and excipients. If available, raw material samples of known impurities should be used to establish the final method. Diode array detection with HPLC is a frequently used technique for providing information on the stability indicating nature of the method.

Robustness should also be considered in the developmental phase. The method should be shown to be reliable when small, but deliberate, variations in analytical conditions (e.g. HPLC pump speed, solvent composition, etc.) are performed. If the analytical results show that the method is not robust with respect to certain analytical condition(s), then the analytical condition(s) should be suitably controlled, or a precautionary statement should be included, in the procedure.

If the developed assay cannot resolve the analyte peak from all impurities or degradation products, additional assays, e.g. TLC, or alternate HPLC assays may be developed to supplement the general assay method.[34]

VI.C.3. Validation of chemical assay

Every analytical method should be validated before it is used in a stability program. Analytical methods can be validated in a number of ways, however, it should be noted that system suitability data alone is considered insufficient for, and does not constitute, method validation.

Validation of an analytical method can be defined as, 'the process of using laboratory studies to establish that a method can meet its intended requirements'. The characteristics of an analytical test method which should be considered during the validation of an analytical method have been described,[34,36] together with recommendations on how to validate the various characteristics for a particular analytical procedure. According to these references the various validation characteristics which should be considered include:[34,36]

- accuracy
- precision

  repeatability
  intermediate precision
  reproducibility

- specificity
- detection limit
- quantification limit
- linearity
- range
- robustness

Each of these validation characteristics is defined by Cartensen.[36]

VII. Evaluation of results

VII.A. Level of acceptable degradation

The purpose of evaluating a stability test is to define how the quality of the product varies with time and environmental conditions with the aim of proposing a shelf life for the product. The proposal would be based upon predicting when the observed degradation profile of the accelerated data intersected an acceptable lower specification limit. In general, most countries accept up to 10% loss in potency. In such cases the acceptable lower specification limit would be defined as 90% of the label content of the product. The latest ICH Guideline suggest a more stringent specification limit of 95%.

VII.B. Statistical evaluation of stability profiles

Statistical evaluation should be provided when proposing a shelf life from accelerated data. An acceptable approach is to determine the time at which the 95% one-sided lower confidence limit for the mean (linear) degradation curve intersects the acceptable lower specification limit.[8,37] When this approach is used, it provides the user with a 95% confidence that the batch will remain within specifications up to the end of the proposed expiration dating period. Note that it is not acceptable to determine the expiration dating period by estimating where the fitted least-square line intersects the acceptable lower specification limit. This approach only provides the user with a 50% confidence that the batch will remain within specification at the proposed expiration date.

If analysis shows that the batch-to-batch variability is very small, it is acceptable to combine the data generated on three batches into one overall estimate. However, this can only be performed after first applying appropriate statistical tests (for example, P values for level of significance of rejection of more than 0.25) to the slopes of the regression lines and zero time intercepts for the individual batches.[8]

Table 2
Deficiencies in stability programs commonly encountered by regulatory authorities

Aspect of stability test	Failure to...
Stability program	Conduct stability testing
	Conduct the stability study according to the regulations
	Write and authorize a stability protocol
	Accumulate stability data on more than one batch of the product
	Use a quantitative figure to express results (instead results were express as 'pass' or 'comply')
	Establish and follow a reliable and meaningful written testing program
	Establish a stability testing program adequate to support the expiration date
	Conduct analysis on the three month test point
	Provide documented evidence that the stability protocol had been approved by Quality Management
	Consistently meet stability timelines (several months overdue)
	Generate adequate long term stability data to support the tentative expiration date which was based on accelerated data
	Assure that lots placed in the stability program were tested at the appropriate intervals
	Store a copy of the stability test protocol at the location where stability testing was conducted
	Conduct stability trials on all products
	Follow Standard Operating Procedures for stability testing and to record and justify procedural deviations
Stability ovens	Ensure that the required humidity and temperature of the stability samples storage room was adequately distributed throughout the room
	Adequately monitor stability oven performance throughout the test
	Control storage conditions within specified limits
Post approval	Place the first production batches on stability as per the commitment in the application
	Select batches for annual stability testing which were representative of the batches manufactured during that year
	Assure that different packaging configurations were included in the stability program on an annual basis
Contract laboratories	Qualify the contract laboratory used for stability testing of the finished product
	Issue approved procedures for pulling, handling and shipping samples to another laboratory
	Establish protocols (between the firm and the contract laboratory) which outlined retesting procedures to follow for samples that failed to meet specifications
Analytical	Determine the specificity of the analytical method
	Identify or evaluate unidentified HPLC peaks found during stability testing of validation lots
	Perform systematic identification of degradation products
	Validate modifications to the analytical methods that were introduced during final product stability testing
	Provide adequate data to demonstrate that the method used for stability testing had been validated as stability indicating with respect to acid and base hydrolysis, photolysis, oxidation and thermal degradation
	Provide adequate data to demonstrate that the method was capable of separating the active ingredient from its major impurities and degradation products
	Provide assurance that the methods used to measure the products shelf life could yield reliable and accurate test results

Table 2 (*continued*)

