DESIGN, DEVELOPMENT AND OPTIMISATION OF VETERINARY INTRAVAGINAL CONTROLLED RELEASE DRUG DELIVERY SYSTEMS

A thesis

submitted in fulfilment

of the requirements for the Degree of

Doctor of Philosophy

at the

University of Waikato

by

COLIN ROGER OGLE

University of Waikato

1999

Errata

Table 2.2.3 (p62) – Formulation A should be 6-up CIDR®1380 Cattle insert not CIDR®1900 Cattle insert.

Tables 2.3.6 to 2.3.9 (pp67-68) – Last row should contain RSD data not SEM.

Table 2.3.6

	Progesterone (g)	Progesterone (%w/w)
RSD	1.23	1.47

Table 2.3.7

	Progesterone (g)	Progesterone (%w/w)
RSD	2.16	2.22

Table 2.3.8

	Progesterone (g)	Progesterone (%w/w)
RSD	3.39	3.05

Table 2.3.9

	Progesterone (g)	Progesterone (%w/w)
RSD	2.82	2.66

Ruggedness (p141) – Should refer to Table 3.3.6 not Table 3.3.8.

Limit of quantitation (p141) – Should refer to Table 3.3.11 not Table 3.3.6.

Specificity (p141) – Should refer to Table 3.3.12 not Table 3.3.7.

Section 4.4.2.1 (p183) – Title should read 'Fitting of existing models describing drug release to invitro data' not 'Fitting of invitro data to existing models describing drug release'.

Eqn 4.4.4 (p185) – Should read $dQ/dt = [AC_pD_p/2t]^{1/2}$ not $dQ/dt = [2AC_pD_p/2t]^{1/2}$.

Section 4.4.3.1 (p190) – Title should read 'Fitting of existing models describing drug release to invivo data' not 'Fitting of invivo data to existing models describing drug release'.

4.5 Conclusions (p197) – Line 9 should read 'Model fitting of the…' not 'Data fitting of the…'.

5.1 Introduction (p198) – Line 2 should read (Figure 5.1.1) not (Figure 5.1).

5.4 Discussion (p222) – Line 21 should read Figure 5.4.5 not Figure 5.4.4.

Figure 6.3.1 (p243) – This should be Figure 6.4.1 not Figure 6.3.1.

6.4 Discussion (p243) – Line 13 should read '..AUC values which were not significantly different to.. not .. AUC values which were significantly different to..'.

Figure 6.3.2 (p246) – This should be Figure 6.4.2 not Figure 6.3.2.

Reference 9 (p249) – Should read New Zealand not New Zealnd.

Reference 226 (p276) – Web address should read http://www.fda.gov/cvm not http://www.fda.gov

Abstract

This Thesis begins by discussing the application of controlled drug delivery to the intravaginal delivery of hormones to control the oestrous cycle of farmed animals, with particular emphasis on the delivery of progestagens to cattle and sheep. The introduction highlights the recent advances made by animal physiologists in their knowledge of the oestrous cycle in cattle and sheep and how these advances have impacted upon the manner in which currently available intravaginal controlled release products are used. In addition it discusses the advances pharmaceutical scientists have made in response to the new knowledge.

The CIDR®1900 Cattle insert is a commercially available silicone based intravaginal delivery system containing the natural steroid progesterone for fertility regulation in cattle. It was designed in 1987 to be inserted for 12 days. Advances made in oestrous cycle understanding by animal scientists have resulted in this product being inserted for much shorter treatment periods of 7 days. Rathbone et al. recently optimised the CIDR®1900 Cattle insert for seven day insertion periods. The outcome of these scientists work was a manufactured prototype which, when compared to the CIDR®1900 Cattle insert, contained a reduced initial drug load and a much lower residual drug load after use. For commercial production the prototype insert needed to be scaled up. This process required detailed pharmaceutical characterisation, invitro release assessment, invivo bioequivalency and chemical and physical stability studies to be performed on the scaled up product (CIDR®1380 Cattle insert) to ensure the success of the scale up process. This characterisation process forms Chapters Two and Three of this Thesis.

Knowledge of the mechanism of release of a drug from a pharmaceutical product provides the formulation scientist with the necessary insight to optimise, further develop or recognise the potential and limitations of a product. Chapter Four explores the mechanism of release of progesterone from the silicone based CIDR® Cattle insert both invitro and invivo. Cumulative release data from both invitro and invivo studies were fitted against conventional mathematical models to determine the mechanism of release of progesterone from the CIDR® Cattle insert. In addition, an

experimental method was developed to assess the validity of the models. The method involved taking thin consecutive horizontal slices from the surface of CIDR® Cattle inserts after various periods of release. Cumulative release of progesterone, both invitro and invivo, was linear when plotted against the square root of time, suggesting that release from the CIDR® Cattle insert occurred in accordance with the square root of time mechanism. However, experimental evidence from the horizontal slicing technique only supported the curve fitting evidence for invitro release. When the insert was investigated invivo, the slicing method indicated that a novel release mechanism was in operation which was better described by a zero order process.

Chapters Five and Six of this Thesis direct their focus to oestrus control in sheep. Chapter Five utilises information gained from both Rathbone et al. and the work performed in this Thesis on the CIDR® Cattle insert to characterise and optimise the commercially available sheep equivalent of the CIDR®1900 Cattle insert (known as the CIDR® Sheep and Goat insert). The Chapter successfully characterises the CIDR® Sheep and Goat insert, defines the parameters of, and tests, an optimised version of this sheep product.

The final Chapter of this Thesis investigates the use of poly-(ε-caprolactone) as a platform for the intravaginal delivery of progesterone to control oestrus in sheep. Silicone has certain limitations as a drug delivery platform, and with animal physiologists recent advances in knowledge, pharmaceutical scientists will need more versatile delivery platforms to develop intravaginal drug delivery systems which fulfil the future demands of the animal scientists.

Acknowledgements

I would like to begin by acknowledging the Foundation for Science, Research and Technology who created the GRIF programme as a strategy for fostering relationships between Industry and Universities to promote the growth of technology in New Zealand and InterAg who saw the value of the GRIF programme and supported me in my academic endeavours.

Special thanks to Mike, not only for his valued expertise in the execution and compilation of this research, but also for his tremendous support of my career development. Thanks to Kim who also provided encouragement and kept a watchful eye on my progress.

There are many people to thank at InterAg. Thanks to all of you who proof read various parts of the text. Thanks Craig for your help with formulation and with statistics. Thanks Shane for your help with analytical issues. Thanks to Justin and John. You guys are great to work with, great to beat at cards and if you hadn't helped me out with my dishes so much, I'd still be washing up now! Thanks to Vaughan for your help on all things manufacturing and to all of your team who helped me out. Thanks to all my other friends at InterAg who have made these three years rewarding socially as well as academically.

Thanks to all the people who participated in the invivo work in this Thesis. Some of you gave up time on weekends to do it and you're all wonderful. Thanks to the members of the Ruakura and the University of Waikato animal ethics committees who reviewed and approved my trial work, especially Tony who came along and checked on the welfare of the cows as part of the protocols. Special thanks Chris for all the organisation and work you put into my trials. Thanks Bid, Stuart, Gwyn, Suzanne for your blood sampling skills and all the farm hands who made sure the cows were handy to the yards. Special thanks John Smith for all the organisation and work on trials in sheep. Thanks to Cowley and John for handling the sheep. Thanks to Trish and Rachel in the RIA laboratory who did a great job of all the plasma analysis. Thanks Ross for loaning me ethanol when my orders were late.

Finally, thanks to all my family, especially my wife Zoe. Your support and encouragement mean a lot.

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List of Abbreviations

%w/w	percentage weight per weight composition
°C	degrees Celcius
ANOVA	ANalysis Of VAriance
AUC	Area Under the Curve
AUFS	Absorbance Units Full Scale
CL	Corpus Luteum
cm	centimetre
DRC	Dairying Research Corporation
E2	Oestradiol
FSH	Follicle Stimulating Hormone
g	gram
GnRH	Gonadotrophin Releasing Hormone
HPLC	High Pressure Liquid Chromatography
hr	hour
I	Intact
LH	Luteinising Hormone
µg	microgram
mg	milligram
mins	minutes
µL	microlitre
mL	millilitre
µm	micrometre
mm	millimetre
ng	nanogram
nm	nanometre
O	Ovariectomised
ODB	Oestradiol Benzoate
P4	Progesterone
PGF	Prostaglandin $F_{2\alpha}$
rpm	revolutions per minute
RSD	Relative Standard Deviation
SDA	Specially Denatured Alcohol
SEM	Standard Error in the Mean
USP	United States Pharmacopeia
UV	Ultra Violet
UV*sec	Ultra-violet light absorbance by time in seconds (integration units)
v/v	volume per volume

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Chapter One - Controlled Release Intravaginal Veterinary Drug Delivery Systems

1.1 Introduction

This Thesis is concerned with the development and optimisation of intravaginal veterinary drug delivery systems in farmed animals. The vagina of animals has been the focus of much research since the beginning of the Century[1, 2], and, since the mid 1960's, has been commercially exploited as a portal for the systemic delivery of drugs to farmed animals[3]. Since that time researchers have focused their efforts on using the vagina to deliver hormones to control the oestrous cycle of cattle, sheep and, to a lesser extent deer and goats. Today, the delivery of hormones to control the oestrous cycle of these animals remains the only application of this route for drug delivery. Indeed, all currently available intravaginal controlled release drug delivery systems contain either progesterone or synthetic progestagens for this purpose.

The development and optimisation of intravaginal veterinary drug delivery systems in farmed animals requires an in depth knowledge of both pharmaceutical science and the natural oestrous cycle of the animal in question. The former is required to rationally develop and optimise the product to ensure the quality, safety and efficacy of the final product. The latter is required to define the type of drugs which need to be delivered and the durations over which they must be administered.

In recent times animal physiologists and endocrinologists have expanded their knowledge on the oestrous cycle of cattle and sheep[3]. New information on how to control the oestrous cycle while maintaining normal fertility has come to light. This new information has impacted on the specifications which products used to control the oestrous cycle must meet (e.g., the delivery period for progesterone has decreased from 12-15 days to 7-10 days to prevent the formation of stale follicles). In addition the new knowledge has highlighted the need for coadministration of other pharmacologic compounds such as oestradiol (for follicular turnover) and prostaglandins (to regress corpus lutea). Animal physiologists and endocrinologists

have introduced fertility programmes which involve the continuous delivery of progesterone from controlled release products coupled with injections of oestradiol and prostaglandin (at appropriate times in the treatment programme) to ensure precise synchrony coupled with normal fertility.

On the pharmaceutical side, several conceptual and commercially available intravaginal veterinary drug delivery systems have been reported in the literature[4, 5] and several attempts have been made to optimise existing products[4, 6]. Only a limited number of polymers have been utilised as platforms for intravaginal drug delivery in farmed animals.

All the above mentioned aspects of intravaginal veterinary drug delivery will be discussed in this Introduction, and the holes in the literature which provided the opportunity for the research performed in this Thesis will be highlighted.

1.2 Fertility Control in Farmed Animals

1.2.1 Introduction

As already mentioned, substantial advances have been made in our understanding of the physiology and endocrinology of the oestrous cycles of livestock. Developments in controlled release drug delivery products have contributed substantially to this progress. The relationship between product availability and scientific advance is symbiotic. The availability of controlled release products has allowed animal scientists to control and study follicular development and ovulation in great depth, while the understanding gained from this research has allowed pharmaceutical scientists to optimise delivery systems which have been in existence for over 30 years. The recent advances in the area of animal physiology and endocrinology of the oestrous cycles of livestock and the impact of these advances on the application of drug delivery to oestrus control in these species has recently been reviewed by Rathbone et al.[3, 4, 5].

Control of the oestrous cycles of farmed animals is desirable for several reasons[5]. These include the synchronisation of oestrus in order to use artificial insemination to

spread desirable genes quickly, synchronisation of donor and recipient animals for embryo transfer, the breeding of synchronised animals as early in the season as possible and the breeding of synchronised animals out of season. These synchronisation practices can result in increased efficiency in production of meat and dairy produce. In addition, oestrous cycle control can contribute to maximisation of the number of offspring a fertile animal may produce within its lifespan. Finally, if all breeding activities can be carried out over a short time period, there are also advantages to the farmer with respect to time management.

The development of theories on how to manipulate the oestrous cycle of farmed animals requires a detailed understanding of the biochemistry involved during a naturally occurring cycle. Once the endogenous hormones which control the oestrous cycle have been identified, together with their pharmacokinetic behaviour and pharmacological interactions, then drug delivery systems for use in treatment programmes to control the cycle can be rationally developed.

1.2.2 Cattle

1.2.2.1 The Oestrous Cycle of Cattle

A complete bovine oestrous cycle occurs over a period of 18-24 days[3, 5]. The pituitary gland in the brain produces follicle stimulating hormone (FSH) which promotes the growth of cohorts of four to ten follicles on the ovaries over a period of 12-36 hours (Figure 1.2.1). Follicles require luteinising hormone (LH), which is released in pulses by the pituitary gland. In mono-ovulating species such as cattle, only one of these follicles is selected to become large and dominant. This process is not well understood but could involve competition for available LH. Follicles also secrete inhibin, which has a negative effect on FSH secretion, to restrict the growth of other follicles[7] (Figure 1.2.1). If the follicles do not receive sufficient LH then follicular atresia occurs. The dominant follicle is then replaced by a new follicular wave, with waves occurring constantly every 8-10 days. The oestrous cycle is coordinated such that a second or third follicular wave is occurring before one follicle survives to become dominant and ovulatory.

The bovine oestrous cycle has been divided into two stages, each with two sub-stages. The follicular phase has a duration of 3-7 days and comprises pro-oestrus and oestrus while the luteal phase completes the rest of the 18-24 day cycle and comprises metoestrus and dioestrus.

Pro-oestrus is a period of 2-6 days while a dominant ovulatory follicle is maturing[8]. The dominant follicle produces oestradiol. When the oestradiol level is high enough, the oestrus phase begins with behavioral oestrus where cows will stand to be mounted by herdmates[9]. Oestradiol also stimulates the formation of oxytocin receptors in the uterus.

Oestrus lasts for 8-30 hours[3, 10] before the high level of oestradiol combined with a low level of progesterone in the system initiates a surge in LH level to >30 ng/mL[5], at which time, ovulation occurs. Ovulation is referred to as day 0 of the oestrous cycle and marks the beginning of the luteal phase.

Days 0-5 of the luteal phase are defined as metoestrus[3, 5]. In the 3-4 days following ovulation a corpus luteum (CL) forms from the remnant theca and granulosa of the ovulatory follicle. The CL comprises small luteal cells which produce progesterone and large luteal cells which secrete oxytocin and develop prostaglandin $F_{2\alpha}$ (PGF) receptors (Figure 1.2.1). At day 4, progesterone levels are typically around 1 ng/mL. The fully formed CL produces increasing quantities of progesterone until, by day 8-9, plasma levels reach around 4-10 ng/mL. Progesterone suppresses LH production and so, during the luteal phase, developing follicles will not receive sufficient LH to ovulate.

The remaining days of the cycle (days 6-18) are termed di-oestrus[5]. During di-oestrus, oxytocin produced by the large luteal cells acts upon the uterine endometrium to stimulate PGF synthesis. At around day 16-17, the CL is destroyed in a process called luteolysis which involves release of PGF from the uterus (Figure 1.2.1). The trigger for PGF release involves oxytocin and oestradiol. It can be prevented by interferon factors produced by a developing embryo (Figure 1.2.1). During luteolysis, there is a precipitous decline in progesterone level and in oxytocin

level. Luteolysis marks the end of the luteal phase and the beginning of the follicular phase.

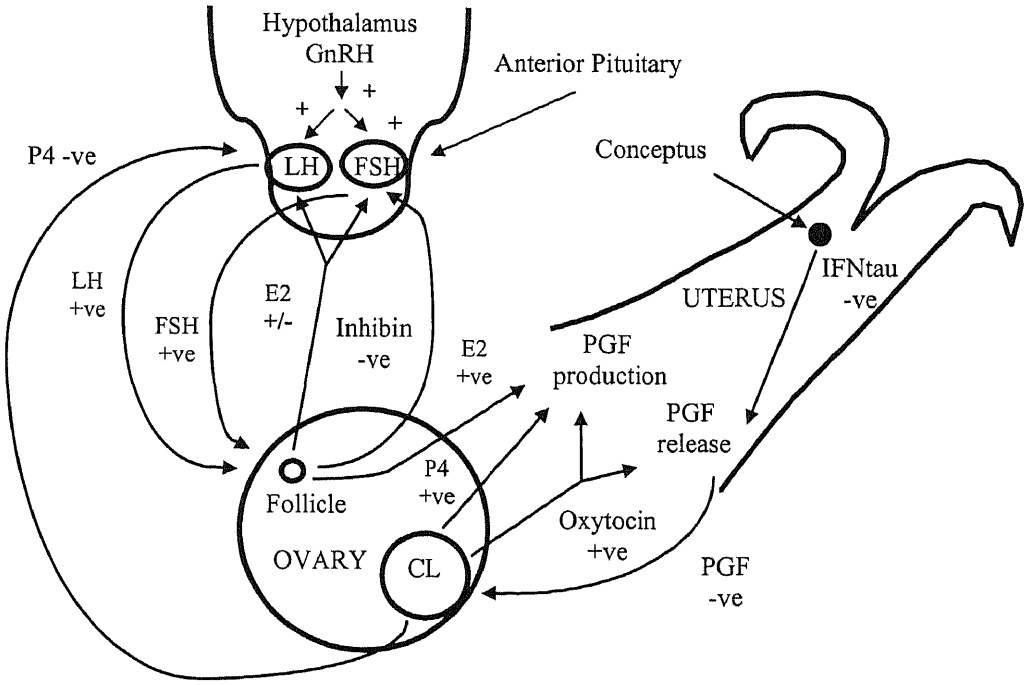


Figure 1.2.1 - Conceptual diagram of a cow and the interactions which control follicular development, ovulation, luteolysis and pregnancy responses (GnRH = gonadotrophin releasing hormone, LH = luteinising hormone, FSH = follicle stimulating hormone, CL = corpus luteum, P4 = progesterone, E2 = oestradiol, PGF = prostaglandin $F_{2\alpha}$, IFNtau = interferon-Tau) (personal communication, Chris Burke, Dairying Research Corporation)

1.2.2.2 Control of Oestrus in Cattle

Concepts for controlling the oestrous cycle have involved follicle wave regulation by either lengthening or shortening the luteal phase or by synchronised luteolysis. The challenges in controlling the oestrous cycle are to achieve a good level of synchronisation without compromising fertility.

1.2.2.3 Single Drug Therapy for Oestrus Control in Cattle

Early hormonal treatments for bovine oestrus control involved the injection of low doses of progesterone for up to three weeks to lengthen the luteal phase. These treatments produced a high degree of synchrony but poor fertility[11, 12] as did higher doses of progesterone per vaginum[13, 14]. Administration of orally active progestagens such as melengestrol acetate in feed resulted in reduced fertility unless progesterone was administered acutely about five days before termination of the feeding

treatment. The large progesterone dose was shown to induce atresia in persistently dominant follicles which form in the ovaries during prolonged periods of low circulating progesterone levels, particularly after spontaneous luteolysis of the CL has occurred[15]. Concentrations of progesterone sufficient to suppress the pro-oestrus pattern of pulsatile LH release must be maintained if persistent follicles and low fertility are to be avoided.

The inter-oestrus period can be shortened by surgical procedures on the CL, thus terminating the luteal phase and initiating pro-oestrus. However, it has long been known that synthetic prostaglandins will lyse the CL without causing any of the tissue damage associated with surgical procedures. PGF was identified as the natural luteolytic hormone first in sheep[16] and then in cattle[17]. A PGF injection is only effective during the luteal phase and as such, a single administration results in 50 to 70% of animals displaying oestrus within the next six days. If two PGF injections are given within ten to fourteen days of each other then, at the second injection, all animals should be in their luteal phase. The resulting synchrony is good in heifers (80% in oestrus from 48 to 80 h after the second injection) but less precise in cows[18,19]. This double PGF injection synchrony method may result in reduced fertility[20]. Attempts have also been made to control oestrus with oestradiol and gonadotrophin releasing hormone (GnRH). Their effects were variable and not successfully adopted into treatment programs[21].

1.2.2.4 Combination Drug Therapy for Oestrus Control in Cattle

Initial combination therapies used progesterone and oestradiol benzoate (ODB)[22] or oestradiol valerate (ODV)[23]. Progesterone should prevent the ovulatory LH surge, while oestradiol compounds were intended to induce premature luteolysis. Using oestradiol in combination with progesterone allowed treatment durations of 10 days with subcutaneous progesterone[23], or 12 days with intravaginal progesterone[14, 22]. These treatments yielded acceptable fertility in heifers but lowered fertility in lactating cows. Availability of synthetic PGF allowed prostaglandins to be used to achieve luteolysis instead of oestradiol. Smith et al.[24] showed that an 8 day progesterone treatment terminated with PGF administration could provide suitable synchrony to allow timed insemination post-treatment with acceptable fertility. Variations on this progesterone/PGF treatment will not always eliminate persistent

follicles and lowered fertility is associated with such follicles[14]. In an effort to control follicle development patterns, a combination of GnRH and PGF was tested. Growing and mature follicles were luteinised or ovulated due to LH release following a GnRH injection[25]. An injection of PGF 7 or 8 days later[26, 27] facilitated onset of oestrus but with poor synchrony as new wave emergence occurs 4 to 7 days after the GnRH administration[26].

Combining follicular phase and luteal phase regulation appears to provide the most successful oestrus regulation to date. Adams[28] and Bo et al.[29] showed that injected oestradiol produced a degree of synchrony in ovarian follicle development, with a new wave forming about four days post injection. Progesterone treatment periods of six to eight days have been shown to minimise the incidence of persistent follicles in heifers[30, 31]. These principles were combined by Day et al.[32] along with the use of PGF as a luteolytic agent. Oestradiol was injected at the beginning of the treatment with progesterone being administered per vaginum for seven days. A luteolytic dose of PGF was administered at removal of the progesterone delivery system and then oestradiol was injected 48 hours after insert removal. In this treatment regime, synchronous follicular development, luteolysis and follicular maturation are combined to achieve synchronised oestrus and a fertile ovulation.

1.2.2.5 Impacts of Current Understanding on Formulation Scientists

Current understanding of oestrus control suggests that synchronisation of cattle with normal fertility rates is best achieved by a 7 day delivery of progesterone and coadministration of shorter acting injectable drugs which control follicular turnover (e.g., oestradiol or GnRH) or induce CL regression (e.g., prostaglandin)[3]. The success of such programmes is evident in that combined treatments regimes are now used in preference to the traditionally used single treatments which involved the prolonged release of progesterone. Such combination programmes have become commonplace in modern farming practice in both Australia and New Zealand.

These new fertility programmes have impacted on drug delivery scientists working on delivery systems used in veterinary fertility regulation. Silicone intravaginal progesterone delivery systems such as CIDR®1900 Cattle inserts were originally developed with high initial drug loads to provide acceptable blood plasma

progesterone levels over 12 day treatments. The new programmes require that the inserts need only be used for shorter 7 day treatment programmes. As a result the intravaginal progesterone delivery systems exhibit a high residual progesterone content after use. This is not only wasteful in terms of drug utilisation and economy of manufacture but may also raise environmental issues. Rathbone et al. have recently addressed this problem and optimised the CIDR®1900 Cattle insert[6] (contains 1.9 g progesterone) to contain a lower initial progesterone load and to exhibit a lower residual load after a 7 day treatment while still maintaining blood plasma progesterone levels equivalent to the original CIDR®1900 Cattle insert. The resultant product is currently marketed under the name CIDR®1380 Cattle insert (contains 1.38 g progesterone). Rathbone et al. performed extensive research on a production made prototype of the CIDR®1380 Cattle insert to justify the steps taken to optimise the insert[6], however, no research has been performed on the large scale commercially manufactured CIDR®1380 Cattle insert and this has provided the opportunity for some of the research performed in this Thesis.

The coadministration of oestradiol benzoate (ODB) and PGF during these fertility programmes has, to date, been achieved by subcutaneous or intramuscular injection. However, in the field of veterinary drug delivery for farmed animals where costs must be minimised to make oestrus control economic to the farmer, the need for coadministration of these agents with the continuous delivery of progesterone presents an opportunity to the drug delivery scientist. The opportunity is to develop a single delivery system which is capable of delivering progesterone continuously, and ODB and PGF as pulsed doses at appropriate times in the treatment. This has been attempted by PLADE Holdings NZ Ltd who have developed an electronic delivery system called the Smartt[1] Intelligent Breeding Device (IBD). There appears to have been little pharmaceutical or biological characterisation of this delivery system. Although a thorough investigation of this product, or the development of a similar product, represents an opportunity for research, this is not pursued in this Thesis. However, it is important to highlight the development of this delivery system since it is a direct outcome of current understanding, and perhaps the future of drug delivery for oestrus control in cattle will involve such a technology.

1.2.3 Sheep

1.2.3.1 The Oestrous Cycle of Sheep

Reproduction in sheep is generally seasonal, with oestrous cycles of New Zealand sheep breeds commencing in late summer and continuing until the following spring if pregnancy does not occur[33]. These ewes are expected, therefore, to be anoestrus during late spring and early summer.

Photoperiod has been established as the environmental cue for annual reproduction to begin. Melatonin is secreted by the pineal gland in response to darkness[34]. Daytime plasma levels are around 25 pg/mL while in the hours of darkness this may rise to between 150 and 300 pg/mL. The changing length of exposure to melatonin as seasons change trigger the sheep's reproductive system. Controlling the photoperiod of a sheep's environment in an appropriated sequence can advance the onset of the breeding season[35].

During the breeding season, the ewe has oestrous cycles lasting 16-17 days. Oestrus itself tends to last 24-36 hours[36] and it is generally believed that ovulation occurs at the end of oestrus and is timed to a surge of LH. GnRH is recognized as important to the generation of the pre-ovulatory LH surge[37-39].

Follicular dynamics are less understood in sheep than in cattle. The ovaries of young ewes contain between 40,000 and 300,000 primordial follicles[40, 41] which are thought to emerge in an ordered sequence but at an unknown rate. It is estimated that development from the resting to pre-ovulatory stage takes about 6 months[42]. A ewe's ovary may contain several hundred follicles at any one time, of which 10-40 are visible[43]. Souza et al.[44] have reported that follicular growth and ovarian steroid secretions occur in waves with two synchronous stages but with a decline in steroid secretion preceding any change in follicular diameter. Other researchers have also observed distinct follicular waves, with up to 5 or 6 occurring between ovulations[45].

1.2.3.2 Control of Oestrus in Sheep

Control of the oestrous cycle in sheep is desirable for the same reasons as it is desirable in cattle: for more rapid genetic advancement of the flock through artificial

insemination and for other advanced breeding activities such as synchronisation of superovulating ewes and embryo recipient ewes for embryo transplantation.

As for cattle, efforts to manipulate oestrus in sheep have focused mainly on delivery of progestagens, as the physiology is essentially the same. A variety of delivery systems have been used for this purpose with some degree of synchrony being attained in flocks after removal of the progestagen releasing device[4, 5].

1.2.3.3 Impacts of Current Understanding on Formulation Scientists

The CIDR® Sheep and Goat insert is a silicone rubber based intravaginal progesterone delivery system which has been impacted by the current understanding of the sheep oestrous cycle and the need for its use in embryo transfer programmes. The CIDR® Sheep and Goat insert was initially developed to deliver progesterone over a 14 day period for the synchronisation of oestrus in New Zealand and Australian sheep breeds. In recent embryo transfer programmes, blood plasma progesterone levels higher than those produced by single CIDR® Sheep and Goat inserts are required. Indeed, in the case of the CIDR® Sheep and Goat insert, its usefulness in these embryo transfer programmes has needed either the replacement of the CIDR® Sheep and Goat insert with a fresh insert on day 8 of a 14 day treatment or the simultaneous insertion of two CIDR® Sheep and Goat inserts for the entire 14 day period[46-48].

The use of the CIDR® Sheep and Goat inserts in this manner has provided the opportunity for some of the research performed in this Thesis. The obvious response to the new applications of the CIDR® Sheep and Goat insert is the development of a single insert which exhibits elevated blood plasma progesterone profile which mimics those seen in the embryo transfer programmes (i.e., two CIDR® Sheep and Goat inserts inserted simultaneously or singly with replacement of the first with the second at day eight of treatment).

1.3 Intravaginal Veterinary Drug Delivery in Farmed Animals

1.3.1 Oestrus Control Products for use in Cattle

Initial studies into methods for the controlled delivery of progesterone to cattle were carried out by Roche in the early 1970's and focused on silicone implants inserted into the dewlap of cattle[49, 50]. The work was reasonably successful but such a large implant was undesirable for commercialisation[51]. This lead Roche to focus his efforts on intravaginal administration, trialling silicone rings and coils, all of which exhibited poor retention characteristics[51]. About the same time Abbott Laboratories independently developed an intravaginal drug delivery system and reported their initial field results in 1975[52]. Their progesterone releasing intravaginal device (PRID®) comprised progesterone in a silicone rubber matrix, moulded onto a stainless steel coil. In 1987 a second silicone/progesterone drug delivery system was commercialised called the CIDR®1900 Cattle insert [personal communication, Dean Epps, InterAg Operations Manager]. Interdispersed over this time was the development of several conceptual intravaginal drug delivery systems for the delivery of progestagens including sponges[4] and rubber tubing devices[4].

1.3.1.1 PRID®

With the collaboration between Abbott and Roche[4], the PRID® became the first commercially available intravaginal progesterone delivery system for cattle in the mid 1970's and is still widely used today.

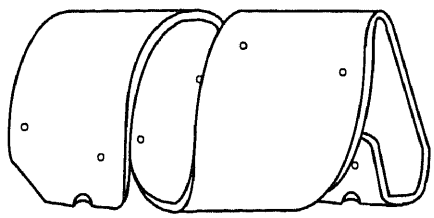


Figure 1.3.1 - Diagram of the PRID® progesterone releasing intravaginal device

The currently available PRID® (Figure 1.3.1) consists of a 3.5 x 28.5 x 0.1 cm stainless steel sheet enclosed in a silicone rubber matrix (which contains either 1.55 g uniformly dispersed micronised progesterone) and is coiled into a spiral of approximately 4 cm diameter and 12 cm length[3, 4, 5]. A hard gelatin capsule

containing 10 mg oestradiol benzoate is glued to the inner surface of the coil and a nylon cord is connected at one end to facilitate removal of the device post-treatment. The PRID® is inserted using a special applicator (Figure 1.3.2).

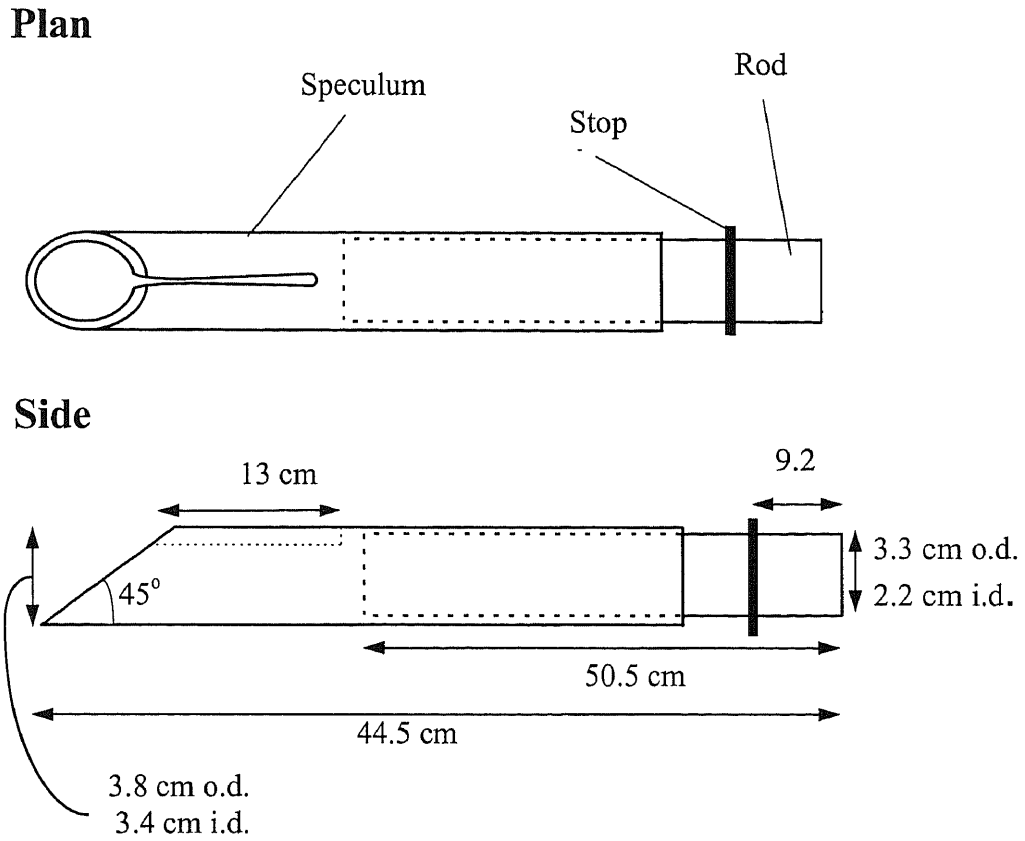


Figure 1.3.2 - Diagram of the specialised PRID® applicator

All reported PRID® retention rates are greater than 92% with an average retention rate in cows and heifers of approximately 95%[4,5].

Following insertion of a PRID®, plasma progesterone levels rise rapidly and then decline gradually over the next 14 days, falling within hours to basal levels after removal of the device. This profile has been reported by several authors[53-70]. The reported values for plasma progesterone vary but it is generally agreed now that steady state progesterone levels are around 3-4 ng/mL. After an insertion of recommended duration, the PRID® still contains a large amount of residual progesterone. McPhee et al. investigated use of the PRID® for up to 3 successive treatments[58]. Two different sterilisation methods were used before reinsertion of the devices including gas sterilisation with ethylene oxide and heat sterilisation by

autoclaving. The plasma progesterone levels produced by gas sterilised PRID® were observed to drop dramatically for each successive insertion. For autoclaved PRID®, a profile similar to that of a fresh PRID® was obtained on the first reinsertion but a drop in progesterone levels occurred with a second reinsertion.

The invitro release of progesterone from PRID® has not been reported although Winkler et al. have done so for hand made progesterone/silicone sheet[52]. The release of progesterone followed a square root of time mechanism (i.e., release was constant with respect to the square root of time).

Winkler et al. also determined invivo release rates by removing PRID® devices at days 2, 6, 9, 12,16 and peeling off the skin of the PRID® and determining the residual progesterone load. Different rates were observed for the outer and inner surfaces (outside 2.597 mg/cm^2/day$^{1/2}$, inner 2.359 mg/cm^2/day$^{1/2}$) but due to different lag times to steady state (outside 1.7 days, inner 4.7 days) the release profile was zero-order overall (i.e., release was constant with respect to time).

1.3.1.2 CIDR®1900 Cattle Insert

The CIDR®1900 Cattle insert was developed in New Zealand for the intravaginal delivery of progesterone to cattle.

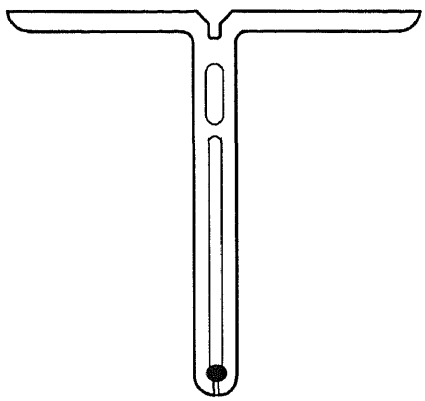


Figure 1.3.3 - Diagram of the CIDR®1900 Cattle insert

The CIDR®1900 Cattle insert (Figure 1.3.3) is described as T-shaped (body with hinged wings) and comprises a supporting nylon spine enclosed in a silicone rubber matrix which contains 1.9 g uniformly dispersed micronised progesterone[3, 4, 5]. The

10% w/w progesterone in silicone matrix is injection moulded over the spine and cured at around 195°C. A groove in the H-cross-section body of the insert allows placement of a gelatin capsule for delivery of oestradiol benzoate. A tail fitted to the end of the insert facilitates its removal at the end of treatment.

The CIDR®1900 Cattle insert is inserted using the special applicator (Figure 1.3.4) with its hinged wings folded forward together.

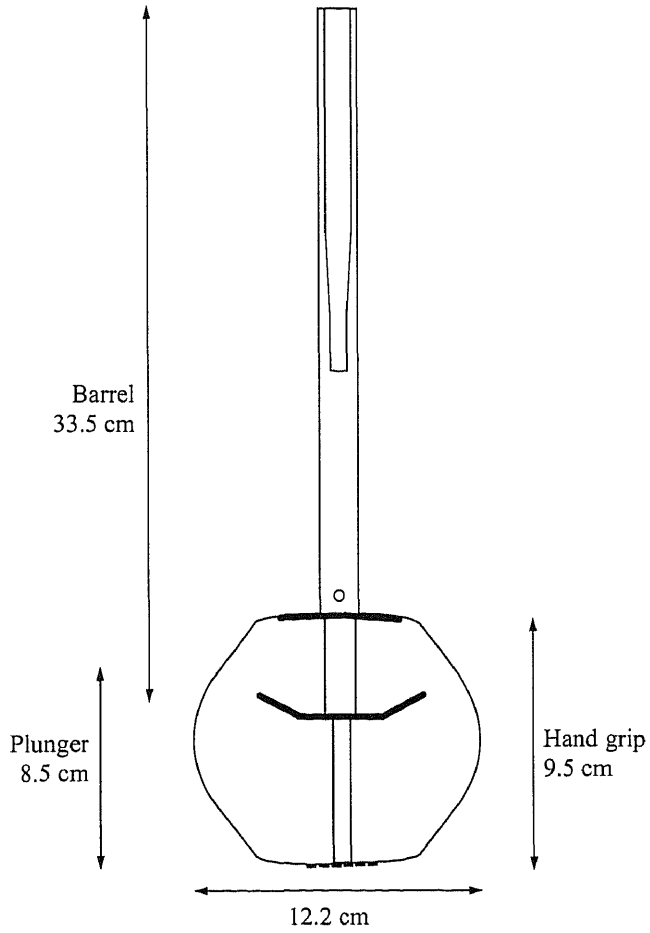


Figure 1.3.4 - Diagram of the specialised CIDR®1900 Cattle insert applicator

When released in the vagina, the hinged wings provide retention by exerting gentle pressure against the vaginal walls as they flex back towards their original T-shape position. Retention rates from 92 to 99.5% have been reported for heifers and cows[71-83].

Following insertion of a CIDR®1900 Cattle insert, there is an initial sharp rise in plasma progesterone level before it declines to around 3 ng/mL where it remains

relatively constant or slowly declines until removal, at which time the level falls rapidly to basal concentration[3, 5]. This typical profile has been reported by several authors[53, 56, 84-95]. Residual progesterone loading in a CIDR®1900 Cattle insert post-treatment is also large. The residual amount has been shown to be dependent upon the initial loading and upon the treatment duration[87, 92, 96]. After a 9 day insertion the CIDR®1900 Cattle insert still contains about 1.1 g and around 0.85 g remains after 15 days. When three CIDR®1900 Cattle insert were inserted simultaneously each insert contained the same residual loading as an insert inserted singly[92]. Due to the high residual in used CIDR®1900 Cattle insert, investigations into reuse of inserts have been carried out[87, 90, 97-100]. Van Cleeff et al. washed, disinfected, dried and reinserted CIDR®1900 Cattle insert which had previously been used for 9 days and showed that the resulting plasma progesterone levels were significantly lower than those obtained for fresh inserts[87].

The invitro release of progesterone from CIDR®1900 Cattle insert has been reported by Bunt et al.[101]. The release experiment was performed in 1100 mL of 60% alcohol to maintain sink conditions and the data was plotted in accordance with the square root of time mechanism. The release rate was shown to increase with the addition of light liquid paraffin to the matrix and with an increase in the initial progesterone loading. The release rate was decreased by the addition of fillers such as calcium carbonate to the matrix and by increasing the Shore hardness of the matrix[102]. All reported observations are consistent with the square root of time mechanism.

No invivo release profile for CIDR®1900 Cattle insert has been reported in the literature.

1.3.1.3 INVAS

The intravaginal application system[3, 4, 5, 103, 104] (INVAS) is a patented delivery system similar in design to the CIDR®1900 Cattle insert in its T-shaped design, but it is produced by a different manufacturing method. A polypropylene spine is used to give INVAS its form and provide retention in the vagina. Progesterone is incorporated with silicone by ball milling. A sheet of milled silicone/progesterone is placed in a split mould. The spine is placed on this bottom sheet before a top sheet is

positioned and the mould is closed. The sheets are then cured around the spine at temperatures between 70 and 120°C. The suggested advantage of curing below 120°C is that this does not exceed the melting point of either the β (121°C) or the α (130°C) crystal habit.

Retention of the device is reported to be 'good'[103] with less instance of irritation than other (unspecified) devices.

General characteristics of plasma progesterone profiles observed for INVAS are very similar to those observed for PRID® or CIDR®1900 Cattle insert, although steady state concentrations of approximately 1.5 ng/mL[3] are a little lower than those achieved with PRID® or CIDR®1900 Cattle insert.

Invitro release from INVAS is reported to follow a nearly zero-order rate[103]. Hornykiewytsch obtained invitro data with INVAS devices in 5000 mL pH 7.4 phosphate buffer, stirred with a blade stirrer, making it difficult to draw comparisons with the CIDR®1900 Cattle insert. The data also correlate well when plotted according to the square root of time model[3].

1.3.1.4 Rajamahendran Device

Rajamehendran and co-workers developed an intravaginal device containing both progesterone and oestradiol-17β for the control of oestrus in cattle[3, 4, 5, 105-107].

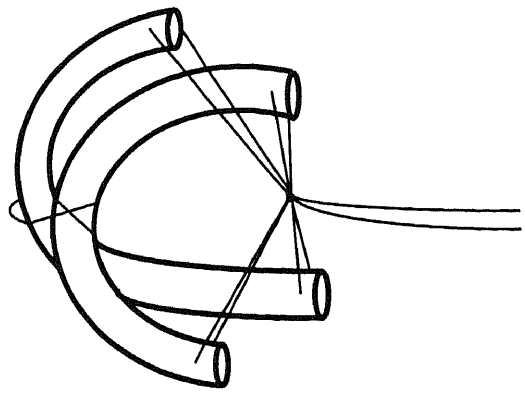


Figure 1.3.5 - Diagram of the Rajamahendran Device

The device (Figure 1.3.5) is 'umbrella-shaped' and is manufactured from silicone rubber tubing (two 20 cm lengths) with inside diameter 0.79 cm and outside diameter 1.27 cm[4, 5]. One end of each tube is sealed and 1 g of progesterone in diethyl ether solution is added to each. The open ends are sealed following evaporation of the solvent and an extra 1 mL of adhesive containing 10 mg oestradiol-17β is coated onto each of the four tube ends. The two tubes are tied at their centres and curved into the 'umbrella' shape. Strings are secured to the free ends to facilitate removal of the device post-treatment. The device has a total surface area of around 160 cm^2 with about 40 cm^2 initially releasing oestradiol-17β[3].

Retention of the Rajamehendran device is good with 21/24 and 11/11 heifers retaining the devices over 12 days in two studies[105]. Vaginal irritation was described as less than that observed for PRID®.

Once again, plasma profiles observed follow those typically observed for the other silicone intravaginal progesterone delivery inserts although progesterone concentration appears to decline gradually over the entire period from day 2 to 12 rather than achieving a plateau for several days. The presence of oestradiol-17β was found not to influence the absorption of progesterone over a 3 day period[105].

1.3.1.5 SPONGES

A number of authors have used sponges to deliver progesterone intravaginally in cattle[108-111]. Sponges are typically a polyurethane foam with a string tail to aid removal (Figure 1.3.6).

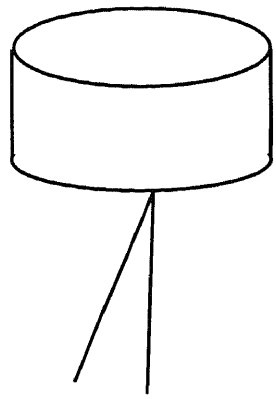


Figure 1.3.6 - Diagram of a polyurethane sponge-type intravaginal insert

Devices of varying size and progesterone loading have been investigated. Sponges have also been impregnated with the more potent progestagens fluorogestone acetate[108], chlormadinone acetate[112], megestrol acetate[112], melengestrol acetate[112], northandrolone[113] and medroxy progesterone acetate[114-116]. Sponges are typically cylindrical, 6-14 cm in diameter and 5-10 cm in length. Impregnation of a sponge with progestagen is typically achieved by applying the required amount of drug in an organic solvent. The solvent would then evaporate, leaving a fine dispersion of the drug within the sponge matrix.

Sponges inserted either singly or numbering up to 12 simultaneously for small devices, exhibit variable retention characteristics in cattle with figures of between 50 and 100% being reported. Factors affecting retention appear to be sponge diameter, length, density, animal age and vaginal size[108, 110, 112, 117-119].

Plasma levels of methyl acetoxy progesterone arising from the insertion of 10 small (50 mg) sponges have been reported[114].

No invitro or invivo release rates have been reported for the sponges used in cattle.

1.3.2 Oestrus Control Products for use in Sheep

1.3.2.1 Sponges

In 1964 and 1965 Robinson reported on the use of progestagen-containing intravaginal polyurethane sponges (Figure 1.3.6) for oestrus control in sheep[120, 121]. Realising there was a market potential, the company GD Searle and Co. began manufacturing prototype 20 mg and 30 mg fluorogestone acetate sponges. Wishart trialed these devices in 2836 British ewes[122] and confirmed Robinson's findings that such a device could be used for ewe synchrony. Vaginal retention rates of synthetic progestagen sponges are high, although their use is associated with a large volume of mucus discharge[121] and, particularly in maiden ewes, some adhesion to the vaginal mucosa[122] which results in difficulty of removal. Removal issues were largely resolved[122] and a number of companies went into sponge manufacture. Commercially available products SYNCRO-MATE[122-144], Veramix[129, 145-147], Repromap[147-162] and Chronogest[148, 149, 152, 159, 163-169] comprise fluorogestone acetate in

polyurethane sponge. Products including Veterdif-Cycle 50 mg[126, 170-173], Spogosan 50 mg[171, 173, 174], Repromap 40 mg[147, 149, 160, 162] and Veramix 60 mg[175] comprise methyl acetoxy progesterone in polyurethane sponge.

Plasma profiles for a single 40 mg fluorogestone acetate sponge were determined by Gaston-Parry et al.[141, 176]. Invivo release was determined for 10, 30 and 90 mg sponges by Allison and Robinson[177].

1.3.2.2 CIDR-S

The CIDR-S was rabbit-eared in shape (Figure 1.3.7), contained 465 mg progesterone in a silicone matrix and was commercially available from 1983 to 1989. Several papers report the use of CIDR-S in sheep[163, 178-181]. The CIDR-S was superseded by the CIDR® Sheep and Goat insert which is easier to insert and remove.

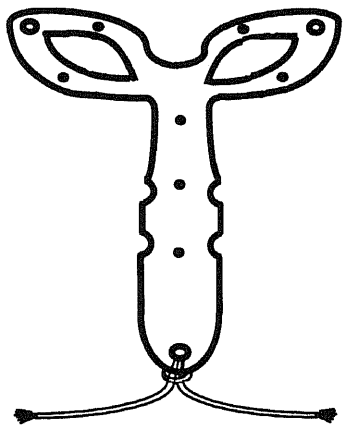


Figure 1.3.7 - Diagram of the CIDR-S intravaginal insert for sheep

1.3.2.3 CIDR® Sheep and Goat Insert

The CIDR® Sheep and Goat insert is a T-shaped insert containing 0.3 g (9% w/w) progesterone homogeneously dispersed throughout silicone for use in sheep and goats (Figure 1.3.8).

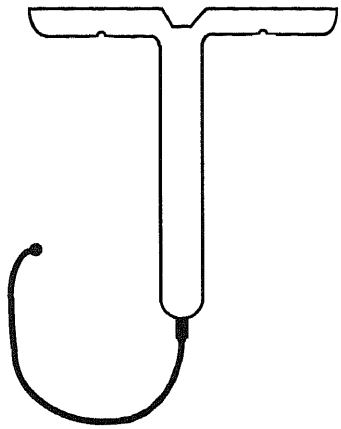


Figure 1.3.8 - Diagram of the CIDR® Sheep and Goat insert

The CIDR® Sheep and Goat insert is approximately 5.4 cm long and 5.8 cm wide with a body diameter of around 9 mm. There is a good deal of literature on the performance of CIDR® Sheep and Goat inserts in sheep[46, 47, 179, 182-190]. Retention is excellent and ewes show no sign of discomfort when inserts are inserted. A typical progesterone profile[191] is similar to that observed in cattle. Neither invitro nor invivo release data have been reported.

1.3.2.4 C-Shaped Plasthyd Device

Mandiki et al.[192] describe a C-shaped intravaginal insert containing progesterone in a 'plasthyd' polymer device which, over a 12 day treatment period, produced steady state plasma progesterone levels of around 0.8-1.0 ng/mL.

1.3.3 Summary

This brief review has highlighted the conceptual and commercially available intravaginal veterinary drug delivery systems. A critical evaluation of these products suggests that there are only a few such delivery systems available, hence providing the opportunity for some of the research performed in this Thesis (i.e., the development of a new intravaginal veterinary drug delivery system).

1.4 Polymers as Platforms for Controlled Release Intravaginal Veterinary Drug Delivery

From the above overview of the different technologies used to manufacture intravaginal veterinary drug delivery systems, a critical evaluation of those products reveals that the only two polymers which have been used as intravaginal drug delivery platforms are silicone and polyurethane. This provides a further opportunity for research to be performed in this Thesis (i.e. the use of a novel polymer, never before used as an drug delivery platform for intravaginal veterinary drug delivery). The following Section will discuss the properties of both the currently used polymers, namely, polyurethane and silicone and a biodegradable polymer, poly-(ε-caprolactone), which has recently been identified as potentially useful as an intravaginal drug delivery platform in farmed animals[193, 194].

1.4.1 Polyurethane

Polyurethane has been used commercially as a platform for intravaginal drug delivery in the form of sponges[3, 4, 5].

1.4.1.1 History of Polyurethanes

The polyurethanes have been exploited for the delivery of progestagens to control the oestrous cycle of sheep (see Section 1.3.2.1). The first urethane polymers were developed in the late thirties in the Leverkusen laboratories of the German Bayer Group[195]. By varying monomers, a large number of polyurethanes were produced and application of these polymers started to flourish in the late forties.

1.4.1.2 Synthesis of Polyurethanes

Polyurethanes are formed by the reaction of a polyhydroxyl compound with an isocyanate by the polyaddition principle[195].

$$ROH + R'NCO \rightarrow R'NH - COOR$$

1.4.1.3 Fabrication Methods Compatible with Polyurethanes

Polyurethane foams are typically fabricated using foaming machines which pump metered amounts of two or more raw materials, mix them together and force the mix into a mould or cavity[195]. The mixture then expands, developing its open cell structure before it hardens and cures in the form of the mould.

1.4.1.4 Properties and Applications of Polyurethanes

As with all polymers, the properties of the polyurethanes are dependent upon the monomers used and the method by which they are processed. Polyurethane foams have an open structure which provides air and water permeability, although their affinity for water is low[195]. Foams may be rigid or flexible. For example, polyurethanes synthesised from polyether based polyols have good acid and alkali resistance and are typically soft and elastic while those synthesised from polyester based polyols are resistant to oxidative degradation, are more rigid and have excellent strength properties[195].

Elastic and thermally stable foams are suited to seating and bedding applications and are well used in furniture, automotive seating, carpet underlay and apparel paddings[195]. Both flexible and rigid foams are commonly used in packaging applications due to their excellent shock absorbing properties[195]. Thermal and sound insulating properties of some polyurethanes have seen them used in the sandwich construction of refrigeration equipment, housing and industrial containment[195]. The foam cell structure of polyurethanes also makes them ideal as air filters and as sponges in cleaning or painting applications[195].

1.4.1.5 Limitations of Polyurethane as an Intravaginal Drug Delivery Platform

As can be seen above polyurethanes have found application in many areas of everyday life. As a drug delivery platform for the intravaginal administration of progestagens to sheep and cattle it has found limitations. Firstly, the polymer comes preformed and exists as a porous open network. This has two limitations; (i) the drug must be incorporated into the polyurethane matrix after it has been formed into shape, thereby requiring an additional step in the manufacturing process, and (ii) the diameter of the pores is variable and the opportunity to control release using this variable does not exist. Secondly the properties of the preformed polymer change

following the addition of drugs into the porous network. The addition of progesterone to a preformed sponge, for example, results in an increase in its density and flexibility resulting in a reduction in its retention rate in sheep[5]. Thirdly, the shape which can be made from polyurethane is limited to cubes and cylinders, which reduces the possibility of insert design. Fourthly, the method of drug incorporation is subject to variability leading to quality control issues. Depending upon the method of manufacture e.g., submersion in solvent, addition of solvent dropwise to the surface of the polyurethane, position solvent is added to the sponge, drying method (temperature, speed, etc.), the drug distribution within the sponge can vary and affect drug release profiles. Finally, polyurethane sponges tend to encourage a mucopurrelent discharge when inserted intravaginally which make them unpleasant to remove at the end of a treatment period.

1.4.2 Silicone

Silicone has been used commercially as a platform for intravaginal drug delivery in CIDR® and PRID® products[3, 4, 5].

1.4.2.1 History of Silicone

Early work on the polysiloxanes (silicones) was done by Kipping and students at the turn of the century. In 1943, the first commercial silicones were manufactured for insulating sparkplugs in warplanes. By 1949, it was widely thought that silicones might be developed as materials for a variety of industrial uses. Then, in the 1950's, investigations into medical applications for silicones began. By 1953, the first silicone specifically for medical application was produced[196, 197].

1.4.2.2 Synthesis of Silicones

The synthesis of silicones can best be described by the following simplified Procedure[197, 198]:

heat silica rock with carbon in an electric furnace

$$SiO_2 + C \longrightarrow Si + CO_2$$

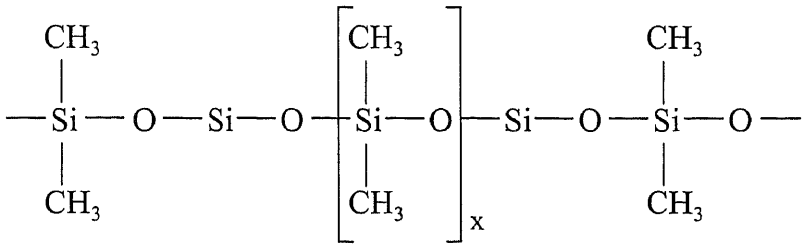
treat the silicon with methyl chloride

$$Si + CH_3Cl \longrightarrow SiCl(CH_3)_2$$

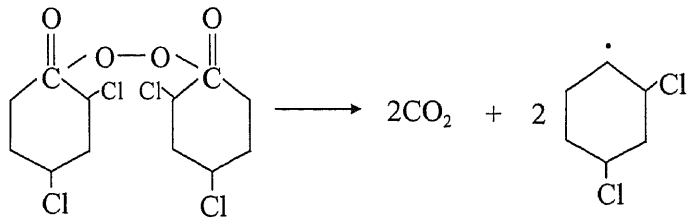
treat the dimethyl dichlorosilane with water

$$SiCl_2(CH)_2 + 2H_2O \longrightarrow Si(OH)_2(CH_3)_2 + 2HCl$$

the extremely unstable dimethyl dihydrosilane immediately condenses to a polymer



silicone rubbers can be made heat curing with radical forming agents such as dichlorobenzoyl peroxide



the free radical extracts H · from a silicone methyl group and two methyl radicals combine to create a silicone crosslink

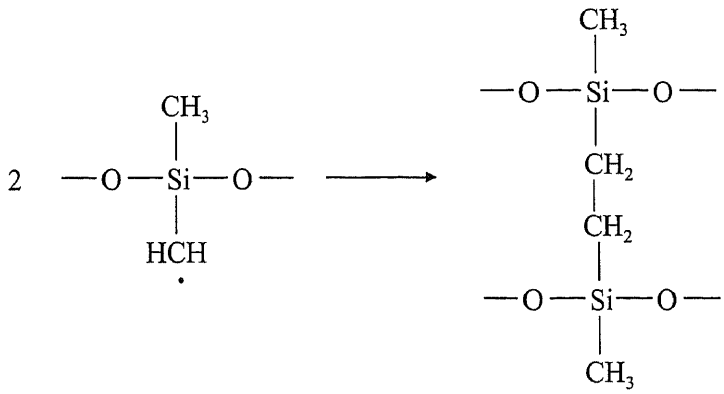


Figure 1.4.1 – Synthesis of silicone polymers

1.4.2.3 Properties of Silicones

The term silicone is extremely general and encompasses a huge variety of silicon based polymers. A wide variety of silicones can be manufactured by controlling the polymer chain length and any cross-linking to give silicone materials ranging from oils to rubbers. In addition, methyl groups can be substituted for other alkyl groups, giving the resulting polymer different properties. As the focus of this Thesis is polydimethylsiloxane rubber, the following discussion on properties of silicones shall refer largely to the silicone rubbers.

1.4.2.3.1 Physical and Chemical Properties [196, 197]

Fabrication: Silicone rubber is easily moulded into shape and the curing (vulcanisation) reaction can be accelerated by applying heat. Typical vulcanising agents include stannous octoate for room temperature cure and dichlorobenzoyl peroxide for heat cure. A variety of organic compounds can inhibit the curing of silicones[199-201]. Inhibitive compounds will contain one of the following functional groups:

Acetylenic or vinylic	(e.g. ethynyl oestradiol)
Carboxylic	(e.g. lauric acid)
Basic amine	(e.g. ephedrine, propranolol)
Pyridine	(e.g. nicotine)
Sulphur	(e.g. cimetidine)

Mechanical properties: The cured polymer has high elongation to failure (100% or better). It also has Shore A hardness of around 40-60 or less and is thus not abrasive to biological tissues. Silicones can be strengthened by the addition of filler in their fabrication. The filler is commonly finely ground silica.

Temperature resistance: Silicones are indifferent to temperatures, retaining their elasticity at low temperature (sub-zero) and retaining their strength at high temperatures. Silicones are thermally and oxidatively stable to 500°F (246°C) or higher. Also, they do not discolour with heat. This combination of properties allow silicones to be sterilized by autoclave for medical use. Silicone rubbers have

approximately twice the thermal conductivity of natural rubber, making them ideal for some electrical insulation applications.

Chemical: Silicones have low solubility in organic solvents. Metals will not corrode in contact with silicone. When implanted in the body, silicone will not deteriorate with time, implying resistance to metabolism, oxidation and ozonization. Gases diffuse more readily through silicone than other polymeric materials.

Table 1.4.1- Gas diffusion through silicone [197]

	CO_2	O_2	N_2	He
Teflon	78	30	14	178
Silastic	6310	1210	600	730

millimetres/minute/atmosphere membranes - 0.001 inch thick at 25°C

Surface properties: With the exception of specialized adhesives, nothing adheres to silicone. Tissues will not grow onto them. The viscosity of silicones is a function of chain length and cross-linking. Silicones differ from hydrocarbon oils due to the free rotation of the methyl groups around silicon. This free rotation leads to large interchain space and thus, low viscosity. This is why some low molecular weight silicones make excellent lubricants. Many silicones are strongly hydrophobic, making them useful as water repellent surface coatings.

Electrical properties: Silicone is an excellent dielectric and may develop static charge on its surface.

Table 1.4.2 - Electrical properties of Silastic® 372 medical grade elastomer [197]

Dielectric Constant	2.85
Dissipation Factor	0.003
Volume Resistivity	5×10^{14} Ohm-cm
Electrical Strength	525 Volts/mL

1.4.2.3.2 Biocompatibility

The monomeric precursors of silicones tend to be highly toxic. Tetraethyl-ortho-silicate is acutely toxic[202]. Alkylchlorosilanes are highly corrosive[203] ($SiCl_4$ was used in warfare as an irritant gas). Ethoxysilanes cause lung and kidney damage[203-205].

However, polymeric silicones from fluids to higher weight solids are chemically inert, have exceedingly low oral toxicity[203] and are well tolerated by tissues[206] provided toxic additives are left out of the manufacturing process. Silicone's chemical inertness also means that actives incorporated into the polymer will not be degraded by their matrix.

Toxic substances are usually reactive chemicals, whereas polymers are normally unreactive. However, reactive chemicals may be used in making polymers and such chemicals are the usual cause of any observed toxicity from polymeric materials[206]. Toxic components within polymers may include:

- Polymer precursors
- Plasticisers
- Sterilising chemicals (e.g. ethylene oxide[207])
- Solvents

A variety of responses may be observed when materials are surgically implanted within the body. The foremost is surgical infection where external pathogens enter the surgical wound and cause an infection. If surgery is successfully sterile then the implant may cause an inflammatory response where the bodies natural defense of phagocytic attack occurs at the implant site. If the implant is degradable, the foreign material will be decomposed and adsorbed for excretion. When an implant causes no inflammation it is said to be biocompatible. In some cases, the body will grow into the implant which becomes part of the biological structure. More commonly (as is the case with silicone), a fibrous tissue will grow around and encapsulate the implant[208-211], sealing it off from the rest of the body.

The definition of medical grade polymer is, therefore, a polymer which has been carefully manufactured to exclude any toxic components which may cause an

inflammatory reaction in biological tissues. Silicone, with its biocompatibility and other excellent properties, has found many applications in medicine[197,212-214].

1.4.2.4 Silicone as a Controlled Drug Delivery System

Of the polymers used as matrices for drug delivery, silicone has played a prominent role. This is because silicone possesses a number of desirable characteristics. When selecting a polymer for use in drug delivery, the requirements are[215]:

- Suitable diffusivity and solubility of the drug in the polymer
- Compatibility with the drug (drug must be stable in the matrix)
- Compatibility of the polymer with the delivery environment (e.g. parenteral body cavity)
- Stability in the delivery environment
- Desirable mechanical properties
- Ease of fabrication
- Cost

So, silicone is an excellent material based on all these criteria and all that remains is for drugs to be identified which have suitable release characteristics from the polymer for practical clinical application.

1.4.2.5 Limitations of Silicone as an Intravaginal Drug Delivery Platform

The curing (vulcanisation) of silicone rubbers generally requires high temperatures to achieve cure in an efficient time frame (i.e. CIDR®1900 Cattle inserts are moulded at 190°C and require approximately 50 seconds to cure at this temperature). Such a moulding temperature requires a high process energy input. Further to this, the high temperature of moulding limits the drug candidates which can be incorporated into silicone rubbers to those which are highly heat stable.

The drug candidate list for incorporation into silicone intravaginal drug delivery systems is further limited by the hydrophobic nature of the polymer as only lipophilic drugs will permeate a hydrophobic matrix. Incorporation of pore-forming agents into polymeric delivery systems allows hydrophilic drugs to diffuse out of the

matrix via the pores that form, but loss of structural integrity tends to occur if pores are created within silicone rubbers.

The elastic nature of silicone impels a requirement for some sort of supporting structure for intravaginal drug delivery systems to give them the rigidity needed to facilitate intravaginal retention (i.e. The CIDR®1900 Cattle insert comprises an inert nylon spine to support its silicone drug delivery matrix). This requirement for support adds an extra component to the manufacture of any silicone based intravaginal drug delivery system, and hence increases the cost of production.

Finally, silicone rubbers are non-biodegradable and therefore, when disposed of by the common New Zealand method of 'land fill' (an excavated dumping ground in an isolated area which will eventually be covered over with earth), they will not decompose.

1.4.3 Poly-(ε-caprolactone): A Potential Polymer for Intravaginal Veterinary Controlled Drug Delivery

The above account of the properties and suitability of polyurethane and silicone indicates that both these materials exhibit certain characteristics which make them ideal for use in the manufacture of intravaginal drug delivery systems. Indeed both these polymers have been successfully used in intravaginal veterinary drug delivery systems for over 30 years. However, as highlighted in the above accounts, both these polymers have limitations as intravaginal drug delivery systems which limit either the drug which can be incorporated into them, the clinical condition they may be used for, their aesthetic nature, pleasantness of use, etc. In light of these limitations, other polymers should be sought which have more ideal properties and exhibit greater flexibility with respect to their potential as intravaginal drug delivery systems. Although the use of new polymers has been and continues to be extensively studied by pharmaceutical scientists interested in controlled release, and many have been identified and are used within both the human and veterinary fields, until recently no attempts have been made to investigate or utilise newer polymers as platforms for intravaginal drug delivery. However, recently Bunt et al. have published the results of some preliminary work which suggested that poly-(ε-

caprolactone) may be usefully employed as a platform for the prolonged delivery of progesterone to cattle[193]. This need for more flexible polymers and the reports by Bunt et al. have provided the opportunity for some of the research performed in this Thesis. In this respect investigations on the polymer poly-(ε-caprolactone) are conducted and its potential as a platform for the delivery of progesterone to sheep is investigated.

