DELIVERY OF A COATED BIOACTIVE FROM A RUMEN CONTROLLED-RELEASE DEVICE

A thesis submitted in fulfilment of the requirements for the degree of

Master of Engineering

by

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Abstract

Ruminants possess a unique digestive system. Using the high metabolic potential of the symbiotic microflora of the rumen, ruminants are capable of digesting plant material and obtaining nutrients and energy from this process. Because of the ruminal fermentation, the most bioactives are not stable in the harsh ruminal environment. Therefore there is a need to improve the bioavailability of a bioactive by protecting it from the ruminal digestion. The formulation of protected bioactive can be delivered in the rumen in a controlled manner and over a long period of time.

In this project the degree of rumen protection was estimated using model substrates (sugar pellets and granules). These materials were coated with the pH-sensitive polymer Eudragit® E. The model bioactive (phloridizin) was coated using the coating methodology adopted from exploratory studies with model substrates. The bioavailability of "protected" (coated) phloridizin was assessed by administering directly into the abomasum of fistulated cows.

Formulation of "protected" phloridizin was used to demonstrate the feasibility of bioactive controlled delivery based on ART ("Active Rumen Technology"). This technology uses an elevated gas pressure created by a hydrogen-producing cell to drive a plunger which extrudes bioactive formulation from an intraruminal controlled-release device.

Four groups of devices filled with formulation containing different amounts of "protected" phloridizin were tested. The bioactive was released in a controlled manner over several days. The formulation release profiles were reproducible suggesting that in principle the technology can be further developed to use in a commercial sense or for research purposes. The limitations of the technology, including formulation issues and gas diffusion through barrel walls, were identified.

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Glossary

Abbreviation / Term	Definition
ACVM	Agricultural Compounds and Veterinary Medicines
ART	"Active Rumen Technology"
Bioactive	Phloridizin (phloridzin, phlorizin) - model bioactive
Eudragit® E	Eudragit® E 100 and Eudragit® E PO - coating polymers of the same chemical composition but different physical form (pellets and powder resp.)
PHZ	Chemically defined entity that maintains and/or improves animal good health status or modifies reproductive parameters
PVP	Polyvinylpyrolidone - granulating agent (binder)
Rumen protected bioactive	Bioactive protected from ruminal degradation by an appropriate physical or chemical treatment

Chapter 1: Overview

1.1 Background and information

The generally moderate climate in New Zealand allows pastoral farming almost the whole year around. There is a very vibrant agricultural industry in which ruminant animals such as cattle, sheep and goats play an important part. As such, animals significantly contribute to the country's economy.

New Zealand is one of the world's largest exporters of dairy products (Dairy industry profile 2005). In the year to June 2007, dairy products were New Zealand's largest export earners and accounted for 21% (NZ$7.5 billion). Other animal products such as meat and wool (13.2% and 6.3% of total merchandise exports respectively) also significantly contribute to overall country's export (New Zealand External Trade Statistics 2007).

In 2007, national live-stock numbers were 9.7 million cattle and 38.6 million sheep (Ministry of Agriculture and Forestry 2008). It is therefore important to ensure good animal health and well-being. All possible measures towards improving the live-stock status will greatly increase animal productivity resulting in lower production end-costs.

Pharmaceutical science possesses considerable knowledge to effectively treat animals. The properties of pharmaceuticals in terms of safety, efficacy and costs are being constantly being improved and delivery of bioactives is being optimized. There is a continuing need for long-term delivery systems for bioactives so animal health and productivity is improved.

Many factors must be considered when developing a bioactive dosage form for ruminants. The most significant include cost, convenience and frequency of administration. Repeated administration to a large number of grazing animals is very labour-intense and expensive. Other issues include tissue residues and environmental and handler safety. Finally, in developing rumen dosage forms, the harsh environment of the ruminant digestive system has to be considered.

Developing a novel bioactive delivery system, which ideally meets all the requirements described while releasing a rumen-protected bioactive in a controlled manner and over the required time is the subject of this project.

1.2 Project objectives

The goals of the project are:

- to protect the model bioactive PHZ (phloridizin) against ruminal degradation by coating it with the polymer Eudragit® E.
- to deliver the rumen-protected bioactive using "Active Rumen Technology" (ART), which uses an elevated gas pressure created by a hydrogen producing cell to drive a plunger which extrudes bioactive formulation.

The milestones to achieve these goals are:

- develop a reliable and effective pan coating method using sugar particles as a model substrate
- manufacture rumen protected model bioactive PHZ (phloridizin) using the developed coating procedure
- assess the degree of (probable) "rumen protection" of coated model bioactive *in vitro*
- assess the degree of rumen protection of coated model bioactive *in vivo*
- deliver the rumen protected bioactive using ART
- identify advantages and constraints in the rumen protected delivery system

1.3 Thesis structure

Chapter 2 reviews diseases of live-stock, challenges that arise in therapy of ruminants, and different pharmaceutical approaches in medicating ruminants including rumen controlled-release devices. The digestive system of ruminants and some approaches to protect bioactives against ruminal environment are described. A brief review of coating technology and the model bioactive phloridizin are given.

The materials used are described in Chapter 3 along with descriptions of the pan coating process, the methods used to assess coated sugar and PHZ particles, and studies on release of coated PHZ particles using a rumen controlled-release device.

Chapter 4 has the results and discussion on characterizing the coating process, the characteristics of coated particles, their *in vitro* and *vivo* assessment and their *in vitro* delivery using a rumen controlled-release device.

The conclusions and recommendations are presented in Chapter 5.

Raw data is summarized in the appendices.

Chapter 2: Delivering Bioactives To Ruminants

2.1 Special features of ruminant health care

Like humans, animals need supplementary nutrients and pharmaceuticals that help to maintain good health and prevent or combat diseases. While the medication of humans is aimed to improve quality of live, intervention with bioactives in ruminants is primarily aimed to increase their production. Thus, all the approaches in medication and/or supplementation using bioactives or feed additives to ruminants have an economical motivation.

The factors that can influence the animal production include:

- Nutritional deficiency
- Infectious diseases
- Parasites (internal and external)
- Metabolic disorders
- Controlling reproduction
- Manipulating animal growth

Nutritional deficiency

Ruminants need nutrients such as vitamins and microelements for their optimal growth and performance. However, in contrast to humans, ruminants are dependent on obtaining microelements from the specific grazing area, which can be deficient in microelements such as cobalt, copper, zinc, iodine etc. To compensate this deficiency the animals would need to be shifted to another grazing area. This way of compensating deficiency in livestock is, in most cases, economically irrational, therefore nutrients need to be supplied to the animal.

Infectious diseases

A variety of microorganisms including viruses, bacteria, protozoa and fungi can affect the animal, interfere with its metabolism and, if untreated, cause severe pathological conditions. The most common infections of ruminants comprise those of the digestive tract, reproductive organs and open wounds.

Parasites (internal and external)

Internal parasites such as helminths are major production limiting factors for ruminants. While therapy with anthelminthic vaccines is promising but at the present not applicable, the anthelminthic gene therapy is still a futuristic vision. Despite the development of anti-drug resistance in the parasite, classical chemotherapy is still the only effective option of treating infections with helminths. Some effective approaches of anthelminthic therapy of ruminants including Captec controlled-release system are described in section 2.4.

A variety of external parasites such as insects, ticks, and mites affect the well-being and productivity of livestock. These parasites can cause blood loss, increase the chance of an infectious disease, or transmit diseases. Irritation of the animal caused by ectoparasites can interfere with feeding and resting patterns, resulting in reduced feed conversion efficiency, reduced weight gains, decreased milk production and decreased wool production. In some cases, damage caused by ectoparasites can decrease the value of hides and carcasses (Miller 2000).

Metabolic disorders

Because many different factors (e. g. changed feed composition) can induce metabolic deviations associated with different symptoms, there are not sufficient commercial products to suit the needs individually. Examples of metabolic disorders of dairy cattle are bloat, and ketosis and acidosis.

Bloat occurs mostly when animals are fed with high-quality fodder such as lucerne, medics, clover etc, which induce intraruminal production of large volumes of gases such as methane and stable foams. The inability of the animal to eructate more rapidly than the gas accumulates can produce high intraruminal gas pressure, which can lead to closure of major blood vessels and obstruction of inner organs (Vandamme and Ellis 2004). To prevent the foam establishing, some effective surfactants such as Pluronic® PE 6200 can be orally administered (Commercial product Bloat Guard®) (Agrimin Ltd., Undated). Some foam de-stabilizing agents can also be delivered with other bioactives from a rumen controlled-release dosage form.

Another metabolic disorder that implies pathological increase of acid and ketotic substances can be caused by forage being too acidic. This results in the buffering capacity of the rumen being over-ridden and the normal ruminal microflora being destroyed. The antibiotic momensin in Rumensin® ABC intraruminal bolus partially inhibits growth of bacteria involved in acetate and propionate production and restores the physiological pH-balance.

Controlling reproduction

It is of great importance to accurately control the reproductive cycle of the cattle. This can be achieved by manipulating the oestrus of selected female animals in a herd by means of hormones. The selected animals can be inseminated at the same time (Burns 1999). This results in shorter lactation-free intervals and therefore to increase in milk yields (Rathbone *et al*. 1997).

Manipulating animal growth

Delivering growth promoters to food-chain animals is a direct approach to increasing their productivity. An overview of different pharmaceutical approaches to deliver growth-promoters to cattle is given in the section 2.2.1.

2.2 Controlled-release systems for ruminants

Some principles of design and function of veterinary controlled-delivery systems were adopted from those for human medicine. However, besides the more obvious differences in anatomy and physiology, there are several factors such as cost, convenience and safety that result in the need to have different approaches when developing controlled-release systems for veterinary use.

The following four major groups of controlled-released delivery systems for livestock are:

- Injectable and implantable systems
- External attachments
- Intravaginal inserts
- Intraruminal dosage forms

2.2.1 Injectable and implantable systems

Injectable and implantable systems represent a practical dosage form for use in animals. The delivery of these parenteral dosage forms requires less labour associated with bioactive administration than conventional immediate-release bioactive delivery.

One of the simplest sustained release injectable systems is a long-lasting formulation of the antibiotic oxytetracycline used by Pfizer for treating pasteurella pneumonia in cattle. Compared with conventional injection, efficacious blood levels of oxytetracycline, released from this formulation, stay almost twice as long (Cardinal and Witchey-Lakshmanan 1992).

Another example for an injectable controlled-release is an intramuscular delivery of testosterone propionate. If delivered in crystals the effect lasts over 8-12 days, and if delivered in oil solution, for 3-9 days. A subcutaneous injection of testosterone propionate pellets is effective over 4-5 weeks (Sinkub 1978).

Another injectable product, Ivomec® contains the anthelmintic bioactive ivermectin, which is dispersed in propyleneglycol and glycerol formal (Merial product information 2006). Long half-life of ivermectin and the nature of the formulation contribute to a prolonged therapeutical effect and necessities only once a month administration.

Implantable systems have been successfully used for promoting growth in cattle. For example, Synovex® S and H (Fort Dodge Animal Health, Kansas, USA), marketed by Syntax to use in steers, release estradiol benzoate/progesterone and estradiol benzoate/testosterone propionate respectively (Cardinal and Witchey-Lakshmanan 1992).

Subcutaneous ear-implants such as Syncro-MATE-B® and SYNCRO-MATE-C®(CEVA, Libourne,France) have been developed for cattle and contain the hormone (norgestomet). Another subcutaneous ear-implant is Melovine® (CEVA) that delivers hormone melatonin to sheep.

An oestrogen containing system COMPUDOSE® (Elanco Animal Health, Indianapolis, IN, USA) (Lewis 1995) consists of a drug-free silastic cylinder

coated with a layer of silicone rubber containing 20 % estradiol-17b. In order to minimize the chances of infection at the site of implantation the dosage from is coated with a small amount of oxytetracycline hydrochloride (Ferguson 1988). COMPUDOSE® has the advantage of giving a more linear release profile due to a thin bioactive layer containing high amount of dispersed bioactive.

2.2.2 External attachments

External controlled delivery systems for livestock are aimed to deliver pesticides over time to repel insects that affect productivity (Miller 2000). These delivery systems can be applied to cattle as ear tags and collars. An example is the Atrobaṇ® Eartag (Schering-Plough Animal Health) (Atroban, Product information, Undated), which releases permethrin for a period of three months. The monolithic structure of many currently available ear tags is responsible for an initially high release rate, which decreases over the time, and can lead to development of resistance against the insecticide due to a prolonged exposure of sublethal insecticide doses (Baggot 1988). To improve the release profiles Herbig *et al.* have developed a membrane-based ear tag that releases insecticide at a constant rate over required period of time (Herbig and Smith 1988). The release of insecticide is determined by the permeability and geometrical parameters of the membrane. Further examples for external attachment are neckband systems which have been developed for the controlled delivery of organophosphorous insecticides to cattle (Miller and Oehler 1988).

2.2.3 Intravaginal inserts

The benefits of intravaginal bioactive delivery include avoidance of tissue damage that is often associated with injections, and a less stressful administration for the animal. A further advantage is the possibility of interrupting the therapeutic effect by withdrawal of an intravaginal dosage form.

A number of intravaginal veterinary bioactive delivery systems have been marketed. These include CIDR® (InterAg, Hamilton, New Zealand), intravaginal polyurethane sponges of various sizes and densities (Figure 1), and PRID

(Progesterone-Releasing Intravaginal Device) (CEVA, Libourne, France) (Figure 2) (Eichman 1999).

Controlled Internal Drug Release dispensers (CIDR®) are intravaginal T-shaped devices constructed of a progesterone-impregnated medical silicone elastomer molded over a nylon core (Bunt *et al.* 1999). The devices were designed for different types of production animals (Figure 1). The winged form of CIDR® devices to facilitate intravaginal retention, resembles some intraruminal devices (section 2.4.1).

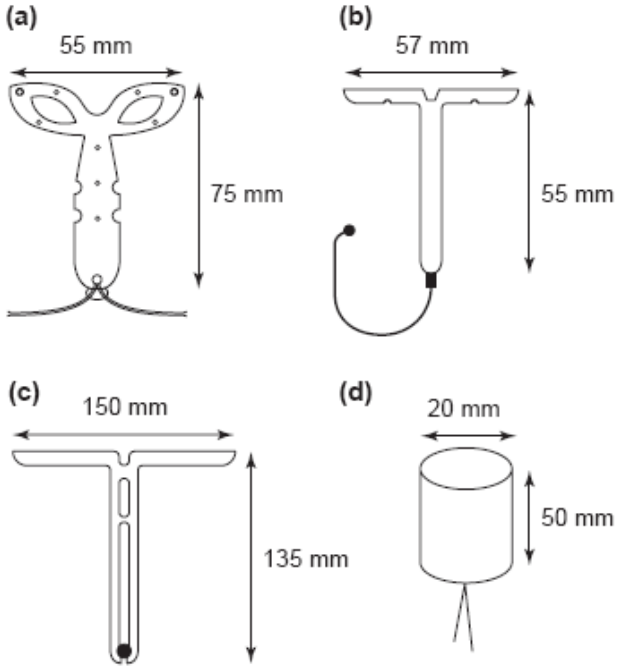

Figure 1: Commercially available oestrus-synchronizing controlled release intravaginal drug delivery systems
CIDR-S® (sheep); (b) CIDR-G® (sheep and goats); (c) CIDR-B® (cattle) (InterAg, Hamilton, New Zealand); (d) Repromap® sponge (sheep) (from Rothen-Weinhold *et al.* 2000).

Intravaginal sponges are cylindrical-shaped polyurethane sponges impregnated with varying quantities of synthetic progestogens. Syncro-Part® and Syncro-Part® MSG (pregnant mares serum gonadotrophin, CEVA) were designed for sheep and cattle, respectively.

The Progesterone-Releasing Intravaginal Device (PRID®, CEVA, Libourne, France) was the first commercially available intravaginal delivery system and was originally developed for use in cattle (Rothen-Weinhold *et al.* 2000). It comprises

micronized progesterone uniformly suspended in an elastomeric silicone matrix. Auxiliary components of the system are gelatin capsule containing oestardiol benzoate and a string to facilitate removal (Figure 2).

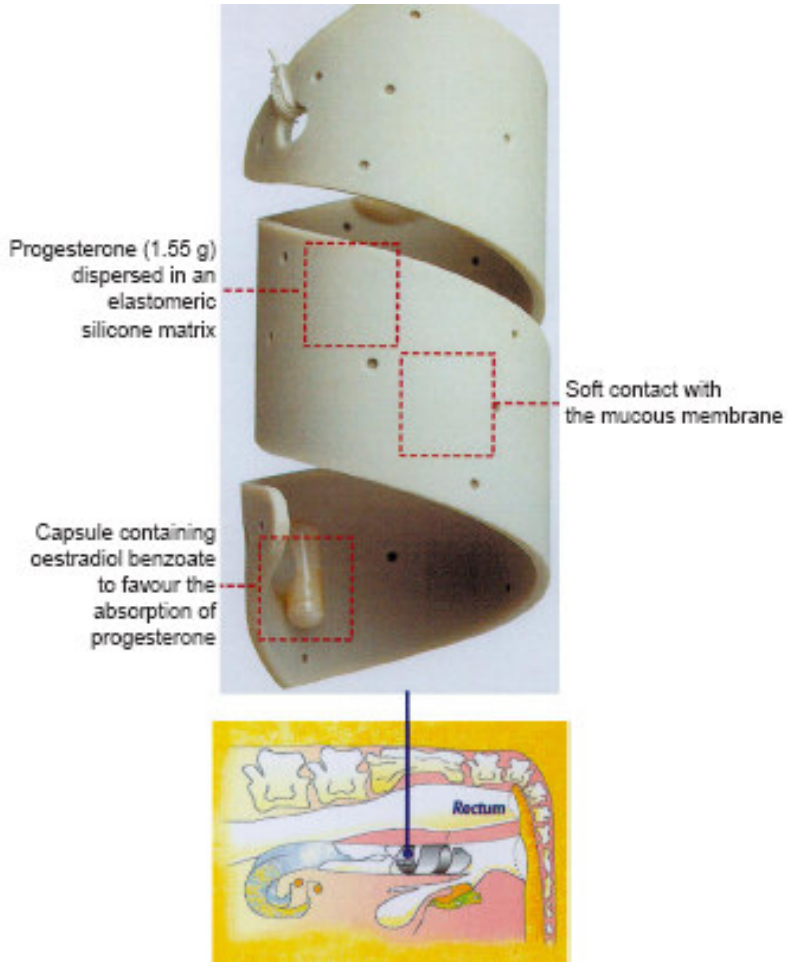

Figure 2: Progesterone-releasing intravaginal device (PRID®) (cattle) (from Rothen-Weinhold *et al.* 2000).

2.2.4 Intraruminal dosage forms

These dosage forms are described in detail in section 2.4.

2.3 The digestive tract of ruminants

Ruminants are herbivores with a unique digestive system adapted to splitting the cellulose in plant structures, thereby making more of the plant components available for use by the host animal. This is achieved by a combination of structural/anatomical features, and the symbiotic relationship with a variety of microflora.

The main anatomical difference from monogastric animals is that ruminant stomach has four distinct compartments: reticulum, rumen, omasum and abomasum (Figures 1 and 2).

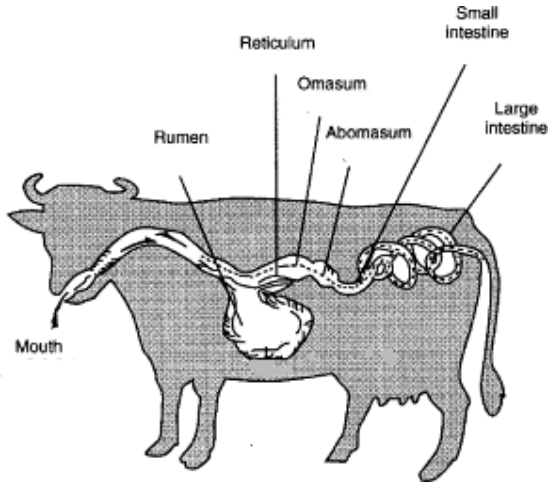

Figure 3: Gastro-intestinal tract of cattle
(Vandamme and Ellis 2004).

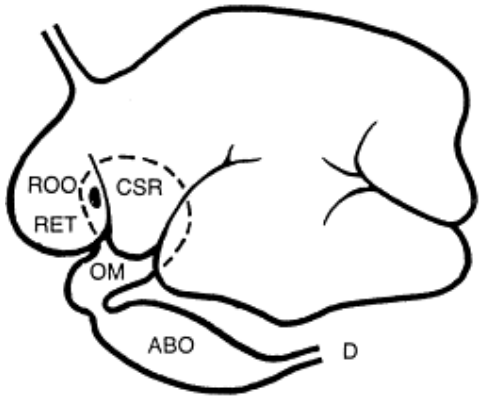

Figure 4: Diagram of the reticulorumen.
ABO, abomasum; OM, omasum; CSR, cranial sac; RET, reticulum; D, duodenum; ROO, reticulo-omasal orifice (from Wu and Papas 1997).

The first and largest is the reticulo-rumen, with a volume of 100 to 225 L in full-grown cattle and 10–25 L in ovine and caprine species (Vandamme and Ellis 2004). In this compartment ingested feed is mixed with water and saliva, and exposed to the rumen microbes, whose main role is to break down the cellular components of the feed and release nutrients. There is continual turbulence that ensures good mixing, provides for some fractionation of particular matter, and promotes in a very complex way for the initiation of rumination (see below).

Cattle produce about 100-190 L of saliva per day (Silanikove 1992). Continuous inflow provides extra fluid for the fermentation and a strong pH buffering of the digesta to optimise the microbial activity.

Rumination is the repeated regurgitation (initiated by ruminal motion and responses) of ingesta from the reticulum, followed by re-mastication and re-swallowing. It provides extra mechanical breakdown of roughage, increasing substrate surface area available to the fermentation microflora. The rumen environment is a unique ecosystem which supports three major types of micro-organisms: bacteria, protozoa and fungi. It is a suitable environment for establishing continuous fermentation because:

- The temperature is relatively constant (approximately 39°C).
- Physical conditions such as pH (6.5–7.0) and osmotic pressure (260–340 mOsm) (Merchen and Hubert 2002) are relatively stable.
- The host animal provides a regular, continuous supply of substrate.
- Fermentation end-products are continuously removed by soluble material being absorbed across the ruminal epithelia and undigested material being expelled to the rest of the digestive system. This prevents end-products accumulating and inhibiting microflora activity.
- Gases such as carbon dioxide, methane, nitrogen (65%, 27% and 7% respectively) and trace amounts of hydrogen and hydrogen sulphide produced in the rumen are expelled by eructation (McArthur and Miltimore 1961). Adult cattle can produce up to 600 L of ruminal gases (Cardinal 1997).

The total number of microbes in rumen contents ranges from 10^{10} - 10^{12} cells g^{-1} (Santos and Huber 2002). The primary aim of these microbes is to pre-digest the plant material yielding short chain volatile fatty acids (VFA) which is the source of energy for the microbes and the host animal. Inactive microbial mass passes to the abomasum where it is digested by host animal's enzymatic system. This process yields a pool of amino acids and short peptides which are utilized by the host animal (Pell *et al.* 2000). Approximately 3 kg/day of microbial protein is produced by lactating cows (Herrera-Saldana *et al.* 1990).

Particles greater than 2 mm (cattle) or 1 mm (sheep) tend to be excluded from passage through the reticular-omasal orifice; this material is recycled through the rumination process.