Aspect of stability test	Failure to...
	Provide sufficient data to show that the method could detect all impurities identified by the drug substance manufacturer
	Provide stability data to support the use of prepared standard solutions over time periods they were claimed to be stable
	Provide procedures to statistically analyze long term stability data points that exhibited abnormal stability patterns
Personnel	Provide adequate training of staff in procedures
	Provide on-going training in cGMP
Out of specification investigations	Perform thorough investigation of OOS results
	Conduct and complete OOS investigations in a timely fashion
	Extend OOS investigations to other packages of failed batches
	Extend OOS investigations to other batches manufactured at the same time as the failed batches
	Provide sufficient information explaining in detail reasons for rejection of analytical data and reassay of samples
	Extend OOS investigations on annual batches to the review or testing of other batches manufactured during that year
	Include corrective actions in the OOS Investigation Report which were necessary to prevent similar recurrences
	Develop and authorize SOPs for conducting product investigations and tracking product failures
	Take corrective action where analyst error was found to be the most probable cause for the OOS
	Provide sufficient data to support conclusions of OOS investigations (particularly for conclusions which cited poorly trained analysts and equipment problems)
Retesting	Assign cause as to why results of an original assay test were discarded
	Perform an investigation to determine the validity of original assay test data which was discarded
	Provide evidence that the original assay test data was not legitimate
	Provide sufficient evidence that reanalysis results (same samples, new standard prepared) invalidated the original results
	Provide an assignable cause for assay results which fell below the alert limit
	Provide adequate reasons to 'invalidate' original assay test data (invalidation was based solely on the basis of two retests)
	Provide an adequate procedure for rejection of analytical data and reassay of samples
	Provide information explaining in detail how the retesting should be conducted
	Provide information explaining at what point testing should end and the product be evaluated
	Investigate, justify and record deviations from written specifications and test procedures when repeated testing is done, due to initial test failures
Trending/review of stability data/ notification	Investigate increasing/decreasing trends in analytical results during stability testing
	Make senior management aware of the presence of several impurities in the finished product
	Explain data submitted in the Annual Reviews which demonstrated that several products had unexpected increases in potency that exceed normal data variability
	Provide a system to assure that upper management is notified of product deficiencies
	Perform supervisory review of stability data in a timely manner
	Undertake an investigation to determine whether highly variable and/or non-linear test results at various stability test stations were due to true product variability or to defects in the testing

Table 2 (*continued*)

Aspect of stability test	Failure to...
Stability reports	Indicate that results submitted in the annual report for the 18 month station were retests of failed samples Indicate in the report that results submitted for the 9 and 12 month stations were retests of failed results or that the problem encountered at the contract laboratory was the reason why the initial tests failed Support conclusions stated in the annual and final reports Provide sufficient stability data to support proposed expiry period Specify the formulations used in the test, and to state which batches were identical to the formulation that will be registered State whether the batches used in the test were pilot or production batches Describe clearly the packaging used in the test and to confirm whether it is identical to the pack which will be registered Define accurately the temperature, lighting and humidity conditions applied during the test Fully describe test methods and sample size used in the stability testing program Include information on the physical characteristics of the product during storage, such as dissolution profiles, homogeneity, viscosity etc. Provide approved calibration reports for stability ovens

VIII. Common deficiencies in the stability program and reports

Deficiencies in stability testing programs are commonly encountered by regulatory authorities. In order to provide assistance in developing and conducting stability testing programs, various regulatory authorities have published their findings. Abbreviated examples of such findings are given in Table 2. These have been sourced from FDA 483 citations, information obtained from various sources (such as GMP Trends, Pink Sheets, Drug GMP Reports) and the list of common deficiencies provided by the Australian Regulatory Authorities.

IX. Conclusions

A well designed stability test conducted using a written and authorized stability testing program is crucial for the generation of accurate stability data from which reliable conclusions can be drawn with respect to the shelf life of the product. All methods used in the stability test for regulatory purposes should be fully validated. In this respect, good science is the order of the day coupled with an appreciation of regulatory requirements.

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CHAPTER 13

USA regulatory aspects pertaining to controlled release veterinary drug delivery systems

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Overview

I. Introduction

The US animal drug registration process is highly legalistic and is based upon the provisions of the US Food, Drug and Cosmetics Act (Act) which relegates enforcement responsibilities to the US Food and Drug Administration (FDA). Extensive and detailed regulations have been promulgated by the FDA pursuant to the authority of the Act.

Key provisions of these regulations include the following:

- That animal drugs must be shown, by quality data, to be safe and effective when used as directed in the product labeling.

- Products must be manufactured according to stringent current Good Manufacturing Practice (cGMP) regulations.
- Safety and effectiveness data must be gathered under rigorous test control conditions to assure data quality.
- Manufacture and use of the product must meet standards which assure that the quality of the human environment is not compromised.
- Products must be shown to remain stable during a defined, acceptable dating period.
- Drugs which are claimed in their labeling to possess a controlled or sustained-release feature, must have appropriate lot release tests to assure that each manufactured lot has the release characteristics claimed in the labeling.

Because of the highly legalistic and involved processes associated with US animal drug registration, sponsors of drug products with controlled or sustained release features who propose to undergo the US animal drug registration process are encouraged to discuss the probable requirements with the Agency early in the development process.