1.4.3.1 History of Poly-(ε-caprolactone)

The ability to form polymers of ε-caprolactone was first discovered in the early 1930's[216]. Poly-(ε-caprolactone) was subsequently identified as a biodegradable polymer which aroused significant interest in its possible application to environmentally friendly packaging and for medical applications[216].

1.4.3.2 Synthesis of Poly-(ε-caprolactone)

Poly-(ε-caprolactone) is a linear aliphatic polyester. It is synthesised by ring opening polymerisation of epsilon-caprolactone. ε-Caprolactone is synthesised by the oxidation of cyclohexanone with peracetic acid. The monomer can then be polymerised by any of three mechanisms including anionic (Figure 1.5.1), cationic (Figure 1.5.2) and coordination (Figure 1.5.3)[216]. Each unique mechanism provides different degrees of control over molecular weight, end-group composition and structure of co-polymers.

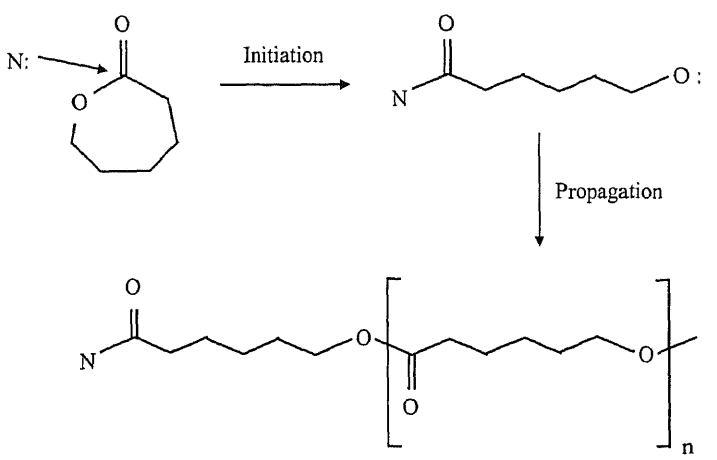


Figure 1.4.2 - Anionic polymerisation of ε-caprolactone to form poly-(ε-caprolactone)

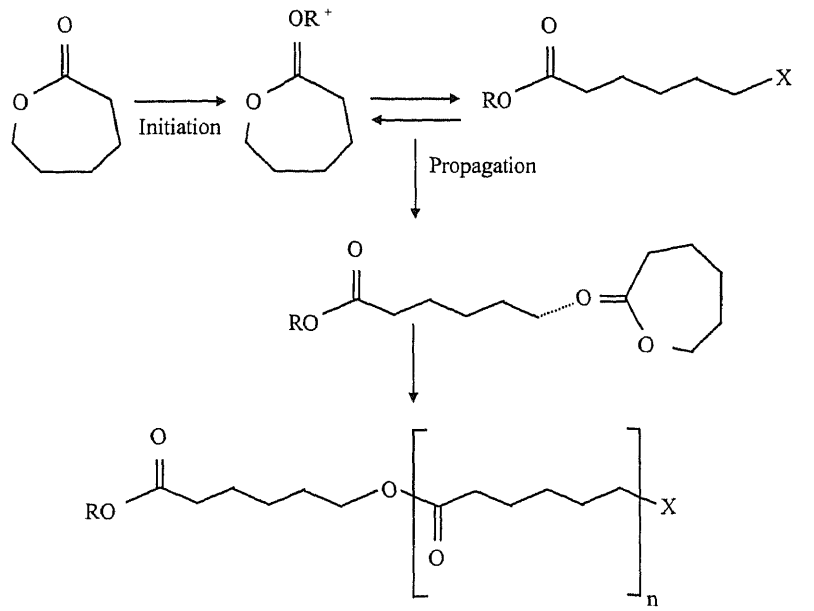


Figure 1.4.3 - Cationic polymerisation of ε-caprolactone to form poly-(ε-caprolactone)

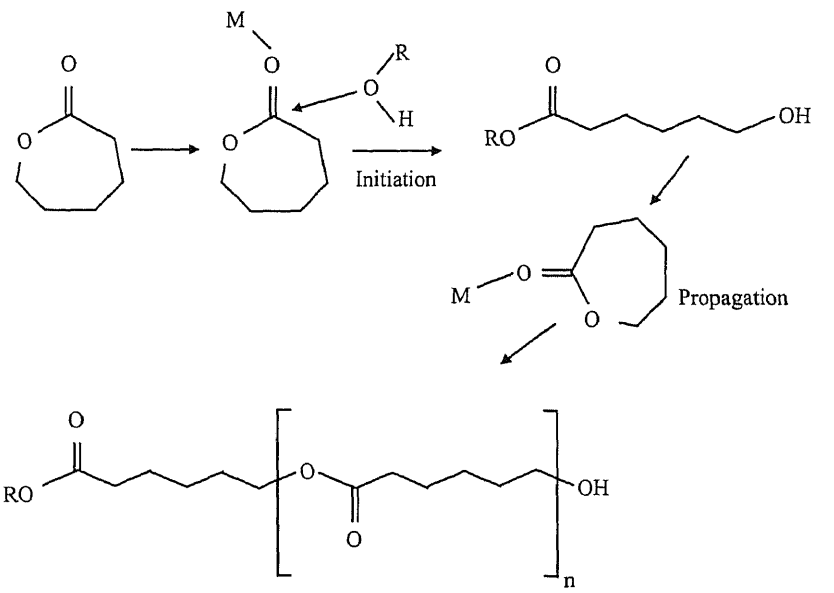


Figure 1.4.4 - Coordination polymerisation of ε-caprolactone to form poly-(ε-caprolactone)

Poly-(ε-caprolactone) can also be synthesised by the radical initiated polymerisation of 2-methylene-1, 3-dioxapane.

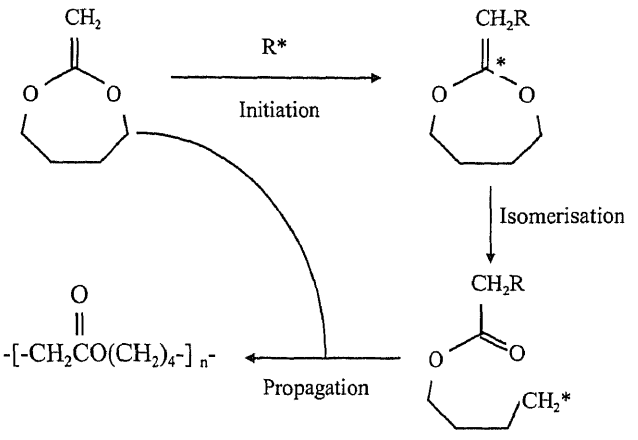


Figure 1.4.5 - Radical polymerisation of 2-methylene-1, 3-dioxapane to form poly-(ε-caprolactone)

1.4.3.3 Fabrication Methods Compatible with Poly-(ε-caprolactone)[216]

Thin films (<0.1 mm) of poly-(ε-caprolactone) and poly-(ε-caprolactone) copolymers can be prepared by casting from solution in one of the many available solvents. More substantial monoliths can be fabricated by various forms of plastic moulding. For example, solid forms have been achieved by compression moulding at temperatures of 100-130°C. Tubing has been prepared by melt extrusion at around 160°C. Fibres have been produced by melt spinning at 161°C. Microcapsules may be prepared by fluidised bed air coating, mechanical or, more commonly, by solution methods involving emulsification of the polymer and drug in a two-phase solvent-nonsolvent mixture in the presence of a surfactant.

1.4.3.4 Properties of Poly-(ε-caprolactone)[216]

Poly-(ε-caprolactone) is a semicrystalline polymer and melts in the range of 59-64°C and has a low glass transition temperature of -60°C. Crystallinity of the polymer increases with decreasing molecular weight. Poly-(ε-caprolactone) of 100,000 molecular weight has about 40% crystallinity, while poly-(ε-caprolactone) of 5000 molecular weight is around 80% crystalline. Crystallinity has a marked effect on both permeability and biodegradation of polymers. Increased crystallinity decreases solute solubility and increases the tortuosity of any diffusional pathways, both of which reduce permeability. Biodegradation is inhibited by increased crystallinity as the bulk crystalline phase is inaccessible to water.

Poly-(ε-caprolactone) is soluble in a number of solvents including tetrahydrofuran, chloroform, methylene chloride, carbon tetrachloride, benzene, toluene, cyclohexanone, dihydropyran and 2-nitropropane. It is insoluble in alcohols, petroleum ether and diethyl ether.

Permeability of poly-(ε-caprolactone) to various drugs has been investigated (Table 1.4.3). Perhaps surprisingly, permeability (J) of poly-(ε-caprolactone) to the model steroid compound progesterone is of similar magnitude as that of silicone (Table 1.4.4). This is because the permeability is the product of the saturation solubility (C_s) and the diffusion coefficient (D).

Table 1.4.3 - Diffusion coefficients of various drugs in poly-(ε-caprolactone)[217]

Drug	Diffusion coefficient (x 10^9 cm^2/sec)
Progesterone	3.6
Testosterone	7.3
Norgestrel	4.1
Norethindrone	6.6
Ethynyl oestradiol	3.3
Naltrexone	2.4
Codeine	3.8
Meperidine	2.5
Methadone	1.9
α-Acetylmethadol	2.6

Table 1.4.4 - Comparison of permeability of silicone and poly-(ε-caprolactone) to progesterone[217]

	C_s (g/cm^3)	D (cm^2/sec)	J (g/cm.sec)
Silicone rubber	0.5×10^{-3}	4.5×10^{-7}	22×10^{-11}
Poly-(ε-caprolactone)	16.9×10^{-3}	3.6×10^{-9}	6.1×10^{-11}

Pitt et al.[218] also studied factors which affect release rates from poly-(ε-caprolactone). Their work showed a linear relationship between initial drug load and

release rate and also an effect due to polymer film thickness. They identified fabrication methods of the films as the reason for this observed effect. One thickness was prepared by solvent casting while two others were prepared by compression moulding. The compression moulded films produced higher release rates. When compression moulded, the thinner film produced similar results to the two thicker films. Clearly, fabrication methods can affect polymer morphology and, subsequently, intrinsic diffusivity of the polymer.

Poly-(ε-caprolactone) is generally regarded as biocompatible and many commercial polycaprolactones have acquired FDA approval for medical uses. Toxicology has been studied extensively in rats, guinea pigs and monkeys with minimal tissue encapsulating response being the worst observed effect of implanted poly-(ε-caprolactone) over trials of 90 days to 2 full years[216]. The human contraceptive system Capronor has also been clinically tested in women[216].

1.4.3.5 Potential for Intravaginal Delivery

Poly-(ε-caprolactone) offers great potential as an intravaginal drug delivery platform. It has both good permeability to a variety of pharmaceutical agents and has physical properties which allow it to be fabricated into controlled drug delivery systems. It may be injection moulded into a variety of shapes, indeed injection moulding is a favoured technique for delivery system fabrication, as it does not require the use of solvents which would then have to be removed from the final product, as well as the occupational health and safety issues for the staff working with the solvents. These characteristics of poly-(ε-caprolactone) coupled with the fact that InterAg specialises in injection moulding of veterinary intravaginal drug delivery products, has provided the opportunity for some of the research performed in this Thesis (i.e. the investigation of poly-(ε-caprolactone) as a platform for the intravaginal delivery of drugs).

1.5 Mechanisms of Release From Intravaginal Veterinary Drug Delivery Systems

The invitro and invivo mechanism of release of progestagens from intravaginal veterinary drug delivery systems has been identified for both the PRID® and sponge delivery systems. The invitro drug release mechanism for the CIDR®1900 Cattle insert has also been reported.

1.5.1 PRID®

The invitro release of progesterone from a commercially manufactured PRID® device has not been reported in the literature. However, in an effort to characterise the invitro release profile of PRID®, Winkler et al.[52] determined the invitro release from a hand manufactured progesterone/silicone sheet[52]. The release medium contained sheep serum to create sink conditions and the invitro release profile was estimated by determining the amount of drug remaining in the sheet and calculating the amount released from knowledge of the initial drug content in the sheet. Although these progesterone containing sheets were not subject to the same manufacturing conditions as a PRID® device, it is likely that the results and conclusions drawn from this study would be applicable. The results suggested that the invitro release of progesterone was shown to occur via the square root of time mechanism (Equation 1.5.1) (Figure 1.5.1)[52].

$$Q = \left[D(2A - C_s)C_s t\right]^{1/2} \qquad \textbf{Equation 1.5.1}$$

where Q is the amount of drug released after time; t, per unit area of exposed area; A is the initial drug concentration; C_s is the solubility of the drug in the matrix; and D is the diffusion coefficient of the drug in the matrix material.

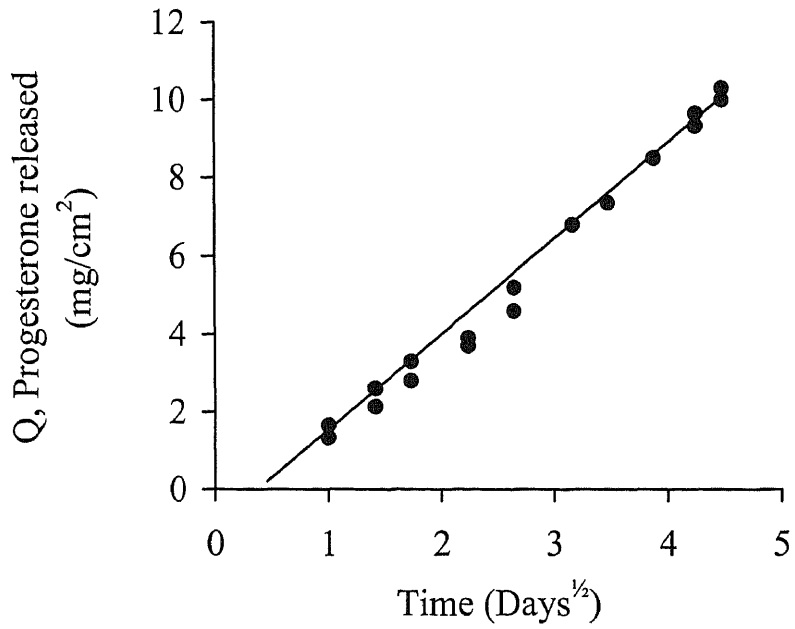


Figure 1.5.1 - PRID® invitro release

The invivo release of progesterone from the PRID® device was also investigated by Winkler et al.[52]. Devices were inserted into the vagina of cattle and then removed after insertion periods of 2, 6, 9, 12 and 16 days. The silicone matrix on the inside and outside surfaces was peeled from the steel and analyzed for progesterone content. The progesterone released invivo was then determined by difference from the initial progesterone concentration of the device. The invivo release of progesterone from the inner and outer surfaces of the PRID® were observed to follow a square root of time mechanism, with similar rate constants for the inner and outer surfaces (outside rate = 2.597 mg/cm^2/day^{½}; inside rate = 2.359 mg/cm^2/day^{½}), but with lag times to steady state (based on extrapolated intercepts) of 1.7 and 4.7 days respectively (Figure 1.5.2).

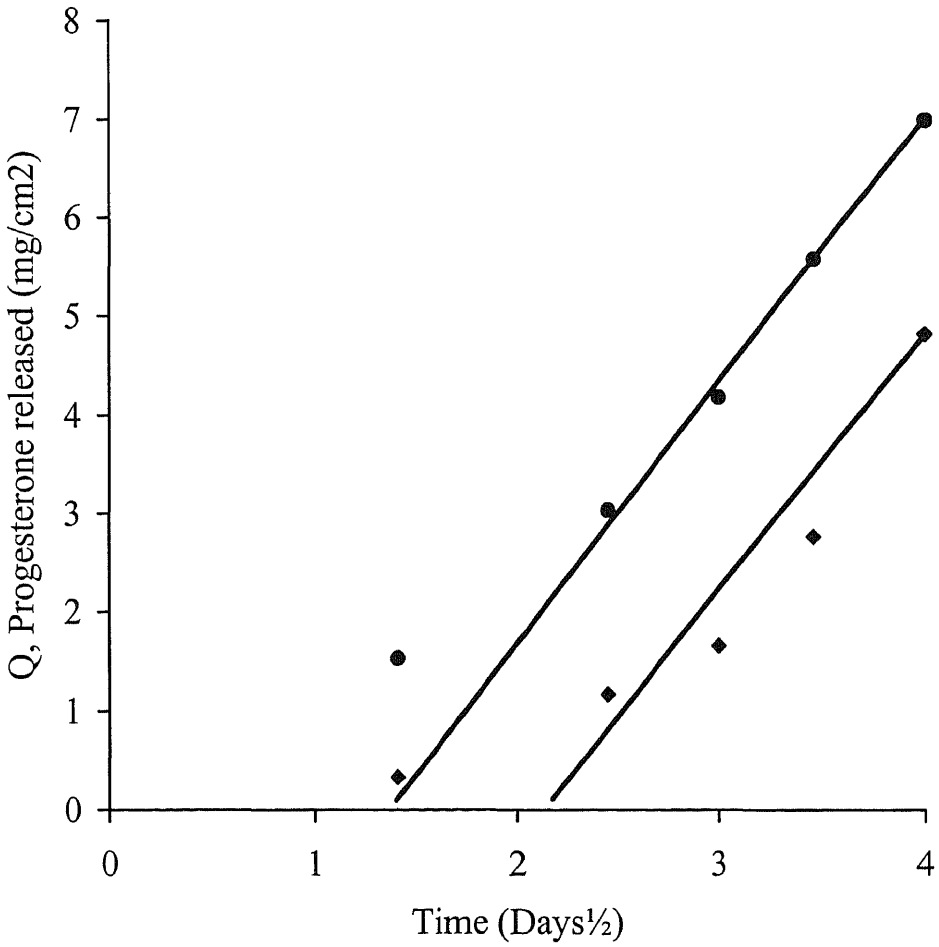


Figure 1.5.2 - Cumulative amount of progesterone released *in vivo* as a function of the square-root-of-time for the inner (◆) and outer (●) surface of the intravaginal PRID® device containing 7.8% initial progesterone concentration in an inert silicone matrix. Outside rate = 2.597 and inside rate = 2.359 mg/cm²/day½. Based on extrapolated intercepts there are 1.7 and 4.7 day lag times before reaching steady state for the outside and inside surfaces, respectively. Drawn from data given in reference 52.

The difference between lag times was attributed to physical phenomena such as wetting at the polymer surface, establishment of steady state diffusion gradient and contact with the vaginal wall. The effect of the time lag difference between the two surfaces resulted in the PRID® releasing progesterone in a pseudo zero-order fashion (Figure 1.5.3).

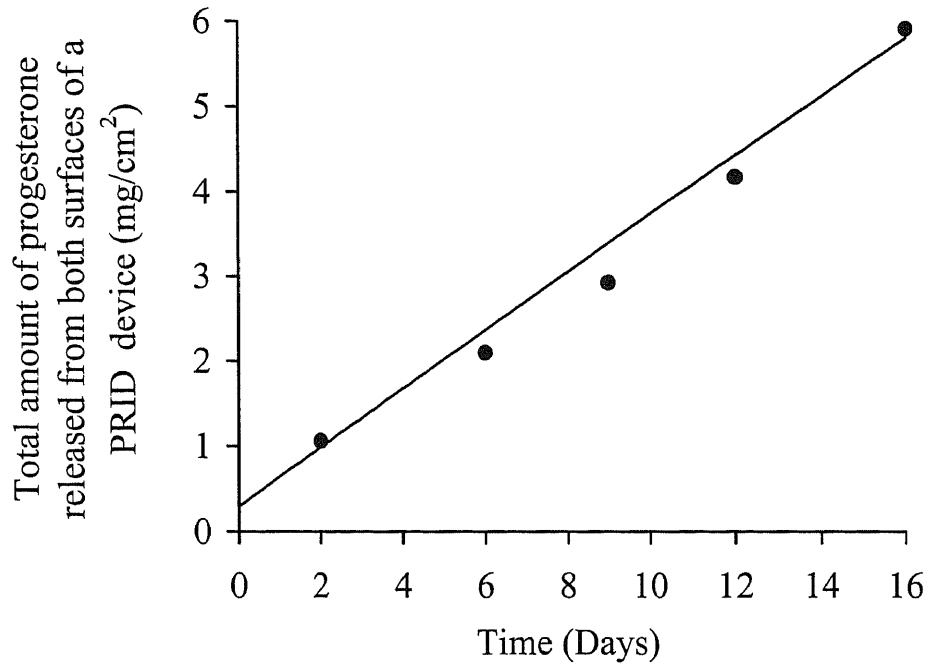


Figure 1.5.3 - PRID® total invivo release (zero-order)

1.5.2 Sponge

Chien reported an invitro system for studying the mechanism of release and intravaginal absorption of fluorogestone acetate from intravaginal sponges[219]. The system determined the rate of fluorogestone acetate release from vaginal sponges and comprised donor and receptor cells which held up to 1 L of elution medium thermostatted to 38°C. A magnetic stirrer assembly thoroughly mixed the elution media from below. In the study an intravaginal sponge was inserted into the flange opening of the donor compartment and an impermeable barrier was sandwiched between the two compartments. The strirrers were turned on and 1000 mL of elution medium was poured into each of the compartments. At pre-selected time points 2 mL samples were taken from both compartments.

Chien's invitro release study suggested that release of fluorogestone acetate from polyurethane sponges containing 40 mg of drug dispersed inside the sponge matrix occurred by a matrix-diffusion controlled mechanism and was characterised by a linear relationship between cumulative amount released and the square root of time when sink conditions prevailed in the elution medium. Figure 1.5.4 shows the

results obtained when either 40%v/v or 50%v/v polyethylene glycol 400 were employed as the elution medium[219].

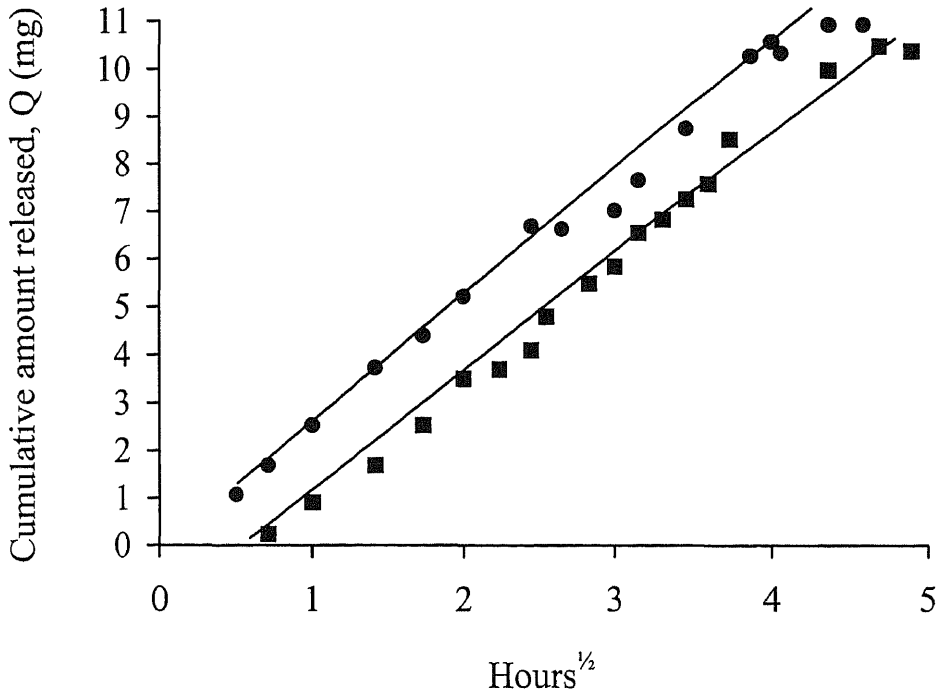


Figure 1.5.4 - Linear relationship between the cumulative amount of fluorogestone acetate (Q) and the square-root-of-time ($t^{½}$) released from a Chronogest sponge (manufactured from 800 grade polyurethane sponge) containing 40 mg fluorogestone acetate under sink conditions into 1000 mL release media comprising (●) 50% polyethylene glycol 400 (slope = 2.55 mg/$h^{½}$) and (■) 40% polyethylene glycol 400 (slope = 2.67 mg/$h^{½}$). Redrawn from reference 219.

The invivo release of fluorogestone acetate from polyurethane sponges has been investigated by several authors[177, 220, 221]. All these authors found that there was a non-linear relationships between the cumulative amount of drug released from polyurethane sponges and time (Figure 1.5.5). Allison and Robinson's results typify those seen by all of the above mentioned authors. These authors inserted flourogesterone acetate sponges containing 10, 30 and 90 mg into the vagina of different sheep for 2, 4, 6, 8, 10, 12 and 14 and 16 days. Analysis of residual drug content and knowledge of the initial drug load allowed them to determine the invivo release profile of fluorogestone acetate from the sponges. Their data indicated that the invivo release of fluorogestone acetate from polyurethane sponges occurred according to the square root of time release mechanism (Figure 1.5.6).

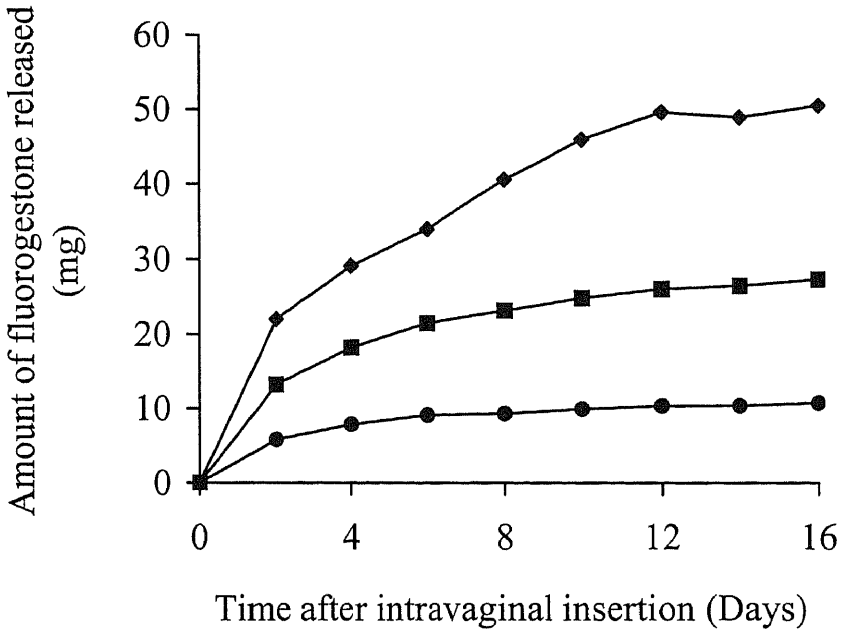


Figure 1.5.5 - Invivo drug release from polyurethane sponges containing 10 mg (●), 30 mg (■) and 90 mg (▲) of fluorogestone acetate. Data from reference 177.

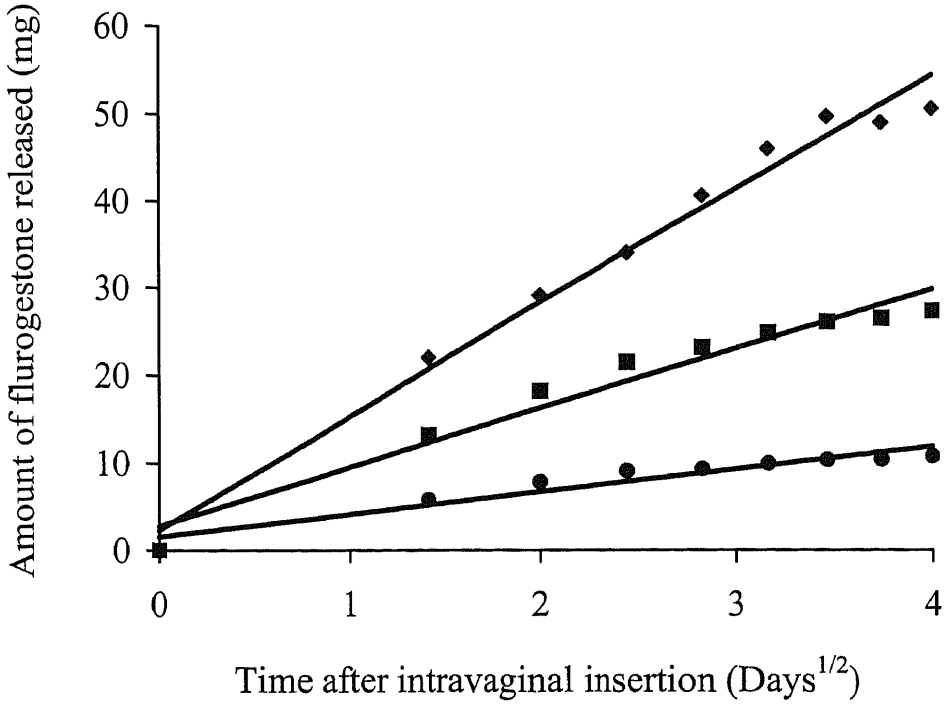


Figure 1.5.6 - Allison and Robinson's invivo data plotted as a square root of time plot. Data from reference 177.

1.5.3 CIDR®1900 Cattle Insert

The invitro release of progesterone from the CIDR®1900 Cattle insert has only recently been reported in the literature[101]. Release of progesterone from CIDR®1900 Cattle insert into 1100 mL 60% alcohol/water mixture was shown to occur via a square root of time release mechanism (Figure 1.5.7).

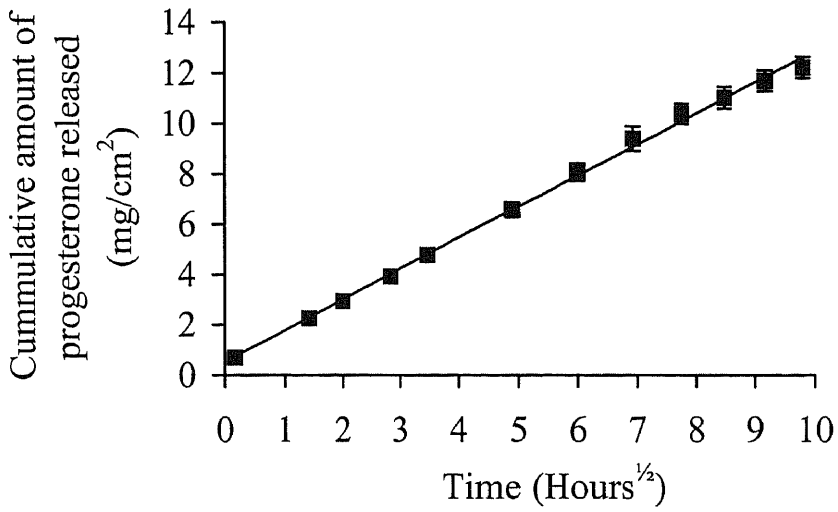


Figure 1.5.7 - Invitro cumulative amount of progesterone release per unit area of CIDR®1900 Cattle insert versus the square root of time[101]

The invivo mechanism of release of progesterone from a CIDR®1900 Cattle insert has not been reported in the literature. This has provided the opportunity for some of the research performed in this Thesis.

1.6 Recent Advances in Controlled Release Intravaginal Veterinary Drug Delivery

Since the early 1990's three significant advances have been made in the field of intravaginal veterinary drug delivery. Optimisation of the CIDR®1900 Cattle insert for the delivery of progesterone by Rathbone and co-workers, the optimisation of the sponge to deliver fluorogestone acetate by Chien and co-workers and the introduction of an electronically controlled drug delivery system which was capable of delivering progesterone, oestradiol and prostaglandin at different times and rates.

1.6.1 Optimisation of the CIDR®1900 Cattle Insert

The CIDR®1900 Cattle insert has been described in Section 1.3.1.2. This insert has recently been optimised by Rathbone and co-workers[6]. The CIDR®1900 Cattle insert was originally developed for synchrony of oestrus and was designed for insertion periods of 12 days. The CIDR®1900 Cattle insert was originally designed to contain 1.9 g (10%w/w) progesterone to assure 12 day effectiveness. In recent times our understanding of the physiology of cattle has increased (as described in Section 1.2.2.5) and when progesterone is continuously administered and used in combination with other drugs progesterone only needs to be delivered for a 7 day period. The outcome of this is that the CIDR®1900 Cattle insert exhibits a high residual content of progesterone in spent devices which have been inserted for periods shorter than 12 days (e.g., 1.33 g of progesterone remains in the existing insert following removal after 7 days). The CIDR®1900 Cattle insert was therefore recently redeveloped so that it initially contained the minimum amount of progesterone for a 7 day treatment period, yet produced the same plasma progesterone profiles and which resulted in the minimum possible amount of progesterone left in the spent insert at the end of the 7 day insertion period.

Initially in the optimisation process the CIDR®1900 Cattle insert was characterised by Rathbone et al. Part of this process identified that the CIDR®1900 Cattle insert had a variable skin thickness ranging from 1 to 5 mm. Next variables which could potentially affect the amount of progesterone released from the intravaginal insert were investigated and included initial drug load and surface area. In addition, the extent of depletion of progesterone from the silicone rubber matrix was determined. It was found that lowering the initial drug load to below 10%w/w resulted in lower blood plasma progesterone levels in cows. However, increasing the initial drug load above 10%w/w did not produce elevated levels above those seen with a 10%w/w load[6, 222]. The surface area of the intravaginal inserts was found to affect the magnitude of blood plasma progesterone levels with increases in surface area producing increases in progesterone levels in a linear fashion[6, 222]. Analysis of spent inserts for progesterone depletion zones of the silicone rubber matrix revealed that

progesterone had only been released from the outer 1 mm of the silicone rubber matrix.

As a result of these studies, Rathbone and co-workers determined that the optimisation criteria could not be met by simply reducing the initial percentage drug load of the silicone rubber matrix as this would result in lower blood plasma progesterone levels. Similarly, the insert could not be optimised by a reduction in physical size as a reduction in surface area would result in a reduction in plasma levels. However, as progesterone was only released from the outer millimetre of silicone rubber matrix, the CIDR®1900 Cattle insert could be successfully optimised by designing an insert to have the same 10%w/w initial drug loading and the same overall surface area but with an even skin thickness of 1 mm over the entire optimised insert. These design criteria resulted in the elimination of the deeper layers of skin which did not contribute to drug release. The optimised design called the CIDR®1380 Cattle insert contained a lower initial progesterone load (1.38 g compared to 1.9 g), produced the same clinical efficacy and contained only 700 mg of progesterone when it was removed after 7 days.

1.6.2 Optimisation of the Sponge

As early as 1967 it was known that sponges were wasteful in their delivery of progestagen[223]. These authors showed that only 12-24% of progestagen released from a polyurethane sponge was absorbed from the vagina and that 98% of the absorbed drug was excreted in urine and faeces.

In the late 1980's, Kabadi and Chien set out to redesign the sponge in order to minimize the loading dose, overcome the declining (square root of time) intravaginal release profile and absorption profiles, as well as to improve systemic bioavailability and dose utilisation[144, 224]. The pair developed two new intravaginal inserts, both employing polyurethane sponge as a mechanical support for retention (Figure 1.6.1).

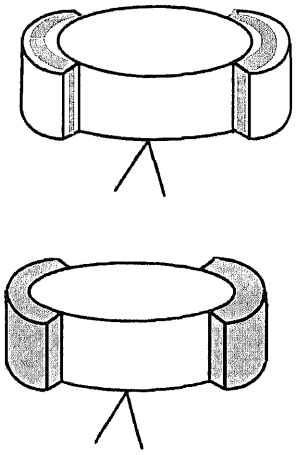


Figure 1.6.1 - Diagram of Type 1 (top) and Type 2 (bottom) prototype sponges

The Type 1 device comprised a progestagen/silicone monolith sandwiched between a rate-limiting non-drug loaded silicone membrane, however, this delivery system released insufficient drug to control the oestrous cycle of sheep. The authors predicted that a rate-limiting membrane thickness of 0.05 mm would have been required to achieve the desired release rate, but fabrication of such a thin coating was beyond their manufacturing capabilities. The Type 2 device simply comprised a progestagen/silicone monolith adhered to the outside of a sponge. The mechanism of release from such a design was shown to be dependent upon initial load. A 2% fluorogestone acetate load resulted in a zero-order release profile; a 0.2% load resulted in a square root of time release profile. The authors demonstrated that the invivo release could be modified by altering the surface area of silicone monolith[225] and used this information to optimise the surface area of their Type 2 silicone drug delivery system to produce a delivery system which was clinically efficacious. The resultant delivery system (18 cm^2 (2% load), 20 mg fluorogestone acetate device) was evaluated in sheep and was found to exhibit comparable efficacy to the conventional polyurethane sponge containing 40 mg fluorogestone acetate. The resultant delivery system successfully reduced the loading dose, exhibited a zero-order intravaginal release profile, as well as demonstrating improved dose utilisation.

1.6.3 Electronically controlled drug delivery system

The Intelligent Breeding Device (IBD) (Figure 1.6.2) is the most recent and technologically advanced controlled release drug delivery system to be developed for oestrus control. It was designed and marketed by PLADE Holdings Limited. It is

used for the control of the cattle oestrous cycle[3, 4, 5]. As discussed in Section 1.2.2.5, current fertility control programmes involve administration of a number of pharmacologically active compounds to ensure precise oestrus synchrony and ovulation coupled with high fertility rates. The continuous administration of progesterone to control the cycle, an initial dose of oestradiol at the beginning of treatment to induce follicular turnover and the administration of a prostaglandin one to two days before cessation of progesterone delivery are common treatment programs used in modern day animal breeding programs. The IBD was designed to contain the three drugs commonly used in fertility treatment programmes (oestradiol, prostaglandin and progesterone) and release these at different rates (oestradiol and prostaglandin-pulsed delivery; progesterone-continuous release) and times (oestradiol-1 hour after insertion; prostaglandin 6 days after insertion; progesterone-continuously for 10 days) following administration.

The delivery system contains a circuit board which controls the timing of release of each of the incorporated drugs. The circuit board, along with drug reservoirs and batteries are enclosed in a plastic sheath which has a tail to aid removal and an eight pronged shaped retention mechanism. The IBD contains four drug reservoirs (one large reservoir at the base of the device which contains progesterone and three smaller reservoirs at the head of the device). The large progesterone reservoir was under pressure due to a loaded spring and plunger arrangement. The progesterone was prevented from being released from the reservoir by a closed orifice. However, a solenoid frequently opened the orifice at intervals to allow the progesterone solution to be released continuously throughout treatment. The smaller reservoirs were located at the head of the device and these were also under pressure by a spring and plunger arrangement. One of the smaller reservoirs contained a single dose of oestradiol and another contained a single dose of prostaglandin. A secured plastic cord held the plunger taught and was secured over a resistor. Release of the plunger occurred by melting the cord on activation of the resistor. The electronic circuitry controlled when these smaller reservoirs delivered their contents. Despite the complexity in design and manufacturing requirements, the IBD offers the advantage of minimising the requirements for stock handling and veterinary visits for farmers in synchrony treatments resulting in lower cost treatment programmes.

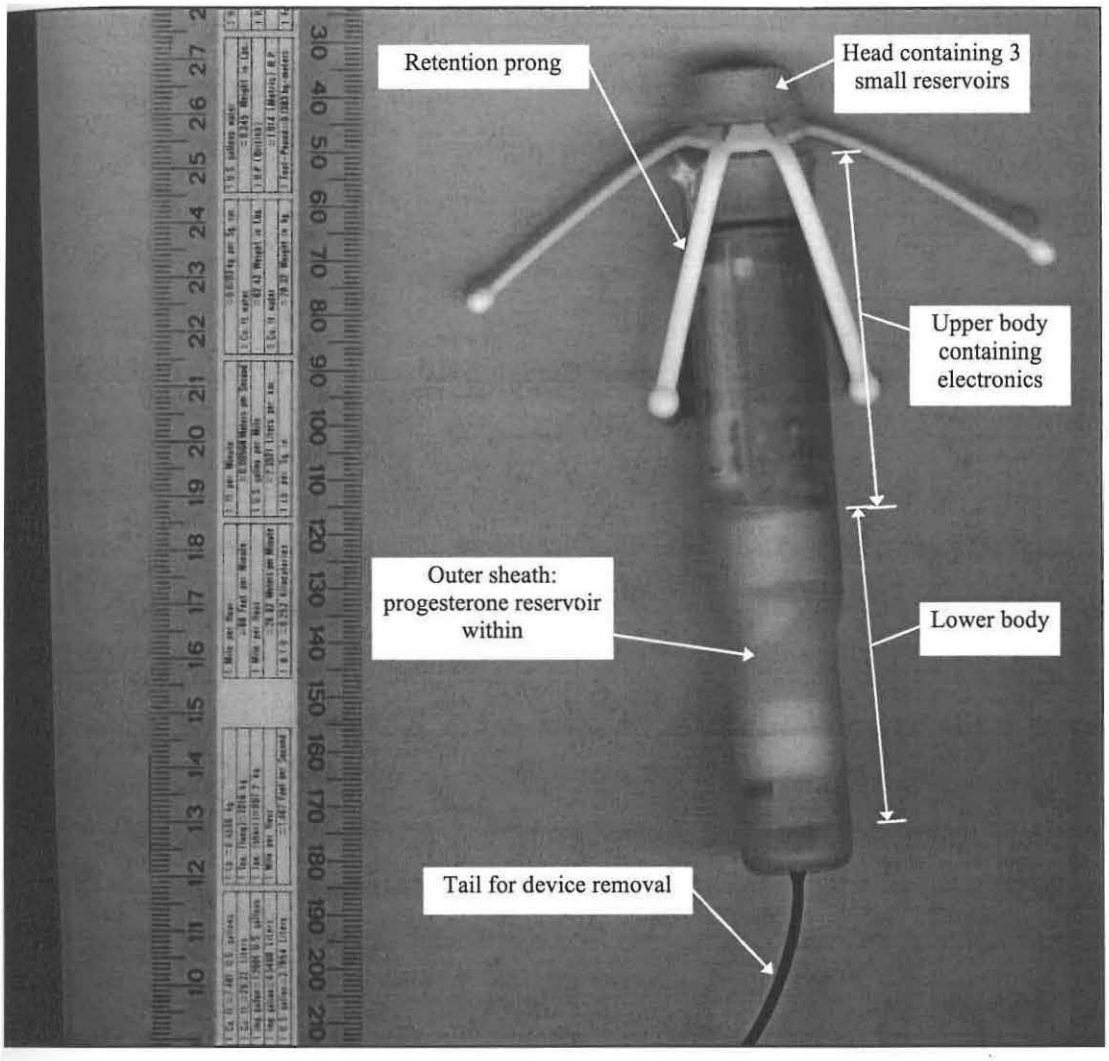


Figure 1.6.2 - External features of the SMARTT*I*™ IBD.

1.7 Aims of Thesis

A review of the current scientific literature has identified several areas where new work could be carried out to add to the knowledge base on intravaginal drug delivery for the control of oestrus in farmed livestock.

Recent advances in our understanding of the control of oestrus in cattle have been discussed and have identified that use of progesterone in fertility programmes should be restricted to shorter treatment periods with the coadministration of other compounds to ensure precise synchrony and high fertility. This knowledge led Rathbone and co-workers to the optimise the CIDR®1900 Cattle insert to produce appropriate plasma progesterone levels while containing both lower initial and residual progesterone contents. This Thesis performs a thorough invitro and invivo

pharmaceutical characterisation of the resultant commercially available (CIDR®1380 Cattle insert) as well as the original CIDR®1900 Cattle insert (Chapters Two and Three). In Chapter Four the invivo mechanism of release of CIDR®1900 Cattle inserts is investigated. In this Chapter a novel mechanism of release is identified and elucidated.

In the Introduction to this Thesis, recent advances in fertility control applications in sheep were discussed. A need to optimise the current commercially available CIDR® Sheep and Goat insert to assure efficacy in more demanding applications was identified. The knowledge gained from the CIDR®1900 Cattle insert characterisation process was used to optimise the silicone CIDR® Sheep and Goat insert technology for use in sheep (Chapter Five).

Only two polymers have been utilised as platforms for the delivery of progestagens via the vagina of sheep (silicone and polyurethane). Although both these polymers have been successfully utilised in commercially available intravaginal veterinary drug delivery systems, they both have inherent limitations and disadvantages. In this Thesis poly-(ε-caprolactone) was identified as an alternate material to these polymers which overcomes their limitations and disadvantages. Chapter Six discusses the development and optimisation of an intravaginal delivery system manufactured from poly-(ε-caprolactone) for the control of oestrus in sheep. The results suggest that poly-(ε-caprolactone) may be a suitable polymer for use as a platform for the intravaginal delivery of progesterone.

Chapter Two - Characterisation of intravaginal drug delivery systems for the delivery of progesterone for control of the bovine oestrous cycle. I. Chemical, physical and biological characteristics of CIDR®1900 Cattle insert and CIDR®1380 Cattle insert.

2.1 Introduction

The introductory Chapter of this Thesis described some limitations of the commercially available CIDR®1900 Cattle insert intravaginal veterinary drug delivery system. These limitations were recognised by Rathbone and his co-workers who have recently reported on the optimisation of the CIDR®1900 Cattle insert[6]. Their work resulted in prototype inserts which exhibited a reduction in the initial progesterone content and reduced residual progesterone content compared to the CIDR®1900 Cattle insert. However, their prototype inserts delivered sufficient progesterone such that plasma profiles were equivalent to those exhibited by the CIDR®1900 Cattle insert.

Optimisation of the CIDR®1900 Cattle insert was achieved following an extensive study which investigated those factors which affected the invitro and invivo release of progesterone from CIDR®1900 Cattle insert[6, 102, 222]. Rathbone and his co-workers used this information to define the procedure which should be undertaken to optimise the CIDR®1900 Cattle insert. They then demonstrated the validity of their optimisation recommendations by manufacturing an intravaginal insert using a prototype mould.

Recently, precision made commercial moulds have been manufactured to produce an intravaginal insert referred to as the CIDR®1380 Cattle insert. Rathbone and his co-workers have not performed any pharmaceutical characterisation experiments on the CIDR®1380 Cattle insert. Hence, this chapter is concerned with the in-depth invitro and invivo pharmaceutical characterisation of the CIDR®1380 Cattle insert and covers both the initial 1-up tooling (a stainless steel tool which allowed only

one insert to be injection moulded at a time) and 6-up tooling processes (a stainless steel tool which allowed six inserts to be injection moulded at a time) which were undertaken as the CIDR®1380 Cattle insert went through its production scale-up to final manufacture. A 1-up tool was manufactured to assure the Company that the theories developed by Rathbone et al. which led to their prototype insert were valid with production made inserts. To assess the performance of the 1-up CIDR®1380 Cattle insert, its invitro and invivo characteristics were compared to the CIDR®1900 Cattle insert. A six-up tool was manufactured to increase manufacturing capacity of the CIDR®1380 Cattle insert. To assess the performance of the 6-up CIDR®1380 Cattle insert, its invitro and invivo characteristics were compared to the 1-up CIDR®1380 Cattle insert.

Invitro characterisation involved determination of initial drug load, surface area, skin thickness, skin weights, percentage drug load and invitro drug release profile. Invivo characterisation involved bioequivalence studies and determination of residual content in spent inserts. The reasons for choosing these characteristics for investigation are shown in Table 2.1.1.

Table 2.1.1 - Pharmaceutical characteristics investigated

Characteristic	Reason for Selection	Reference
Initial drug load	Optimisation aim was to reduce drug load	6, 222
Skin weight	Shot weight variation occurs in manufacturing which creates possible variation in drug load and is required to determine percentage drug load	6
Percentage drug load	Because of shot weight variation, percentage drug load is a more consistent variable. Rathbone et al. demonstrated that biological effect is more dependent on percentage drug load rather than total drug load	6
Surface area	Bunt et al. demonstrated that surface area is an important determinant of the magnitude of plasma profiles	222
Skin thickness	Rathbone et al. reported progesterone is only released from the first 1 mm of silicone skin from an intravaginal insert	6
Invitro drug release profile	A rapid measure of the release characteristics of a controlled release product	101
Bioequivalence	A definitive measure of the comparative performance of the test insert with the original	6
Residual content in spent inserts	Reduction in residual content was a goal of the optimisation process	6
Amount released	In conjunction with the bioequivalence study this gives a measure of the comparative performance of the test insert with the original	6

2.2 Experimental Methods

2.2.1 Ultra-Violet (UV) Spectrophotometric Assay for Progesterone in CIDR® Cattle Inserts Following Extraction from Silicone Skin or Released During an Invitro Drug Release Test

2.2.1.1 Method

Progesterone content in ethanolic solutions was determined by UV Spectrophotometry at 240 nm (DU650i, Beckman, USA). This method was applied to determine progesterone content of CIDR® cattle inserts after extraction of the progesterone from the silicone skin, or after its release during an invitro drug release test.

2.2.1.2 Standard Preparation

Standards were prepared by transferring 0.1000 g progesterone standard (USP micronised, Pharmacia & Upjohn , USA) into a 100 mL volumetric flask followed by the addition of 50 mL alcohol (SDA-3A, Mobil, New Zealand). The mixture was sonicated until the progesterone completely dissolved, made up to volume with alcohol (SDA-3A, Mobil, New Zealand) and mixed by inversion. 200 µL, 500 µL, 1000 µL, 1500 µL and 2000 µL aliquots were then pipetted into 50 mL volumetric flasks, made up to volume with alcohol (SDA-3A, Mobil, New Zealand) and mixed by inversion.

2.2.1.3 Sample Preparation (Extraction Procedure)

CIDR® Cattle insert samples were prepared for progesterone content determination by a Soxhlet extraction procedure. Drug loaded silicone skin was removed from the nylon spine and weighed on a four figure analytical balance (BP110S, Sartorius, Germany). The skin was cut into 5-8 cm lengths using a scalpel and the lengths were placed into a Soxhlet apparatus, ensuring that all lengths sat below the siphon level. 350 mL ethanol (SDA-3A, Mobil, New Zealand) was added to the round bottom flask of the Soxhlet apparatus and then the flask, Soxhlet and water cooled condenser were assembled in a heating mantle (Electromantle ME, Electrothermal, Britain). Skins were extracted for at least 12 hours and allowed to cool before transferring (with rinsing) to a 500 mL

volumetric flask and making the volume up to the mark with ethanol (SDA-3A, Mobil, New Zealand). This solution was then diluted (0.5 mL to 100 mL) with alcohol (SDA-3A, Mobil, New Zealand) prior to analysis by UV Spectrophotometry at 240 nm.

2.2.1.4 Sample Preparation (Invitro Drug Release Test)

Samples collected during invitro drug release testing of CIDR® Cattle inserts were prepared for analysis by evaporating off the hydro-alcoholic release media in a drying oven (SERIES FIVE OVEN, Contherm, UK) at 80°C over 24 hours and then reconstituting the samples in 5 mL ethanol (SDA-3A, Mobil, New Zealand). The reconstitution step involved addition of the 5 mL ethanol aliquot from a dispenser (dosipet 30 mL, Kartell) followed by 10 seconds vortexing, sonication for at least 5 minutes (SONOREX SUPER RK 510H, Bandelin Electronic, Germany) and a further 10 seconds vortexing. A 0.5 mL aliquot of the reconstituted solution was then diluted by addition of 5 mL ethanol (SDA-3A, Mobil, New Zealand) before analysis by UV spectrophotometry at 240 nm. The sample preparation by reconstitution method was validated by performing the procedure as written on replicate 1mL aliquots of standard solutions at low (0.22 mg/mL) and high (1.10 mg/mL) concentrations and analysing the reconstituted validation standards by the UV spectrophotometric method.

2.2.1.5 Validation of the UV Spectrophotometric Assay

The UV spectrophotometric assay was validated by assessment of its linearity and range, accuracy, precision, ruggedness, limit of quantitation and specificity[226-228].

2.2.1.5.1 Linearity and Range

The linearity of an analytical method is defined as its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range; the range being the interval between the upper and lower levels of analyte that have been demonstrated to be determined with precision, accuracy, and linearity using the method as written. Linearity was determined by analysing two sets of calibration standards (4, 10, 20, 30 and 40 μg/mL) and performing linear regression

upon the results. The range was determined by analysing validation standards at 4 and 40 μg/mL and determining their concentrations against the linear regression.

2.2.1.5.2 Accuracy

Accuracy is defined as the nearness of a result to the true or accepted value. Accuracy was determined by analysing validation standards at 8, 15 and 35 μg/mL, calculating their concentrations against the linear regression and assessing the deviation of the calculated results from their true concentrations.

2.2.1.5.3 Precision

Precision is defined as the agreement of repeat observations made under the same conditions. Precision occurs on different levels including repeatability (multiple absorbance readings of the same solution), and reproducibility (determination of multiple known samples). Repeatability was assessed by executing repeat absorbance readings of a single standard solution and determining the relative standard deviation of the results about their mean value. Reproducibility was determined in association with accuracy. The concentrations of replicate 8, 15 and 35 μg/mL validation standards were calculated against the linear regression and the relative standard deviations of the results about their means were determined.

2.2.1.5.4 Ruggedness

Ruggedness is an element of precision and is defined as agreement in the determination of multiple known samples using the same method but under different conditions such as day of analysis and analyst carrying out the experiment. Ruggedness was determined by replicate analyses of CIDR® inserts on multiple days and with multiple analysts.

2.2.1.5.5 Limit of quantitation

The limit of quantitation is defined as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The limit of quantitation was determined by determining the background response of the method from multiple blank determinations, followed by analysis of standards near the determined limit.

2.2.1.5.6 Specificity

Specificity is defined as the ability of the method to resolve a substance from its impurities and any other substances such as degradation products or placebo ingredients of the product. Specificity of the UV spectrophotometric assay was assessed by analysis of progesterone standards in the presence of any unknown compounds extractable from the silicone CIDR® matrix.

2.2.2 Invitro Drug Release Test

2.2.2.1 Invitro Drug Release Test Method

Release of progesterone from CIDR® Cattle inserts was assessed using a modified USP dissolution apparatus (Hanson SRII8 Dissolution Test Station, Hanson Instruments, USA). Inserts were held in specially manufactured stirrer paddles (Figure 2.2.1) so that they could be centralised in the flasks (Figure 2.2.2) and rotated at 150 rpm. Lids were specially designed (Figure 2.2.3) to accommodate the specially designed stirrer paddles and the standard autosampler probes while minimising the evaporative loss of release media over the duration of each invitro drug release assessment test. The release media used comprised 1100 mL of 62.5% ethanol (SDA-3A, Mobil, New Zealand) and 37.5% double distilled water (AUTOSTILL™ Auto Four WS SYSTEM, Jencons Scientific Ltd., UK) maintained at 37°C. Release media composition was selected based on the requirement for maintenance of perfect sink conditions in the release media throughout the duration of the invitro drug release assessment test. The volume of 1100 mL was identified as a suitable volume which could be used to fully cover the CIDR® with the vortexing created by rotating the stirring system at 150 rpm. Sink conditions are maintained provided the concentration of drug in the release media does not exceed 10% of its saturation solubility in the release media. Solubility of progesterone in alcohol:water media was determined by Rathbone et al. (personal communication) (Table 2.2.1). If all 1900 mg progesterone were released into 1100 mL release media of the selected composition then progesterone would be present at approximately 6% of its saturation concentration. Samples (1 mL) were collected automatically (Hanson SIP Control, Hanson Instruments, USA), evaporated to dryness and reconstituted prior to analysis by the UV spectrophotometric assay described in Section 2.2.1. Predefined sample collection times were 2 minutes, 2, 4, 8, 12, 24, 48, 72 and 96 hours. Since

the CIDR®1380 Cattle insert approached exhaustion of its load sooner than the CIDR®1900 Cattle insert, rate constants were calculated using data up to and including 48 hours for both inserts. Data to this time was linear as a function of the square root of time and allowed direct comparison of all inserts.

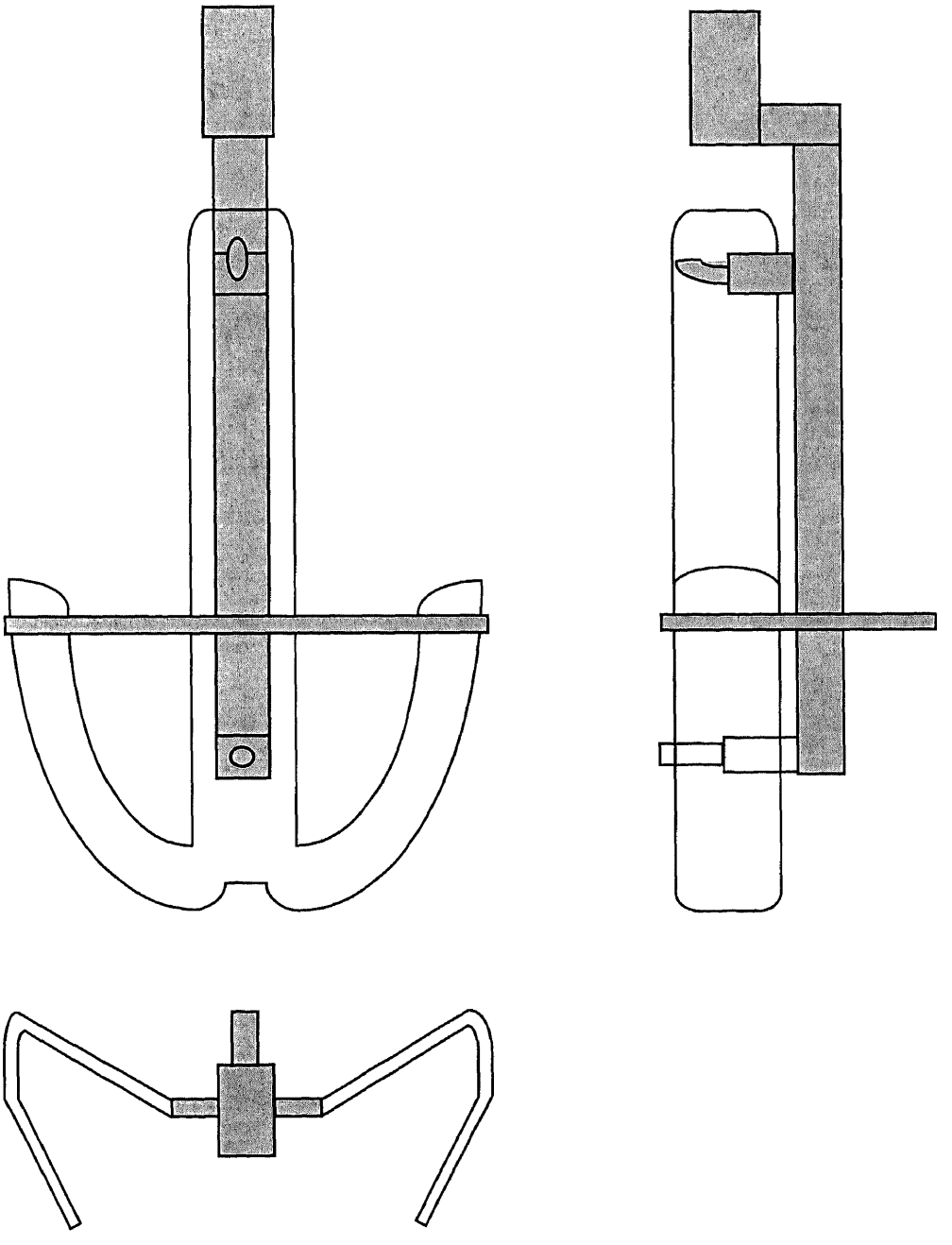


Figure 2.2.1 - Front elevation (top left), side elevation (top right) and plan view of mounted existing CIDR®1900 Cattle insert in specially designed holder (bottom left).

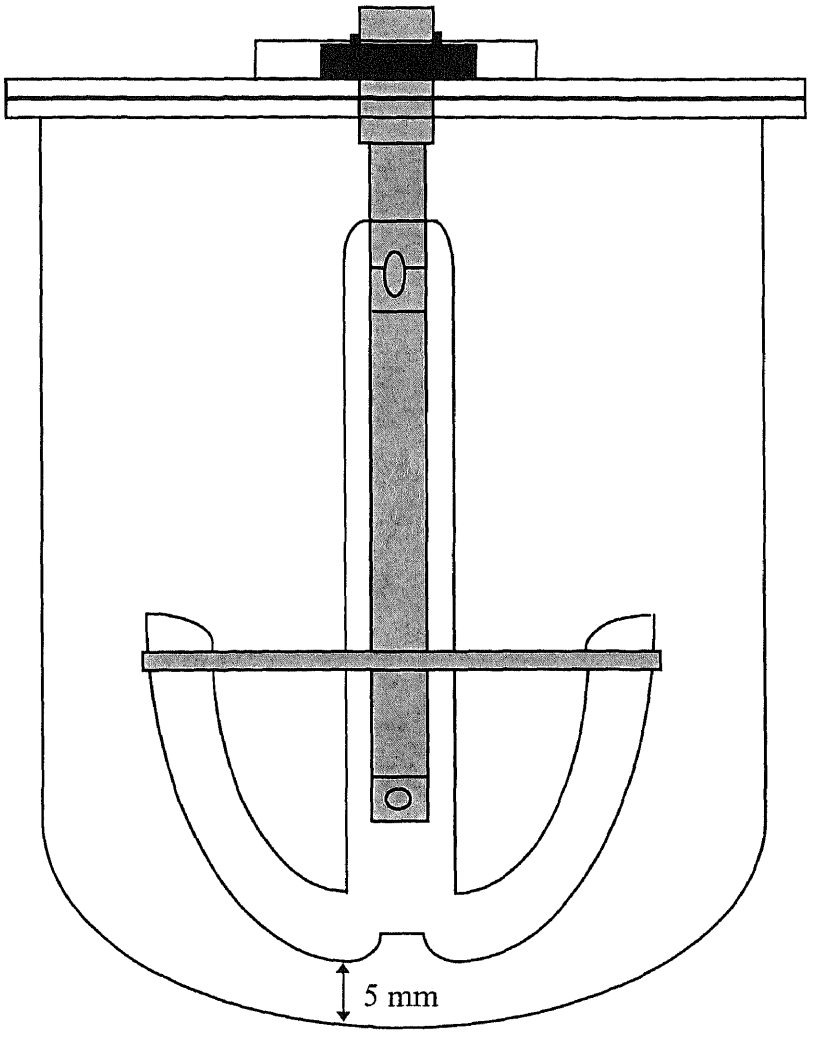


Figure 2.2.2 - Front view of a mounted existing CIDR®1900 Cattle insert and position in flask.

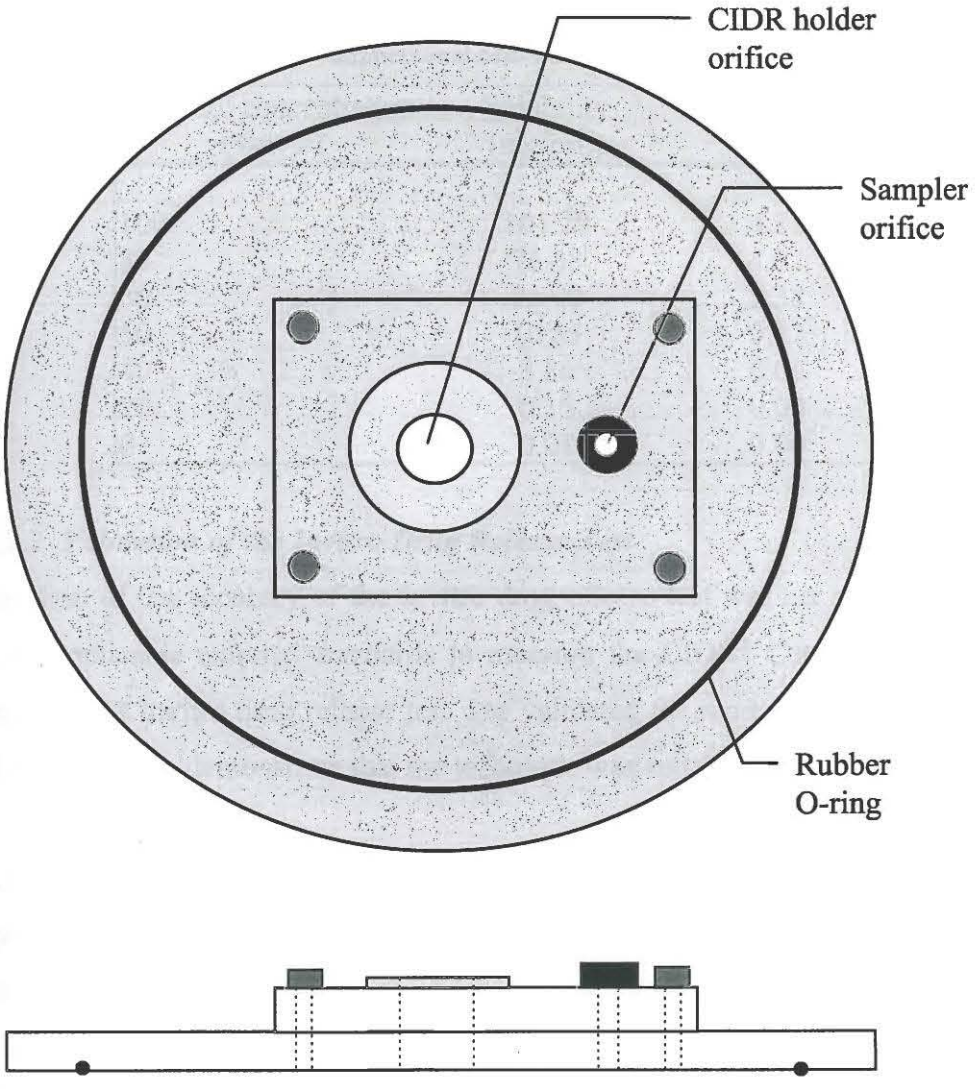


Figure 2.2.3 - Lid design for release assessment apparatus.