The abomasum is a true, glandular stomach, which secretes acid and functions in as a manner similar to the stomach of a monogastric. Ingesta are finally subjected to the ruminant's own digestive enzymes (Harfoot 1978).

2.4 Intraruminal bioactive delivery

The delivery of pharmaceutical or nutrient supplements to livestock present several challenges different from those associated with either humans or with other monogastric animals. At the same time, the rumen offers unique opportunities and advantages:

- Cost/benefit ratio: grazing animals cannot always be mustered for bioactive administration over the entire grazing season at the most (drug related) beneficial time – long acting delivery systems are therefore required.
- Longevity of application: properly designed devices can remain in the rumen over required (extended) time period.
- Compliance: the host animal apparently does not recognize the presence of a intraruminal device in the rumen.
- Regular mixing of the rumen contents and the presence of vast amount of water. This facilitates disintegration of the intraruminal device and dissolution of the bioactive.
- Opportunities to use the ruminal microflora to convert a bioactive precursor (pro-bioactive) into a more potent derivative.
- Easier oral administration of intraruminal devices compared with to injectables.

On the other hand, when designing any intraruminal device, one has to consider following issues:

- Bacterial fermentation: the formulation or/and the bioactive can be degraded by ruminal microflora.
- Regurgitation: due to regurgitation the intraruminal device can be propelled into the oral cavity and expelled.
- Dilution factor: due to the large rumen volume and water content the bioactive has to be either very potent or be administered in larger amounts.
- Monitoring of the intraruminal device: the device has to function reliably as its monitoring in most cases is not feasible.

2.4.1 Intraruminal devices

Intraruminal devices are solid preparations that deliver the bioactive at required release rate and over required period of time. There are several main design features that are required for any intraruminal devices:

- A size and shape making them capable of being readily administered orally.
- Reliability of long term retention in the rumen.
- A controlled and long term release of the bioactive.

Theoretically there should be no limit to the duration of retention and release, but in practice the device lifetime should match the health condition for which it is designed. For example, treating an infection might need only days or weeks of exposure, an antiparasite drug might be needed for the duration of the grazing season, while a nutrient might be needed for 365 days a year. In practice, most current commercial products have a lifetime of around 100 days, and another device is re-administered if longer term treatment is required. Some intraruminal devices are presented in Table 1.

Table 1: Some marketed intraruminal devices
(from Rothen-Weinhold *et al.* 2000).

Trade name	Bolus type	Manufacturer	Drug	*In vivo* duration
Paratect Flex® Bolus	Expanding device	Pfizer Animal Health (New York, NY, USA)	Morantel tartrate	3 months
Rumensin ABC®	Expanding device	Elanco Animal Health, Lilly (Indianapolis, IN, USA)	Monensin sodium	3 months
Monensin RDD® (no longer marketed)	High-density device	Elanco Animal Health, Lilly (Indianapolis, IN, USA)	Monensin sodium	3 months
Spanbolet® II	High-density device	Norden Laboratories (Lincoln, NE, USA)	Sulfmethazine	3–50 days
Dura SE®	High-density device	Schering-Plough Animal Health (Mundelein, IL, USA)	Sodium selenite	4 months
Ivomec SR®	High-density device	Merial (Knightsbridge, London, UK)	Ivermectin	4–5 months
Panacur SR®	High-density device	Intervet/Hoechst Roussel Vet (Boxmeer, The Netherlands)	Fenbendazole	4–5 months

2.4.2 Ruminal retention

To avoid regurgitation during rumination, intraruminal devices have to be properly designed. This can generally be achieved either by increasing the density (high density devices) or modifying geometrical parameters of the device (expanding devices) (Table 1). Both approaches can also be combined in a single device.

2.4.3 Bioactive release mechanism

Various physical and/or chemical processes can be used to release the bioactive from ruminal devices. The simplest mechanism is the erosion of a bolus due to the permanent agitation of the ruminal contents. Another approach uses simple diffusion of a water-soluble bioactive out of the matrix, while an even more sophisticated process relies on osmosis as the release controlling process. All of these types of device can be referred to as "passive" release systems. On the other hand, there are "active" release mechanisms whereby some force is generated to mechanically force bioactive from the device.

Passive release

The intraruminal devices that release the active ingredient in passive manner tend to be the simplest and the cheapest. These combine the active ingredient into formulation that can determine the release pattern of a bioactive and which can give the intraruminal device the required properties of appropriate density and/or geometry.

One of the earliest approaches using physical disintegration of an intraruminal device was done using iron oxide as matrix with incorporated elemental selenium or cobalt (Dewey *et al.* 1958). Similarly minerals and vitamins can be incorporated into silicate glasses (Telfer *et al.* 1984) that disintegrate in the rumen over the time and slowly release the bioactive. Other examples of passive heavy density devices are Agrimin® Rumbul (Hemingway and Ritchie 1969) and SireSine® magnesium capsule (Ridley, Australasia) (Laby 1973).

Both consist of a magnesium alloy. Release occurs not because of abrasion but because of electrochemical activity. In the Ridley product, two half cylinders

made of magnesium and aluminium alloy are connected by a rubber hinge. The device is administered in form of the cylinder but when in the rumen the device unfolds ensuring ruminal retention by both density and variable geometry.

More complex are intraruminal devices that combine a bioactive with inert and ruminally stable matrix, but in such a way that the matrix material determines the release rate of the bioactive from the device. One of the marketed intraruminal devices uses this concept is Paratect Flex® bolus (Pfizer Inc.). This device is a trilaminate sheet that is administered in a rolled shape but once in the rumen the rolled sheet uncoils providing retention in the rumen (Ranade and Curtis 1992, 1994) (Figure 5). The bioactive morantel is delivered through perforation in the sheet consisting of 50:50 ratio of bioactive and ethylene vinyl acetate (EVAc) (Brewer and Griffin 1980; Boettner and Aguiar 1988).

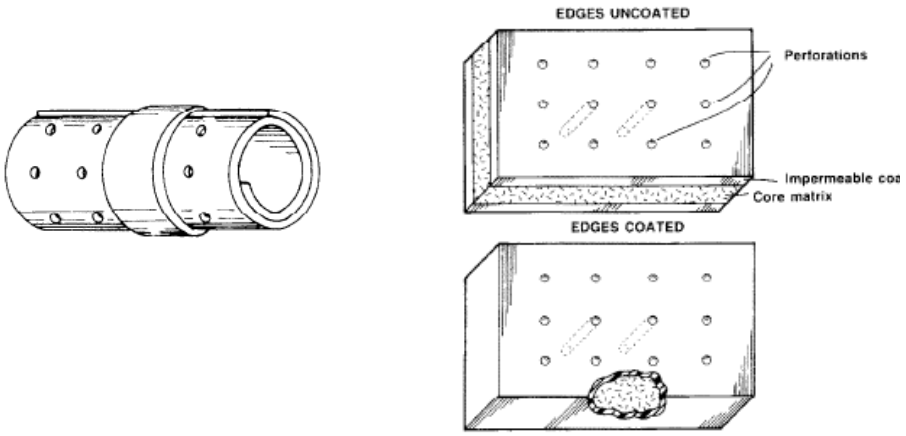

Figure 5: Diagram of Paratect Flex® bolus in a rolled and unrolled shape (from Boettner and Aguiar 1988).

The efficacy of some drugs (e.g. anthelmintics, antibiotics) can be improved by a pulsed dosing regime. There are several products that use this strategy.

The Holloway (Holloway 1982) device has a series of bioactive compartments separated by degradable cellulose segments. Once in the ruminal environment, the cellulose segments are degraded by ruminal microflora, which result in the adjacent bioactive compartments dissolving. The duration of the pulse can be modulated by the thickness of the cellulose compartments. In the Vandamme development (Figure 6) the drug compartments are separated by biodegradable

monofilaments. The duration between pulses depends on the nature of microfilaments.

The Castex system (Figure 7) marketed as Autoworm® or Synanthic Multidose® 130 (Synthex, Janssen Pharmaceutica) and Repidose® (Coopers) has a central magnesium alloy core with five surrounding rings of oxfendazole formulation encased in PVC caps. When exposed to ruminal environment, electrolysis causes sequential disintegration of the core which releases the drug tablets.

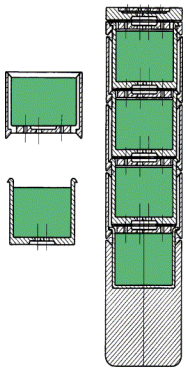

Figure 6: Cross-section of the intermittent system of Vandamme (from Vandamme and Ellis 2004).

Figure 7: Castex system (from Vandamme and Ellis 2004).

A feature of passive release devices is that they have a great dependency on the rumen environment, and therefore are prone to variable release, depending on the animal's grazing pattern.

Active release

From this type of intraruminal devices the bioactive is released mechanically by either a flexible membrane or a plunger driven by some mechanical force, commonly either osmotic, spring or gas driven. Although the bioactive release of the devices can sometimes depend on the parameters of the ruminal environment, there are inherently more reliable as regards inter-animal and intraruminal variations.

The Laby spring driven system (Figure 8) (Laby 1974) was commercialized by Elanco in their Rumensin® ABC devices and by Captec (Nufarm Pty., N.Z.) for a variety of products.

The Rumensin® ABC device consists of a solid matrix core of formulation contained in a plastic cylinder. The formulation is a dispersion of surfactant mix containing the polyether antibiotic monensin sodium for the treatment of bloat and for the enhanced rate of growth. When in the rumen, the formulation absorbs water and softens so the formulation can be released through an orifice under the influence of the spring. The device is retained in the rumen by means of an expanding pair of plastic wings.

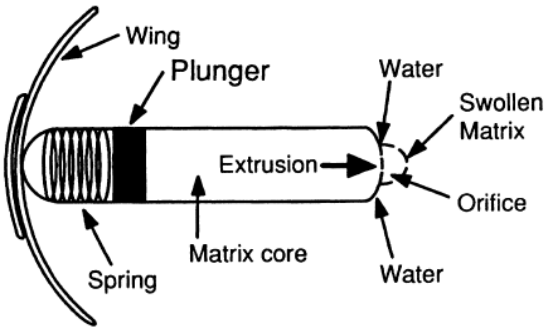

Figure 8: Laby spring driven system. The wings are held in place by a piece of tape during administration and passage down the esophagus
(from Cardinal 2000).

The Captec technology differs only by using a series of tablets instead of a single core of formulation used by Elanco (Laby 1987). This makes possible to vary bioactives and their dose to provide pulsed delivery.

The Ivomec® SR Bolus uses the osmotic technology originally developed by Alza (Mountain View, CA, USA) for the Oros® and Alzet® mini pumps. This device consists of three elements combined in one capsule: a reservoir consisting of osmotic ingredient dispersed in a gel, an adjacent reservoir separated by an inert flexible membrane, and a density element (Figure 9). When in the rumen, water diffuses across the semipermeable membrane. The osmotic gel formulation increases in volume, which causes the bioactive formulation to be extruded through the delivery orifice. Another example of an osmotic rumen bolus is

Dura SE® which delivers sodium selenite to selenium-deficient cattle for up to four months (Rothen-Weinhold *et al.* 2000). A major disadvantage of the technology is its limitation due to the low bioactive pay-load. Therefore, only highly potent bioactives can be used.

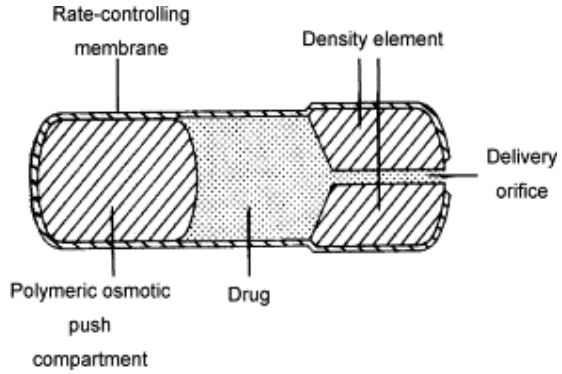

Figure 9: Cross-section of the ruminal delivery system Ivomec® SR Bolus (from Vandamme and Ellis 2004).

2.4.4 Controlled bioactive delivery using ART

Not yet commercially available, but central to this thesis is another plunger driven system, ART (Active Rumen Technology). It uses a galvanic cell to release up to 160 mL of hydrogen (Simatec gas-cell product information, Undated). Elevated pressure resulted causes the plunger to extrude bioactive formulation. Previous work (McLellan 2007) has demonstrated the feasibility of linear and reproducible release of placebo formulation out of an ART device over an extended period of time.

The first commercialized medical controlled-release system using elevated gas pressure of a hydrogen producing cell was the Theratron® (Fresenius) infusion system which is made of a syringe that injects up to 60 mL of an infusion liquid (Winsel 1993; Winsel and Sauer 2000) . Another example using this approach is an injection-moulded intravaginal device that delivers to cattle over 8 days (Rathbone *et al.* 2002; Ismail 2006) and could also deliver a pulse of a second bioactive at a predetermined time when a second reservoir of formulation was forced open by plunger movement.

The Active Rumen Technology may have a number of advantages over existing controlled-release technologies:

- The gas cell occupies a very small volume in the device so a larger bioactive payload is possible.
- Gas production rate is independent of the ruminal environment.
- There is a potential to modulate the bioactive release rate by remote control.

2.5 Ruminal protection

Rumen fermentation can destroy or modify bioactives and many drug delivery systems used to prevent or treat diseases or increase of livestock productivity. As a consequence, there is a need for a rumen-stable delivery system with the capability of delivering rumen protected bioactives. This will increase efficacy and economics because less bioactive would be required. Initial efforts to develop ruminal protection were focused on protecting proteins and amino acids to supplement normal animal feeds.

Chemical treatment

Chemically treating protein can decrease its solubility and thus increase the amount of protein that by-passes the ruminal fermentation process. Several chemical agents have been evaluated including: aldehydes, acetic acid and tannins (Ames and Robeson 1976; Vicini *et al.* 1983). Another approach is to synthesise analogues that are considerably less soluble than the original molecules (Papas *et al.* 1974).

In a further approach, the pH difference between reticulo-rumen and abomasum was used. While being relatively inert in the ruminal environment, copper oxide, after having passed into the abomasum, can be trapped in the abomasal rugae where it slowly dissolves delivering biological active copper ions (Vandamme and Ellis 2004).

Heat treatment

Heat treatment can promote a reaction between amino acids and carbohydrates in feed stuffs, reducing rumen degradation rates.

Lipid-based formulations

Formulating small pellets of bioactive that are coated with inexpensive food-grade lipid can partially protect the bioactive from degradation in the rumen. However, major limitations of this technology result from the low (ca. 30%) bioactive payload that can be routinely achieved, and from the relatively low post ruminal absorption rates of these materials. Despite the disadvantages, several lipid-based, rumen protected products are available. One example is Mepron®, which is 85% DL-methionine protected by a fat-fibre-ash coating. This product can provide 85% protection of the DL-methionine payload after 5–6 h of rumen incubation (Degussa 1995).

pH-sensitive enteric coating

An elegant approach to developing a rumen-stable delivery system is using a pH-sensitive polymer which is stable at rumen pH but which dissolves quickly at lower pH values.

2.6 pH-sensitive coating for ruminal drug delivery

2.6.1 Physiological considerations

Ingested feed material may be retained, on average for 6 h (and up to ~18 h) in the rumen and for approximately 30 min in the abomasum before it is transported into intestine. The pH of rumen fluid is normally 6.5–7.0 and that of abomasal fluid is variable but often 2.0–3.0. This pH difference is the basis for developing a pH-dependent rumen-stable delivery system.

2.6.2 pH-sensitive polymers

The pH-sensitive polymers for coating bioactives that will be used in ruminants need to have the following properties:

- Insoluble in rumen fluid, but soluble in abomasal fluid
- Physiologically inert, non-absorbable
- Thermally stable or non-degradable at temperatures used during process and storage.

A more effective delivery system was developed using copolymers of vinyl pyridine and styrene (reverse-enteric coating system) (Eastman Chemical Company, Undated). The coating used a basic polymer such as poly (2-vinylpyridine–co-styrene in ratio 80:20) (2VP–ST, 80:20). Methionine and glucose pellets were coated in a fluidized bed. Protection of coated pellets *in vitro* was assessed using simulated rumen fluid pH 5.4 buffer for 24 h followed by pH 2.9 buffer for 1 h to simulate release in the abomasums (Figure 10).

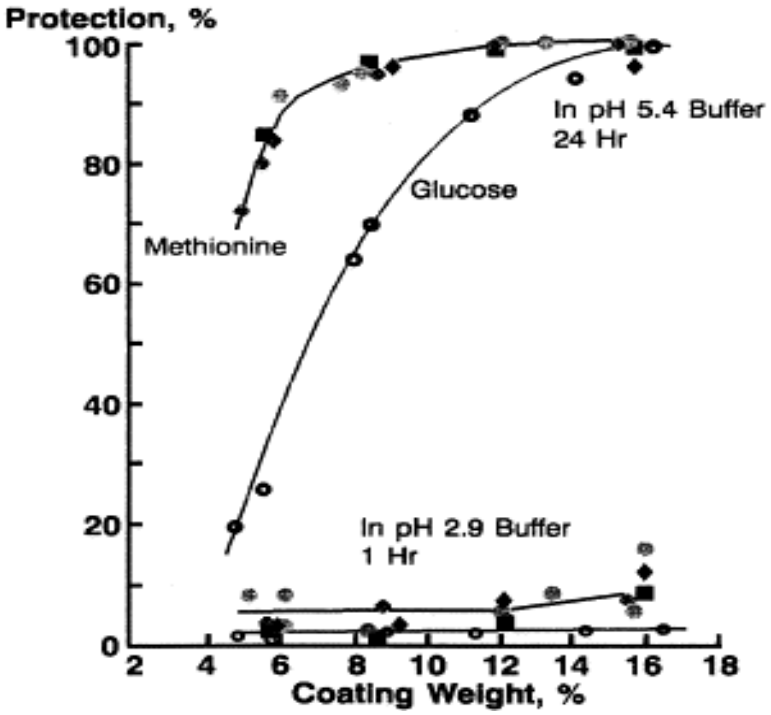

Figure 10: *In vitro* rumen protection (pH 5.4) and abomasal release (pH 2.9) of reverse-enteric protected methionine and glucose pellets
(from Wu and Papas 1997).

Further studies have investigated different coating materials. A study at Kyushu University (Yoshimaru et *al.* 1999) used a methacrylate copolymer Eudragit E® 100. This commercial pH-sensitive copolymer has potential for ruminal protection. Porous starch was used as core material to produce 20-30 µm microcapsules, which were separately coated with Eudragit® E 100, Aqoat® AS-HF (Shin-Etsu) and shellac. *In vitro* dissolution assays showed that the microcapsules were stable in pH 6.5 phosphate buffer and that 85 % of the contents were released over 30 min in pH 3 the citrate buffer. Incubating the

particles with ruminal microorganisms showed that the particles had 65% protection.

2.6.3 Eudragit® E

Eudragit® E ("Basic butylated methacrylate copolymer" Ph. Eur.) is a pH-sensitive film coating material which has been used in various pharmaceutical products since 1959 (Roehm GmbH, Darmstadt, Germany).

This cationic copolymer (~150,000 Da) is based on dimethylaminoethyl methacrylate and neutral methacrylic esters (Figure 11) and is commercially available in following forms:

- Eudragit® E 100 - pellets
- Eudragit® E PO – powder obtained from Eudragit® E 100
- Eudragit® E 12,5 – solution of Eudragit® E 100 with 12.5% (w/w) dry substance in 60% (w/w) isopropyl alcohol

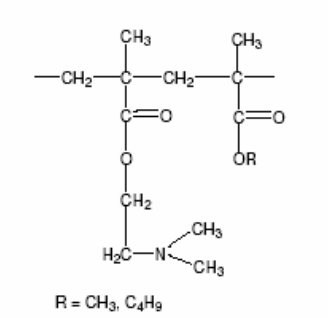

Figure 11: Chemical structure of Eudragit® E monomer.

Eudragit® E 100 is soluble in methanol, ethanol, isopropyl alcohol, acetone, ethyl acetate, methylene chloride (about 1 part of polymer in 7 parts of the solvent) and practically insoluble in petroleum ether and water. The most important feature of Eudragit® E that is relevant to this project is its pH dependent solubility. The polymer is easily soluble under pH 3 but stable at pH values higher than six. This can be used to protect bioactives from ruminal degradation by film coating with the Eudrgit® E polymer.

Toxicological studies with Eudragit® E proved its safety (Degussa 2004).

2.7 Coating bioactives

Initially, the coating process was used to preserve food but later on it was used to mask the taste of medicines. In 1953, a dramatic change was made in tablet coating when Abbott Laboratories marketed the first film-coated pharmaceutical product (Wurster 1953). This initiated interest in using polymers to physically or chemically protect the bioactive and/or modify its release.

There are several major reasons for coating the bioactive:

- to protect from the environment of the digestive tract with an appropriate pH-sensitive polymer
- to provide physical and chemical protection
- to control the release
- to incorporate another bioactive or excipient in the coating to avoid chemical incompatibilities or to provide sequential bioactive release

There are three primary features of coating process:

- Properties of the substrate
- Coating process
- Coating compositions

Several substrate types can be used for coating, ranging from amorphous granules to spherical particles (pellets). Each offer different levels of ease and convenience in coating. One of the most convenient substrates is sugar pellets, which are inert spherical particles composed of sucrose and flour, and are used as bioactive vehicle for controlled or sustained delivery technologies. Figure 12 demonstrates the mechanism of film coating formation.

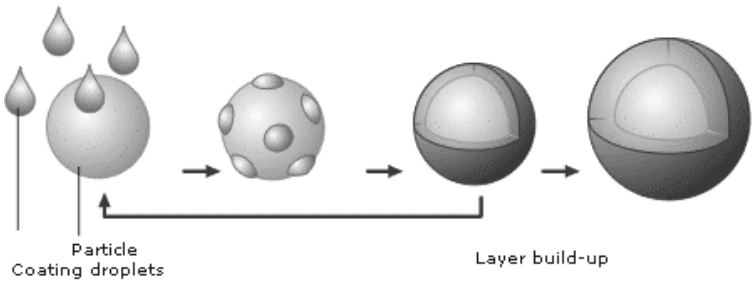

Figure 12: Schematic of film coating
(from Glatt, Undated).

2.8 Phloridizin (PHZ) as a model bioactive

Phloridizin (Figure 13) is a natural product found in a number of fruit trees. It inhibits sodium-glucose transporters located in the proximal renal tubule and mucosa of the small intestine, resulting in increased renal glucose elimination (Ehrenkranz *et al.* 2005).

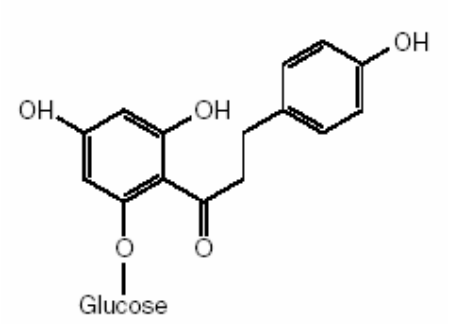

Figure 13: Chemical structure of phloridizin.

Phloridizin can be analytically detected using HPLC (Escarpa and Gonzalez 1998) and by measuring UV absorbance at about 280 nm (Lin *et al*. 2000). Molecular weight of phloridizin is 436.44 g/mol. The molar extinction coefficient of pure phloridizin is approximately 18,000 $m^2 \cdot mol^{-1}$.