II. Federal Food Drug and Cosmetics Act

The US Federal Food, Drug and Cosmetics Act sets forth the basic legal requirements for both human and animal drugs. It was adopted to protect the consuming public by attempting to provide drugs of consistent quality, purity and efficacy. It is a living document in that it is amended frequently and interpreted constantly. The Act delegates to the US Secretary of Health and Human Services the responsibility of administering and enforcing its provisions. It also directs the Secretary to promulgate appropriate regulations which have the force of law and which further define detailed provisions for interpreting and enforcing the intent of the Act.

The responsibility for enforcement of the Federal Food Drug and Cosmetic Act has been delegated to the Food and Drug Administration, which is a part of the Department of Health and Human Services. The agency is administered by a Commissioner, and six Associate Commissioners. It is broken down, at the headquarters level, into five Centers, namely Foods (which includes cosmetics), Drugs, Devices, Radiological Health, and Veterinary Drugs.

FDA maintains a headquarters building in the District of Columbia, but its main headquarters activities are at 5600 Fishers Lane, Rockville, MD. There are ten regions and 17 district offices located throughout the United States. These local offices have the prime responsibility for the day-to-day monitoring of manufacturers of products falling within their jurisdiction.

The Center for Veterinary Medicine is located at 7500 Standish Place, Rockville, MD and has the responsibility for reviewing new animal drug applications and establishing and coordinating policies regarding the safe and effective use of drugs used in animals.

Pursuant to the mandate of the Federal Food Drug and Cosmetics Act, the FDA has

promulgated an extensive set of regulations which have the force of law. Each of these was published in the Federal Register as a proposal and following public review and comment became incorporated into the US Code of Federal Regulations (CFR). Many provisions of the resulting 21 CFR affect both human and animal drugs, however specific regulations affecting animal drugs exclusively have been promulgated. For example 21 CFR Parts 500–599 currently consist of approximately 572 pages of detailed regulations which are specific to animal drugs, feeds and related products.

III. US regulations and requirements

The US animal drug regulation process is probably the most legalistic of any nation. Each new animal drug approval requires publication of a regulation describing the formal conditions of use allowed with the approval. These regulations are incorporated into 21 CFR Part 500. In addition to the official regulations, the agency has developed extensive written policies and procedures which are intended to provide uniformity and fairness to the regulatory process. Associated with this legalistic formality is a strenuous set of requirements for data development and reporting which assure that the new animal drug product meet high standards for safety and effectiveness including the public health safety of food derived from treated animals. The products must also meet rigid standards for strength, purity and quality. Essentially the manufacturing and controls provisions for new animal drugs are the same as for human drugs. Prior to an approval the manufacturing and control process must be thoroughly validated and the validation records along with the facility and equipment are subject to a pre-approval inspection. This inspection is usually performed by the same local FDA staff members which perform this function for human drugs.

IV. Public policy and animal drug regulation in the US

Public policy as related to animal drug regulation in the US can be likened to a three-legged stool. The three legs would consist of science, law and social values. When there is a balance among the emphases placed upon these and the 'legs' are of essentially the same length, the regulatory 'stool' is relatively level and functional. However, when inordinate emphasis is placed upon one facet or the 'leg' becomes lengthened, the surface of the regulatory 'stool' becomes tilted and unbalanced. For example, there is an occasional tendency for a not well informed public to become inordinately concerned regarding a social value aspect such as animal residue or environmental safety and those concerns become reflected in inappropriate requirements. Fortunately the legal and scientific aspects are (usually) eventually brought to bear and tend to correct such excesses.

V. US animal drug regulation process

It would be impossible in this chapter to thoroughly describe all aspects of veterinary

drug regulation. However, it may be instructive to begin by citing some definitions as described by Section 201 of the Act.[1]

V.A. Definitions

V.A.1. New animal drug

The term 'new animal drug' means[2] 'any drug intended for use for animals other than man, including any drug intended for use in animal feed but not including such animal feed (1) the composition of which is such that such drug is not generally recognized, among experts qualified by scientific training and experience to evaluate the safety and effectiveness of animal drugs, as safe and effective for use under the conditions prescribed, recommended, or suggested in labeling thereof; except that such a drug is not so recognized shall not be deemed to be a 'new animal drug' if at any time prior to June 25, 1938, it was the subject to the Food and Drug act of June 30, 1906, as amended, and if at such time its labeling contained the same representations concerning the conditions of its use; or (2) the composition of which is such that such drug, as a result of investigations to determine its safety and effectiveness for use under such conditions, has become so recognized but which has not, otherwise than in such investigations, been used to a material extent for a material time under such conditions; or (3) which drug is composed wholly or partly of any kind of penicillin, streptomycin, chlortetracycline, chloramphenical, or bacitracin, or any derivation thereof, except when there is in effect a published order of the Secretary declaring such drug not to be new animal drug on the grounds that (A) the requirement of certification batches of such drug, as provided for in section 512(n), is not necessary to insure that the objectives specified in paragraph (3) thereof are achieved and (B) that neither subparagraph (1) nor (2) of this paragraph (w) applies to such drug'.