Table 2.2.1 - Solubility of progesterone in various alcohol:water mixtures at 37°C (Rathbone et al., personal communication).

Alcohol concentration (%w/w)	Solubility (mg/mL)	sem (N=3)
100	101.29	4.13
80	76.68	4.02
60	25.00	2.38
50	10.85	0.47
40	3.86	0.06
20	0.15	0.00
0	0.01	0.00

2.2.2.2 Validation of the Invitro Drug Release Test

Validation of the accuracy of the invitro drug release test was not possible as there are no applicable external standards to measure the CIDR® Cattle inserts against. However, the invitro drug release test was validated for reproducibility, ruggedness and robustness to demonstrate that the technique was suitable for use.

2.2.2.2.1 Reproducibility and Ruggedness

Reproducibility is an element of precision (i.e., the agreement of repeat observations made under the same conditions) and is defined as the determination of multiple known samples. Ruggedness is an element of precision and is defined as agreement in the determination of multiple known samples using the same method but under different conditions such as day of analysis.

Reproducibility of the invitro drug release test was determined by testing a number of 1-up CIDR®1380 Cattle insert from the same production batch. Ruggedness was determined by subjecting further inserts from each of a second and third production batch to the invitro drug release test.

2.2.2.2.2 Robustness

Robustness is defined as the ability of the method to produce accurate, reproducible determinations in spite of deliberate deviations from the method as written. Robustness was assessed by inducing deviations from both method variables and sample variables. Several different parameters were assessed. The speed at which samples were rotated or stirred was examined by reprogramming the dissolution test

apparatus. The alcohol concentration of the release media was examined by mixing different ratios of ethanol (SDA-3A, Mobil, New Zealand) and double distilled water. The ionic strength of the release media was examined by the addition of appropriate quantities of sodium chloride to the release media (AnalaR®, BDH, New Zealand). The pH of the release media was examined by the dropwise addition of either concentrated hydrochloric acid (AnalaR®, BDH, New Zealand) or 2M sodium hydroxide (AnalaR®, BDH, New Zealand), measuring pH (GP353 ATC pH METER, EDT Instruments) after each addition until the desired pH was achieved. Finally, the initial drug load of CIDR® inserts was examined using progesterone loaded 1-up CIDR®1380 Cattle insert containing 2.5, 5, 7.5, 10, 15 and 25 %w/w.

2.2.3 Manufacture of CIDR® inserts

All CIDR® Cattle inserts were manufactured using InterAg's patented high temperature injection moulding process. Progesterone (USP micronised, Pharmacia & Upjohn , USA) was mixed at 10% w/w in both parts A and B silicone (Silastic® Q7-4840, Dow Corning, USA) in a dynamic epicentric mixer (InterAg, Hamilton, New Zealand) for a minimum of 2 hours. The mixture was then evacuated for a minimum of 2 hours at 60 kilopascals. Nylon (Ultramid A3K, BASF, Germany) spines were annealed in a water bath at 70°C for 7.5 hours. Spines were then placed in a tool cavity in an injection moulding machine (LIM-100-35V, Sanjo Seiki Co., Ltd., Japan; Type PS40E5A, Nissei Plastic Industrial Co., Ltd., Japan; 320M-500-210, Arburg GmbH & Co., Germany) and drug loaded silicone parts A and B were simultaneously injected through a pre-mixing static mixer around the nylon spines and cured for approximately 50 seconds at around 190°C.

2.2.4 Invitro Characterisation of CIDR® Cattle Inserts

2.2.4.1 Location of Progesterone in CIDR® Cattle Inserts

The location of progesterone in CIDR® Cattle inserts after moulding was determined by separating the silicone skin from the nylon spine of CIDR®1900 Cattle inserts and performing a separate Soxhlet extraction on both the skin and spine. Drug content was determined by the UV assay described in Section 2.2.1.

2.2.4.2 *CIDR® Cattle Insert Skin Weight*

The weight of the progesterone containing silicone skin of CIDR® Cattle inserts was determined by first removing the skin from the nylon spine using a scalpel and then weighing the removed skin on a four figure analytical balance (BP110S, Sartorius, Germany).

2.2.4.3 *Initial Drug Load*

The amount of drug in CIDR® Cattle inserts was determined by Soxhlet extraction of the skin with ethanol (SDA-3A, Mobil, New Zealand) and analysis of the resulting extracts by UV Spectrophotometry (See 2.2.1). The amount of drug in CIDR® Cattle inserts was also determined as a percentage of the total weight of the injection moulded skin by dividing the amount of drug extracted from the skin by the initial skin weight and multiplying by one hundred.

In cases where initial loads were not directly determined by Soxhlet extraction and UV spectrophometry due to insufficient supply of CIDR® Cattle inserts from the same manufacturing batch, initial load was estimated mathematically (defined below) from the mean residual determination, the total residual skin weight and the known percentage initial load. This applied to both the CIDR® 1900 Cattle insert and CIDR®1380 Cattle insert, each manufactured to contain 10%w/w progesterone.

$R + r = I$ and....

$R + W_s = I / 10\% = W_f$

$\Rightarrow \quad I - r = (I / 0.1) - W_s$

$\Rightarrow \quad (I / 0.1) - I = W_s - r$

$\Rightarrow \quad (I - 0.1I) / 0.1 = W_s - r$

$\Rightarrow \quad 9I = W_s - r$

$\Rightarrow \quad I = (W_s - r) / 9$

where

I = Initial progesterone load in CIDR® Cattle insert

R = Amount of progesterone released (g)

r = Residual progesterone in spent CIDR® Cattle insert (g)

W_s = Weight of skin containing progesterone of spent CIDR® Cattle insert (g)

W_f = Weight of skin containing progesterone of fresh CIDR® Cattle insert (g)

2.2.4.4 Surface Area

The surface area of CIDR® Cattle inserts was determined by wrapping inserts in aluminium foil of known density per unit area (0.0034 g/cm^2) and weighing (BP110S, Sartorius, Germany) the foil required to cover the insert. The surface area was calculated by dividing the foil weight by its density per square centimetre.

2.2.4.5 Skin Thickness

The skin thickness of CIDR® Cattle inserts was measured at a series of pre-determined positions (Figure 2.2.4) using an outside micrometer (Moore and Wright, Sheffield).

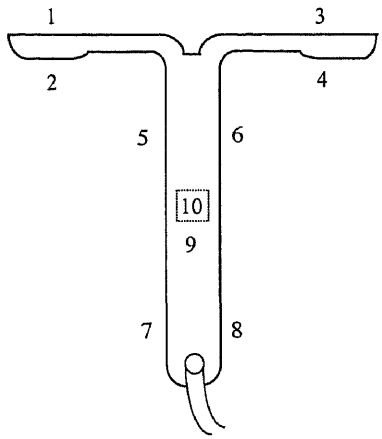


Figure 2.2.4 - CIDR® Cattle insert with pre-chosen positions depicted

2.2.5 Invivo Characterisation of CIDR® Cattle inserts

2.2.5.1 Plasma Progesterone Profiles

Ethical approval was obtained from both the AgResearch, Ruakura Animal Ethics Committee and from the University of Waikato Animal Ethics Committee on the Welfare of Experimental Animals. All animal work was carried out with supervision from a veterinarian.

The invivo plasma progesterone profiles were determined in the following manner. CIDR® Cattle inserts were inserted into the vagina of bilaterally ovariectomised

Friesian cows maintained by Dairying Research Corporation (DRC) using a standard CIDR® Cattle insert applicator. Treatments were terminated by removing the insert from the vagina. Plasma samples were collected by trained AgResearch personnel from the coccygeal vein of the cows on predetermined days over the insertion period. For more intensive bleeding schedules, cows were cannulated in the jugular vein by a veterinarian. The cannulation procedure was accompanied by antibiotic treatment and cows were kept in stalls while cannulated. Plasma was separated by centrifugation and stored at -20°C for subsequent progesterone analysis. Concentrations of progesterone in plasma were determined by direct radio immuno assay by trained technicians at DRC laboratories using a commercial solid phase I^{125} label (Coat-a-count, DPC, USA).

The liveweight and liver function of all ovariectomised cows was monitored to ensure no bias was introduced to the data.

The experimental design of the invivo trials carried out in this chapter are shown in Tables 2.2.2 to 2.2.5.

Table 2.2.2 - 7 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to CIDR®1900 Cattle insert

Formulations A versus B		Insertion Time (days)	Number of Animals (N)	Crossover Trial design (Yes or No)
CIDR®1900 Cattle insert	1-up CIDR®1380 Cattle insert	7	8	Yes

Table 2.2.3 - 10 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to 6-up CIDR®1380 Cattle insert

Formulations A versus B		Insertion Time (days)	Number of Animals (N)	Crossover Trial design (Yes or No)
CIDR®1900 Cattle insert	1-up CIDR®1380 Cattle insert	10	10	Yes

Table 2.2.4 - 20 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to CIDR®1900 Cattle insert

Formulations A versus B		Insertion Time (days)	Number of Animals (N)	Crossover Trial design (Yes or No)
CIDR®1900 Cattle insert	1-up CIDR®1380 Cattle insert	20	10	Yes

Table 2.2.5 - 10 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to CIDR®1900 Cattle insert

Formulations A versus B		Insertion Time (days)	Number of Animals (N)	Crossover Trial design (Yes or No)
1-up CIDR®1380 Cattle insert	6-up CIDR®1380 Cattle insert	10	24	No

2.2.5.2 Pharmacokinetic Analysis of Invivo Plasma Profiles Following Insertion of CIDR® Cattle Inserts

The Area Under the Curve (AUC) was determined from the blood plasma progesterone levels determined at given time points over the duration of treatment with intravaginal inserts for each individual cow treated. The AUC were determined by simple numeric estimation using the trapezoidal rule[229] using the following equations:

$$AUC_i = \frac{C_k + C_j}{2} \times T_i \qquad \textbf{Equation 2.2.1}$$

$$AUC = \sum AUC_i \qquad \textbf{Equation 2.2.2}$$

where:

AUC_i is the area under the curve over the i^{th} time interval (i.e., Area under curve between the j^{th} sample time and the k^{th} sample time).

C_k is the plasma level (ng/mL) at the k^{th} sample time.

C_j is the plasma level at the j^{th} sample time.

T_i is the numerical value of the i^{th} time interval (days) (i.e., Time at which the k^{th} sample was taken minus time at which the j^{th} sample was taken).

2.2.5.3 Residual Progesterone Content Remaining in CIDR® Cattle Inserts Following Intravaginal Insertion

The residual progesterone content remaining in CIDR® Cattle inserts following intravaginal insertion was determined by Soxhlet extraction of the skin with ethanol (SDA-3A, Mobil, New Zealand) and analysis of the resulting extracts by UV Spectrophotometry at 240 nm (Beckman DU650i, USA) (See Section 2.2.1). Prior to extraction, the silicone skin of each used CIDR® Cattle insert was removed from its nylon spine, washed in cold water to remove any vaginal mucus deposits and dried with paper towels.

2.2.5.4 Amount of Progesterone Released from CIDR® Cattle Inserts

The amount of progesterone released from CIDR® Cattle inserts over a particular insertion period was determined by subtracting the experimentally determined residual progesterone load from the initial load in the CIDR® Cattle inserts.

2.2.6 Statistical Analysis

The statistical significance of each and every comparison between AUC of different treatments, initial progesterone loads, residual progesterone loads and amounts of progesterone released during intravaginal insertion was determined by Oneway Unstacked ANOVA (MINITAB 8.2 Statistical Software).

2.3 Results

2.3.1 UV Assay Validation

2.3.1.1 Linearity and Range

Data for two sets of calibration standards are presented in Table 2.3.1. Combined regression analysis of the two curves is presented in Table 2.3.2.

Table 2.3.1 - Analysis of Calibration Working Standards for validation of the UV assay

Concentration (μg/mL)	Standard A Absorbance	Standard B Absorbance
4	0.2174	0.2203
10	0.5385	0.5421
20	1.0800	1.0871
30	1.6278	1.6262
40	2.1590	2.1677
Slope	0.0541	0.0541
Intercept	0.0002	0.0029
R^2	1.0000	1.0000

Table 2.3.2 - Combined Regression Analysis of both A and B Working Standards for validation of the UV assay

Parameter	Result	S.E	± 95% C.L	± 99% C.L
B1	0.0538	0.000116	0.000259	0.000368
B0	0.00726	0.00260	0.00581	0.00826

Data acquired to confirm the range of the assay is presented in Table 2.3.3.

Table 2.3.3 - Analysis of Range Validation Standards for validation of the UV assay

QC Sample	Absorbance	Calculated concentration (μg/mL)
4 μg/mL	0.2224	4.00
4 μg/mL	0.2189	3.93
40 μg/mL	2.1393	39.63
40 μg/mL	2.1366	39.58

2.3.1.2 *Accuracy*

Data for the method validation parameter of accuracy is presented in Table 2.3.4.

Table 2.3.4 - Analysis of Accuracy Validation Standards (8, 15 & 35 µg/mL) for validation of the UV assay

8 µg/mL	day 0	day 1	day 2	day 3	mean	SD	%RSD	%deviation
Rep. A	7.91	7.88	7.92	7.84	7.89	0.04	0.46	1.41
Rep. B	7.81	8.01	7.98	7.89	7.92	0.09	1.14	0.97
mean	7.86	7.95	7.95	7.87	7.91	0.05	0.62	1.19
SD	0.07	0.09	0.04	0.04	0.02			
%RSD	0.90	1.16	0.53	0.45	0.31			
%Dev.	1.75	0.69	0.62	1.69	1.19			
15 µg/mL	**day 0**	**day 1**	**day 2**	**day 3**	**mean**	**SD**	**%RSD**	**%deviation**
Rep. A	14.83	14.71	14.91	14.90	14.84	0.09	0.62	1.08
Rep. B	14.86	14.76	15.05	14.90	14.89	0.12	0.81	0.72
mean	14.85	14.73	14.98	14.90	14.87	0.10	0.69	0.90
SD	0.02	0.04	0.10	0.00	0.04			
%RSD	0.14	0.27	0.68	0.00	0.26			
%Dev.	1.03	1.77	0.13	0.68	0.90			
35 µg/mL	**day 0**	**day 1**	**day 2**	**day 3**	**mean**	**SD**	**%RSD**	**%deviation**
Rep. A	34.76	34.73	35.02	34.64	34.79	0.16	0.47	0.61
Rep. B	34.81	34.71	35.03	34.76	34.83	0.14	0.40	0.49
mean	34.79	34.72	35.03	34.70	34.81	0.15	0.43	0.55
SD	0.04	0.02	0.01	0.08	0.03			
%RSD	0.10	0.05	0.03	0.24	0.08			
%Dev.	0.61	0.80	0.07	0.86	0.55			

2.3.1.3 *Precision*

Reproducibility precision was determined in conjunction with the accuracy experiment and relative standard deviation data for this parameter is presented in Table 2.3.4. Repeatability precision data is presented in Table 2.3.5.

Table 2.3.5 - Repeatability (Replicate Readings) for validation of the UV assay

Sample	Absorbance	Calculated concentration (µg/mL)
20 µg/mL	1.0835	20.00
20 µg/mL	1.0837	20.01
20 µg/mL	1.0839	20.01
20 µg/mL	1.0835	20.00
20 µg/mL	1.0833	20.00

2.3.1.4 Ruggedness

Data pertaining to the ruggedness of the UV spectrophotometric assay is presented in Tables 2.3.6 through 2.3.9.

Table 2.3.6 - Ruggedness data for validation of the UV assay: 1-up CIDR®1380 Cattle insert Batch 7246227 - Day 1

Sample (1-up CIDR®1380 Cattle insert)	Skin Weight (g)	Absorbance 1	Absorbance 2	Progesterone (g)	Progesterone (% w/w)
1	13.4218	0.7792	0.7683	1.32	9.81
2	13.2927	0.7753	0.7861	1.33	9.99
3	13.4532	0.8013	0.8023	1.36	10.14
4	13.3506	0.7947	0.7934	1.35	10.12
5	13.4464	0.7814	0.7794	1.33	9.87
Mean				1.34	9.98
SEM				0.01	0.07

Table 2.3.7 - Ruggedness data for validation of the UV assay: 1-up CIDR®1380 Cattle insert Batch 7246227 - Day 2

Sample (1-up CIDR®1380 Cattle insert)	Skin Weight (g)	Absorbance 1	Absorbance 2	Progesterone (g)	Progesterone (% w/w)
1	13.4392	0.8090	0.8108	1.35	10.01
2	13.3861	0.8298	0.8209	1.37	10.24
3	13.4228	0.7852	0.7815	1.30	9.70
4	13.3131	0.8195	0.8156	1.36	10.20
5	13.4316	0.8224	0.8260	1.37	10.19
Mean				1.35	10.07
SEM				0.01	0.10

Table 2.3.8 - Ruggedness data for validation of the UV assay: 1-up CIDR®1380 Cattle insert Batch 7246227 - Day 3

Sample (1-up CIDR®1380 Cattle insert)	Skin Weight (g)	Absorbance 1	Absorbance 2	Progesterone (g)	Progesterone (% w/w)
1	13.4662	0.8175	0.8196	1.32	9.82
2	13.4348	0.8245	0.8207	1.33	9.89
3	13.3475	0.7600	0.7546	1.22	9.16
4	13.4027	0.8023	0.8040	1.30	9.68
5	13.3921	0.8117	0.8128	1.31	9.80
Mean				1.30	9.67
SEM				0.02	0.13

Table 2.3.9 - Ruggedness data for validation of the UV assay: 1-up CIDR®1380 Cattle insert

Batch 7246227 - Day 3 analyst two

Sample (1-up CIDR®1380 Cattle insert)	Skin Weight (g)	Absorbance 1	Absorbance 2	Progesterone (g)	Progesterone (% w/w)
1	13.4662	0.8328	0.8320	1.34	9.92
2	13.4348	0.8375	0.8382	1.34	10.01
3	13.3475	0.7734	0.7798	1.25	9.34
4	13.4027	0.8169	0.8166	1.31	9.78
5	13.3921	0.8200	0.8241	1.32	9.85
Mean				1.31	9.78
SEM				0.02	0.12

2.3.1.5 Limit of Quantitation

Data acquired for the determination of the limit of quantitation is presented in Table 2.3.10.

Table 2.3.10 - Limit of quantitation data obtained in the validation of the UV assay

Sample	Absorbance	Calculated concentration (µg/mL)
blank	0.0154	0.15
blank	0.0153	0.15
blank	0.0152	0.15
blank	0.0154	0.15
blank	0.0153	0.15
1 µg/mL	0.0837	1.42
0.1 µg/mL	0.0377	0.57
0.01 µg/mL	0.0258	0.34

2.3.1.6 Specificity

Data pertaining to the specificity of the UV spectrophotometric assay with respect to placebo components of CIDR® inserts is presented in Table 2.3.11.

Table 2.3.11 - Specificity data obtained to validate the UV assay

Sample	Absorbance	Calculated concentration (µg/mL)
20 µg/mL in specially denatured alcohol	1.0953	20.22
20 µg/mL in silicone extract (normal dilution)	1.0919	20.16
Silicone extract (normal dilution)	0.0004	-0.13

2.3.1.7 Soxhlet extraction Validation

The efficiency of the Soxhlet extraction method is 99 ± 1%, as determined from the data obtained in the ruggedness experiment where replicate CIDR® Cattle inserts were extracted on four different occasions by two analysts (see Section 2.3.1.4).

2.3.1.8 Invitro Drug Release Test Sample Reconstitution Validation

Recovery data determined to validate the reconstitution of invitro drug release test samples is shown in Table 2.3.12.

Table 2.3.12 - Absorbance and calculated progesterone recovery data for low and high level spikes analysed following reconstitution (N=5)

Spike Level (mg)	Absorbance (Mean ± SEM)	Progesterone Recovered (g) (Mean ± SEM)
0.22	0.216 ± 0.001	0.216 ± 0.001
1.10	1.06 ± 0.01	1.09 ± 0.01

2.3.2 Invitro Drug Release Test Validation

2.3.2.1 Reproducibility and Ruggedness

Release rate data acquired for 1-up CIDR®1380 Cattle inserts from three manufacturing batches are presented in Table 2.3.13.

Table 2.3.13 - Intra-batch and inter-batch release rates for 1-up CIDR®1380 Cattle inserts

Batch #	N	Release Rate ($\mu g/cm^2/hr^{1/2}$)		Intercept ($\mu g/cm^2$)	
		Mean	SEM	Mean	SEM
7317	15	1426	62	955	143
7313	5	1142	34	1337	125
7307	5	1455	74	303	152

2.3.2.2 *Robustness*

Invitro drug release rate data for 1-up CIDR®1380 Cattle inserts determined with variations to the standard method are presented in Table 2.3.14.

Table 2.3.14 - Effect of system dependent variables upon release rates for 1-up CIDR®1380 Cattle insert (N=3)

Parameter*	Release Rate ($\mu g/cm^2/hr^{1/2}$) Mean ± SEM
Rotation speed 50 rpm	1239 ± 13
Rotation speed 100 rpm	1329 ± 23
Rotation speed 150 rpm	1361 ± 15
Release media 40% ethanol	1192 ± 53
Release media 50% ethanol	1276 ± 94
Release media 60% ethanol	1361 ± 15
Release media 80% ethanol	1695 ± 97
Release media ionic strength 0.00	1361 ± 15
Release media ionic strength 0.15	1426 ± 21
Release media ionic strength 0.30	1474 ± 86
Release media ionic strength 0.50	1256 ± 84
Release media pH 4	1343 ± 102
Release media pH 6	1326 ± 92
Release media pH 8	1391 ± 21

*Only parameter listed was varied from the standard conditions. (N.B. Standard conditions are rotation speed = 150 rpm, 62.5% ethanol, ionic strength = 0, pH = 5-6)

Invitro drug release rate data for 1-up CIDR®1380 Cattle inserts with different initial drug loads are presented in Table 2.3.15.

Table 2.3.15 - Effect of initial drug load upon release rates for 1-up CIDR®1380 Cattle insert under standard release assessment test conditions (N=2)

Initial Drug Load (%w/w)	Release Rate ($\mu g/cm^2/hr^{1/2}$) (Mean ± SEM)
2.5	321 ± 8
5.0	826 ± 8
7.5	1193 ± 61
15.0	1838 ± 30
20.0	2508 ± 108
25.0	2855 ± 79

These results are also shown in Figure 2.3.1.

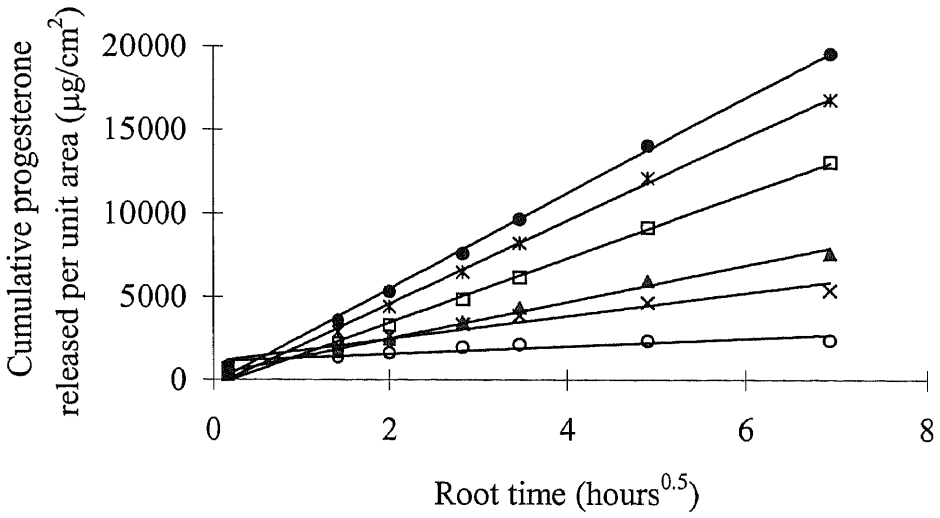


Figure 2.3.1 - Effect of initial drug load as seen by the cumulative progesterone released per unit area per square root of time (O = 2.5% load; × = 5% load; ▲ = 7.5% load; = 10% load; ✻ = 15% load; ● = 20% load)

2.3.3 Invitro Characterisation (CIDR®1900 Cattle insert)

2.3.3.1 Location of Drug in CIDR® Cattle Inserts

The levels of progesterone determined in both the silicone skin and in the nylon spine of CIDR®1900 Cattle inserts are presented in Table 2.3.16.

Table 2.3.16 - Levels of progesterone determined in both the silicone skin and nylon spine of CIDR® Cattle insert

Location	Progesterone content (g) (Mean ± SEM)
Silicone Skin (N=30)	1.90 ± 0.01
Nylon Spine (N=2)	0.011 ± 0.002

2.3.3.2 Skin Weight and Initial Load

Determinations of the skin weight and drug load of CIDR®1900 Cattle insert are presented in Table 2.3.17.

Table 2.3.17 - Skin Weight and Total Initial Drug Load of a CIDR®1900 Cattle insert (N=21)

Parameter	Result Mean ± SEM
Skin Weight (g)	19.97 ± 0.03
Drug Weight (g)	1.90 ± 0.01
Drug Load (%w/w)	9.51 ± 0.03

2.3.3.3 Surface area

The surface area of CIDR®1900 Cattle insert (N=3) was determined to be 120 ± 1.6 cm^2.

2.3.3.4 Skin thickness

The skin thickness of CIDR®1900 Cattle insert (N=5) ranged from 1 to 5 mm.

2.3.3.5 Invitro Drug Release

The invitro drug release rate for CIDR®1900 Cattle inserts (N=17) was 1302 ± 33 $\mu g/cm^2/hr^{1/2}$. A summary of the data is presented in Table 2.3.18.

Table 2.3.18 - Cumulative amount of progesterone released per unit area at given time and the resulting release rate for CIDR®1900 Cattle inserts

	Cumulative amount of progesterone released per unit area at given time ($\mu g/cm^2$)									Rate ($\mu g/cm^2/hr^{1/2}$)*
Replicate	0.03	2	4	8	12	24	48	72	96	
1	392	1774	2556	3761	4260	6453	9035	10619	11953	1294
2	368	1805	2438	3442	4389	5934	8171	9607	10837	1165
3	622	2000	2749	3921	4915	6740	9215	10910	12335	1298
4	456	1847	2529	3576	4614	6248	8705	10363	11681	1238
5	557	1854	2665	3868	4724	6864	9381	10999	12494	1342
6	642	2103	2864	4043	5028	7041	9446	10993	12077	1333
7	1187	1893	2613	3717	4621	6716	9584	11255	12647	1298
8	1012	1647	2331	3360	4217	6183	8886	10464	11755	1219
9	1108	1775	2473	3526	4393	6360	8846	10608	11837	1203
10	1072	1719	2401	3418	4332	6134	8550	10308	11687	1162
11	1118	1797	2479	3579	4518	6518	9219	10917	12414	1258
12	1100	1693	2402	3432	4313	6128	8598	10435	11755	1167
13	992	1994	2719	3835	4830	6867	9337	11065	12615	1287
14	964	2570	3471	4709	5660	7636	10256	11932	13045	1393
15	974	1825	2624	3736	4667	6697	9189	11155	12182	1272
16	536	2321	3301	4794	5845	7693	10557	12478	13353	1495
17	814	2530	3414	5186	6465	8779	12015	13583	15218	1702
Mean										1302
SEM										33

*Calculated based on data up to 48 hours

2.3.4 Invitro Characterisation (1-up CIDR®1380 Cattle insert)

2.3.4.1 Skin Weight and Initial Load

Skin weight and initial drug load data for the 1-up CIDR®1380 Cattle insert is presented in Table 2.3.19.

Table 2.3.19 - Skin Weight and Drug Load of a 1-up CIDR®1380 Cattle insert (N=15)

Parameter	Result (Mean ± SEM)
Skin Weight (g)	13.87 ± 0.01
Drug Weight (g)	1.38 ± 0.004
Drug Load (%w/w)	9.93 ± 0.03

2.3.4.2 Surface Area

The surface area of the 1-up CIDR®1380 Cattle insert (N=3) were determined to be 123 ± 1.4 cm^2.

2.3.4.3 Skin Thickness

The skin thickness of the 1-up CIDR®1380 Cattle insert was determined (N=5) to be approximately 1 mm over the entire insert except for an area on the wing tips which was deliberately made thicker for animal comfort. These areas were approximately 1.6 mm thick.

2.3.4.4 Invitro Drug Release

The invitro drug release rate for the 1-up CIDR®1380 Cattle insert was determined (N=30) to be 1320 ± 22 $\mu g/cm^2/hr^{1/2}$. The data is summarised in Table 2.3.20.

Table 2.3.20 - Cumulative amount of progesterone released per unit area at given time and the resulting release rate for 1-up CIDR®1380 Cattle inserts

	Cumulative amount of progesterone released per unit area at given time (μg/cm²)									Rate (μg/cm² /hr$^{1/2}$)*
Replicate	0.03	2	4	8	12	24	48	72	96	
1	1189	3796	4902	6077	7196	8686	10351	11371	11695	1332
2	430	1767	2514	3731	4713	6673	8981	10647	11100	1304
3	599	2293	3054	4337	5245	7211	9498	11121	11516	1337
4	660	2429	3268	4603	5730	7704	9810	10817	11169	1387
5	787	2541	3401	4698	5799	7642	9740	10988	11809	1351
6	345	1883	2858	4203	5253	7300	9698	12035	11871	1422
7	804	2979	3850	5463	6393	8379	10336	11168	11419	1427
8	515	1889	2657	3816	4754	6893	9103	11913	11093	1312
9	1112	3446	3999	4926	6176	7476	9443	11242	10962	1205
10	1428	4115	5012	6064	6989	8673	10039	11848	12045	1246
11	577	2161	3101	4242	5191	7058	9240	9315	10127	1303
12	692	2202	2989	4140	5059	6748	8888	11142	11669	1232
13	1048	3153	3857	4979	5905	7553	9418	10995	11487	1230
14	1254	3187	3853	5051	5757	7518	9340	10733	11423	1194
15	1323	3824	4614	5570	6359	7798	9595	10788	11179	1176
16	1267	3026	4840	6018	6672	9062	11187	10597	11275	1489
17	802	2128	3114	3955	4877	6893	8931	10181	10667	1234
18	429	1631	2315	3270	4081	5882	7767	11721	13307	1118
19	684	1956	2716	3764	4631	6526	8515	11188	11246	1194
20	507	1783	3093	4218	4922	6401	8170	11697	12207	1156
21	1567	2830	3732	5073	6130	8100	10337	10907	11443	1351
22	1474	2737	3504	4822	5849	7599	9654	10839	11009	1258
23	1779	3298	4029	5328	6425	8010	10230	7772	8370	1277
24	1856	3191	4058	5418	6724	8330	10086	10766	11120	1278
25	1132	2290	3067	4348	5402	7584	9896	9242	10019	1359
26	866	2995	4049	5663	6904	8749	10798	11537	11752	1497
27	854	2454	3218	4733	5575	7277	9603	9331	10128	1315
28	847	1626	4070	5750	6870	8694	11054	10956	11458	1600
29	352	1303	2778	4340	5334	6918	10110	11789	11795	1485
30	640	1887	2855	4480	5631	7964	10537	11868	12177	1539
Mean										1320
SEM										22

*Calculated based on data up to 48 hours

2.3.5 Invitro Characterisation (6-up CIDR®1380 Cattle insert)

2.3.5.1 Skin Weight and Initial Load

Skin weights and initial loads for three individual manufacturing batches of 6-up CIDR®1380 Cattle insert are presented in Table 2.3.21, by batch and by cavity position.

Table 2.3.21 - Skin weights and drug loads for three production batches of 6-up CIDR®1380 Cattle insert with results presented by batch and by tool cavity position

Parameter	Skin Weight (g) (Mean ± SEM)	Drug Load (g) (Mean ± SEM)	Drug Load (%w/w) (Mean ± SEM)
Batch 8065 (N=18)	13.92 ± 0.03	1.32 ± 0.003	9.49 ± 0.02
Batch 8067 (N=18)	13.95 ± 0.02	1.34 ± 0.002	9.58 ± 0.01
Batch 8069 (N=18)	13.87 ± 0.02	1.32 ± 0.002	9.54 ± 0.01
Cavity 1 (N=9)	13.87 ± 0.03	1.33 ± 0.006	9.56 ± 0.03
Cavity 2 (N=9)	13.91 ± 0.02	1.33 ± 0.003	9.56 ± 0.02
Cavity 3 (N=9)	13.83 ± 0.02	1.32 ± 0.003	9.55 ± 0.02
Cavity 4 (N=9)	13.96 ± 0.02	1.33 ± 0.002	9.54 ± 0.02
Cavity 5 (N=9)	14.02 ± 0.02	1.33 ± 0.004	9.49 ± 0.03
Cavity 6 (N=9)	13.89 ± 0.04	1.32 ± 0.004	9.50 ± 0.02

2.3.5.2 Surface Area

The surface area of the 6-up CIDR®1380 Cattle insert were determined to be 123 ± 1 cm^2 (N = 30). Mean results for the three batches are presented in Table 2.3.22, by batch and by tool cavity position.

Table 2.3.22 - Surface areas (cm^2) for three production batches of 6-up CIDR®1380 Cattle insert with results presented by batch and by tool cavity position

Parameter	Surface Area (cm^2) (Mean ± SEM)
Batch 8065 (N=6)	120.7 ± 0.7
Batch 8067 (N=6)	125.8 ± 1.0
Batch 8069 (N=18)	123.6 ± 0.7
Cavity 1 (N=5)	126.6 ± 1.5
Cavity 2 (N=5)	122.2 ± 1.3
Cavity 3 (N=5)	121.8 ± 1.6
Cavity 4 (N=5)	122.5 ± 0.9
Cavity 5 (N=5)	122.7 ± 0.3
Cavity 6 (N=5)	124.9 ± 1.4

2.3.5.3 Skin Thickness

The skin thickness of 6-up CIDR®1380 Cattle insert (N=30) was determined to be approximately 1 mm over the entire insert except for an area on the wing tips which was deliberately made thicker for animal comfort. These areas were approximately 1.6 mm thick.

2.3.5.4 *Invitro Drug Release*

The invitro release rate for the 6-up CIDR®1380 Cattle insert was determined (N=30) to be 1236 ± 9 $\mu g/cm^2/hr^{1/2}$. The data is shown in Table 2.3.23 and summarised on an individual cavity position basis in Table 2.3.24.

Table 2.3.23 - Cumulative amount of progesterone released per unit area at given time and the resulting release rate for 6-up CIDR®1380 Cattle inserts

Cavity / Replicate	Cumulative amount of progesterone released per unit area at given time ($\mu g/cm^2$)								Rate ($\mu g/cm^2/hr^{1/2}$)*
	0.03	2	4	8	12	24	48	72	
1/1	371	1646	2325	3265	4026	5769	7811	9609	1121
2/1	323	1733	2427	3482	4320	6229	8332	9462	1210
3/1	311	1712	2401	3480	4333	6176	8380	9628	1216
4/1	293	1639	2313	3370	4173	5976	8081	9164	1175
5/1	270	1650	2358	3477	4267	6092	8303	9315	1210
6/1	292	1566	2240	3205	3983	5683	7885	9111	1140
1/2	424	1822	2584	3762	4686	6726	8831	9797	1283
2/2	430	1907	2742	3834	4735	6761	8926	9951	1286
3/2	418	2016	2914	4095	5094	7173	9322	10296	1350
4/2	369	1775	2561	3706	4677	6742	8805	9648	1290
5/2	358	1843	2726	3844	4822	6922	9028	9952	1320
6/2	389	1807	2598	3670	4582	6503	8702	9765	1257
1/3	331	1711	2386	3465	4296	6164	8269	9450	1200
2/3	304	1794	2508	3634	4508	6409	8650	9789	1257
3/3	311	1753	2490	3635	4540	6465	8768	9984	1277
4/3	302	1745	2456	3589	4447	6270	8392	9414	1221
5/3	311	1746	2448	3587	4509	6388	8573	9668	1250
6/3	343	1644	2287	3316	4147	5915	8184	9440	1181
1/4	441	1827	2559	3556	4338	6169	8350	9534	1187
2/4	427	1902	2630	3737	4575	6480	8596	9771	1232
3/4	426	1876	2608	3695	4559	6525	8824	10063	1265
4/4	392	1800	2531	3621	4459	6315	8475	9529	1219
5/4	382	1804	2520	3565	4378	6282	8541	9789	1226
6/4	369	1801	2517	3572	4406	6308	8535	9647	1229
1/5	373	1549	2275	3364	4161	5980	8316	9540	1203
2/5	330	1735	2450	3519	4362	6356	8656	⇧	1256
3/5	310	1780	2552	3696	4577	6512	8864	10129	1288
4/5	298	1686	2430	3529	4383	6287	8460	10059	1235
5/5	287	1679	2432	3526	4418	6336	8681	10006	1267
6/5	339	1668	2402	3430	4258	6095	8426	9645	1217
Mean									1236
SEM									9

*Calculated based on data up to 48 hours
⇧Lost sample

Table 2.3.24 - Release rates for 6-up CIDR®1380 Cattle inserts from each of the individual cavity positions (N=5)

Cavity	Rate ($\mu g/cm^2/hr^{1/2}$) (Mean ± SEM)
1	1199 ± 26
2	1248 ± 13
3	1279 ± 22
4	1228 ± 18
5	1255 ± 19
6	1205 ± 20

2.3.6 Comparative Invivo Characterisation (CIDR®1900 Cattle insert versus 1-up CIDR®1380 Cattle insert)

2.3.6.1 Plasma Profiles

Plasma progesterone data obtained from the 7 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to CIDR®1900 Cattle insert invivo trial are presented in Tables 2.3.25 through 2.3.27. Data which comprises the absorption profile for the two CIDR® formulations is presented in Table 2.3.25. Data which comprises the clearance profile following removal of the two CIDR® formulations is presented in Table 2.3.26 and the daily data acquired for the two CIDR® formulations over the course of the seven day insertion is presented in Table 2.3.27.

Table 2.3.25 - Absorption data for 7 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to CIDR®1900 Cattle insert

Formulation	0	10 mins	20 mins	30 mins	45 mins	1 h	2 h	4 h	6 h	8 h
CIDR®1900 Cattle insert	0.04	1.36	1.98	4.00	4.01	5.09	4.48	4.97	5.58	6.08
	0.10	4.62	6.08	5.87	6.42	7.45	5.77	4.72	5.01	5.33
	0.18	2.57	2.91	2.43	2.26	2.11	1.35	1.19	1.40	1.85
	0.03	2.99	5.06	6.44	6.82	6.88	6.15	5.64	5.63	5.88
	0.00	4.94	6.30	6.46	5.91	6.16	6.81	4.85	5.71	5.62
	0.03	3.15	3.70	4.31	4.66	4.78	5.23	4.25	7.25	4.65
	0.10	4.7	4.36	⇧	4.99	4.50	5.27	4.29	4.87	4.53
	0.00	4.56	2.79	4.90	4.84	5.08	4.94	4.82	4.21	4.85
1-up CIDR®1380 Cattle insert	0.00	4.38	5.31	5.77	6.43	5.69	6.61	5.36	5.75	6.26
	0.17	1.66	3.35	3.72	3.65	4.16	4.54	4.28	4.84	4.53
	0.09	3.47	4.45	4.39	4.70	4.72	4.90	3.76	4.19	4.22
	0.05	2.36	3.59	3.74	4.38	4.99	4.31	3.66	4.00	3.82
	0.00	3.62	3.16	2.60	3.10	3.39	3.85	3.78	3.70	3.87
	0.05	4.02	4.13	4.41	4.27	4.41	3.86	3.43	4.18	3.83
	0.41	2.93	5.38	6.14	5.21	4.41	3.99	4.09	4.18	4.53
	0.00	3.39	4.63	4.73	5.77	5.50	4.06	3.36	4.33	4.36

⇧Lost sample

Table 2.3.26 - Clearance data for 7 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to CIDR®1900 Cattle insert

Time after removal	0	15 mins	30 mins	1 h	2 h	4 h	8 h	24 h
CIDR®1900 Cattle insert	3.21	2.37	1.73	0.99	0.63	0.39	0.17	0.21
	2.92	2.10	1.53	0.79	0.59	0.33	0.20	0.13
	3.28	2.51	1.66	0.80	0.60	0.26	0.23	0.08
	3.00	2.12	1.38	0.82	0.47	0.19	0.14	0.05
	2.93	1.85	1.35	0.78	0.59	0.41	0.37	0.22
	2.42	1.33	0.76	0.67	0.51	0.29	0.26	⇧
	2.25	1.39	0.86	0.77	0.36	0.27	0.11	0.09
	2.67	1.21	0.72	0.66	0.55	0.40	0.09	0.01
1-up CIDR®1380 Cattle insert	3.54	2.40	2.00	1.22	0.88	0.41	0.24	0.28
	2.67	1.91	1.47	0.91	0.67	0.35	0.22	0.28
	2.57	1.78	1.13	0.69	0.52	0.30	0.17	0.11
	2.50	1.74	1.00	0.53	0.27	1.82	0.16	0.07
	2.57	1.52	1.16	0.77	0.40	0.34	0.22	0.16
	1.98	1.04	0.55	0.52	0.31	0.37	0.27	0.10
	3.78	1.72	0.82	0.57	0.63	0.24	0.11	0.00
	2.70	1.54	0.94	0.66	0.34	0.20	0.16	0.00

⇧Lost sample

Table 2.3.27 - Daily data for 7 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to CIDR®1900 Cattle insert

Time after insertion (days)	1 day	2	2.25	3	4	5	6	7
CIDR®1900 Cattle insert	5.04	3.49	3.94	2.82	2.84	3.48	2.77	3.21
	5.33	4.23	3.63	2.71	2.80	2.51	2.53	2.92
	4.98	3.74	3.78	2.66	2.69	3.14	3.35	3.28
	6.36	4.20	3.57	2.90	2.77	3.13	3.33	3.00
	6.03	6.10	3.82	3.98	4.00	3.52	3.49	2.93
	5.63	8.56	3.61	3.68	3.31	2.92	3.31	2.42
	3.42	4.34	3.42	3.59	3.60	3.03	3.10	2.25
	3.44	4.18	3.40	3.87	3.09	3.22	3.27	2.67
1-up CIDR®1380 Cattle insert	5.85	4.67	4.39	3.51	3.50	3.45	3.38	3.54
	4.85	4.03	3.59	2.76	2.82	2.51	2.24	2.67
	4.36	3.44	2.65	2.47	2.70	2.75	2.99	2.57
	3.44	3.44	2.27	3.22	3.56	3.43	2.84	2.50
	4.46	4.05	3.86	3.81	3.69	3.52	2.82	2.57
	4.46	3.43	4.03	3.19	2.59	2.78	1.97	1.98
	4.87	4.12	3.06	3.18	3.39	2.69	2.55	3.78
	4.37	3.66	2.90	3.21	2.81	2.76	3.25	2.70

Table 2.3.28 contains data obtained in the 10 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to CIDR®1900 Cattle insert.

Table 2.3.28 - Data for 10 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to CIDR®1900 Cattle insert

Time (days)	0	0.25	1	2	3	4	5	6	7	8	9	10	10.3
1900	0.04	6.21	4.81	4.23	4.69	3.77	3.27	3.53	3.02	2.51	2.76	2.62	0.31
	0.06	5.77	4.13	2.77	3.92	2.51	3.30	2.80	2.69	2.25	2.34	2.41	0.20
	0.05	5.92	3.93	3.05	4.84	2.92	3.65	3.45	3.32	2.10	2.10	2.21	0.16
	0.04	4.69	4.16	2.70	3.20	2.77	3.50	3.85	3.69	1.93	1.81	1.76	0.06
	0.00	1.52	2.02	2.37	3.56	1.89	2.66	2.88	2.19	1.84	1.55	1.92	0.22
	0.00	6.03	4.42	3.93	2.89	2.30	2.57	2.87	2.16	1.91	1.97	1.81	0.27
	0.00	4.54	4.19	2.46	2.47	2.05	2.00	1.98	1.77	1.37	1.42	1.74	0.17
	0.00	4.10	3.67	3.59	3.32	2.62	2.88	2.95	2.62	2.41	2.57	2.70	0.27
	0.92	6.71	5.38	4.49	2.80	4.50	5.01	2.53	3.27	4.26	2.73	2.33	0.25
	0.00	5.07	2.99	3.00	1.91	2.22	4.68	1.47	1.51	1.74	1.65	1.34	0.24
1380	0.09	5.26	3.69	2.85	3.81	3.47	3.49	3.00	3.62	2.33	2.41	2.12	0.30
	0.09	4.41	4.17	3.05	3.29	2.21	2.87	2.38	2.68	1.95	2.11	1.71	0.21
	0.00	3.99	3.90	4.11	6.89	2.64	3.42	2.99	2.60	1.95	1.93	1.94	0.56
	0.82	6.20	6.50	4.41	7.25	5.11	5.80	3.77	6.95	5.00	3.78	3.56	0.25
	0.03	3.81	3.75	3.23	3.10	2.88	2.21	2.59	4.11	2.24	2.05	2.03	0.04
	0.01	6.20	4.91	3.48	4.35	3.92	4.04	3.50	3.40	1.82	2.14	2.11	0.18
	0.04	5.04	5.65	3.82	3.29	3.12	3.16	2.48	2.19	2.75	1.76	2.55	0.23
	0.00	5.45	4.41	3.81	3.33	3.27	2.84	2.52	2.82	1.75	1.91	3.48	0.26
	0.00	6.16	4.69	4.02	7.81	2.40	1.93	3.31	2.63	2.06	1.83	2.13	0.38
	0.04	4.02	3.99	2.92	3.05	2.90	2.81	1.97	2.33	1.62	1.68	2.25	0.17

Tables 2.3.29 and 2.3.30 contain data obtained in the 20 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to CIDR®1900 Cattle insert.

Table 2.3.29 - Data for 20 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to CIDR®1900 Cattle insert (days 0-10)

Time (days)	0	0.25	1	2	3	4	5	6	7	8	9	10
1900	0.03	5.20	4.39	3.17	3.44	2.41	2.74	2.70	2.67	2.08	1.85	1.88
	0.00	7.26	3.96	4.67	4.22	2.68	3.65	3.86	3.59	2.55	2.52	3.06
	0.07	2.89	3.16	3.37	4.65	2.96	3.47	3.28	2.86	1.90	1.88	2.10
	0.00	13.1	4.47	4.25	4.39	3.65	4.08	3.01	3.08	2.70	2.41	3.08
	0.00	6.33	3.85	4.32	3.40	3.23	3.22	3.87	2.68	2.47	1.99	2.21
	0.00	4.65	4.48	3.94	2.57	2.62	3.22	2.46	2.52	1.73	1.82	2.26
	0.04	3.98	3.33	2.69	2.55	2.58	1.94	2.73	2.37	1.61	2.00	2.29
	0.02	3.54	2.32	2.07	2.84	2.17	2.14	2.75	2.01	2.16	1.59	2.70
	0.03	4.46	3.64	3.52	2.82	2.46	2.53	2.42	2.17	1.65	1.80	1.93
	0.00	5.51	4.72	4.03	2.85	3.13	3.50	2.57	2.94	2.45	1.71	3.40
1380	0.00	4.90	4.31	2.80	3.55	2.84	2.84	3.49	3.37	1.68	2.05	1.67
	0.03	5.68	4.82	3.59	4.00	2.75	2.32	3.03	2.54	1.57	1.36	1.57
	0.02	3.79	3.49	2.81	3.55	3.54	2.48	2.62	2.89	1.79	1.61	1.43
	0.05	5.19	3.15	4.45	4.16	2.02	2.50	2.37	2.03	1.60	1.72	2.57
	0.15	4.80	4.57	3.72	3.92	2.91	2.89	3.63	2.36	1.84	2.67	1.81
	0.02	4.31	3.83	4.52	3.23	2.78	4.18	2.64	2.12	2.41	1.57	2.37
	0.02	5.64	4.85	4.03	3.39	3.06	2.70	2.50	2.87	2.10	2.99	3.58
	0.12	3.79	4.47	3.65	4.06	3.17	2.52	1.69	2.30	1.44	1.81	1.13
	0.00	7.66	4.36	4.80	4.10	4.49	4.13	2.81	2.96	3.13	2.77	3.27
	0.02	4.17	3.73	2.35	1.84	3.12	2.69	2.15	2.48	2.70	2.20	1.91

Table 2.3.30 - Data for 20 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to CIDR®1900 Cattle insert (days 11 - 20)

Time (days)	11	12	13	14	15	16	17	18	19	20	20.25
1900	1.86	2.10	2.44	1.78	1.67	1.72	1.37	1.07	1.06	1.17	0.12
	2.95	3.19	3.05	2.88	2.46	2.46	1.68	2.16	1.95	1.70	0.11
	1.73	1.81	1.97	1.23	1.54	1.64	1.64	1.80	1.24	1.06	0.10
	2.60	2.26	4.18	2.15	1.73	2.72	1.80	1.67	1.99	1.25	0.13
	1.75	4.67	3.57	1.22	1.42	2.30	2.15	3.01	2.02	1.25	0.13
	2.26	1.56	1.32	1.21	0.98	1.66	1.57	1.65	1.36	1.21	0.13
	2.68	1.34	1.50	1.09	1.39	1.17	1.60	✞	✞	✞	✞
	2.69	2.14	1.08	1.40	1.62	1.32	2.04	2.80	2.11	1.34	0.28
	4.29	1.42	1.58	1.37	1.16	1.45	1.90	1.96	1.46	1.55	0.10
	2.84	1.68	1.37	1.30	1.49	1.23	2.13	1.57	1.47	1.86	0.13
1380	2.14	2.96	2.57	2.50	1.60	1.21	0.92	0.83	0.73	0.65	0.38
	1.42	1.17	1.82	0.97	0.77	1.29	0.87	0.65	0.82	0.50	0.37
	2.01	1.67	1.98	1.21	1.27	1.39	0.78	0.86	1.20	0.58	0.12
	3.97	6.03	4.10	2.88	0.96	1.71	1.30	1.17	1.97	0.62	0.09
	1.57	2.14	1.94	1.62	1.57	1.42	1.18	1.92	0.87	0.77	0.09
	2.21	1.26	0.96	1.27	1.12	0.75	1.08	1.40	0.98	0.66	0.10
	3.21	1.68	1.54	1.24	1.70	2.07	1.56	✞	✞	✞	✞
	1.71	1.67	1.03	0.89	1.17	0.72	1.05	0.92	0.73	0.64	0.08
	3.85	1.62	1.85	1.36	1.48	1.65	2.01	1.18	1.02	0.79	0.14
	3.34	1.47	2.06	1.61	1.28	1.91	1.40	1.47	1.07	1.29	0.12

✞Lost sample

2.3.6.2 Pharmacokinetic analysis of Invivo Plasma Profiles of CIDR® Cattle Inserts

Areas under the Curve calculated for the invivo plasma profiles of CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert in the 7 day trial are presented in Table 2.3.31. The corresponding AUC data for the 10 day trial and the 20 day trial are presented in Tables 2.3.32 and 2.3.33, respectively.

Table 2.3.31 - Area under the curve data for invivo plasma profiles of CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert in a 7 day insertion (N=8)

Formulation	Area Under Curve (AUC)		
	0-4 hours (Average ± SEM)	Day 7-8 (Average ± SEM)	Day 0-8 (Average ± SEM)
CIDR®1900 Cattle insert	0.77 ± 0.08	0.26 ± 0.03	26.17 ± 0.96
CIDR®1380 Cattle insert	0.70 ± 0.04	0.27 ± 0.04	24.10 ± 0.90

Table 2.3.32 - Area under the curve data for invivo plasma profiles of CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert in a 10 day insertion (N=10)

Formulation	Area Under Curve (AUC) (Average ± SEM)
CIDR®1900 Cattle insert	29.85 ± 1.78
CIDR®1380 Cattle insert	33.39 ± 2.39

Table 2.3.33 - Area under the curve data for invivo plasma profiles of CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert in a 20 day insertion (N=10)

Formulation	Area Under Curve (AUC)	
	Day 0-15 (Average ± SEM)	Day 15-20 (Average ± SEM)
CIDR®1900 Cattle insert	40.56 ± 2.30	8.35 ± 0.70
CIDR®1380 Cattle insert	39.79 ± 1.66	5.81 ± 0.38

2.3.6.3 Residual Progesterone Load and Amount Released (CIDR®1900 Cattle insert versus 1-up CIDR®1380 Cattle insert)

Residual progesterone load and amount released data for CIDR®1900 Cattle insert used in the 7 day, 10 day and 20 day trials and CIDR®1380 Cattle insert used in the 7 day, 10 day and 20 day trials are presented in Tables 2.3.34 and 2.3.35 respectively.

Table 2.3.34 - Residual progesterone load in spent CIDR®1900 Cattle insert and the calculated amount released in the 7, 10 and 20 day trials

Trial Length (days)	Residual (g) (Mean ± SEM)	Amount Released (g) (Mean ± SEM)
7 (N=8)	1.38 ± 0.01	0.61 ± 0.01
10 (N=10)	1.19 ± 0.03	0.78 ± 0.03
20 (N=10)	0.79 ± 0.04	1.20 ± 0.04

Table 2.3.35 - Residual progesterone load in spent CIDR®1380 Cattle insert and the calculated amount released in the 7, 10 and 20 day trials

Trial Length (days)	Residual (g) (Mean ± SEM)	Amount Released (g) (Mean ± SEM)
7 (N=8)	0.72 ± 0.02	0.62 ± 0.02
10 (N=10)	0.48 ± 0.01	0.87 ± 0.01
20 (N=10)	0.20 ± 0.01	1.16 ± 0.01

2.3.7 Comparative Invivo Characterisation (1-up CIDR®1380 Cattle insert versus 6-up CIDR®1380 Cattle insert)

2.3.7.1 Plasma Profiles

Plasma progesterone data obtained from the 10 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to 6-up CIDR®1380 Cattle insert are presented in Table 2.3.36.

Table 2.3.36 - Absorption data for 10 day bioequivalency trial comparing 1-up CIDR®1380 Cattle insert to 6-up CIDR®1380 Cattle insert

		Plasma progesterone level (ng/mL) over days 0 to 10										
CIDR	Cavity	0	1	2	3	4	5	6	7	8	9	10
8927	1	0.23	3.89	3.15	2.39	3.16	2.55	2.02	2.63	2.03	2.66	2.40
8931	1	0.14	3.08	3.42	4.29	2.61	2.35	1.98	2.71	2.72	2.31	2.56
8934	1	0.24	2.74	2.52	3.94	2.30	2.33	1.78	2.26	1.73	1.53	1.52
8927	2	0.51	4.89	5.09	4.61	4.49	3.48	2.31	3.06	3.27	2.22	2.44
8931	2	0.20	3.14	2.78	4.16	3.13	2.43	2.37	2.75	2.20	2.52	2.80
8934	2	0.13	3.52	3.33	2.65	3.15	3.71	2.61	2.97	2.66	2.07	2.75
8927	3	0.17	2.65	2.03	1.70	1.89	1.67	1.46	1.84	1.72	1.68	2.59
8934	3	0.14	3.08	4.00	4.37	3.15	3.16	3.06	2.86	2.09	3.16	3.48
8927	4	0.27	3.16	3.96	2.47	5.00	1.98	1.44	1.95	1.92	1.71	2.18
8931	4	0.15	4.85	3.66	4.49	3.54	2.88	2.51	3.43	2.42	2.55	3.10
8934	4	0.25	3.24	2.41	3.66	1.86	2.72	1.65	1.62	2.05	1.85	2.09
8927	5	0.18	3.29	2.09	2.55	2.67	2.79	1.68	2.73	1.98	2.72	3.04
8934	5	0.16	4.06	5.18	⇧	3.85	2.87	2.44	2.77	2.30	2.45	2.58
8931	6	0.48	3.17	3.70	4.47	3.54	2.55	2.08	2.62	1.82	2.00	1.80
8934	6	0.45	2.68	2.57	2.90	3.17	2.17	2.11	1.12	1.08	1.98	1.67
1-up	-	0.68	2.37	2.72	2.87	2.35	2.37	1.27	1.48	1.42	2.52	2.29
1-up	-	0.37	2.65	3.06	2.75	2.92	3.00	2.69	2.00	1.94	2.01	2.15

⇧Lost sample

2.3.7.2 *Pharmacokinetic analysis of Invivo Plasma Profiles of CIDR® Cattle Inserts*

Areas under the Curve calculated for the invivo plasma profiles of 1-up CIDR®1380 Cattle insert and 6-up CIDR®1380 Cattle insert in the 10 day trial comparing the 1-up CIDR®1380 Cattle insert with the 6-up CIDR®1380 Cattle insert are presented in Table 2.3.37.

Table 2.3.37 - Area under the curve data for invivo plasma profiles of 1-up CIDR®1380 Cattle insert (N=4) and 6-up CIDR®1380 Cattle insert overall (N=16), by batch { batch 8927 (N=6), batch 8931 (N=4), batch 8934 (N=6)} and by cavity {cavity 1 (N=3), cavity 2 (N=3), cavity 3 (N=2), cavity 4 (N=3), cavity 5 (N=2), cavity 6 (N=3)} in a 10 day insertion

Formulation	Area Under Curve (AUC) Day 0-10 (Average ± SEM)
1-up CIDR®1380 Cattle insert	26.50 ± 2.45
6-up CIDR®1380 Cattle insert	26.56 ± 1.15
6-up CIDR®1380 Cattle insert Batch 8927	26.82 ± 2.56
6-up CIDR®1380 Cattle insert Batch 8931	28.20 ± 1.25
6-up CIDR®1380 Cattle insert Batch 8934	25.20 ± 1.65
6-up CIDR®1380 Cavity 1	24.87 ± 1.47
6-up CIDR®1380 Cavity 2	29.98 ± 2.47
6-up CIDR®1380 Cavity 3	24.38 ± 6.37
6-up CIDR®1380 Cavity 4	26.33 ± 2.91
6-up CIDR®1380 Cavity 5	25.69 ± 1.59
6-up CIDR®1380 Cavity 6	27.07 ± 3.60

2.3.7.3 *Residuals*

Residual progesterone load and amount released data for 1-up CIDR®1380 Cattle insert and 6-up CIDR®1380 Cattle insert used in the 10 day trial are presented in Table 2.3.37.

Table 2.3.38 - Residual and amount released data for 1-up CIDR®1380 Cattle insert (N=4) and 6-up CIDR®1380 Cattle insert batch 8927 (N=6), batch 8931 (N=4), and batch 8934 (N=6) in a 10 day insertion

CIDR® insert	Residual (g) (Mean ± SEM)	Amount Released (g) (Mean ± SEM)
1-up CIDR®1380	0.68 ± 0.03	0.66 ± 0.03
6-up CIDR®1380 batch 8927	0.72 ± 0.02	0.63 ± 0.02
6-up CIDR®1380 batch 8931	0.76 ± 0.02	0.58 ± 0.02
6-up CIDR®1380 batch 8934	0.68 ± 0.02	0.65 ± 0.02

2.4 Discussion

The aim of this chapter was to characterise the CIDR®1900 Cattle insert and its optimised counterpart, the CIDR®1380 Cattle insert, as it passed through the two stages of manufacturing scale-up (1-up CIDR®1380 Cattle insert and 6-up CIDR®1380 Cattle insert). Within this aim was the objective to experimentally determine evidence that the CIDR®1380 Cattle insert was bioequivalent to the original CIDR®1900 Cattle insert and released drug invitro at the same rate. A further objective of the characterisation was to document the physical differences between the CIDR®1900 Cattle insert and the CIDR®1380 Cattle insert.

2.4.1 Validation of Experimental Methods

2.4.1.1 Validation of the UV Spectrophotometric Assay for Progesterone in CIDR® Inserts

Guidelines posted on the world wide web by the United States Food and Drug Administration Centre for Veterinary Medicine[226] recommend that an assay can be considered validated if the method exhibits a linearity of R^2>0.999, intercept ≈ 0, an accuracy and specificity (% bias) of <4%, a precision (%RSD) of <2% and a ruggedness (%RSD) of <4%.

Using standards at 27, 67, 133, 200 and 267% of the target value, the developed method yielded linear regressions with R^2 values of >0.999 and acceptably low intercept values (Table 2.3.1). The regression line produced accurate (<2% deviation at low and <1% deviation at high level) determinations of the concentrations of validation standards at the extremes of the calibration range. These results validated the method to be linear within the range of 4 to 40 μg/mL (Table 2.3.3). The regression line also produced accurate (<2% deviation at 8 μg/mL and <1% deviation at 15 and 35 μg/mL) determinations of 8, 15 and 35 μg/mL standards, validating the accuracy of the method (Table 2.3.4). Results for 5 replicate analyses of the same standard using the developed method exhibited a 0.03% relative standard deviation from their mean result (Table 2.3.5). Intra and inter-day repeat determinations of 8, 15 and 35 μg/mL standards yielded mean results with relative

standard deviations <2% (Table 2.3.4) which validated the precision (repeatability and reproducibility) of the developed method. The method also proved to be rugged. Interday and interanalyst determinations of progesterone in 1-up CIDR®1380 Cattle insert yielded results with relative standard deviations <3% (Tables 2.3.6 to 2.3.9). Progesterone standards were accurately quantitated in the presence of placebo ingredients from the silicone matrix of CIDR® inserts (Table 2.3.11) thereby confirming that the developed method was specific. In addition, the limit of quantitation, although not absolutely necessary for a potency assay which is developed to measure relatively high levels of analyte, was determined to be 1 μg/mL (Table 2.3.10) which is suitably sensitive for the intended studies.

2.4.1.2 Validation of the Extraction Method of Progesterone from CIDR® Inserts

It is desirable that an extraction method should reproducibly recover as close to 100% of the analyte to be measured as possible to enable accurate analyte determination. The developed method was shown to recover 99 ± 1% of the progesterone in CIDR® inserts (Tables 2.3.6 to 2.3.9) thereby validating the suitability of the Soxhlet extraction method.

2.4.1.3 Validation of the Reconstitution Method of Progesterone from Invitro Drug Release Test Samples

As for an extraction method, the reconstitution of samples for analysis should provide close to 100% recovery of analyte to allow accurate analyte determination. Reconstituted samples were determined within 2% of their known values (Table 2.3.12), validating the suitability of the reconstitution method.

2.4.1.4 Validation of the Invitro Drug Release Test for CIDR® Inserts

The effect of both system dependent and manufacturing variables on the release of progesterone from the 1-up CIDR®1380 Cattle insert were investigated in order to validate the reproducibility and ruggedness of the release assessment test and enable the setting of limits to enable comparison of different inserts.

To be considered validated, an invitro drug release test should be capable of reproducibly demonstrating the release characteristics of a controlled release drug

product yet rugged enough to do so consistently at different times. It should be robust enough to not be heavily influenced by system dependent variables but be sensitive enough to be capable of distinguishing between different products. Experiments conducted to validate the release assessment test used in this Thesis showed acceptable intra-day and inter-day reproducibility and ruggedness. Robustness experiments showed that the test could reproduce typical results independent of some system dependent variables. However, the results also showed that the test method was sensitive to differentiate between formulation variables such as drug load.

To allow for the comparison of different products, based on the release rates determined in the validation procedure, specifications were set for the invitro release rate and intercept value of the square root of time release curve. The specifications were set, based upon the mean release rate and twice the standard deviation from the reproducibility and ruggedness experiment. The specification for the release rate was set at 1375 ± 463 $\mu g/cm^2/hr^{0.5}$. Specifications for the intercept value was set at 900 ± 1143 $\mu g/cm^2$.

2.4.2 Invitro and Invivo Characterisation of the 1-up CIDR®1380 Cattle Insert, 6-up CIDR®1380 Cattle Insert and CIDR®1900 Cattle Insert

2.4.2.1 Confirmation that Tools Produced Inserts Which Fulfilled Defined Criteria

The design parameters for an optimised CIDR® insert for oestrus synchronisation in cattle were defined by Rathbone and co-workers[C315]. These parameters were based on an insert with a surface area of 120 cm^2 and an even 1 mm thick skin of silicone matrix impregnated with progesterone at an initial load of 10%w/w. Once defined InterAg's manufacturing team progressively applied those criteria to the design of several stainless steel tools which were capable of producing inserts which fulfilled the defined parameters. Initially a 1-up tool (a stainless steel tool which allowed only one insert to be injection moulded at a time) was made to assure the Company that the theories developed by Rathbone et al. were valid with production made inserts. Then a 6-up tool (a stainless steel tool which allowed six inserts to be

injection moulded at a time) was made. The 6-up tool was manufactured to increase manufacturing capacity, thus ensuring a commercially viable process.