Due to its safety and the ease of measuring a physiological response**,** phloridizin has been used as a research tool for over 150 years. Apart from use in laboratory animals, phloridizin has been also used to explore metabolic adaptations associated with irreversible glucose loss in cattle (Meier *et al.* 2008).

Because of its safety (ACVM approval #A009723), pharmacological efficacy, good dose-response correlation and ease in measuring physiological response, phloridizin was selected as a model bioactive for this project. It can be readily granulated and therefore has sufficient physical stability to be coated with an organic coating polymer such as Eudragit® E.

2.9 Summary and objectives

Coated phloridizin particles, which should withstand ruminal degradation, can be suspended in appropriate medium, and delivered from a rumen controlled-release device (McLellan 2007). A reliable, reproducible and controllable release of rumen protected phloridizin particles is the goal of this project.

Chapter 3: Materials and Methods

3.1 Materials

The following chemicals and materials were used in the study:

Acetone (BDH Laboratory Supplies, England)

Citric acid (BDH Laboratory Supplies, England)

di-Sodium hydrogen orthophosphate 2-hydrate ($Na_2HPO_4 \cdot 2H_2O$)
(VWR International Ltd., England)

Eudragit® E (Degussa Coatings & Colorants Pty Ltd, Dandenong, Australia)

Hydrochloric acid (HCl) 36% w/w (Ajax Finechem, Australia)

Hydroxypropyl methylcellulose (HPMC) (Metolose®, Shin-Etsu, Japan)

Phloridizin (PHZ) 80% purity (Green Way, China)

Polyvinylpyrrolidone (PVP) (VWR International Ltd., England)

Sodium citrate (BDH Laboratory Supplies, England)

Sodium dihydrogen orthophosphate 1-hydrate ($NaH_2PO_4 \cdot H_2O$)
(VWR International Ltd., England)

Sugar granules (JK Settler, pure cane white sugar)

Sugar pellets (Golden Bridge Marketing Ltd, Auckland, New Zealand)

3.2 Equipment

Spray applicators - Spray application for granulating was achieved using a hand-held 500-mL spray bottle delivering about 0.7 mL per trigger action. For coating, a touch-up spray gun (T100, Airpower Superworks) connected to an air compressor was operated between 2 and 3 atmospheres to deliver a fine mist of spraying solution.

Pan Coater – The pan (Figure 14) was made by joining the rims of two strong 295-mm diameter plastic bowls (THBN, The Warehouse, Hamilton). The base of

one bowl had been removed (to provide an opening) and a 5-mm diameter bolt had been inserted into the base of the other. The bolt was then attached to a Heidolph RZR 1 variable speed motor. To minimize particles being lost from the pan, the opening was covered with cotton fabric with 0.3 mm pores. The fabric had a 5-cm diameter orifice to allow the spray and air streams into the coater.

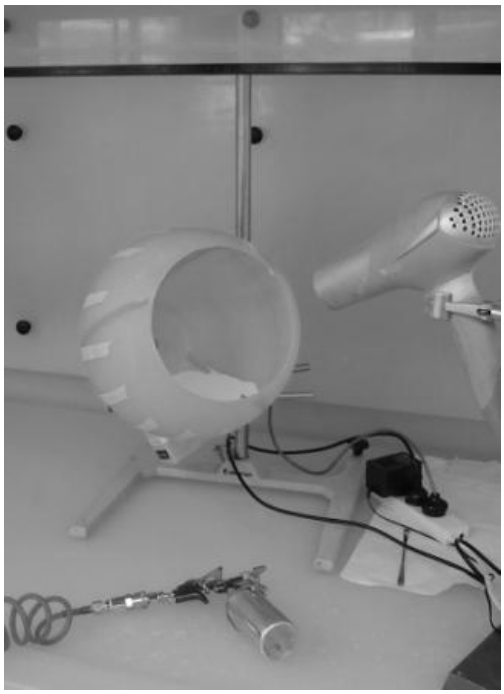

Figure 14: Coating equipment.

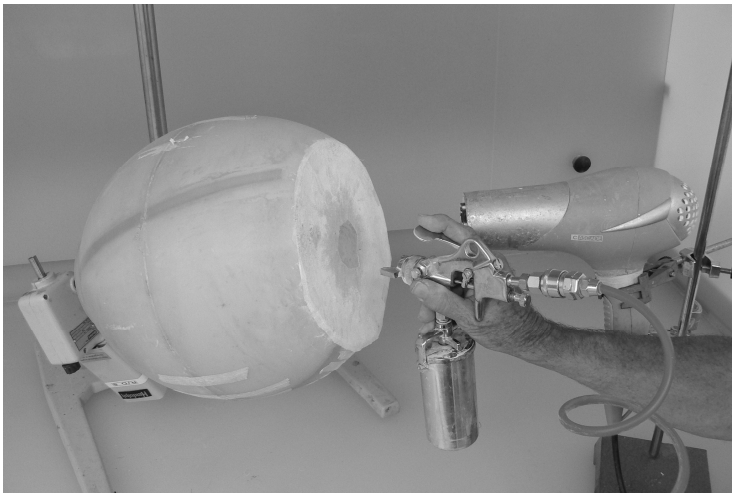

Figure 15: Spraying with a touch-up spray gun.

3.3 General laboratory methods

When required, samples were air dried in a 60°C oven and cooled to room temperature in a dessicator with silica gel.

MilliQ water (deionized reverse osmosis purified) was used for making all solutions and suspension formulations. To ensure complete dissolution, solutions were vortexed or stirred. Suspensions of material were clarified either by centrifugation or by filtration using 12.5-mm filter papers (Whatman® No. 2) or 0.45-μm PVDF microfilters (Biolab, Australia).

The following dissolution media were used:

Phosphate buffer, pH 6.5: 0.1 M phosphate buffer was made by weighing appropriate amounts of sodium dihydrogen orthophosphate 1-hydrate ($NaH_2PO_4 \cdot H_20$) and di-sodium hydrogen orthophosphate 2-hydrate ($Na_2HPO_4 \cdot 2H_2O$) and making to a standard volume with water. The stock buffer was then diluted to obtain lower molarity phosphate buffers.

Citrate buffer, pH 3: 0.1 M citrate buffer was made by weighing appropriate amounts of citric acid and sodium citrate and making a standard volume with water. The stock buffer was then diluted to obtain lower molarity citrate buffers.

Hydrochloric acid HCl, pH 3: 0.1 M HCl was prepared by making an appropriate volume of 36% (w/w) HCl to a standard volume with water. The stock solution was diluted with water to obtain 10^{-3} M HCl (pH 3).

3.4 Analytical methods

Dry matter determinations were made by weighing samples into pre-dried and weighed containers, drying in the 60°C oven and cooling as above.

Sugar concentrations were measured in Brix (1 degree Brix or °Bx corresponds to 1% (w/w) sugar of total) using a SR 400 refractometer (Bell Technology Ltd., Auckland, New Zealand) with auto-calibration mode. Calibration was checked with standard solutions made from commercial sugar.

UV-spectral characteristics of PHZ were measured by Libra 12 (Biochrom Ltd., Cambridge, England). The UV absorbance of PHZ at 285 nm was used for analysis. The instrument had to be warmed up for at least a one hour and re-zeroed regularly to minimize "drift" in either the lamp or sensors. To obtain the calibration curve, a series of standards were made in water, 0.05 M phosphate buffer (pH 6.5) or 10^{-3}M HCl (pH 3) by diluting a stock solution.

Sugar in blood and urine of cattle was tested using Precision Plus™ (Medisence Pty. Ltd., Australia) blood glucose monitor and disposable electrodes

3.5 Solubility, bioactive content and release rate

Solubility of PHZ powder at saturation concentration was measured by sequentially adding known amounts to fixed volumes of medium (water, buffer or acid) with stirring until no more material dissolved.

To measure the dissolved bioactive content, samples were prepared from known amounts of medium and raw materials or coated (or uncoated) granules. The resulting concentration in solution was measured by Brix or UV. Coated or granulated material had been disrupted by grinding. Care was needed to distinguish between undissolved material and the insoluble ground coating, which generally would sediment more quickly due to the size and weight of the fragments.

Dissolution rates were measured by adding known weights of material to a given weight or volume of medium in a sealed container that was rotated at approximately 60 r.p.m. continually on the bottle roller at 40^{o} C.

Small samples were taken at known times and assayed using the appropriate method for the solute being dissolved.

3.6 Physical characteristics of particles

3.6.1 Average particle weight

Average particle weight was obtained by measuring the total weight for a known number (~100) of sugar pellets.

3.6.2 Average particle size

The diameters of 50 randomly chosen sugar pellets were measured with callipers (Mitutoyo Corp., Japan) and averaged. Measuring average particle sizes of sugar granules were obtained by sieving as in section 3.6.3.

3.6.3 Particle size distribution

Approximately 100 g of either sugar pellets or granules was shaken using an electromagnetic shaker (EMS-8, Mumbai, India) using a nest of sieves ranging from 0.25 to 2.00 mm mesh size. The separated material fractions were expressed as a weight percentage of the total sample.

3.7 Coating amount and/or model bioactive content

3.7.1 Direct method

The thickness of Eudragit® E coating was measured by comparing the size of coated and uncoated sugar pellets. Coating thickness was calculated from the equation:

$$\text{Coating thickness} = 0.5 \cdot (D_c - D_u)$$

Where D_c and D_u represent diameter of coated and uncoated particles respectively.

3.7.2 Theoretical (maximal) value

The spray-gun bottle containing a known amount of spraying solution was weighed before and after each coating cycle. The amount of Eudragit® E used was calculated from the weight loss of the bottle and known concentration of Eudragit® E. This was compared to the weight of substrate used.

3.7.3 Assay for model bioactive content (sugar and PHZ)

Prior to measuring the content of poorly soluble PHZ, a known amount of either raw PHZ or ground PHZ granules was suspended in a known weight of water and stirred until dissolution was considered to be complete.

An appropriate amount of either ground sugar material or an aqueous suspension of PHZ obtained as described above was weighed into a tarred 15-mL centrifuge tube. About 15 mL of water was added and the tube was re-weighed. The tube was vortexed for 5 min and then centrifuged at 10 000 G for 5 min. The solution was assayed by Brix or UV. The amount of Eudragit® E was calculated as the difference between the total weight of particles and analyzed bioactive content.

3.8 Manufacturing and processing trials

3.8.1 Granulating PHZ

The process was a simplified version of the industrial process.

A 2% w/w aqueous solution of PVP was sprayed at about 1 trigger action per second onto a known amount of PHZ spread on a 38 x 25 x 3 cm plastic flat tray placed at a 30^{o} angle to the horizontal. After 20 trigger action, the particles were mixed and left to dry for 15 min before repeating the process. After the final spraying phase, the tray was placed into the 60^{o}C oven and dried for 12 h (to constant weight).

Monitoring the amount of binder

The difference in weight of original tray with sample and dried weight after spraying indicated the amount of binder added

The amount of binder was also estimated by weighing the spray container (and contents) after each spraying phase to measure the amount of solution that had been applied.

3.8.2 Developing a coating procedure

The standard procedure used a stock solution of 6% w/w Eudragit® E in acetone. The pan was 25^{o} to the horizontal axis. The procedure involved coating about 150 g of pellets (diameter 1.4-1.7 mm) at a spraying rate of about 3.5 g/min (4.4 mL/min), with the pan turning at 80 r.p.m. The spray gun nozzle was set to give the narrowest and strongest spray stream possible, and operated at about 10 trigger action per min (1 trigger action per 8 pan rotations). The distance between the spray gun nozzle and the bottom of the pan averaged 21 cm. The dryer was placed behind the spray gun and at setting 2 (maximum speed) to give a stream of warm air at a 10^{o} angle from the spray gun stream.

The coating process was continuous except when taking measurements or overnight. After coating was completed, pellets were dried with the fan in a static pan for about 30 min to remove any acetone.

3.8.3 Initial coating study – comparisons of core particles

To investigate the effect of particle size and shape, and the degree of abrasion during the coating process, initial coating trials using sugar pellets and sugar granules were performed. The sugar pellets contain about 15% flour (a binder to increase particle robustness). Their spherical shape minimizes contact between particles, reducing agglomeration during the coating process. The sugar pellets were sieved to obtain the 1.7-2.0 mm diameter fraction. The sugar granules were sieved to obtain the 0.71-1.00 mm diameter fraction to simulate PHZ granules.

To assess the extent of abrasion, approximately 160 g of sugar pellets were coated with total of about 50 g of Eudragit® E. Small samples of coated sugar pellets were taken progressively and the average particle weight was determined.

Batches of material were made with varying amounts of Eudragit® E. The amount of coating on material was determined (section 3.7.3 above) and the dissolution rate of coated material was measured in various media (section 3.5).

3.8.4 Coated PHZ for *in vitro* trials

Three batches of PHZ granules containing 2.7, 1.3 and 0.7% (w/w) PVP respectively were separately granulated and coated. The dissolution rates of

coated and uncoated PHZ granules and PHZ powder were measured in pH 6.5, 0.05 M phosphate buffer and HCl 10^{-3} M.

3.9 Animal trials

In a related project, a small series of animal trials was conducted by staff at Dexcel Limited using material prepared during this study. This work is included in this thesis because the data are of direct relevance to assessing the coating technology and to the overall project that the research in this thesis contributes to. Individual credit for data in this section is not claimed by the candidate.

3.9.1 Animal ethics

Animal trials were approved independently by the AgResearch Ruakura Animal Ethics Committee. The use of PHZ was approved by Agricultural Compounds and Veterinary Medicines group (ACVM approval #A009723, Expiring 1 June 2009). Eudragit® E 100 / E PO (an excipient in human pharmaceuticals) and guar gum (GRAS Register, CAS No 9000-30-0) were approved ingredients.

3.9.2 Unprotected PHZ

The objective of these studies was to assess effectiveness of the physiological response of unprotected (i.e. uncoated) PHZ. Single doses of unprotected PHZ powder were administered as a suspension in 60 mL water, with 0.5 - 1.0 g guar gum to aid in suspension, directly into the abomasum of fistulated cows (Table 2) via a 1.2-m flexible PVC tube with a 200-mm, 10-mm internal diameter stainless steel tube on one end.

Table 2: Animal studies with unprotected PHZ.

	Trial 1	Trial 2
Number of animals	2	5
PHZ single dose (g)	4 or 8	0, 2, 4, 8, 16

Samples of urine were collected at known time intervals and screened for glucose (section 3.4).

3.9.3 Protected PHZ

The objective was to compare the physiological effectiveness of protected and unprotected PHZ when administered into the abomasum. The trial involved administration of PHZ directly to the abomasum of fistulated cows with a 16 g single dose of unprotected and protected PHZ. Process was repeated 2 days later with treatments being over-crossed.

The product was suspended in water (day 1) or in 5% w/w HPMC in water gel (day 2). Samples of urine were collected at 2-h intervals for up to 12 h and screened for glucose (section 3.4).

3.10 Active Rumen Technology (ART)

The concept of Active Rumen Technology, which involves intraruminal bioactive delivery using elevated gas pressure, is described in section 2.4.4.

This section describes controlled *in vitro* delivery of protected PHZ. To explore the relation between *in vitro* release rate and the payload of coated bioactive a series of devices were made up of aqueous 6% w/w HPMC gel with 0%, 5%, 10% and 15% (w/w) of coated PHZ. For each concentration, five devices were assembled.

3.10.1 Preparing the bioactive formulation

HPMC gel was centrifuged in 50-mL tubes for 5 min at 5000 G to remove air. Known amounts of coated protected PHZ particles were carefully mixed into in the gel to obtain the required content by weight. Devices were carefully filled with the formulation so no aeration resulted.

3.10.2 Manufacturing the devices

Syringe barrels with low gas permeability and low friction plungers were used. The barrel orifice of each was extended to 6 mm diameter.

The following components (Figure 16) for manufacturing the devices were used:

- 7-mL BD Epilor® barrels and plungers
- hydrogen producing gas cell (Varta 4690 11.6-mm by 5.4-mm gas-producing cell, Simatec, Switzerland)
- resistors 1800 Ω
- sealing components: rubber plugs, plastic holding plates

Figure 16: Components of the ART device.

After carefully filling with formulation (Figure 17), the plunger was placed into the barrel followed by a gas cell. Each device was sealed by attaching a holding plate to the base of the barrel.

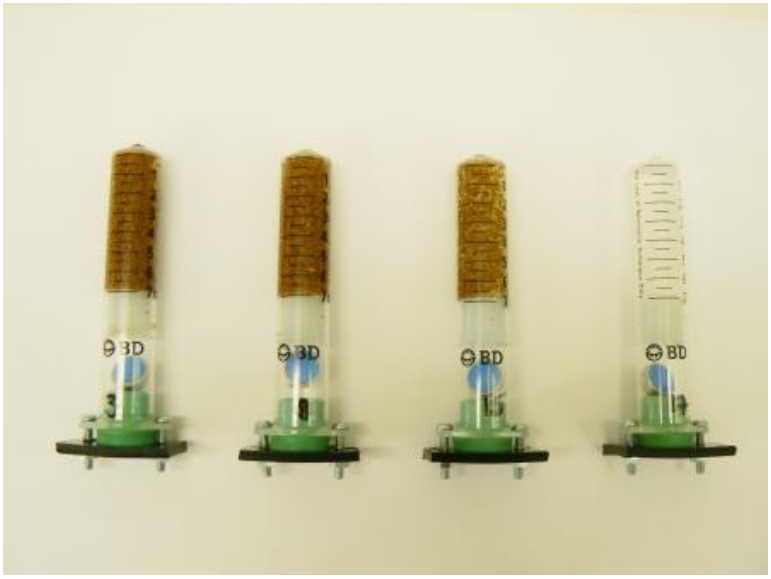

Figure 17: ART devices filled with bioactive formulation.

3.10.3 Measuring release rate

All devices were placed horizontally on the test tube rack and pre-weighed 5-mL sample tubes were placed under the orifices of the barrels to collect expelled material. At regular times, devices were weighed and the plunger position measured on two opposite sides using callipers. To measure the amount of solid material released, the test tubes with collected material were dried and weighed.

Chapter 4: Results and Discussion

4.1 Granulating PHZ

4.1.1 Analysis of PHZ powder

UV absorbance

The UV spectra of PHZ in the different media at concentrations of about 30 µg/mL showed that the spectra are essentially independent of medium and/or pH in the spectral region of analytical interest (Figure 18). Routine analysis used the maximum absorbance at 285 nm.

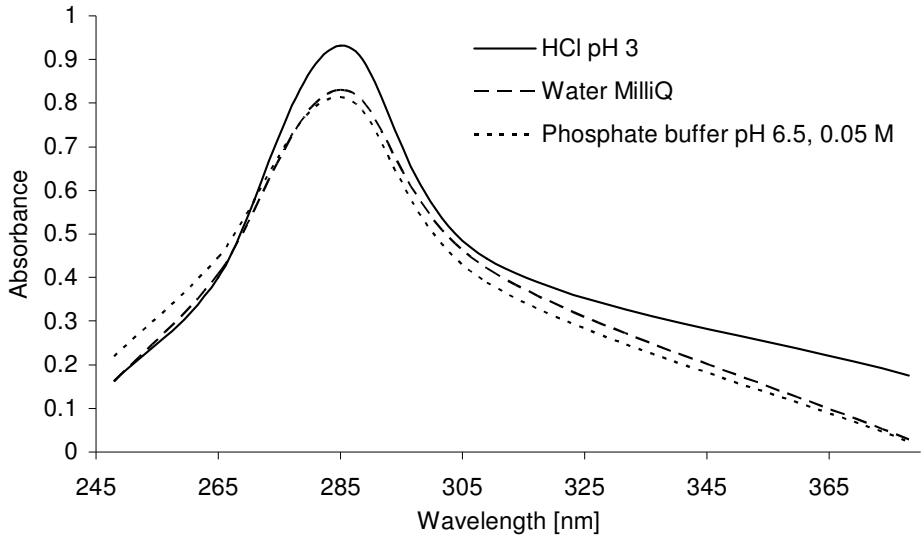

Figure 18: UV spectra in different media.

Beer's law plots in all media were linear, yielding a common slope (Figure 19). This was use in all subsequent assays.

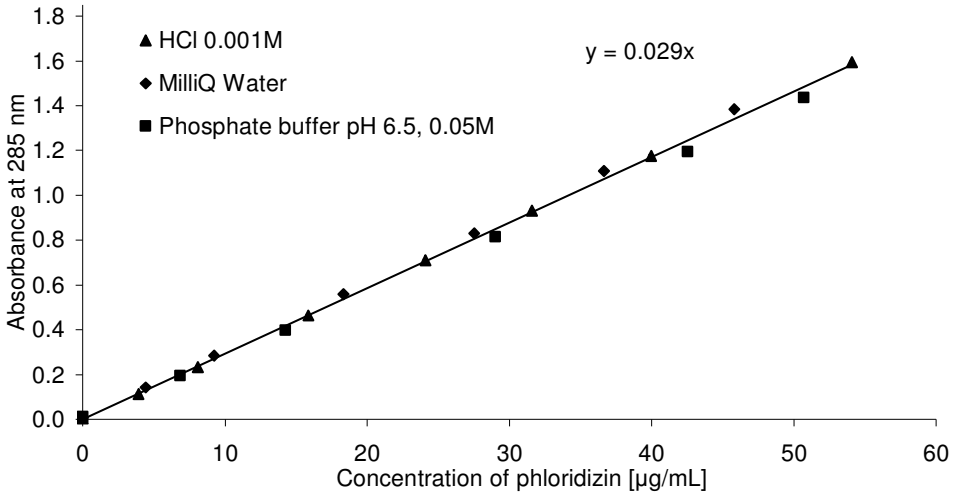

Figure 19: UV absorbance of PHZ in different media.

The molar extinction coefficient for powdered PHZ was calculated using the mean regression line (Figure 19) and is of the order of 12,600 $m^2 \cdot mol^{-1}$. Considering the PHZ used was only 80% pure (section 3.1), the calculated value (15,800 $m^2 \cdot mol^{-1}$) is within about 15% of the literature value of about 18,000 $m^2 \cdot mol^{-1}$.

Solubility determination

Duplicated assays demonstrated that solubility of PHZ was dependent on pH, but apparently also affected by the ionic nature of the medium (Table 3).

Table 3: Solubility of PHZ powder.

Medium	Solubility (g/L)
Water	1.15
Phosphate buffer pH 6.5, 0.05M	1.94
HCl pH 3	0.91

4.1.2 The effect of PVP

PVP was used as a binding material for granulating PHZ. Three batches of 0.71 - 1.00 mm diameter PVP granules with 2.7, 1.3 and 0.7% (w/w) PVP content respectively were manufactured.

The dissolution of granules in phosphate buffer showed that PVP had only a small effect on initial dissolution rate (Figure 20). All granules reached 85-95% dissolution after 180 min.