Attempting to interpret this definition gives one an appreciation of the regulatory complexity of the Act. A key provision which deserves some elaboration is the clause '*safe and effective for use under the conditions prescribed, recommended or suggested in the labeling*'. This clause, in turn, calls for an understanding of the Acts definition of a label and labeling.

V.A.2. Label

The term 'label' means[3] 'a display of written, printed or graphic matter upon the immediate container of any article; and a requirement made by or under authority of this Act that any word, statement or other information appearing on the label shall not be considered to be complied with unless such word, statement, or other information also appear(s) on the outside container or wrapper, if any there be, of the retail package of such article, or is easily legible through the outside container or wrapper'.

V.A.3. Labeling

The term 'labeling' means[4] 'all labels and other written, printed, or graphic matter (1) upon any article or any of its containers or wrappers, or (2) accompanying such article'.

V.B. Provisions of the act

Specific requirements of the Act for new animal drugs are contained in Section 512 which begins[5] 'Sec. 512 (a)(1) A new animal drug shall, with respect to any particular use or intended use of such drug, be deemed unsafe for the purposes of section 501 (a)(5) and section 402 (a)(2)(D) unless (A) there is in effect an approval of an application filed pursuant to subsection (b) of this section with respect to such use or intended use of such drug, (B) such drug, its labeling, and such use conform to such approved application, and (C) in the case of a new animal drug subject to subsection (n) of this section and not exempted therefrom by regulations it is from a batch with respect to which a certificate or release issued pursuant to subsection (n) is in effect with respect to such drug'.

It is important to note that an **investigational exemption** to the above provision is provided in Sec. 512 (a)(3) which states[6] 'A new animal drug or an animal feed bearing or containing a new animal drug shall not be deemed unsafe for the purposes of section 501 (a)(5) or (6) if such article is for investigational use and conforms to the terms of an exemption in effect with respect thereto under section 512(j)'.

The introductory provisions to Section 512 of the Act described above provide for applications to be filed and approved. Section 512 goes on to provide additional instructions for filing of applications and establishing basic requirements for their approval as follows[7]: 'Sec. 512 (b)(1) Any person may file with the Secretary an application with respect to any intended use or uses of a new animal drug. Such person shall submit to the Secretary as a part of the application (A) full reports of investigations which have been made to show whether or not such drug is safe and effective for use; (B) a full list of articles used as components of such drug; (C) a full statement of the composition of such drug; (D) a full description of the methods used in, and the facilities and controls used for, the manufacture, processing, and packing of such drug; (E) such samples of such drug and of the articles used as components thereof, of any animal feed for use in or on which such drug is intended, and of the edible portions or products (before or after slaughter) of animals to which such drug (directly or in or on animal feed) is intended to be administered, as the Secretary may require; (F) specimens of the labeling proposed to be used for such drug, or in case such drug is intended for use in animal feed, proposed labeling appropriate for such use, and specimens the labeling for the drug to be manufactured, packed or distributed by the applicant; (G) a description of practicable methods for determining the quantity, if any, of such drug in or on food, and any substance formed in or on food, because of its use; and (H) the proposed tolerance or withdrawal period or other use restrictions for such drug if any tolerance or withdrawal period or other use restrictions are required in order to assure that the proposed use of such drug will be safe'.

V.C. Provisions of the Regulations (applications)

The provisions of the regulations which have been promulgated pursuant to the Act regarding applications for approval of veterinary drugs are described in 21 CFR subpart 514.1 as follows [8]: '(a) Applications to be filed under section 512(b) of the

act shall be submitted in the form described in paragraph (b) of this section. If any part of the application is in a foreign language, an accurate and complete English translation shall be appended to such part. Translations of literature printed in a foreign language shall be accompanied by copies of the original publication. The application must be signed by the applicant or by an authorized attorney, agent, or official. If the applicant or such authorized representative does not reside or have a place of business within the United States, the application must also furnish the name and post office address of, and must be countersigned by, an authorized attorney, agent or official residing or maintaining a place of business within the United States. Pertinent information may be incorporated in, and will be considered as part of, an application on the basis of specific reference to such information, including information submitted under the provisions of 511.1 of this chapter, in the files of the Food and Drug Administration; however, the reference must be specific in identifying the information. Any reference to information furnished by a person other than the applicant may not be considered unless its use is authorized in a written statement signed by the person who submitted it'.

Paragraph (b) which is described in the above paragraph reads as follows: '(b) Applications for a new animal drugs shall be submitted in triplicate and assembled in the manner prescribed by paragraph (b)(15) of this section, and shall include the following information [9]:

(1) *Identification.* Whether the submission is an original or supplemental application; the name and address of the applicant; the date of the application; the tradename(s) (if one has been proposed) and chemical name(s) of the new animal drug. Upon receipt, the application will be assigned a number NADA——, which shall be used for all correspondence with respect to the application.