Initially the physical characteristics of each insert were compared to ensure that the design criteria had been met and to characterise the inserts. These included skin weight, surface area, initial drug load and skin thickness. In addition the invitro drug release characteristics of the product were determined.

Preliminary experiments were performed to determine where the progesterone was located within the insert following its manufacture (i.e., within the skin, spine, or both). Although it had always been assumed with the CIDR®1900 Cattle insert that all the progesterone is located in the silicone skin, this had never been proved experimentally. Experimental results showed that the progesterone was located within the silicone skin after manufacture with insignificant quantities being determined within the nylon spine material.

Table 2.4.1 shows the experimentally determined skin weight, initial drug load, surface area and skin thicknesses of the different inserts.

Table 2.4.1 - Table - skin weight, initial load, surface area and skin thickness

	CIDR®1900 Cattle insert	1-up CIDR®1380 Cattle insert	6-up CIDR®1380 Cattle insert
Surface Area (cm^2)	120	123	123
Skin Weight (g)	19.97	13.87	13.91
Initial Load (g)	1.90	1.38	1.34
Initial Load (%w/w)	9.51	9.92	9.53
Skin thickness range (mm)	1 - 5	≈ 1.0 over entire insert*	≈ 1.0 over entire insert*

*Except for wing tips which were deliberately made thicker for animal comfort

Examination of Table 2.4.1 indicate that both the 1-up and 6-up tooling designs produced inserts in accord with the specified design criteria. Each exhibited a uniform skin thickness of 1 mm, had a surface area of 120 cm^2 and contained a 10%w/w initial load.

2.4.2.2 Invitro drug release profile of the CIDR® Cattle inserts

Table 2.4.1 indicated that both the 1-up and 6-up tooled CIDR®1380 Cattle inserts exhibited a uniform skin thickness of 1 mm, had a surface area of 120 cm^2 and contained a 10%w/w initial load. However, Table 2.4.1 also shows that the amount of progesterone in the 1-up and 6-up tooled CIDR®1380 Cattle inserts is considerably lower compared to the CIDR®1900 Cattle insert. We therefore set out to assess the drug release properties of each of the inserts to determine how this reduction in initial drug load affected the release of drug from the inserts. The results are shown in Figure 2.4.1.

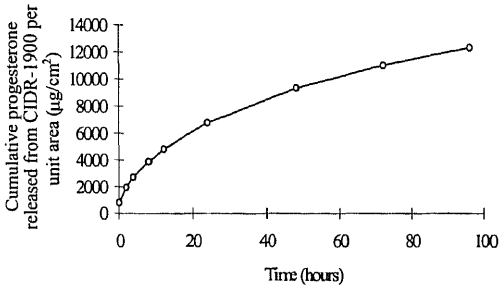
Examination of Figure 2.4.1 reveals that the CIDR®1900 Cattle insert, 1-up CIDR®1380 Cattle insert and 6-up CIDR®1380 Cattle insert all release progesterone at the same release rates in accord with the same mechanism of release, despite the reduction in the amount of progesterone in each insert. This can be explained when one examines the equations relating to the square root of time mechanism. When drug is released from a matrix containing an homogenous dispersion drug in excess of its solubility it will be released according to the following equation[230].

$$Q = \left[\left(2A - C_p\right)C_p D_p t\right]^{1/2} \qquad \textbf{Equation 2.4.1}$$

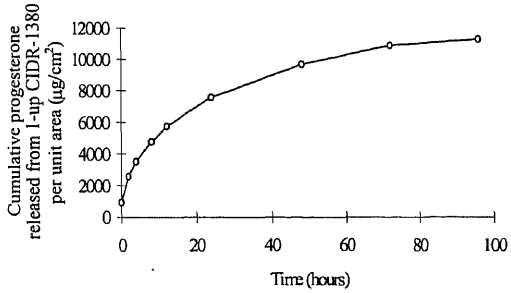
where Q is the amount of drug released after time, t, per unit area of exposed area; A is the initial drug concentration (amount per unit volume); C_p is the solubility of the drug in the matrix; and D_p is the diffusion coefficient of the drug in the matrix material.

This equation shows that the rate of release is dependent not upon the absolute amount of drug in the matrix, but its concentration. The reason why all three inserts give the same release rate, despite differences in initial amount of progesterone content, is because they contain that amount of progesterone in the same concentration (10%w/w).

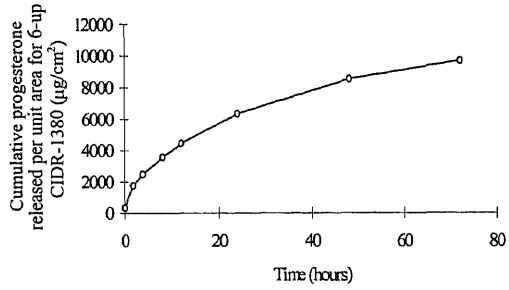
Release profiles



CIDR®1900 Cattle insert

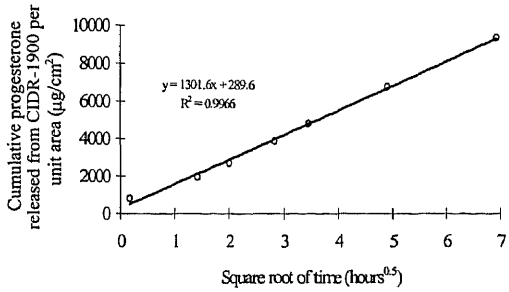


1-up CIDR®1380 Cattle insert

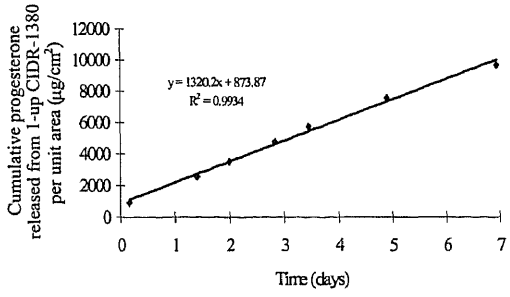


6-up CIDR®1380 Cattle insert

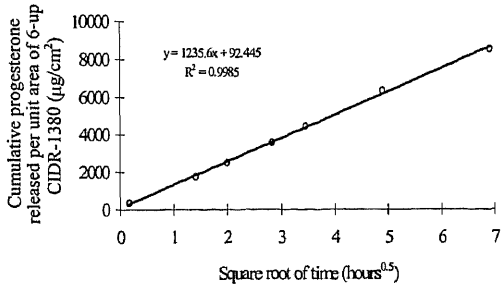
Data plotted against the square root of time



CIDR®1900 Cattle insert



1-up CIDR®1380 Cattle insert



6-up CIDR®1380 Cattle insert

Figure 2.4.1- Invitro release from the CIDR®1900 Cattle insert, 1-up and 6-up CIDR®1380 Cattle insert

2.4.2.3 Invivo Equivalency Determination and Characterisation

Following confirmation that the physical characteristics of each insert met the design criteria and the invitro release characteristics were identical, the invivo performance of the inserts was compared.

This was performed in several ways which reflected the progressive nature of the design and manufacture of the 1-up and 6-up tools. Initially, a crossover bioequivalency trial over a 7 day treatment period (standard anoestrus cow synchrony programme) was carried out using 8 ovariectomised cows which compared the 1-up CIDR®1380 Cattle insert with the CIDR®1900 Cattle insert. This trial included a rigorous sampling upon insertion and removal of the inserts to obtain detailed drug absorption and clearance data. Figure 2.4.2 shows the entire profile while Figure 2.4.3 and 2.4.4 show the detailed absorption and clearance portions of the profile, respectively.

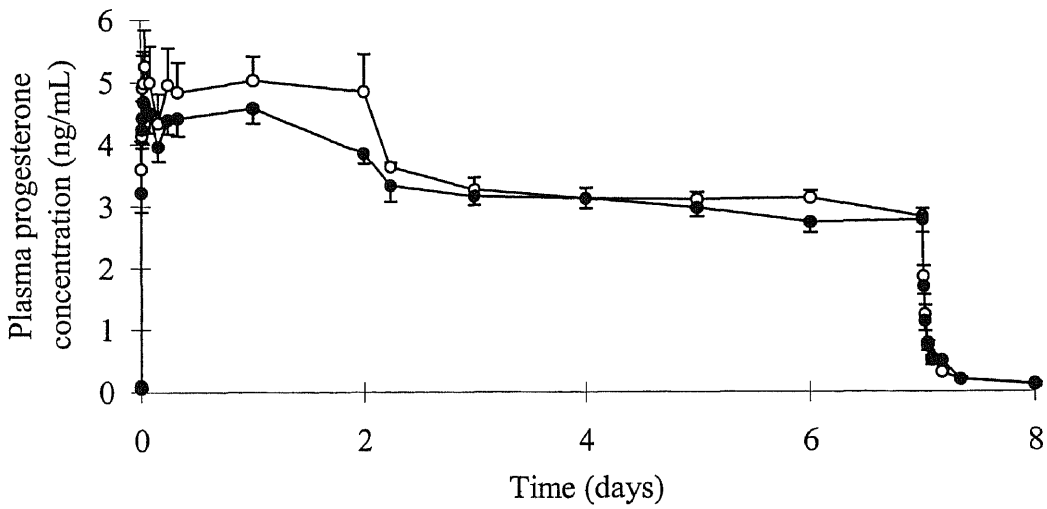


Figure 2.4.2 - Entire blood plasma progesterone profile for CIDR®1900 Cattle insert versus 1-up CIDR®1380 Cattle insert (O = CIDR®1900 Cattle insert, • = CIDR®1380 Cattle insert, N = 8, error bars = SEM)

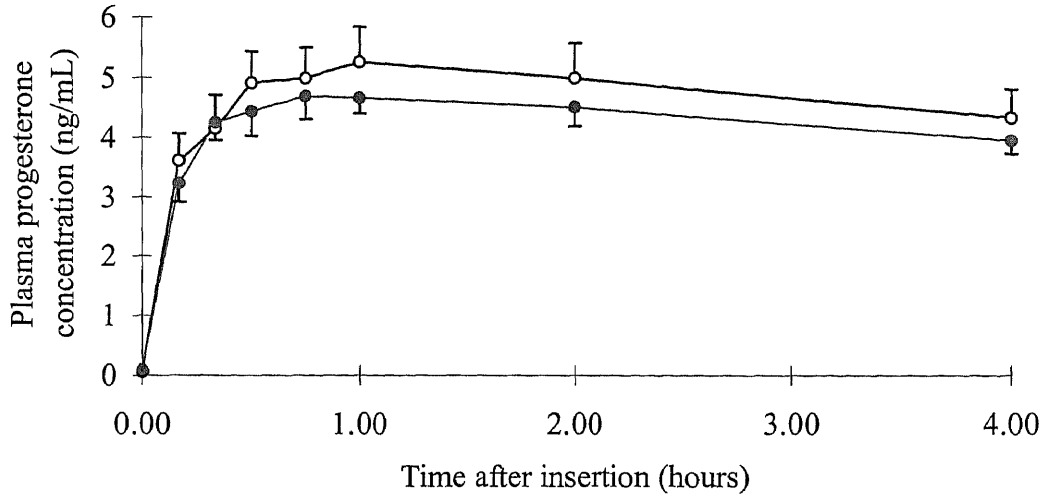


Figure 2.4.3 - Blood plasma progesterone absorption profile for the CIDR®1900 Cattle insert (O) and the 1-up CIDR®1380 Cattle insert (•) (n = 8; error bars = SEM)

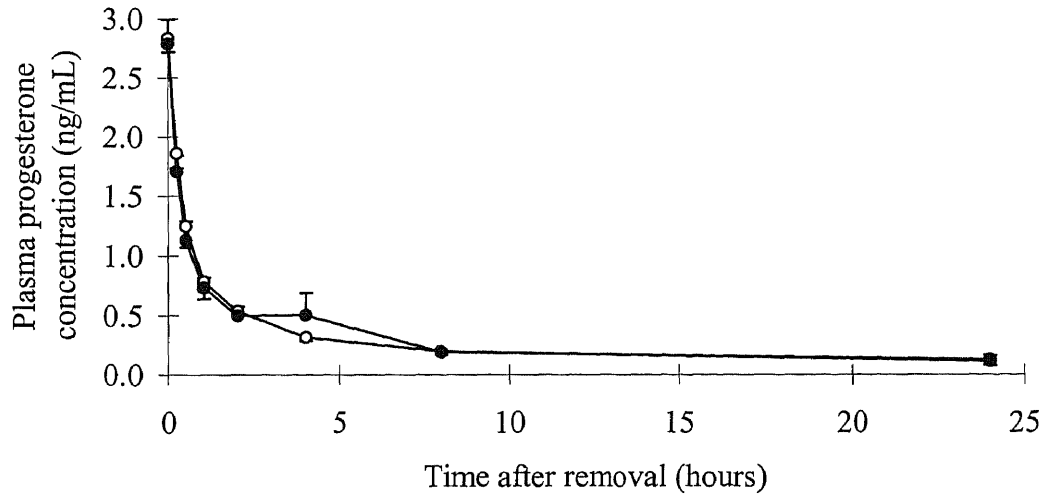


Figure 2.4.4 - Blood plasma progesterone clearance profile for the CIDR®1900 Cattle insert (O) and the 1-up CIDR®1380 Cattle insert (●) (n = 8; error bars = SEM)

Statistical analysis of the areas under the curve (AUC) (Table 2.3.30) for Figures 2.4.2, 2.4.3 and 2.4.4 suggest that the CIDR®1380 Cattle insert and CIDR®1900 Cattle insert were bioequivalent over a 7 day insertion in respect to both the absorption profile ($AUC_{0\text{-}4hr}$; p=0.452), clearance profile ($AUC_{7\text{-}8day}$; p=0.702) as well as the overall profile ($AUC_{0\text{-}8day}$; p=0.137).

The second crossover bioequivalency trial again compared the 1-up CIDR®1380 Cattle insert with the CIDR®1900 Cattle insert, but on this occasion it was carried out in 10 ovariectomised Friesian cows over a 10 day treatment period (which corresponded to a standard heifer synchrony programme). Results are shown in Figure 2.4.5.

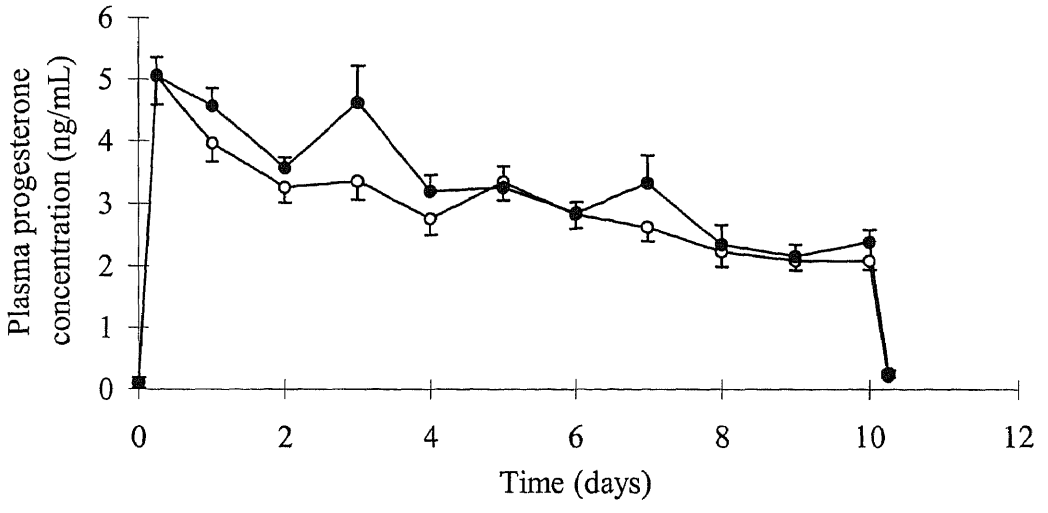


Figure 2.4.5 - Blood plasma progesterone levels in ovariectomised cows produced by CIDR®1900 Cattle insert (O) and 1-up CIDR®1380 Cattle insert (•) over a 10 day treatment crossover study (N=10; error bars = SEM)

The profiles obtained can be seen to be overlapping throughout the ten day treatment period and statistical analysis of AUC's (Table 2.3.31) for the two CIDR® Cattle inserts over the ten days yielded p=0.251. Therefore, the CIDR®1900 Cattle insert and CIDR®1380 Cattle insert were shown to be bioequivalent over a ten day insertion period (Figure 2.4.5) as well as a 7 day period (Figure 2.4.2).

A third crossover bioequivalency trial was conducted comparing the CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert in 10 ovariectomised Friesian cows over a 20 day treatment period to determine how long an insertion would be possible before the 1-up CIDR®1380 Cattle insert would fail to maintain plasma progesterone levels equivalent to the CIDR®1900 Cattle insert. Results are presented in Figure 2.4.6.

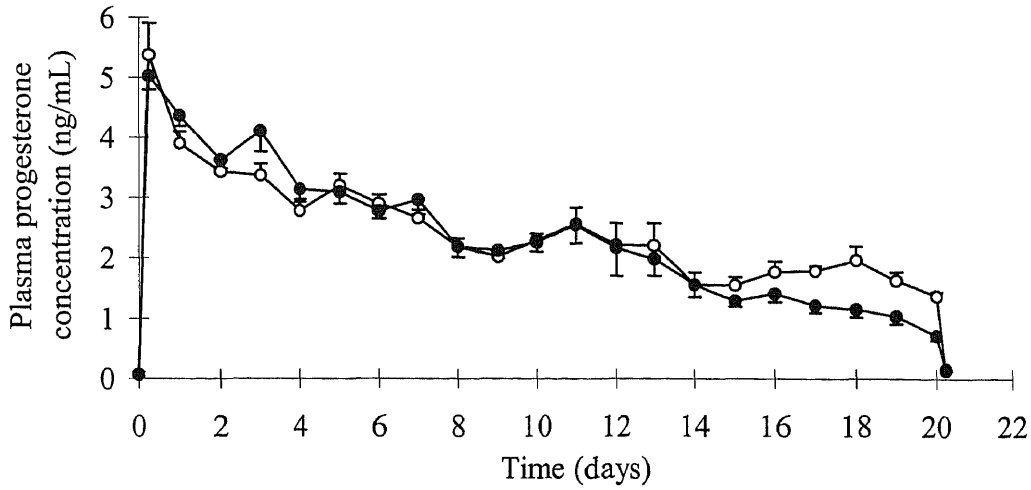


Figure 2.4.6 - Blood plasma progesterone levels in ovariectomised cows produced by CIDR®1900 Cattle insert (O) and 1-up CIDR®1380 Cattle insert (•) over a 20 day treatment crossover study (N=10; error bars = SEM)

Statistical analysis of AUC values (Table 2.3.32) showed that there was no difference between the two inserts between days 0-15 ($p=0.789$), however, there was a significant difference between the inserts over days 15-20 ($p<0.001$) of the insertion period. This is a reflection of the lower amount of progesterone originally incorporated into the 1-up CIDR®1380 Cattle insert.

A final bioequivalency trial was performed when the 6-up tool had been manufactured. This trial compared the 1-up CIDR®1380 Cattle insert with the 6-up CIDR®1380 Cattle insert. The bioequivalency trial involved 24 ovariectomised Friesian cows comparing three production batches of 6-up CIDR®1380 Cattle insert with 1-up CIDR®1380 Cattle insert was carried out over a 10 day treatment period. Six animals per cell were used to assess the variables. Figure 2.4.7 shows the mean profile of the three 6-up CIDR®1380 Cattle insert batches versus the 1-up CIDR®1380 Cattle insert.

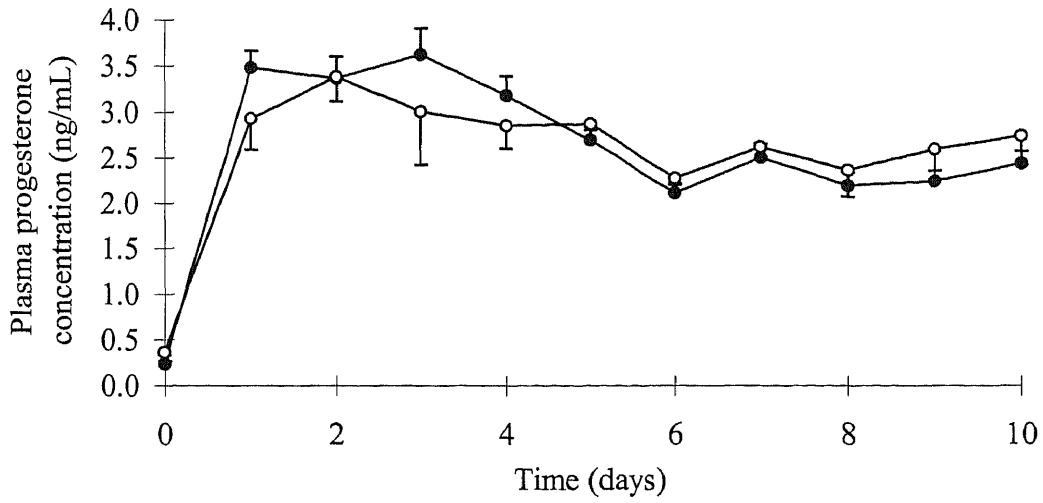


Figure 2.4.7 - Blood plasma progesterone profiles for 1-up CIDR®1380 Cattle insert (O; N = 4) versus 6-up CIDR®1380 Cattle insert (●; N = 16) (error bars = SEM)

Figure 2.4.7 shows that the 6-up CIDR®1380 Cattle insert produced overlapping plasma progesterone profiles with those produced by the 1-up CIDR®1380 Cattle insert. Statistical analysis of AUC data (Table 2.3.36) suggests that these inserts are indeed bioequivalent (p=0.810) over a ten day insertion period.

Figure 2.4.8 shows the individual batch comparisons of the 6-up CIDR®1380 Cattle insert to the 1-up CIDR®1380 Cattle insert.

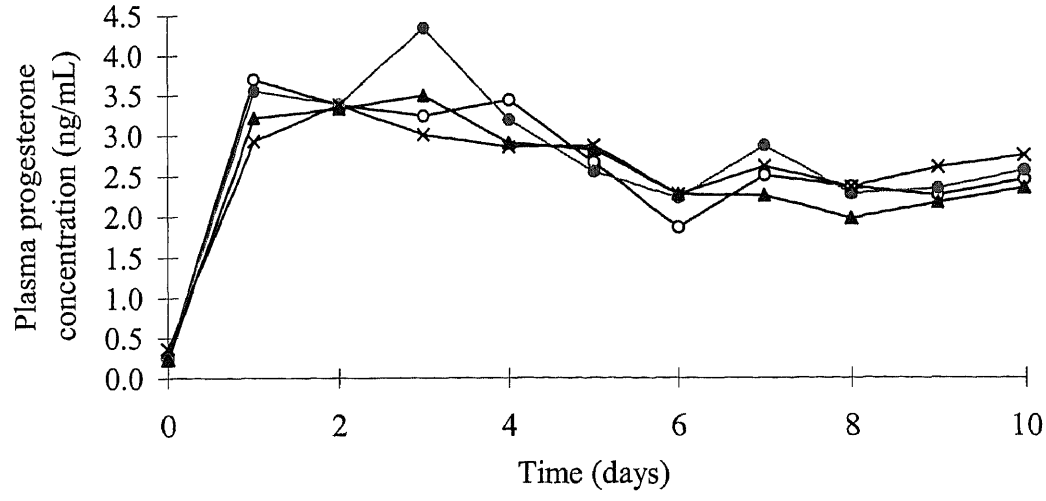


Figure 2.4.8 - Blood plasma progesterone profiles for 1-up CIDR®1380 Cattle insert (×, N = 4) and three batches of 6-up CIDR®1380 Cattle insert (O= batch 8927, N =6; ● = batch 8931, N = 4; ▲ = batch 8934, N = 6)

Figure 2.4.8 shows that each of the three separate production batches of 6-up CIDR®1380 Cattle insert produce overlapping plasma progesterone profiles with those produced by the 1-up CIDR®1380 Cattle insert. Statistical analysis of AUC data (Table 2.3.36) suggests that these inserts are each bioequivalent (p=0.810) over a ten day insertion period to the 1-up CIDR®1380 Cattle insert.

A further analysis is shown in Figure 2.4.9 which shows the mean profile of each of the individual cavities which make up the 6-up CIDR®1380 Cattle insert batches compared to the 1-up CIDR®1380 Cattle insert.

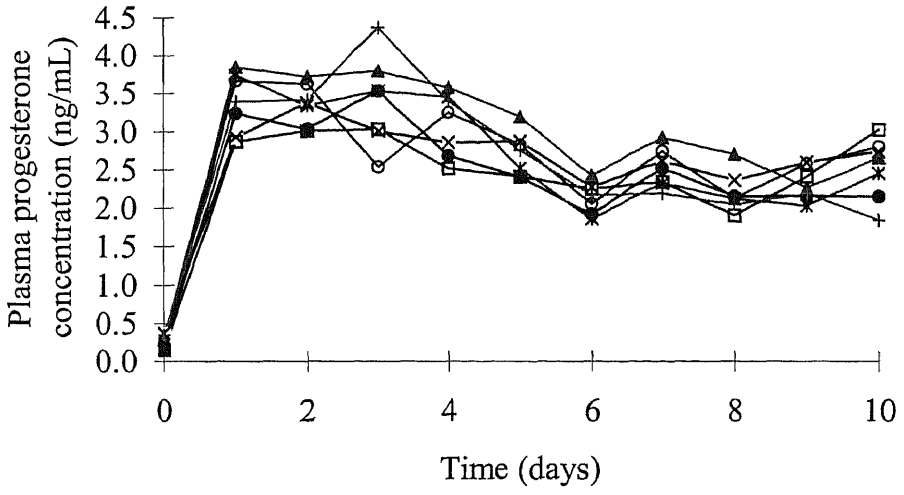


Figure 2.4.9 - Blood plasma progesterone profiles for 1-up CIDR®1380 Cattle insert (×, N = 4) and 6-up CIDR®1380 Cattle insert from each of the six tool cavities (● = cavity 1, N = 3; ▲ = cavity 2, N = 3; = cavity 3, N = 2; ✻ = cavity 4, N = 3; O= cavity 5, N = 2; | = cavity 6, N = 3)

Figure 2.4.9 shows that each of the six individual cavities which make up the 6-up tool produce overlapping plasma progesterone profiles with those produced by the 1-up CIDR®1380 Cattle insert. Statistical analysis of AUC data (Table 2.3.36) suggests that each cavity produces inserts which are each bioequivalent (p=0.892) over a ten day insertion period to the 1-up CIDR®1380 Cattle insert.

2.4.2.4 Residual Progesterone Content in Spent Inserts

The residual content of progesterone in CIDR® Cattle inserts inserted for various insertion periods is summarised in Table 2.4.2.

Table 2.4.2 - Initial and residual progesterone loads in CIDR® Cattle inserts inserted for various insertion periods and the calculated amount of progesterone released over that period

Insert	Insertion period (days)	Initial amount of progesterone (g)	Residual progesterone (g)	Amount released (g)
CIDR®1900 Cattle insert	7	1.99	1.38 ± 0.01	0.61 ± 0.01
CIDR®1900 Cattle insert	10	1.97	1.19 ± 0.03	0.78 ± 0.03
CIDR®1900 Cattle insert	20	1.99	0.79 ± 0.04	1.20 ± 0.04
1-up CIDR®1380 Cattle insert	7	1.34	0.72 ± 0.02	0.62 ± 0.02
1-up CIDR®1380 Cattle insert	10	1.35	0.48 ± 0.01	0.87 ± 0.01
1-up CIDR®1380 Cattle insert	20	1.36	0.20 ± 0.01	1.16 ± 0.01
1-up CIDR®1380 Cattle insert	10	1.34	0.68 ± 0.03	0.66 ± 0.03
6-up CIDR®1380 Cattle insert Batch 8927	10	1.35	0.72 ± 0.02	0.63 ± 0.02
6-up CIDR®1380 Cattle insert Batch 8931	10	1.34	0.76 ± 0.02	0.58 ± 0.02
6-up CIDR®1380 Cattle insert Batch 8934	10	1.33	0.68 ± 0.02	0.65 ± 0.02

Table 2.4.2 shows that the residual amount of progesterone remaining in a used CIDR® Cattle insert is dependent upon the length of insertion and the type of insert used (CIDR®1900 Cattle insert versus 1-up and 6-up CIDR®1380 Cattle insert). The CIDR®1900 Cattle insert contains the highest residual compared to either the 1-up or 6-up CIDR®1380 Cattle insert, whereas the 1-up and 6-up CIDR®1380 Cattle inserts contain the same residual after a given insertion period in the same trial ($p<0.1$).

2.4.2.5 Amount released

Table 2.4.2 also shows the amount of progesterone released from each insert following insertion. Over seven days, the CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert released the same amount of drug ($p=0.614$) which was approximately 0.6 g. Over 20 days these two inserts also released the same amount of drug ($p=0.370$), approximately 1.2 g. In contrast, the amounts of progesterone released for the CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert over ten days was shown to be statistically different ($p=0.024$), however, this seems an unusual observation given the overlapping plasma progesterone profiles. Determination of the amount of progesterone released from 1-up CIDR®1380 Cattle insert and the three batches of 6-up CIDR®1380 Cattle insert during a ten day insertion showed that these inserts all released the same amount of progesterone ($p>0.1$).

2.5 Conclusions

Table 2.5.1 highlights both the successful implementation by the manufacturing team of the recommendations of Rathbone et al. and the successful nature of these workers' optimisation process for the CIDR®1900 Cattle insert.

Table 2.5.1 - Comparison of the characteristics of the CIDR®1900 Cattle insert, 1-up CIDR®1380 Cattle insert and 6-up CIDR®1380 Cattle insert.

Parameter	CIDR®1900 Cattle insert	1-up CIDR®1380 Cattle insert	6-up CIDR®1380 Cattle insert
initial loads (g)	1.90	1.38	1.34
Amount released 7 days (g)	0.61	0.62	not determined
Amount released 10 days (g)	0.78	0.87[a] / 0.66[b]	0.58-0.65[d]
Amount released 20 days (g)	1.20	1.16	not determined
Skin thickness (mm)	1-5	≈ 1.0 over entire insert*	≈ 1.0 over entire insert*
Residual 7 days (g)	1.38	0.72	not determined
Residual 10 days (g)	1.19	0.48[a] / 0.68[b]	0.68-0.76[d]
Residual 20 days (g)	0.79	0.20	not determined
Bioequivalency[c] 7 days	26	24	not determined
Bioequivalency[c] 10 days	30	33[a] / 27[b]	25-28[d]
Bioequivalency[c] 20 days	49	45	not determined
Invitro release rate ($\mu g/cm^2/hr^{1/2}$)	1302	1320	1236
Surface area (cm^2)	120	123	123

a = result from 10 day trial #1; b = result from 10 day trial #2; c = quantified in terms of AUC (ng.day/mL); d = range of results between each of the 3 batches and or 6 cavity positions determined

* = Except for wing tips which were deliberately made thicker for animal comfort

1-up and 6-up tools were successfully designed and manufactured which resulted in the production of CIDR® inserts which exhibited a uniform skin thickness of 1 mm, contained a 10%w/w initial load and had a surface area of 120 cm^2 (Table 2.5.1). The resultant CIDR®1380 Cattle inserts manufactured with these tools were shown to release progesterone invitro at the same rate as the CIDR®1900 Cattle insert and to release the same amount of progesterone over a given insertion period, resulting in

invivo bioequivalence to the CIDR®1900 Cattle insert up to a 15 day insertion period (Table 2.5.1). However, the redevelopment of a CIDR®1380 Cattle insert with a uniform 1 mm skin thickness resulted in a reduction in the initial progesterone load (Table 2.5.1). Since the same percentage load was included in the CIDR®1380 Cattle insert as the CIDR®1900 Cattle insert (Table 2.5.1) the release characteristics of the insert were equivalent and this was reflected in the two inserts exhibiting bioequivalence. In addition all inserts were shown to release the same amount of progesterone over a given insertion period (irrespective of initial load or skin thickness) (Table 2.5.1). Thus, because the CIDR®1380 Cattle insert contained a lower initial load (1.34 g) compared to the CIDR®1900 Cattle insert (1.90 g), the residual progesterone content of the CIDR® 1380 Cattle insert (0.7 g) was lower than that of the CIDR®1900 Cattle insert (1.32 g).

The CIDR®1380 Cattle insert therefore exhibits a lower initial load, lower residual drug load and is bioequivalent to the CIDR®1900 Cattle insert (Table 2.5.1). In addition to these favourable attributes of the CIDR®1380 Cattle insert because of the elimination of wasted drug loaded silicone skin, it achieves a significant improvement in material utilisation over the CIDR®1900 Cattle insert.

Chapter Three - Characterisation of intravaginal drug delivery systems for the delivery of progesterone for control of the bovine oestrous cycle. II. Physical and chemical stability characteristics of CIDR®1900 Cattle inserts and 1-up CIDR®1380 Cattle inserts.

3.1 Introduction

Stability testing is an integral component of any drug product profile. It provides experimental evidence for the length of time the chemical and physical characteristics of a drug product remain within acceptable limits of quality, safety and efficacy after its date of manufacture[226].

Stability studies should be conducted in accord with a predefined stability programme. Essential components of a stability programme include a fully validated stability indicating chemical assay, well controlled temperature and humidity conditions, statements which specify pre-defined sample points, limits on temperature and humidity parameters and definitions of the quality characteristics of the product. In addition, for a controlled release product, the stability programme should include an assessment of the invitro drug release profile over the duration of the trial to demonstrate that the release characteristics of the drug do not change detrimentally upon storage[231].

In a stability study, a product would be considered stable over a given period of time if the level of active contained within product stored for that time period remained greater than 90% of the stated label claim. A tentative shelf life for a product can be proposed on the basis of short term (6-12 months) data obtained for product stored under accelerated time (elevated temperature and humidity; 40°C, 75%RH) conditions. This tentative shelf life would then be confirmed using real time (ambient temperature and humidity; 25°C, 60%RH) data generated over the time period corresponding to the proposed shelf life.

In this chapter the chemical and physical stability of the CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert were determined under real time and accelerated time conditions. The predefined parameters which describe the quality characteristics of the product are shown in Table 3.1.1. The selection of these product quality parameters was based on InterAg's previous experience with the product and were known to influence the quality, safety and efficacy of the product.

Table 3.1.1 - Product variables assessed in the stability study testing programme

Potential changes on storage	**Potential chemical and physical changes based on previous experience**	**Rationale for the selection of product variables included in the testing programme**	**Action in stability trial**
Chemical changes			
Loss of potency	Decrease in potency profile with time	Potential loss of efficacy	Determination of degree of chemical change to establish storage conditions and determine shelf life
Increase in degradation products	Increase in degradation profile with time	Potential loss of efficacy	Determination of change in degradation profile
Physical changes			
Colour	Very slight and gradual change from white to a duller white colour	Loss of pharmaceutical elegance	Visually assess colour change and ensure change falls within specified limits
Surface progesterone	Gradual appearance of progesterone on the surface of the insert over the first three months	Loss of pharmaceutical elegance	Determination of degree of change of initial burst effect in the invitro drug release test and ensure change falls within specified limits
Change in release profiles	Change in potency profile with time could result in change in release characteristics	Potential loss of efficacy	Determination of degree of change of release characteristics and ensure change falls within prespecified limits

3.2 Methods

3.2.1 Stability Trial

3.2.1.1 Stability-Indicating and Potency HPLC Assay

3.2.1.1.1 Method

HPLC System:	Waters™ LC Module I Plus, Degasys DG2410	
Reagents:	Double distilled water (AUTOSTILL™ Auto Four WS SYSTEM, Jencons Scientific Ltd.) and methanol (HiperSolv™ for HPLC, BDH, N.Z.) filtered and degassed, Progesterone (USP micronised, Pharmacia & Upjohn, USA)	
Column:	Waters Symmetry™ C18 3.9 x 150 mm, 5 micron HPLC Cartridge Column	
Guard Column:	Waters Sentry™ Guard Column	
Column Conditions:	Mobile Phase:	70:30v/v (methanol:water)
	Mobile Phase Flow:	1 mL per minute
	Injection Volume:	20 µL
	AUFS:	1.000
	Progesterone Retention Time:	9.2 ± 0.4 minutes
	Detection Wavelength:	240 nm
	Integration:	Peak Area

3.2.1.1.2 Standard Preparation

Standards were prepared by transferring 0.1000 g progesterone standard (USP micronised, Pharmacia &Upjohn, USA) into a 100 mL volumetric flask followed by the addition of 50 mL alcohol (SDA-3A, Mobil, New Zealand). The mixture was sonicated until the progesterone completely dissolved, made up to volume with alcohol (SDA-3A, Mobil, New Zealand) and mixed by inversion. 200 µL, 500 µL, 1000 µL, 1500 µL and 2000 µL aliquots were then pipetted into 50 mL volumetric flasks, made up to volume with alcohol (SDA-3A, Mobil, New Zealand) and mixed by inversion.

3.2.1.1.3 Sample Preparation

Drug loaded silicone skin was removed from the nylon spine and weighed on a four figure analytical balance (BP110S, Sartorius, Germany). The skin was cut into 5-8 cm lengths using a scalpel and the lengths were placed into a Soxhlet apparatus, ensuring that all lengths sat below the siphon level. 350 mL ethanol (SDA-3A, Mobil, New Zealand) was added to the round bottom flask of the Soxhlet apparatus and the flask, Soxhlet and condenser were assembled. Skins were extracted for 12 hours and allowed to cool before transferring (with rinsing) to a 500 mL volumetric flask and making the volume up to the mark with ethanol (SDA-3A, Mobil, New Zealand). This solution was then diluted (0.5 mL to 100 mL) in ethanol (SDA-3A, Mobil, New Zealand) prior to analysis using the stability-indicating HPLC assay.

3.2.1.2 Validation of the Stability-Indicating and Potency HPLC Assay

The HPLC-UV assay was validated by assessment of its linearity and range, accuracy, precision, limit of quantitation, stability indicating nature, and ruggedness[226-228].

3.2.1.2.1 Linearity and Range

The linearity of an analytical method is defined as its ability to elicit test results that are directly proportional to the concentration of analyte in samples within a given range, the range being the interval between the upper and lower levels of analyte that have been demonstrated to be determined with precision, accuracy, and linearity using the method as written. Linearity was determined by analysing two sets of calibration standards (4, 10, 20, 30 and 40 μg/mL) and performing linear regression upon the results. The range was determined by analysing validation standards at 4 and 40 μg/mL and determining their concentrations against the linear regression.

3.2.1.2.2 Accuracy

Accuracy is defined as the nearness of a result to the true or accepted value. Accuracy was determined by analysing validation standards at 8, 15 and 35 μg/mL, calculating their concentrations against the linear regression and assessing the deviation of the calculated results from their true concentrations.

3.2.1.2.3 Precision

Precision is defined as the agreement of repeat observations made under the same conditions. Precision occurs on different levels including repeatability (multiple injections of the same solution), reproducibility (determination of multiple known samples). Repeatability was assessed by executing repeat injections of a single standard solution and determining the relative standard deviation of the results about their mean value. Reproducibility was determined in association with accuracy. The concentrations of replicate 8, 15 and 35 μg/mL validation standards were calculated against the linear regression and the relative standard deviations of the results about their means were determined.

3.2.1.2.4 Ruggedness

Ruggedness is an element of precision and is defined as agreement in the determination of multiple known samples using the same method but under different conditions such as day of analysis and analyst carrying out the experiment. Ruggedness was determined by replicate analyses of CIDR® inserts on multiple days and with multiple analysts.

3.2.1.2.5 Limit of quantitation

The limit of quantitation is defined as the lowest concentration of analyte in a sample that can be determined with acceptable precision and accuracy under the stated experimental conditions. The limit of quantitation was determined by estimating the background response of the method from multiple blank injections, followed by analysis of standards near the determined limit.

3.2.1.2.6 Specificity

Specificity is defined as the ability of the method to resolve a substance from its impurities and any other substances such as degradation products or placebo ingredients of the product. Specificity of the stability-indicating assay was assessed by analysis of progesterone standards in the presence of analogous compounds (Figure 3.2.1) and any unknown compounds extractable from the silicone CIDR® matrix.

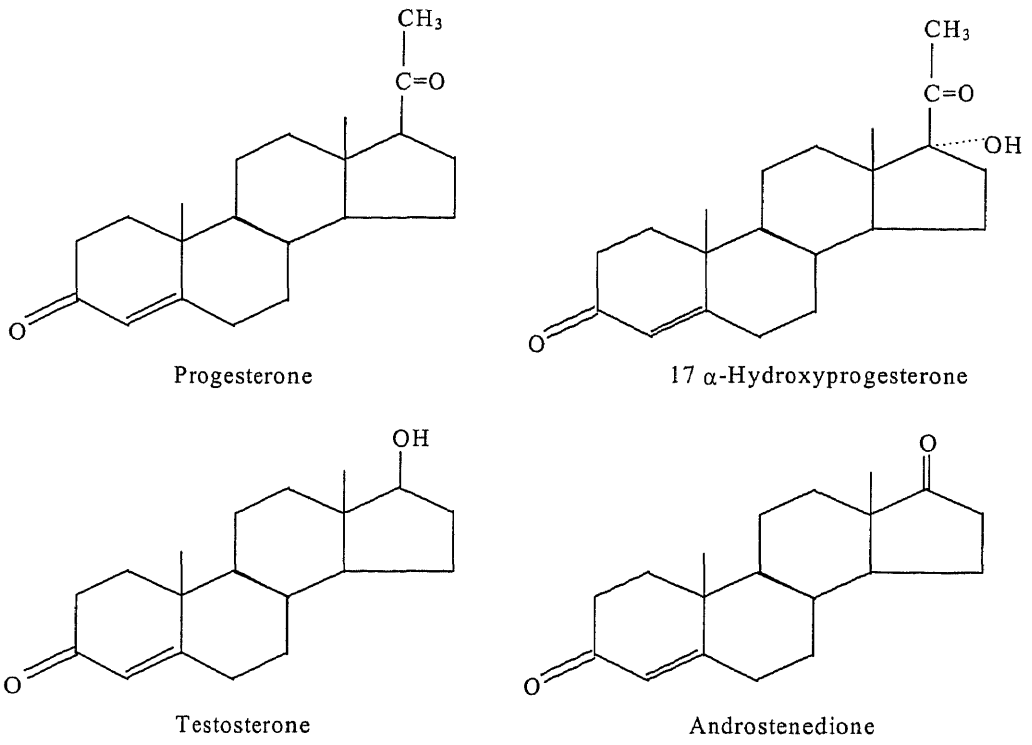


Figure 3.2.1 - Chemical structure of progesterone and some of its structurally related analogues

3.2.2 Real and Accelerated Time Stability Study

3.2.2.1 Manufacture of CIDR®1900 Cattle Inserts and 1-up CIDR®1380 Cattle Inserts

All CIDR inserts were manufactured as described in Chapter Two (see Section 2.2.3).

3.2.2.2 Stability Trial Protocol

3.2.2.2.1 Batch production and sample selection procedure

Three production batches each of both CIDR®1900 Cattle inserts and 1-up CIDR®1380 Cattle inserts were manufactured under large scale manufacturing conditions. As the inserts were manufactured they were placed into large polyethylene bags and labeled according to CIDR® type and batch number. CIDR® inserts were then selected at random from the large poly-bags and packed in lots of two in the closure system. Once packaged, bags of CIDR®1900 Cattle inserts and 1-up CIDR®1380 Cattle inserts from each production batch were randomly selected,

allocated a sample time point, closure condition (open or closed bags) and storage condition (real or accelerated time) and then labeled accordingly.

3.2.2.2.2 Closure

The closure used in the studies was the same as that which is used for the packaging of retailed product. The packaging comprised opaque white resealable (Minigrip® zipseal) 60 μm polyethylene bags with tamper evident perforated closure strips.

Inserts were stored in both open and closed (heat sealed) closures during the trial.

3.2.2.2.3 Storage conditions

Inserts were stored under conditions representing 'real time' (25°C/60%RH) and 'accelerated time' (40°C/75%RH) storage conditions[227]. Precise temperature control (±2°C) was achieved using thermoregulated incubators (Contherm, UK). Humidity was controlled to within ±5% of nominated values within the incubators by use of saturated salt solutions; 60% humidity at 25°C was achieved with the presence of a saturated solution of NaBr (GPR™, BDH, New Zealand) while 75% humidity at 40°C was achieved with the use of a saturated NaCl (GPR™, BDH, New Zealand) solution[232]. No natural or artificial lighting was present within the incubators.

3.2.2.2.4 Sampling Plan

The predefined sampling schedule for the CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert stability trial is presented in Table 3.2.1. Ticks represent the time points at which samples stored under real time and accelerated time conditions, in open and in closed bags, were sampled for analysis.

Table 3.2.1 - Sampling protocol for potency, invitro drug release and physical stability during the stability trial

	Potency		Invitro release assessment		Physical stability assessment	
Time (months)	CIDR®1900	1-up CIDR®1380	CIDR®1900	1-up CIDR®1380	CIDR®1900	1-up CIDR®1380
0	✔	✔	✔	✔	✔	✔
0.5		✔				
1		✔				
2		✔				
2.5		✔				
3	✔	✔		✔		
6	✔	✔		✔	✔	✔
9	✔	✔				
12	✔	✔	✔	✔	✔	✔
18	✔	✔				
24	✔	✔	✔	✔	✔	✔

3.2.2.3 Assessment of Chemical Changes

3.2.2.3.1 Potency Assay

It was previously shown in Chapter Two, Section 2.3.3.1, that following manufacture of CIDR® inserts using InterAg's standard manufacturing technique and materials, drug was contained in the skin. To determine the potency of each sample, two samples were removed from their controlled storage conditions at each allocated time point and silicone skins were prepared for analysis by a Soxhlet extraction technique using ethanol (SDA-3A, Mobil, New Zealand) (see Chapter Two, Section 2.2.1.3). They were then analysed to determine drug potency by a validated stability-indicating and potency HPLC assay (see Section 3.2.1).

3.2.2.3.2 Degradation Products

The presence of degradation products was determined by visually examining each of the HPLC chromatograms generated during the potency assay determinations for the presence of new peaks which were not originally apparent in time zero chromatograms.

3.2.2.4 Assessment of Physical Changes

3.2.2.4.1 Physical Observations

The colour of the inserts was subjectively assessed by visual examination of two inserts from each batch at each storage condition employed in the study.

The amount of progesterone powder appearing on the surface with time was subjectively assessed by looking at the surface and subjectively estimating the extent of surface progesterone.

3.2.2.4.2 Invitro Drug Release

CIDR inserts removed from controlled storage conditions at each predefined time point were subjected to a validated invitro drug release test which was developed based upon the criteria described in the Drug Release monograph of the 23rd United States Pharmacopeia[233] (see Chapter Two, Sections 2.2.2 and 2.3.2).

3.2.2.5 Analysis of Stability Data

3.2.2.5.1 Accelerated Time Data

Accelerated time potency data was plotted as the average percent label claim of the duplicate samples from each production batch at each sample time point and least squares regression was performed on the data. A one sided lower 95% confidence interval about this regression line was used to estimate the time frame in which potency would decline to 90% of the label claim. The one-sided lower 95% confidence interval about the regression line was calculated from the following equations[234]. The least squares line (y = a + bx) constants 'a' and 'b' are given by:

$$b = \left[\sum xy - \left(\left(\sum x\right)\left(\sum y\right)/N\right)\right] / \left[\sum\left(x^2\right) - \left(\left(\sum x\right)^2/N\right)\right] \qquad \textbf{Equation 3.2.1}$$

where

b = the slope of the least squares line

Σxy is the sum of the cross-products of corresponding x and y values

Σx = the sum of all x (time) values

Σy = the sum of all y (potency) values

N = the total number of data points (sets of x,y data)

$\Sigma(x^2)$ = the sum of all squared x (time) values

$$a = \left[\sum y - \left(b\sum x\right)\right]/N \qquad \textbf{Equation 3.2.2}$$

where

a = the intercept of the least squares line with the y axis

There will be a β confidence interval, z, about a predicted value y (i.e. $y_i \pm z_i$) where

$$z_i = t_{N-2,\beta} \cdot s_{xy} \cdot \left[(1/N) + \left\{\left(x_i - x_{avg}\right)^2 / \left\{\left(\sum x^2\right) - \left(\left(\sum x\right)^2 / N\right)\right\}\right\}\right]^{1/2} \qquad \textbf{Equation 3.2.3}$$

where:

$t_{N-2,\beta}$ = the tabulated student's t value with N - 2 degrees of freedom and β confidence

s_{xy} = the square root of s_{xy}^2

$$s_{xy}^2 = \frac{\left[\left\{\sum\left(y^2\right) - \left(\sum y\right)^2/N\right\} - \left\{b^2\left(\sum\left(x^2\right) - \left(\sum x\right)^2/N\right)\right\}\right]}{(N-2)} \qquad \textbf{Equation 3.2.4}$$

where

Σy^2 is the sum of the squared y values

3.2.2.5.2 Real Time Data

Real time potency data was plotted as the average percent label claim of the duplicate samples from each production batch at each sample time point. The curve was then visually assessed to ascertain if values determined under real time conditions remained above the 90% label claim specification.

3.2.3 Manufacturing Study

The "manufacturing study" investigated manufacturing factors that affected the rate and extent of appearance of progesterone on the surface of the 1-up CIDR®1380

Cattle insert. In this study, only the 1-up CIDR®1380 Cattle insert was investigated as InterAg planned for the CIDR®1380 Cattle insert to supersede the CIDR®1900 Cattle insert in the marketplace. The 1-up CIDR®1380 Cattle inserts investigated in this manufacturing study were not subjected to a stability trial, instead, the amount of progesterone appearing on the surface was quantified as a function of storage time at 40°C/75%RH using a surface wash technique.

3.2.3.1 Manufacture of 1-up CIDR®1380 Cattle Inserts

A range of 1-up CIDR®1380 Cattle inserts were fabricated under different manufacturing conditions (Table 3.2.2) in order to assess the effect of moulding temperature, curing time and spine annealing time on the rate and extent to which progesterone appeared on the surface of the product. The 1-up CIDR®1380 Cattle inserts were manufactured with the same equipment normally employed by InterAg to manufacture CIDR® inserts, however, modified manufacturing conditions were impelled as described in Table 3.2.2. In all cases Dow Silastic® Q7-4840 silicone and Pharmacia & Upjohn USP micronised progesterone were used in the formulations.

Table 3.2.2 - Variables in the fabrication of 1-up CIDR®1380 Cattle inserts which were investigated in the manufacturing study

Test	Manufacturing condition		
	Mould temperature (°C)	Time in mould (s)	Spine anneal time (hours)
1	130	50	7.5
2	130	110	7.5
3	160	50	7.5
4	190	35	7.5
5	190	50	7.5
6	190	65	7.5
7	190	80	7.5
8	210	50	7.5
9	210	65	7.5
10	190	50	0
11	190	50	2.5
12	190	50	4.5

3.2.3.2 Trial Protocol

3.2.3.2.1 Batch Production and Sample Selection Procedure

A single production batch of 1-up CIDR®1380 Cattle inserts were manufactured, altering manufacturing variables (Table 3.2.2) as the production run progressed to produce the different inserts. As the inserts were manufactured they were placed into large polyethylene bags and labeled according to their manufacturing variation. CIDR® inserts from each manufacturing variant were then selected at random from their large polyethylene bag and packed in lots of two in the closure system. Once packaged, bags of 1-up CIDR®1380 Cattle inserts were randomly selected, allocated a sample time point and labeled accordingly.

3.2.3.2.2 Closure

Product closure was the same as that used in the Real and Accelerated Stability Trial (see Section 3.2.2.2.2). Product was stored in open bags.

3.2.3.2.3 Storage Conditions

Samples were stored under accelerated time conditions (40°C, 75% RH) using the equipment and chemicals described in Section 3.2.2.2.3.

3.2.3.2.4 Sampling Plan

Two inserts were sampled at time points of 0, 3 and 12 months.

3.2.3.3 Assessment of Surface Progesterone

3.2.3.3.1 Determination of Surface Progesterone

In this part of the study attempts to quantify the amount of progesterone appearing on the surface were made (in the stability study this parameter was only subjectively assessed). Quantification of the amount of progesterone on the surface of the 1-up CIDR®1380 Cattle insert was achieved by rinsing the skin of the CIDR® insert with between 45 and 50 mL of ethanol (SDA-3A, Mobil, New Zealand). The ethanolic wash was collected in a 400 mL beaker, transferred to a 100 mL volumetric flask and made up to volume using ethanol. Progesterone content was then determined by UV Spectrophotometry using the assay described in Section 2.2.1.

3.2.3.3.2 Determination of Progesterone in Silicone Skin

Following the skin rinsing, each skin was subjected to soxhlet extraction as described in Chapter Two, Section 2.2.1.3 to determine the remainder of its progesterone content by UV Spectrophotometry using the assay described in Chapter Two, Section 2.2.1.

3.2.4 Potential Future Manufacturing Strategies

In this part of the study, a stability study was used to determine the ability of two potential manufacturing strategies to decrease the appearance of progesterone on the surface of the 1-up CIDR®1380 Cattle insert. These strategies included the use of a water-free (non-annealed) spine material and a silicone that allows manufacture of the CIDR® insert at low curing temperatures.

3.2.4.1 Alternate Spine Material

3.2.4.1.1 Manufacture of 1-up CIDR®1380 Cattle Inserts

1-up CIDR®1380 Cattle inserts were manufactured with InterAg's standard injection moulding technique using Dow Silastic® Q7-4840 silicone and Pharmacia & Upjohn USP micronised progesterone (see Chapter Two, Section 2.2.3). However, in this part of the study, inserts were manufactured with a non-annealed polyester (Hoescht™ Riteflex® 677) spine.

3.2.4.1.2 Trial Protocol

3.2.4.1.2.1 Batch Production and Sample Selection Procedure

A single production batch of 1-up CIDR®1380 Cattle inserts was manufactured. As the inserts were manufactured they were placed into large polyethylene bags. CIDR® inserts were then selected at random from the large polyethylene bags and packed in lots of two in the closure system. Once packaged, bags of CIDR® inserts were labeled appropriately for storage.

3.2.4.1.2.2 Closure

Product closure was the same as that used in the Real and Accelerated Stability Study (see Section 3.2.2.2.2). Product was stored in open bags.

3.2.4.1.2.3 Storage Conditions

Samples were stored under real time conditions (25°C, 60% RH) using the equipment and chemicals described in Section 3.2.2.2.3.

3.2.4.1.2.4 Sampling Plan

No predefined sampling plan was specified and samples were removed from storage for analysis at convenient times.

3.2.4.1.3 Assessment of Chemical Changes

3.2.4.1.3.1 Potency Assay

Drug content in the silicone skins of the 1-up CIDR®1380 Cattle inserts which were manufactured with a polyester spine was determined by Soxhlet extraction of the silicone skins with ethanol (SDA-3A, Mobil, New Zealand) (see Section 3.2.1.2) followed by analysis by UV assay (see Chapter Two, Section 2.2.1).

3.2.4.1.3.2 Determination of Progesterone in the Polyester Spine

Drug content in the polyester spine was determined after the silicone skin had been removed from the polyester spine. After removal of the silicone skin, the spine was accurately weighed (BP110S, Sartorius, Germany) and cut into 5-8 cm lengths using an hacksaw. The lengths were placed into a Soxhlet apparatus, ensuring that all lengths sat below the siphon level. 350 mL ethanol (SDA-3A, Mobil, New Zealand) was added to the round bottom flask of the Soxhlet apparatus and the flask, Soxhlet and condenser were assembled. Spines were extracted for 12 hours and allowed to cool before transferring (with rinsing) to a 500 mL volumetric flask and making the volume up to the mark with ethanol (SDA-3A, Mobil, New Zealand). This solution was then diluted 1 to 15 and analysed by HPLC (see Section 3.2.1).

3.2.4.2 Low Temperature Cure Silicone

3.2.4.2.1 Manufacture of 1-up CIDR®1380 Cattle Inserts

1-up CIDR®1380 Cattle inserts were manufactured using a low temperature curing, two part (A and B) silicone elastomer (Wacker Chemicals Elastosil® LR3004-40). Progesterone (USP micronised, Pharmacia & Upjohn, USA) was thoroughly mixed at 10% w/w into each of the silicone parts A and B, degassed and then the two parts injection moulded over an annealed nylon spine and cured at 130°C for approximately 35 seconds.

3.2.4.2.2 Stability Trial Protocol

3.2.4.2.2.1 Batch Production and Sample Selection Procedure

A single production batch of 1-up CIDR®1380 Cattle inserts was manufactured with Wacker Elastosil® LR3004-40 silicone instead of Dow Silastic® Q7-4840 silicone. As the inserts were manufactured they were placed into large polyethylene bags. CIDR® inserts were then selected at random from the large polyethylene bags and packed in lots of two in the closure system. Once packaged, bags of CIDR® inserts were randomly selected, allocated a sample time point and storage condition (real or accelerated) and labeled accordingly.

3.2.4.2.2.2 Closure

Product closure was the same as that used in the Real and Accelerated Stability Trial (see Section 3.2.2.2.2). Product was stored in open bags.

3.2.4.2.2.3 Storage Conditions

Samples were stored under real time (25°C, 60% RH) and accelerated time conditions (40°C, 75% RH) using the equipment and chemicals described in 3.2.2.2.3.

3.2.4.2.2.4 Sampling Plan

Sufficient samples were allocated to real time and accelerated time storage for predefined periods of 0, 3, 6, 9, 12, 18, 24 and 36 months.

3.2.4.2.3 Assessment of Chemical Changes

3.2.4.2.3.1 Potency Assay

Low temperature cure 1-up CIDR®1380 Cattle inserts were prepared for analysis as described in Section 3.2.1.2 and their potency (drug content in skin) was determined as described in Chapter Two, Section 2.2.1 (UV Assay).

3.2.4.2.4 Assessment of Physical Changes

3.2.4.2.4.1 Invitro Drug Release

Low temperature cure 1-up CIDR®1380 Cattle inserts were subjected to the invitro drug release test as described in Chapter Two, Section 2.2.2).

3.2.4.2.5 Invivo Performance

A bioequivalency study was carried out to compare the low temperature cure silicone 1-up CIDR®1380 Cattle insert with the conventional silicone CIDR®1900 Cattle insert over a 10 day insertion period. The study involved 12 ovariectomised Friesian cows (n = 6 per treatment). The study followed the invivo protocol as described in Chapter Two, Section 2.2.5 and plasma samples were collected prior to CIDR® insertion, daily for the 10 days and finally, 6 hours following CIDR® insert removal.

3.3 Results

3.3.1 Stability-Indicating and Potency HPLC Assay Validation

3.3.1.1 Linearity and Range

Calibration curve data obtained on day one of the validation is presented in Table 3.3.1. The combined regression analysis of this data is presented in Table 3.3.2. Determinations of range validation standards are presented in Table 3.3.3.

Table 3.3.1 - Peak areas and regression analysis of A and B calibration working standards in the stability-indicating and potency HPLC assay validation

Concentration (μg/mL)	Standard A Peak Area (UV*sec)	Standard B Peak Area (UV*sec)
4	229212	231802
10	578548	576392
20	1145270	1171457
30	1742761	1737551
40	2326720	2327017
Slope	58268	58170
Intercept	-7477	-1101
r^2	0.9999	1.0000

Table 3.3.2 - Combined regression analysis of both A and B working standards in the stability-indicating and potency HPLC assay validation

	Value	Standard Error	± 95% Confidence Limit	± 99% Confidence Limit
B1	58201.558	141.409	315.342	448.266
B0	-3926.185	3170.424	7070.046	10050.245

Table 3.3.3 - Peak areas and calculated concentrations of range validation standards in the stability-indicating and potency HPLC assay validation

QC Sample	Peak Area (UV*sec)	Calculated Concentration (μg/mL)
4 μg/mL	233900	4.09
4 μg/mL	230280	4.02
40 μg/mL	2337437	40.23
40 μg/mL	2300335	39.59

3.3.1.2 Accuracy

Determinations of accuracy validation standards are presented in Table 3.3.4.

Table 3.3.4 - Analysis of accuracy validation standards in the stability-indicating and potency HPLC assay validation

8 µg/mL	day 0	day 1	day 2	day 3	mean	SD	%RSD	%deviation
Rep. A	7.97	7.75	8.02	7.85	7.90	0.12	1.54	1.28
Rep. B	7.99	7.8	7.93	7.93	7.91	0.08	1.01	1.09
mean	7.98	7.78	7.98	7.89	7.91	0.10	1.21	1.19
SD	0.01	0.04	0.06	0.06	0.01			
%RSD	0.18	0.45	0.80	0.72	0.13			
%Dev.	0.25	2.81	0.31	1.38	1.19			
15 µg/mL	day 0	Day 1	day 2	day 3	mean	SD	%RSD	%deviation
Rep. A	14.83	14.72	14.84	14.82	14.80	0.05	0.37	1.30
Rep. B	15.00	14.75	14.96	14.81	14.88	0.12	0.80	0.79
mean	14.92	14.74	14.90	14.82	14.84	0.08	0.56	1.05
SD	0.12	0.02	0.08	0.00	0.05			
%RSD	0.81	0.12	0.56	0.03	0.36			
%Dev.	0.57	1.76	0.65	1.21	1.05			
35 µg/mL	day 0	day 1	day 2	day 3	mean	SD	%RSD	%deviation
Rep. A	34.83	34.65	34.99	34.63	34.78	0.17	0.49	0.64
Rep. B	34.86	34.74	35.46	34.75	34.95	0.34	0.98	0.14
mean	34.85	34.69	35.23	34.69	34.86	0.25	0.72	0.39
SD	0.02	0.06	0.32	0.09	0.12			
%RSD	0.06	0.18	0.93	0.25	0.36			
%Dev.	0.44	0.88	0.64	0.88	0.39			

3.3.1.3 Precision

Reproducibility precision data (%RSD) are presented in Table 3.3.4 along with accuracy data. Data on the injection repeatability precision are presented in Table 3.3.5.

Table 3.3.5 - Determination of injection repeatability in the stability-indicating and potency HPLC assay validation

Sample	Absorbance	Concentration
15 µg/mL	857114	14.79
15 µg/mL	857229	14.80
15 µg/mL	857844	14.81
15 µg/mL	856535	14.78
15 µg/mL	855794	14.77
15 µg/mL	859369	14.83

3.3.1.4 Ruggedness

Results for calibration curves derived on different days and by different analysts are presented in Table 3.3.6. Results obtained from the analysis of 1-up CIDR®1380 Cattle inserts on multiple days with multiple analysts are presented in Table 3.3.7 to Table 3.3.10.

Table 3.3.6 - Calibration curve ruggedness data from the stability-indicating and potency HPLC assay validation

Regression	Correlation (R^2)	Slope (B1) ($UV.sec.mL.\mu g^{-1}$)	Intercept (B0) (UV.sec)
Analyst 1 Day 1	0.999	55784	-4852
Analyst 1 Day 2	0.999	57093	-993
Analyst 1 Day 3	0.999	57781	-3289
Analyst 2 Day 3	0.999	58999	-13662

Table 3.3.7 - Reproducibility data (1-up CIDR®1380 Batch 7246227 - Day 1) from the stability-indicating and potency HPLC assay validation

Sample	Skin Weight (g)	Peak Area 1 (UV*sec)	Peak Area 2 (UV*sec)	Progesterone (g)	Progesterone % w/w
1	13.4218	840373	826963	1.32	9.80
2	13.2927	838075	846379	1.33	10.00
3	13.4532	863742	966529	1.36	10.14
4	13.3506	859681	858364	1.36	10.02
5	13.4464	844145	846854	1.33	9.92
Mean				**1.34**	**10.00**
SEM				**0.01**	**0.06**
%RSD				**1.50**	**1.50**

Table 3.3.8 - Reproducibility data (1-up CIDR®1380 Batch 7246227 - Day 2) from the stability-indicating and potency HPLC assay validation

Sample	Skin Weight (g)	Peak Area 1 (UV*sec)	Peak Area 2 (UV*sec)	Progesterone (g)	Progesterone % w/w
1	13.4392	857928	856878	1.32	9.79
2	13.3861	868648	884091	1.34	10.04
3	13.4228	859985	826621	1.29	9.64
4	13.3131	883781	868091	1.34	10.10
5	13.4316	867452	881429	1.34	9.99
Mean				**1.33**	**9.91**
SEM				**0.01**	**0.09**
%RSD				**1.70**	**1.93**

Table 3.3.9 - Reproducibility data (1-up CIDR®1380 Batch 7246227 - Day 3) from the stability-indicating and potency HPLC assay validation

Sample	Skin Weight (g)	Peak Area 1 (UV*sec)	Peak Area 2 (UV*sec)	Progesterone (g)	Progesterone % w/w
1	13.4662	862838	864153	1.31	9.75
2	13.4348	874595	865350	1.32	9.84
3	13.3475	812535	808310	1.23	9.23
4	13.4027	842169	850939	1.29	9.60
5	13.3921	854666	857114	1.30	9.72
Mean				**1.29**	**9.63**
SEM				**0.02**	**0.11**
%RSD				**2.75**	**2.47**

Table 3.3.10 - Reproducibility data (1-up CIDR®1380 Batch 7246227 - analyst two) from the stability-indicating and potency HPLC assay validation

Sample	Skin Weight (g)	Peak Area 1 (UV*sec)	Peak Area 2 (UV*sec)	Progesterone (g)	Progesterone % w/w
1	13.4662	895953	877396	1.34	9.92
2	13.4348	886536	894320	1.34	9.98
3	13.3475	815976	820556	1.23	9.24
4	13.4027	861366	859335	1.30	9.67
5	13.3921	865533	871212	1.31	9.77
Mean				**1.30**	**9.72**
SEM				**0.02**	**0.13**
%RSD				**3.29**	**2.99**

3.3.1.5 Limit of quantitation

Data acquired for determination of the limit of quantitation are presented in Table 3.3.11.