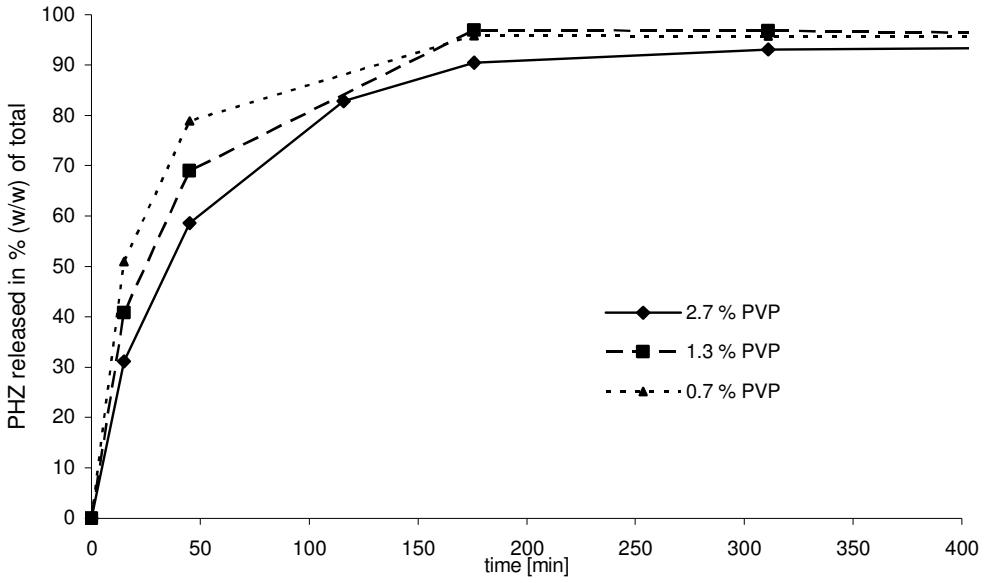

Figure 20: Dissolution profiles of uncoated PHZ granules containing different amounts of PVP (% w/w) in pH 6.5, 0.05 M phosphate buffer.

4.2 Developing a coating procedure – practical observations

Preliminary studies used sugar pellets to explore the effect of varying different parameters, and to gain experience in using the equipment.

Rotation speed

Industry tends to use for coating tablets rotation speeds of the order of less than 15 r.p.m. (Lachman *et al.* 1986). This rotation speed appeared to be too low for

small (2 mm) particles as they tend to agglomerate. If pan rotation speed exceeded 90 r.p.m, the particles were centrifugally forced out and glued to the pan coater walls. The optimal rotation speed for 150 g of 1.4 - 1.7 mm diameter sugar pellets was 80 r.p.m.

Angular position

The optimum angular position, when coating about 150 g of 1.4 - 1.7 mm diameter sugar pellets was on average 30º.

Composition of the spray solution

The initial composition of the spray solution was adapted from the Degussa Eudragit® E specification sheets (Degussa, Undated). However it was found that the coated material dried too slowly, causing increased gluing of the material. This appeared to be due to the water and isopropyl alcohol content so they were therefore omitted from the spray.

Talc is normally added as a glidant to prevent gluing of the particles. However, exploratory trials showed that talc did not increase the coating efficiency but could interfere with analytical methods because it was incorporated in the coating. Therefore, talc was excluded from the spray solution.

The composition of spraying solution adopted for routine studies was 6% (w/w) Eudragit® E in acetone.

Spraying rate

A spraying rate of 1 trigger action per 8 pan rotations was found to be the most efficient in terms of drying rate. The weight of spraying solution expelled depends on the spray gun settings but on average corresponded to about 3.5 g of spraying solution per minute.

Spray gun settings

The spray gun was set to produce a spray that would reach the epicentre of the pan coater but not to the pan wall behind the epicentre.

Pan rotation time

When the particles rotated in the pan, they were exposed to high mechanical forces between the particles and also between themselves and the pan walls. This facilitated abrasion (section 3.8.3) of particle surface resulting in decreased particle weight and size, which is demonstrated in the Figure 21. There was a small weight loss at the start of the process (i.e. when there was only a small amount of added Eudragit® E), but this stopped after 15 g of Eudragit® E had been added and average particle weight increased.

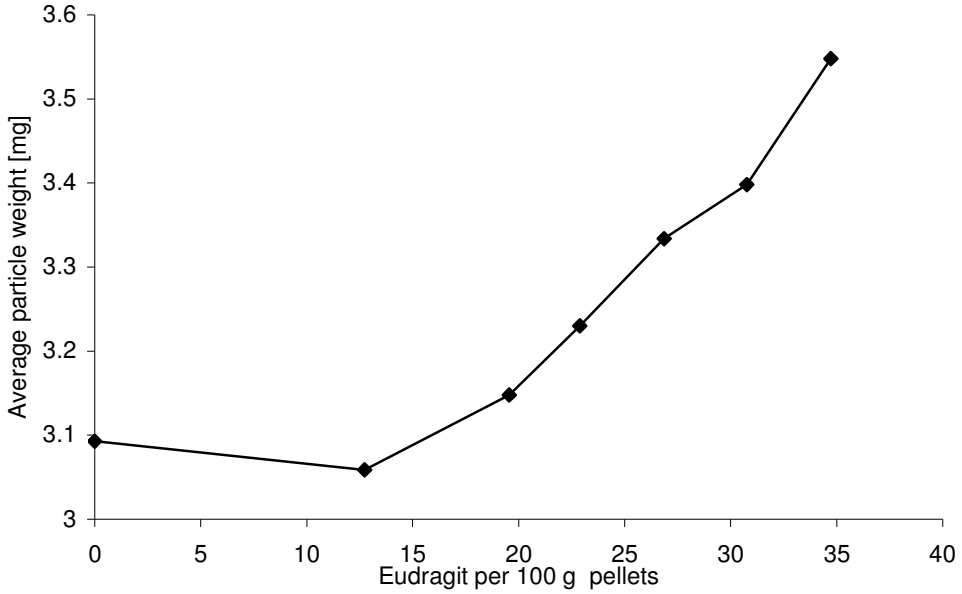

Figure 21: Effect of processing time (expressed as added Eudragit® E) on average particle weight.

4.3 Coating using different model substrates

4.3.1 Sugar pellets

Coating efficiency

To estimate the efficiency of the coating process and degree of protection *in vitro,* two batches of coated sugar pellets with varying amounts of Eudragit® E. were produced. Particle weight, thickness and % coating of each batch were determined (Table 4).

Table 4: Eudragit® E coating content of sugar pellets
(UP - uncoated sugar pellets; CP 2 – medium coating; CP 3 – extended coating).

	Theoretical (max.) value			Measuring particle size			Weighing particle		Measuring Brix	
Particle	Weight spray applied, g	Eudragit® E applied , g	Theoretical (max.) E % (w/w)	Average particle size,	Difference, mm	Coating thickness, µm	Weight, g	Increase in weight in %	Sugar % (w/w)	Eudragit® E % (w/w)
UP	0.0	0.0	0.0	1.52	0.0	0	3.09	0.0	100.0	0.0
CP2	661	39.6	20.9	1.63	0.11	55	3.58	13.6	86.7	13.3
CP3	1557	93.4	38.4	1.81	0.29	145	4.35	29.8	73.5	26.5

The coating of CP3 was about twice that on CP2, whether measured by theoretical amount of Eudragit® E added, diameter of particles or increase in weight. Comparison of the theoretical values to those calculated by measuring Brix, deliver coating efficiency values of about 65%.

In vitro dissolution rates

Eudragit®E coated sugar pellets dissolve only slowly in water (Figure 22). It took about 24 hours for 10% of the sugar to be solubilised from CP2 and much longer for the more heavily coated CP3.

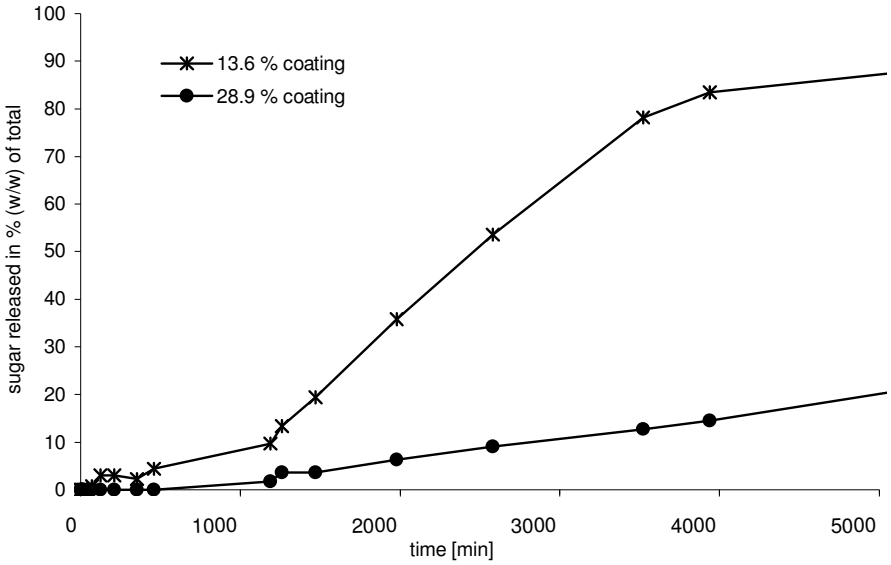

Figure 22: Effect of Eudragit® E coating content on dissolution of coated sugar pellets in water.

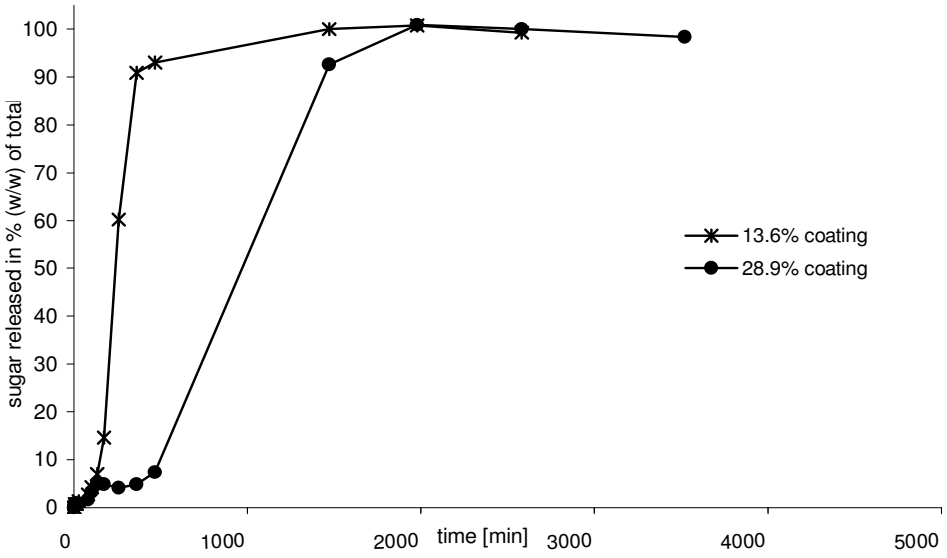

Figure 23: Effect of Eudragit® E coating content on dissolution of coated sugar pellets in pH 6.5, 0.05 M phosphate buffer.

Assuming that phosphate buffer is representative of rumen fluid, 90% protection would be afforded for up to about 9 hours (about 540 min) for extended coating (28.9% Eudragit® E) and only 2 hours (about 120 min) for medium coating (13.6% Eudragit® E).

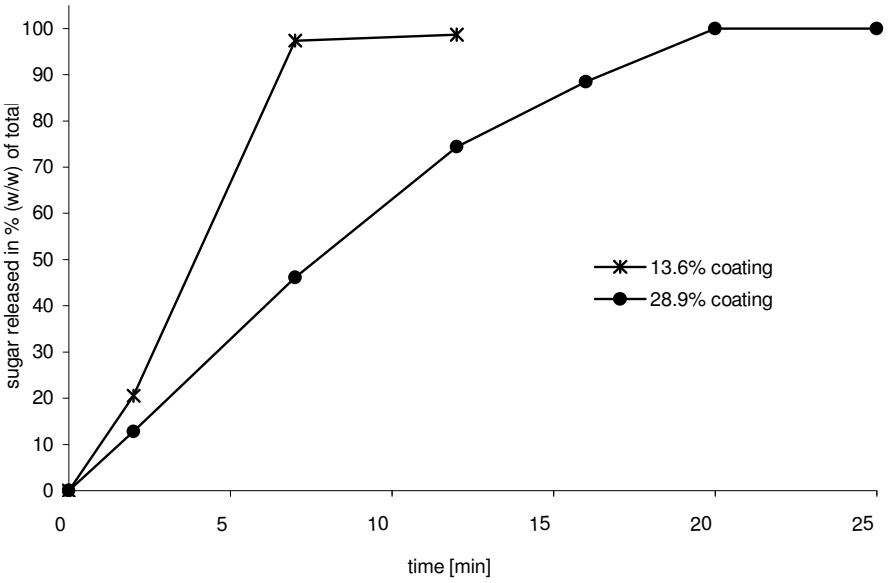

Figure 24: Effect of Eudragit® E coating content on dissolution of coated sugar pellets in pH 3, 0.05 M citrate buffer.

The time to release 50% of sugar from coated sugar pellets for both 13.6 and 28.9% E coating (CP2 and CP3) in the pH 3, 0.05 M citrate buffer is on average 80 times shorter than in phosphate buffer and on average 350 times shorter than when in water (Figure 24).

Molarity of the medium

Because the particles dissolved more rapidly in neutral phosphate buffer than in water at a similar pH, it was thought that the presence of ions (ionic strength) also influenced disintegration of the coating. This was investigated by comparing dissolution rates of coated material in pH 3 citrate buffers of different molarities (Figure 25).

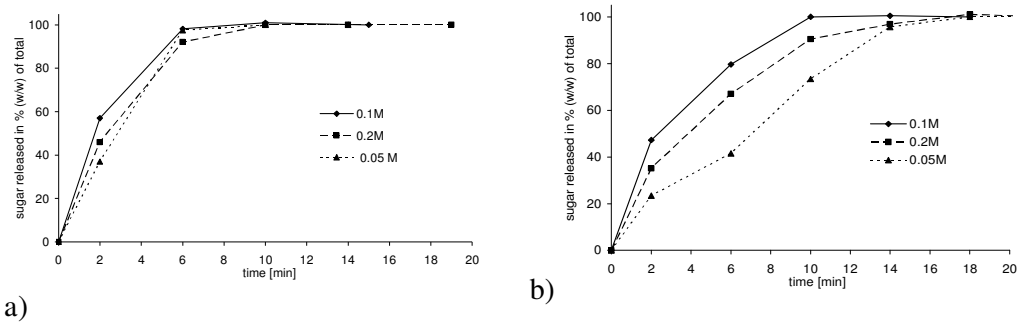

Figure 25: Effect of citrate buffer molarity on dissolution of coated sugar pellets

a) 13.6% Eudragit® E coating (CP2)
b) 28.9% Eudragit® E coating (CP3).

While not as extensive as the effect of water and phosphate buffer, both different coating degrees of particles (i.e. different coating levels) did dissolve more quickly at the higher molarity.

4.3.2 Sugar granules

Coating efficiency

To estimate the efficiency of the coating process and degree of protection *in vitro,* three batches of coated 0.50 – 0.71 mm sugar granules with varying amounts of Eudragit® E were produced. Percentage of Eudragit® E coating of each batch was determined.

Table 5: Eudragit® E content of 0.50-0.71 mm sugar granules
(UG - uncoated sugar granules, CG 1 – light coating, CG 2 – medium coating, CG 3 - extended coating)

Product	Theoretical (max.) value (% w/w)	Calculated by measuring Brix	
		Sugar % (w/w)	Eudragit® E % (w/w)
UG	0.0	100.0	0.0
CG1	5.9	97.0	3.0
CG2	19.9	88.4	11.6
CG3	40.0	75.2	24.8

Comparison of the values for applied (theoretical) and calculated by measuring Brix percentage of Eudragit® E coating (Table 5) indicates that coating efficiency of 0.50-0.71 mm sugar granules was of the order of 60%.

In vitro dissolution rates

Similarly to the dissolution rates of sugar pellets, sugar granules with more Eudragit® E coating dissolve slower than those with less Eudragit® E coating (Figure 26). Particles with a very thin coating (3% coating) were 90% dissolved within 60 min but those with the thickest coating (27.4% coating) were only 10% dissolved after 180 min.

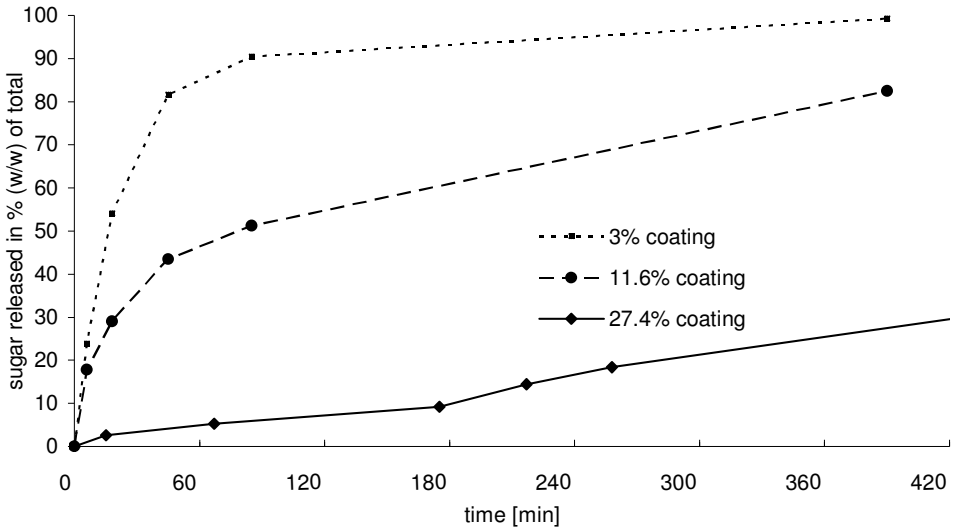

Figure 26: Effect of Eudragit® E coating content on dissolution of coated sugar granules in water.

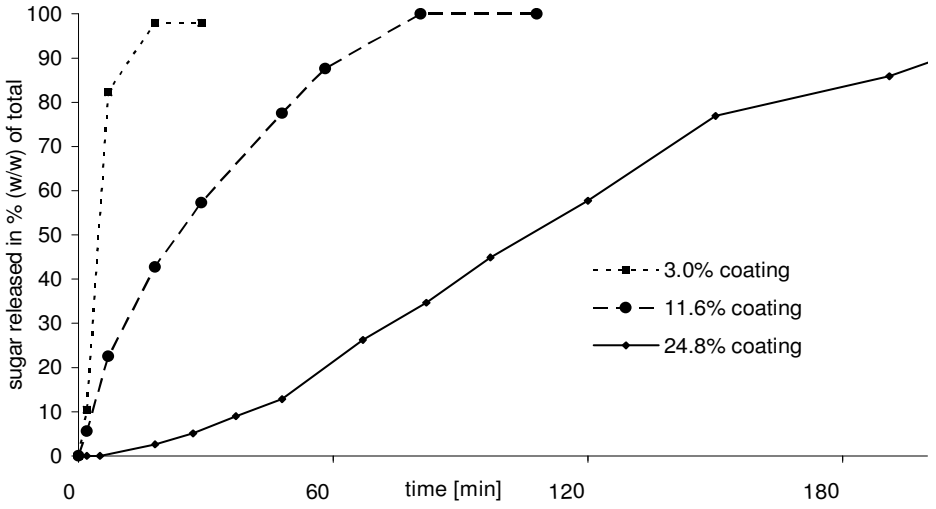

Figure 27: Effect of Eudragit® E coating content on dissolution of coated sugar granules in pH 6.5, 0.05 M phosphate buffer.

Coating thickness also affected dissolution in pH 6.5, 0.05 M phosphate buffer (Figure 27). However, particles of similar coating thickness dissolved more quickly in phosphate buffer than in water. For example, particles with 24.8% coating were 50% dissolved after 120 min compared with only 5% when in water.

All particles were completely dissolved within 16 minutes when immersed in pH 3, 0.05 M citrate buffer (Appendix A.6.3).

4.4 Coating PHZ granules

Coating efficiency

To estimate the likely coating efficiency, three different (25g) batches (2.7, 1.3 and 0.7% (w/w) PVP respectively) of 0.71 – 1.00 mm PHZ granules were coated in a normal fashion except for small samples were taken progressively during this process for quantitative analysis and calculating coating efficiency.

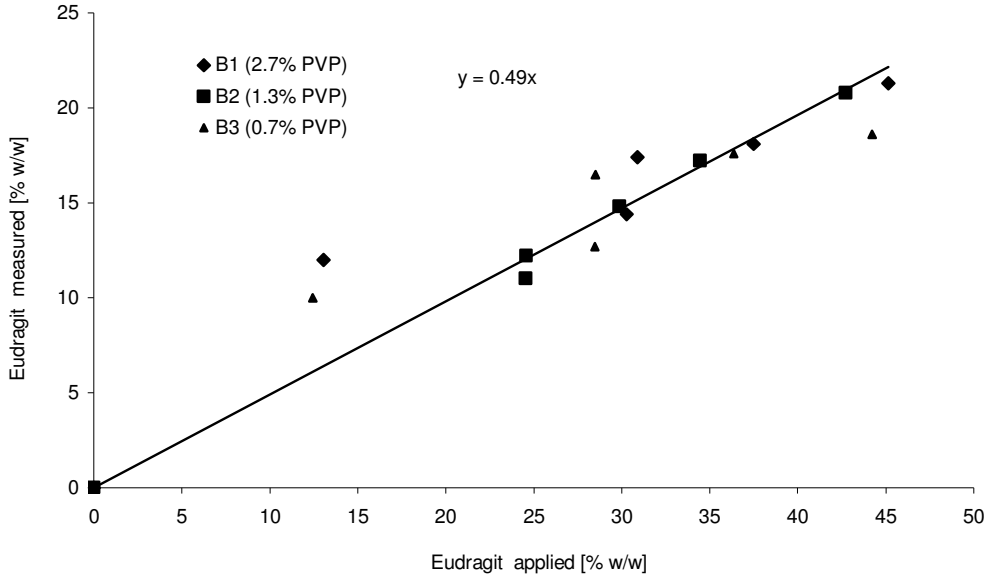

Figure 28: Correlation between measured and applied Eudragit® E of three batches (B1, B2, B3) of 0.71 – 1.00 mm PHZ granules.

The data indicated little difference between the batches (Figure 28). The coating efficiency is only about of the order of 50%, which was lower than either granules (60%) or pellets (65%). These differences are possibly due to particle properties such as size, shape, abrasion and tendency to stick together and fragment.

In vitro dissolution rates

The dissolution rate profiles of coated PHZ granules of two different particle sizes were measured.

The dissolution rate profiles of small (0.50-0.71 mm) 21.5% Eudragit® E coated PHZ granules in different media were compared (Figure 29). Because the smaller PHZ granules were subject to static electricity they readily became attached to the walls of the pan coater walls, which complicated the coating process.

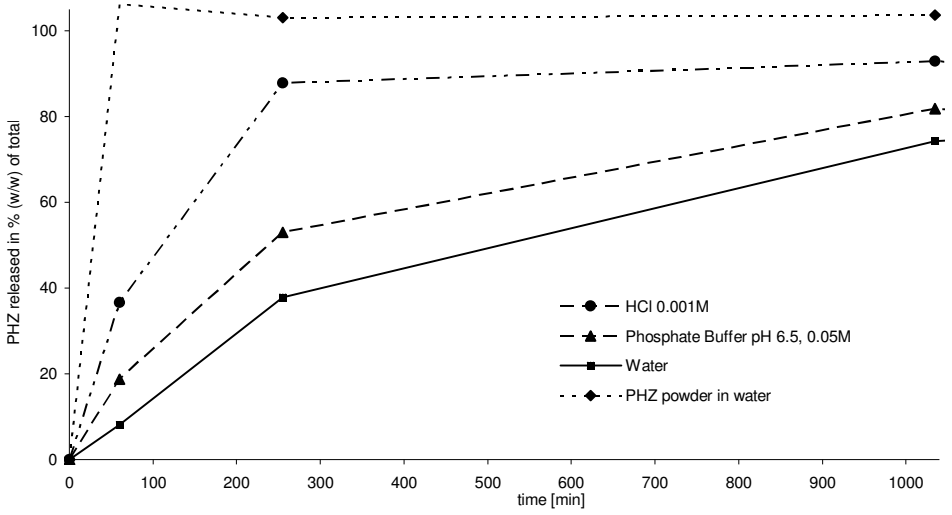

Figure 29: pH dependent release of PHZ from small (0.71-1.00 mm) 21.5% Eudragit® E coated PHZ granules.