(2) *Table of contents and summary.* The application shall be organized in a cohesive fashion, shall contain a table of contents which identifies the data and other material submitted, and shall contain a well organized summary and evaluation of the data in the following form:

(i) Chemistry

(a) chemical structural formula or description for any new animal drug substance;
(b) relationship to other chemically or pharmacologically related drugs;
(c) description of dosage form and quantitative composition;

(ii) Scientific rationale and purpose the new animal drug is to serve:

(a) clinical purpose;
(b) highlights of laboratory studies: the reasons why certain types of studies were done or omitted as related to the proposed conditions of use and to information already known about this class of compounds. Emphasize any unusual or particularly significant pharmacological effects or toxicological findings;
(c) highlights of clinical studies: the rationale of the clinical study plan showing why types of studies were done, amended, or omitted as related to laboratory studies and prior clinical experience;

(d) conclusions: a short statement of conclusions combining the major points of effectiveness and safety as they relate to the use of the new animal drug.

(3) *Labeling.*Three copies of each piece of all labeling to be used for the article (total of nine).

(i) All labeling should be identified to show its position on, or the manner in which it is to accompany the market package.
(ii) Labeling for non-prescription new animal drugs should include adequate directions for use by the layman under all conditions of use for which the new animal drug is intended, recommended or suggested in any of the labeling or advertising sponsored by the applicant.
(iii) Labeling for prescription veterinary drugs should bear adequate information for use under which veterinarians can use the new animal drug safely and for the purposes for which it is intended, including those purposes for which it is to be advertised or represented, in accord with 201.105 of this chapter.
(iv) All labeling for prescription or nonprescription new animal drugs shall be submitted with any necessary use restrictions prominently and conspicuously displayed.
(v) Labeling for new animal drugs intended for use in the manufacture of medicated feeds shall include:

(a) Specimens of labeling to be used for such new animal drug with adequate directions for the manufacture and use of finished feeds for all conditions for which the new animal drug is intended, recommended, or suggested in any of the labeling, including advertising sponsored by the applicant. Ingredient labeling may utilize collective names as provided in 501.110 of this chapter.
(b) Representative labeling proposed to be used for Type B and Type C medicated feeds containing the new animal drug.

(4) *Components and composition.* A complete list of the articles used for production of the new animal drug including a full list of the composition of each article:

(i) A full list of the articles used as components of the new animal drug. This list should include all substances used in the synthesis, extraction, or other method of preparation of any new animal drug and in the preparation of the finished dosage form, regardless of whether they undergo chemical change or are removed in the process. Each component should be identified by its established name, if any, or complete chemical name, using structural formulas when necessary for specific identification. If any proprietary name is used, it should be followed by a complete quantitative statement of composition. Reasonable alternatives for any listed component may be specified.
(ii) A full statement of the composition of the new animal drug. The statement shall set forth the name and amount of each ingredient, whether active or not, contained in a stated quantity of the new animal drug in the form in which it is to be distributed (for example, amount per tablet or milliliter) and a batch formula representative of that to be employed for the manufacture of the finished dosage form. All compo-

nents should be included in the batch formula regardless of whether they appear in the finished product. Any calculated excess of an ingredient over label declaration should be designated as such and percent excess shown. Reasonable variation may be specified.

(iii) If it is a new animal drug produced by fermentation:

(a) source and type of microorganism used to produce the new animal drug;
(b) composition of the media used to produce the new animal drug;
(c) type of precursor used, if any, to guide or enhance production of the antibiotic during fermentation;
(d) name and composition of preservative, if any, used in the broth;
(e) a complete description of the extraction and purification processes including the names and compositions of the solvents, precipitants, ion exchange resins, emulsifiers, and all other agents used;
(f) if the new animal drug is produced by a catalytic hydrogenation process (such as tetracycline from chlortetracycline), a complete description of each chemical reaction with graphic formulas used to produce the new animal drug, including the names of catalyst used, how it is removed, and how the new animal drug is extracted and purified.

(5) *Manufacturing methods, facilities and controls.* A full description of the methods used in, and the facilities and controls used for, the manufacture, processing, and packing of the new animal drug. This description should include full information with respect to any new animal drug in sufficient detail to permit evaluation of the adequacy of the described methods of manufacture, processing, and packing, and the described facilities and controls to determine and preserve the identity, strength, quality, and purity of the new animal drug, and the following:

(i) If the applicant does not himself perform all the manufacturing, processing, packaging, labeling, and control operations for any new animal drug, he shall:

identify each person who will perform any part of such operations and designate the part; and provide a signed statement from each such person fully describing, directly or by reference, the methods, facilities, and controls he will use in his part of the operation. The statement shall include a commitment that no changes will be made without prior approval by the Food and Drug Administration, unless permitted under 514.8.

(ii) A description of the qualifications, including educational background and experience, of the technical and professional personnel who are responsible for assuring that the new animal drug has the identity, strength, quality, and purity it purports or is represented to possess, and a statement of their responsibilities.

(iii) A description of the physical facilities including building and equipment used in manufacturing, processing, packaging, labeling, storage, and control operations.

(iv) The methods used in the synthesis, extraction, isolation, or purification of any new animal drug. When the specifications and controls applied to such new animal

drugs are inadequate in themselves to determine its identity, strength, quality, and purity, the methods should be described in sufficient detail, including quantities used, times, temperature, pH, solvents, etc., to determine these characteristics. Alternative methods within reasonable limits that do not affect such characteristics of the new animal drug may be specified. A flow sheet and indicated equations should be submitted when needed to explain the process.