Table 3.3.11 - Limit of quantitation determination data from the stability-indicating and potency HPLC assay validation

Sample	Peak Area (UV*sec)	Concentration (μg/mL)
blank	0	0.07
blank	0	0.07
blank	0	0.07
blank	0	0.07
blank	0	0.07
1 μg/mL	56024	1.03
0.1 μg/mL	5769	0.17
0.01 μg/mL	0	0.07

3.3.1.6 Specificity

Determinations of progesterone standards in the presence of analogous compounds or placebo ingredients extracted from silicone are presented in Table 3.3.12.

Table 3.3.12 - Specificity data from the stability-indicating and potency HPLC assay validation

Sample	Peak area (UV*sec)	Concentration (μg/mL)
20 μg/mL in specially denatured alcohol	1156097	19.93
20 μg/mL in silicone extract (normal dilution)	1175375	20.26
Blank silicone extract (normal dilution)	0	0.07
20 μg/mL in analogue mixture	1160259	20.00

3.3.2 Stability Study

3.3.2.1 Manufacture of CIDR®1900 Cattle Inserts and 1-up CIDR®1380 Cattle Inserts

Table 3.3.13 and 3.3.14 contain manufacturing details for CIDR®1900 Cattle inserts and 1-up CIDR®1380 Cattle inserts, respectively, manufactured for the real and accelerated time stability study.

Table 3.3.13 - Manufacturing details of CIDR®1900 Cattle inserts produced for the stability trial

Batch Number	Manufacturing Date
7313	March 1997
7317	March 1997
7324	March 1997

Table 3.3.14 - Manufacturing details of 1-up CIDR®1380 produced for the stability trial

Batch Number	Manufacturing Date
7307	March 1997
7313	March 1997
7317	March 1997

3.3.2.2 Chemical changes

3.3.2.2.1 Potency

3.3.2.2.1.1 CIDR®1900 Cattle Inserts

Results from the analysis by stability-indicating and potency HPLC assay of three production batches of CIDR®1900 Cattle inserts stored under real time and accelerated time conditions in open and closed bags are shown in Tables 3.3.15 through 3.3.26.

Table 3.3.15 - Potency data for CIDR®1900 batch 7313 stored under real time conditions (25°C/60%RH) in open bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.87	1.91	98.21	100.52	99.36
3	1.93	1.93	101.61	101.49	101.55
6	1.84	1.86	96.94	97.85	97.40
9	1.82	1.81	95.81	95.48	95.65
12	1.76	1.83	92.52	96.19	94.36
18	1.91	1.93	100.30	101.65	100.98

Table 3.3.16 - Potency data for CIDR®1900 batch 7317 stored under real time conditions (25°C/60%RH) in open bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.91	1.96	100.64	103.01	101.83
3	1.86	1.93	98.06	101.63	99.85
6	1.93	1.90	101.72	99.87	100.80
9	1.82	1.85	95.67	97.30	96.49
12	1.82	1.80	95.67	94.83	95.25
18	1.89	1.91	99.74	100.32	100.03

Table 3.3.17 - Potency data for CIDR®1900 batch 7324 stored under real time conditions (25°C/60%RH) in open bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.93	1.92	101.84	101.19	101.51
3	1.92	1.90	100.83	99.95	100.39
6	1.86	1.87	98.06	98.35	98.21
9	1.76	1.80	92.57	94.67	93.62
12	1.79	1.80	94.05	94.67	94.36
18	1.88	1.89	99.11	99.46	99.28

Table 3.3.18 - Potency data for CIDR®1900 batch 7313 stored under real time conditions (25°C/60%RH) in closed bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.87	1.91	98.21	100.52	99.36
3	1.93	1.92	101.36	101.09	101.23
6	1.92	1.92	101.25	100.92	101.09
9	1.83	1.82	96.34	95.83	96.09
12	1.83	1.83	96.42	96.53	96.47
18	1.92	1.89	101.21	99.70	100.46

Table 3.3.19 - Potency data for CIDR®1900 batch 7317 stored under real time conditions (25°C/60%RH) in closed bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.91	1.96	100.64	103.01	101.83
3	1.95	1.91	102.62	100.35	101.49
6	1.83	1.88	96.12	99.05	97.59
9	1.83	1.79	96.16	94.26	95.21
12	1.80	1.85	94.92	97.49	96.20
18	1.92	1.86	100.84	97.81	99.33

Table 3.3.20 - Potency data for CIDR®1900 batch 7324 stored under real time conditions (25°C/60%RH) in closed bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.93	1.92	101.84	101.19	101.51
3	1.87	1.91	98.42	100.48	99.45
6	1.83	1.90	96.40	99.98	98.19
9	1.83	1.83	96.21	96.35	96.28
12	1.75	1.79	91.95	94.00	92.97
18	1.92	1.90	100.93	99.99	100.46

Table 3.3.21 - Potency data for CIDR®1900 batch 7313 stored under accelerated time conditions (40°C/75%RH) in open bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.87	1.91	98.21	100.52	99.36
3	1.97	1.94	103.64	101.97	102.81
6	1.82	1.81	95.88	95.44	95.66
9	1.84	1.91	96.88	100.39	98.63
12	1.87	1.86	98.44	97.64	98.04
18	1.83	1.87	96.27	98.32	97.29

Table 3.3.22 - Potency data for CIDR®1900 batch 7317 stored under accelerated time conditions (40°C/75%RH) in open bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.91	1.96	100.64	103.01	101.83
3	1.98	1.90	103.95	99.91	101.93
6	1.80	1.82	94.62	95.82	95.22
9	1.85	1.79	97.37	93.98	95.68
12	1.84	1.85	96.94	97.18	97.06
18	1.90	1.85	100.14	97.50	98.82

Table 3.3.23 - Potency data for CIDR®1900 batch 7324 stored under accelerated time conditions (40°C/75%RH) in open bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.93	1.92	101.84	101.19	101.51
3	1.91	1.90	100.56	99.82	100.19
6	1.78	1.70	93.89	89.54	91.72
9	1.80	1.82	94.92	95.77	95.34
12	1.77	1.78	93.30	93.80	93.55
18	1.85	1.89	97.15	99.51	98.33

Table 3.3.24 - Potency data for CIDR®1900 batch 7313 stored under accelerated time conditions (40°C/75%RH) in closed bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.87	1.91	98.21	100.52	99.36
3	1.91	1.95	100.61	102.54	101.58
6	1.79	1.79	93.95	94.39	94.17
9	1.86	1.89	97.71	99.56	98.63
12	1.84	1.90	97.01	99.86	98.43
18	1.86	1.89	97.87	99.60	98.73

Table 3.3.25 - Potency data for CIDR®1900 batch 7317 stored under accelerated time conditions (40°C/75%RH) in closed bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.91	1.96	100.64	103.01	101.83
3	1.92	1.97	100.99	103.46	102.23
6	1.79	1.87	93.98	98.28	96.13
9	1.80	1.84	94.74	96.76	95.75
12	1.82	1.89	95.92	99.26	97.59
18	1.90	1.88	100.23	98.69	99.46

Table 3.3.26 - Potency data for CIDR®1900 batch 7324 stored under accelerated time conditions (40°C/75%RH) in closed bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.93	1.92	101.84	101.19	101.51
3	1.92	1.88	101.30	99.18	100.24
6	1.84	1.78	96.72	93.60	95.16
9	1.83	1.83	96.29	96.57	96.43
12	1.80	1.82	94.88	95.93	95.41
18	1.90	1.85	99.94	97.13	98.53

3.3.2.2.1.2 1-up CIDR®1380 Cattle Inserts

Results from the analysis by a stability-indicating and potency HPLC assay of three production batches of 1-up CIDR®1380 Cattle inserts stored under real time and accelerated time conditions in open and closed bags are shown in Tables 3.3.27 through 3.3.38.

Table 3.3.27 - Potency data for 1-up CIDR®1380 batch 7307 stored under real time conditions (25°C/60%RH) in open bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.39	1.39	100.46	100.68	100.57
0.5	1.38	1.39	100.01	100.77	100.39
1	1.32	1.35	95.83	97.81	96.82
2	1.32	1.38	95.91	100.04	97.98
2.5	1.34	1.34	97.19	96.92	97.06
3	1.40	1.39	101.12	100.55	100.84
6	1.36	1.32	98.89	95.52	97.21
9	1.32	1.36	95.41	98.52	96.96
12	1.29	1.26	93.54	91.03	92.28
18	1.26	1.30	91.24	94.28	92.76

Table 3.3.28 - Potency data for 1-up CIDR®1380 batch 7313 stored under real time conditions (25°C/60%RH) in open bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.36	1.42	98.23	102.72	100.47
0.5	1.40	1.38	101.69	100.08	100.89
1	1.39	1.35	100.53	98.08	99.31
2	1.36	1.38	98.40	100.04	99.22
2.5	1.37	1.33	99.53	96.17	97.85
3	1.35	1.36	97.83	98.32	98.07
6	1.31	1.34	95.23	97.01	96.12
9	1.36	1.35	98.30	97.65	97.98
12	1.27	1.29	92.04	93.16	92.60
18	1.29	1.27	93.77	91.68	92.72

Table 3.3.29 - Potency data for 1-up CIDR®1380 batch 7317 stored under real time conditions (25°C/60%RH) in open bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.40	1.39	101.39	100.58	100.98
0.5	1.37	1.36	98.95	98.34	98.64
1	1.37	1.35	99.01	98.19	98.60
2	1.37	1.36	99.47	98.73	99.10
2.5	1.38	1.36	99.94	98.54	99.24
3	1.38	1.39	99.84	100.61	100.22
6	1.34	1.34	97.20	97.15	97.17
9	1.37	1.39	99.14	100.66	99.90
12	1.27	1.26	92.07	91.65	91.86
18	1.28	1.25	93.09	90.69	91.89

Table 3.3.30 - Potency data for 1-up CIDR®1380 batch 7307 stored under real time conditions (25°C/60%RH) in closed bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.39	1.39	100.46	100.68	100.57
0.5	1.38	1.39	99.67	101.07	100.37
1	1.39	1.37	100.92	99.16	100.04
2	1.35	1.35	97.97	97.69	97.83
2.5	1.37	1.35	99.38	97.95	98.66
3	1.39	1.38	100.48	99.99	100.24
6	1.34	1.37	96.79	99.28	98.04
9	1.32	1.34	95.65	96.93	96.29
12	1.28	1.27	92.47	91.68	92.07
18	1.28	1.27	92.65	91.70	92.18

Table 3.3.31 - Potency data for 1-up CIDR®1380 batch 7313 stored under real time conditions (25°C/60%RH) in closed bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.36	1.42	98.23	102.72	100.47
0.5	1.37	1.39	99.28	100.92	100.10
1	1.36	1.37	98.80	99.44	99.12
2	1.40	1.35	101.20	97.62	99.41
2.5	1.38	1.37	99.87	98.99	99.43
3	1.38	1.29	100.34	93.64	96.99
6	1.33	1.36	96.49	98.24	97.36
9	1.34	1.34	96.87	96.85	96.86
12	1.29	1.29	93.80	93.16	93.48
18	1.31	1.31	95.21	94.77	94.99

Table 3.3.32 - Potency data for 1-up CIDR®1380 batch 7317 stored under real time conditions (25°C/60%RH) in closed bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.40	1.39	101.39	100.58	100.98
0.5	1.38	1.40	100.16	101.33	100.75
1	1.38	1.40	99.93	101.70	100.82
2	1.41	1.39	102.48	100.96	101.72
2.5	1.37	1.38	98.98	99.96	99.47
3	1.38	1.39	99.80	100.41	100.10
6	1.30	1.34	94.34	97.05	95.70
9	1.36	1.34	98.68	97.46	98.07
12	1.28	1.29	92.90	93.53	93.21
18	1.29	1.31	93.61	95.28	94.45

Table 3.3.33 - Potency data for 1-up CIDR®1380 batch 7307 stored under accelerated time conditions (40°C/75%RH) in open bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.39	1.39	100.46	100.68	100.57
0.5	1.36	1.39	98.59	100.42	99.51
1	1.30	1.36	94.14	98.51	96.33
2	1.36	1.34	98.40	96.94	97.67
2.5	1.33	1.37	96.64	99.29	97.97
3	1.26	1.29	91.27	93.46	92.37
6	1.31	1.33	95.16	96.48	95.82
9	1.27	1.24	92.26	89.98	91.12
12	1.16	1.20	84.32	87.31	85.82
18	1.26	1.30	91.42	93.89	92.65

Table 3.3.34 - Potency data for 1-up CIDR®1380 batch 7313 stored under accelerated time conditions (40°C/75%RH) in open bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.36	1.42	98.23	102.72	100.47
0.5	1.37	1.35	99.53	97.70	98.62
1	1.38	1.36	99.98	98.68	99.33
2	1.37	1.36	99.17	98.20	98.69
2.5	1.36	1.36	98.29	98.54	98.41
3	1.37	1.36	99.27	98.25	98.76
6	1.34	1.25	96.81	90.38	93.60
9	1.29	1.31	93.21	94.90	94.05
12	1.23	1.21	89.01	87.78	88.40
18	1.25	1.34	90.70	97.18	93.94

Table 3.3.35 - Potency data for 1-up CIDR®1380 batch 7317 stored under accelerated time conditions (40°C/75%RH) in open bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.40	1.39	101.39	100.58	100.98
0.5	1.33	1.33	96.64	96.49	96.56
1	1.32	1.33	95.39	96.09	95.74
2	1.35	1.31	97.92	95.13	96.53
2.5	1.31	1.27	95.22	91.82	93.52
3	1.32	1.25	95.70	90.90	93.30
6	1.27	1.36	92.33	98.56	95.44
9	1.27	1.31	92.29	95.08	93.68
12	1.16	1.22	84.09	88.57	86.33
18	1.29	1.32	93.12	96.01	94.57

Table 3.3.36 - Potency data for 1-up CIDR®1380 batch 7307 stored under accelerated time conditions (40°C/75%RH) in closed bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.39	1.39	100.46	100.68	100.57
0.5	1.31	1.37	94.99	98.95	96.97
1	1.36	1.36	98.59	98.27	98.43
2	1.35	1.32	97.57	95.96	96.76
2.5	1.30	1.35	94.43	98.15	96.29
3	1.31	1.34	95.15	97.18	96.17
6	1.29	1.34	93.81	97.13	95.47
9	1.29	1.32	93.69	95.59	94.64
12	1.22	1.21	88.64	87.74	88.19
18	1.31	1.29	94.66	93.58	94.12

Table 3.3.37 - Potency data for 1-up CIDR®1380 batch 7313 stored under accelerated time conditions (40°C/75%RH) in closed bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.36	1.42	98.23	102.72	100.47
0.5	1.37	1.36	99.04	98.56	98.80
1	1.30	1.36	94.19	98.33	96.26
2	1.35	1.39	97.59	100.88	99.24
2.5	1.32	1.32	95.90	95.44	95.67
3	1.34	1.32	97.38	95.35	96.36
6	1.27	1.23	91.97	88.79	90.38
9	1.31	1.31	94.73	94.71	94.72
12	1.26	1.28	91.42	92.92	92.17
18	1.31	1.29	94.67	93.42	94.05

Table 3.3.38 - Potency data for 1-up CIDR®1380 batch 7317 stored under accelerated time conditions (40°C/75%RH) in closed bags determined using the stability-indicating and potency HPLC assay

Time (months)	Absolute Potency (g)		% label claim		
	Rep 1	Rep 2	Rep 1	Rep 2	Mean
0	1.40	1.39	101.39	100.58	100.98
0.5	1.40	1.40	101.15	101.76	101.46
1	1.40	1.34	101.22	96.99	99.11
2	1.33	1.35	96.61	98.16	97.39
2.5	1.30	1.34	93.99	97.38	95.68
3	1.32	1.32	95.45	95.88	95.66
6	1.32	1.36	95.88	98.62	97.25
9	1.28	1.31	93.10	94.74	93.92
12	1.24	1.20	89.83	87.21	88.52
18	1.26	1.33	91.45	96.10	93.78

3.3.2.2.2 Degradation Products

3.3.2.2.2.1 CIDR®1900 Cattle Inserts

No degradation products were observed on any of the HPLC chromatograms.

3.3.2.2.2.2 1-up CIDR®1380 Cattle Inserts

No degradation products were observed on any of the HPLC chromatograms.

3.3.2.3 Physical Changes

3.3.2.3.1 Physical Observations

3.3.2.3.1.1 CIDR®1900 Cattle Inserts

The three production batches of CIDR®1900 Cattle inserts stored under real time and accelerated time conditions in open and closed bags were all the same in appearance. Typical observations of their physical characteristics are shown in Table 3.3.39.

Table 3.3.39 - Physical observations for CIDR®1900, batches 7313, 7317 and 7324 stored under both real time conditions (25°C/60%RH) and accelerated time conditions (40°C/75%RH) in open and in closed bags

Sample Time (Months)	Sample	Colour	Surface Progesterone
0	1	Off White	Absent
0	2	Off White	Absent
6	1	Off White	Slight
6	2	Off White	Slight
12	1	Off White	Slight
12	2	Off White	Slight

3.3.2.3.1.2 1-up CIDR®1380 Cattle Inserts

The three production batches of 1-up CIDR®1380 Cattle inserts stored under real time and accelerated time conditions in open and closed bags were all the same in appearance. Typical observations of their physical characteristics are shown in Table 3.3.40.

Table 3.3.40 - Physical observations for 1-up CIDR®1380, batches 7307, 7313 and 7317, stored under both real time conditions (25°C/60%RH) and accelerated time conditions (40°C/75%RH) in both open and closed bags

Sample Time (Months)	Sample	Colour	Surface Progesterone
0	1	Off White	Absent
0	2	Off White	Absent
6	1	Off White	Slight
6	2	Off White	Slight
12	1	Off White	Slight
12	2	Off White	Slight

3.3.2.3.2 Invitro Drug Release

3.3.2.3.2.1 CIDR®1900 Cattle Inserts

Results from the invitro drug release testing of three production batches of CIDR®1900 Cattle inserts stored under real time and accelerated time conditions in open and closed bags are shown in Tables 3.3.41 through 3.3.44.

Table 3.3.41 - Invitro drug release test results for CIDR®1900 stored under real time conditions in open bags

Batch	Replicate	Initial		12 Months	
		Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)
7313	Replicate 1	1231	173	1256	684
7313	Replicate 2	1098	341	1211	724
7313	**Mean**	**1165**	**257**	**1234**	**704**
7317	Replicate 1	1240	425	1049	1141
7317	Replicate 2	1187	282	1269	1116
7317	**Mean**	**1214**	**353**	**1159**	**1129**
7324	Replicate 1	1273	280	1371	1785
7324	Replicate 2	1225	583	1129	1946
7324	**Mean**	**1249**	**431**	**1250**	**1865**

Table 3.3.42- Invitro drug release test results for CIDR®1900 stored under real time conditions in closed bags

Batch	Replicate	Initial		12 Months	
		Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)
7313	Replicate 1	1231	173	1247	528
7313	Replicate 2	1098	341	1113	826
7313	**Mean**	**1165**	**257**	**1180**	**677**
7317	Replicate 1	1240	425	1083	529
7317	Replicate 2	1187	282	1299	1290
7317	**Mean**	**1214**	**353**	**1191**	**910**
7324	Replicate 1	1273	280	1053	708
7324	Replicate 2	1225	583	1220	934
7324	**Mean**	**1249**	**431**	**1161**	**821**

Table 3.3.43 - Invitro drug release test results for CIDR®1900 stored under accelerated time conditions in open bags

Batch	Replicate	Initial		12 Months	
		Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)
7313	Replicate 1	1231	173	1263	433
7313	Replicate 2	1098	341	1169	861
7313	**Mean**	**1165**	**257**	**1216**	**647**
7317	Replicate 1	1240	425	1077	1008
7317	Replicate 2	1187	282	1158	1064
7317	**Mean**	**1214**	**353**	**1118**	**1036**
7324	Replicate 1	1273	280	1270	1440
7324	Replicate 2	1225	583	1259	1380
7324	**Mean**	**1249**	**431**	**1264**	**1410**

Table 3.3.44 - Invitro drug release test results for CIDR®1900 stored under accelerated time conditions in closed bags

Batch	Replicate	Initial		12 Months	
		Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)
7313	Replicate 1	1231	173	1328	497
7313	Replicate 2	1098	341	1182	825
7313	**Mean**	**1165**	**257**	**1255**	**661**
7317	Replicate 1	1240	425	1107	583
7317	Replicate 2	1187	282	1230	956
7317	**Mean**	**1214**	**353**	**1169**	**770**
7324	Replicate 1	1273	280	1269	1971
7324	Replicate 2	1225	583	1209	825
7324	**Mean**	**1249**	**431**	**1239**	**1398**

3.3.2.3.2.2 1-up CIDR®1380 Cattle Inserts

Results from the invitro drug release testing of three production batches of 1-up CIDR®1380 Cattle inserts stored under real time and accelerated time conditions in open and closed bags are shown in Tables 3.3.45 through 3.3.48.

Table 3.3.45 - Invitro drug release test results for 1-up CIDR®1380 stored under real time conditions in open bags

Batch	Replicate	Initial		3 Month		6 Month		12 Month	
		Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)
7307	1	1586	1402	1661	1057	1343	336	1211	443
7307	2	1345	-9	1292	446	1275	268	1297	447
7307	**Mean**	**1465**	**697**	**1476**	**751**	**1309**	**302**	**1254**	**445**
7313	1	1410	318	1613	2678	1364	1301	1373	1754
7313	2	1512	341	1162	72	1405	67	1374	1495
7313	**Mean**	**1461**	**329**	**1388**	**1375**	**1385**	**684**	**1373**	**1624**
7317	1	1474	513	1250	301	1429	1668	1328	1274
7317	2	1503	-62	1291	307	1470	1094	1456	1515
7317	**Mean**	**1489**	**225**	**1271**	**304**	**1449**	**1381**	**1392**	**1394**

Table 3.3.46 - Invitro drug release test results for 1-up CIDR®1380 stored under real time conditions in closed bags

Batch	Replicate	Initial		3 Month		6 Month		12 Month	
		Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)
7307	1	1586	1402	1619	658	1619	2234	1399	879
7307	2	1345	-9	1361	67	1500	1518	1374	1156
7307	**Mean**	**1465**	**697**	**1490**	**363**	**1560**	**1876**	**1387**	**1017**
7313	1	1410	318	1334	1237	1360	1511	1300	1904
7313	2	1512	341	1499	1687	1332	1052	1235	1625
7313	**Mean**	**1461**	**329**	**1417**	**1462**	**1346**	**1281**	**1268**	**1765**
7317	1	1474	513	1434	614	1467	727	1265	1528
7317	2	1503	-62	1312	468	1524	1147	1163	846
7317	**Mean**	**1489**	**225**	**1373**	**541**	**1495**	**937**	**1214**	**1187**

Table 3.3.47 - Invitro drug release test results for 1-up CIDR®1380 stored under accelerated time conditions in open bags

Batch	Replicate	Initial		3 Month		6 Month		12 Month	
		Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)
7307	1	1586	1402	1450	351	1386	804	1470	1603
7307	2	1345	-9	1292	400	1257	383	1238	544
7307	**Mean**	**1465**	**697**	**1371**	**376**	**1322**	**594**	**1354**	**1074**
7313	1	1410	318	1258	315	1252	925	1330	1427
7313	2	1512	341	1372	588	1327	1077	1239	982
7313	**Mean**	**1461**	**329**	**1315**	**451**	**1290**	**1001**	**1285**	**1205**
7317	1	1474	513	1396	1412	1392	1485	1341	887
7317	2	1503	-62	1459	484	1398	816	1330	1176
7317	**Mean**	**1489**	**225**	**1428**	**948**	**1395**	**1151**	**1336**	**1031**

Table 3.3.48 - Invitro drug release test results for 1-up CIDR®1380 stored under accelerated time conditions in closed bags

Batch	Replicate	Initial		3 Month		6 Month		12 Month	
		Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)
7307	1	1586	1402	1388	303	1467	1164	1594	1348
7307	2	1345	-9	1301	433	1446	640	1323	710
7307	**Mean**	**1465**	**697**	**1345**	**368**	**1456**	**902**	**1458**	**1029**
7313	1	1410	318	1367	1048	1471	1401	1274	1816
7313	2	1512	341	1315	1197	1435	1267	1233	1629
7313	**Mean**	**1461**	**329**	**1341**	**1123**	**1453**	**1334**	**1254**	**1723**
7317	1	1474	513	1337	1622	1410	1004	1275	1186
7317	2	1503	-62	1480	4066	1463	905	1235	1377
7317	**Mean**	**1489**	**225**	**1409**	**2844**	**1437**	**954**	**1255**	**1281**

3.3.3 Manufacturing Study

3.3.3.1 Manufacture of 1-up CIDR®1380 Cattle Inserts

Table 3.3.49 contains manufacturing details for 1-up CIDR®1380 Cattle inserts fabricated for use in the manufacturing study.

Table 3.3.49 - Manufacturing details for 1-up CIDR®1380 fabricated for use in the manufacturing study

Batch Number	Date of Manufacture
7D22	December 1997

3.3.3.2 Surface Progesterone

Tables 3.3.50 and 3.3.51 contain surface progesterone data determined in the manufacturing study.

Table 3.3.50 - Amount of progesterone determined on the surface of 1-up CIDR®1380 manufactured at different temperatures and cure times

Time (Months)	Replicate	Amount of surface progesterone (mg)							
		130 opt	130 + 60s	160 opt	190 - 15s	190 opt	190 + 15s	190 + 30s	210 opt
0	1	26	37	18	22	29	28	69	70
0	2	44	23	21	23	35	30	104	81
0	**mean**	**35**	**30**	**19**	**23**	**32**	**29**	**86**	**76**
3	1	50	110	138	145	233	196	306	348
3	2	44	135	192	244	362	192	315	247
3	**mean**	**47**	**123**	**165**	**195**	**297**	**194**	**311**	**297**
12	1	79	126	178		213	185	270	188
12	2	46	124	148	219		207	250	190
12	**mean**	**62**	**125**	**163**	**219**	**213**	**196**	**260**	**189**

Table 3.3.51 - Amount of progesterone determined on the surface of 1-up CIDR®1380 manufactured with spines annealed for different lengths of time

Time (Months)	Replicate	Amount of surface progesterone (mg)			
		no anneal	2.5h anneal	4.5h anneal	7.5h anneal
0	1	40	35	40	29
0	2	37	41	39	35
0	**mean**	**39**	**38**	**39**	**32**
3	1	79	221	309	233
3	2	104	145	290	
3	**mean**	**91**	**183**	**299**	**233**
12	1	182	179	308	213
12	2	124	170	331	
12	**mean**	**153**	**175**	**319**	**213**

3.3.3.3 Progesterone in Silicone Skin

Tables 3.3.52 and 3.3.53 contain skin progesterone data determined in the manufacturing study.

Table 3.3.52 - Amount of progesterone determined in the skin of 1-up CIDR®1380 manufactured at different temperatures and cure times

Time (Months)	Replicate	Observed skin progesterone for each variant (mg)							
		130 opt	130 + 60s	160 opt	190 - 15s	190 opt	190 + 15s	190 + 30s	210 opt
0	1	1351	1341	1359	1358	1350	1351	1310	1310
0	2	1333	1355	1358	1357	1344	1348	1277	1297
0	**mean**	1342	1348	1359	1357	1347	1350	1293	1303
3	1	1329	1260	1231	1216	1110	1163	1038	991
3	2	1334	1236	1169	1107	977	1163	1034	1105
3	**mean**	1332	1248	1200	1161	1043	1163	1036	1048
12	1	1300	1246	1187	1046	1141	1180	1068	1173
12	2	1332	1248	1220	1121	1199	1154	1093	1173
12	**mean**	1316	1247	1203	1083	1170	1167	1081	1173

Table 3.3.53 - Amount of progesterone determined in the skin of 1-up CIDR®1380 manufactured with spines annealed for different lengths of time

Time (Months)	Replicate	Amount of skin progesterone (mg)			
		no anneal	2.5h anneal	4.5h anneal	7.5h anneal
0	1	1339	1344	1340	1350
0	2	1341	1337	1341	1344
0	**mean**	1340	1341	1341	1347
3	1	1290	1137	1036	1110
3	2	1263	1220	1056	977
3	**mean**	1277	1179	1046	1043
12	1	1176	1184	1042	1141
12	2	1238	1190	1020	1199
12	**mean**	1207	1187	1031	1170

3.3.4 Potential Future Manufacturing Strategies

3.3.4.1 Alternate Spine Material

3.3.4.1.1 Manufacture of 1-up CIDR®1380 Cattle Inserts (polyester spines)

Table 3.3.54 contains manufacturing details for 1-up CIDR®1380 Cattle inserts manufactured for the study.

Table 3.3.54 - Manufacturing details for 1-up CIDR®1380 moulded for the alternate spine material study

Batch Number	Date of Manufacture
7313	March 1997

3.3.4.1.2 Chemical Changes

3.3.4.1.2.1 Potency

Results from potency assay of the silicone skin of the 1-up CIDR®1380 Cattle inserts manufactured using polyester spines are shown in Table 3.3.55.

Table 3.3.55 - Amount of progesterone determined in the skins of 1-up CIDR®1380 manufactured with polyester spines as a function of time

Time (Days)	Skin Progesterone Content (mg)			
	Replicate 1	Replicate 2	Replicate 3	Mean
0	1359	1321	1302	1327
1	1319	1319	1320	1319
2	1330	1313	1258	1300
4	1327	1342	1309	1326
10	1274	1371	1260	1302
14	1289	1280	1332	1300
18	1286	1336	1288	1303
24	1361	1271	1363	1332
31	1307	1320	1310	1312
74	1334	1330	1229	1298
128	1383	1352	1362	1366
257	1344	1356	1382	1361
376	1350	1331	1327	1336

3.3.4.1.2.2 Progesterone in the Polyester Spine

Results from the assessment of progesterone in the polyester spine of 1-up CIDR®1380 Cattle inserts manufactured with polyester spines are shown in Table 3.3.56.

Table 3.3.56 - Amount of progesterone determined in the polyester spine as a function of time

Time (Days)	Polyester Spine Progesterone Content (mg)			
	Replicate 1	Replicate 2	Replicate 3	Mean
0	58	58	63	60
1	63	60	62	62
2	58	60	54	57
4	59	60	50	56
10	53	55	57	55
14	53	53	51	52
18	49	52	61	54
24	52	50	54	52
31	53	48	52	51
74	47	46	49	48
128	56	53	54	55
257	48	43	44	45
376	44	46	46	45

3.3.4.2 Low Temperature Cure Silicone

3.3.4.2.1 Manufacture of 1-up CIDR®1380 Cattle Inserts (low temperature cure silicone)

Table 3.3.57 contains manufacturing details for 1-up CIDR®1380 Cattle inserts manufactured for this study.

Table 3.3.57 - Manufacturing details for 1-up CIDR®1380 moulded for the low temperature cure silicone study

Batch Number	Date of Manufacture
8902	September 1998

3.3.4.2.2 Chemical Changes

3.3.4.2.2.1 Potency

Results from potency assay of the low temperature cure silicone 1-up CIDR®1380 Cattle inserts stored under real and accelerated conditions are shown in Tables 3.3.58 and 3.3.59. Note that only twelve months data was available at the time of writing this thesis.

Table 3.3.58 - Results from the potency assay of low temperature cure 1-up CIDR®1380 stored under real time conditions (25°C/60%RH) in open bags

Time (months)	Absolute load (g)			% Label claim			
	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Mean
0	1.42	1.41	1.42	102.73	102.03	102.69	102.49
3	1.40	1.38	1.41	101.72	99.97	102.07	101.25
6	1.42	1.44		102.97	104.04		103.51
9	1.40	1.42		100.05	101.29		100.67
12	1.40	1.38		99.67	98.79		99.23

Table 3.3.59 - Results from the potency assay of low temperature cure 1-up CIDR®1380 stored under accelerated time conditions (40°C/75%RH) in open bags

Time (months)	Absolute load (g)			% Label claim			
	Replicate 1	Replicate 2	Replicate 3	Replicate 1	Replicate 2	Replicate 3	Mean
0	1.42	1.41	1.42	102.73	102.03	102.69	102.49
3	1.42	1.38	1.39	103.00	99.91	100.56	101.16
6	1.41	1.39		102.23	100.94		101.59
9	1.36	1.42		97.27	101.31		99.29
12	1.39	1.42		99.52	101.43		100.48

3.3.4.2.3 Physical Changes

3.3.4.2.3.1 Invitro Drug Release

Tables 3.3.60 and 3.3.61 contain invitro drug release data determined for 1-up CIDR®1380 Cattle inserts manufactured using low temperature cure silicone. Note that only six months data was available at the time of writing this thesis.

Table 3.3.60 - Invitro drug release test results for low temperature cure 1-up CIDR®1380 stored under real time storage conditions in open bags

Replicate	Initial		3 Months		6 Months	
	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)
1	1085	24	942	216	1083	236
2	1011	19	999	56	1096	254
Mean	1048	21	971	136	1089	245

Table 3.3.61 - Invitro drug release test results for low temperature cure 1-up CIDR®1380 stored under accelerated time storage conditions

Replicate	Initial		3 Months		6 Months	
	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)	Rate ($\mu g/cm^2/hr^{0.5}$)	Intercept ($\mu g/cm^2$)
1	1085	24	976	46	1041	491
2	1011	19	1198	40	1254	172
Mean	1048	21	1087	43	1148	332

3.3.4.2.4 Invivo performance

Table 3.3.62 contains plasma progesterone data determined in the invivo trial comparing the performance of the low temperature cure silicone 1-up CIDR®1380 Cattle insert with the CIDR®1900 Cattle insert.

Table 3.3.62 - Plasma progesterone levels in ovariectomised cows following insertion of CIDR®1900 (Dow silicone) and low temperature cure 1-up CIDR®1380 (Wacker silicone)

CIDR type	Plasma progesterone level on given day (ng/mL)										
	0	1	2	3	4	5	6	7	8	9	10
Dow	0.00	3.33	3.27	4.03	4.37	2.76	3.26	2.64	3.09	2.70	2.15
Dow	0.00	3.75	3.56	4.27	4.53	4.61	3.40	3.49	3.76	2.43	3.38
Dow	0.00	3.22	2.43	2.69	2.77	3.74	2.38	2.51	2.43	2.06	1.99
Dow	0.00	4.12	3.51	3.38	3.06	3.05	2.44	2.10	2.51	2.25	1.70
Dow	0.00	2.89	2.81	3.30	2.61	3.06	2.18	2.50	2.34	2.38	2.35
Dow	0.00	2.86	2.98	3.32	2.74	2.92	2.97	2.41	1.75	2.86	2.27
Wacker	0.00	4.62	4.56	4.02	3.55	2.88	3.27	2.76	3.35	2.35	2.42
Wacker	0.00	3.67	3.34	3.36	3.24	3.65	2.50	2.68	2.23	2.08	2.95
Wacker	0.00	3.55	2.84	3.56	3.59	2.74	2.59	2.30	3.21	2.07	1.90
Wacker	0.00	3.58	3.24	3.83	3.47	2.93	2.90	1.94	2.36	2.86	2.23
Wacker	0.04	4.44	5.21	3.42	3.01	3.13	2.77	2.45	2.84	2.21	2.18
Wacker	0.00	4.47	4.09	3.28	3.08	3.28	3.25	2.45	1.89	2.89	2.14

3.4 Discussion

3.4.1 Validation of Stability-indicating HPLC Assay

3.4.1.1 Linearity and range

A calibration curve should be linear and proportional, therefore validation specifications for acceptable linearity are based upon both the correlation coefficient and the intercept of the regression line[226]. Correlation coefficients should be >0.999 and intercept values should be close enough to zero to allow an acceptable limit of quantitation[226]. In terms of the range, for the method to be considered validated, standards at the high and low levels which define the method range should be determined with a bias of <4%[226]. Using standards at 27, 67, 133, 200 and 267% of the typical target value, the developed method yielded linear regressions with r^2 values of >0.999 and acceptably low intercept values (Table 3.3.1). The combined regression analysis line (Table 3.3.2) produced accurate (2.25% deviation at low level and 0.58% deviation at high level) determinations of the concentrations of the validation standards at the extremities of the calibration range. These results validated the range of the developed method to be 4 to 40 μg/mL (Table 3.3.3).

3.4.1.2 Accuracy

An accurate method is defined as one which allows the determination of analyte at between 96 and 104% of its true value[226]. Determinations of 8, 15 and 35 μg/mL validation standards using the developed method yielded results for each standard which exhibited <3% deviation from its known concentration. These results validated the accuracy of the developed method.

3.4.1.3 Precision

A precise method should be repeatable and reproducible with relative standard deviations about the mean result of <2%[226]. Results for 5 replicate analyses of the same standard using the developed method exhibited a 0.15% relative standard deviation from their mean result (Table 3.3.5). Intra and inter-day repeat determinations of 8, 15 and 35 μg/mL standards yielded mean results with relative standard deviations <2% (Table 3.3.4). These results validated the precision (repeatability and reproducibility) of the developed method.

3.4.1.4 Ruggedness

A rugged method should allow determination of results for the same product on any day and by any analyst with a relative standard deviation about the mean result of <4%[226]. Calibration curves derived on different days and by different analysts using the developed method were linear and proportional (Table 3.3.8). Interday and interanalyst potency determinations of 1-up CIDR®1380 Cattle inserts yielded results with observed relative standard deviations of <3%. These results confirmed that the developed method was rugged.

3.4.1.5 Limit of quantitation

The limit of quantitation of a stability-indicating method should be sufficiently low to determine significant quantities of degradation or impurity compounds should they occur in the analyte. Limit of quantitation was determined to be 1 μg/mL (Table 3.3.6) which is suitably sensitive for the intended study.

3.4.1.6 Specificity

A stability-indicating method should allow quantitation of the target compound without interference from any degradation, impurity or placebo components of the analyte. Absence of interference is confirmed by the accurate (<2% bias) determination of a standard in the presence of possible interferences[226]. Results indicated that progesterone standards could be quantitated with <2% bias in the presence of analogous compounds or placebo ingredients of the silicone matrix (Table 3.3.7). In addition, analysis of blank silicone extracts showed no interfering peaks. These results confirm that the developed method is stability-indicating and specific.

3.4.2 Prediction of Shelf Life

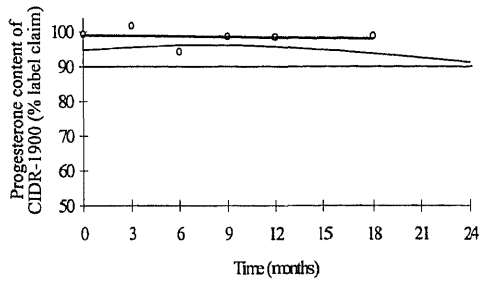
A veterinary product will typically be considered stable over a given period of time if its drug content does not deviate ±10% from its labeled potency[226, 234]. The prediction of the shelf life of a veterinary product can be estimated from data generated under accelerated (40°C, 75% RH) conditions using a one-sided lower 95% confidence interval[234]. The potency data is plotted against time and subjected to linear regression. The one-sided lower 95% confidence interval is

then applied to this regression line. The time at which the one-sided lower 95% confidence interval line crosses the 90% of labeled potency line is multiplied by two to give the predicted shelf life[235].

This approach assumes that the degradation process occurs in accordance with zero order kinetics. In our studies, degradation of the progesterone may follow kinetics of a more complex nature. However, in our case, the validity of using an alternate degradation kinetics equation as a predictor for the product shelf life would be difficult to justify given the limited number of data points and the limited amount of degradation that has occurred over the period of investigation.

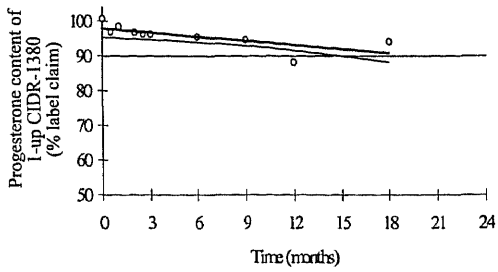
Potency data for three batches each of CIDR®1900 Cattle inserts and 1-up CIDR®1380 Cattle inserts stored under accelerated conditions in closed bags ("in use conditions") are shown in Figure 3.4.1. Each profile has been subjected to linear regression and a one-sided lower 95% confidence interval has been applied to the regression line. The predicted shelf-life expiration times for each batch have been calculated from the intercept of the one-sided lower 95% confidence interval line with the 90% of labeled potency line. These are shown in Table 3.4.1.

CIDR®1900 Cattle insert accelerated time closed bag

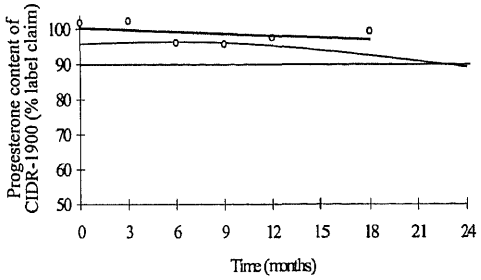


Batch 7313

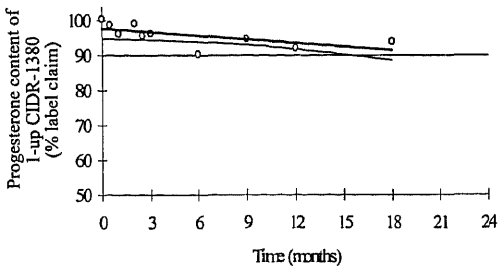
1-up CIDR®1380 Cattle insert accelerated time closed bag



Batch 7307



Batch 7317



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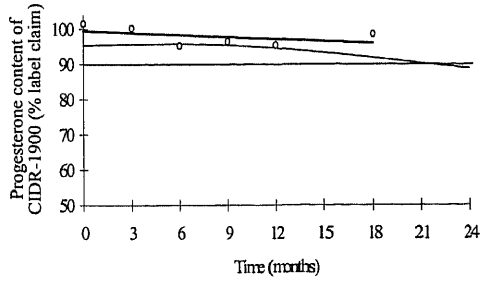
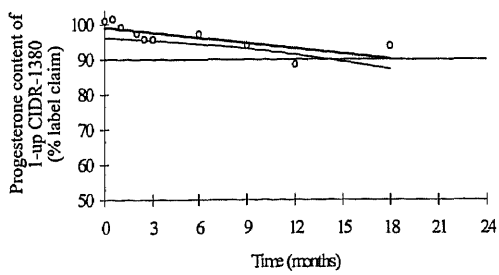


Batch 7324



Batch 7317

Figure 3.4.1 - Effect of time on the potency of CIDR®1900 Cattle inserts and 1-up CIDR®1380 Cattle inserts (expressed as % label claim) stored under accelerated time conditions (40°C/75%RH) in closed bags

Table 3.4.1 – One sided lower 95% confidence interval data for CIDR®1900 Cattle inserts and 1-up CIDR®1380 Cattle inserts stored under accelerated conditions (40°C/75%RH) in closed bags

CIDR® Formulation	Batch Number	Calculated Intercept Time (months)	Predicted Expiry Time (months)	Average Predicted Expiry Time (months)
CIDR®1900 Cattle	7313	27.0	54.0	47.4
CIDR®1900 Cattle	7317	22.6	45.2	
CIDR®1900 Cattle	7324	21.5	43.0	
1-up CIDR®1380 Cattle	7307	14.8	29.6	29.6
1-up CIDR®1380 Cattle	7313	15.5	31.0	
1-up CIDR®1380 Cattle	7317	14.1	28.2	

Examination of Table 3.4.1. shows that the CIDR®1900 Cattle insert exhibits a significantly longer predicted shelf life (48 months) than the 1-up CIDR®1380 Cattle insert (30 months) ($p = 0.007$). However, both these products exhibit acceptable shelf life values (personal communication, James Anderson, Marketing Manager). However, chemical stability is not the only factor to consider when estimating a shelf life for a controlled release veterinary product. The drug release characteristics must also be considered[226], and be shown not to change upon storage. Tables 3.4.2 and 3.4.3 contain pooled data from the invitro drug release testing of CIDR®1900 Cattle inserts and 1-up CIDR®1380 Cattle inserts.

Table 3.4.2 - Pooled invitro drug release test data for CIDR®1900 stored under accelerated conditions (40°C/75%RH) in closed bags

Time (Months)	Average Rate ($\mu g/cm^2/hr^{0.5}$)	SEM	Average Intercept ($\mu g/cm^2$)	SEM
0	1209.1	24	347.2	57
12	1221.0	30	942.8	216

Table 3.4.3 - Pooled invitro drug release test data for 1-up CIDR®1380 stored under accelerated conditions (40°C/75%RH) in closed bags

Time (Months)	Average Rate ($\mu g/cm^2/hr^{0.5}$)	SEM	Average Intercept ($\mu g/cm^2$)	SEM
0	1472	34	417	217
3	1365	27	1445	561
6	1449	9	1063	112
12	1322	56	1344	156

Inspection of pooled invitro drug release test data for CIDR®1900 Cattle inserts (Table 3.4.2) and for 1-up CIDR®1380 Cattle inserts (Table 3.4.3) reveals that the release rates for both formulations remained constant over the testing period. The intercept values for the 1-up CIDR®1380 Cattle inserts displayed a larger increase above their initial values than those for the CIDR®1900 Cattle inserts as the stability trial progressed, but intercept values for both formulations remained within specifications (see Chapter Two, Section 2.4.1.4). Therefore the invitro drug release test data supports the shelf life predictions drawn from the chemical stability data.

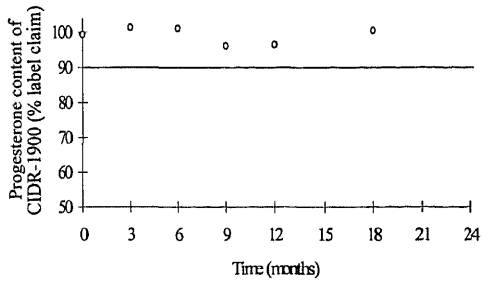
Another factor which can influence the shelf life of a veterinary product is its physical stability. The term 'physical stability' encompasses a variety of parameters which should be visually assessed during a stability trial to ensure product integrity. Physical stability parameters may not affect either the potency or release characteristics of the product, but may be detrimental to the products aesthetic quality and hence marketability. Subjective observations made on the CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert during the stability trial (see Section 3.3.2.3.1) showed a slight appearance of powder on the surface of the inserts with time. These observations are the likely cause for the increase in intercept values observed in the invitro drug release test. Matrix type controlled release veterinary drug delivery systems are typically associated with a 'burst effect'. This is where the drug particles in the very outermost layer of the delivery matrix are exposed to the environment and therefore, are available for immediate release when they come in contact with a release media. This results in a high initial value in an invitro drug release test. In our study, the typical initial value determined at the first time point (t = 2 minutes) for a freshly made CIDR® (i.e. one which was subjected to a release

assessment test within a few days of fabrication) was approximately 400 $\mu g/cm^2$ (see Table 3.3.41). Upon storage higher initial values were observed in the invitro drug release test. This observation was due to the migration of progesterone to the surface of the CIDR® inserts; these increased values being the result of the traditional inherent burst effect plus the migrated progesterone. However, migration of progesterone does not have a detrimental effect on the quality, safety or efficacy of the product since the burst effect value falls within specifications for the invitro drug release test. Therefore, the study demonstrates that the product is physically stable over the time course of the investigation supporting the shelf life predictions drawn from the chemical stability data.

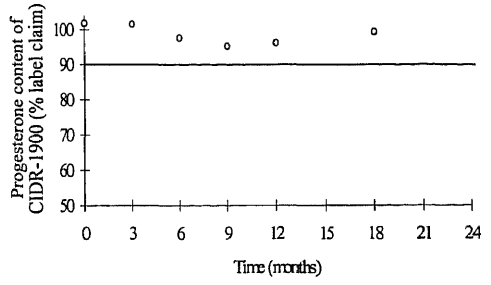
3.4.3 Confirmation of Shelf Life

Accelerated storage conditions are employed so that tentative expiration times can be proposed to regulatory bodies based on limited data e.g., 6-12 months data. This expedites the drug registration process, allowing companies to submit registration documents before the actual shelf life of the product has been established (e.g. 3 years). Following submission the proposed expiration time is verified using real time stability data obtained over the entire period of the proposed shelf life. In this study the accelerated time stability data predicted a product shelf life of greater than 2 years. Figure 3.4.2 presents the real time stability data generated to date for the CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert.

CIDR®1900 Cattle insert real time closed bag



Batch 7313



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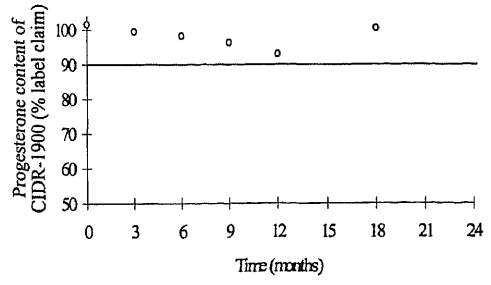
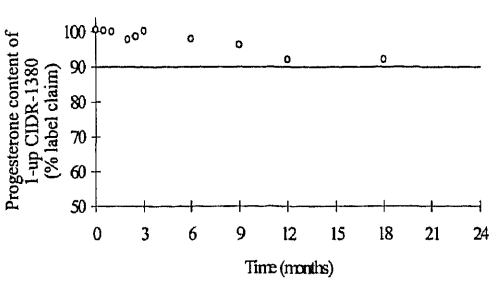
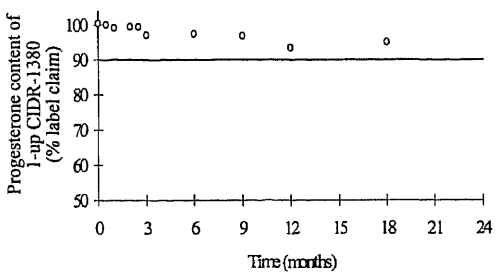


Batch 7324

1-up CIDR®1380 Cattle insert real time closed bag



Batch 7307



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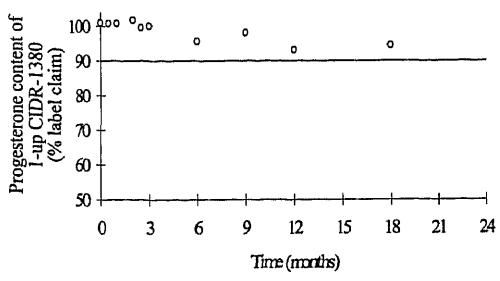

Batch 7317

Figure 3.4.2 - Effect of time on the potency of CIDR®1900 Cattle inserts and 1-up CIDR®1380 Cattle inserts (expressed as % label claim) stored under real time conditions (25°C/60%RH) in closed bags

Examination of Figure 3.4.2 reveals that all data points up to the 18 month time point lie above the 90% label claim limit for veterinary pharmaceutical product stability. The real time data generated to date therefore supports the shelf life predictions drawn from the accelerated stability study.

Tables 3.4.4 and 3.4.5 show the pooled invitro drug release test data for the CIDR® inserts stored under real time conditions in closed bags.

Table 3.4.4 - Pooled invitro drug release data for CIDR®1900 Cattle inserts stored under real time conditions (25°C/60%RH) in closed bags

Time (Months)	Average Rate ($\mu g/cm^2/hr^{0.5}$)	SEM	Average Intercept ($\mu g/cm^2$)	SEM
0	1209	25	347	58
12	1169	41	803	118

Table 3.4.5 - Pooled invitro drug release test data for 1-up CIDR®1380 Cattle inserts stored under real time conditions (25°C/60%RH) in closed bags

Time (Months)	Average Rate ($\mu g/cm^2/hr^{0.5}$)	SEM	Average Intercept ($\mu g/cm^2$)	SEM
0	1472	34	417	217
3	1426	48	789	237
6	1467	44	1365	212
12	1289	36	1323	175

Inspection of Table 3.4.4 and Table 3.4.5 reveals that the release rates for both formulations remained constant across the observed time frame. The intercept values for the 1-up CIDR®1380 Cattle insert exhibited a larger increase above their initial values than those for the CIDR®1900 Cattle insert as the stability trial progressed, however, intercept values for both formulations remained below the predetermined specifications set for the burst effect. Overall, the chemical, physical and invitro drug release test data observed during the real time study supported the shelf life predictions drawn from the accelerated stability trial.

3.4.4 Observations on Degradation Products During Accelerated and Real Time Stability Trials

At no time during the accelerated or real time stability studies was there any appearance of degradation products of progesterone observed in the HPLC chromatograms. This is in agreement with the literature, which suggests that progesterone is a very stable compound[236]. This experimental observation led us to question why, if progesterone was chemically stable, did we observe a declining potency profile in the stability curves (Figures 3.4.1 and 3.4.2.) for these products?

It was reasoned that the answer to this question lay in the subjective physical stability observations which were made on the CIDR® inserts and in the experimentally determined increase in intercept values seen during the invitro drug release test which were observed on storage. It was concluded that upon storage, a small quantity of progesterone migrated to the surface of the CIDR® inserts. This resulted in the slight powdery appearance of the CIDR® inserts and was experimentally reflected in a higher intercept value of the invitro drug release curve due to the ready availability of this surface progesterone for release.

Further it was reasoned that the observed loss of potency of CIDR® inserts was a result of the dislodgment (onto gloves) of this surface progesterone during the extensive manipulations required to prepare CIDR® inserts for analysis by soxhlet extraction. To extract the progesterone from the silicone skin, it had to be removed from the spine so that it could be cut into pieces which would fit into the soxhlet extraction apparatus. These extensive manipulations were unfortunately unavoidable and, on reflection, were an obvious disadvantage of the method.

3.4.5 Factors Affecting the Appearance of Progesterone on the Surface of CIDR® Inserts Upon Storage

3.4.5.1 Post-Manufacture Factors (Storage Conditions)

3.4.5.1.1 Temperature and Humidity

A comparison of the results obtained when the inserts were stored under different storage conditions revealed that higher storage temperatures and humidities promote the appearance of progesterone on the surface of the insert. The pooled real time and pooled accelerated time data for the 1-up CIDR®1380 Cattle insert (Figure 3.4.3) shows this effect. The potency of the 1-up CIDR®1380 Cattle inserts stored under accelerated time conditions appears to decline more rapidly compared to the 1-up CIDR®1380 Cattle inserts stored under real time conditions (Figure 3.4.3).

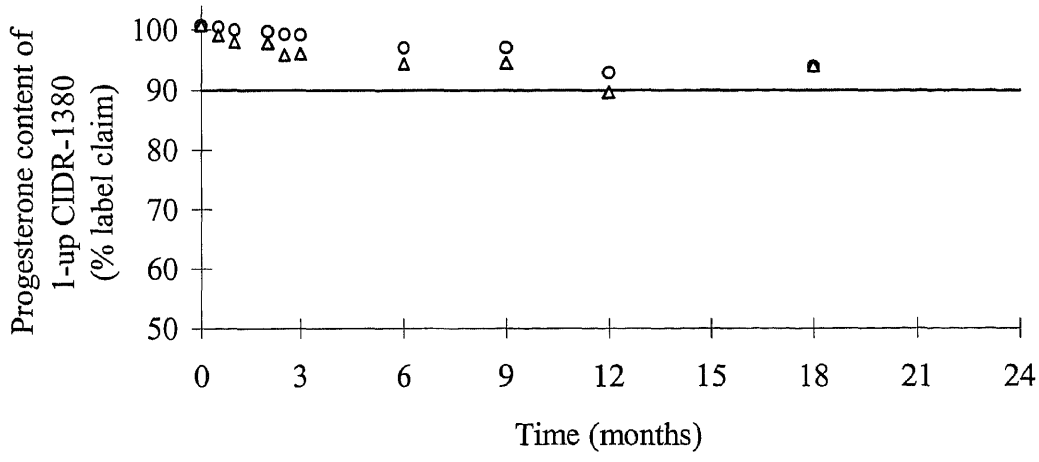

Figure 3.4.3 - Pooled potency data for 1-up CIDR®1380 Cattle insert stored under real time (o) and accelerated time (Δ) conditions

An examination of the pooled invitro drug release test data for 1-up CIDR®1380 Cattle inserts stored under real time conditions (Table 3.4.6) and under accelerated time conditions (Table 3.4.7) supports these observations. Although the release rate remains relatively constant throughout the observed time frame, the intercept value was seen to increase more rapidly when the 1-up CIDR®1380 Cattle insert was stored under accelerated time conditions than for samples stored under real time conditions.

These observations suggest that an increase in temperature and humidity promotes progesterone to migrate to the surface of the insert.

Table 3.4.6 - Pooled invitro drug release test data for 1-up CIDR®1380 Cattle inserts stored under real time conditions (25°C/60%RH) in closed bags

Time (Months)	Average Rate ($\mu g/cm^2/hr^{0.5}$)	SEM	Average Intercept ($\mu g/cm^2$)	SEM
0	1472	34	417	217
3	1426	48	789	237
6	1467	44	1365	212
12	1289	36	1323	175

Table 3.4.7 - Pooled invitro drug release test data for 1-up CIDR®1380 Cattle inserts stored under accelerated time conditions (40°C/75%RH) in closed bags

Time (Months)	Average Rate ($\mu g/cm^2/hr^{0.5}$)	SEM	Average Intercept ($\mu g/cm^2$)	SEM
0	1472	34	417	217
3	1365	27	1445	561
6	1449	9	1063	112
12	1322	56	1344	156

3.4.5.1.2 Product Closure (Open versus Closed)

A comparison of the results obtained when the inserts were stored in closures which were open or closed revealed that the appearance of progesterone on the surface of the insert remained independent of closure condition (Figures 3.4.4 and 3.4.5). From these Figures, one-sided lower confidence interval expiry times were determined and are presented in Table 3.4.8.

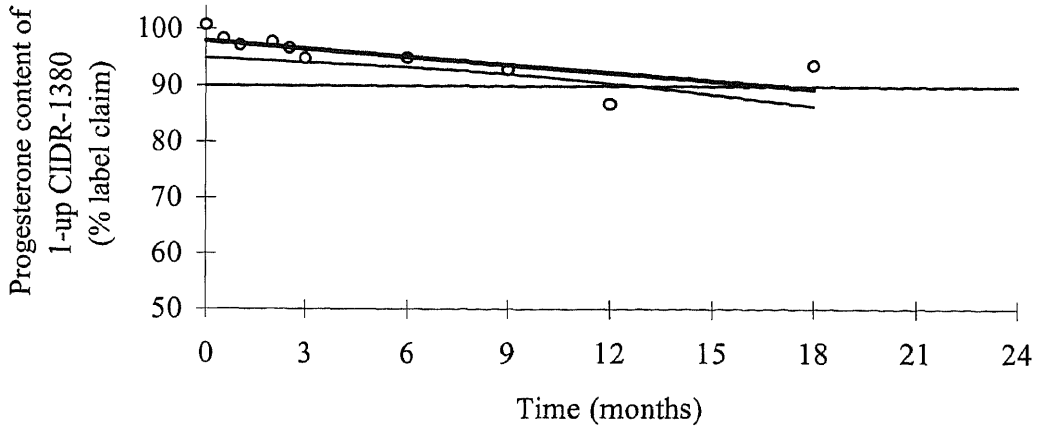

Figure 3.4.4 - Pooled potency data for 1-up CIDR®1380 Cattle inserts stored under accelerated time conditions (40°C/75%RH) in open bags

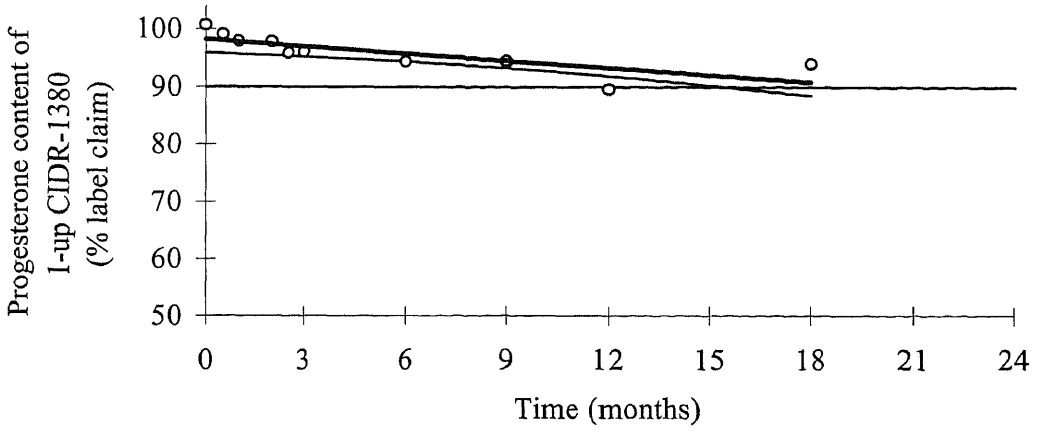

Figure 3.4.5 - Pooled potency data for 1-up CIDR®1380 Cattle inserts stored under accelerated time conditions (40°C/75%RH) in closed bags

Table 3.4.8 - Calculated expiry times based on one sided lower 95% confidence interval data for 1-up CIDR®1380 Cattle inserts stored under accelerated time conditions (40°C/75%RH) in open and in closed bags

Batch Number	Closure	Calculated Expiry Time (months)
7307	Open	10.7
7313	Open	14.2
7317	Open	12.4
Pooled	Open	12.7
7307	Closed	14.8
7313	Closed	15.5
7317	Closed	14.1
Pooled	Closed	15.4

There was no statistical difference in the predicted expiry times of the three batches of 1-up CIDR®1380 Cattle inserts stored in closed bags compared to the stability of those stored in open bags ($p = 0.095$). The reason for this observation in this study probably lies in the fact that polyethylene is moisture permeable and thus, whether the closure was closed or open, there would have been free access of moisture to the product.

3.4.5.2 Factors During Manufacture (Manufacturing Conditions)

Several manufacturing conditions were modified and investigated in this study. These included silicone curing temperature, curing cycle time and water content of the nylon spine (annealing times). In contrast to the stability study, these experiments were designed to quantify the amount of progesterone on the surface of the insert as a function of the different manufacturing variables. As InterAg planned for the CIDR®1380 Cattle insert to supersede the CIDR®1900 Cattle insert, in contrast to the stability study, these investigations were carried out specifically on the 1-up CIDR®1380 Cattle insert. Results of these determinations are shown in Figure 3.4.6.

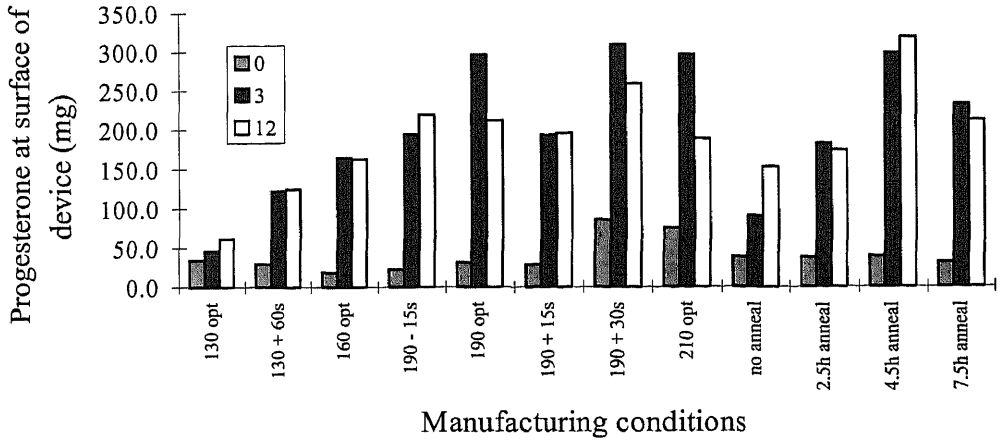

Figure 3.4.6 - Effect of manufacturing conditions on the amount of progesterone determined on the surface of a 1-up CIDR®1380 Cattle insert as a function of time under accelerated storage conditions (40°C/75%RH) in open bags

Figure 3.4.6 shows that in for all manufacturing conditions, the amount of progesterone at the surface of the 1-up CIDR®1380 Cattle inserts at the time of

manufacture was relatively low (left bar of each set of three). The Figure further shows that progesterone migration to the surface occurs within the first three months of storage (middle bar). After three months storage, progesterone migration appears to cease. This is evident from the approximate equivalence of the 12 month and 3 month values (compare middle and right bars).