Dissolution rates of two batches of larger (0.71-1.00 mm) PHZ granules varying in Eudragit® E amount and raw PHZ were measure in phosphate buffer (Figure 30).

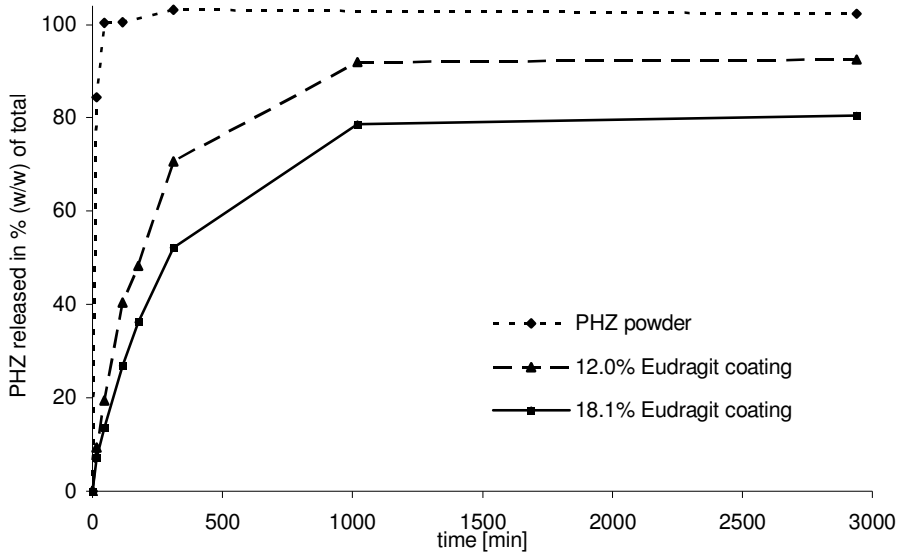

Figure 30: Effect of Eudragit® E coating content on PHZ release from 0.71 - 1.00 mm coated PHZ granules in pH 6.5, 0.05 M phosphate buffer.

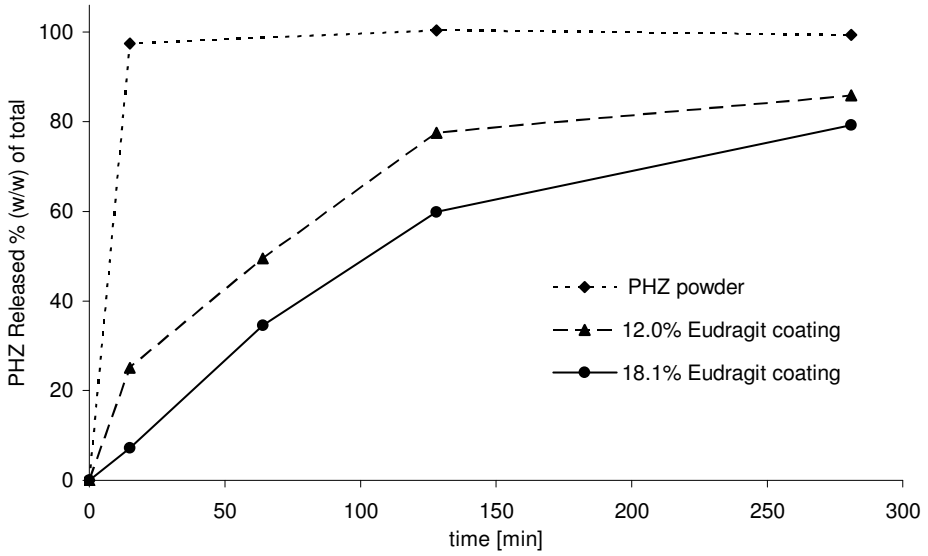

Figure 31: Effect of Eudragit® E coating content on PHZ release from 0.71 - 1.00 mm PHZ granules in pH 3 HCl.

Once again it is seen that the higher the level of coating, the slower the dissolution rate of the coated material and dissolution rate in pH 3 is faster than in pH 6.5 (Figures 30 and 31).

The profiles obtained showed a similar trend with the previous studies using sugar pellets and granules. However, the presence of PVP in the PHZ granules apparently contributes to generally slower dissolution rates than those of sugar products.

4.5 PHZ granules for in vivo trials

Coating efficiency

A new 170-g batch of 0.71-1.00 mm coated PHZ granules was manufactured by using a standard coating procedure except for intervals at which the spraying was interrupted in order to weigh the coated material.

The total amount of Eudragit® E sprayed was plotted with increase in weight of coated product (Figure 32). The amount of particles that were lost from the pan coater was considered in the calculation.

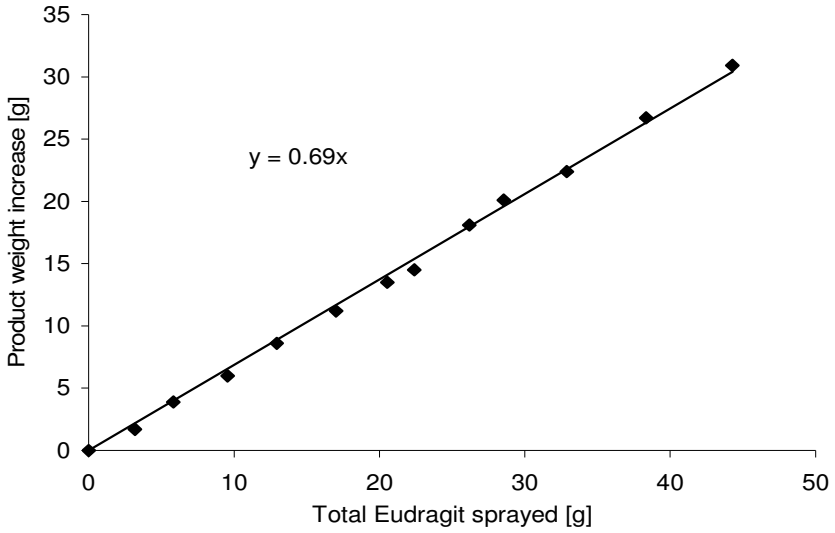

Figure 32: Effect of total Eudragit® E applied on increase in total weight of coated product.

Using this approach coating efficiency is about 70%. This is higher than the previous observation with smaller batches of PHZ granules (section Coating efficiency in 4.4)

Accurate measure of Eudragit® E coating

The final product was assayed by UV absorbance and the amount of Eudragit® E coating was calculated as the difference between the amount of PHZ in coated and uncoated particles (Table 6).

Table 6: Amount of Eudragit® E applied to the PHZ powder.

Product	n	% (w/w) PHZ	St. dev.	% Eudragit® E
Uncoated granules	4	96.0	0.8	0
Coated granules	4	73.2	1.8	22.8

In vitro dissolution rates

The pH of the environment affected the dissolution of coated PHZ that were to be used in the *in vivo* trials (Figure 33), showing that PHZ release through the Eudragit® E coating was pH dependant.

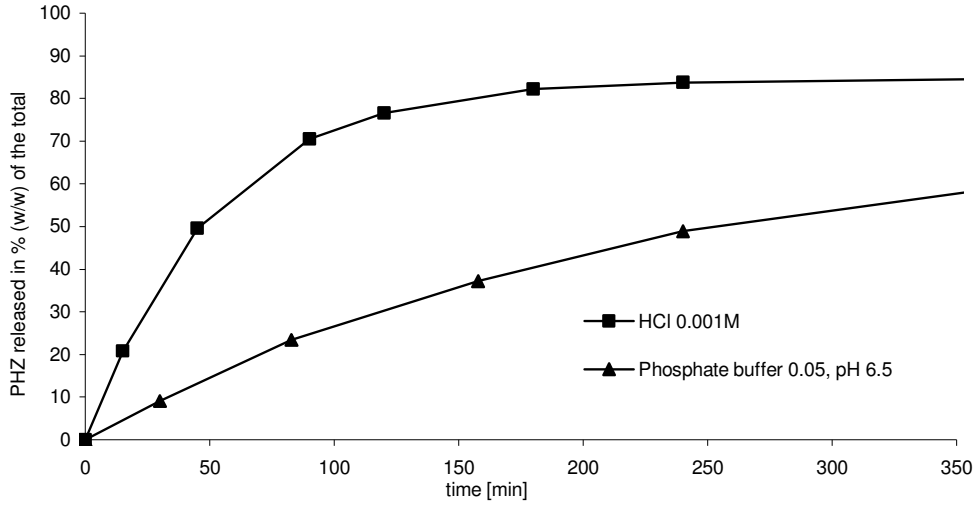

Figure 33: Effect of pH on PHZ release (pH 3 HCl vs. pH 6.5, 0.05 M phosphate buffer).

4.6 Animal trials

The trials using PHZ as a model bioactive were only partly successful. In the first instance, (Trial 1) doses of 8 and 16 g PHZ elicited responses in urinary sugar levels, while low doses were not detected. There was no demonstrated effect on blood sugar levels.

In the second trial using five cows in a Latin square design, there was a relatively consistent response from the 8 and 16 g doses, but not from the lower dose levels.

The results of the final trial (Trial 3) comparing coated and uncoated material was less convincing. Of the six cows used, three gave urinary sugar responses to coated PHZ, while only one responded to uncoated material.

The final reports on these trials indicate concern about the adequacy of the intra-abomasal infusion technique. It appears probable that much of the dose is not introduced either adequately, or sufficiently deeply, into the omasum for it to be carried automatically into the abomasum, and so much of it may "reflux" back into the rumen.

None-the-less, the data indicate that PHZ can elicit an easily measured response from the host animal, and it could therefore be a suitable model compound for further studies of granulated and coated bioactive.

4.7 Active Rumen Technology (ART)

Release rates of bioactive formulation from ART devices are presented in Figure 34.

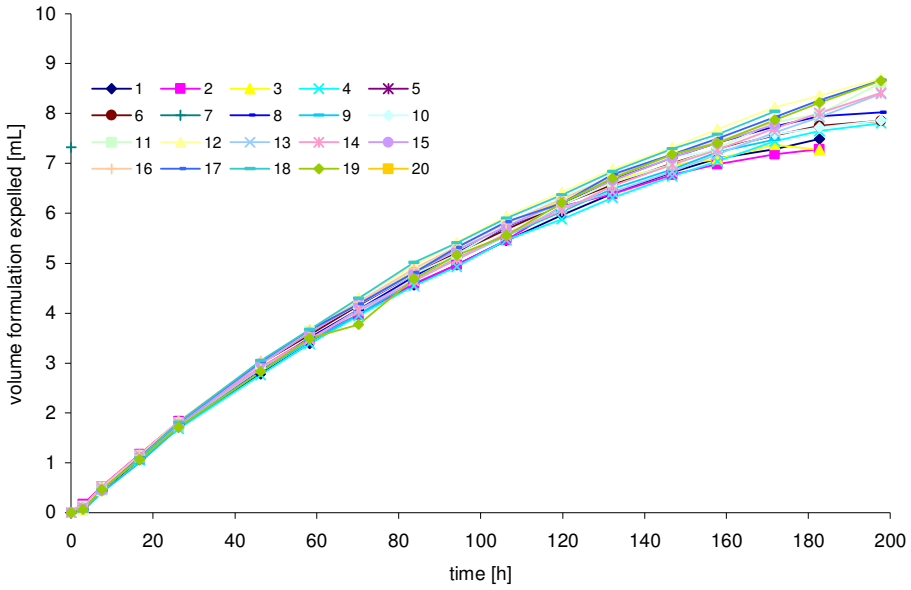

Figure 34: Release of the formulation from the ART devices
(devices 1-5: 15 % bioactive, 6-10: 10 % bioactive, 11-15: 5 % bioactive, 16-20: no bioactive).

The curvature of the profiles was thought to be due to the outward gas diffusion through the device barrel walls (McLellan 2007). It is shown that contents release from devices with the high bioactive payload (devices 1-10) slowed down sooner than those with no bioactive (devices 16-20) or low payload (devices 11-15). However, this slow down is observed in the final stage of the release, suggesting that generally the content of bioactive in form of granules does not considerably affect the releasing performance of the ART device. Due to the physical properties of the formulation based on HPMC gel, PHZ granules tended to stick together and clog at the device orifice as the gel formulation was drying out.

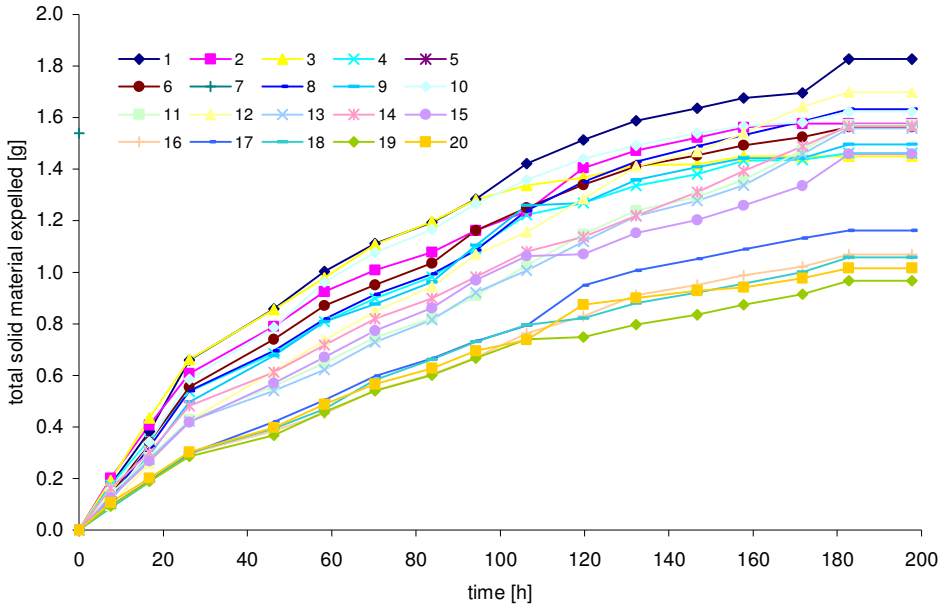

Figure 35: Release of solid material consisting of PHZ granules and HPMC (devices 1-5: 15 % bioactive, 6-10: 10 % bioactive, 11-15: 5 % bioactive, 16-20: no bioactive).

The previous results (Figure 34) are consistent with those presented in Figure 35. However, in this case the difference between four populations of used ART devices is more obvious, and the terminal amounts of solid material released is consistent with the content of PHZ granules in the formulation.

The release profiles from Figure 37 demonstrate that sustained and controlled *in vitro* bioactive release using Active Rumen Technology is in principle feasible. However, further research has to be done.

The release rate can be modified by applying different resistors and the linearity of the profiles can be achieved by using an appropriate barrel wall material.

Chapter 5: Conclusions and Recommendations

5.1 Conclusions

Pan coating process

The pan coating method used in this study requires considerable optimization of the material to be coated, and of the equipment. The efficiency of the method depends on following parameters:

- Material characteristics

Particle size and shape – Generally, smaller rectangular particles (sugar granules) tended to more agglomeration and adhering to the pan coater walls, resulting in extensive gluing together of particles.

Starting weight of material - The efficiency of applied pan coating procedure decreases with lower amounts of starting material.

- Process parameters

Composition of spray solution - The most efficient spray composition was shown to be 6% w/w of coating material (Eudragit® E) in acetone. Inclusion of water, talc (glidant) and/or isopropylalcohol did not contribute to better coating and, on the contrary, slowed down the process and led to more particle adhering together. In addition, talc tended to become incorporated in the coating and interfered with analytical methods.

Pan rotation speed - Coating at pan rotation speed of about 80 r.p.m. provided better fluidizing of particles and resulted in better efficiency and quality of coating.

Spraying rate - Spraying rates of about 3.5 g/min showed to be the most suitable in terms of optimal ratio of drying and rotation time.

Dissolution/disintegration of Eudragit® E coated material

Measuring *in vitro* dissolution rate by rotating the material in a vial is a quick and convenient method and can be applied as an alternative to standard dissolution rate methods specified by USP (The United States Pharmacopoeia).

Each type of coated substrates that have been used has shown a delay in dissolution rates in comparison to uncoated material, and a pH-dependent release profile.

In vitro dissolution profiles of both sugar pellets and granules suggest that the amount of Eudragit® E coating is not directly proportional to dissolution time of material.

Bioactive release through Eudragit® E coating depends on following factors:

- pH of medium
- molarity of medium

Bioactive controlled delivery using ART

In this study it was shown that in principle the controlled and reproducible delivery of a particulate, rumen-protected bioactive from a gas pressure driven rumen device is possible.

The release profiles of a larger number of devices (n=20) were reproducible and generally independent of the bioactive content over several days, confirming that the coating and release technologies are compatible. However, a limitation in bioactive payload was observed: the ART devices loaded with 15% bioactive payload released only about 80% of the content, which was thought to be due to drying out of HPMC gel.

Controlled and linear bioactive delivery over longer period of time could be expected to result from optimizing the bioactive formulation and the parameters of the ART device.

5.2 Recommendations

In this section recommendations for a generic rumen controlled delivery system with a protected bioactive are given.

Rumen protection

Ideally, spherical bioactive particles will be either microencapsulated or coated using fluidized bed coating. Microencapsulation is a preferential option as it will yield much smaller protected bioactive particles, which could be better suspended and homogenized in a formulation, and easier released through an ideally very small orifice of the ART device.

The protective coating could be a combination of different coating materials to delay microbial digestion. To augment the efficiency of rumen protection, traces of an appropriate and safe antibiotic agent could be added into the coating.

Formulation

The formulation will be preferentially an inorganic matrix (e.g. bentonite gels) to prevent its degradation by ruminal bacteria, which are capable of migrating into the device and digesting the bioactive formulation. The addition of small amounts of a safe antibiotic agent into the formulation will minimize the chance of bacterial colonization. Appropriate viscosity and consistency of the matrix will ensure more uniform and long-term suspension of bioactive particles.

Rumen controlled release device

To ensure the linearity of the bioactive release, the barrel of the device will be manufactured from material that ideally is impermeable to hydrogen and ruminal gases. A low friction plunger could be designed to have multiple points of contact with barrel walls to minimize chances of gas or formulation passing between the plunger and the barrel walls.

Future vision

Controlled intraruminal release of an effectively protected bioactive would reveal new opportunities for administration of a wide range of bioactives which, when unprotected, are unstable in the rumen. This might result in new approaches for improving health care and the productivity of livestock.

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Appendix

Contents

A.1 Calibrating UV spectrophotometer

MilliQ water

Dilution	stock solution [g]	diluted stock solution [g]	Conc. PHZ [µg/ml]	Absorbance @ 285 nm
	5.101	5.101	45.80	1.384
1	4.021	5.024	36.66	1.108
2	3.011	5.012	27.52	0.831
3	2.018	5.042	18.34	0.558
4	1.012	5.015	9.24	0.286
5	0.488	5.047	4.43	0.143
0	0.000	5.739	0.00	0.002

Phosphate buffer pH 6.5. 0.05

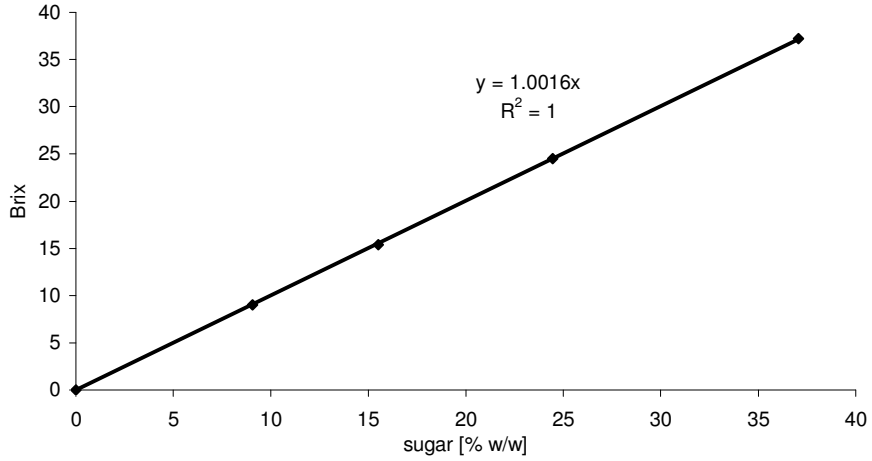
Dilution	stock solution [g]	diluted stock solution [g]	Conc. PHZ [µg/ml]	Absorbance @ 285 nm
1	1.021	5.745	50.70	1.437
2	3.069	5.057	42.55	1.195
3	2.087	5.038	29.03	0.814
4	1.034	5.085	14.26	0.397
5	0.490	5.013	6.86	0.195
0	0.000	5.183	0.00	0.001

* Stock solution was diluted down to 70.1 µg/ml

HCl 0.001M

Dilution	stock solution [g]	diluted stock solution [g]	Conc. PHZ [µg/ml]	Absorbance @ 285 nm
1	2.730	5.415	39.96	1.177
2	2.003	5.029	31.57	0.932
3	1.539	5.058	24.11	0.711
4	1.003	5.008	15.88	0.464
5	0.514	5.029	8.11	0.234
6	0.251	5.081	3.91	0.113
0	0.000	5.087	0.00	0.000

A.2 Calibrating Brix device



sample	sugar [g]	Sugar + water [g]	Sugar [% w/w]	Brix
0	0.000	10.235	0.00	0.00
1	0.914	10.072	9.07	9.00
2	1.802	11.605	15.53	15.40
3	2.569	10.507	24.45	24.50
4	3.349	9.037	37.06	37.20

A.3 PHZ solubility

	Number measurement	weight saturated solution [g]	weight after dilution [g]	Absorption @ UV285	conc. diluted sample [mcg/ml]	conc. saturated stock solution[g/l]	average [g/L]
MIlliQ water	1	1.316	46.094	0.987	32.57	1.143	
slope: y = 0.0282x	2	1.259	45.636	0.967	31.91	1.159	**1.15**
Phosphate buffer, pH 6.5, 0.05M	1	0.896	28.996	1.664	59.43	1.923	
slope: y = 0.0303x	2	1.012	33.106	1.676	59.86	1.957	**1.94**
HCl 0.001M	1	0.492	5.776	2.276	77.15	0.905	
slope: y = 0.0295x	2	0.314	6.532	1.300	44.07	0.916	**0.91**