(v) Precautions to insure proper identity, strength, quality, and purity of the raw materials, whether active or not, including:

(a) the specifications for acceptance and methods of testing for each lot of raw material;

(b) a statement as to whether or not each lot of raw materials is given a serial number to identify it, and the use made of such numbers in subsequent plant operations.

(vi) The instructions used in the manufacturing, processing, packaging, labeling of each dosage form of the new animal drug, including:

(a) the method of preparation of the master formula records and individual batch records and the manner in which these records are used;

(b) the number of individuals checking weight or volume of each individual ingredient entering into each batch of the new animal drug;

(c) a statement as to whether or not the total weight or volume of each batch is determined at any stage of the manufacturing process subsequent to making up a batch according to the formula card and, if so, at what stage and by whom it is done;

(d) the precautions used in checking the actual package yield produced from a batch of the new animal drug with the theoretical yield. This should include a description of the accounting for such items as discards, breakage, etc., and the criteria used in accepting or rejecting batches of drugs in the event of an unexplained discrepancy;

(e) the precautions used to assure that each lot of the new animal drug is packaged with the proper label and labeling, including provisions for labeling storage and inventory control;

(f) any special precautions used in the operations.

(vii) The analytical controls used during the various stages of the manufacturing, processing, packaging, and labeling of the new animal drug, including a detailed description of the collection of samples and the analytical procedures to which they are subjected. The analytical procedures should be capable of determining the active components within a reasonable degree of accuracy and of assuring the identity of such components:

(a) a description of practicable methods of analysis of adequate sensitivity to determine the amount of the new animal drug in the final dosage form should be included. The dosage form may be a finished pharmaceutical product, a Type A medicated article, a Type B or a Type C medicated feed, or a product for use in

animal drinking water. Where two or more active ingredients are included, methods should be quantitative and specific for each active ingredient;
(b) if the article is one that is represented to be sterile, the same information with regard to the manufacturing, processing, packaging, and the collection of samples of the drug should be given for sterility controls. Include the standards used for acceptance of each lot of the finished drug.

(viii) An explanation of the exact significance of any batch control numbers used in the manufacturing, processing, packaging, and labeling of the new animal drug, including such control numbers that may appear on the label of the finished article. State whether these numbers enable determination of the complete manufacturing history of the product. Describe any methods used to permit determination of the distribution of any batch if its recall is required.
(ix) Adequate information with respect to the characteristics of and the test methods employed for the container, closure, or the component parts of the drug package to assure their suitability for the intended use.
(x) A complete description of, and data derived from, studies of the stability of the new animal drug in the final dosage form, including information showing the suitability of the analytical methods used. A description of any additional stability studies underway or planned. Stability data for the finished dosage form of the new animal drug in the container in which it is to be marketed, including any proposed multiple dose container, and, if it is to be put into solution at the time of dispensing, for the solution prepared as directed. If the new animal drug is intended for use in the manufacture of Type C medicated feed as defined in 558.3 of this chapter, stability data derived from studies in which representative formulations of the medicated feed articles are used. Similar data may be required for Type B medicated feeds as determined by the Food and Drug Administration on a case-by-case basis. Expiration dates shall be proposed for the finished pharmaceutical dosage forms and Type A medicated articles. If the data indicate that an expiration date is needed for Type B or Type C medicated feeds, the applicant shall propose such expiration date. If no expiration date is proposed for Type B or Type C medicated feeds, the applicant shall justify its absence with data.
(xi) Additional procedures employed which are designed to prevent contamination and otherwise assure proper control of the product. An application may be refused unless it includes adequate information showing that the methods used in, and the facilities and controls used for, the manufacturing, processing, and packaging of the new animal drug are adequate to preserve its identity, strength, quality, and purity in conformity with good manufacturing practice and identifies each establishment, showing the location of the plant conducting these operations.

(6) *Samples.* Samples of the new animal drug and articles used as components and information concerning them may be requested by the Center for Veterinary Medicine as follows:

(i) Each sample shall consist of four identical, separately packaged subdivisions, each containing at least three times the amount required to perform the laboratory

test procedures described in the application to determine compliance with its control specifications for identity and assays. Each of the samples submitted shall be appropriately packaged and labeled to preserve its characteristics, to identify the material and the quantity in each subdivision with the name of the applicant and the new animal drug application to which it relates.

(7) *Analytical methods for residues.* Applicants shall include a description of practicable methods for determining the quantity, if any, of the new animal drug in or on food, and any substance formed in or on food because of its use, and the proposed tolerance or withdrawal period or other use restrictions to ensure that the proposed use of this drug will be safe. When data or other adequate information establish that it is not reasonable to expect the new animal drug lot become a component of food at concentrations considered unsafe, a regulatory method is not required.