Further examination of Figure 3.4.6 suggests that increasing moulding temperature (across temperatures of 130, 160, 190 and 210°C; bar charts 1, 3, 5 and 8) has an effect upon the quantity of progesterone migrating to the surface of CIDR® inserts over time. This effect is most clearly observed when the data is expressed as the sum of the total amount of progesterone recovered from the surface of CIDR® inserts over the study period (i.e., the amount determined at t = 0 plus the amount determined at t = 3 plus the amount determined at t = 12 month analysis time). This is shown in Figure 3.4.7.

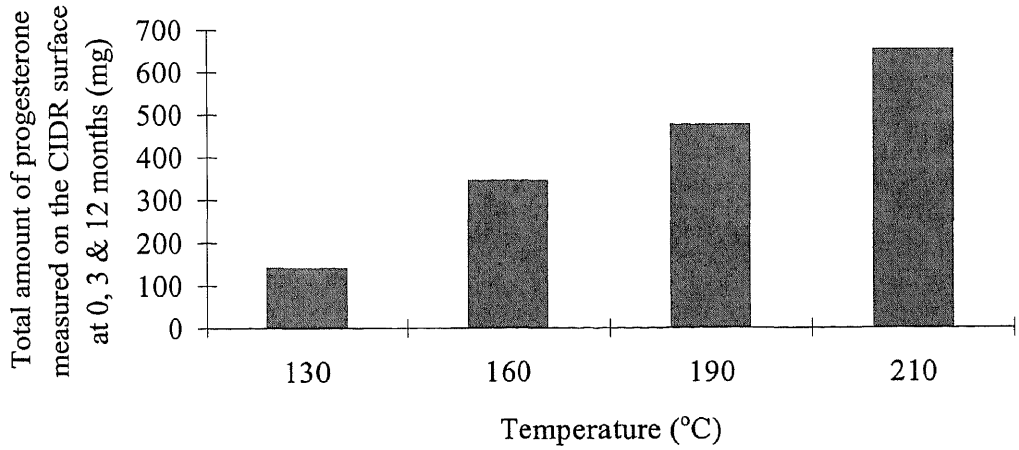

Figure 3.4.7 - Effect of moulding temperature on the total amount of progesterone determined on the surface of 1-up CIDR®1380 Cattle inserts during the study (sum of t = 0, 3 and 12 months analysis)

Further examination of Figure 3.4.6 (bar charts 9, 10, 11 and 12) suggests that increasing the annealing time, and hence water content of the spine, (across annealing times of 0, 2.5, 4.5 and 7.5 hours) affects the quantity of progesterone migrating to the surface of 1-up CIDR®1380 Cattle inserts upon storage. Again, this effect is more apparent when the data is expressed as the sum of the total amount of

progesterone recovered from the surface of 1-up CIDR®1380 Cattle inserts over the study period. This is shown in Figure 3.4.8.

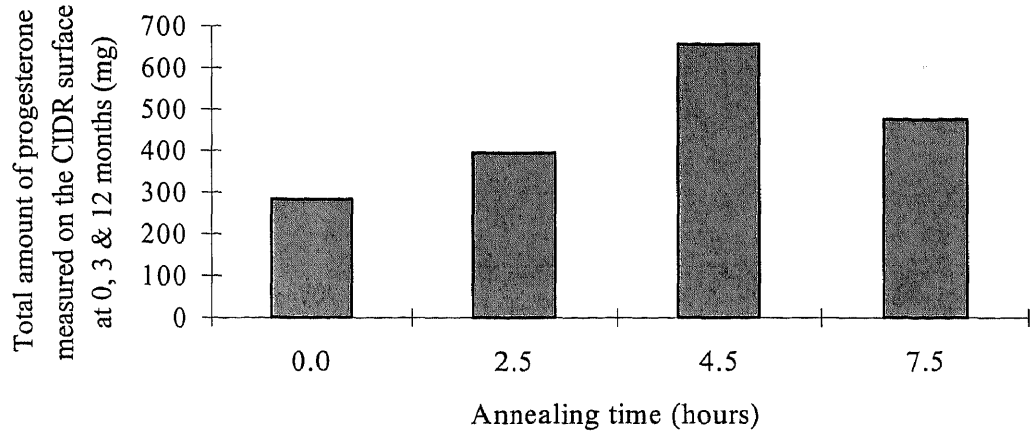

Figure 3.4.8 - Effect of spine annealing time on the total amount of progesterone determined on the surface of 1-up CIDR®1380 Cattle inserts during the study (sum of t = 0, 3 and 12 months analysis)

Figure 3.4.6 (bar charts 4, 5, 6 and 7 from the left) also shows that changing the time the 1-up CIDR®1380 Cattle insert is in the mould (across the range -15 to +30 seconds around the optimum cure time) affects the quantity of progesterone migrating to the surface of CIDR® inserts upon storage. Again, this effect is more clearly observed when the data is expressed as the sum of the total amount of progesterone recovered from the surface of CIDR® inserts over the study period. This is shown in Figure 3.4.9.

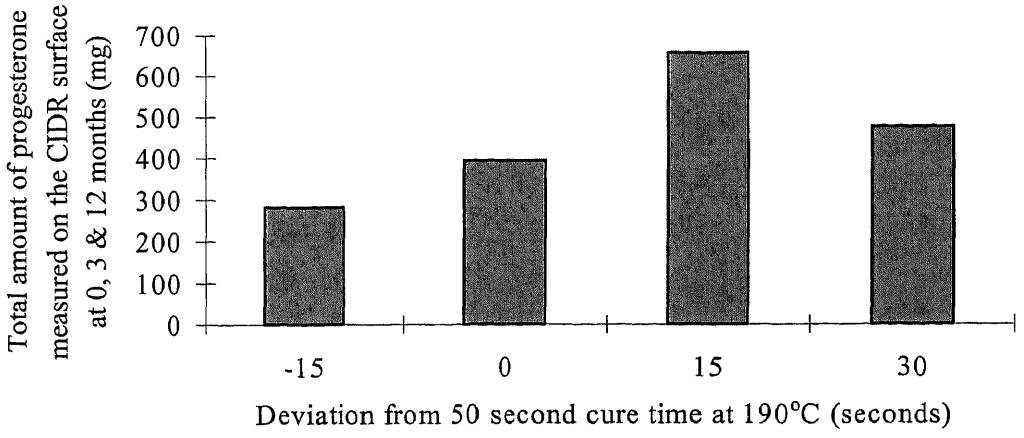

Figure 3.4.9 - Effect of cure time deviations at 190°C curing temperature on the total amount of progesterone determined on the surface of 1-up CIDR®1380 Cattle inserts during the study (sum of t = 0, 3 and 12 months analysis)

The amount of progesterone remaining within the skin was also quantified for mass balance. The results of these determinations are presented in Figure 3.4.10.

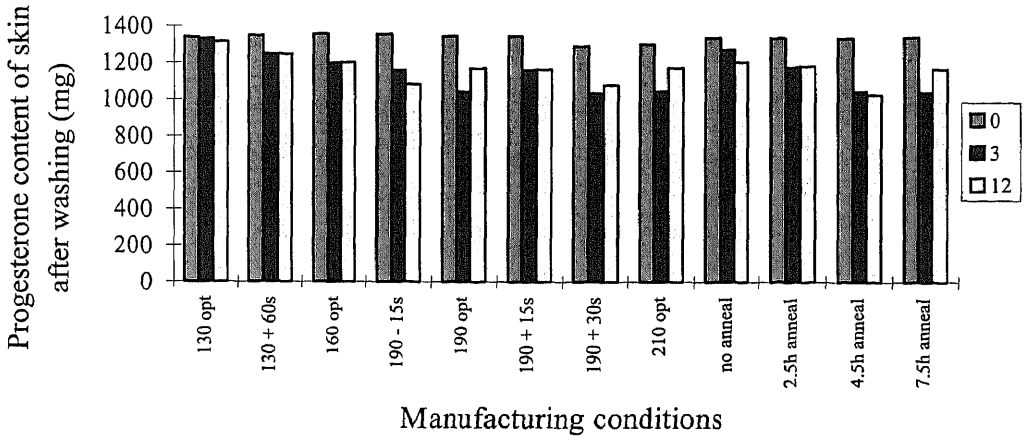

Figure 3.4.10 - Total amount of progesterone remaining in the skin of 1-up CIDR®1380 Cattle inserts after removal of surface progesterone

The amount of progesterone determined to be remaining in the skin was a reflection of the amount determined on the surface. Progesterone content in the skin was observed to decline rapidly between zero and three months and then to hold relatively steady at that level out to twelve months. Mass balance was achieved when summing the quantities observed in the skin with the quantities observed on the skin surface.

3.4.6 Potential Future Manufacturing Strategies

The conclusions drawn from the manufacturing study which determined the rate and extent of appearance of progesterone on the surface of the insert using a surface wash technique, were that both annealing conditions (presence of water in the spine) and the temperature of manufacture affect the extent of migration of progesterone to the surface of the 1-up CIDR®1380 Cattle insert. As a result of these conclusions two further studies were initiated to determine the feasibility of utilising this knowledge to advantage in the manufacturing process. The first study investigated the use of a spine material which did not require annealing, thereby eliminating the presence of water in the inert spine. The second study investigated the use of a low temperature cure silicone.

3.4.6.1 Alternate Spine Material

Nylon possesses an inherent ambient water content which impels flexibility and toughness. These properties, along with excellent heat stability, were the attributes for which nylon was originally selected for use as the spine material of CIDR® inserts (personal communication, Vaughan Woodward, Production Manager). However, the fabrication of nylon spines by injection moulding requires the dehydrated nylon to avoid steam formation. To restore the nylon spine's inherent properties, its ambient water content is restored by annealing in hot water prior to use in the manufacture of CIDR® inserts. An alternate polymer (polyester) was identified for fabrication into spines which still had desirable mechanical properties but would not require annealing (personal communication, Vaughan Woodward, Production Manager). Therefore polyester was used to replace nylon in the manufacture of the 1-up CIDR®1380 Cattle inserts for this study.

Following manufacture of the 1-up CIDR®1380 Cattle inserts at 190°C with the polyester spines, low initial drug loads were observed when a potency assay was undertaken (See Table 3.3.55). Further studies identified that the missing progesterone was contained in the polyester spine. It was surmised that during manufacture the high temperatures promoted the transfer of progesterone from the silicone skin into the polyester spine. A study was therefore initiated which investigated the fate of the progesterone in the spine material and, if it left the spine with time, whether it re-entered into the silicone skin. The results of that study are shown in Figure 3.4.11 together with potency determinations of the silicone skin (Figure 3.4.12).

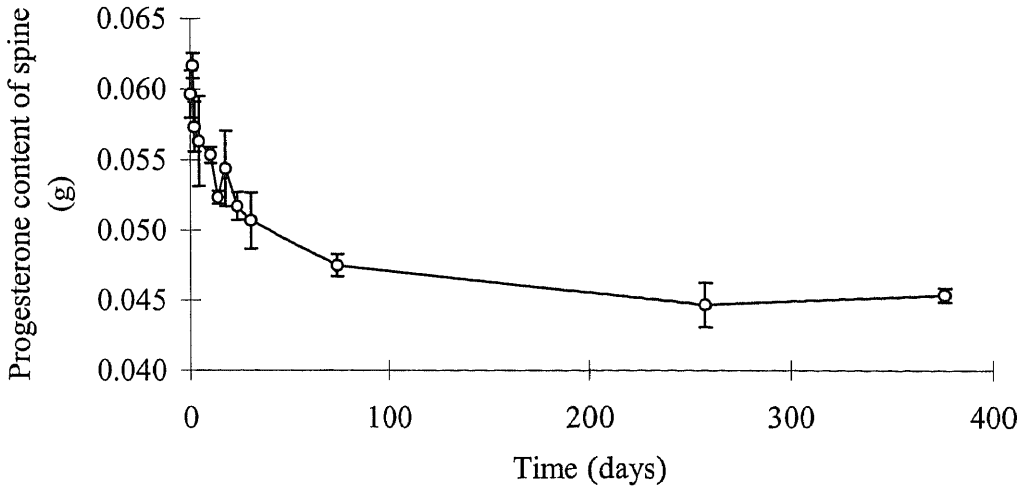

Figure 3.4.11 - Amount of progesterone in spines of 1-up CIDR®1380 Cattle inserts manufactured with polyester spines as a function of time under real time storage conditions (25°C/60%RH) in open bags

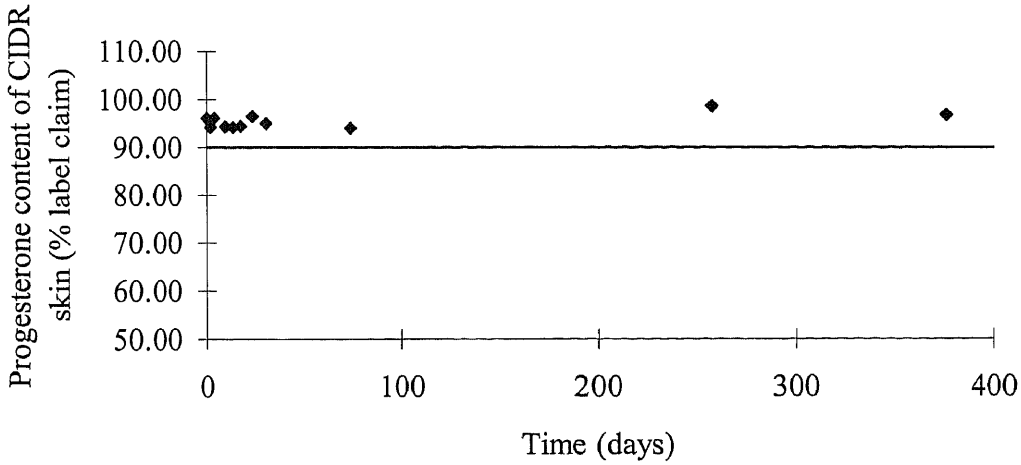

Figure 3.4.12 - Amount of progesterone in skins of 1-up CIDR®1380 Cattle inserts manufactured with polyester spines as a function of time under real time storage conditions (25°C/60%RH) in open bags

Figure 3.4.11 shows that approximately 60 mg of progesterone transfers from the silicone into the polyester spine during curing at 190°C, and that upon storage approximately 15 mg leaves the spine and either partitions into the silicone skin or adheres to the surface of the silicone skin, since it was not visually apparent on the surface of the spine. The skin content data (Figure 3.4.12) does not confirm this observation since the analytical technique was not precise enough to detect such a

small change in drug content. However, the real time potency data obtained to approximately twelve months (Figure 3.4.12) suggested that the use of polyester spines to replace the annealed nylon spines has reduced the amount of progesterone migrating to the surface of the 1-up CIDR®1380 Cattle insert. Figure 3.4.12 does not appear to exhibit the rapid decline in potency over the early months of storage which was observed in the previous real and accelerated time stability study. However, despite this apparent improvement in product quality, we conclude from this study that polyester is not an acceptable substitute for nylon as a spine material when 1-up CIDR®1380 Cattle inserts are manufactured using the current high moulding temperature due to the quantity of drug which is lost from the skin into the spine material.

3.4.6.2 Low temperature cure silicone

1-up CIDR®1380 Cattle inserts are currently manufactured using Dow Corning Silastic® Q7-4840. To ensure commercial manufacturing efficiencies, CIDR® inserts manufactured using this polymer are cured at a high temperature (190°C) which results in a cycle time of less than 50 seconds per insert (i.e., it takes 50 seconds to injection mould and cure the CIDR® inserts). This high curing temperature and associated 50 second cycle time allows InterAg to produce 3-up CIDR®1900 Cattle inserts (three CIDR®1900 Cattle inserts are injection moulded in one machine cycle) at a rate that allows their manufacture to be a profitable venture. It is possible for CIDR® inserts to be cured at lower temperatures but this would mean longer cure times and hence, the process would be less profitable. Recently a new medical grade of silicone has become commercially available which has a lower cure enthalpy. This silicone is Elastosil® LR3004-40 and is manufactured by Wacker Chemicals Ltd. It can be fully cured within 35 seconds at 130°C. In this study this new polymer (Elastosil® LR3004-40) was used to manufacture 1-up CIDR®1380 Cattle inserts to investigate the effect of low temperature curing on the migration of progesterone to the surface of 1-up CIDR®1380 Cattle inserts under both real time (Figure 3.4.14) and accelerated time conditions (Figure 3.4.13).

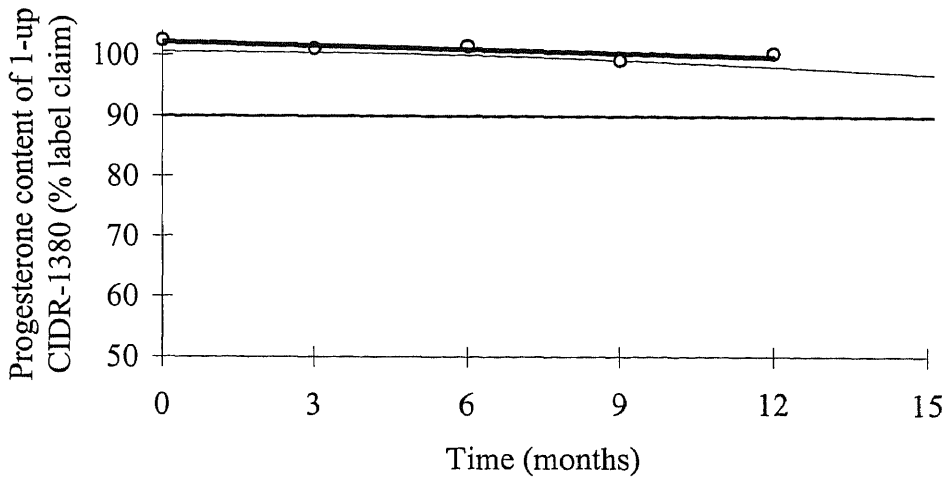

Figure 3.4.13 - Potency data for low temperature cure 1-up CIDR®1380 Cattle inserts stored under accelerated time conditions (40°C/75%RH) in open bags

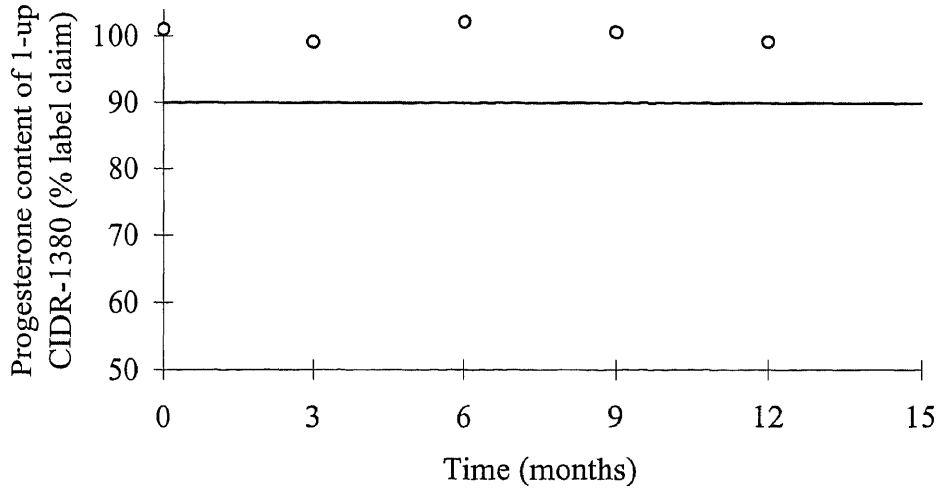

Figure 3.4.14 - Potency data for low temperature cure 1-up CIDR®1380 Cattle inserts under real time conditions (25°C/60%RH) in open bags

At the time of writing this thesis, only 12 months of data had been collected. However, Figures 3.4.13 and 3.4.14 clearly show improvements in the potency profile. The one-sided lower 95% confidence interval calculated around the regression line of the accelerated time data yielded a tentative expiry time of 32 months. The real time stability profile also shows no significant reduction in potency over the observed time frame. Therefore we conclude that the moulding of 1-up CIDR®1380 Cattle inserts at a lower temperature has reduced the migration of progesterone to the surface of the inserts. Invitro drug release rates for the low

temperature cure 1-up CIDR®1380 Cattle inserts (see Tables 3.3.60 and 3.3.61) did not change significantly over the observed time frame and exhibited low intercept values at each time point. We noted, however, that the observed invitro drug release rates for the low temperature cure silicone 1-up CIDR®1380 Cattle inserts, although within specification (See Section 2.4.2) were at the lower end (≈1000 $\mu g/cm^2/hr^{0.5}$) of the range for the release rate of the conventional high temperature cured 1-up CIDR®1380 Cattle insert (≈900-1840 $\mu g/cm^2/hr^{0.5}$). Given these observations, and the potential application of this new low temperature cure silicone in production, we thought it would be prudent to question whether the low temperature cure 1-up CIDR®1380 Cattle insert would be equivalent to the high temperature cure product invivo.

3.4.6.3 Invivo Performance of Low Temperature Cure Silicone 1-up CIDR®1380 Cattle Inserts

An invivo comparison of the high temperature cure silicone CIDR®1900 Cattle insert and low temperature silicone 1-up CIDR®1380 Cattle insert showed (Figure 3.4.15) that the inserts were, in fact, bioequivalent. This finding supports the validity of the release test specifications as CIDR® inserts displaying invitro drug release rates at the lower end of the specifications still exhibit blood plasma profiles which are known to possess clinical efficacy.

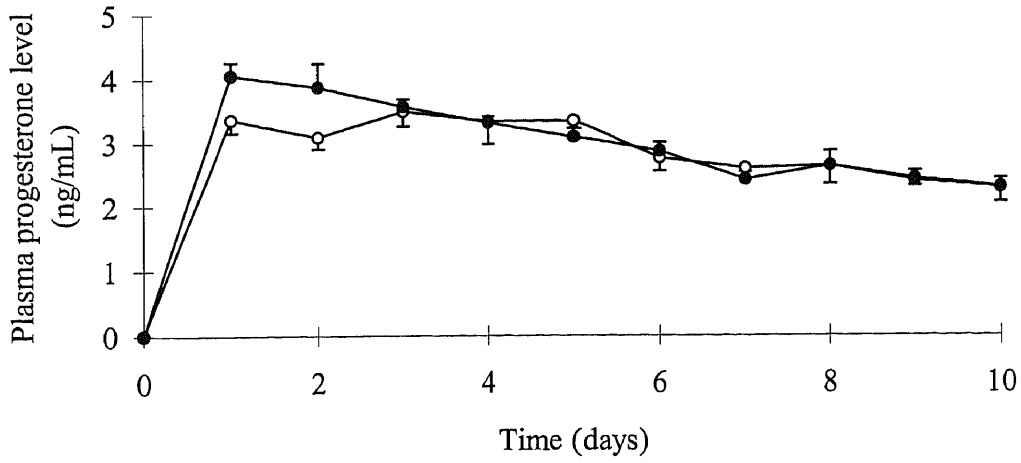

Figure 3.4.15 - Plasma profile for CIDR®1900 Cattle inserts (o) and low temperature cure 1-up CIDR®1380 Cattle inserts (•) in ovariectomised cows (n = 6, error bars = SEM) over a ten day insertion

3.5 Conclusions

In this chapter the chemical and physical stability of the CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert were determined under real and accelerated time conditions. We concluded from the results of the potency analysis and release assessment testing of samples stored under controlled conditions that both the CIDR®1900 Cattle insert and 1-up CIDR®1380 Cattle insert were chemically very stable over the storage durations. HPLC chromatograms showed no sign of the appearance of any degradation products upon storage, and release rates did not change with time. A decline in potency was observed with time for both storage conditions but was not sufficient to cause the values to fall below the 90% label claim limit which is used to define acceptable stability of a product. We concluded that the fall in potency was not due to chemical degradation but was the result of the migration of progesterone powder to the surface of the insert over the first three months of storage, after which time the level of powder on the surface remained constant for the remainder of the study. We concluded that the decline in observed potency of the products was a reflection of the dislodgment of progesterone powder from the surface of the inserts and that the extensive manipulation of CIDR® inserts during sample preparation is the cause of the progesterone powder dislodgment and is a clear limitation of our experimental technique. The higher intercept value in the invitro drug release test reflected the immediate availability of the progesterone present at the surface of the insert, however, the intercept remained within acceptable specifications and release rates remained unchanged over the course of the study, indicating that the changes caused by the physical instability of the product were only minor, and did not affect the quality, safety and efficacy of the product.

As a result of these observations, although the stability trial indicated a satisfactory product, investigations were initiated to examine if any manufacturing procedures could be readily manipulated to reduce the rate and extent of migration of progesterone to the surface of the product. We concluded from the results of the investigation that water content of the spine material and curing temperature were potential factors affecting the rate and extent of migration of progesterone to the surface of 1-up CIDR®1380 Cattle inserts Cattle inserts. Given this information 1-up

CIDR®1380 Cattle inserts were manufactured at the same curing temperature (190°C) but using a spine made from polyester (in which there is no water content) and further 1-up CIDR®1380 Cattle inserts were manufactured with the traditional annealed nylon spine but using a low temperature cure silicone.

The 1-up CIDR®1380 Cattle inserts manufactured with polyester spines exhibited a reduction of progesterone migration to the surface of the 1-up CIDR®1380 Cattle insert. However, progesterone was observed to penetrate the spine material during manufacture, resulting in a low initial load in the silicone skin. We concluded from these results that the 1-up CIDR®1380 Cattle inserts manufactured with polyester spines resulted in less progesterone migrating to the surface, but was not of practical use because of the transfer of progesterone into the spine during manufacture at high temperatures.

The 1-up CIDR®1380 Cattle inserts manufactured with annealed nylon spines but using a low temperature cure silicone exhibited a reduction of progesterone migration to the surface of the 1-up CIDR®1380 Cattle insert. We concluded from these results that the manufacture of 1-up CIDR®1380 Cattle inserts with low temperature cure silicone resulted in a product which exhibited a reduction in the rate and extent to which progesterone appears on the surface of the product. This may have practical application in manufacturing, since cycle times for the low temperature cure silicone were comparable to those currently employed with the high temperature cure silicone. Manufacture of CIDR® inserts with low temperature cure silicone would, therefore, be a commercially viable process.

Chapter Four - Characterisation of intravaginal drug delivery systems for the delivery of progesterone for control of the bovine oestrus cycle. III. Invitro and invivo release mechanisms of CIDR® intravaginal drug delivery systems.

4.1 Introduction

4.1.1 Mechanism of Release and the Drug Delivery Scientist

Knowledge of the mechanism of drug release from a veterinary pharmaceutical provides the formulation scientist with the tool to rationally optimise a controlled release veterinary drug delivery system[237]. Many different mechanisms of release have been identified in the literature which describe the release of drugs from veterinary pharmaceuticals. Complex equations which mathematically describe these mechanisms have been derived[238-241]. In their complete form, such equations are cumbersome to use and difficult to understand, requiring the determination of parameters which are often difficult, time consuming or impractical to determine. However, simplified versions of these equations have been developed and are widely published in the scientific literature[240, 242, 243]. These simplified versions permit elucidation of the mechanism of drug release from a controlled release veterinary product by comparing experimentally determined release data to the simplified mathematical model. Thus, the modern veterinary formulation scientist does not need a detailed knowledge of mathematics to elucidate a mechanism of release from a veterinary pharmaceutical. He or she merely needs to be aware of the mathematical models which are available, generate appropriate release data and curve fit to the models to establish which mechanism of release is the most likely to describe release from their delivery system. Confidence in the correct model is assured using statistical techniques which provide an indication of the goodness of fit. Once armed with knowledge of the mechanism of release the veterinary pharmaceutical scientist can examine the appropriate equation and develop or optimise their product through rational modification of the parameters which make up that equation.

Within the pharmaceutical literature there are three major mechanisms by which drugs are released from controlled release dosage forms. These are referred to as 1st order, zero order and square root of time mechanisms[243-246].

4.1.1.1 The First Order Mechanism

The first order mechanism occurs where the concentration of drug in the delivery system is insufficient to maintain a constant flux over the delivery period. This results in a diminishing amount of drug released per unit time from the delivery system with time in proportion to the concentration of the drug in the reservoir. This type of release mechanism is described by an exponential equation of the type $Q = Ae^{-kt}$ where Q = amount released per unit area, A = constant, k = rate constant and t = time and provides a characteristic profile shown in Figure 4.1.1.

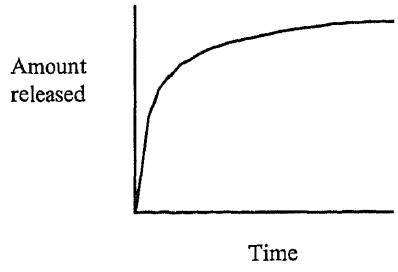

Figure 4.1.1- Plot of first order release

4.1.1.2 The Zero Order Mechanism

The zero order mechanism typically occurs where the drug is in excess of its saturation concentration in a reservoir which is in contact with a rate limiting membrane. In this case the amount of drug released per unit time from the delivery system is constant. This type of release mechanism is described by a linear equation of the type Q = At and provides a characteristic profile shown in Figure 4.1.2.

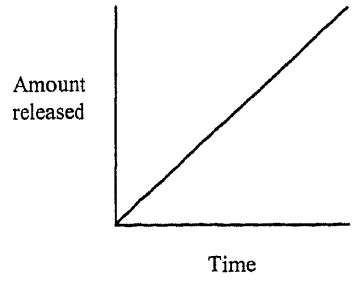

Figure 4.1.2- Plot of zero order release

4.1.1.3 The Square Root of Time Mechanism (Dispersed Systems)

The square root of time release mechanism typically occurs where the drug is homogeneously distributed throughout a solid matrix in excess of its solubility as fine particles. In this case the amount of drug released per unit time from the delivery system declines with time (in contrast to the zero order release but in a similar fashion as the first-order release mechanism). However, in contrast to the first order mechanism it does not decline in an exponential fashion, but as a function of the square root of time. This type of release mechanism is described by an equation of the type $Q = At^{½}$ and provides a characteristic profile shown in Figure 4.1.3.

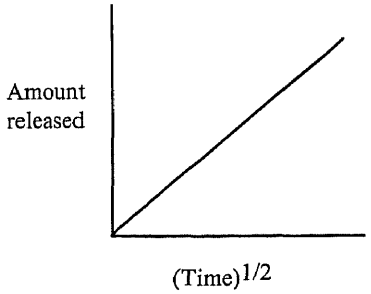

Figure 4.1.3 - Plot of $t^{1/2}$ release

In this chapter we investigated the invitro and invivo release mechanism for CIDR® intravaginal drug delivery systems.

4.2 Experimental Methods

4.2.1 Determination of Invitro Drug Release

The invitro drug release profiles from CIDR®1900 Cattle insert used in this Chapter are those determined in Chapter Two using a modified USP dissolution procedure described in Chapter Two, Section 2.2.2.

4.2.2 Determination of Drug Content in Horizontal Plane of CIDR®1900 Cattle insert

4.2.2.1 Slicing Method

A segment of CIDR®1900 Cattle insert of approximately 50 mm^2 area was removed from the body of the device directly above the capsule socket where the skin thickness exists at its greatest (5 mm thickness). The segment was mounted on a sample block using cyanoacrylate ester adhesive in preparation for thin slicing along the horizontal plane using a vibrating microtome (Vibratome® Series 1000 sectioning system, Technical Products International Inc., USA). Consecutive slices of approximately 100 μm each were taken, starting from the outer surface of the CIDR® Cattle insert matrix and gradually reaching the inner layers to a depth of approximately 1 mm.

Figure 4.2.1 - Slicing of CIDR®1900 Cattle insert segments with the Vibratome® Series 1000 sectioning system

4.2.2.2 Extraction of Drug from Horizontal Plane of CIDR®1900 Cattle insert

Individual slices of CIDR®1900 Cattle insert matrix were placed in glass sample bottles with rubber lined aluminium screw caps. The slices were then extracted with approximately 5 mL dichloromethane (HiPerSolv for HPLC™, BDH, New Zealand) for 2 hours after which time the slice was removed from the solvent with stainless steel tweezers. The dichloromethane was then allowed to evaporated to dryness overnight. The extracts were then reconstituted by addition of approximately 5 mL alcohol (SDA-3A, Mobil, New Zealand). With the screw caps on, the samples were shaken vigorously and then transferred to a sonic bath (SONOREX SUPER RK 510H, Bandelin) for 5 to 7 minutes of sonication to ensure complete reconstitution. The 5 mL reconstituted samples were then transferred to volumetric flasks. The glass sample bottles were rinsed with four consecutive 3 mL aliquots of ethanol (SDA-3A, Mobil, New Zealand), each aliquot being transferred to the same volumetric flask. After rinsing, the volumetric flasks were made up to volume (25 or 50 mL) with ethanol (SDA-3A, Mobil, New Zealand) and analysed directly.

4.2.2.3 Analytical Method

Analysis of samples for progesterone content was carried out by a validated UV spectrophotometric assay (See Section 2.2.1).

4.2.3 Determination of Invivo Drug Release

4.2.3.1 Invivo Methodology

The CIDR®1900 Cattle insert invivo drug release profile was determined using intact, normally cycling Friesian cows. The trial involved insertion of a CIDR®1900 Cattle insert into the vagina of each of 40 cows at a time denoted day 0. On each subsequent day for 10 days, the cows were herded into yards and four cows were selected at random to have their CIDR®1900 Cattle insert removed. The collected inserts were rinsed with water, padded dry with paper towels and placed in a plastic bag labeled with the insertion duration. The bagged inserts were then stored at -20°C until analysis. At a later date, one of the four inserts collected at each insertion duration was subjected to the slicing technique and two were subjected to residual drug load determination.

4.2.3.2 Determination of Residual Drug Load

Residual progesterone content in devices inserted for time periods encompassing 1 through 10 days was determined by soxhlet extraction and UV spectrophotometry (see Chapter Two, Section 2.2.1).

4.2.3.3 Determination of the Amount of Drug Released

Cumulative amount of progesterone released for each day over the treatment period was calculated by subtracting the residual progesterone content as determined for each day of insertion from the known initial content of the CIDR®1900 Cattle inserts (i.e. cumulative progesterone released in 5 days equals the initial load of a CIDR®1900 Cattle insert minus the residual progesterone load determined for a CIDR®1900 Cattle after an insertion of 5 days).

4.2.4 Determination of Drug Content in Horizontal Plane of CIDR®1900 Cattle insert

4.2.4.1 Slicing Method

See Section 4.2.2.1.

4.2.4.2 Extraction of Drug from Horizontal Plane of CIDR®1900 Cattle Insert

See Section 4.2.2.2.

4.2.4.3 Analytical Method

See Section 4.2.2.3.

4.2.5 Determination of Invivo Blood Profile Following Insertion of CIDR®1900 Cattle Insert

Plasma progesterone profiles resulting from the insertion of CIDR®1900 Cattle insert over 7, 10 and 20 days were determined in Chapter Two, Section 2.2.5. Data from the 7, 10 and 20 day studies was collated to form the invivo blood profile of the CIDR®1900 Cattle insert reported in this chapter.

4.2.6 Statistical Treatment of Data

Linear regression analysis of both invitro and invivo release data was performed in Microsoft Excel, Version 5.0a. Testing for non-linearity by comparing the lack-of-fit mean square with the pure error (between replicates) mean square, was performed utilising statistical calculations derived from (Bolton, 1984; Draper and Smith, 1966).

4.3 Results

4.3.1 Invitro Drug Release

Data from the determination of invitro drug release from CIDR®1900 Cattle insert is presented in Table 4.3.1 (Data reprinted from Chapter Two, Section 2.3.3.5).

Table 4.3.1 - Invitro drug release test data for CIDR®1900 Cattle insert (n = 17)

	Cumulative amount of progesterone released per unit area at given time ($\mu g/cm^2$)								
Rep	**0.03**	**2**	**4**	**8**	**12**	**24**	**48**	**72**	**96**
1	392	1774	2556	3761	4260	6453	9035	10619	11953
2	368	1805	2438	3442	4389	5934	8171	9607	10837
3	622	2000	2749	3921	4915	6740	9215	10910	12335
4	456	1847	2529	3576	4614	6248	8705	10363	11681
5	557	1854	2665	3868	4724	6864	9381	10999	12494
6	642	2103	2864	4043	5028	7041	9446	10993	12077
7	1187	1893	2613	3717	4621	6716	9584	11255	12647
8	1012	1647	2331	3360	4217	6183	8886	10464	11755
9	1108	1775	2473	3526	4393	6360	8846	10608	11837
10	1072	1719	2401	3418	4332	6134	8550	10308	11687
11	1118	1797	2479	3579	4518	6518	9219	10917	12414
12	1100	1693	2402	3432	4313	6128	8598	10435	11755
13	992	1994	2719	3835	4830	6867	9337	11065	12615
14	964	2570	3471	4709	5660	7636	10256	11932	13045
15	974	1825	2624	3736	4667	6697	9189	11155	12182
16	536	2321	3301	4794	5845	7693	10557	12478	13353
17	814	2530	3414	5186	6465	8779	12015	13583	15218
Mean	818	1950	2708	3877	4811	6764	9352	11041	12346
SEM	69	68	87	129	152	174	219	223	229

4.3.2 Drug Distribution in Horizontal Plane of CIDR®1900 Cattle Insert

4.3.2.1 Validation of Slice Extraction Method

Table 4.3.2 contains recovery data for slices of CIDR®1900 Cattle insert extracted for five hours.

Table 4.3.2 - Drug recovery data for a 5 hour dichloromethane extraction of CIDR®1900 Cattle insert slices

Replicate	Recovery (%)
1	103.74
2	99.79
3	96.71
4	99.19
5	96.51
6	98.17
7	97.24
8	96.39
9	98.70
10	98.32
11	100.35
12	98.48
13	97.35
14	98.55
Mean Recovery	98.54
Standard Error in the Mean	0. 51

Table 4.3.3 contains recovery data for one, two and three hour extractions.

Table 4.3.3 - Drug recovery data for 1, 2 and 3 hour dichloromethane extractions of CIDR®1900 Cattle insert slices

	Recovery (%) after given extraction time		
Replicate	1 hr	2 hr	3 hr
1	97.06	100.02	96.66
2	100.73	96.02	98.44
3	100.74	96.75	114.58
4	95.51	96.50	91.77
5	95.14	97.29	99.38
Mean Recovery	97.83	97.32	100.17
SEM	1.23	0.71	3.83

Table 4.3.4 contains data acquired from the analysis of non-loaded CIDR®1900 Cattle insert.

Table 4.3.4 - Results for a 2 hour dichloromethane extraction of non-drug loaded (sample blank) CIDR®1900 Cattle insert slices

Replicate	UV Absorbance @ 240 nm	Implied Progesterone level (% w/w load)
1	0.043	0.263
2	0.030	0.284
3	0.029	0.299
4	0.031	0.619
5	0.021	0.307
6	0.023	0.325
Mean	0.029	0.349
SEM	0.003	0.055

Table 4.3.5 contains data for extractions carried out in the absence of any sample.

Table 4.3.5 - Results for a 2 hour dichloromethane extraction in the absence of CIDR®1900 Cattle insert slices (solvent blank)

Replicate	UV Absorbance @ 240 nm
1	0.006
2	0.005
3	0.008
4	0.011
Mean	0.008
SEM	0.001

4.3.2.2 Invitro Release

Tables 4.3.6 through 4.3.13 contain progesterone loadings determined along the horizontal plane of CIDR®1900 Cattle insert at different stages of an invitro drug release test.

Table 4.3.6 - Progesterone loads along the horizontal plane in a fresh CIDR®1900 Cattle insert

Median depth into CIDR®1900 Cattle insert (μm)	Progesterone load (% w/w)
80	10.56
240	10.80
420	9.92
600	10.22
760	10.68
920	9.91
1075	10.54
1235	10.37
1395	10.31
1545	10.04

Table 4.3.7 - Progesterone loads along the horizontal plane in a CIDR®1900 Cattle insert after 2 hours invitro release

Median depth into CIDR®1900 Cattle insert (μm)	Progesterone load (% w/w)
60	3.12
175	7.72
280	10.59
380	10.07
480	10.05
580	10.28
680	10.33
780	10.10
880	10.64
980	10.79

Table 4.3.8 - Progesterone loads along the horizontal plane in a CIDR®1900 Cattle insert after 4 hours invitro release

Median depth into CIDR®1900 Cattle insert (μm)	Progesterone load (% w/w)
75	1.36
200	6.68
300	9.24
400	9.78
500	9.98
600	9.58
700	9.77
800	10.30
900	10.37
1000	10.00

Table 4.3.9 - Progesterone loads along the horizontal plane in a CIDR®1900 Cattle insert after 8 hours invitro release

Median depth into CIDR®1900 Cattle insert (μm)	Progesterone load (% w/w)
35	0.82
120	0.51
225	2.08
335	6.14
440	9.07
540	10.38
640	10.92
740	10.34
840	10.82
940	11.08

Table 4.3.10 - Progesterone loads along the horizontal plane in a CIDR®1900 Cattle insert after 12 hours invitro release

Median depth into CIDR®1900 Cattle insert (μm)	Progesterone load (% w/w)
60	0.41
170	0.61
285	1.53
400	6.27
500	9.54
600	9.57
700	9.58
800	9.93
900	9.78
1000	9.98

Table 4.3.11 - Progesterone loads along the horizontal plane in a CIDR®1900 Cattle insert after 24 hours invitro release

Median depth into CIDR®1900 Cattle insert (μm)	Progesterone load (% w/w)
50	0.65
155	0.45
275	0.47
390	1.10
500	2.93
610	7.25
710	9.11
810	9.70
910	9.92
1010	9.86

Table 4.3.12 - Progesterone loads along the horizontal plane in a CIDR®1900 Cattle insert after 71 hours invitro release

Median depth into CIDR®1900 Cattle insert (μm)	Progesterone load (% w/w)
95	0.48
305	0.33
520	0.39
710	0.35
875	0.85
1025	5.00
1175	8.78
1340	9.60
1505	9.92
1655	9.85

Table 4.3.13 - Progesterone loads along the horizontal plane in a CIDR®1900 Cattle insert after 137 hours invitro release

Median depth into CIDR®1900 Cattle insert (μm)	Progesterone load (% w/w)
100	0.22
330	0.23
570	0.27
790	0.24
1000	0.27
1200	4.28
1410	8.94
1595	9.76
1745	9.83
1895	10.06

4.3.3 Invivo Drug Release

Table 4.3.14 contains residual determinations and mean amount of progesterone released from CIDR®1900 Cattle insert on each day of a ten day insertion period.

Table 4.3.14 - Residual drug loads and average amount of progesterone released from CIDR®1900 Cattle insert invivo

Time (days)	Determined residual load (g)			Amount Released (g)	
	Rep 1	Rep 2	Mean	Mean	SEM
1	1.80	1.80	1.80	0.12	0.001
2	1.72	1.71	1.72	0.20	0.005
3	1.63	1.63	1.63	0.29	0.002
4	1.60	1.57	1.59	0.33	0.015
5	1.51	1.53	1.52	0.40	0.008
6	1.45	1.47	1.46	0.46	0.006
7	1.42	1.35	1.38	0.54	0.035
8	1.36	1.32	1.34	0.58	0.021
9	1.30	1.27	1.29	0.63	0.020
10	1.29	1.23	1.26	0.66	0.030

4.3.4 Determination of Drug Content in the Horizontal Plane

4.3.4.1 Invivo Release

Tables 4.3.15 through 4.3.25 contain progesterone loadings determined through the horizontal plane of CIDR®1900 Cattle insert on days one through ten of an invivo insertion.

Table 4.3.15 - Progesterone load along the horizontal plane in a fresh CIDR®1900 Cattle insert

Replicate 1		Replicate 2	
Median depth into CIDR®1900 Cattle insert (μm)	% Load	Median depth into CIDR®1900 Cattle insert (μm)	% Load
80	9.76	80	9.03
240	10.00	260	8.31
420	9.12	440	9.10
600	9.42	600	9.37
760	9.88	755	10.05
920	9.11	905	9.87
1075	9.74	1055	9.98

Table 4.3.16 - Progesterone load along the horizontal plane in a CIDR®1900 Cattle insert after release for 1 day invivo

Replicate 1		Replicate 2	
Median depth into CIDR®1900 Cattle insert (μm)	% Load	Median depth into CIDR®1900 Cattle insert (μm)	% Load
75	8.09	100	12.77
225	8.23	275	8.62
375	8.98	410	8.89
500	8.51	530	8.99
625	9.29	650	8.92
765	9.61	770	9.09
895	9.31	890	9.87
1020	9.60	1010	9.30
1140	9.72	1130	9.95
1260	9.88	1250	9.95

Table 4.3.17 - Progesterone load along the horizontal plane in a CIDR®1900 Cattle insert after release for 2 days invivo

Replicate 1		Replicate 2	
Median depth into CIDR®1900 Cattle insert (μm)	% Load	Median depth into CIDR®1900 Cattle insert (μm)	% Load
95	7.29	95	7.29
240	8.00	240	8.00
340	8.77	340	8.77
440	8.85	440	8.85
540	8.76	540	8.76
640	8.93	640	8.93
740	9.25	740	9.25
840	9.46	840	9.46
940	9.71	940	9.71
1040	9.53	1040	9.53

Table 4.3.18 - Progesterone load along the horizontal plane in a CIDR®1900 Cattle insert after release for 3 days invivo

Replicate 1		Replicate 2	
Median depth into CIDR®1900 Cattle insert (μm)	% Load	Median depth into CIDR®1900 Cattle insert (μm)	% Load
70	9.12	75	5.52
190	8.44	205	5.86
300	8.53	315	7.56
420	8.80	425	8.85
490	9.24	525	9.15
555	9.10	620	9.39
670	9.12	720	9.61
785	9.27	820	9.89
890	9.36	920	10.46
990	9.39	1020	10.18

Table 4.3.19 - Progesterone load along the horizontal plane in a CIDR®1900 Cattle insert after release for 4 days invivo

Replicate 1		Replicate 2	
Median depth into CIDR®1900 Cattle insert (μm)	% Load	Median depth into CIDR®1900 Cattle insert (μm)	% Load
100	6.17	125	5.84
270	7.09	305	7.26
400	8.11	410	7.25
515	8.61	510	8.23
620	9.48	610	10.01
720	9.92	710	10.10
825	10.07	810	9.61
935	9.30	910	9.82
1045	9.70	1005	9.80
1150	8.87	1100	9.79

Table 4.3.20 - Progesterone load along the horizontal plane in a CIDR®1900 Cattle insert after release for 5 days invivo

Replicate 1		Replicate 2	
Median depth into CIDR®1900 Cattle insert (μm)	% Load	Median depth into CIDR®1900 Cattle insert (μm)	% Load
55	4.34	65	4.10
165	2.81	195	4.46
270	3.83	315	4.86
370	5.71	420	6.32
475	6.83	520	7.48
580	8.41	620	8.63
680	8.87	720	9.29
790	9.71	820	9.20
965	9.04	920	9.41
1205	9.68	1020	9.44

Table 4.3.21 - Progesterone load along the horizontal plane in a CIDR®1900 Cattle insert after release for 6 days invivo

Replicate 1		Replicate 2	
Median depth into CIDR®1900 Cattle insert (μm)	% Load	Median depth into CIDR®1900 Cattle insert (μm)	% Load
50	6.60	55	3.05
155	6.52	160	4.29
265	6.70	260	4.94
370	7.33	360	6.17
470	7.82	455	8.11
570	8.84	550	8.68
670	10.01	645	9.19
770	10.03	740	8.89
870	9.73	840	9.13
970	9.48	940	9.69

Table 4.3.22 - Progesterone load along the horizontal plane in a CIDR®1900 Cattle insert after release for 7 days invivo

Replicate 1		Replicate 2	
Median depth into CIDR®1900 Cattle insert (μm)	% Load	Median depth into CIDR®1900 Cattle insert (μm)	% Load
60	3.87	60	2.97
170	3.41	185	5.29
270	4.83	295	5.10
370	6.59	390	5.42
470	7.55	490	6.46
570	8.85	590	7.56
670	9.58	690	8.52
770	9.46	790	9.71
865	9.40	890	9.36
960	9.73	990	9.79

Table 4.3.23 - Progesterone load along the horizontal plane in a CIDR®1900 Cattle insert after release for 8 days invivo

Replicate 1		Replicate 2	
Median depth into CIDR®1900 Cattle insert (μm)	% Load	Median depth into CIDR®1900 Cattle insert (μm)	% Load
60	4.07	65	3.13
170	3.93	170	4.54
270	5.05	265	5.25
370	6.06	370	5.61
480	7.23	470	7.55
590	8.47	575	8.78
690	9.59	685	9.16
790	9.40	790	9.34
890	9.29	890	9.91
990	9.46	990	10.16

Table 4.3.24 - Progesterone load along the horizontal plane in a CIDR®1900 Cattle insert after release for 9 days invivo

Replicate 1		Replicate 2	
Median depth into CIDR®1900 Cattle insert (μm)	% Load	Median depth into CIDR®1900 Cattle insert (μm)	% Load
100	2.64	70	3.51
255	3.19	185	3.57
360	3.81	280	3.95
460	4.70	380	4.64
560	6.22	480	5.18
660	6.70	585	6.46
760	8.22	695	7.69
860	9.31	800	8.56
960	9.66	900	9.26
1060	9.29	1000	9.50

Table 4.3.25 - Progesterone load along the horizontal plane in a CIDR®1900 Cattle insert after release for 10 days invivo

Replicate 1		Replicate 2	
Median depth into CIDR®1900 Cattle insert (μm)	% Load	Median depth into CIDR®1900 Cattle insert (μm)	% Load
65	3.75	70	1.47
180	3.48	190	2.06
280	4.45	290	3.34
380	5.33	390	4.53
480	7.17	490	6.12
580	8.17	595	7.16
680	8.63	705	8.35
780	9.02	810	8.44
880	8.94	910	8.84
980	9.24	1010	9.56

4.3.5 Invivo Blood Profile Resulting from Insertion of CIDR®1900 Cattle Insert

Plasma progesterone levels determined over the course of seven, ten and twenty day CIDR®1900 Cattle insert insertions are presented in Table 4.3.26 (Data reprinted from Chapter Two, Section 2.3.6).

Table 4.3.26 - Plasma progesterone levels in ovariectomised Friesian cows determined during 10 and 20 day insertions of CIDR®1900 Cattle insert

Trial and Replicate	Plasma progesterone level (ng/mL) on given day (d)										
	0d	1d	2d	3d	4d	5d	6d	7d	8d	9d	10d
7 Day Trial #1	0.04	5.04	3.49	2.82	2.84	3.48	2.77	3.21	-	-	-
#2	0.10	5.33	4.23	2.71	2.80	2.51	2.53	2.92	-	-	-
#3	0.18	4.98	3.74	2.66	2.69	3.14	3.35	3.28	-	-	-
#4	0.03	6.36	4.20	2.90	2.77	3.13	3.33	3.00	-	-	-
#5	0.00	6.03	6.10	3.98	4.00	3.52	3.49	2.93	-	-	-
#6	0.03	5.63	8.56	3.68	3.31	2.92	3.31	2.42	-	-	-
#7	0.10	3.42	4.34	3.59	3.60	3.03	3.10	2.25	-	-	-
#8	0.00	3.44	4.18	3.87	3.09	3.22	3.27	2.67	-	-	-
10 Day Trial #1	0.04	4.81	4.23	4.69	3.77	3.27	3.53	3.02	2.51	2.76	2.62
#2	0.06	4.13	2.77	3.92	2.51	3.30	2.80	2.69	2.25	2.34	2.41
#3	0.05	3.93	3.05	4.84	2.92	3.65	3.45	3.32	2.10	2.10	2.21
#4	0.04	4.16	2.70	3.20	2.77	3.50	3.85	3.69	1.93	1.81	1.76
#5	0.00	2.02	2.37	3.56	1.89	2.66	2.88	2.19	1.84	1.55	1.92
#6	0.00	4.42	3.93	2.89	2.30	2.57	2.87	2.16	1.91	1.97	1.81
#7	0.00	4.19	2.46	2.47	2.05	2.00	1.98	1.77	1.37	1.42	1.74
#8	0.00	3.67	3.59	3.32	2.62	2.88	2.95	2.62	2.41	2.57	2.70
#9	0.92	5.38	4.49	2.80	4.50	5.01	2.53	3.27	4.26	2.73	2.33
#10	0.00	2.99	3.00	1.91	2.22	4.68	1.47	1.51	1.74	1.65	1.34
20 Day Trial #1	0.03	4.39	3.17	3.44	2.41	2.74	2.70	2.67	2.08	1.85	1.88
#2	0.00	3.96	4.67	4.22	2.68	3.65	3.86	3.59	2.55	2.52	3.06
#3	0.07	3.16	3.37	4.65	2.96	3.47	3.28	2.86	1.90	1.88	2.10
#4	0.00	4.47	4.25	4.39	3.65	4.08	3.01	3.08	2.70	2.41	3.08
#5	0.00	3.85	4.32	3.40	3.23	3.22	3.87	2.68	2.47	1.99	2.21
#6	0.00	4.48	3.94	2.57	2.62	3.22	2.46	2.52	1.73	1.82	2.26
#7	0.04	3.33	2.69	2.55	2.58	1.94	2.73	2.37	1.61	2.00	2.29
#8	0.02	2.32	2.07	2.84	2.17	2.14	2.75	2.01	2.16	1.59	2.70
#9	0.03	3.64	3.52	2.82	2.46	2.53	2.42	2.17	1.65	1.80	1.93
#10	0.00	4.72	4.03	2.85	3.13	3.50	2.57	2.94	2.45	1.71	3.40

4.4 Discussion

4.4.1 Validation of Slicing Technique

Extraction of progesterone from the thin horizontal slices produced by the slicing technique was shown to be 98% efficient using dichloromethane as the solvent and when subjecting the slices to an extraction time of 2 hours. The extraction method was shown to be specific with minor interference (<4% bias) from unknown extractables from the silicone matrix and very low interference from the solvent. Overall the slicing technique was accurate and efficient for the purpose for which it was being applied.

4.4.2 Invitro Drug Release Profile for the CIDR®1900 Cattle Insert

4.4.2.1 Fitting of Invitro Data to Existing Models Describing Drug Release

Pharmaceutically, the CIDR®1900 Cattle insert can be described as comprising a homogenous dispersion of progesterone particles throughout a solid silicone matrix. This description is typically used within the pharmaceutical literature to describe monolithic matrix controlled release drug delivery systems[247]. The physical model which describes the release of drug from such delivery systems was first described by Higuchi[248] and is depicted in Figure 4.4.1[249, 250]. The type of release mechanism that this physical model describes is often referred to as the square root of time release mechanism.

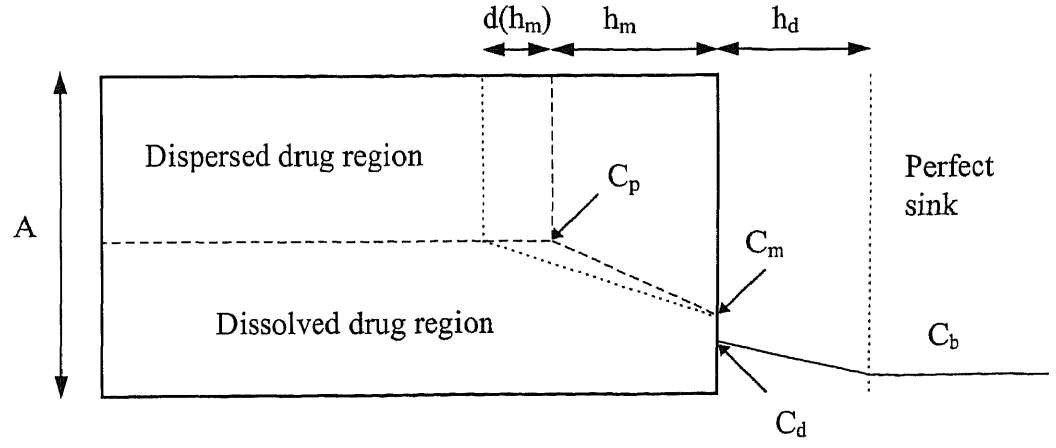

Figure 4.4.1 - Theoretical physical model for monolithic controlled release drug delivery systems in contact with a perfect sink

In Figure 4.4.1, A is the initial amount of drug impregnated in a unit volume of 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 the elution solution; h_d and h_m are the thickness of the hydrodynamic diffusion layer on the immediate surface of the matrix and of the depletion zone, respectively; and $d(h_m)$ is the differential thickness of the depletion zone when more solid drug is released.

Equations describing release from a monolithic matrix controlled release drug delivery system have been derived from the physical model depicted in Figure 4.4.1. Such equations state that the cumulative amount of progesterone delivered by the monolithic matrix controlled release drug delivery system would be defined by:

$$Q = \left[\left(2A - C_p\right)C_p D_p t\right]^{1/2} \qquad \textbf{Equation 4.4.1}$$

Where:

Q = cumulative amount of drug released from a unit area of matrix (g/cm^2)
A = the initial amount of drug incorporated into a unit volume of matrix (g/cm^3)
C_p = the solubility of the drug in the polymer phase (g/cm^3)
D_p = the diffusion coefficient of the drug in the polymer phase (cm^2hr^{-1})
t = time (hours)

Assuming that drug load >> C_p then Equation 1 reduces to:

$$Q = \left[2AC_p D_p t\right]^{1/2} \qquad \textbf{Equation 4.4.2}$$

Thus the release profile of progesterone from the matrix is defined by:

$$Q/t^{1/2} = \left[2AC_p D_p\right]^{1/2} \qquad \textbf{Equation 4.4.3}$$

and the rate of release becomes time dependent as defined by:

$$\frac{dQ}{dt} = \left[\frac{2AC_pD_p}{2t}\right]^{1/2}$$ **Equation 4.4.4**

Equation 4.4.2 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 $[2AC_pD_p]^{1/2}$.

Figure 4.4.2 shows the release profile for the CIDR®1900 Cattle insert resulting from the pooled data acquired for seventeen individual devices over 94 hours of assessment.

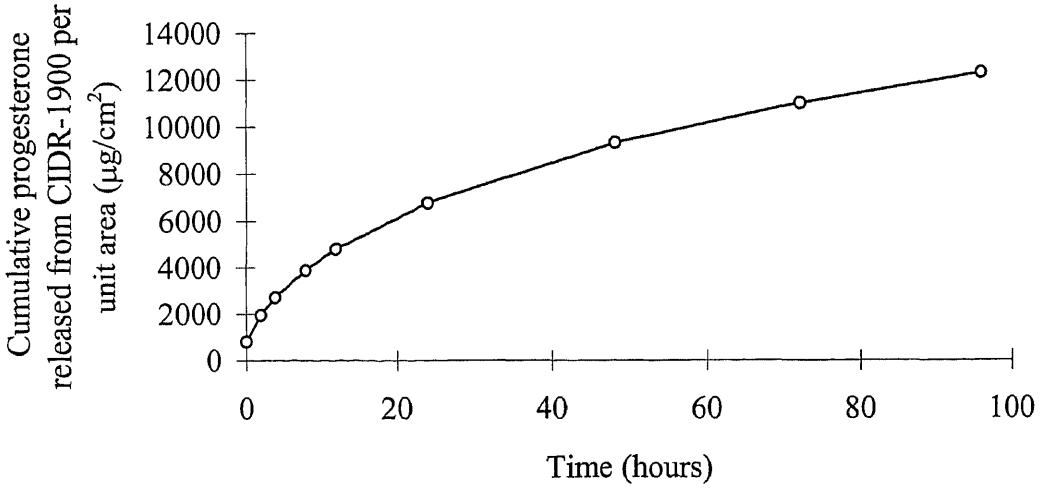

Figure 4.4.2 - Invitro release profile for CIDR®1900 Cattle insert (N = 17)

Replotting the data shown in Figure 4.4.2 against the square root of time in accord with Equation 4.4.2 we obtain Figure 4.4.3.

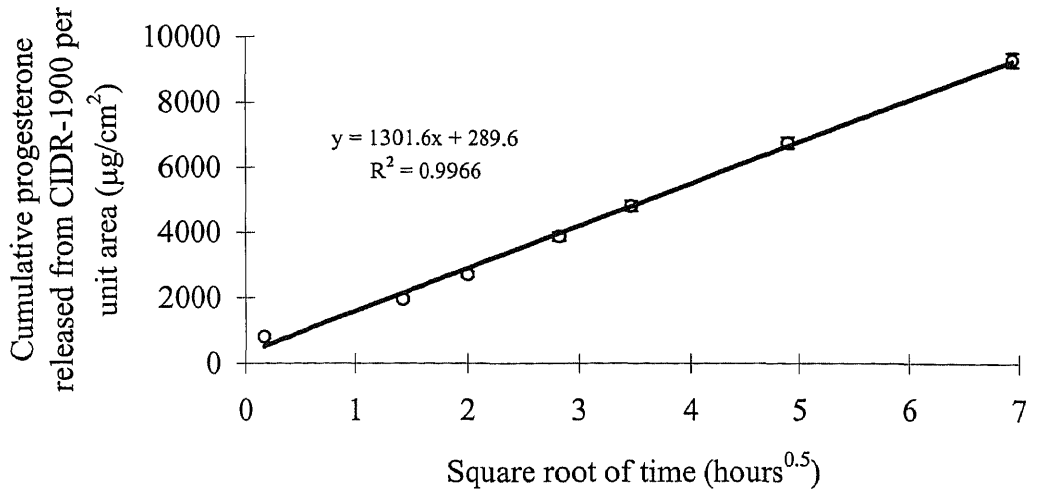

Figure 4.4.3 - Invitro release profile for CIDR®1900 Cattle insert plotted against the square root of time (N=17, error bars = SEM)

Figure 4.4.3 shows a linear dependence (F=1.867; not significant at α=0.05) of cumulative amount released on the square root of time (R^2 = 0.997), suggesting that the invitro release of progesterone from the CIDR®1900 Cattle insert is described by a square root of time release mechanism and attests to the appropriateness of this model for our experimentally generated data. This analysis and conclusion agrees with data reported by Rathbone et al.[101].

4.4.2.2 Experimental Confirmation that Invitro Drug Release from CIDR®1900 Cattle Insert Occurs via a Square Root of Time Mechanism

The model fitting exercise suggests that the release of progesterone from the CIDR®1900 Cattle insert occurs according to a square root of time mechanism. If this is so, then the release of progesterone from the CIDR®1900 Cattle insert can be visualized as follows:

1. The CIDR®1900 Cattle insert delivery system comprises progesterone dispersed homogeneously throughout a silicone matrix formed by cross-linking of individual silicone polymer chains. The progesterone exists both in solution in the silicone and as discrete solid particles which are evenly distributed throughout the silicone polymer network.

2. Solid progesterone particles are fixed in position during fabrication of the CIDR®1900 Cattle insert and can not delocalize throughout the silicone following manufacture. As a result only solubilized drug can be released from the CIDR®1900 Cattle insert (i.e., solid particles cannot be released from the CIDR®1900 Cattle insert).

3. Release of progesterone therefore occurs through a series of steps. These are:
    - dissolution of solid progesterone into the silicone that immediately surrounds the drug particle
    - diffusion of the dissolved progesterone from the area of dissolution through the silicone to the surface of the CIDR®1900 Cattle insert
    - partitioning of solubilized progesterone from the surface of the silicone into the surrounding aqueous environment
    - movement of released progesterone away from the surface of the CIDR®1900 Cattle insert[a].

This process results in solid drug near the surface being eluted first. As more and more silicone becomes depleted of solid progesterone a zone which is void of solid drug becomes apparent. This is referred to as the 'depletion zone'.

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 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 progesterone from the CIDR®1900 Cattle insert it is assumed that the diffusion of progesterone through the silicone matrix is the slowest step in the whole process.

[a] microscopically a thin layer of stagnant fluid termed the hydrodynamic diffusion boundary layer will exist on the immediate surface of the CIDR®1900 Cattle insert. Within this layer, drug molecules move, under a concentration gradient, from the surface of the CIDR®1900 Cattle insert in to the bulk of the receptor fluid. In our experiments we increased the stirring speed to one which effectively eliminates this layer. This layer does not, therefore, play any role in drug release in our invitro studies.

It should be noted that diffusion through the polymer occurs because the dissolved progesterone sets up a concentration gradient between where it has dissolved and the surface of the CIDR®1900 Cattle insert. The magnitude of the concentration gradient is dependent upon the inherent solubility of the progesterone in the silicone polymer (i.e., its saturation solubility) and remains constant for each successive layer of progesterone particles. However, as the depletion zone becomes larger, the diffusional distance that the next dissolving drug particles 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 the non-linear dependence of progesterone release with time.