A.4 The effect of PVP

time [min]	material	weight vial [g]	weight totla [g]	weight medium [g]	weight material [g]	weight solution taken for analysis [g]	weight after dilution [g]	Absortption diluted sample @ UV285	Absorption original solution @ UV285	conc. phloridizin [mcg/g]	conc PHZ theor. [mcg/g]	% PHZ released
15	B1	159.57	264.721	105.151	76.8	0.828	14.942	0.361	6.515	227.782	729.845	31.2
	B2	159.305	266.522	107.217	80.1	0.925	16.905	0.477	8.717	304.451	746.525	40.8
	B3	159.093	267.495	108.402	81.3	0.916	15.277	0.656	10.941	382.186	749.424	51.0
45	B1	159.57	264.721	105.151	76.8	0.638	15.463	0.505	12.240	427.599	729.845	58.6
	B2	159.305	266.522	107.217	80.1	0.595	15.308	0.573	14.742	515.098	746.525	69.0
	B3	159.093	267.495	108.402	81.3	0.546	15.787	0.585	16.915	591.064	749.424	78.9
116	B1	159.57	264.721	105.151	76.8	0.547	14.233	0.665	17.291	604.210	729.845	82.8
	B2	159.305	266.522	107.217	80.1	0.558	13.995	1.013	25.393	887.512	746.525	
	B3	159.093	267.495	108.402	81.3	0.575	14.290	0.879	21.845	763.457	749.424	
176	B1	159.57	264.721	105.151	76.8	0.686	14.487	0.894	18.880	659.768	729.845	90.4
	B2	159.305	266.522	107.217	80.1	0.452	15.064	0.621	20.696	723.291	746.525	96.9
	B3	159.093	267.495	108.402	81.3	0.629	14.399	0.898	20.557	718.417	749.424	95.9
311	B1	159.57	264.721	105.151	76.8	0.709	15.415	0.894	19.437	679.267	729.845	93.1
	B2	159.305	266.522	107.217	80.1	0.551	15.585	0.731	20.676	722.591	746.525	96.8
	B3	159.093	267.495	108.402	81.3	0.643	14.255	0.926	20.529	717.440	749.424	
1020	B1	159.57	264.721	105.151	76.8	0.445	10.117	0.874	19.870	694.407	729.845	95.1
	B2	159.305	266.522	107.217	80.1	0.437	9.984	0.880	20.105	702.618	746.525	94.1
	B3	159.093	267.495	108.402	81.3	0.295	10.397	0.580	20.442	714.383	749.424	95.3
2940	B1	159.57	264.721	105.151	76.8	0.442	10.075	0.852	19.412	678.398	729.845	93.0
	B2	159.305	266.522	107.217	80.1	0.578	9.598	1.235	20.496	716.291	746.525	96.0
	B3	159.093	267.495	108.402	81.3	0.278	10.056	0.571	20.661	722.070	749.424	96.4

A.5 Sugar pellets

A.5.1 Effect of processing time

Intermidiate product	Sugar Pellets	P0	P10	P15	P2	P3	P4	P5	P6
weight pellets beforecoating [g]		156.00	126.00	127.23	121.00	125.70	127.39	126.00	128.63
m Eudragit [g] total	0.00	0.80	15.40	24.07	28.10	33.11	38.08	43.08	50.49
m Eudragit [g]/100g pellets	0.00	0.51	12.22	6.81	3.33	3.99	3.90	3.97	5.76
m Eudragit [g]/100g cummulative	0.00	0.51	12.73	19.55	22.88	26.86	30.76	34.73	40.49
average particle size [mm]	1.59			1.63	1.61	1.61	1.66	1.66	
average particle weight [mg]	3.09	3.01	3.02	3.06	3.15	3.23	3.33	3.40	3.55
diff. from initial particle mass in %	0.00	-0.03	-0.02	-0.01	0.02	0.04	0.07	0.09	0.13

A.5.2 Eudragit® E content - theoretical (max.) value

A.5.2.1 Coating CP2 (medium coating)

Starting material [g]	spraying solution [g]	Eudragit [g]	coating time [min]	spraying rate [ml/min]	average particle weight [mg]	Eudragit % [w/w]
150	62.3	3.738	18	3.46		
	100	6			3.212	3.71
	72.1	4.326	19	3.79	3.178	2.68
	117.4	7.044	31	3.79		
	98.7	5.922	23	4.29	3.36	7.95
	110.5	6.63	25	4.42		
	99.6	5.976	26	3.83	3.7	16.41
total [g]	660.6	39.636				

A.5.2.2 Coating CP3 (extended coating)

Starting material [g]	spraying solution [g]	Eudragit [g]	coating time [min]	spraying rate [ml/min]	average particle weight [mg]	Eudragit % [w/w]
150	117	7.02	29			
	118	7.08	30			
	134.7	8.082	31			
	126.4	7.584				
	121	7.26			3.5789	13.58
	44	2.64	11	4		
	130.7	7.842				
	73.2	4.392				
	121.6	7.296	29.9	4.07		
	116.3	6.978	25.5	4.56		
	79.2	4.752				
	87	5.22			4.061	23.84
	99	5.94				
	40	2.4			4.04	23.44
	73	4.38			4.179	25.99
	76	4.56			4.3543	28.97
total [g]	1557.1	93.426				

A.5.3 Average particle size

UP (uncoated sugar pellets) (n=50)

1.67	1.7	1.68	1.7	1.56
1.64	1.53	1.58	1.56	1.65
1.51	1.51	1.45	1.54	1.79
1.7	1.55	1.49	1.68	1.64
1.72	1.66	1.55	1.69	1.62
1.47	1.64	1.4	1.57	1.56
1.57	1.51	1.58	1.65	1.39
1.6	1.58	1.65	1.65	1.62
1.6	1.53	1.62	1.77	1.58
1.5	1.55	1.58	1.52	1.63

CP2 (sugar pellets with medium coating) (n=50)

1.58	1.57	1.71	1.55	1.60
1.59	1.72	1.46	1.68	1.60
1.68	1.67	1.76	1.65	1.61
1.58	1.68	1.62	1.58	1.70
1.57	1.57	1.63	1.63	1.58
1.70	1.73	1.48	1.64	1.65
1.62	1.67	1.64	1.68	1.59
1.53	1.57	1.64	1.69	1.65
1.71	1.67	1.55	1.56	1.74
1.68	1.67	1.68	1.60	1.59

CP3 (sugar pellets with extended coating) (n=50)

1.82	1.88	1.74	1.94	1.80
1.88	1.86	1.66	1.88	1.92
1.87	1.79	1.75	1.89	1.82
1.73	1.72	1.78	1.80	1.83
1.80	1.77	1.82	1.90	1.89
1.83	1.78	1.71	1.67	1.69
1.75	1.81	1.87	1.80	1.86
1.65	1.70	1.97	1.94	1.81
1.72	1.88	1.83	1.84	1.80
1.71	1.84	1.78	1.91	1.77

A.5.3.1 Particle weight

	number of particles	total sample weight [g]	average particle weight [mg]	coating % [w/w]
Uncoated sugar pellets	100	0.309	3.093	0.0
Coated 13.6 % sugar pellets	100	0.358	3.58	13.6
Coated 28.9 % sugar pellets	100	0.435	4.354	28.9

A.5.4 Eudragit® E content - measuring Brix

	weight material [g]	weight sugar in material [g]	weight total [g]	sugar % [w/w] theor.	brix	sugar % [w/w] brix measured	Eudragit coating % [w/w]
Uncoated pellets (UP)							
0	0	0.000	10.212	0.00	0.0	0.0	
1	0.561	0.482	10.185	4.73	4.8	100.6	
2	1.125	0.967	10.643	9.08	8.9	98.5	
3	1.751	1.504	11.307	13.31	13.3	99.8	
4	2.299	1.975	11.286	17.50	17.7	101.1	
					av.	100.0	0.00
13.6% pellets (CP2)							
0	0	0.000	9.876	0.00	0.0	0.0	
1	0.545	0.468	10.134	4.62	4.1	88.0	
2	1.122	0.964	9.283	10.38	8.7	84.2	
3	1.773	1.523	9.833	15.49	13.5	87.3	
4	2.383	2.047	9.783	20.93	18.3	87.4	
					av.	86.7	13.27
28.9% pellets (CP3)							
0	0	0.000	10.231	0.00	0.0	0.0	
1	0.554	0.476	9.687	4.91	3.5	70.6	
2	1.163	0.999	10.746	9.30	6.7	72.1	
3	1.76	1.512	9.388	16.11	12.1	75.3	
4	2.368	2.035	9.857	20.64	15.7	75.9	
					av.	73.5	26.54

A.5.5 *In vitro* dissolution rates

	MilliQ water				Phosphate buffer				Citrate buffer				
	28.9% Eudragit coating		13.6% Eudragit coating		28.9% Eudragit coating		13.6% Eudragit coating		28.9% Eudragit coating		13.6% Eudragit coating		
time [min]	brix	% of max	brix	% of max	brix	% of max	brix	% of max	brix	% of max	brix	% of max	time [min]
0	0.0	0.0	0.0	0.0	0.7	0.0	0.7	0.0	1.0	0.0	1.0	0.0	0
1													1
2					0.7	0.0	0.7	0.0	2.0	12.8	2.6	20.5	2
6	0.0	0.0	0.0	0.0									6
7					0.8	0.8	0.8	0.7	4.6	46.2	8.6	97.4	7
12									6.8	74.4	8.7	98.7	12
16									7.9	88.5			16
17					0.8	0.8	0.8	0.7					17
18	0.0	0.0	0.0	0.0									18
20									8.8	100.0			20
23											8.8	100.0	23
25									8.8	100.0			25
26													26
27					0.8	0.8	0.8	1.4					27
42					0.8	1.6	0.8	1.4					42
43													43
45	0.0	0.0	0.0	0.0									45
57					0.9	2.5	0.8	1.4					57
71	0.0	0.0	0.1	0.7									71
82					0.8	1.6	0.9	2.8					82
102					0.9	3.3	1.0	4.2					102
126	0.0	0.0	0.2	3.0									126
135					1.0	4.9	1.2	7.0					135
175					1.0	4.9	1.8	14.7					175
210	0.0	0.0	0.2	3.0									210
217													217
220					1.1	5.7	3.2	35.0					220
258					1.0	4.1	5.0	60.1					258
353	0.0	0.0	0.2	2.2									353
360					1.0	4.9	7.2	90.9					360
458	0.0	0.0	0.3	4.5									458
468					1.2	7.4	7.4	93.0					468
1187	0.1	1.8	0.7	9.7									1187
1192					5.4	77.0	7.8	98.6					1192
1260	0.2	3.6	0.9	13.4									1260
1470	0.2	3.6	1.3	19.4	6.4	92.6	7.9	100.0					1470
1980	0.4	6.4	2.4	35.8	6.9	100.8	7.9	100.7					1980
2580	0.5	9.1	3.6	53.7	6.8	100.0	7.8	99.3					2580
3520	0.7	12.7	5.3	78.2	6.7	98.4							3520
3940	0.8	14.5	5.6	83.5									3940
7720	1.9	34.5	6.5	96.9									7720

A.5.6 Effect of molarity

	CP 3		CP 2		CP 3		CP 2		CP 3		CP 2	
	0.05M		0.05M		0.1M		0.1M		0.2M		0.2M	
time [min]	brix	% of max	brix	% of max	brix	% of max	brix	% of max	brix	% of max	brix	% of max
0	1.1	0.0	1.1	0.0	2.0	0.0	2.0	0.0	3.8	0.0	3.8	0.0
1												
2	2.2	23.4	3.0	37.0	3.3	35.1	4.0	46.0	5.1	47.2	6.1	57.0
3												
4												
5												
6	3.9	41.5	7.9	97.5	6.3	67.0	8.0	92.0	8.6	79.6	10.5	98.1
7												
8												
9												
10	6.9	73.4	8.1	100.0	8.5	90.4	8.7	100.0	10.8	100.0	10.8	100.9
11												
12												
13												
14	9.0	95.7	8.1	100.0	9.1	96.8	8.7	100.0	10.9	100.5		
15											10.7	100.0
16												
17												
18	9.4	100.0			9.5	101.1			10.8	100.0		
19							8.7	100.0				
20												
21												
22	9.4	100.0			9.4	100.0						

A.6 Sugar granules

A.6.1 Eudragit® E content - theoretical (max) value

Product	starting material [g]	spraying solution spent [g]	Eudragit [g]	Eudragit [g/100g]	Eudragit theroetical % [w/w]
CG1	103.0	38	2.3	2.2	5.88
CG2	65	151	9.1	13.9	19.90
CG3	18	89.7	5.4	29.9	39.98

A.6.2 Eudragit® E content - measuring Brix

product	sample ground	product + water [g]	Sugar % (w/w) of total	Brix measured	Eudragit coating % w/w	
CG1	1	4.2657	9.7	9.4	2.8	
	2	4.3757	9.2	8.9	3.1	
					av. =	3.0
CG2	1	8.8988	10.2	9.0	11.2	
	2	8.8358	9.8	8.6	12.1	
					av. =	11.6
CG3	1	7.1036	9.0	6.7	24.8	
	2	7.254	9.2	6.9	24.1	
					av. =	24.4

A.6.3 *In vitro* dissolution rates

	MilliQ water						Phosphate buffer pH 6.5, 0.05M						Citrate buffer pH 3, 0.05M						
	24.8% Eudragit coatin		11.6% Eudragit coatin		3.0% Eudragit coating		24.8% Eudragit coatin		11.6% Eudragit coatin		3.0% Eudragit coating		24.8% Eudragit coatin		11.6% Eudragit coatin		3.0% Eudragit coating		
time [min]	brix	% of max	brix	% of max	brix	% of max	brix	% of max	brix	% of max	brix	% of max	brix	% of max	brix	% of max	brix	% of max	
0	0.0	0.0	0.0	0.0	0.0	0.0	0.8	0.0	0.8	0.0	0.8	0.0	1.1	0.0	1.3	0.0	1.3	0.0	0
1																			1
2							0.8	0.0	1.3	5.6	1.6	10.4	2.6	14.3	4.3	30.3	7.4	87.2	2
3																			3
4																			4
5	0.3	4.4					0.8	0.0					10.3	87.6			7.4	87.2	5
6			1.6	17.8	1.9	23.9									10.6	93.0			6
7									2.8	22.5	7.1	82.2							7
8																			8
10													10.4	88.6					10
14													11.6	100.0					14
15	0.2	2.9																	15
16																			16
17																			17
18			2.6	29.0	4.3	54.0	1.0	2.6	4.6	42.7	8.3	97.9			11.3	100.0	8.3	100.0	18
19																			19
20																			20
27							1.2	5.1											27
28																			28
29									5.9	57.3	8.3	97.9							29
33																			33
37							1.5	9.0											37
45			3.9	43.4	6.5	81.7													45
48							1.8	12.8	7.7	77.5									48
49																			49
50																			50
58.2									8.6	87.6									51
54																			54
55																			55
60																			60
67	0.2	2.9					2.9	26.3											67
71																			71
80.6									9.7	100.0									75
79																			79
82							3.5	34.6											82
85			4.6	51.2	7.2	90.5													85
91																			91
92																			92
97							4.3	44.9											97
102																			102
108									9.7	100.0									108
120							5.3	57.7											120
135	1.4																		135
140																			140
150							6.8	76.9											150
175	0.8	11.8																	175
180																			180
191							7.5	85.9											191
210																			210
217	1.3	19.1																	217
233							8.6	100.0											233
235																			235
258	1.6	23.5																	258
276							9.1												276
295																			295
336							9.4												336
390			7.4	82.4	7.9	99.2													390
430	2.6	38.2																	430
453							9.8	100.0											453
458																			458
468																			468
1147																			1147
1175	5.4	79.4																	1175

A.7 Coating PHZ granules

A.7.1 Coating efficiency

B1 (2.7% PVP)

Subbatch	measured Eudragit % [w/w]	material in the pan [g]	spraying solution [g]	Eudragit [g]	E per material [g/100g]	Eudragit theoretical % [w/w]
B1.1	12.0	24.0	60	3.6	15.0	13.0
B1.2	14.4	18.3	80	4.8	26.2	30.3
B1.3	17.4	15.1	60	3.6	23.8	30.9
B1.4	18.1	11.2	60	3.6	32.1	37.5
B1.5	21.3	7.3	60	3.6	49.3	45.1

B2 (1.3% PVP)

Subbatch	measured Eudragit % [w/w]	material in the pan [g]	spraying solution [g]	Eudragit [g]	E per material [g/100g]	Eudragit theoretical % [w/w]
B2.1	11.0	24.0	130	7.8	32.5	24.5
B2.2	12.2	20.0	60	3.6	18.0	24.6
B2.3	14.8	16.2	68	4.1	25.2	29.9
B2.4	17.2	12.4	62	3.7	30.0	34.5
B2.5	20.8	7	52	3.1	44.6	42.7

B3 (0.7% PVP)

Subbatch	measured Eudragit % [w/w]	material in the pan [g]	spraying solution [g]	Eudragit [g]	E per material [g/100g]	Eudragit theoretical % [w/w]
B3.1	10.0	25.3	60	3.6	14.2	12.5
B3.2	12.7	18.6	80	4.8	25.8	28.5
B3.3	16.5	16.3	60	3.6	22.1	28.5
B3.4	17.6	12.5	65	3.9	31.2	36.4
B3.5	18.6	8.3	66	4.0	47.7	44.2

A.7.2 *In vitro* dissolution rates

A.7.2.1 Small coated PHZ granules in different media

Coated Granules in HCl 0.001M

	product [mg]	medium [g]		concentration product [µg/g]		
	54.2	123.77	437.703	437.703		
time [min]	stock solution[g]	diluted solution [g]	Absorption @ 285 nm calculated	Absorption @ 285 nm measured	concentration PHZ [µg/g]	PHZ released % (w/w) of total
0	0.0000	0.0000	0	0.001	0.0	0.0
60	0.4988	5.7508	0.418	4.819	160.3745	36.6
255	0.5680	5.2630	1.245	11.536	384.2659	87.8
1035	0.3140	6.1340	0.625	12.209	406.7132	92.9
1335	0.5460	6.0340	1.085	11.991	399.4214	91.3
1635	0.3020	6.6301	0.541	11.877	395.6367	
2235	0.3090	6.1150	0.663	13.121	437.0845	99.9

Coated Granules in Phosphate Buffer

0	product [mg]	medium [g]		concentration product [µg/g]		
0	49.4	112.77	437.86	437.86		
0						
time [min]	stock solution[g]	diluted solution [g]	Absorption @ 285 nm calculated	Absorption @ 285 nm measured	concentration PHZ [µg/g]	PHZ released % (w/w) of total
0	0.0000	0.0000	0	0.000	0.0	0.0
60	0.6150	5.1420	0.295	2.466	81.94959	18.7
255	0.1970	3.7650	0.365	6.976	232.2587	53.0
1035	0.4470	6.0870	0.79	10.758	358.3262	81.8
1335	0.8650	6.3230	1.432	10.468	348.6557	79.6
1635	0.3400	6.4560	0.529	10.045	334.5592	
2235	0.4520	6.1760	0.798	10.904	363.1882	82.9

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Coated Granules in Water

		product [mg]	medium [g]	concentration	concentration product [µg/g]		
		49.8	119.35	417.083	417.083		
time [min]	stock solution[g]	diluted solution [g]	Absorption @ 285 nm calculated		Absorption @ 285 nm measured	concentration PHZ [µg/g]	PHZ released % (w/w) of total
0	0.0000	0.0000	0		0.002	0.0	0.0
60	1.1150	6.3380	0.181		1.029	34.02864	8.2
255	0.9120	5.8290	0.742		4.742	157.8151	37.8
1035	0.4690	6.0440	0.722		9.304	309.8803	74.3
1335	0.4530	6.4420	0.684		9.727	323.9664	77.7
1635	0.3070	6.4000	0.427		8.902	296.4543	
2235	0.3920	6.0480	0.669		10.322	343.7905	82.4

PHZ as Powder in Water

		product [mg]	medium [g]	concentration	concentration product [µg/g]		
		49	126.44	387.401			
time [min]	stock solution[g]	diluted solution [g]	Absorption @ 285 nm calculated		Absorption @ 285 nm measured	concentration PHZ [µg/g]	PHZ released % (w/w) of total
0	0.0000	0.0000	0		0.0	0.0	0.0
60	0.1330	6.4200	0.256		12.357	411.6431	106.3
255	0.4360	6.0100	0.87		11.992	399.481	103.1
1035	0.2720	5.9960	0.547		12.058	401.6711	103.7

A.7.2.2 Larger coated PHZ granules in phosphate buffer.

time [min]	material	weight vial [g]	weight totla [g]	weight medium [g]	weight material [mg]	weight solution taken for analysis [g]	weight after dilution [g]	Absortption diluted sample @ UV285	Absorption original solution @ UV285	conc. PHZ [µg/g]	conc PHZ theor. [µg/g]	% PHZ released
15												
	PHZ Powder	147.572	256.688	109.116	60.1	0.698	14.388	0.645	13.296	464.521	550.487	84.4
	B1.1	163.039	271.732	108.693	84.2	0.91	14.872	0.127	2.076	72.215	774.059	9.3
	B2.1	158.108	266.019	107.911	80.5	0.937	14.67	0.106	1.660	57.670	745.429	7.7
	B3.1	159.418	262.371	102.953	68.4	1.014	14.467	0.291	4.152	144.810	663.940	21.8
	B1.2	159.325	265.162	105.837	75.2	1.033	14.983	0.109	1.581	54.922	710.022	7.7
	B2.2	160.566	264.276	103.71	70.9	1.352	14.555	0.116	1.249	43.308	683.170	6.3
	B3.2	159.332	267.118	107.786	67.6	1.836	15.021	0.16	1.309	45.413	626.776	7.2
	B1.3	159.309	265.816	106.507	82.2	1.555	4.7357	0.461	1.404	48.733	771.185	6.3
	B2.3	157.872	263.373	105.501	79.1	1.2751	4.8672	0.329	1.256	43.553	749.194	5.8
	B3.3	162.088	265.558	103.47	77.3	1.3027	5.0672	0.509	1.980	68.870	746.519	9.2
	B1.4	160.534	263.258	102.724	88.5	0.9312	3.9451	0.418	1.771	61.563	860.790	7.2
	B2.4	160.082	263.17	103.088	78.6	1.0257	4.134	0.342	1.378	47.839	761.874	6.3
	B3.4 (1)	158.734	265.181	106.447	61.1	1.2948	3.9847	0.481	1.480	51.401	573.665	9.0
	B3.4 (2)	164.059	264.074	100.015	62.1	1.1189	4.0498	0.428	1.549	53.809	620.522	8.7
	B3.4 (3)	159.022	260.921	101.899	59.7	1.1213	3.8593	0.437	1.504	52.233	585.531	8.9
	B1.5	158.869	262.245	103.376	72	1.0769	4.0138	0.294	1.096	37.958	696.002	5.5
	B2.5	158.919	258.09	99.171	78.7	1.2492	4.0383	0.438	1.416	49.151	792.950	6.2
	B3.5	160.445	263.046	102.601	77.6	1.0063	4.7546	0.414	1.956	68.038	755.756	9.0
45												
	PHZ Powder	147.572	256.688	109.116	60.1	0.349	14.489	0.381	15.818	552.703	550.487	100.4
	B1.1	163.039	271.732	108.693	84.2	1.046	12.431	0.364	4.326	150.898	774.059	19.5
	B2.1	158.108	266.019	107.911	80.5	1.14	11.988	0.234	2.461	85.682	745.429	11.5
	B3.1	159.418	262.371	102.953	68.4	1.122	12.848	0.597	6.836	238.673	663.940	35.9
	B1.2	159.325	265.162	105.837	75.2	0.916	12.669	0.249	3.444	120.058	710.022	16.9
	B2.2	160.566	264.276	103.71	70.9	1.111	11.645	0.333	3.490	121.684	683.170	17.8
	B3.2	159.332	267.118	107.786	67.6	1.162	11.239	0.273	2.640	91.968	626.776	14.7
	B1.3	159.309	265.816	106.507	82.2	0.918	12.165	0.248	3.286	114.553	771.185	14.9
	B2.3	157.872	263.373	105.501	79.1	1.031	11.811	0.217	2.486	86.564	749.194	11.6
	B3.3	162.088	265.558	103.47	77.3	0.978	10.923	0.318	3.552	123.827	746.519	16.6
	B1.4	160.534	263.258	102.724	88.5	1.08	11.409	0.319	3.370	117.471	860.790	13.6
	B2.4	160.082	263.17	103.088	78.6	1.185	8.184	0.346	2.390	83.195	761.874	10.9
	B3.4 (1)	158.734	265.181	106.447	61.1	1.074	8.855	0.27	2.226	77.480	573.665	13.5
	B3.4 (2)	164.059	264.074	100.015	62.1	1.185	8.651	0.35	2.555	88.984	620.522	14.3
	B3.4 (3)	159.022	260.921	101.899	59.7	1.263	9.144	0.335	2.425	84.446	585.531	14.4
	B1.5	158.869	262.245	103.376	72	1.327	8.221	0.369	2.286	79.574	696.002	11.4
	B2.5	158.919	258.09	99.171	78.7	1.074	8.341	0.304	2.361	82.194	792.950	10.4
	B3.5	160.445	263.046	102.601	77.6	0.986	8.349	0.34	2.879	100.306	755.756	13.3
116												
	PHZ Powder	147.572	256.688	109.116	60.1	0.427	14.561	0.464	15.823	552.897	550.487	100.4
	B1.1	163.039	271.732	108.693	84.2	1.064	14.689	0.65	8.974	313.404	774.059	40.5
	B2.1	158.108	266.019	107.911	80.5	1.282	14.438	0.612	6.892	240.636	745.429	32.3
	B3.1	159.418	262.371	102.953	68.4	1.178	14.734	0.949	11.870	414.670	663.940	62.5
	B1.2	159.325	265.162	105.837	75.2	1.179	14.636	0.467	5.797	202.346	710.022	28.5
	B2.2	160.566	264.276	103.71	70.9	1.169	15.379	0.33	4.341	151.440	683.170	22.2
	B3.2	159.332	267.118	107.786	67.6	1.172	14.87	0.408	5.177	180.643	626.776	28.8
	B1.3	159.309	265.816	106.507	82.2	1.291	14.717	0.534	6.087	212.491	771.185	27.6
	B2.3	157.872	263.373	105.501	79.1	1.174	13.691	0.4	4.665	162.746	749.194	21.7
	B3.3	162.088	265.558	103.47	77.3	1.279	15.134	0.588	6.958	242.917	746.519	32.5
	B1.4	160.534	263.258	102.724	88.5	1.005	10.156	0.655	6.619	231.080	860.790	26.8
	B2.4	160.082	263.17	103.088	78.6	1.228	10.201	0.604	5.017	175.078	761.874	23.0
	B3.4 (1)	158.734	265.181	106.447	61.1	1.075	10.605	0.447	4.410	153.829	573.665	26.8
	B3.4 (2)	164.059	264.074	100.015	62.1	1.041	14.409	0.366	5.066	176.776	620.522	28.5
	B3.4 (3)	159.022	260.921	101.899	59.7	1.084	10.358	0.467	4.462	155.670	585.531	26.6
	B1.5	158.869	262.245	103.376	72	1.012	14.47	0.373	5.333	186.123	696.002	26.7
	B2.5	158.919	258.09	99.171	78.7	1.272	11.404	0.56	5.021	175.190	792.950	22.1
	B3.5	160.445	263.046	102.601	77.6	0.985	10.075	0.564	5.769	201.351	755.756	26.6