(i) The kind of information required by this subdivision may include: Complete experimental protocols for determining drug residue levels in the edible products, and the length of time required for residues to be eliminated from such products following the drug's use; residue studies conducted under appropriate (consistent with the proposed usage) conditions of dosage, time, and route of administration to show levels, if any, of the drug and/or its metabolites in test animals during and upon cessation of treatment and at intervals thereafter in order to establish a disappearance curve; if the drug is used in combination with other drugs, possible effects of interaction demonstrated by the appropriate disappearance curve or depletion patterns after drug withdrawal under appropriate (consistent with the proposed usage) conditions of dosage, time, and route of administration; if the drug is given in the feed or water, appropriate performance data in the treated animal; if the drug is to be used in more than one species, drug residue studies or appropriate metabolic studies conducted for each species that is food-producing. To provide these data, a sufficient number of birds or animals should be used at each sample interval. Appropriate use of labeled compounds (e.g. radioactive tracers) may be utilized to establish metabolism and depletion curves. Drug residue levels ordinarily should be determined in muscle, liver, kidney, and fat and where applicable, in skin, milk and eggs (yolk and egg white). As a part of the metabolic studies, levels of the drug or metabolite should be determined in blood where feasible. Samples may be combined where necessary. Where residues are suspected or known to be present in litter from treated animals, it may be necessary to include data with respect to such residues becoming components of other agricultural commodities because of use of litter from treated animals.

(ii) A new animal drug that has the potential to contaminate the human food with residues whose consumption could present a risk of cancer to people must satisfy the requirements of subpart E of part 500 of this chapter.

(8) Evidence to establish safety and effectiveness.

(i) An application may be refused unless it contains full reports of adequate tests by

all methods reasonably applicable to show whether or not the new animal drug is safe and the effective use as suggested in the proposed labeling.
(ii) An application may be refused unless it includes substantial evidence, consisting of adequate and well-controlled investigations, including field investigation, by experts qualified by scientific training and experience to evaluate the effectiveness of the new animal drug involved, on the basis of which it could fairy and reasonably be concluded by such experts that the new animal drug will have the effect it purports or is represented to have under the conditions of use prescribed, recommended, or suggested in the proposed labeling.
(iii) An application may be refused unless it contains detailed reports of the investigations, including studies made on laboratory animals, in which the purpose, methods, and results obtained are clearly set forth of acute, subacute, and chronic toxicity, and unless it contains appropriate clinical laboratory results related to safety and efficacy. Such information should include identification of the person who conducted each investigation, a statement of where the investigations were conducted, and where the raw data are available in the application.
(iv) All information pertinent to an evaluation of the safety and effectiveness of the new animal drug received or otherwise obtained by the applicant from any source, including information derived from other investigations or commercial marketing (for example, outside the United States), or reports in the scientific literature, both favorable and unfavorable, involving the new animal drug that is the subject of the application and related new animal drugs shall be submitted. An adequate summary may be acceptable in lieu of a reprint of a published report that only supports other data submitted. Include any evaluation of the safety or effectiveness of the new animal drug that has been made by the applicant's veterinary or medical department, expert committee, or consultants.
(vi) An application shall include a complete list of the names and post office addresses of all investigators who received the new animal drug. This may be incorporated in whole or in part by reference to information submitted under the provisions of 511.1 of this chapter.
(vii) Explain any omission of reports from any investigator to whom the investigational new animal drug has been made available. The unexplained omission of any reports of investigations made with the new animal drug by the applicant or submitted to him by an investigator or the unexplained omission of any pertinent reports of investigations or clinical experience received or otherwise obtained by the applicant from published literature or other sources that would bias an evaluation of the safety of the new animal drug for its effectiveness in use, constitutes grounds for the refusal or withdrawal of the approval of an application.
(viii) If a sponsor has transferred any obligations for the conduct of any clinical study to a contract research organization, the application is required to include a statement containing the name and address of the contract research organization, identifying the clinical study, and listing the obligations transferred. If all obligations governing the conduct of the study have been transferred, a general statement of this transfer – in lieu of a listing of the specific obligations transferred – may be submitted.

(ix) If original subject records were audited or reviewed by the sponsor in the course of monitoring any clinical study to verify the accuracy of the case reports submitted to the sponsor, a list identifying each clinical study so audited or reviewed.

(9) *Reserved.*

(10) *Not applicable.*

(11) *Applicants commitment.* It is understood that the labeling and advertising for the new animal drug will prescribe, recommend, or suggest its use only under the conditions stated in the labeling which is part of this application and if the article is a prescription new animal drug, it is understood that any labeling which furnishes or purports to furnish information for use or which prescribes, recommends, or suggest a dosage for use of the new animal drug will also contain, in the same language and emphasis, information for its use including indications, effects, dosages, routes, methods and frequency and duration of administration, any relevant hazards, contraindications, side effects, and precautions contained in the labeling which is part of this application. It is understood that all representations in this application apply to the drug produced until changes are made in conformity with 514.8.

(12) *Additional commitments.*

(i) New animal drugs as defined in 510.3 of this chapter, intended for use in the manufacture of animal feeds in any State will be shipped only to persons who may receive such drugs in accordance with 510.7 of this chapter.

(ii) The methods, facilities, and controls describe under item (5) of this application conform to the current good manufacturing practice regulations in subchapter C of this chapter.

(iii) With respect to each non-clinical laboratory study contained in the application, either a statement that the study was conducted in compliance with good laboratory practice regulations set forth in part 58 of this chapter, or, if the study was not conducted in compliance with such regulations, a brief statement of the reason for the non compliance.