This description suggests that experimental confirmation of the square root of time mechanism could be achieved by taking consecutive horizontal slices to determine the change in drug content of each slice as a function of time. If the square root of time mechanism was in operation, the technique should show the presence of a drug depletion zone. The results from the horizontal slicing technique appeared to confirm that the progesterone was being released from the CIDR®1900 Cattle insert in accord with the previously described square root of time mechanism (Figure 4.4.4). Prior to commencement of release, the CIDR®1900 Cattle insert exhibited a uniform distribution of progesterone throughout the silicone at the 10% w/w level at which progesterone and silicone are combined during manufacturing (0 hours; Figure 4.4.4). As release occurred, the outer layers of the CIDR®1900 Cattle insert skin became depleted of progesterone and this depletion zone continued to grow larger with time (Figure 4.4.4).

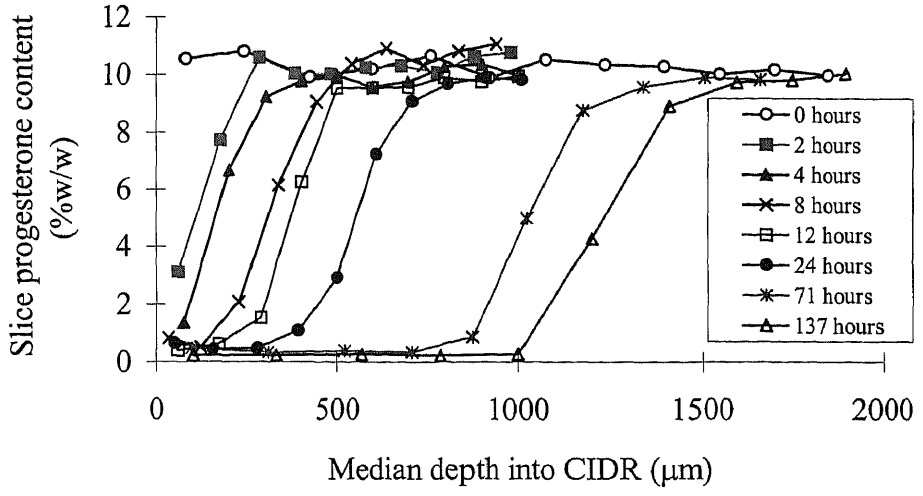

Figure 4.4.4 - Progesterone loads along the horizontal plane measured by the slicing technique in CIDR®1900 Cattle insert subjected to the invitro drug release test for different lengths of time

Further evidence can be provided by comparing the experimentally observed depletion zone with the theoretical depletion zone depth calculated from the cumulative mass of progesterone released (as predicted by the linear relationship between the average cumulative release from the CIDR®1900 Cattle insert and the square root of time) at each time point, the mass of CIDR®1900 Cattle insert matrix required to deliver this quantity of progesterone, the density of CIDR®1900 Cattle insert matrix and the surface area of the CIDR®1900 Cattle insert using equation 4.4.5.

$$h_m = \frac{S(mt+c)}{1000000} \times \frac{1}{A} \times \frac{1}{\rho} \times \frac{1}{S} \times \frac{10000}{1} = \frac{m\sqrt{t}+c}{10\rho} \qquad \text{Equation 4.4.5}$$

Where

h_m = thickness of the depletion zone (μm)

S = surface area of the CIDR®1900 Cattle insert (120 cm^2)

m = slope of the average cumulative progesterone released per unit area versus root time line (1301.6 $\mu g.cm^{-2}.hr^{-0.5}$)

c = intercept of the average cumulative progesterone released per unit area versus root time line (298.6 $\mu g.cm^{-2}$)

t = time (hr)

A = initial progesterone concentration in CIDR matrix (10 %w/w)

ρ = density of 10 %w/w progesterone loaded silicone (1.09 $g.cm^{-3}$)

1000000 = µg to g conversion factor ($\mu g.g^{-1}$)

10000 = cm to µm conversion factor ($\mu m.cm^{-1}$)

Calculated values of the depletion depth are tabulated against the observed depletion depths in Table 4.4.1 where the observed depletion depth was taken to be the mid-point of the 'S-shaped' curve in Figure 4.4.4.

Table 4.4.1 - Theoretical versus observed depletion depths for the invitro slicing experiment

Time (hours)	Square root of time ($hours^{0.5}$)	Theoretical depletion depth (µm)	Observed depletion depth (µm)
2	1.41	195	150
4	2.00	265	220
8	2.83	364	350
12	3.46	440	450
24	4.90	612	600
71	8.43	1033	1000
137	11.70	1424	1250

The calculated and observed values for depletion depth agree well, adding further evidence that the invitro release of progesterone from the CIDR®1900 Cattle insert is described by the square root of time release mechanism.

4.4.3 Invivo Drug Release Profile

4.4.3.1 Fitting of Invivo Data to Existing Models Describing Drug Release

The invivo release profile determined from the residual load of CIDR®1900 Cattle inserts following their intravaginal insertion in Friesian cows for periods of 1-10 days is shown in Figure 4.4.5.

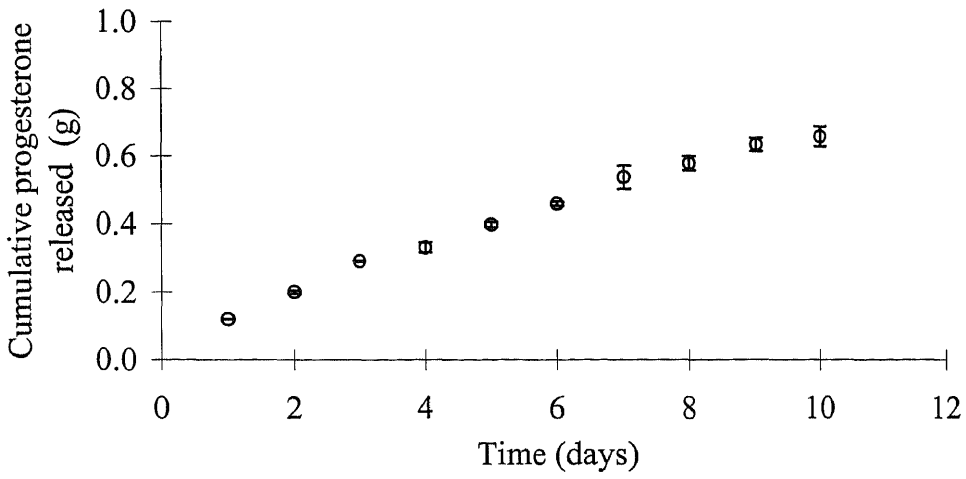

Figure 4.4.5 - Invivo release profile for CIDR®1900 Cattle insert (n = 2, error bars = SEM)

When the data used to generate Figure 4.4.5 is plotted against the square root of time the linear appearance of the curve and high R^2 value ($R^2 = 0.992$) appear to suggest that progesterone was being released invivo in accord with the square root of time mechanism (Figure 4.4.6). Testing for non-linearity confirms this observation ($F=0.747$; not significant at $\alpha=0.05$).

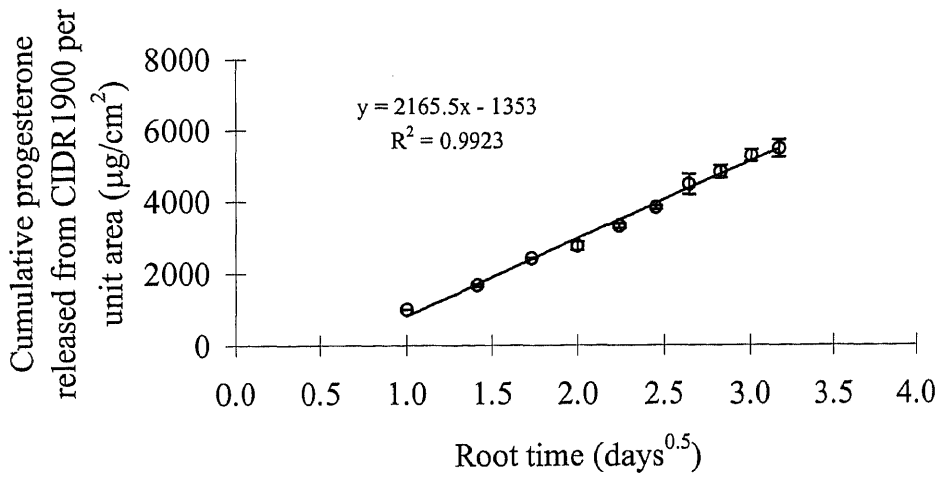

Figure 4.4.6 - Invivo release from CIDR®1900 Cattle insert - square root of time plot (N=2, error bars = SEM)

4.4.3.2 Experimental Evidence that Invivo Drug Release from CIDR®1900 Cattle Insert does not Occur via a Square Root of Time Mechanism

The model fitting exercise on the invivo data suggested that the release of progesterone from the CIDR®1900 Cattle insert occurs according to a square root of time mechanism. However, the horizontal slicing data obtained on CIDR®1900 Cattle insert inserted intravaginally for various days, appeared to contradict the outcomes of the model fitting exercise (Figure 4.4.7).

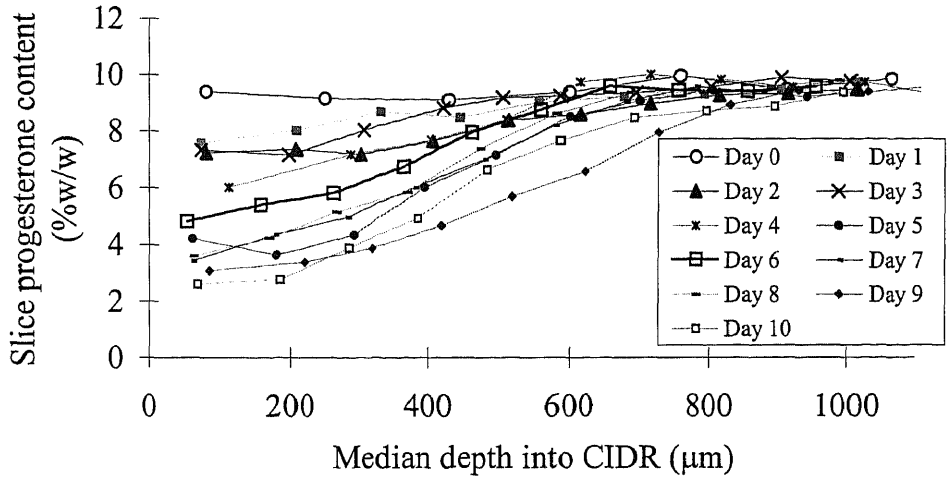

Figure 4.4.7 - Progesterone loads along the horizontal plane measured by the slicing technique in CIDR®1900 Cattle insert inserted in intact Friesian cows for different lengths of time

Figure 4.4.7 shows that a depletion zone void of any particulate drug does not appear to form. The question arises therefore, is a depletion zone not observed because it is too small to be detected using the horizontal slicing technique? (i.e., if the theoretical depletion zones were <100 μm, then the slicing technique would not be capable of detecting them). To answer this query, the magnitude of the depletion zones were theoretically calculated based on the square root of time regression equation generated from the invivo data (Figure 4.4.6) using equation 4.4.5.

The theoretical depletion zones (Table 4.4.2) suggested that if the square root of time mechanism was in operation, they would have been easily detected with the horizontal slicing technique. For example, the square root of time model predicted a theoretical depletion zone of 438 μm at day 7. A depletion zone of such depth would easily have been determined by the slicing technique. Proof of this can be seen in

Figure 4.4.4 where the theoretical depletion depth of 440 μm which should occur after 12 hours of invitro drug release testing, is easily detected using the horizontal slicing technique.

Table 4.4.2 - Theoretical depletion depth according to square root of time mechanism for invivo release from CIDR®1900 Cattle insert

Time (days)	Square root of time ($days^{0.5}$)	Theoretical depletion depth (μm)
1	1.00	124
4	2.00	315
7	2.65	438
10	3.16	504

Examination of Figure 4.4.7 shows that progesterone is lost from the CIDR®1900 Cattle insert during intravaginal insertion. While progesterone is being lost from the insert invivo, an unusual phenomenon appears to occur within the silicone matrix which contrasts to that which was observed when the insert was examined invitro. During invitro release, a drug depletion zone formed as drug was lost from the insert and the amount of drug particles per unit volume remained constant beyond the depletion zone in accord with the square root of time mechanism (Figure 4.4.4). In the invivo case, as drug was being lost, two differences were observed. Firstly, a depletion zone did not form. Secondly, progesterone was lost from deeper within the silicone matrix than those depths predicted by the square root of time mechanism (Table 4.4.2). The result of these differences is a continuously changing gradient in which the number of progesterone particles per volume of silicone on any day was lowest at the interface and progressively increased with increasing depth into the silicone skin. As each day passed, the gradient changed in a characteristic manner, with progesterone particle content declining near the surface of the insert (but not forming a depletion zone) and simultaneously extending into the deeper layers of the silicone skin. This can be seen more clearly in Figure 4.4.8 when selected days are plotted.

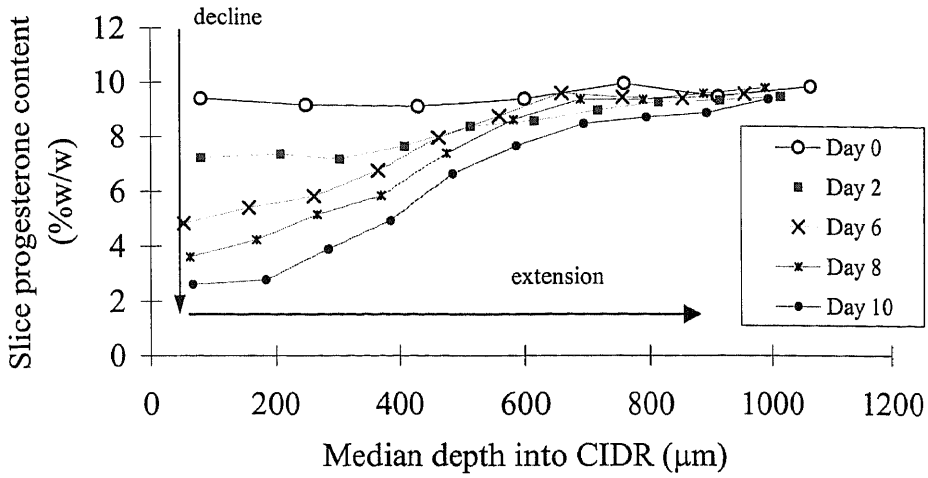

Figure 4.4.8 - Progesterone loads along the horizontal plane measured by the slicing technique in CIDR®1900 Cattle insert inserted in intact Friesian cows - selected insertion times

The observed change in progesterone profile within the silicone matrix with time conflicts with the predictions of the square root of time mechanism and does not support the conclusion drawn from simple curve fitting (Figure 4.4.6) that release occurs via a square root of time mechanism. The depleted zone and rapid increase to full initial particulate progesterone loading observed in the invitro experiment (Figure 4.4.4) is clearly not reproduced in the invivo model (Figure 4.4.7).

It is apparent, therefore, that even though the invivo release data can be fitted to a square root of time mechanism plot, the square root of time mechanism does not appear to be an appropriate model to describe the invivo release of progesterone from the CIDR®1900 Cattle insert based on the results obtained from the horizontal slicing technique.

Having discounted the square root of time release mechanism as a descriptor of the invivo release of progesterone from the CIDR®1900 Cattle insert, the task remains to propose a description which more appropriately describes the experimentally determined invivo release profile and which concurs with the experimental observations.

An outcome of the phenomenon described above which occurs during invivo release of progesterone from the silicone matrix (Figures 4.4.7 and 4.4.8) is that solid

particles are always present in the immediate vicinity of the polymer/solution interface and the resultant dissolution of such particles into the silicone within this vicinity instantly replaces any drug lost from the insert by the process of partitioning. This results in the concentration of drug at the polymer/solution interface on the polymer side being always equal to the saturation solubility of the drug in the silicone matrix. Thus, invivo release of progesterone is controlled via a partitioning process rather than a diffusion controlled mechanism. Such a theory would therefore predict a constant release of drug from the CIDR®. Replotting the invivo data as a function of time and curve fitting a linear regression to that data (Figure 4.4.9) reveals a good fit ($F=1.183$; not significant at $\alpha=0.05$) with a reasonable correlation coefficient ($R^2 = 0.9891$). Based on the good fit and the results from the slicing technique, we suggest that invivo release of progesterone from the CIDR® occurs via a zero order release mechanism.

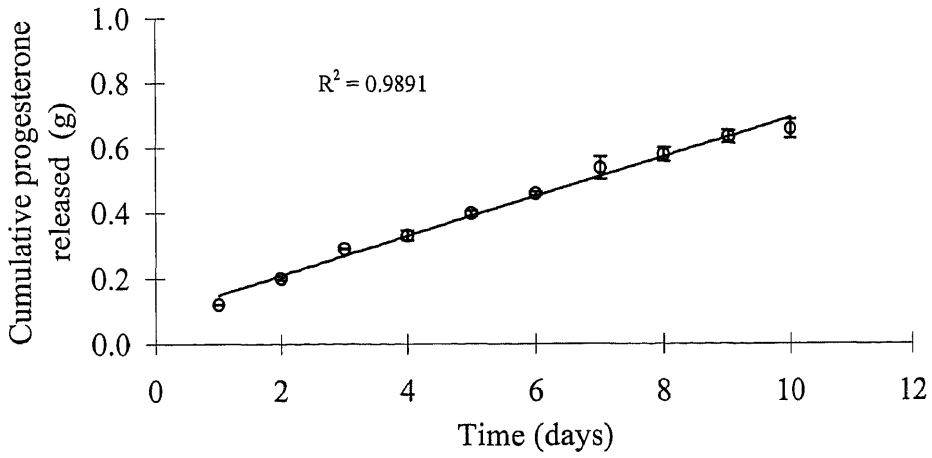

Figure 4.4.9 - Invivo release profile for CIDR®1900 Cattle insert with linear regression applied

4.4.4 Correlation of Invivo Release Mechanism with Invivo Blood Profile

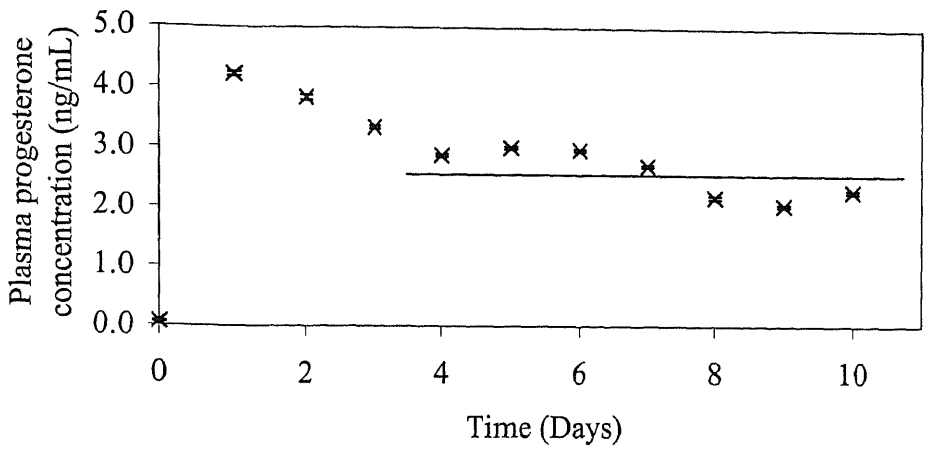

Figure 4.4.10 – Plasma progesterone profile resulting from insertion of CIDR®1900 Cattle insert - combined data (N=28, error bars = SEM)

Following insertion of a CIDR®1900 Cattle insert an initial spike in plasma progesterone level is observed which falls over the next 2-3 days to levels which were constant or diminished very slightly over the remaining days of the insertion period. The apparent steady-state levels occurring over the latter days of the insertion period reflect the constant delivery of progesterone to the cows arising from the zero order release of progesterone from the CIDR®1900 Cattle insert. Therefore the observed invivo plasma profile appears to support the proposal that the invivo release of progesterone from the CIDR®1900 Cattle insert occurs via a zero order mechanism.

4.5 Conclusions

In this chapter the invitro and invivo mechanism of release of progesterone from the CIDR®1900 Cattle insert was investigated.

Invitro release of progesterone from the CIDR®1900 Cattle insert was found to occur in accordance with the square root of time mechanism as predicted for a matrix type drug delivery system and in agreement with the square root of time release mechanism model commonly found in the literature[249, 250]. A horizontal slicing technique supported this finding and provided evidence of depletion zone formation as the drug release process proceeded.

Data fitting of the experimentally determined invivo release of progesterone from the CIDR®1900 Cattle insert suggested that invivo release of progesterone from the CIDR®1900 Cattle insert also followed a square root of time mechanism. However, this data fitting exercise was not supported by experimental evidence obtained using the slicing technique, which produced evidence that depletion zones did not form as drug release occurred. Contrary to the square root of time mechanism, when the CIDR®1900 Cattle insert was inserted into the vagina of cattle, progesterone appeared to redistribute through the silicone matrix to maintain a level of progesterone at the surface of the matrix which sustained a saturation concentration of progesterone in the layers of silicone in the immediate vicinity of the polymer/solution interface. Since a saturation concentration was maintained in the silicone at the interface, drug release was partitioning controlled resulting in a zero order release of progesterone from the CIDR®1900 Cattle insert.

Hopefully, one day, mathematicians will examine the experimental data presented in this Thesis and will develop a model to describe the novel release mechanism observed.

Chapter Five - Optimisation of a Commercially Available Silicone/Progesterone Intravaginal Veterinary Drug Delivery System for the Control of the Ovine Oestrous Cycle

5.1 Introduction

The CIDR® Sheep and Goat insert is a commercially available T-shaped intravaginal insert used in sheep (Figure 5.1)[4, 5]. It contains 0.3 g of progesterone which is homogeneously dispersed throughout a silicone matrix which is cured over a preformed T-shaped nylon spine. In these respects it is similar to the CIDR®1380 Cattle insert and CIDR®1900 Cattle insert which are used in cattle (and which have been extensively described in the preceding chapters of this Thesis), however, its dimensions are much smaller (Figure 5.1.1) in order to allow it to be easily inserted into sheep.

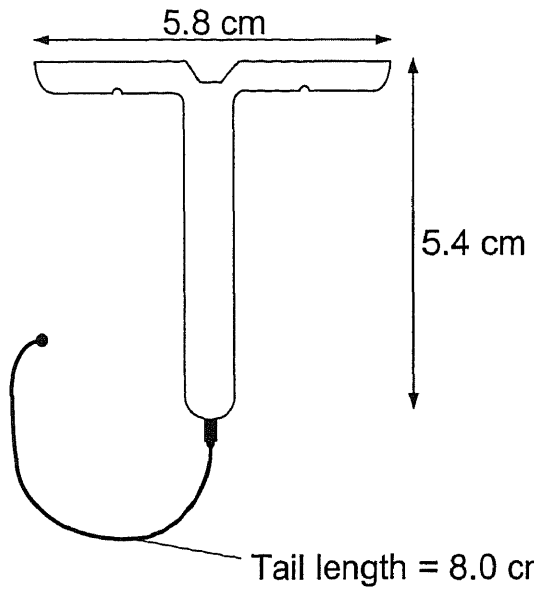

Figure 5.1.1 - Diagram of the CIDR® Sheep and Goat insert detailing dimensions

The CIDR® Sheep and Goat insert has been successfully used in treatment regimes in sheep for seasonal synchronization of oestrus in Australia and New Zealand[164, 178]. In this case, a single CIDR® Sheep and Goat insert is inserted into the vagina of sheep and removed 14 days later. It has also been used in embryo transfer fertility

applications including superovulating donor synchrony and recipient synchrony programmes. In these cases, the use of the CIDR® Sheep and Goat insert involves either simultaneous insertion of two CIDR® Sheep and Goat insert for the entire 14 day treatment period (simultaneous programme, Figure 5.1.2) or insertion of a single CIDR® Sheep and Goat insert into the vagina of sheep for 8 days, at which point it is removed and replaced with a fresh one for 6 more days (replacement programme, Figure 5.1.2)[46-48]. These protocols have been designed to provide plasma progesterone levels which are elevated above those seen when a single CIDR® Sheep and Goat insert is intravaginally inserted.

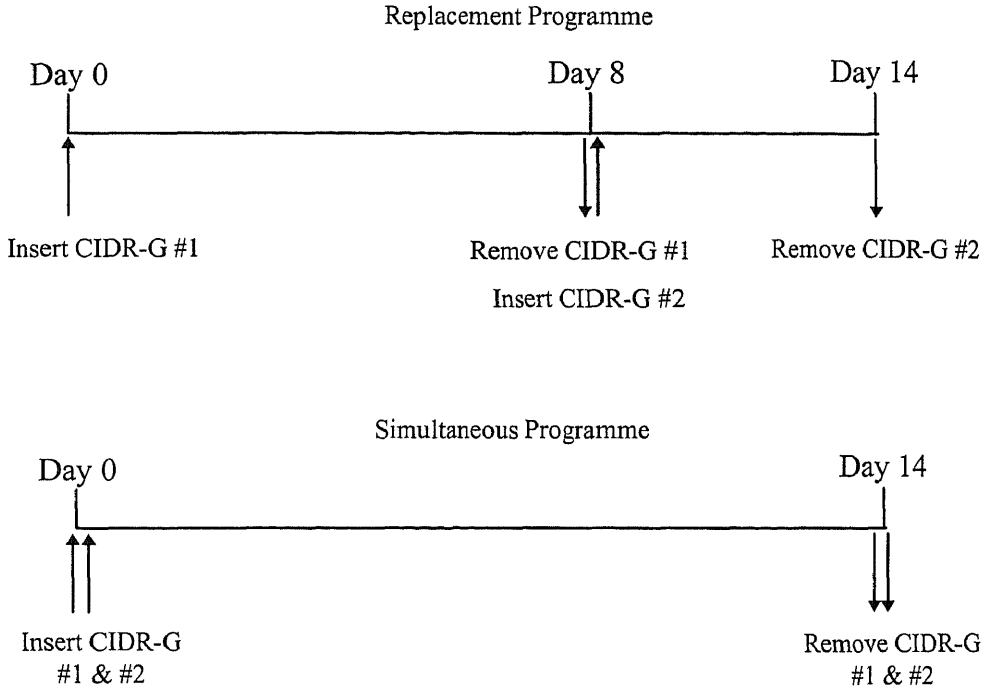

Figure 5.1.2 - Schematics of specialist fertility programmes

Although the simultaneous insertion of two CIDR® Sheep and Goat insert results in successful treatments, the insertion of two CIDR® Sheep and Goat insert simultaneously into the vagina of sheep is challenging to the end user, and it would be easier if a single silicone/progesterone insert could be developed which produces the same plasma levels as those observed when two CIDR® Sheep and Goat insert are inserted simultaneously. Likewise the increased time and manpower required to removed a CIDR® Sheep and Goat insert at day 8 and replace it with a fresh one could be overcome with a single insert which achieved the same clinical effect but

which required only a single administration and removal procedure. In this Chapter the CIDR® Sheep and Goat insert was optimised to achieve these goals.

The optimisation criteria were:

1. The optimised CIDR® Sheep and Goat insert should produce plasma profiles which mimicked those seen when the two CIDR® Sheep and Goat insert treatment regimes are used (Figure 5.1.2).
2. The optimised CIDR® Sheep and Goat insert should utilise the same manufacturing technology as the CIDR® Sheep and Goat insert.
3. The optimised CIDR® Sheep and Goat insert be no more expensive to manufacture than a single currently available CIDR® Sheep and Goat insert.

To optimise the CIDR® Sheep and Goat insert it was pharmaceutically characterised with respect to its physical characteristics and invivo performance and thereafter, knowledge from literature reports of extensive investigations on the CIDR®1900 Cattle insert[6, 102, 222] were used to define the parameters used to produce a prototype silicone intravaginal insert. Extensive invivo studies were then performed on the prototype silicone intravaginal insert to determine if the optimisation process had been successful.

5.2 Methods

5.2.1 Manufacture of CIDR® Sheep and Goat insert and Prototype Silicone Intravaginal Inserts

CIDR® Sheep and Goat insert were manufactured using InterAg's patented high temperature injection moulding technique Progesterone (USP micronised, Pharmacia & Upjohn, USA) was mixed at 9%w/w in both parts A and B silicone (Silastic® Q7-4840, Dow Corning, USA) in a dynamic epicentric mixer (InterAg, Hamilton, New Zealand) for a minimum of 2 hours. The mixture was then evacuated for a minimum of 2 hours at -60 kilopascals. Nylon (Ultramid A3K, BASF, Germany) spines were annealed in a water bath at 70°C for 7.5 hours. Spines were then placed in a 4 cavity tool mounted into an injection moulding machine (LIM-100-35V, Sanjo Seiki Co., Ltd., Japan; Type PS40E5A, Nissei Plastic Industrial Co., Ltd., Japan) and drug loaded silicone parts A and B were simultaneously injected through a pre-mixing static mixer around the nylon spines and cured for approximately 50 seconds at around 190°C.

Prototype silicone intravaginal inserts were manufactured with the same equipment and materials used to manufacture the CIDR® Sheep and Goat insert (See Section 5.2.1 above) but with the following modifications. Firstly, silicone intravaginal inserts were fabricated using a prototype tool which produced inserts with increased wing span (7.3 cm) and increased body length (13.4 cm) (Figure 5.2.1). The prototype tool allowed the moulding of a silicone skin approximately 1 mm thick over the nylon spine. Also silicone intravaginal inserts with different initial drug loads were manufactured (10, 15, 25% w/w progesterone in silicone).

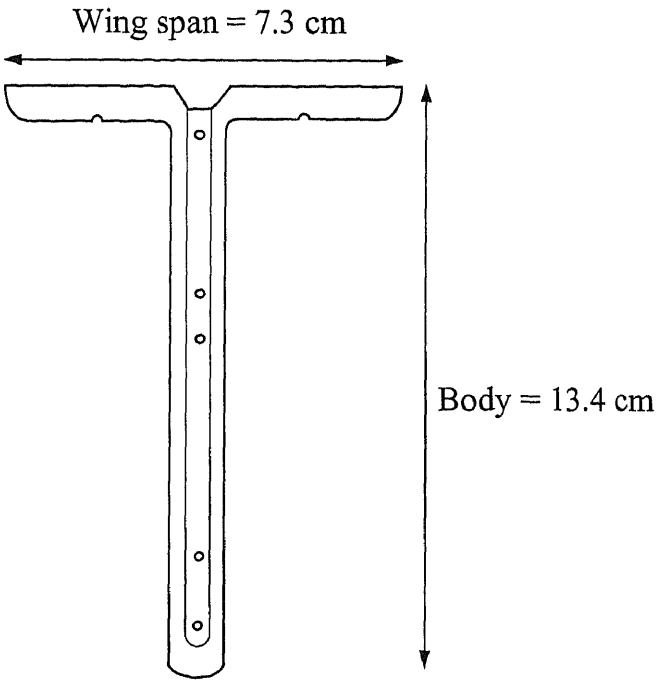

Figure 5.2.1 - Diagram detailing dimensions of the silicone intravaginal insert manufactured using the prototype tool

To produce the prototype silicone intravaginal inserts, the bodies of the manufactured inserts were cut down to appropriate dimensions (3, 5.5 and 8 cm) to give surface areas of 28, 40 and 50 cm^2. Finally, any exposed nylon spine was sealed off with RTV (Room Temperature Vulcanising) silicone and polyester tails were fitted to the end of the body opposite the wings to facilitate removal post-treatment.

5.2.2 Characterisation of Physical Dimensions of the Sheep Vagina

The depth of the sheep vagina was characterised using an artificial insemination rod which was marked in 0.5 cm graduations. Romney ewes were restrained in a bail, the graduated rod was dipped into veterinary lubricant and then carefully inserted into the vaginal cavity until the head of the rod contacted the cervical entrance. The graduation just visible at the vulva was then recorded. This measurement was taken as the vaginal depth for each ewe.

The measured vaginal lengths of the sheep were used to define the maximum length of the prototype silicone intravaginal inserts.

5.2.3 Characterisation of Physical Dimensions of the CIDR® Sheep and Goat insert and Prototype Silicone Intravaginal Inserts

Wing spans and body lengths of the CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts were determined using a calibrated ruler (300 mm, TOLEDO Steel International, Japan).

5.2.4 Determination of Initial and Residual Drug Load in CIDR® Sheep and Goat insert and Prototype Silicone Intravaginal Inserts

The amount of progesterone in the inserts immediately after manufacture, or following their removal after insertion into the vagina of test animals was determined as follows. The silicone skins of the CIDR® Sheep and Goat insert were removed from their nylon spines with a scalpel. The skins were then cut into 2-5 cm lengths, weighed on a four figure balance (BP110S, Sartorius, Germany) and placed into 100 mL capacity soxhlet apparatus, ensuring that the pieces of skin were all below the siphon level. 350 mL ethanol (SDA-3A, Mobil, New Zealand) was added to the round bottom flask of the Soxhlet apparatus before the flask, Soxhlet and water cooled condenser were assembled in a heating mantle (Electromantle ME, Electrothermal, Britain). The silicone skins were extracted for approximately 12 hours and allowed to cool to room temperature. The ethanolic extract was then transferred to a 500 mL volumetric flask with rinsing to ensure complete transfer before making the volume up to the mark with ethanol (SDA-3A, Mobil, New Zealand). The solution was then diluted (1 in 25) in ethanol (SDA-3A, Mobil, New Zealand) before analysis by UV spectrophotometry (DU 650i, Beckman, USA) at 240 nm using a validated assay (See 2.2.1).

5.2.5 Determination of Surface Area of CIDR® Sheep and Goat insert and Prototype Silicone Intravaginal Inserts

The surface area of the intravaginal inserts was determined using pieces of aluminium foil of known weight per unit surface area (0.0034 g/cm^2). These were adapted over the insert, trimmed and weighed, and surface areas determined from the weights on a four figure analytical balance (BP110S, Sartorius, Germany). Care was

taken when the foil was being adapted to the insert to avoid stretching which would have caused thinning of the foil. This was overcome by using small individual pieces of foil.

5.2.6 Determination of Skin Weight of CIDR® Sheep and Goat insert and Prototype Silicone Intravaginal Inserts

Silicone skins of CIDR® Sheep and Goat inserts were removed from the spines using a scalpel and weighed on a four figure analytical balance (BP110S, Sartorius, Germany).

5.2.7 Determination of Skin Thickness of CIDR® Sheep and Goat insert and Prototype Silicone Intravaginal Inserts

The thickness of the silicone skin of the intravaginal inserts was measured at a series of positions over the insert. Silicone skins were removed from the spine using a scalpel and the thickness of the skin was then carefully determined using an outside micrometer (Moore and Wright, Sheffield, England) (See Section 2.2.3) taking care not to compress the silicone skin during the process.

5.2.8 Determination of Invivo Plasma Profiles of CIDR® Sheep and Goat insert and Prototype Silicone Intravaginal Inserts

Ethical approval to perform the animal experiments in this part of the Thesis was obtained from both the AgResearch, Ruakura Animal Ethics Committee and from the University of Waikato Animal Ethics Committee on the Welfare of Experimental Animals.

Details of the insert formulations used in the study and the trial details are given in Tables 5.2.1 and 5.2.2.

Table 5.2.1 - Trial #1 - determination of the effect of surface area and drug load on plasma progesterone levels following intravaginal insertion of various CIDR® Sheep and Goat insert treatment regimes and various prototype inserts

Formulation	Insertion Time (days)	Number of Animals (N)
CIDR® Sheep and Goat insert: 9%w/w, 28 cm^2	14	6
Two CIDR® Sheep and Goat insert, replacement programme	14	6
Prototype I: 10%w/w, 29 cm^2	14	6
Prototype II: 10%w/w, 40 cm^2	14	6
Prototype III: 10%w/w, 50 cm^2	14	6
Prototype IV: 15%w/w, 40 cm^2	14	6
Prototype V: 25%w/w, 40 cm^2	14	6

Table 5.2.2 - Trial #2 - supplementary trial protocol to re-examine the effect of surface area on plasma progesterone levels following intravaginal insertion into larger ewes

Formulation	Insertion Time (days)	Number of Animals (N)
CIDR® Sheep and Goat insert: 9%w/w 28 cm^2	14	6
Two Simultaneous CIDR® Sheep and Goat insert	14	6
Prototype II: 10%w/w, 40 cm^2	14	6
Prototype III: 10%w/w, 50 cm^2	14	6

In each trial, CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts were inserted into the vagina of anoestrus Romney ewes using either a standard CIDR® Sheep and Goat insert applicator or modified applicator which had a slightly larger barrel bore size. Applicators were dipped in veterinary lubricant prior to administration of inserts. Treatments were allocated to the ewes in a random manner in Trial #1, however, larger ewes were selected in Trial #2 for insertion of prototype III which had a large surface area. Trials were terminated by removing the insert from the vagina by gently, but firmly, pulling on the nylon tail which was attached to the inserts and which protruded beyond the vulva of the animals. At the termination of trials, sheep underwent vaginal examination to assess for irritation or damage caused by the intravaginal inserts during their insertion. In each of the trials, plasma samples were collected immediately prior to insertion on day 0, during insertion on days 2, 4, 6, 8, 9, 10, 11, 12, 13, 14 and 24 hours following removal of

the inserts. Plasma samples were collected by trained AgResearch personnel from the jugular vein of the sheep. Immediately following sampling, the plasma was separated by centrifugation and stored at -20°C for subsequent progesterone analysis. Concentrations of progesterone in plasma were determined by direct radio immuno assay by trained technicians at DRC laboratories using a commercial solid phase I^{125} label (Coat-a-Count, DPC, USA).

5.2.9 Pharmacokinetic Analysis of Invivo Plasma Profiles of CIDR® Sheep and Goat Insert and Prototype Silicone Intravaginal Inserts

Area Under the Curve (AUC) data were determined from the blood plasma progesterone levels determined at given time points over the duration of treatment with intravaginal inserts for each individual ewe treated. The AUC were determined by simple numeric estimation using the trapezoidal rule[229] using the following equations:

$$AUC_i = \frac{C_k + C_j}{2} \times T_i \qquad \textbf{Equation 5.2.1}$$

$$AUC = \sum AUC_i \qquad \textbf{Equation 5.2.2}$$

where:

AUC_i is the area under the curve over the i^{th} time interval (ie. Area under curve between the j^{th} sample time and the k^{th} sample time).

C_k is the plasma level (ng/mL) at the k^{th} sample time.

C_j is the plasma level at the j^{th} sample time.

T_i is the numerical value of the ith time interval (days) (i.e. Time at which the k^{th} sample was taken minus time at which the j^{th} sample was taken).

The AUC for the different treatments were then compared by Oneway Unstacked ANOVA (MINITAB 8.2 Statistical Software).

5.3 Results

5.3.1 Manufacture of CIDR® Sheep and Goat insert and Optimised CIDR Inserts

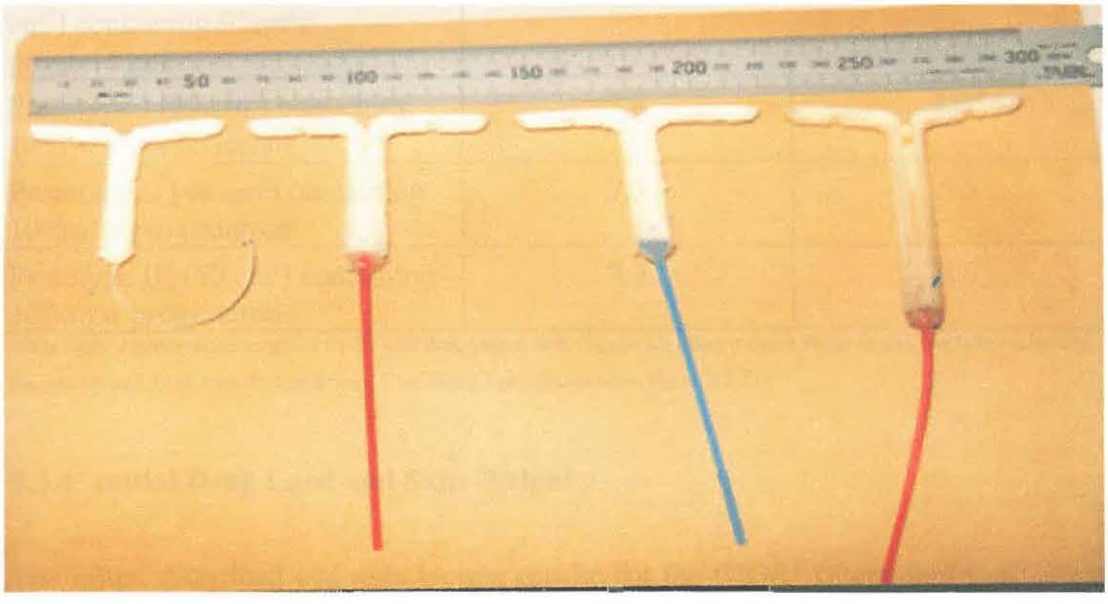

Figure 5.3.1 - Photograph of the CIDR® Sheep and Goat insert and the prototype I, II and III silicone intravaginal inserts used in the trials (left to right, respectively)

5.3.2 Physical Dimensions of the Sheep Vagina

The average vaginal depth of the sheep was 13.4 ± 0.3 cm.

5.3.3 CIDR® Sheep and Goat insert and Prototype Silicone Intravaginal Insert Dimensions

Dimensions of the CIDR® Sheep and Goat insert and prototype silicone intravaginal insert are shown in Table 5.3.1.

Table 5.3.1 - Dimensions of the CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts (N=3)

Insert type	Wing Span (± 0.1 cm)	Length (± 0.1 cm)
CIDR® Sheep and Goat insert (28 cm^2) containing 9%w/w progesterone	5.8	5.4
Prototype I (29 cm^2) containing 10%w/w progesterone	7.3	3.0*
Prototype II (40 cm^2) containing 10%w/w progesterone	7.3	5.5
Prototype III (50 cm^2) containing 10%w/w progesterone	7.3	8.0

*This figure represents the length of CIDR with drug loaded skin - to prevent insert rotation in the vagina, the actual length of the insert was 5.5 cm, with the additional 2.5 cm being blank silicone (see Figure 5.3.1).

5.3.4 Initial Drug Load and Skin Weight

The initial drug load and skin weight results for the CIDR® Sheep and Goat insert and the prototype silicone intravaginal inserts are presented in Table 5.3.2.

Table 5.3.2 - Skin weight and drug load of CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts (N=3)

Formulation	Skin Weight (g) Average ± SEM	Drug Load (g) Average ± SEM	Drug Load (%w/w) Average ± SEM
CIDR® Sheep and Goat insert (28 cm^2) containing 9%w/w progesterone	4.17 ± 0.01	0.37 ± 0.003	8.77 ± 0.05
Prototype I (29 cm^2) containing 10%w/w progesterone	3.68 ± 0.02	0.35 ± 0.002	9.54 ± 0.09
Prototype II (40 cm^2) containing 10%w/w progesterone	4.80 ± 0.01	0.46 ± 0.003	9.53 ± 0.05
Prototype III (50 cm^2) containing 10%w/w progesterone	6.11 ± 0.02	0.59 ± 0.01	9.67 ± 0.09
Prototype IV (40 cm^2) containing 15%w/w progesterone	4.95 ± 0.002	0.76 ± 0.003	15.39 ± 0.06
Prototype V (40 cm^2) containing 25%w/w progesterone	4.87 ± 0.03	1.21 ± 0.02	24.79 ± 0.35

5.3.5 Surface Area

The surface area of the CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts is shown in Table 5.3.3.

Table 5.3.3 - Surface areas of CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts (N=3)

Formulation	Surface area (cm^2) Average ± SEM
CIDR® Sheep and Goat insert (28 cm^2) containing 9%w/w progesterone	28.2 ± 0.6
Prototype I (29 cm^2) containing 10%w/w progesterone	29.1 ± 0.3
Prototype II (40 cm^2) containing 10%w/w progesterone	39.7 ± 1.5
Prototype III (50 cm^2) containing 10%w/w progesterone	50.1 ± 0.6
Prototype IV (40 cm^2) containing 15%w/w progesterone	39.7 ± 1.5
Prototype V (40 cm^2) containing 25%w/w progesterone	39.7 ± 1.5

5.3.6 Skin Thickness

Skin thickness of the CIDR® Sheep and Goat insert was measured and was found to be 3 mm over the insert body, 2 mm on the underside of the wings and 1 mm on the top of the wings.

Skin thickness of the prototype silicone intravaginal inserts was measured and was found to vary between 1 and 1.3 mm dependent upon the position the measurement was made on the insert.

5.3.7 Invivo Plasma Profiles

5.3.7.1 Trial #1 Plasma Progesterone Data

Table 5.3.4 contains blood plasma progesterone levels determined in sheep during a 14 day insertion of a single CIDR® Sheep and Goat insert. Table 5.3.5 contains blood plasma progesterone levels determined in sheep during the insertion of two CIDR® Sheep and Goat insert over 14 days in a replacement programme (Figure 5.1.2). Tables 5.3.6 to 5.3.8 contain blood plasma progesterone levels determined in sheep during the insertion of prototype silicone intravaginal inserts with surface

areas of 29, 40 and 50 cm^2, respectively. Tables 5.3.9 and 5.3.10 contain blood plasma progesterone levels determined in sheep during the insertion of prototype silicone intravaginal inserts with a surface area of 40 cm^2 containing 15%w/w and 25%w/w initial progesterone loadings, respectively.

Table 5.3.4 - Blood levels resulting from insertion of a single CIDR® Sheep and Goat insert in Trial #1

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.22	2.97	2.81	1.90	2.20	1.36	1.28	1.04	1.00	0.85	1.22	0.08
2	0.06	1.99	1.73	2.10	1.56	1.64	1.09	1.22	1.14	1.15	0.95	0.08
3	0.09	2.25	1.88	2.01	1.56	1.53	1.39	1.46	1.42	1.78	1.56	0.19
4	0.07	1.93	1.53	1.34	1.17	1.04	1.00	0.93	1.07	1.02	1.17	0.30
5	0.07	2.29	2.49	2.53	1.89	1.33	1.58	1.29	1.53	0.93	1.34	0.14
6	0.03	1.87	1.45	1.70	1.34	0.89	0.80	0.96	1.34	1.01	0.95	0.21
mean	0.09	2.22	1.98	1.93	1.62	1.30	1.19	1.15	1.25	1.12	1.20	0.17
SEM	0.03	0.17	0.22	0.16	0.15	0.12	0.12	0.08	0.09	0.14	0.10	0.03

Table 5.3.5 - Blood levels resulting from insertion of two CIDR® Sheep and Goat insert in a replacement programme (Figure 5.1.2) in Trial #1

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.31	2.49	2.50	2.64	2.78	2.01	2.25	1.92	1.96	1.97	1.40	0.09
2	0.19	2.05	1.94	1.88	1.52	2.52	1.68	1.39	1.18	1.26	1.29	0.20
3	0.07	2.77	2.63	2.92	1.90	2.41	2.54	1.59	1.59	2.85	1.64	0.16
4	0.20	2.68	2.14	2.78	1.49	2.99	2.76	2.03	1.67	1.86	1.65	0.13
5	0.06	2.34	1.83	2.52	1.35	1.71	1.92	1.43	1.25	1.52	1.29	0.09
6	0.17	2.64	2.98	2.54	1.85	3.84	2.59	2.15	1.34	1.37	1.37	0.30
mean	0.17	2.49	2.34	2.54	1.81	2.58	2.29	1.75	1.50	1.81	1.44	0.16
SEM	0.04	0.11	0.18	0.15	0.21	0.31	0.17	0.13	0.12	0.24	0.07	0.03

Table 5.3.6 - Blood levels resulting from insertion of prototype silicone intravaginal inserts with a surface area of 29 cm^2 in Trial #1

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.04	1.71	1.53	2.38	1.32	1.12	1.22	6.84	1.17	1.02	1.06	0.13
2	0.20	3.00	2.47	3.15	2.26	1.70	1.35	1.15	0.85	1.18	1.39	0.19
3	0.08	1.29	1.44	1.86	1.32	1.16	1.06	1.06	0.99	1.25	1.18	0.16
4	0.09	1.58	1.87	1.69	1.42	1.12	0.91	0.74	0.66	0.72	0.74	0.06
5	0.04	2.11	2.16	2.03	2.32	1.76	1.46	1.15	1.30	1.37	1.44	0.09
6	0.06	2.90	2.23	1.66	1.44	0.86	0.98	1.02	1.14	1.24	1.36	0.13
mean	0.09	2.10	1.95	2.13	1.68	1.29	1.16	1.02	1.02	1.13	1.19	0.13
SEM	0.02	0.29	0.17	0.23	0.19	0.15	0.09	0.97	0.10	0.09	0.11	0.02

Table 5.3.7 - Blood levels resulting from insertion of prototype silicone intravaginal inserts with a surface area of 40 cm^2 in Trial #1

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.09	3.30	3.37	3.64	2.67	2.31	2.44	2.04	2.12	2.34	2.05	0.17
2	0.17	4.41	3.49	3.54	3.12	2.31	2.42	1.97	2.25	1.98	1.53	0.12
3	0.04	3.74	2.69	3.76	2.20	2.70	2.33	1.99	1.97	1.90	1.69	0.23
4	0.07	4.17	4.18	3.75	3.24	3.65	2.59	2.62	2.17	2.52	2.02	0.85
5	0.06	3.18	2.38	2.40	1.91	1.80	1.82	1.99	2.09	1.89	1.91	0.12
6	0.04	4.52	4.92	3.45	2.93	2.31	2.19	2.38	1.93	1.95	1.70	0.09
mean	0.08	3.89	3.50	3.42	2.68	2.51	2.30	2.17	2.09	2.10	1.82	0.26
SEM	0.02	0.23	0.38	0.21	0.22	0.26	0.11	0.11	0.05	0.11	0.08	0.12

Table 5.3.8 - Blood levels resulting from insertion of prototype silicone intravaginal inserts with a surface area of 50 cm^2 and 10%w/w initial load in Trial #1

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.04	4.20	3.86	4.17	3.20	2.37	2.57	1.72	1.92	2.06	1.91	0.19
2	0.19	3.86	3.45	4.10	2.69	2.38	2.41	1.43	1.66	1.98	2.04	0.19
3	0.10	3.03	2.17	3.94	2.82	2.65	2.45	2.43	2.01	1.90	2.07	0.14
4	0.05	2.76	3.38	2.86	2.56	2.28	2.10	1.56	1.29	1.31	1.22	0.04
5	0.04	3.72	3.44	3.48	2.98	2.46	2.38	2.21	2.05	1.87	1.88	0.07
6	0.01	4.30	3.77	3.82	2.76	2.02	1.69	2.28	2.07	2.02	2.08	0.48
mean	0.07	3.64	3.34	3.73	2.83	2.36	2.27	1.94	1.83	1.86	1.87	0.18
SEM	0.03	0.25	0.25	0.20	0.09	0.08	0.13	0.17	0.13	0.11	0.13	0.06

Table 5.3.9 - Blood levels resulting from insertion of prototype silicone intravaginal inserts with a surface area of 40 cm^2 and 15%w/w initial load in Trial #1

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.06	3.70	4.00	3.77	2.42	2.88	2.80	2.01	2.22	1.96	1.93	0.17
2	0.10	2.93	2.95	2.42	1.56	1.57	1.79	1.31	1.61	1.70	1.70	0.11
3	0.04	3.60	1.66	1.45	1.85	1.28	1.14	1.20	1.25	1.64	0.98	0.05
4	0.30	3.60	3.24	3.51	3.18	2.36	2.47	1.84	1.81	2.35	1.99	0.10
5	0.10	4.10	1.88	2.10	1.86	1.38	1.20	1.40	1.83	1.86	1.53	0.22
6	0.10	2.98	2.66	2.78	2.73	1.76	1.85	2.13	1.93	1.48	1.59	0.12
mean	0.12	3.49	2.73	2.67	2.27	1.87	1.87	1.65	1.77	1.83	1.62	0.13
SEM	0.04	0.18	0.36	0.36	0.25	0.25	0.27	0.16	0.13	0.12	0.15	0.02

Table 5.3.10 - Blood levels resulting from insertion of prototype silicone intravaginal inserts with a surface area of 40 cm^2 and 25%w/w initial load in Trial #1

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	1.27	4.45	4.45	6.08	4.90	4.26	4.84	3.51	3.33	3.39	3.81	0.61
2	0.03	2.82	2.58	2.11	2.44	1.38	1.36	1.29	1.33	1.40	1.50	0.07
3	0.05	2.52	2.03	2.79	3.08	2.13	2.16	1.76	2.14	1.83	2.06	0.06
4	0.06	2.21	2.64	2.59	2.42	1.49	1.60	1.49	1.60	1.51	1.66	0.21
5	0.18	2.72	3.15	3.53	3.06	3.72	2.50	2.13	2.08	2.29	1.78	0.12
6	0.02	3.27	3.42	2.16	1.79	1.51	1.21	1.41	1.55	1.37	1.25	0.15
mean	0.27	3.00	3.05	3.21	2.95	2.41	2.28	1.93	2.00	1.97	2.01	0.20
SEM	0.20	0.32	0.34	0.61	0.44	0.51	0.55	0.34	0.29	0.32	0.38	0.08

5.3.7.2 Trial #2 Plasma Progesterone Data

Table 5.3.11 contains blood plasma progesterone levels determined in sheep during a 14 day insertion of a single CIDR® Sheep and Goat insert insert. Table 5.3.12 contains blood plasma progesterone levels determined in sheep during the insertion of two CIDR® Sheep and Goat insert inserts in a simultaneous manner (Figure 5.1.2). Tables 5.3.13 and 5.3.14 contain blood plasma progesterone levels determined in sheep during the insertion of 40 cm^2 and 50 cm^2 prototype silicone intravaginal inserts both with 10%w/w initial drug loads, respectively.

Table 5.3.11 - Blood levels resulting from insertion of a single CIDR® Sheep and Goat insert in Trial #2

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	1.30	5.99	7.75	7.60	7.39	9.34	3.66	1.98	1.21	1.04	1.54	0.15
2	0.14	1.44	1.44	1.46	1.42	1.08	1.36	1.12	1.31	1.30	1.40	0.22
3	0.14	2.29	2.32	2.07	2.41	1.91	1.92	2.20	1.35	1.56	1.35	0.17
4	0.83	4.50	5.60	7.50	6.29	5.09	2.51	2.48	1.86	1.64	1.83	0.16
5	0.15	1.39	1.36	1.38	1.31	1.47	1.27	1.79	1.30	1.25	1.05	0.09
6	0.06	2.40	2.70	2.75	3.22	3.18	3.00	2.62	1.81	2.01	2.47	0.14
mean	0.12	1.88	1.96	1.91	2.09	1.91	1.89	1.93	1.44	1.53	1.57	0.16
SEM	0.13	0.51	0.70	1.04	0.83	0.66	0.30	0.25	0.12	0.12	0.23	0.02

Table 5.3.12 - Blood levels resulting from insertion of two simultaneous CIDR® Sheep and Goat insert in a simultaneous manner (Figure 5.1.2) in Trial #2

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.18	4.14	4.72	4.17	3.66	3.95	3.87	4.60	2.79	2.82	3.24	0.34
2	0.15	6.00	4.76	4.83	5.26	4.38	4.00	4.50	3.90	3.45	3.81	0.33
3	0.41	3.41	2.39	1.54	1.63	1.31	1.80	2.03	1.97	2.67	1.96	0.47
4	0.11	4.79	4.66	3.70	4.34	4.00	4.16	3.34	3.14	2.48	2.96	0.17
5	0.09	3.56	3.26	2.42	2.39	2.48	2.52	2.86	2.68	2.41	3.15	0.46
6	0.05	3.21	4.63	2.98	3.63	2.77	2.84	2.48	2.16	2.32	2.84	0.22
mean	0.16	4.18	4.07	3.27	3.49	3.15	3.20	3.30	2.77	2.69	2.99	0.33
SEM	0.05	0.43	0.41	0.49	0.53	0.48	0.39	0.43	0.28	0.17	0.25	0.05

Table 5.3.13 - Blood levels resulting from insertion of prototype silicone intravaginal inserts with a surface area of 40 cm^2 and 10%w/w initial load in Trial #2

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.06	3.55	3.14	3.15	3.07	2.50	1.76	2.25	1.57	1.56	2.11	0.16
2	0.25	2.40	3.16	2.88	2.78	2.40	3.00	3.07	2.59	2.52	2.04	0.26
3	0.15	4.57	3.66	3.60	3.34	3.41	1.95	2.18	2.06	1.40	2.21	0.17
4	0.07	4.35	3.99	3.48	3.44	2.89	2.77	3.10	3.45	2.54	2.80	0.14
5	0.12	4.16	3.49	3.14	2.44	2.29	1.98	2.27	2.37	2.03	2.28	0.17
6	0.19	2.74	3.18	3.03	3.02	2.46	2.31	2.09	2.11	1.93	2.83	0.37
mean	0.14	3.63	3.44	3.21	3.01	2.66	2.29	2.49	2.36	2.00	2.38	0.21
SEM	0.03	0.37	0.14	0.11	0.15	0.17	0.20	0.19	0.26	0.19	0.14	0.04

Table 5.3.14 - Blood levels resulting from insertion of prototype silicone intravaginal inserts with a surface area of 50 cm^2 and 10%w/w initial load in Trial #2

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.17	6.03	3.86	4.04	3.72	2.22	3.24	3.31	2.90	2.63	2.95	0.29
2	0.33	6.44	4.54	3.90	3.90	4.12	3.56	3.43	2.89	2.55	2.69	0.33
3	0.12	5.31	3.68	3.31	3.74	3.81	3.03	4.06	2.84	2.81	3.13	0.20
4	0.00	4.60	3.91	3.44	3.18	2.64	2.87	3.41	2.38	2.32	2.52	0.12
5	0.97	6.19	7.86	8.01	9.38	4.90	3.16	2.95	2.67	2.48	3.01	0.15
6	0.06	3.39	3.53	2.49	2.87	2.98	2.40	2.96	2.07	2.39	2.21	0.12
mean	0.14	5.15	3.90	3.43	3.48	3.15	3.02	3.43	2.61	2.54	2.70	0.21
SEM	0.05	0.50	0.16	0.25	0.18	0.32	0.18	0.16	0.15	0.08	0.15	0.04

5.3.8 Pharmacokinetic analysis of Invivo Plasma Profiles of CIDR® Sheep and Goat insert and Prototype Silicone Intravaginal Inserts

Areas under the Curve calculated for the invivo plasma profiles of CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts in Trial #1 and Trial #2 are shown in Table 5.3.15.

Table 5.3.15 - Area under the curve data for invivo plasma profiles of CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts in Trial #1 and Trial #2

Trial	Formulation	Area Under Curve (AUC)		
		Day 0-8 Average ± SEM	Day 8-15 Average ± SEM	Day 0-15 Average ± SEM
1	CIDR® Sheep and Goat insert	-	-	22.06 ± 1.45
1	2 CIDR® Sheep and Goat insert replacement	16.73 ± 0.88	12.35 ± 0.75	29.08 ± 1.59
1	Prototype I	14.12 ± 1.33	8.69 ± 1.06	22.81 ± 1.80
1	Prototype II	24.38 ± 1.62	14.45 ± 0.70	38.83 ± 2.11
1	Prototype III	24.33 ± 1.18	13.63 ± 0.56	37.96 ± 1.48
2	Prototype IV	-	-	31.96 ± 2.64
2	Prototype V	-	-	35.90 ± 5.47
2	CIDR® Sheep and Goat insert	-	-	25.11 ± 4.13
2	2 CIDR® Sheep and Goat insert simultaneous	-	-	46.71 ± 4.97
2	Prototype II	-	-	39.51 ± 1.66
2	Prototype III	-	-	52.99 ± 5.62

5.3.9 Vaginal Inspection After Removal

Examination of all ewes immediately following removal of each insert used in the study showed that no damage and minimal vaginal discharge was present.

5.3.10 Residual Drug Load After Removal

5.3.10.1 Trial #1

Residual drug loads in CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts determined following 14 day insertion are shown in Table 5.3.16 along with initial loads and the calculated amount of progesterone released during the 14 day insertion.

Table 5.3.16 - Residual drug loads in CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts determined following 14 day insertion (N=6) in Trial #1

Formulation	Initial Load (g)	Residual Load (g)	Amount Released	
			(g)	SEM
CIDR® Sheep and Goat insert	0.38	0.21	0.17	0.01
Prototype I	0.35	0.16	0.19	0.01
Prototype II	0.46	0.19	0.27	0.01
Prototype III	0.59	0.26	0.33	0.01
Prototype IV	0.76	0.47	0.29	0.01
Prototype V	1.21	0.90	0.31	0.01

5.3.10.2 Trial #2

Residual drug loads in CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts determined following 14 day insertion are shown in Table 5.3.17 along with initial loads and the calculated amount of progesterone released during the 14 day insertion.

Table 5.3.17 - Residual drug loads in CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts determined following 14 day insertion (N=3) in Trial #2

Formulation	Initial Load (g)	Residual Load (g)	Amount Released	
			(g)	SEM
CIDR® Sheep and Goat insert	0.37	0.16	0.22	0.01
Two Simultaneous CIDR® Sheep and Goat insert	0.74	0.34	0.40	0.02
Prototype II	0.46	0.16	0.30	0.02
Prototype III	0.59	0.25	0.34	0.01

5.4 Discussion

Research in this Chapter set out to optimise the commercially available CIDR® Sheep and Goat insert so that a single intravaginal insert could be used in donor/recipient synchrony programmes for embryo transfer. Such treatment regimes currently require the use of two CIDR® Sheep and Goat insert inserted in either a simultaneous or replacement programme (Figure 5.4.1)[46-48] to provide plasma progesterone levels which are elevated above those seen when a single CIDR® Sheep and Goat insert is inserted.

Plasma progesterone levels in sheep following insertion of a single CIDR® Sheep and Goat insert has been reported by several investigators[46, 47, 179, 182, 183]. However, a review of the published research in this area revealed that plasma profiles resulting from the administration of two CIDR® Sheep and Goat insert either in a simultaneous or replacement programme was sparse[46-48], and where it had been reported, it was performed in intact ewes and the resultant profiles were the sum of both exogenous and endogenous progesterone. Since the success of the optimisation process was to be based on plasma profiles, the first step in the optimisation process involved the determination and documentation of plasma profiles resulting from the administration of two CIDR® Sheep and Goat insert in both a simultaneous and replacement programme (Figure 5.1.2). Figure 5.4.1 shows the resultant plasma progesterone levels in anoestrus sheep for a single CIDR® Sheep and Goat insert, two CIDR® Sheep and Goat insert inserted simultaneously and a single CIDR® Sheep and Goat insert inserted for 8 days, removed and immediately replaced by insertion of a second, fresh, CIDR® Sheep and Goat insert. The use of anoestrus sheep in this trial provided the opportunity to determine plasma progesterone levels resulting from the intravaginal insertion of progesterone-containing inserts while endogenous progesterone was absent.

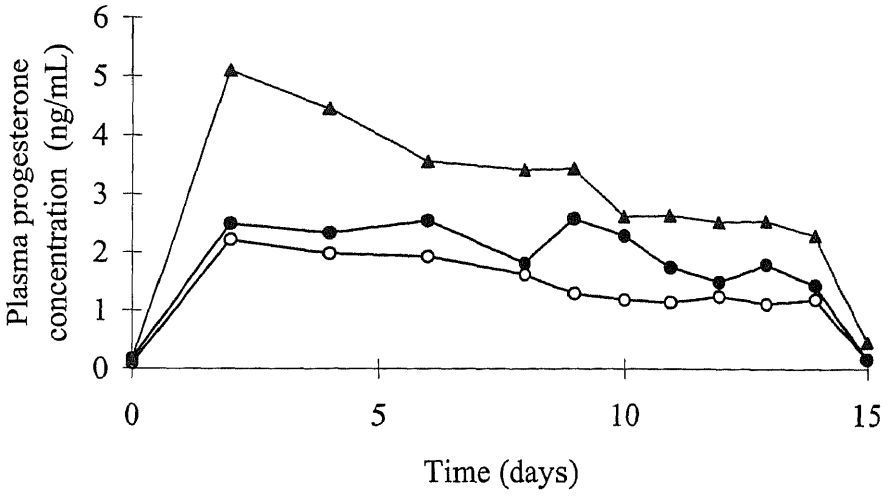

Figure 5.4.1 - Plasma progesterone levels in sheep resulting from insertion of a single CIDR® Sheep and Goat insert (O), simultaneous insertion of two CIDR® Sheep and Goat insert (▲; simultaneous programme, Figure 5.1.2) and consecutive insertion of two CIDR® Sheep and Goat insert (●; replacement programme, Figure 5.1.2)

The profile for the single CIDR® Sheep and Goat insert is typical of those already reported in the literature[46, 47, 179, 182, 183] (Figure 5.4.1). The profile is characterised by an initial increase in plasma progesterone (which occurs as a result of the absorption of exogenous progesterone from the intravaginal insert) followed by slowly declining plasma levels over the insertion period. Once the CIDR® Sheep and Goat insert is removed, progesterone levels fall rapidly to basal values (Figure 5.4.1). When a single CIDR® Sheep and Goat insert is replaced by a second fresh CIDR® Sheep and Goat insert at day 8 of treatment, an increase in plasma progesterone is observed over days 8 through 14. The elevation is probably due to an initial burst release of progesterone from the CIDR® Sheep and Goat insert which is a characteristic of matrix-type drug delivery systems. The plasma progesterone level is then observed to slowly decline to those levels observed when a single CIDR® Sheep and Goat insert is inserted (Figure 5.4.1). When two CIDR® Sheep and Goat inserts are inserted simultaneously for a 14 day insertion period, the same progesterone profile trend is observed as that observed for a single CIDR® Sheep and Goat insert, except that the progesterone levels are higher than those observed for a single CIDR® Sheep and Goat insert over the entire treatment period (Figure 5.4.1). These profiles formed the basis for the optimisation process which was based upon

developing a prototype insert which produced plasma progesterone levels which mimicked those seen in these programmes (Figure 5.4.1).