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time [min]	material	weight vial [g]	weight totla [g]	weight medium [g]	weight material [mg]	weight solution taken for analysis [g]	weight after dilution [g]	Absortption diluted sample @ UV285	Absorption original solution @ UV285	conc. PHZ [µg/g]	conc PHZ theor. [µg/g]	% PHZ released
176	PHZ Powder	147.572	256.688	109.116	60.1	0.761	13.795	0.9	16.315	570.088	550.487	103.6
	B1.1	163.039	271.732	108.693	84.2	0.787	14.032	0.601	10.716	374.317	774.059	48.4
	B2.1	158.108	266.019	107.911	80.5	0.888	14.35	0.569	9.195	321.146	745.429	43.1
	B3.1	159.418	262.371	102.953	68.4	0.989	10.606	1.314	14.091	492.346	663.940	74.2
	B1.2	159.325	265.162	105.837	75.2	0.965	11.023	0.656	7.493	261.649	710.022	36.9
	B2.2	160.566	264.276	103.71	70.9	0.895	10.164	0.524	5.951	207.712	683.170	30.4
	B3.2	159.332	267.118	107.786	67.6	1.067	10.384	0.729	7.095	247.706	626.776	39.5
	B1.3	159.309	265.816	106.507	82.2	0.69	9.815	0.512	7.283	254.294	771.185	33.0
	B2.3	157.872	263.373	105.501	79.1	0.739	10.288	0.443	6.167	215.281	749.194	28.7
	B3.3	162.088	265.558	103.47	77.3	0.501	9.902	0.472	9.329	325.826	746.519	43.6
	B1.4	160.534	263.258	102.724	88.5	0.461	10.231	0.403	8.944	312.364	860.790	36.3
	B2.4	160.082	263.17	103.088	78.6	0.937	10.596	0.59	6.672	232.929	761.874	30.6
	B3.4 (1)	158.734	265.181	106.447	61.1	0.867	10.454	0.508	6.125	213.815	573.665	37.3
	B3.4 (2)	164.059	264.074	100.015	62.1	0.934	9.958	0.628	6.696	233.753	620.522	37.7
	B3.4 (3)	159.022	260.921	101.899	59.7	0.888	10.331	0.51	5.933	207.103	585.531	35.4
	B1.5	158.869	262.245	103.376	72	1.322	10.351	0.888	6.953	242.751	696.002	34.9
	B2.5	158.919	258.09	99.171	78.7	1.028	11.02	0.579	6.207	216.664	792.950	27.3
	B3.5	160.445	263.046	102.601	77.6	1.061	10.649	0.843	8.461	295.482	755.756	39.1
311												
	PHZ Powder	147.572	256.688	109.116	60.1	0.581	14.814	0.637	16.242	567.540	550.487	103.1
	B1.1	163.039	271.732	108.693	84.2	0.685	14.664	0.731	15.649	546.802	774.059	70.6
	B2.1	158.108	266.019	107.911	80.5	0.823	15.165	0.744	13.709	478.990	745.429	64.3
	B3.1	159.418	262.371	102.953	68.4	0.752	14.524	0.851	16.436	574.331	663.940	86.5
	B1.2	159.325	265.162	105.837	75.2	0.759	14.592	0.579	11.131	388.855	710.022	54.8
	B2.2	160.566	264.276	103.71	70.9	0.713	14.418	0.468	9.464	330.542	683.170	48.4
	B3.2	159.332	267.118	107.786	67.6	0.814	14.774	0.603	10.944	382.314	626.776	61.0
	B1.3	159.309	265.816	106.507	82.2	0.957	10.092	1.116	11.769	411.137	771.185	53.3
	B2.3	157.872	263.373	105.501	79.1	1.187	12.145	0.903	9.239	322.692	749.194	43.1
	B3.3	162.088	265.558	103.47	77.3	1.047	10.561	1.373	13.849	483.886	746.519	64.8
	B1.4	160.534	263.258	102.724	88.5	0.764	10.398	0.945	12.861	449.343	860.790	52.2
	B2.4	160.082	263.17	103.088	78.6	1.059	10.302	1.041	10.127	353.731	761.874	46.4
	B3.4 (1)	158.734	265.181	106.447	61.1	0.978	10.816	0.866	9.577	334.516	573.665	58.3
	B3.4 (2)	164.059	264.074	100.015	62.1	0.989	11.688	0.883	10.435	364.514	620.522	58.7
	B3.4 (3)	159.022	260.921	101.899	59.7	1.063	10.371	0.949	9.259	323.377	585.531	55.2
	B1.5	158.869	262.245	103.376	72	1.054	11.289	0.974	10.432	364.404	696.002	52.4
	B2.5	158.919	258.09	99.171	78.7	0.989	10.817	0.877	9.592	335.029	792.950	42.3
	B3.5	160.445	263.046	102.601	77.6	1.101	11.128	1.187	11.997	419.126	755.756	55.5
1020												
	PHZ Powder	147.572	256.688	109.116	60.1	0.412	9.29	0.758	17.092	597.259	550.487	108.5
	B1.1	163.039	271.732	108.693	84.2	0.316	9.77	0.659	20.375	712.048	774.059	92.0
	B2.1	158.108	266.019	107.911	80.5	0.504	10.279	0.925	18.865	659.267	745.429	88.4
	B3.1	159.418	262.371	102.953	68.4	0.659	9.862	1.173	17.554	613.422	663.940	92.4
	B1.2	159.325	265.162	105.837	75.2	0.45	10.509	0.741	17.305	604.707	710.022	85.2
	B2.2	160.566	264.276	103.71	70.9	0.512	10.363	0.824	16.678	582.789	683.170	85.3
	B3.2	159.332	267.118	107.786	67.6	0.548	10.351	0.835	15.772	551.114	626.776	87.9
	B1.3	159.309	265.816	106.507	82.2	0.3634	10.426	0.648	18.591	649.692	771.185	84.2
	B2.3	157.872	263.373	105.501	79.1	0.3938	9.864	0.699	17.509	611.837	749.194	81.7
	B3.3	162.088	265.558	103.47	77.3	0.4109	9.9049	0.748	18.031	630.092	746.519	84.4
	B1.4	160.534	263.258	102.724	88.5	0.6932	10.327	1.301	19.381	677.307	860.790	78.7
	B2.4	160.082	263.17	103.088	78.6	0.5897	10.12	1.022	17.539	612.889	761.874	80.4
	B3.4 (1)	158.734	265.181	106.447	61.1	0.5926	9.9803	0.796	13.406	468.380	573.665	81.6
	B3.4 (2)	164.059	264.074	100.015	62.1	0.4849	11.526	0.634	15.071	526.588	620.522	84.9
	B3.4 (3)	159.022	260.921	101.899	59.7	0.5248	9.8392	0.737	13.818	482.777	585.531	82.5
	B1.5	158.869	262.245	103.376	72	0.5789	10.031	0.905	15.682	547.955	696.002	78.7
	B2.5	158.919	258.09	99.171	78.7	0.4984	9.9754	0.888	17.773	621.083	792.950	78.3
	B3.5	160.445	263.046	102.601	77.6	0.4871	9.921	0.822	16.742	585.030	755.756	77.4

2940

A.7.2.3 Larger coated PHZ granules in HCl

product	time [min]	medium [g]	product [mg]	sample [g]	diluted sample [g]	Absorbance @ 285 nm measured	Absorbance @ 285 nm calculated	concentartion PHZ dissolved [µg/g]	concentartion PHZ total [µg/g]	% PHZ released
PHZ	0	0.000	0.0	0.000	0.000	0.001	0.000	0.000	0.000	0.0
powder	15	93.636	72.7	0.580	9.738	1.329	22.313	756.364	775.808	97.5
	64	93.636	72.7	0.435	9.936	1.057	24.143	818.395	775.808	
	128	93.636	72.7	0.469	9.738	1.106	22.964	778.425	775.808	100.3
	281	93.636	72.7	0.412	10.271	0.912	22.736	770.682	775.808	99.3
B1.1	0	0.000	0.0	0.000	0.000	0.000	0.000	0.000	0.000	0.0
	15	99.167	83.5	0.634	8.988	0.439	6.224	210.944	841.306	25.1
	64	99.167	83.5	0.493	10.420	0.582	12.301	416.963	841.306	49.6
	128	99.167	83.5	0.559	10.026	1.073	19.245	652.346	841.306	77.5
	281	99.167	83.5	0.685	13.051	1.119	21.320	722.682	841.306	85.9
B1.2	0	0.000	0.0	0.000	0.000	0.001	0.000	0.000	0.000	0.0
	15	97.455	89.7	0.507	9.787	0.231	4.459	151.134	919.578	16.4
	64	97.455	89.7	0.435	9.819	0.836	18.871	639.656	919.578	
	128	97.455	89.7	0.586	9.753	1.076	17.908	607.035	919.578	66.0
	281	97.455	89.7	0.630	13.735	1.063	23.175	785.572	919.578	85.4
B1.3	0	0.000	0.0	0.000	0.000	0.000	0.000	0.000	0.000	0.0
	15	100.481	84.9	0.665	9.949	0.241	3.606	122.199	844.223	14.5
	64	100.481	84.9	0.485	10.003	0.455	9.384	318.087	844.223	37.7
	128	100.481	84.9	0.614	9.847	0.976	15.653	530.572	844.223	62.8
	281	100.481	84.9	0.607	12.515	1.010	20.824	705.874	844.223	83.6
B1.4	0	0.000	0.0	0.000	0.000	0.002	0.000	0.000	0.000	0.0
	15	102.271	81.0	0.570	10.158	0.095	1.693	57.366	791.387	7.2
	64	102.271	81.0	0.499	9.778	0.411	8.054	272.980	791.387	34.5
	128	102.271	81.0	0.713	10.075	0.990	13.989	474.184	791.387	59.9
	281	102.271	81.0	0.499	12.485	0.739	18.490	626.749	791.387	79.2

A.8 PHZ granules for in vivo trials

A.8.1 Coating efficiency

total material in the pan [g]	total weight increase [g]	material loss [g]	recycled lost material [g]	sprayin solution applied [g]	Eudragit applied [g]
170.1	0.0	0.0	0.0	0.0	0.0
171.8	1.7	0.6		53.0	3.2
174.0	3.9	0.5		44.0	5.8
176.1	6.0	1.2		62.2	9.6
178.7	8.6	0.8		56.6	12.9
181.3	11.2	0.8	0.4	67.7	17.0
183.6	13.5			58.7	20.5
184.6	14.5			31.1	22.4
188.2	18.1			63.1	26.2
190.2	20.1	1.2	0.5	39.2	28.5
192.5	22.4			72.6	32.9
196.8	26.7	0.6		90.7	38.3
201.0	30.9	0.5		99.0	44.3
	total [g]	6.2	0.9		

		weight increase [% w/w]
total weight increase [g]	30.9	15.4
total weight increase considering recycled material [g]	36.2	17.5

A.8.2 Measuring PHZ content

Uncoated PHZ granules 0.7-1.0mm

Nr Measurement Vial ID	product [g]	product + water [ml]	conc product [µg/mL]	sample [g]	sample diluted [g]	A @ 285 nm mesured	A @ 285 nm calculated	conc PHZ [µg/mL]	conc. PHZ [% w/w]
1	0.196	500	392	0.767	15.501	0.555	11.216	373.617	95.4
2	0.139	500	278	0.759	15.701	0.385	7.964	265.209	95.4
3	0.199	500	398	0.785	15.420	0.591	11.609	386.707	97.1
4	0.196	500	392	0.819	15.146	0.610	11.281	375.763	96.0
								average=	**96.0**
								st. dev.=	**0.8**

Coated PHZ granules 0.7-1.0mm

Nr Measurement Vial ID	product [g]	product + water [ml]	conc product [µg/mL]	sample [g]	sample diluted [g]	A @ 285 nm mesured	A @ 285 nm calculated	conc PHZ [µg/mL]	conc. PHZ [% w/w]
1	0.207	500	415	0.750	14.790	0.470	9.268	308.680	74.4
2	0.197	500	394	0.828	14.789	0.499	8.913	296.823	75.3
3	0.198	500	396	0.854	15.230	0.476	8.489	282.695	71.4
4	0.202	500	404	0.807	15.169	0.465	8.741	291.083	72.1
								average=	**73.2**
								st. dev.=	**1.8**

A.8.3 *In vitro* dissolution rates

A.8.3.1 Phosphate buffer (0.05M, pH 6.5) - 100 mL-vials

time [h]	sample	product [mg]	medium [g]	sample [g]	diluted sample [g]	A @ 285 nm	A @ 285 nm calculated	conc PHZ measured[µg/g]	conc PHZ calculated [µg/g]	PHZ released [% w/w]
0.5										
	1	85.3	996.6	1.54	6.277	0.06	0.245	8.734	85.584	10.2
	2	82	996.1	1.427	6.209	0.065	0.283	10.101	82.314	12.3
	3	78.4	990.1	1.319	6.741	0.044	0.225	8.031	79.178	10.1
1.38										
	1	85.3	996.6	1.357	3.205	0.252	0.595	21.256	85.584	24.8
	2	82	996.1	1.224	3.685	0.183	0.551	19.677	82.314	23.9
	3	78.4	990.1	1.429	3.493	0.22	0.538	19.206	79.178	24.3
2.63										
	1	85.3	996.6	1.151	5.126	0.208	0.926	33.083	85.584	38.7
	2	82	996.1	1.325	6.284	0.179	0.849	30.319	82.314	36.8
	3	78.4	990.1	1.321	6.214	0.175	0.823	29.400	79.178	37.1
4										
	1	85.3	996.6	1.242	6.049	0.246	1.198	41.535	85.584	48.5
	2	82	996.1	1.43	5.801	0.276	1.120	38.791	82.314	47.1
	3	78.4	990.1	1.056	5.589	0.207	1.096	37.950	79.178	47.9
7										
	1	85.3	996.6	1.336	6.605	0.321	1.587	56.678	85.584	66.2
	2	82	996.1	1.31	5.518	0.352	1.483	52.954	82.314	64.3
	3	78.4	990.1	1.352	5.869	0.326	1.415	50.541	79.178	63.8
20										
	1	85.3	996.6	1.041	6.831	0.284	1.864	66.557	85.584	77.8
	2	82	996.1	1.381	6.08	0.397	1.748	62.423	82.314	75.8
	3	78.4	990.1	1.173	6.751	0.295	1.698	60.636	79.178	76.6
30										
	1	85.3	996.6	1.173	6.849	0.306	1.787	63.811	85.584	74.6
	2	82	996.1	1.331	6.968	0.335	1.754	62.635	82.314	76.1
	3	78.4	990.1	1.185	12.212	0.165	1.700	60.729	79.178	76.7
50										
	1	85.3	996.6	1.25	7.266	0.308	1.790	63.941	85.584	74.7
	2	82	996.1	1.146	6.757	0.299	1.763	62.963	82.314	76.5
	3	78.4	990.1	1.157	6.546	0.298	1.686	60.214	79.178	76.0
75										
	1	85.3	996.6	5	5	1.78	1.780	63.571	85.584	74.3
	2	82	996.1	5	5	1.754	1.754	62.643	82.314	76.1
	3	78.4	990.1	5	5	1.678	1.678	59.929	79.178	75.7

A.8.3.2 Phosphate buffer (0.05M, pH 6.5) - 1000 mL-vials

time [h]	sample	product [mg]	medium [g]	sample [g]	diluted sample [g]	A @ 285 nm	A @ 285 nm calculated	conc PHZ measured[µg/g]	conc PHZ calculated [µg/g]	PHZ released [% w/w]
0.5										
	1	85.3	996.6	1.54	6.277	0.06	0.245	8.73423	85.58368	10.2
	2	82	996.1	1.427	6.209	0.065	0.283	10.10074	82.31428	12.3
	3	78.4	990.1	1.319	6.741	0.044	0.225	8.031084	79.17765	10.1
1.38										
	1	85.3	996.6	1.357	3.205	0.252	0.595	21.25645	85.58368	24.8
	2	82	996.1	1.224	3.685	0.183	0.551	19.67656	82.31428	23.9
	3	78.4	990.1	1.429	3.493	0.22	0.538	19.20574	79.17765	24.3
2.63										
	1	85.3	996.6	1.151	5.126	0.208	0.926	33.08328	85.58368	38.7
	2	82	996.1	1.325	6.284	0.179	0.849	30.31903	82.31428	36.8
	3	78.4	990.1	1.321	6.214	0.175	0.823	29.40008	79.17765	37.1
4										
	1	85.3	996.6	1.242	6.049	0.246	1.198	41.53535	85.58368	48.5
	2	82	996.1	1.43	5.801	0.276	1.120	38.79138	82.31428	47.1
	3	78.4	990.1	1.056	5.589	0.207	1.096	37.95004	79.17765	47.9
7										
	1	85.3	996.6	1.336	6.605	0.321	1.587	56.67785	85.58368	66.2
	2	82	996.1	1.31	5.518	0.352	1.483	52.95354	82.31428	64.3
	3	78.4	990.1	1.352	5.869	0.326	1.415	50.54137	79.17765	63.8
20										
	1	85.3	996.6	1.041	6.831	0.284	1.864	66.55702	85.58368	77.8
	2	82	996.1	1.381	6.08	0.397	1.748	62.42268	82.31428	75.8
	3	78.4	990.1	1.173	6.751	0.295	1.698	60.63649	79.17765	76.6
30										
	1	85.3	996.6	1.173	6.849	0.306	1.787	63.81056	85.58368	74.6
	2	82	996.1	1.331	6.968	0.335	1.754	62.63497	82.31428	76.1
	3	78.4	990.1	1.185	12.212	0.165	1.700	60.72875	79.17765	76.7
50										
	1	85.3	996.6	1.25	7.266	0.308	1.790	63.9408	85.58368	74.7
	2	82	996.1	1.146	6.757	0.299	1.763	62.96257	82.31428	76.5
	3	78.4	990.1	1.157	6.546	0.298	1.686	60.21447	79.17765	76.0

A.8.3.3 HCl (10^{-3} M) - 100 ml vials

time [h]	sample	product [mg]	medium [g]	sample [g]	diluted sample [g]	A @ 285 nm	A @ 285 nm calculated	conc PHZ measured[µg/g]	conc PHZ calculated [µg/g]	PHZ released [% w/w]
0.25										
	1	77.7	111.687	0.701	7.254	0.395	4.087	138.3217	695.2106	19.90
	2	78.6	108.018	0.893	7.688	0.503	4.330	146.5566	727.1274	20.16
	3	81.6	105.083	0.937	8.055	0.595	5.115	173.1515	775.9265	22.32
0.75										
	1	77.7	111.687	0.723	9.849	0.733	9.985	338.2449	695.2106	48.65
	2	78.6	108.018	0.72	10.101	0.729	10.227	346.4496	727.1274	47.65
	3	81.6	105.083	0.74	10.081	0.881	12.002	406.6047	775.9265	52.40
1.5										
	1	77.7	111.687	0.615	9.931	0.889	14.356	486.3913	695.2106	69.96
	2	78.6	108.018	0.609	10.081	0.902	14.931	505.9029	727.1274	69.58
	3	81.6	105.083	0.703	10.328	1.122	16.484	558.531	775.9265	71.98
2										
	1	77.7	111.687	0.675	10.026	1.053	15.641	529.9512	695.2106	76.23
	2	78.6	108.018	0.896	10.342	1.403	16.194	548.7119	727.1274	75.46
	3	81.6	105.083	0.794	11.19	1.271	17.912	606.9646	775.9265	78.22
3										
	1	77.7	111.687	0.807	10.573	1.285	16.836	570.46	695.2106	82.06
	2	78.6	108.018	0.724	10.627	1.2	17.614	596.8411	727.1274	82.08
	3	81.6	105.083	0.568	10.793	0.993	18.869	639.3813	775.9265	82.40
4										
	1	77.7	111.687	0.547	12.074	0.779	17.195	582.6429	695.2106	83.81
	2	78.6	108.018	0.68	12.287	0.996	17.997	609.8251	727.1274	83.87
	3	81.6	105.083	0.609	12.662	0.919	19.107	647.4696	775.9265	83.44
6										
	1	77.7	111.687	0.478	10.518	0.787	17.317	586.7896	695.2106	84.40
	2	78.6	108.018	0.468	11.687	0.727	18.155	615.1798	727.1274	84.60
	3	81.6	105.083	0.403	11.608	0.673	19.385	656.8838	775.9265	84.66