(13) *Reserved.*

(14) *Environmental assessment.* The applicant is required to submit either a claim for a categorical exclusion under 25.30 or 25.33 of this chapter or an environmental assessment under 25.40 of this chapter.[10]

V.D. Cross referencing with other regulations

While the above regulations are comprehensive in the stated requirements, numerous references are made to other regulations such as Labeling; Good Manufacturing Practices (GMP)[11]; Good Laboratory Practices (GLP) [12]; and Environmental Impact Considerations.[10] Each of these contains extensive and detailed provisions regarding that particular subject and persons considering obtaining approval of an application for a controlled release new animal drug are advised to read and appreciate the impact and importance of complying with these provisions, since they have a significant impact on their registration process.

V.E. Requirements for controlled release veterinary drugs

The Center for Veterinary Medicines Division of Manufacturing Technologies is responsible for review and approval of information contained in new animal drug applications regarding Parts 514.1 (b)(4) Components and Composition and (5) Manufacturing Methods Facilities and Controls. Any special testing requirement for a sustained release product would be the responsibility of this group.

Other than the following provisions included in the CVM Stability Testing Guidelines[13] there are currently no known formally written policies regarding the Center for Veterinary Medicines requirements for controlled release animal drugs:

> 'J. SUSTAINED-RELEASE PRODUCTS: In addition to the specific stability tests that are required for the particular dosage form, the stability study should include assays for the release pattern of the active ingredient. Because of the nature of these types of drugs, drug release patterns or rates should be measured by dissolution tests. When microencapsulated sustained-release dosage forms are to be studied, measurement of the capsule particle size range distribution, including ratios of core to wall thickness, may be necessary'.[13]

Based upon experience and discussions with CVM officials, a critical element regarding specific requirements for controlled-release products is a statement on the label for the product which make special reference to controlled release or specific claims for duration of action or effectiveness. Whenever such references or claims are made, CVM can be expected to insist that appropriate analytical methods and finished product release specifications be included in the new animal drug application to assure that each manufactured lot or batch of the product will possess the claimed release characteristic.[14]

While CVM has few formal unique policies for control release new animal drugs, it can be expected to rely heavily upon related requirements for human drugs. Controlled release dosage forms are defined by the FDA as *'those formulations designed to release an active ingredient(s), at rates which differ significantly from their corresponding immediate release forms'*. Many terms have been used to describe these forms, e.g. delayed action, extended action, gradual release, prolonged release, protracted release, repeat action, slow release, sustained release, depot, retard, and timed release dosage forms. Any of these terms, or similar terms which impart the same idea, may be used in labeling to designate a controlled release product. However, the Agency generally uses the following terminology:

A. Oral dosage:

1. delayed release drug products;
2. prolonged release drug products.

B. Intramuscular dosage:

1. depot injections;
2. water immiscible injections, i.e. oils.

C. Cutaneous/subcutaneous dosage:

1. implants;
2. transdermal preparations.

D. Targeted dosage form:

1. ocusert;
2. IUDs;
3. pumps.

Preliminary consideration is being given by the Center for Veterinary Medicine to including the term 'intravaginal insert' as a targeted dosage form for products to be inserted into the vagina of domestic animals for purposes of providing sustained delivery of progesterone for estrus synchronization and ancillary actions.

Approval of such drug delivery systems requires submission of data to substantiate the clinical safety and efficacy (if not otherwise known), of the product and demonstration of its controlled drug release characteristics. Controlled clinical studies may be required to demonstrate the safety and efficacy of the drug(s) depending upon what is known about the drug substance. Additionally, drug bioavailability data on the controlled release formulation is required. Such data may, on rare occasions, be acceptable in lieu of clinical trials.

VI. Summary

In summary, the US animal drug registration process is probably the most highly legalistic in the world. It is based upon the provisions of the US Food, Drug and Cosmetics Act. This Act relegates enforcement responsibilities to the US Food and Drug Administration. The FDA have promulgated extensive and detailed regulations pursuant to the authority of the Act. Persons considering obtaining approval of an application for a controlled release new animal drug are advised to share, in advance, the characteristics of the drug and its proposed use with appropriate Center for Veterinary Medicine officials and to obtain their advice regarding appropriate safety and effectiveness testing requirements as well as special requirements for stability testing and batch release requirements.

References

1. Section 201. US Food, Drug and Cosmetics Act.
2. Section 201 (w).
3. Section 201 (k).
4. Section 201 (m).
5. Section 512 (a)(1).
6. Section 512 (a)(3).
7. Section 512 (b)(1).
8. Part 514.1 (a). Title 21 US Code of Federal Regulations (21 CFR).
9. Part 514.1 (b) (1)–(14).
10. Part 25–(21 CFR).

11. Part 211–(21 CFR).
12. Part 58–(21 CFR).
13. FDA Centre for Veterinary Medicine Drug Stability Guidelines; Fourth Revision, 12/1/90; Section 2. J. Sustained Release Products. Published by US Department of Health and Human Services, Public Health Service, Food and Drugs Administration. 1990. pp. 2–25.
14. Arnold RG. Controlled release new animal drugs. J Control Release 1988;8:85–90.

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