In the scientific literature, Rathbone et al has recently reported on the optimisation of a silicone/progesterone intravaginal drug delivery system for the control of the oestrous cycle in cattle, namely, the CIDR®1900 Cattle insert[6]. The CIDR®1900 Cattle insert is manufactured using the same technology as the CIDR® Sheep and Goat insert, however, the CIDR®1900 Cattle insert is larger to facilitate its use in cattle. Rathbone et al's results suggested that the only way to increase blood progesterone levels in cows was to increase the surface area of the CIDR®1900 Cattle insert[6] and that release of progesterone occurred only from the first 1 mm of skin. Variables such as drug load, or the inclusion of various formulation additives to the silicone, had no effect on plasma progesterone levels in cattle[6, 102, 222]. These authors also found that the mechanism of invivo release of progesterone from silicone was different to the invitro mechanism of release [Rathbone, Personal communication]. Chapter Four of this Thesis investigated the release of progesterone from the CIDR®1900 Cattle insert and confirmed that mechanistic differences exist between the invitro and invivo cases. Therefore, invitro formulation development work performed on silicone/progesterone intravaginal inserts does not correlate well with the invivo performance of such drug delivery systems. As a result of this knowledge, in this Chapter, we opted not to perform any invitro experimentation, but rather, to focus on conducting invivo experiments to assess the effectiveness of any prototype intravaginal inserts.

Based on the work of Rathbone et al[6], we modified both the surface area and skin thickness of the CIDR® Sheep and Goat insert and assessed if these parameters could be manipulated to produce an optimised version of the CIDR® Sheep and Goat insert. In addition, even though the results of Rathbone et al[6] indicated that drug load had no effect on plasma progesterone levels from silicone based intravaginal drug delivery systems, we also investigated the effect of drug load (10%, 15% and 25%w/w) upon the magnitude of the plasma progesterone profiles in sheep, in case of any interspecies differences in the intravaginal release of progesterone from silicone.

Our results suggested that initial drug load had no effect on plasma progesterone levels following insertion of silicone/progesterone intravaginal inserts (Figure 5.4.2). For each of the inserts, the AUC values were not significantly different from each other (p=0.442). Therefore when silicone/progesterone intravaginal inserts with the same surface area but different initial loads were inserted into the vagina of sheep, the same plasma progesterone levels resulted (Figure 5.4.2). These observations supported those previously reported by Rathbone et al in cattle[6].

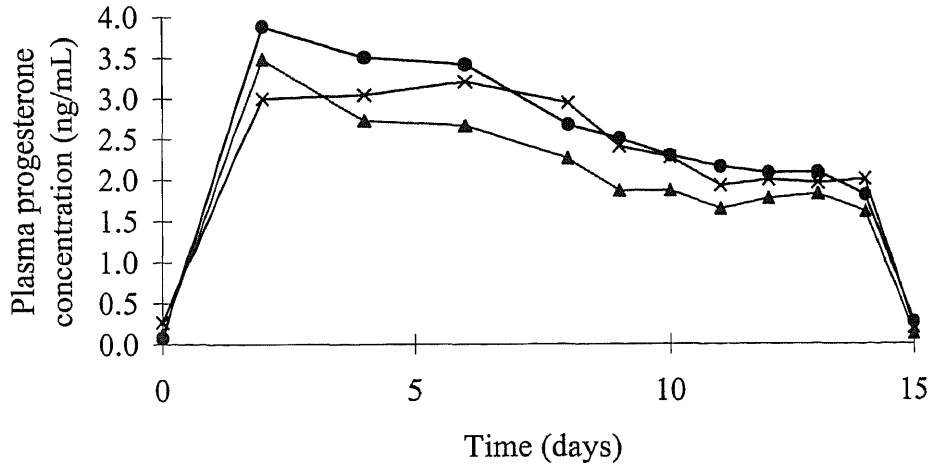

Figure 5.4.2 - Plasma progesterone levels in sheep resulting from the insertion of prototype silicone intravaginal inserts of the same surface area but with initial drug loads of 10%w/w (●), 15%w/w (▲) and 25%w/w (X) progesterone in silicone

In contrast, if prototype silicone intravaginal inserts with the same initial drug load but different surface areas were inserted into the vagina of sheep, significantly different plasma profiles were observed (p<0.001) (Figure 5.4.3).

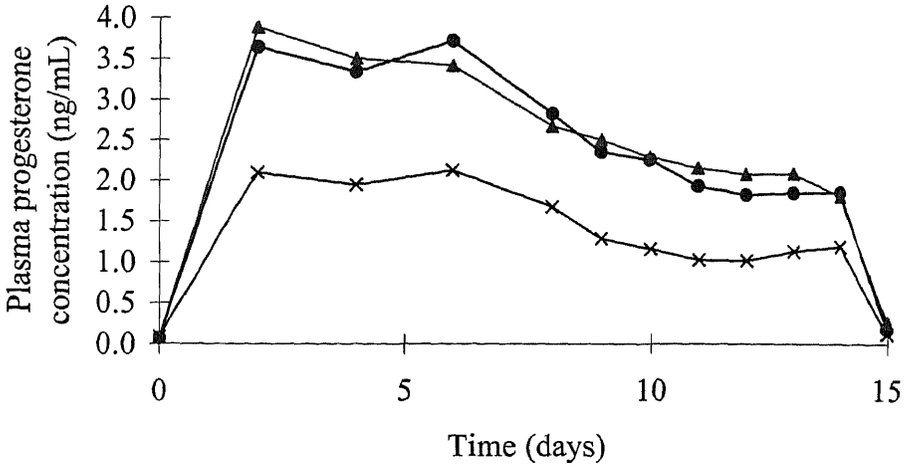

Figure 5.4.3 - Plasma progesterone levels in sheep resulting from insertion of prototype silicone intravaginal inserts with surface areas of 29 cm^2 (X), 40 cm^2 (▲) and 50 cm^2 (●) and initial drug loads of 10%w/w

Statistical analysis of the area under the curves of each plasma progesterone curve revealed a significant difference ($p<0.001$) between the 29 cm^2 insert and the 40 cm^2 and 50 cm^2 intravaginal inserts. However, statistical analysis of the AUC of the plasma progesterone curves for the 40 cm^2 and 50 cm^2 intravaginal inserts showed that these were not significantly different ($p=0.744$). This latter result was not expected; Rathbone et al had previously shown that the elevation in plasma progesterone levels was not limited by insert size[6]. Upon further investigation, the lack of an increase in plasma levels produced by the 50 cm^2 insert may be explained by subjective reports from the field hands that, as the trial progressed, they observed that most of the 50 cm^2 inserts were protruding from the vaginas of the ewes. It would appear that some backward migration of the inserts was occurring, resulting in the inserts protruding from the vagina. Although the plasma progesterone profiles for the 40 cm^2 and 50 cm^2 overlapped (Figure 5.4.3), the amount of progesterone released from the 50 cm^2 insert over the 14 day treatment (0.33 g) was higher than the amount released from the 40 cm^2 insert (0.27 g). This observation may be explained by postulating that the inserts spent several days completely within the vagina before they began to protrude and, whilst protruding, they would have been regularly flushed with urine, which may have promoted drug release.

Comparison of the profiles obtained following insertion of the prototype inserts to those obtained when the CIDR® Sheep and Goat insert is used in its various programmes is shown in Figure 5.4.4.

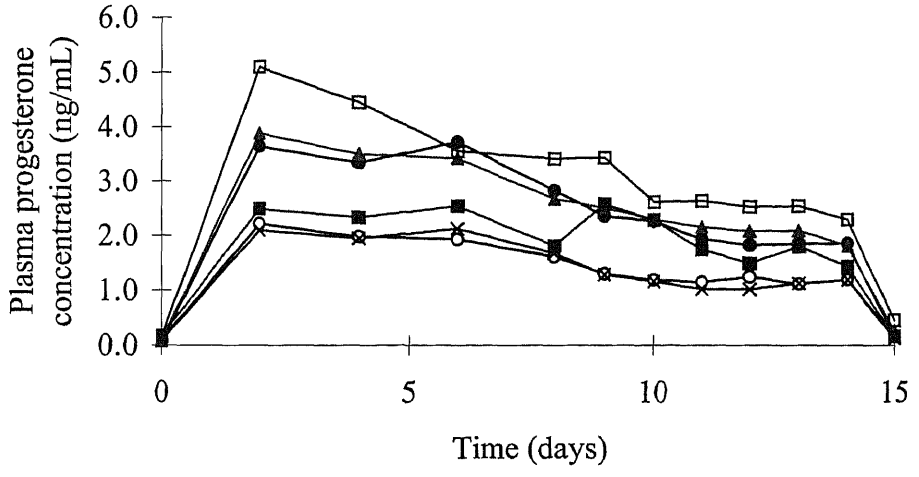

Figure 5.4.4 - Plasma progesterone levels in sheep resulting from insertion of a single CIDR® Sheep and Goat insert (O), insertion of two CIDR® Sheep and Goat insert in a replacement programme (■), insertion of 10%w/w initial drug loaded prototype silicone intravaginal inserts with surface areas of 29 (X), 40 (▲) and 50 cm^2 (●) and insertion of two CIDR® Sheep and Goat insert in a simultaneous programme (□)

The prototype silicone intravaginal inserts which had the same surface area as the commercially available CIDR® Sheep and Goat insert produced plasma progesterone profiles which mimicked those of the CIDR® Sheep and Goat insert (Figure 5.4.3) and provided AUC values which were not statistically different ($p=0.754$). The 40 cm^2 and 50 cm^2 prototype silicone intravaginal inserts exhibited an increase in plasma levels and amount released (0.27 g and 0.33 g released respectively) over the single CIDR® Sheep and Goat insert (0.17 g released) (Figure 5.4.3) and provided AUC values which were statistically different ($p<0.001$). However, the prototype silicone intravaginal inserts did not give the same plasma levels as two CIDR® Sheep and Goat insert in either the simultaneous or replacement treatment programmes ($p=0.004$). These inserts provided AUC values which were larger than the two CIDR® Sheep and Goat insert replacement programme treatment regime and smaller than those observed during the two CIDR® Sheep and Goat insert simultaneous programme. However, examination of the profiles (Figure 5.4.4) suggests that the

difference between the profiles of Prototype II and Prototype III and the two CIDR® Sheep and Goat insert replacement programme occurs at the beginning of the treatment period but each of these profiles are very similar towards the end of the treatment period. Their AUC values for the first 8 days were significantly different ($p=0.001$), however, they were not significantly different from days 8 to 15 of insertion ($p=0.119$). Since the second CIDR® Sheep and Goat insert was inserted to elevate plasma levels over the last 6 days of insertion in the programme, it is proposed that the 40 cm^2 insert could be considered an optimised product for the replacement programme, but does not provide sufficiently high enough plasma levels to be considered a product which would mimic, and therefore could be considered optimised for the simultaneous insertion treatment programme.

From Figure 5.4.4 it would appear that optimisation of the CIDR® Sheep and Goat insert to mimic the two CIDR® Sheep and Goat insert simultaneous programme is not possible. However, as indicated above, the lack of difference between plasma profiles produced by the 40 cm^2 and 50 cm^2 inserts was unexpected and possibly due to the observation by field hands that most of the 50 cm^2 inserts were protruding from the vaginas of the ewe. Consequently, a second trial was initiated with the 50 cm^2 inserts, however, on this occasion they were deliberately inserted into ewes with longer vaginas to ensure that they were fully contained within the vaginal cavity over the time course of the insertion period. The resulting plasma progesterone profiles are presented in Figure 5.4.4. In this trial the 50 cm^2 intravaginal inserts produced elevated plasma progesterone levels and statistical analysis of the AUC showed a significant difference between the 40 cm^2 and 50 cm^2 prototype intravaginal inserts ($p=0.044$). Indeed, Figure 5.4.4 shows that when the 50 cm^2 inserts were contained within the vagina of sheep for the entire administration period, they produced plasma levels which were equivalent to the plasma levels produced following insertion of two CIDR® Sheep and Goat insert in a simultaneous programme and this was confirmed by the AUC values ($p=0.423$). In the same trial the 40 cm^2 prototype silicone intravaginal insert and single CIDR® Sheep and Goat insert treatments gave the same plasma profiles as in Trial #1 and similar AUC values (Table 5.3.14). Interestingly, the quantities of progesterone released by the two CIDR® Sheep and Goat insert in a simultaneous programme and the 50 cm^2 prototype silicone

intravaginal insert were 0.40 g and 0.34 g, respectively. The amounts released were significantly different (p=0.050) suggesting that insertion of a single large delivery system resulted in a superior bioavailability of progesterone to that arising from the delivery of progesterone from two simultaneously inserted inserts.

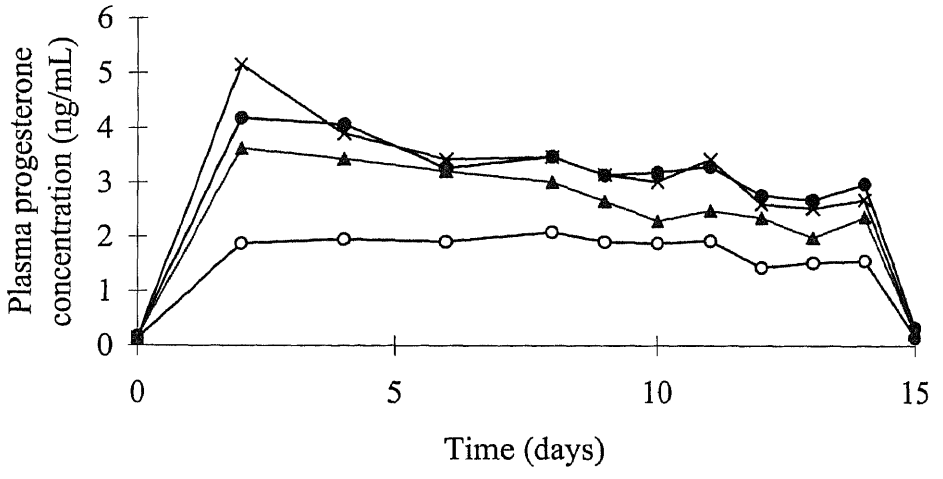

Figure 5.4.5 - Plasma progesterone levels in sheep resulting from insertion of a single CIDR® Sheep and Goat insert (O), insertion of two CIDR® Sheep and Goat insert in a simultaneous programme (●) and insertion of prototype silicone intravaginal inserts with surface areas of 40 cm^2 (▲) and 50 cm^2 (X) with initial drug loads of 10%w/w

These latter results suggested that a 50 cm^2 insert could be considered an optimised product for the simultaneous programme. Indeed, since both the simultaneous and replacement programmes are designed to elevate plasma progesterone levels above those observed for the single CIDR® Sheep and Goat insert, the final design parameters of an optimised CIDR® Sheep and Goat insert were based on the 50 cm^2 since this insert could not only elevate plasma progesterone levels which mimicked those observed with the two CIDR® Sheep and Goat insert simultaneous programme, but provided plasma levels which were elevated above those seen in the two CIDR® Sheep and Goat insert replacement programme. These parameters are summarised in Table 5.4.1.

Table 5.4.1 - Design parameters of an optimised CIDR® Sheep and Goat insert

Wing Span (cm)	Length (cm)	Surface Area (cm^2)	Skin Thickness (mm)	Drug load
7.3	5.5< length <8.0	50	1	10%w/w

Clearly, however, from the results presented in the first trial (Trial #1) some design work would have to be performed in order to produce an optimised intravaginal insert with a surface area of 50 cm^2 but with a body length that is more appropriate with the available length of the vagina of the majority of the flock e.g., the body of the optimised insert may be shorter but have a larger diameter.

The question to be answered before such an optimised insert would be considered for commercialisation is whether or not the insert would be cost effective. This question can be answered by comparing the physical characteristics of the optimised insert to the characteristics of the CIDR® Sheep and Goat insert.

Table 5.4.2 - Comparative surface areas, skin thicknesses and progesterone loaded silicone skin volumes for CIDR® Sheep and Goat insert and optimised insert

Parameter	Characteristics of CIDR® Sheep and Goat insert	Proposed Characteristics of Optimised Insert
Surface area	28 cm^2	50 cm^2
Skin thickness	Variable: 0.1-0.3 cm	0.1 cm
Volume of drug loaded skin	4.2 cm^{3*}	5.0 cm^{3**}

*Experimentally determined from known skin weight (Table 5.3.2) and the density of progesterone loaded silicone = 1.09 (personal communication, Vaughan Woodward, Production Manager)

**Calculated from [(required surface area) x (required skin thickness)]

Table 5.4.2 shows that there is the potential for an optimised insert to be fabricated from a similar amount of drug loaded skin as the current commercially available CIDR® Sheep and Goat insert. Since progesterone and silicone are the most expensive components of the CIDR® Sheep and Goat insert, an optimised insert which has a larger surface area, but a reduced skin thickness would be comparable in price to a single CIDR® Sheep and Goat insert. Having such an optimised insert would subsequently improve the end user cost-effectiveness of the intravaginal insert since they would only need to purchase one optimised insert per animal to be treated, rather than two of the currently available CIDR® Sheep and Goat insert.

5.5 Conclusions

It can be concluded that the commercially available CIDR® Sheep and Goat insert could be optimised to meet the criteria discussed in the introduction to this Chapter. In this Chapter, prototype silicone intravaginal inserts were manufactured whose insertion into anoestrus Romney ewes resulted in plasma progesterone profiles which mimicked those seen for the two CIDR® Sheep and Goat insert treatment regimes (Figure 5.1.2). An optimised insert could therefore be fabricated using existing manufacturing technology and as such, would require no major capital expenditure by the Company. Further to this, the optimised insert would be of a similar manufacturing cost to the currently available CIDR® Sheep and Goat insert to manufacture. In addition, the ability to use a single insert in embryo transfer fertility programmes at a similar cost to that of a single CIDR® Sheep and Goat insert would improve the marketability of the product and represent savings for the end user.

Chapter Six - Development and Optimisation of a New Intravaginal Veterinary Drug Delivery System Containing Progesterone for the Control of the Ovine Oestrous Cycle

6.1 Introduction

Chapter Five discussed the optimisation of the commercially available CIDR® Sheep and Goat insert, a silicone based intravaginal insert for control of oestrus in sheep. The optimisation criteria stipulated that the optimised product must utilise the same manufacturing technology as the CIDR® Sheep and Goat insert. This was to ensure that, if successful optimisation was achieved and the parameters of the optimised insert could be defined, then by using an existing in-house manufacturing technology, such a product could be rapidly developed to full scale production without significant expense to the Company. This would provide a short term solution to the product optimisation challenges outlined in Chapter Five. However, the silicone injection moulding technology used to manufacture CIDR® Sheep and Goat insert has certain limitations. Therefore a more long term strategy should also be considered which involves the introduction of a new, improved technology with the potential to overcome the current limitations of the silicone technology whilst remaining cost effective. From a Company perspective, such technologies should involve exploitation of the Manufacturing Team's core competencies i.e., injection moulding, but should not be limited to thermosetting polymers (even though the Company has invested much time and expense in thermosetting equipment). Such a strategy may involve up front investment costs for new machinery, but this would be weighed against the potential benefits and new products which could arise from the introduction of the new technology.

The main limitation of the silicone injection moulding technology mentioned above is the temperature of 190°C which is required to cure the polymer in an efficient time

frame. This high temperature limits the application of this technology as only the most heat stable drugs can withstand the high cure temperatures. As a result the silicone based technology supports a limited number of drug candidates and therefore has limited clinical applications. A further disadvantage of silicone is that it is a non-biodegradable polymer. If spent silicone inserts are disposed of by burial they will not decompose. Therefore, the purpose of this Chapter was to identify an injection mouldable, biodegradable polymer which can be fabricated into intravaginal inserts using low moulding temperatures. Once identified, a further purpose of the Chapter was to investigate the effect of formulation variables on plasma profiles of intravaginal inserts fabricated using the biodegradable polymer. In this Chapter progesterone was used as a model drug to investigate the potential of the biodegradable polymer as an intravaginal drug delivery platform in sheep. In this respect, intravaginal inserts containing progesterone were formulated to determine if an insert fabricated with the biodegradable polymer could mimic the plasma profiles observed when a single CIDR® Sheep and Goat insert was inserted.

The biodegradable polymer chosen for the study was poly-(ε-caprolactone). It was chosen because it exhibited certain desirable properties which include:

- Biocompatibility: This is well documented in the literature[216] and allows poly-(ε-caprolactone) to be used in products which contact animal tissues.

- Biodegradability: This is also well documented in the literature[216, 251] and contributes to increased environmental acceptability of any poly-(ε-caprolactone) product in comparison to non-biodegradable polymers.

- Low glass transition temperature[216]: This allows the polymer to be flexible under conditions of use, even in winter months, thereby offering the potential that the final insert will not need an internal spine to provide form for the insert and will not become brittle and break when flexed during use.

- Melting point of 60°C[216]: This allows poly-(ε-caprolactone) to be injection moulded at relatively low temperatures, making it possible to incorporate drug candidates with low tolerance for heat.

- Permeability to lipophilic drugs: Poly-(ε-caprolactone) is hydrophobic and therefore lipid soluble drugs can permeate though it by diffusion[216].

- Permeability to hydrophilic drugs: Unlike silicone, if channel forming agents are incorporated into and leached out of a poly-(ε-caprolactone) insert, the insert can maintain its structural integrity. This mechanical strength allows the incorporation of hydrophilic drugs into poly-(ε-caprolactone) for release by diffusion through the formed pores and channels.

- There is evidence in the literature that poly-(ε-caprolactone) can be fabricated into intravaginal inserts which have the potential for use as a platform for drug delivery in cattle[193].

6.2 Methods

6.2.1 Manufacture of CIDR® Sheep and Goat insert

CIDR® Sheep and Goat insert were manufactured as described in Chapter Five, Section 5.2.1.

6.2.2 Manufacture of Poly-(ε -caprolactone) Intravaginal Inserts

Poly-(ε-caprolactone) intravaginal inserts were manufactured by hand. This was achieved by mixing progesterone (USP micronised, Pharmacia & Upjohn, USA) and granular poly-(ε-caprolactone) (Tone® P-767, Union Carbide, USA) in the required ratio (8% and 9%w/w progesterone) in a glass beaker at a temperature of approximately 130°C (Sunbeam Bake 'n' Grill Oven). Following thorough mixing the resultant melt was compressed into flat sheets (140 x 90 x 2 mm) in a specially cut aluminium tool (Figure 6.2.1) which had previously been pre-warmed to 60°C (Contherm SERIES FIVE INCUBATOR, Contherm Scientific Ltd., New Zealand).

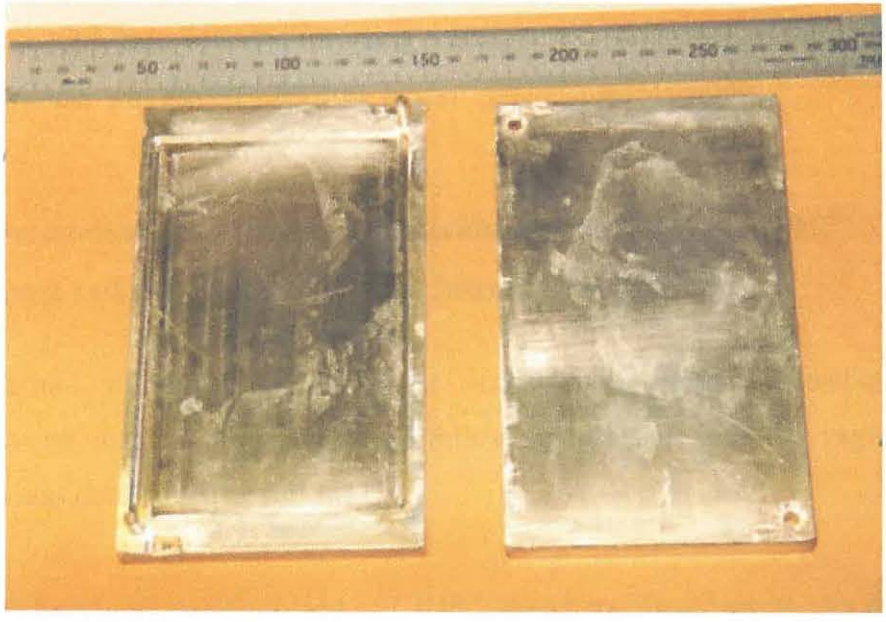

Figure 6.2.1 - Photo of aluminium tool used to cast flat sheets of progesterone loaded poly-(ε-caprolactone)

After cooling to ambient temperature, the resultant flat sheets were removed from the aluminium tool and then cut into pieces of suitable dimensions for plastic welding into the desired shape of the poly-(ε-caprolactone) intravaginal inserts. This involved the following:

- From the 2 mm thick drug loaded poly-(ε-caprolactone) sheet, 15 mm by 63 mm strips were cut to form the wings and 15 mm by 50 mm segments were cut to form the bodies of the intravaginal inserts. The insert was then fabricated by thermo-welding one end of the body to the centre of the wing, creating a T-shaped insert (Insert I). The corners were then rounded off using a scalpel and sandpaper.

- A further insert was then constructed by thermo-welding 0.65 mm by 50 mm segments of 2 mm sheet to each side of the body providing a second insert with a larger surface area (Insert II).

- A third insert was also fabricated (Insert III). In this case a strip of 45 mm by 48 mm was cut from the flat sheet and was formed into a cylinder before thermo-welding one of its ends onto the middle of a wing (17 mm by 63 mm). The corners were then rounded off using a scalpel and sandpaper and the open end of the cylinder was sealed with drug loaded poly-(ε-caprolactone).

6.2.3 Determination of Initial and Residual Drug Load in CIDR® Sheep and Goat insert and Poly-(ε-caprolactone) Intravaginal Inserts

The amount of progesterone in the intravaginal inserts immediately after manufacture, and after their removal following insertion into the vagina of test animals was determined as follows.

The initial and residual drug load of CIDR® Sheep and Goat inserts was determined as described in Chapter Five, Section 5.2.4.

The initial and residual drug load of poly-(ε-caprolactone) intravaginal inserts was determined as follows. The intravaginal inserts were cut using engineer's side

cutters into small pieces (≈2 x 5 x 5 mm) and placed in 150 mL glass medicine bottles. The progesterone was then extracted from the poly-(ε-caprolactone) with three consecutive 100 mL aliquots of ethanol (SDA-3A, Mobil, New Zealand), each aliquot being maintained in a waterbath at approximately 37°C (waterbath) for 16-24 hours before being replaced with the next aliquot. Successive extracts were collected and combined in a 500 mL volumetric flask. The medicine bottle was rinsed with 50 mL ethanol (SDA-3A, Mobil, New Zealand) following each extraction before the fresh aliquot was added. These rinses were also collected and combined into the volumetric flask. When the third extract and rinse was collected, the 500 mL flask was made up to the mark with ethanol (SDA-3A, Mobil, New Zealand). This solution was then diluted (1 in 25) in ethanol (SDA-3A, Mobil, New Zealand) before analysis by UV spectrophotometry (DU 640, Beckman, USA) at 240 nm using a validated assay (see Chapter Two, Section 2.2.1).

6.2.4 Determination of Weight of Poly-(ε-caprolactone) Intravaginal Inserts

The weight of each poly-(ε-caprolactone) intravaginal insert was determined on a four decimal place analytical balance (BP110S, Sartorius, Germany).

6.2.5 Determination of Surface Area of CIDR® Sheep and Goat insert and Poly-(ε-caprolactone) Intravaginal Inserts

The surface area of CIDR® Sheep and Goat inserts and poly-(ε-caprolactone) intravaginal inserts was determined as described in Chapter Five, Section 5.2.5).

6.2.6 Determination of Invivo Plasma Profiles of CIDR® Sheep and Goat insert and Poly-(ε-caprolactone) Intravaginal Inserts

Ethical approval to perform the animal experiments in this part of the Thesis was obtained from both the AgResearch, Ruakura Animal Ethics Committee and from the University of Waikato Animal Ethics Committee on the Welfare of Experimental Animals.

Details of the inserts used in the study and the trial details are given in Tables 6.2.1 and 6.2.2.

Table 6.2.1 - Surface Area Trial protocol

Insert	Insertion Time (days)	Number of Animals (N)
CIDR® Sheep and Goat insert (28 cm^2) containing 9%w/w progesterone	14	8
Two Simultaneous CIDR® Sheep and Goat insert	14	8
Insert I (40 cm^2) containing 8% w/w progesterone	14	8
Insert I (40 cm^2) containing 9% w/w progesterone	14	8
Insert II (51 cm^2) containing 8% w/w progesterone	14	8
Insert II (51 cm^2) containing 9%w/w progesterone	14	8

Table 6.2.2 - Shape Trial protocol

Insert	Insertion Time (days)	Number of Animals (N)
Two Simultaneous CIDR® Sheep and Goat insert	14	8
Insert II (51 cm^2) containing 9%w/w progesterone	14	8
Insert III (50 cm^2) containing 9%w/w progesterone	14	8

In each trial, CIDR® Sheep and Goat insert and poly-(ε-caprolactone) intravaginal inserts were inserted into the vagina of anoestrus Romney ewes using either a standard CIDR® Sheep and Goat insert applicator or modified applicator which had a slightly larger barrel bore size. Treatments were allocated to the ewes in a random manner. Sufficient animals were treated in each trial to detect significant treatment effects. Treatment was terminated by removal of the insert from the vagina by gently, but firmly, pulling on the nylon tail which was attached to the intravaginal inserts and which protruded beyond the vulva of the animals. At the termination of trials, sheep underwent vaginal examination to inspect for any irritation or damage

caused by the intravaginal inserts during their insertion. In the Surface Area Trial (Table 6.2.1), plasma samples were collected immediately prior to insertion on day 0, during insertion on days 2, 4, 6, 8, 9, 10, 11, 12, 13, 14 and 24 hours following removal of the intravaginal inserts. In the Shape Trial (Table 6.2.2), plasma samples were collected immediately prior to insertion on day 0, during insertion on days 2, 4, 7, 9, 12, 14 and 24 hours following removal of the intravaginal inserts. Plasma samples were collected by trained AgResearch personnel from the jugular vein of the sheep. Immediately following sampling, the plasma was separated by centrifugation and stored at -20°C for subsequent progesterone analysis. Concentrations of progesterone in plasma were determined by direct radio immuno assay by trained technicians at DRC laboratories using a commercial solid phase I^{125} label (Coat-a-Count, DPC, USA).

6.2.7 Pharmacokinetic Analysis of Invivo Plasma Profiles of CIDR® Sheep and Goat insert and Poly-(ε-caprolactone) Intravaginal Inserts

Area Under the Curve (AUC) data were determined from the blood plasma progesterone levels determined at given time points over the duration of treatment with intravaginal inserts for each individual ewe treated. The AUC were determined by simple numeric estimation using the trapezoidal rule[229] using equations 5.2.1 and 5.2.2 as described in Chapter Five, Section 5.2.9. The AUC for the different treatments were then compared by Oneway Unstacked ANOVA (MINITAB 8.2 Statistical Software).

6.3 Results

6.3.1 Manufacture of Poly-(ε-caprolactone) Intravaginal Inserts

The poly-(ε-caprolactone) intravaginal inserts resulting from the fabrication process are shown in Figure 6.3.1.

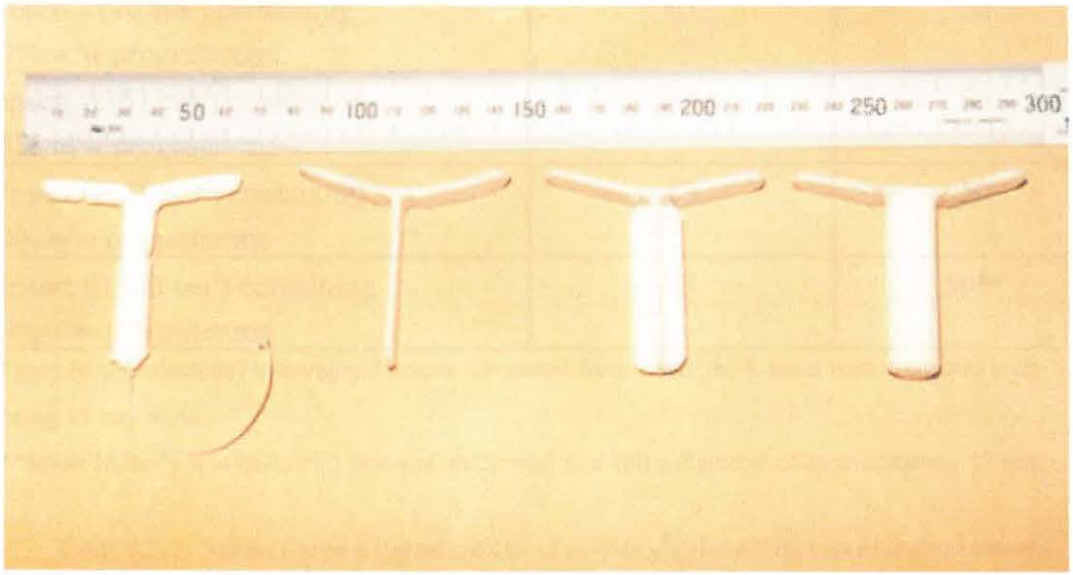

Figure 6.3.1- Photograph of the CIDR® Sheep and Goat insert and poly-(ε -caprolactone) intravaginal inserts (CIDR® Sheep and Goat insert; left, Insert I; centre left, Insert II; centre right, Insert III; right)

6.3.2 Insert Dimensions, Surface Area, Initial Skin Weight and Drug Load

Insert dimensions, surface area, initial skin weight and drug load of the poly-(ε-caprolactone) intravaginal inserts are shown in Tables 6.3.1, 6.3.2 and 6.3.3, respectively.

Table 6.3.1 - Physical dimensions of CIDR® Sheep and Goat insert and poly-(ε -caprolactone) intravaginal inserts

Poly-(ε-caprolactone) intravaginal inserts	Wing* length (cm)	Body* length (cm)
CIDR® Sheep and Goat insert (28 cm^2) containing 9%w/w progesterone	58	54
Insert I (40 cm^2) containing 8% w/w progesterone	63	50
Insert I (40 cm^2) containing 9% w/w progesterone	63	50
Insert II (51 cm^2) containing 8% w/w progesterone	63	50
Insert II (51 cm^2) containing 9%w/w progesterone	63	50
Insert III (50 cm^2) containing 9%w/w progesterone	63	50**

*poly-(ε-caprolactone) intravaginal inserts fabricated from 2 mm thick sheet with wing and body being 15 mm wide

**Insert III body was hollow (2 mm wall thickness) and had a diameter of approximately 17 mm

Table 6.3.2 - Surface area determinations of poly-(ε -caprolactone) intravaginal inserts

Replicate	Insert I	Insert II	Insert III
1	38.36	50.57	49.92
2	40.79	51.25	49.15
3	42.69	51.13	49.94
4	40.40	50.76	51.16
5	41.25	51.50	Not determined
Mean	40.70	51.04	50.04

Table 6.3.3 - Weights and initial drug loads of poly-(ε -caprolactone) intravaginal inserts (n=2)

Insert	Insert Weight (g)	Drug Load (g)	Drug Load (%w/w)
Insert I (40 cm^2) containing 8%w/w progesterone	4.68	0.37	7.82
Insert II (51 cm^2) containing 8%w/w progesterone	5.92	0.47	7.98
Insert I (40 cm^2) containing 9%w/w progesterone	4.36	0.38	8.58
Insert II (51 cm^2) containing 9%w/w progesterone	5.69	0.53	9.25
Insert III (50 cm^2) containing 9%w/w progesterone	8.90	0.80	9.03

6.3.3 Invivo Plasma Profiles of CIDR® Sheep and Goat insert and Poly-(ε-caprolactone) Intravaginal Inserts

6.3.3.1 Surface Area Trial

Blood plasma progesterone levels determined during insertion of a CIDR® Sheep and Goat insert for 14 days are shown in Table 6.3.4. Blood plasma progesterone levels determined during insertion of 8% w/w progesterone loaded Insert I and Insert II poly-(ε-caprolactone) intravaginal inserts for 14 days are shown in Tables 6.3.5 and 6.3.6, respectively. Blood plasma progesterone levels determined during insertion of 9% w/w progesterone loaded Insert I and Insert II poly-(ε-caprolactone) intravaginal inserts for 14 days are shown in Tables 6.3.7 and 6.3.8, respectively.

Table 6.3.4 - Blood plasma progesterone levels resulting from a 14 day insertion of a CIDR® Sheep and Goat insert in Surface Area Trial

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.07	3.18	2.42	2.00	1.72	1.96	1.48	1.81	2.56	1.58	1.69	0.19
2	0.19	2.46	1.84	1.62	1.69	1.79	1.75	1.69	1.72	1.60	1.29	0.14
3	0.24	2.58	2.55	1.63	1.01	1.33	1.06	1.82	1.13	0.95	1.37	0.06
4	0.03	2.21	2.00	2.22	1.96	1.89	1.48	1.45	1.86	1.22	1.50	0.10
5	0.03	2.40	1.93	1.93	2.46	2.35	1.56	1.50	1.28	1.42	1.33	0.20
6	0.05	2.86	2.41	1.66	1.36	1.46	1.35	1.93	1.94	1.69	1.24	0.08
7	0.11	2.32	2.10	1.90	1.42	1.75	1.69	1.14	0.95	1.03	0.97	0.15
8	0.10	2.58	2.83	2.24	2.18	2.75	1.72	1.47	1.51	1.41	1.49	0.14
mean	0.10	2.57	2.26	1.90	1.72	1.91	1.51	1.60	1.62	1.36	1.36	0.13
SEM	0.03	0.11	0.12	0.09	0.17	0.16	0.08	0.09	0.18	0.10	0.08	0.02

Table 6.3.5 - Blood plasma progesterone levels resulting from a 14 day insertion of a Insert I (poly-(ε -caprolactone) intravaginal insert containing 8% w/w progesterone) in Surface Area Trial

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.03	2.84	2.02	1.86	1.71	1.52	1.42	0.83	0.97	1.09	1.01	0.10
2	0.19	2.82	1.76	1.38	1.10	1.01	1.02	0.83	0.91	0.79	0.78	0.07
3	0.14	3.40	2.40	1.94	1.12	1.39	1.33	1.37	1.10	0.84	1.37	0.13
4	0.11	2.72	2.00	1.57	1.62	1.36	1.02	1.19	1.06	0.98	0.98	0.08
5	0.02	1.75	1.97	0.80	1.13	0.88	0.91	0.56	0.67	0.59	0.77	0.01
6	0.05	2.40	1.49	1.01	0.98	1.27	0.88	1.11	0.71	0.73	0.91	0.06
7	0.09	3.28	2.72	1.58	1.79	1.38	1.25	1.17	1.07	0.88	1.02	0.12
8	0.06	✞	1.32	1.26	1.10	1.39	1.17	1.25	1.19	0.87	0.90	0.07
mean	0.09	2.74	1.96	1.42	1.32	1.27	1.13	1.04	0.96	0.85	0.97	0.08
SEM	0.02	0.20	0.16	0.14	0.12	0.08	0.07	0.10	0.07	0.05	0.07	0.01

✞Lost sample

Table 6.3.6 - Blood plasma progesterone levels resulting from a 14 day insertion of a Insert II (poly-(ε -caprolactone) intravaginal insert containing 8% w/w progesterone) in Surface Area Trial

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.11	4.12	3.47	2.83	2.11	2.50	1.65	1.62	⇧	1.67	1.57	0.24
2	0.04	3.06	2.14	1.60	1.64	1.65	1.24	0.94	1.27	1.32	1.32	0.12
3	0.04	3.94	2.63	2.07	1.60	1.83	1.19	1.28	1.29	1.91	1.30	0.08
4	0.19	4.03	3.42	1.79	1.73	1.80	1.39	1.46	1.45	1.29	1.37	0.12
5	0.05	3.05	2.58	2.05	1.51	2.00	1.37	1.14	1.26	1.07	1.19	0.13
6	0.06	2.52	2.19	1.47	1.60	0.90	1.01	1.38	1.44	0.91	0.84	0.12
7	0.00	3.07	2.10	1.53	1.47	2.39	1.91	0.97	1.18	1.14	1.20	0.08
8	0.18	2.72	2.71	2.15	2.35	1.80	1.52	1.54	1.25	0.97	1.42	0.12
mean	0.08	3.32	2.65	1.93	1.75	1.86	1.41	1.29	1.30	1.29	1.28	0.13
SEM	0.02	0.22	0.19	0.16	0.11	0.17	0.10	0.09	0.04	0.12	0.08	0.02

⇧Lost sample

Table 6.3.7 - Blood plasma progesterone levels resulting from a 14 day insertion of a Insert I (poly-(ε -caprolactone) intravaginal insert containing 9% w/w progesterone) in Surface Area Trial

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.44	3.80	3.25	1.88	1.78	2.16	1.92	1.68	1.72	1.18	1.40	0.23
2	0.08	2.37	1.75	1.51	1.11	1.45	1.59	1.15	0.83	0.91	0.65	0.06
3	0.03	3.00	3.57	2.06	1.67	1.76	1.34	1.11	1.16	1.05	1.26	0.14
4	0.02	2.52	3.08	1.87	1.70	1.37	1.16	1.23	1.15	1.02	1.05	0.10
5	0.08	3.36	2.22	1.92	1.63	1.91	1.46	1.15	1.25	1.30	0.85	0.05
6	0.15	3.95	2.48	2.40	2.41	2.11	1.95	1.57	1.32	1.20	1.23	0.29
7	0.16	2.47	3.48	2.46	1.79	2.68	1.64	1.67	1.53	1.34	1.43	0.10
8	0.00	2.47	1.99	1.80	1.45	1.58	0.92	0.98	1.17	0.77	1.09	0.16
mean	0.12	2.99	2.73	1.99	1.69	1.88	1.50	1.32	1.27	1.10	1.12	0.14
SEM	0.05	0.23	0.25	0.11	0.13	0.15	0.13	0.10	0.09	0.07	0.10	0.03

Table 6.3.8 - Blood plasma progesterone levels resulting from a 14 day insertion of a Insert II (poly-(ε -caprolactone) intravaginal insert containing 9% w/w progesterone) in Surface Area Trial

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)											
	0	2	4	6	8	9	10	11	12	13	14	15
1	0.08	4.16	2.83	1.89	2.04	2.08	1.27	1.34	1.70	1.46	1.32	0.10
2	0.08	2.86	2.78	1.58	1.18	1.27	1.31	1.10	1.51	1.20	1.18	0.09
3	0.16	3.86	3.42	2.67	2.57	2.91	1.97	2.05	1.60	1.14	1.64	0.06
4	0.04	4.05	3.14	2.49	2.10	2.15	1.54	1.76	1.88	2.04	1.56	0.10
5	0.07	3.78	3.05	2.49	2.00	2.07	1.36	1.26	1.28	1.27	1.22	0.11
6	0.14	3.37	1.86	1.82	1.61	2.03	1.22	1.08	1.29	1.24	1.19	0.12
7	0.20	4.59	3.78	2.76	3.10	4.21	2.31	1.96	2.09	1.71	2.05	0.18
8	0.42	4.84	3.60	2.58	2.34	3.47	2.08	1.64	1.79	1.40	1.44	0.15
mean	0.15	3.94	3.06	2.28	2.12	2.52	1.63	1.52	1.64	1.43	1.45	0.11
SEM	0.04	0.22	0.21	0.16	0.21	0.33	0.15	0.13	0.10	0.11	0.11	0.01

6.3.3.2 Shape Trial

Blood plasma progesterone levels determined during insertion of two CIDR® Sheep and Goat insert in a simultaneous programme (Figure 5.1.2) for 14 days are shown in Table 6.3.9. Blood plasma progesterone levels determined during insertion of 9% w/w progesterone loaded Insert II and Insert III poly-(ε-caprolactone) intravaginal inserts for 14 days are shown in Tables 6.3.10 and 6.3.11, respectively.

Table 6.3.9 - Blood plasma progesterone levels resulting from a 14 day insertion of two CIDR® Sheep and Goat insert simultaneously in Shape Trial

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)							
	0	2	4	7	9	12	14	15
1	0.64	7.93	8.01	6.65	5.07	5.48	2.90	0.20
2	0.15	4.01	4.20	3.13	2.60	2.77	2.12	0.25
3	0.10	2.50	4.38	3.07	2.14	2.98	2.00	0.24
4	0.09	5.96	5.30	3.64	2.79	2.51	1.81	0.19
5	0.11	3.62	2.88	2.33	2.44	2.41	1.94	0.25
6	0.33	3.40	5.21	3.75	2.43	2.73	2.36	0.09
7	0.03	2.62	5.64	3.85	3.57	3.68	3.20	0.30
8	0.41	2.69	4.17	2.90	3.00	2.30	2.31	0.16
mean	0.23	4.09	4.98	3.67	3.01	3.11	2.33	0.21
SEM	0.09	0.78	0.61	0.53	0.38	0.43	0.20	0.03

Table 6.3.10 - Blood plasma progesterone levels resulting from a 14 day insertion of a Insert II (poly-(ε -caprolactone) intravaginal insert containing 9% w/w progesterone) in Shape Trial

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)							
	0	2	4	7	9	12	14	15
1	0.79	1.80	3.82	2.07	2.37	1.57	1.18	0.12
2	0.11	2.28	3.76	2.12	2.03	1.12	1.36	0.21
3	0.09	2.68	3.64	2.76	2.31	1.47	1.48	0.28
4	0.56	3.67	3.17	2.39	2.27	1.58	1.10	0.09
5	0.15	3.08	4.31	2.90	2.21	1.85	1.80	0.37
6	1.11	3.80	4.11	2.59	2.54	1.36	1.09	0.22
mean	0.47	2.88	3.80	2.47	2.29	1.49	1.33	0.22
SEM	0.17	0.32	0.16	0.14	0.07	0.10	0.11	0.04

Table 6.3.11 - Blood plasma progesterone levels resulting from a 14 day insertion of a Insert III (poly-(ε -caprolactone) intravaginal insert containing 9% w/w progesterone) in Shape Trial

Replicate	Plasma progesterone concentration (ng/mL) at each time following insertion (days)							
	0	2	4	7	9	12	14	15
1	0.10	2.52	4.36	2.47	2.36	2.42	1.50	0.16
2	0.10	2.87	3.22	2.72	2.23	2.17	2.17	0.27
3	0.08	5.51	6.16	4.87	3.18	1.94	1.79	0.37
4	0.02	3.86	3.19	2.46	2.19	2.80	1.58	0.14
5	0.80	4.26	5.23	5.45	3.38	2.03	1.86	0.21
6	0.15	2.27	4.28	4.85	4.77	2.62	1.91	0.30
mean	0.23	3.55	4.41	3.80	3.02	2.33	1.86	0.26
SEM	0.12	0.50	0.47	0.57	0.41	0.14	0.10	0.04

6.3.4 Pharmacokinetic Analysis of Invivo Plasma Profiles of CIDR® Sheep and Goat insert and Poly-(ε-caprolactone) Intravaginal Inserts

Areas under the Curve calculated for the invivo plasma profiles of CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts in the Surface Area Trial and the Shape Trial are shown in Table 6.3.12.

Table 6.3.12 - Area under the curve data for invivo plasma profiles of CIDR® Sheep and Goat insert and prototype silicone intravaginal inserts in Surface Area Trial and Shape Trial

Trial	Insert	Area Under Curve (AUC)		
		Day 0-10 Average ± SEM	Day 10-15 Average ± SEM	Day 0-15 Average ± SEM
Surface Area	CIDR® Sheep and Goat insert	18.82 ± 0.64 21.98 ± 0.70*	6.76 ± 0.37 3.60 ± 0.23*	25.58 ± 0.87
Surface Area	2 CIDR® Sheep and Goat insert simultaneous	-	-	47.83 ± 5.92
Surface Area	Insert I, 8%w/w	-	-	20.77 ± 1.43
Surface Area	Insert II, 8%w/w	-	-	25.93 ± 1.13
Surface Area	Insert I, 9%w/w	-	-	26.31 ± 1.55
Surface Area	Insert II, 9%w/w	25.22 ± 1.77	6.92 ± 0.45	32.14 ± 2.17
Shape	2 CIDR® Sheep and Goat insert simultaneous	-	-	48.89 ± 5.38
Shape	Insert II, 9%w/w	29.87 ± 1.21*	3.60 ± 0.25*	33.47 ± 1.31
Shape	Insert III, 9%w/w	-	-	44.02 ± 3.61

*No day 10 sample was taken in Shape Trial so these calculations performed were AUC_{0-12} and AUC_{12-15}

6.3.5 Vaginal Inspection After Removal

Examination of all ewes immediately following removal of each insert used in the study showed that no damage and minimal vaginal discharge was present.

6.3.6 Residual Drug Load after Vaginal Insertion

6.3.6.1 Surface Area Trial Residual Drug Loads

Residual drug loads in CIDR® Sheep and Goat insert and poly-(ε-caprolactone) intravaginal inserts determined following 14 day insertion are shown in Table 6.3.13 along with initial loads and the calculated amount of progesterone released during the 14 day insertion.

Table 6.3.13 - Residual drug loads in CIDR® Sheep and Goat insert and poly-(ε-caprolactone) intravaginal inserts determined following 14 day insertion in sheep (n=3) in Surface Area Trial

Insert	Initial Load (g)	Residual Load (g)	Amount Released	
			(g)	SEM
CIDR® Sheep and Goat insert	0.38	0.17	0.21	0.01
Insert I (40 cm^2) containing 8%w/w progesterone	0.37	0.18	0.19	0.01
Insert II (51 cm^2) containing 8%w/w progesterone	0.47	0.27	0.20	0.01
Insert I (40 cm^2) containing 9%w/w progesterone	0.38	0.20	0.18	0.02
Insert II (51 cm^2) containing 9%w/w progesterone	0.53	0.30	0.23	0.01

'.6.2 *Shape Trial Residual Drug Loads*

sidual drug loads in CIDR® Sheep and Goat insert and poly-(ε-caprolactone) ravaginal inserts determined following 14 day insertion are shown in Table 6.3.14)ng with initial loads and the calculated amount of progesterone released during : 14 day insertion.

'able 6.3.14 - Residual drug loads in CIDR® Sheep and Goat insert and poly-(ε-caprolactone) intravaginal inserts determined following 14 day insertion in sheep (n=5) in Shape Trial

sert	**Initial Load (g)**	**Residual Load (g)**	**Amount Released**	
			(g)	**SEM**
wo simultaneous CIDR® ieep and Goat insert	0.74	0.37	0.37	0.01
isert II (51 cm^2) containing %w/w progesterone	0.53	0.32	0.21	0.02
isert III (50 cm^2) containing %w/w progesterone	0.80	0.40	0.40	0.04

6.4 Discussion

The design of the poly-(ε-caprolactone) intravaginal inserts used in this study was based upon two criteria; (i) the constraints imposed upon the length of intravaginal inserts due to the depth of the ovine vaginal anatomy reported in Chapter Five, Section 5.3.2 and (ii) the need to retain the insert over the duration of the insertion period. Since the currently available CIDR® Sheep and Goat insert fits comfortably within the vagina of even small or maiden ewes, and it exhibits very high retention rates[4] the design of the poly-(ε-caprolactone) intravaginal inserts were based on the shape and dimensions of the currently available CIDR® Sheep and Goat insert (Figure 6.3.1). The design of the poly-(ε-caprolactone) intravaginal inserts (Figure 6.3.1) proved to be very appropriate since during our trials all of the poly-(ε-caprolactone) intravaginal inserts were retained within the vagina of the ewes used in the trials (100% retention rate) for the duration of the 14 day insertion period, and post-removal vaginal inspection revealed only minor membrane irritation and minimal mucopurrelant reaction.

In the Surface Area Trial, plasma progesterone levels following insertion of poly-(ε-caprolactone) intravaginal inserts with two different initial drug loads (8% and 9% w/w) and two different surface areas (40 cm^2 and 50 cm^2) were determined and compared to those observed following insertion of a single CIDR® Sheep and Goat insert (9% w/w; 28 cm^2) and the insertion of two CIDR® Sheep and Goat insert simultaneously. The resultant plasma progesterone profiles obtained are shown in Figure 6.4.1.

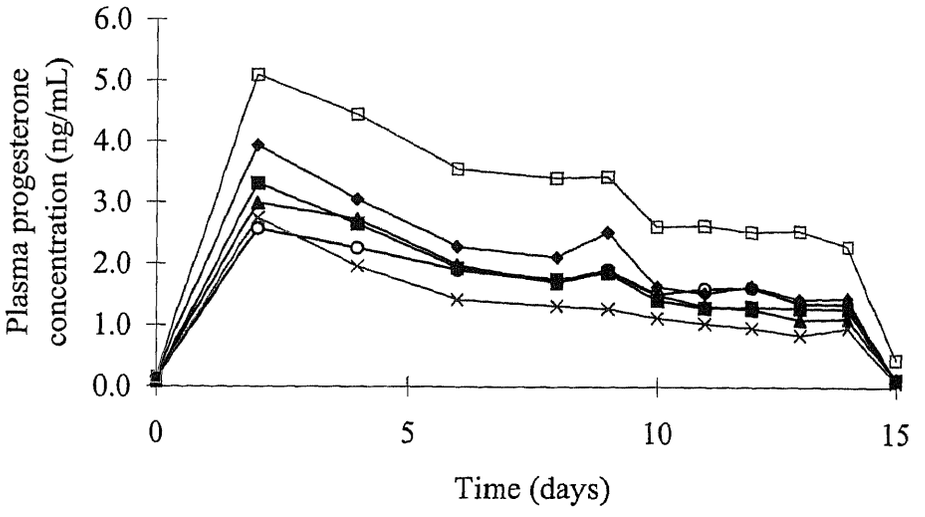

Figure 6.3.1 - Plasma progesterone profiles resulting from the insertion of a single CIDR® Sheep and Goat insert (O), two CIDR® Sheep and Goat insert simultaneously (□), Insert I containing 8%w/w progesterone (X), Insert I containing 9%w/w progesterone (▲), Insert II containing 8%w/w progesterone (■) and Insert II containing 9%w/w progesterone (◆)

In Figure 6.4.1 the typical plasma progesterone profile for the CIDR® Sheep and Goat insert as described in the literature and seen in Chapter 5 (Figure 5.4.1) was observed[179]. In the typical CIDR® Sheep and Goat insert profile, plasma progesterone levels rise following its intravaginal insertion and then slowly decline over the treatment period and then drops to basal values rapidly once the CIDR® Sheep and Goat insert is removed (Figure 6.4.1). The plasma profiles obtained following insertion of the poly-(ε-caprolactone) intravaginal inserts appeared to mimic the same trend seen for the CIDR® Sheep and Goat insert. Insert I containing 8%w/w progesterone produced a profile which was slightly lower than that of the CIDR® Sheep and Goat insert and gave an AUC value which was significantly different to that of the CIDR® Sheep and Goat insert ($p=0.013$), Insert II containing 8%w/w progesterone and Insert I containing 9%w/w progesterone both produced plasma profiles which overlapped with that of the CIDR® Sheep and Goat insert and had AUC values which were significantly different to that of the CIDR® Sheep and Goat insert ($p=0.912$). Insert II containing 9%w/w progesterone gave elevated plasma levels above those of the CIDR® Sheep and Goat insert during the early stages of treatment (AUC_{0-10} significantly different; $p=0.004$) but not over the final four days of insertion (AUC_{11-14} not significantly different; $p=0.794$). These

observations were supported by the residual determination data which indicated that the Insert I containing 8%w/w progesterone released less progesterone that a CIDR® Sheep and Goat insert, Insert II containing 8%w/w progesterone and Insert I containing 9%w/w progesterone both released approximately the same amount of progesterone as the CIDR® Sheep and Goat insert ($p=0.337$) and Insert II containing 9%w/w progesterone released slightly more progesterone than a CIDR® Sheep and Goat insert over the 14 day insertion period ($p=0.043$).

Figure 6.4.1 shows that none of the poly-(ε-caprolactone) intravaginal inserts achieved plasma levels which mimicked those produced following the simultaneous insertion of two CIDR® Sheep and Goat insert. This observation was supported by residual determinations which indicated that each of the inserts studied released much less progesterone than that which was released by two CIDR® Sheep and Goat inserted simultaneously and by differences in AUC ($p<0.001$). As indicated in Chapter 5, it would be desirable to develop the new technology to produce plasma progesterone levels which mimicked those seen when two CIDR® Sheep and Goat insert are inserted simultaneously, to enable its use in specialised fertility programmes.

Factors such as initial drug load and surface area are well documented in the literature as parameters which influence the release of drugs from poly-(ε-caprolactone)[193, 218]. Figure 6.4.1 shows the effect of initial drug load on plasma profiles. At a fixed surface area, initial drug load influenced the magnitude of the plasma profiles. At fixed surface areas of both 40 and 50 cm^2 an increase in initial load resulted in a significant increase in AUC (Table 6.3.12) ($p=0.027$). However, this increase was not sufficient to elevate plasma progesterone levels to mimic those seen following insertion of two CIDR® Sheep and Goat insert simultaneously.

Figure 6.4.1 also shows the effect of surface area on plasma profiles. At a fixed initial drug load, surface area influenced the magnitude of the plasma profiles. At a fixed initial drug load both the 40 and 50 cm^2 intravaginal inserts resulted in a significant increase in AUC (Table 6.3.12) (at 8%w/w $p=0.018$, at 9%w/w $p=0.046$). However, this increase was not sufficient to elevate plasma progesterone levels to

mimic those seen following insertion of two CIDR® Sheep and Goat insert simultaneously. This was a surprising result based on observations made in Chapter Five with silicone based intravaginal drug delivery systems where a similar increase in surface area produced very different (elevated) plasma profiles. We had expected to see a significant difference between plasma levels for different surface area poly-(ε-caprolactone) intravaginal inserts. Indeed the 9% loaded Insert II poly-(ε-caprolactone) intravaginal insert was theoretically designed to mimic the plasma profile produced following the simultaneous insertion of two CIDR® Sheep and Goat insert.

Recently Bunt observed that when intravaginal inserts with the same surface area but with different shapes were placed into cattle vaginas they produced different plasma progesterone levels depending upon the shape of the insert (Bunt; Unpublished observation). He reasoned that higher plasma progesterone levels would be achieved using inserts whose shape allowed more surface area to contact with the vaginal mucosa. Applying this theory to our case, one can imagine the vagina as a collapsed tube which, following the insertion of an intravaginal insert, would contact more surface area of an insert with a cylindrical-shaped body. In contrast less contact might be envisaged with an insert manufactured with a flat-shaped body. In this case the vaginal mucosa would contact with the edges of the insert, but would be hindered from completely contacting the flat surface of the insert. Therefore we postulated that if we manufactured a further insert which had a cylindrically-shaped body and a surface area of 50 cm^2, then it should produce higher plasma progesterone levels than its flat bodied counterpart, even though the surface area of both inserts are identical. A Shape Trial was therefore initiated to test this theory. The resulting plasma profiles for the Shape Trial are shown in Figure 6.4.2.

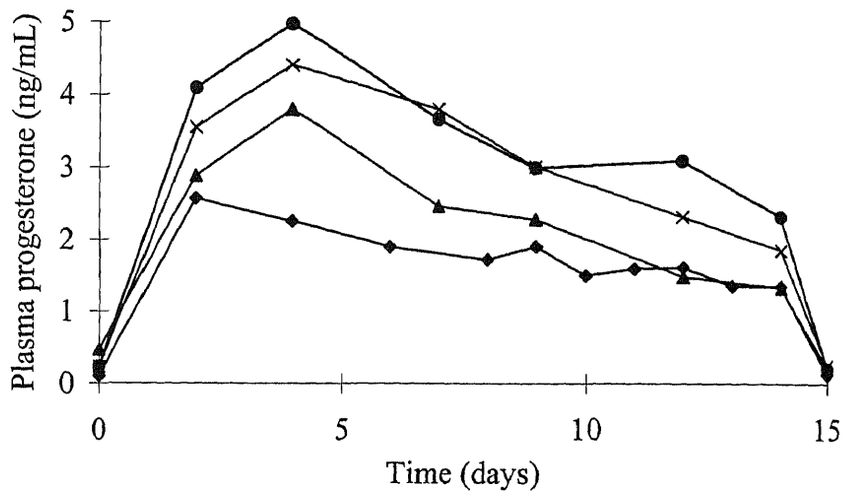

Figure 6.3.2 - Plasma progesterone profiles resulting from the insertion of a single CIDR® Sheep and Goat insert (◆), two CIDR® Sheep and Goat insert simultaneously (●), Insert II poly-(ε-caprolactone) 9% w/w initial load intravaginal inserts (▲) and Insert III poly-(ε-caprolactone) 9% w/w initial load intravaginal inserts (X)

In the Shape Trial we again observed a profile for Insert II containing 9%w/w progesterone which was elevated initially above that of the CIDR® Sheep and Goat insert (AUC_{0-12} significantly different; $p<0.001$) but which was not significantly different to that of the CIDR® Sheep and Goat insert towards the end of treatment (AUC_{12-15} not significantly different; $p=0.994$). In contrast, Insert III containing 9%w/w progesterone and having an equivalent surface area to Insert II but of cylindrical design produced elevated plasma progesterone levels. AUC values for Insert III were significantly different to those obtained following insertion of Insert II. Indeed, the AUC produced by Insert III were not significantly different to the levels produced when two CIDR® Sheep and Goat insert are inserted simultaneously ($p=0.500$). Given this observation we conclude that it may be possible to produce a poly-(ε-caprolactone) intravaginal insert which produces plasma progesterone profiles which mimic those seen when two CIDR® Sheep and Goat inserts are inserted simultaneously through the modification of the surface area of an insert which is appropriately shaped.

The new poly-(ε-caprolactone) technology offers many advantages over the currently used silicone technology as an intravaginal drug delivery platform. Poly-(ε-

caprolactone) is a thermoplastic and can be processed at lower temperatures than silicone. Being a thermoplastic, poly-(ε-caprolactone) can be reprocessed if required. The main advantage of low temperature processing is that it allows the opportunity to incorporate drugs other than progesterone which are less heat-stable. A further advantage of low temperature processing is the energy saving it provides. The innate rigidity of poly-(ε-caprolactone) eliminates the need for an internal inert spine to provide form to the insert (which is currently required with the silicone technology). The poly-(ε-caprolactone) also provides the opportunity to incorporate channel forming agents within the formulation which would enable hydrophilic drugs to be incorporated and delivered even though they cannot permeate through the polymer itself in the same manner as lipophilic drugs can. This latter property of poly-(ε-caprolactone) greatly increases the potential list of drug candidates which could be incorporated within a poly-(ε-caprolactone) intravaginal drug delivery system. Although poly-(ε-caprolactone) has sufficient rigidity to be self supporting, it is also flexible enough to be fabricated into a 'T-shaped' insert which can be retained intravaginally by exerting gentle pressure on the vaginal membrane. The low glass transition temperature of -60°C means that this flexibility is retained by inserts fabricated from poly-(ε-caprolactone), even in the winter months of the colder geographic locations. Finally, poly-(ε-caprolactone) offers the advantage of biodegradability. Although an intravaginal insert fabricated from poly-(ε-caprolactone) does not degrade invivo over the duration of a fertility treatment, when spent devices are buried they degrade substantially within 6 months[193]. This biodegradability offers advantages for the disposal of intravaginal inserts made of poly-(ε-caprolactone) after use.

6.5 Conclusions

It can be concluded from the work presented in this Chapter that the application of poly-(ε-caprolactone) as an intravaginal controlled drug delivery insert technology is feasible. The poly-(ε-caprolactone) intravaginal inserts used in this study were proven to be capable of elevating and sustaining plasma progesterone in sheep at levels similar to those achieved with the CIDR® Sheep and Goat insert. Formulation factors such as drug load, surface area and even the shape of poly-(ε-caprolactone) intravaginal inserts containing progesterone were shown to have an effect on the magnitude of blood plasma progesterone levels following their insertion into sheep. These parameters could be rationally modified (optimised) to fabricate a poly-(ε-caprolactone) intravaginal insert which produces plasma progesterone profiles which mimic those seen when two CIDR® Sheep and Goat insert are inserted simultaneously.

The cited advantages of poly-(ε-caprolactone) as both an intravaginal drug delivery system and as a technology to replace the current silicone technologies, coupled with the promising results of the feasibility studies described in this chapter open up the opportunity for further development and implementation of this technology in the future.

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