A.9 Active rumen technology (ART)

Device numbering:
1-5: 15% bioactive payload
6-10: 10% bioactive payload
11-15: 5% bioactive payload
16-20: 0% bioactive payload

time [h]	***0.00***					***time [h]***	***3.00***			
Device	side 1	side 2	average	weight of the device [g]	volume expelled [ml]	Device	side 1	side 2	average	volume expelled [ml]
1	27.82	27.82	27.82	23.181	0.00	1	28.22	28.35	28.29	0.08
2	28.54	28.54	28.54	23.416	0.00	2	29.55	29.54	29.55	0.17
3	27.74	27.78	27.76	23.539	0.00	3	28.21	28.22	28.22	0.08
4	27.89	27.95	27.92	23.549	0.00	4	28.33	28.59	28.46	0.09
5	27.94	28.06	28.00	23.740	0.00	5	28.37	28.51	28.44	0.07
6	27.58	27.54	27.56	23.821	0.00	6	28.20	28.27	28.24	0.11
7	27.75	27.77	27.76	23.603	0.00	7	28.42	28.39	28.41	0.11
8	27.85	28.08	27.97	23.482	0.00	8	28.24	28.35	28.30	0.05
9	27.94	27.98	27.96	23.341	0.00	9	28.24	28.24	28.24	0.05
10	27.65	27.88	27.77	23.569	0.00	10	28.12	28.27	28.20	0.07
11	27.93	28.08	28.01	23.483	0.00	11	28.74	28.88	28.81	0.13
12	28.19	28.01	28.10	23.448	0.00	12	28.67	28.56	28.62	0.08
13	28.35	28.37	28.36	23.486	0.00	13	29.02	28.91	28.97	0.10
14	28.12	27.97	28.05	23.692	0.00	14	28.59	28.77	28.68	0.10
15	28.33	28.13	28.23	23.521	0.00	15	28.77	28.80	28.79	0.09
16	27.94	28.14	28.04	23.705	0.00	16	28.75	28.53	28.64	0.10
17	28.23	28.35	28.29	23.788	0.00	17	28.69	28.70	28.70	0.07
18	27.55	27.68	27.62	23.708	0.00	18	27.80	27.79	27.80	0.03
19	27.95	28.26	28.11	23.750	0.00	19	28.45	28.53	28.49	0.06
20	28.36	28.48	28.42	23.636	0.00	20	28.34	29.22	28.78	0.06

Continued next page…

time [h]	***7.50***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	30.47	30.52	30.50	0.44	22.756	0.425	2.469	2.524	0.055
2	31.70	31.75	31.73	0.53	22.921	0.495	2.470	2.533	0.063
3	30.49	30.81	30.65	0.48	23.100	0.439	2.435	2.495	0.060
4	30.60	30.69	30.65	0.45	23.106	0.443	2.433	2.514	0.081
5	30.47	30.52	30.50	0.41	23.368	0.372	2.472	2.513	0.041
6	30.34	30.45	30.40	0.47	23.369	0.452	2.432	2.474	0.042
7	30.71	30.66	30.69	0.48	23.134	0.469	2.436	2.490	0.054
8	30.81	30.59	30.70	0.45	23.073	0.409	2.431	2.470	0.039
9	30.43	30.31	30.37	0.40	22.950	0.391	2.445	2.482	0.037
10	30.67	30.80	30.74	0.49	23.121	0.448	2.438	2.488	0.050
11	30.97	31.22	31.10	0.51	22.987	0.496	2.441	2.485	0.044
12	30.83	30.70	30.77	0.44	23.040	0.408	2.937	2.967	0.030
13	31.32	31.04	31.18	0.47	23.017	0.469	2.442	2.481	0.039
14	31.13	31.22	31.18	0.52	23.179	0.513	2.435	2.486	0.051
15	30.90	30.85	30.88	0.44	23.093	0.428	2.435	2.474	0.039
16	31.13	30.96	31.05	0.50	23.247	0.458	2.416	2.445	0.029
17	31.07	31.00	31.04	0.45	23.325	0.463	2.432	2.461	0.029
18	30.18	30.43	30.31	0.44	23.258	0.450	2.474	2.501	0.027
19	30.80	31.05	30.93	0.47	23.279	0.471	2.441	2.470	0.029
20	31.64	31.60	31.62	0.53	23.105	0.531	2.437	2.470	0.033

time [h]	***26.25***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	34.22	34.3	34.26	1.06	22.167	1.014	2.438	2.518	0.080
2	35.62	35.58	35.6	1.16	22.297	1.119	2.447	2.526	0.079
3	34.5	34.57	34.535	1.12	22.484	1.055	2.441	2.535	0.094
4	34.55	34.69	34.62	1.10	22.483	1.066	2.956	3.047	0.091
5	34.19	34.13	34.16	1.02	22.799	0.941	2.473	2.555	0.082
6	34.29	34.31	34.3	1.11	22.741	1.08	2.466	2.546	0.080
7	34.29	33.99	34.14	1.05	22.562	1.041	2.443	2.511	0.068
8	34.36	34.69	34.525	1.08	22.469	1.013	2.432	2.508	0.076
9	34.01	34.04	34.025	1.00	22.363	0.978	2.957	3.024	0.067
10	34.76	34.72	34.74	1.15	22.506	1.063	2.441	2.511	0.070
11	35.03	34.94	34.985	1.15	22.378	1.105	2.445	2.499	0.054
12	35.22	34.95	35.085	1.15	22.391	1.057	2.444	2.507	0.063
13	35.01	35.03	35.02	1.10	22.426	1.06	2.437	2.495	0.058
14	35.05	35.07	35.06	1.16	22.57	1.122	2.445	2.498	0.053
15	35.13	34.79	34.96	1.11	22.476	1.045	2.442	2.497	0.055
16	35.15	34.81	34.98	1.14	22.623	1.082	2.444	2.483	0.039
17	35.14	34.79	34.965	1.10	22.71	1.078	2.432	2.47	0.038
18	34.26	34.22	34.24	1.09	22.624	1.084	2.436	2.475	0.039
19	34.53	34.57	34.55	1.06	22.686	1.064	2.441	2.478	0.037
20	35.55	35.54	35.545	1.17	22.487	1.149	2.444	2.481	0.037

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time [h]	***46.25***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	38.34	38.11	38.23	1.72	21.516	1.665	2.44	2.549	0.109
2	39.62	39.63	39.63	1.83	21.651	1.765	2.436	2.515	0.079
3	38.72	38.59	38.66	1.80	21.787	1.752	2.414	2.503	0.089
4	38.56	38.58	38.57	1.76	21.802	1.747	2.437	2.537	0.100
5	38.09	38.27	38.18	1.68	22.134	1.606	2.419	2.509	0.090
6	38.25	38.33	38.29	1.77	22.061	1.76	2.472	2.556	0.084
7	37.89	37.98	37.94	1.68	21.928	1.675	2.431	2.506	0.075
8	38.75	38.58	38.67	1.76	21.811	1.671	2.414	2.56	0.146
9	38.29	38.27	38.28	1.70	21.68	1.661	2.471	2.552	0.081
10	38.54	38.79	38.67	1.80	21.804	1.765	2.44	2.536	0.096
11	38.9	38.95	38.93	1.80	21.71	1.773	2.398	2.456	0.058
12	39.29	39.09	39.19	1.83	21.68	1.768	2.438	2.504	0.066
13	38.78	38.97	38.88	1.73	21.744	1.742	2.434	2.492	0.058
14	38.79	39.09	38.94	1.80	21.891	1.801	2.448	2.52	0.072
15	38.9	38.94	38.92	1.76	21.781	1.74	2.445	2.505	0.060
16	39.01	39.31	39.16	1.83	21.942	1.763	2.45	2.491	0.041
17	39.24	39.25	39.25	1.81	22.021	1.767	2.957	2.999	0.042
18	38.62	38.63	38.63	1.82	21.923	1.785	2.474	2.5189	0.045
19	38.6	38.43	38.52	1.72	22.044	1.706	2.419	2.457	0.038
20	39.64	39.65	39.65	1.85	21.817	1.819	2.47	2.51	0.040

time [h]	***58.25***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	44.73	44.66	44.70	2.78	20.413	2.768	2.448	2.613	0.165
2	46.11	46.35	46.23	2.92	20.607	2.809	2.447	2.6	0.153
3	45.65	45.84	45.75	2.97	20.6	2.939	2.434	2.596	0.162
4	45.72	45.4	45.56	2.91	20.695	2.854	2.954	3.065	0.111
5	45.12	44.89	45.01	2.80	21.03	2.71	2.442	2.544	0.102
6	45.81	45.59	45.70	2.99	20.874	2.947	2.413	2.568	0.155
7	44.51	44.52	44.52	2.76	20.829	2.774	2.958	3.08	0.122
8	45.19	45.22	45.21	2.84	20.703	2.779	2.936	3.065	0.129
9	44.87	45.32	45.10	2.83	20.536	2.805	2.955	3.104	0.149
10	46.02	46.15	46.09	3.02	20.603	2.966	2.953	3.122	0.169
11	45.96	46.11	46.04	2.97	20.518	2.965	2.442	2.551	0.109
12	46.53	46.52	46.53	3.04	20.456	2.992	2.922	3.08	0.158
13	45.88	45.94	45.91	2.89	20.63	2.856	2.448	2.547	0.099
14	45.52	45.9	45.71	2.91	20.805	2.887	2.419	2.528	0.109
15	46.25	46.41	46.33	2.98	20.594	2.927	2.439	2.564	0.125
16	46.4	46.43	46.42	3.03	20.732	2.973	2.43	2.503	0.073
17	46.65	46.57	46.61	3.02	20.801	2.987	2.927	3.03	0.103
18	46.08	46.07	46.08	3.04	20.689	3.019	2.45	2.528	0.078
19	44.98	45.5	45.24	2.83	20.883	2.867	2.445	2.604	0.159
20	46.94	47.25	47.10	3.08	20.598	3.038	2.442	2.521	0.079

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time [h]	***58.25***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	48.11	48.59	48.35	3.39	19.784	3.397	2.954	3.027	0.073
2	49.69	49.76	49.73	3.49	20.017	3.399	2.415	2.482	0.067
3	49.66	49.68	49.67	3.61	19.959	3.58	2.432	2.494	0.062
4	19.32	49.2	34.26	1.05	20.051	3.498	2.445	2.502	0.057
5	48.82	48.83	48.83	3.43	20.401	3.339	2.952	2.999	0.047
6	49.17	49.26	49.22	3.57	20.231	3.59	2.436	2.501	0.065
7	48.16	48.43	48.30	3.39	20.243	3.36	2.434	2.496	0.062
8	49.14	49.37	49.26	3.51	20.073	3.409	2.469	2.531	0.062
9	48.77	48.99	48.88	3.45	19.9	3.441	2.43	2.496	0.066
10	49.76	49.91	49.84	3.64	19.961	3.608	2.41	2.497	0.087
11	49.94	50.1	50.02	3.63	19.901	3.582	2.951	2.996	0.045
12	50.27	50.4	50.34	3.67	19.826	3.622	2.954	3.014	0.06
13	49.476	49.23	49.35	3.46	20.072	3.414	2.955	2.996	0.041
14	49.08	49.2	49.14	3.48	20.231	3.461	2.934	2.986	0.052
15	50.09	50.12	50.11	3.61	19.986	3.535	2.43	2.481	0.051
16	50.13	50.29	50.21	3.66	20.128	3.577	2.951	2.984	0.033
17	50.5	50.46	50.48	3.66	20.173	3.615	2.435	2.477	0.042
18	49.89	49.81	49.85	3.67	20.069	3.639	2.447	2.484	0.037
19	49.37	49.24	49.31	3.50	20.255	3.495	2.956	3.001	0.045
20	50.86	50.69	50.78	3.69	19.973	3.663	2.951	2.996	0.045

time [h]	***70.25***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	51.92	51.84	51.88	3.97	19.288	3.893	2.924	2.978	0.054
2	52.64	52.57	52.61	3.97	19.579	3.837	2.435	2.477	0.042
3	53.01	53.2	53.11	4.18	19.438	4.101	2.41	2.474	0.064
4	52.7	52.69	52.70	4.09	19.543	4.006	2.44	2.482	0.042
5	52.19	51.81	52.00	3.96	19.921	3.819	2.466	2.512	0.046
6	52.75	52.77	52.76	4.16	19.735	4.086	2.413	2.453	0.04
7	51.9	51.48	51.69	3.95	19.747	3.856	2.925	2.97	0.045
8	52.71	52.63	52.67	4.07	19.538	3.944	2.934	2.982	0.048
9	51.9	52.29	52.10	3.98	19.407	3.934	2.953	2.987	0.034
10	53.54	53.45	53.50	4.24	19.446	4.123	2.971	3.028	0.057
11	53.31	53.32	53.32	4.17	19.375	4.108	2.956	3.005	0.049
12	54.23	54.22	54.23	4.31	19.238	4.21	2.943	2.999	0.056
13	53.19	52.96	53.08	4.08	19.526	3.96	2.966	3.019	0.053
14	52.49	52.59	52.54	4.04	19.686	4.006	2.96	3.011	0.051
15	53.7	53.7	53.70	4.20	19.421	4.1	2.961	3.012	0.051
16	53.64	53.59	53.62	4.22	19.542	4.163	3.002	3.047	0.045
17	53.62	53.65	53.64	4.18	19.598	4.19	2.955	3.001	0.046
18	53.62	53.66	53.64	4.29	19.475	4.233	2.962	3.019	0.057
19	49.37	52.62	51.00	3.77	19.714	4.036	2.997	3.038	0.041
20	50.86	54.69	52.78	4.02	19.4	4.236	2.956	2.995	0.039

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time [h]	***83.75***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	55.47	55.51	55.49	4.56	18.64	4.541	2.9958	3.0422	0.0465
2	56.37	56.38	56.38	4.59	18.941	4.475	2.9725	3.0118	0.0393
3	57.14	57.04	57.09	4.84	18.768	4.771	2.9898	3.0401	0.0503
4	56.49	56.79	56.64	4.74	18.9	4.649	2.9808	3.0162	0.0354
5	55.8	55.87	55.84	4.59	19.339	4.401	2.9653	3.0028	0.0375
6	56.55	56.52	56.54	4.78	19.093	4.728	3.0409	3.0887	0.0478
7	55.33	55.29	55.31	4.54	19.145	4.458	2.9751	3.0218	0.0468
8	56.76	56.63	56.70	4.74	18.885	4.597	3.0138	3.0579	0.0442
9	56.26	56.08	56.17	4.65	18.792	4.549	2.9832	3.0314	0.0482
10	57.39	57.26	57.33	4.87	18.786	4.783	2.9856	3.0360	0.0505
11	56.98	56.97	56.98	4.78	18.811	4.672	2.9623	3.0052	0.0429
12	58.32	58.05	58.19	4.96	18.627	4.821	2.9681	3.0230	0.0549
13	56.53	56.54	56.54	4.65	18.961	4.525	3.0037	3.0529	0.0492
14	56.13	56.41	56.27	4.65	19.103	4.589	2.9642	3.0082	0.0440
15	57.27	57.58	57.43	4.81	18.814	4.707	2.9819	3.0312	0.0493
16	57.73	57.74	57.74	4.90	18.935	4.77	2.9728	3.0091	0.0363
17	57.17	57.77	57.47	4.81	18.985	4.803	2.9469	2.9846	0.0378
18	58.29	57.77	58.03	5.02	18.834	4.874	2.9706	3.0144	0.0438
19	56.6	56.51	56.56	4.69	19.127	4.623	2.9505	2.9854	0.0349
20	58.26	58.28	58.27	4.92	18.799	4.837	3.0022	3.0366	0.0344

time [h]	***94.25***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	58.18	57.73	57.96	4.97	18.293	4.888	2.940	2.9789	0.0389
2	58.59	58.75	58.67	4.97	18.592	4.824	2.968	3.0047	0.0367
3	59.7	60.17	59.94	5.31	18.364	5.175	2.997	3.0337	0.0367
4	59.4	59.39	59.40	5.19	18.509	5.040	2.999	3.0278	0.0288
5	58.45	58.13	58.29	4.99	18.951	4.789	3.000	3.0290	0.0290
6	59.12	59.23	59.18	5.21	18.709	5.112	2.948	3.0036	0.0556
7	57.59	57.69	57.64	4.93	18.757	4.846	2.965	3.0135	0.0485
8	59.52	59.5	59.51	5.20	18.480	5.002	2.945	2.9853	0.0403
9	58.96	58.75	58.86	5.09	18.425	4.916	2.980	3.0425	0.0625
10	60.22	59.93	60.08	5.33	18.380	5.189	3.000	3.0439	0.0439
11	59.46	59.27	59.37	5.17	18.448	5.035	3.005	3.0419	0.0369
12	60.96	60.97	60.97	5.42	18.187	5.261	2.943	2.9968	0.0538
13	59.11	59.07	59.09	5.07	18.558	4.928	2.970	3.0154	0.0454
14	58.82	59.02	58.92	5.09	18.696	4.996	2.973	3.0100	0.0370
15	60.13	60.16	60.15	5.26	18.382	5.139	2.957	3.0046	0.0476
16	60.37	60.41	60.39	5.33	18.491	5.214	3.003	3.0306	0.0276
17	60.48	60.55	60.52	5.31	18.539	5.249	3.000	3.0295	0.0295
18	60.4	60.36	60.38	5.40	18.395	5.313	2.948	2.9787	0.0307
19	59.46	59.33	59.40	5.16	18.676	5.074	3.003	3.0318	0.0288
20	60.85	61.05	60.95	5.36	18.358	5.278	2.954	2.9838	0.0298

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time [h]	***106.25***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	60.88	61.11	61.00	5.47	17.818	5.363	2.968	3.0370	0.0690
2	61.68	62.02	61.85	5.49	18.091	5.325	2.974	3.0116	0.0376
3	63.03	62.86	62.95	5.80	17.881	5.658	2.999	3.0272	0.0282
4	62.23	62.19	62.21	5.65	18.036	5.513	2.956	2.9989	0.0429
5	60.93	60.83	60.88	5.42	18.506	5.234	2.975	3.0197	0.0447
6	62.00	62.14	62.07	5.69	18.226	5.595	2.950	2.9932	0.0432
7	60.88	60.98	60.93	5.47	18.258	5.345	2.957	3.0223	0.0653
8	62.71	62.69	62.70	5.73	17.951	5.531	2.972	3.0483	0.0763
9	61.63	61.68	61.66	5.56	17.961	5.380	2.953	3.0303	0.0773
10	62.87	62.99	62.93	5.80	17.886	5.683	2.979	3.0252	0.0462
11	62.94	62.43	62.69	5.72	17.936	5.547	2.959	3.0188	0.0598
12	64.09	64.03	64.06	5.93	17.693	5.755	2.953	2.9969	0.0439
13	62.01	62.05	62.03	5.55	18.108	5.378	3.002	3.0449	0.0429
14	61.95	62.02	61.99	5.60	18.124	5.568	2.966	3.0145	0.0485
15	63.06	63.12	63.09	5.75	17.891	5.630	3.001	3.0477	0.0467
16	63.19	63.44	63.32	5.82	18.014	5.691	2.955	3.0024	0.0474
17	63.64	63.39	63.52	5.81	18.045	5.743	2.971	3.0018	0.0308
18	63.39	63.37	63.38	5.90	17.897	5.811	2.970	3.0020	0.0320
19	61.28	62.28	61.78	5.63	18.187	5.563	2.966	3.0020	0.0360
20	63.24	63.50	63.37	5.76	17.962	5.674	2.973	2.9946	0.0216

time [h]	***119.75***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	64.01	63.88	63.945	5.96	17.350	5.831	2.975	3.0271	0.0521
2	65.73	65.82	65.775	6.14	17.674	5.742	2.933	3.0270	0.0940
3	65.66	66.08	65.87	6.28	17.413	6.126	2.960	2.9773	0.0173
4	64.66	64.84	64.75	6.07	17.608	5.941	2.971	3.0028	0.0318
5	63.48	63.49	63.485	5.85	18.103	5.637	2.968	3.0050	0.0370
6	65.07	54.77	59.92	5.34	17.782	6.039	2.950	3.0009	0.0509
7	63.45	63.42	63.435	5.88	17.815	5.788	2.966	2.9911	0.0251
8	65.52	65.26	65.39	6.17	17.504	5.978	2.954	3.0173	0.0633
9	64.53	64.53	64.53	6.03	17.480	5.861	2.990	2.9947	0.0047
10	66.03	65.79	65.91	6.29	17.410	6.159	2.999	3.0455	0.0465
11	65.46	65.38	65.42	6.17	17.461	6.022	2.976	3.0436	0.0676
12	67.07	67.08	67.075	6.43	17.222	6.226	2.961	3.0337	0.0727
13	65.43	64.61	65.02	6.05	17.671	5.815	2.949	3.0124	0.0634
14	64.73	64.75	64.74	6.05	17.754	5.938	2.963	2.9961	0.0331
15	65.97	66.15	66.06	6.24	17.442	6.079	2.999	3.0026	0.0036
16	66.13	66.14	66.135	6.28	17.548	6.157	3.001	3.0383	0.0373
17	65.87	66.19	66.03	6.22	17.565	6.223	2.946	3.0332	0.0872
18	66.23	66.45	66.34	6.39	17.397	6.311	2.969	2.9840	0.0150
19	65.28	65.32	65.3	6.21	17.701	6.049	3.001	3.0059	0.0049
20	66.35	66.42	66.385	6.26	17.497	6.139	2.955	3.0313	0.0763

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time [h]	***132.25***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	66.58	66.66	66.62	6.40	16.945	6.236	2.966	3.0050	0.0390
2	67.29	67.02	67.16	6.37	17.270	6.146	2.972	3.0076	0.0356
3	68.15	68.19	68.17	6.66	17.021	6.518	2.963	2.9881	0.0251
4	67.41	67.83	67.62	6.55	17.197	6.352	2.966	2.9956	0.0296
5	65.88	65.70	65.79	6.23	17.775	5.965	2.937	2.9645	0.0275
6	67.40	67.73	67.57	6.60	17.369	6.452	2.963	3.0001	0.0371
7	66.03	66.16	66.10	6.32	17.412	6.191	2.950	2.9847	0.0347
8	68.42	68.44	68.43	6.67	17.058	6.424	2.937	2.9778	0.0408
9	67.30	67.38	67.34	6.49	17.065	6.276	2.985	3.0324	0.0474
10	68.67	68.57	68.62	6.74	17.013	6.556	2.960	2.9872	0.0272
11	68.17	68.16	68.16	6.62	17.011	6.472	2.962	3.0097	0.0477
12	69.84	70.23	70.04	6.92	16.724	6.724	2.951	3.0154	0.0644
13	67.29	67.29	67.29	6.42	17.238	6.248	2.962	3.0137	0.0517
14	67.72	67.49	67.61	6.52	17.317	6.375	2.971	3.0133	0.0423
15	68.59	69.15	68.87	6.70	16.948	6.573	2.951	2.9943	0.0433
16	68.88	69.19	69.04	6.76	17.113	6.592	2.958	3.0006	0.0426
17	69.42	69.33	69.38	6.77	17.143	6.645	2.941	2.9711	0.0301
18	69.07	69.30	69.19	6.85	16.961	6.747	2.941	2.9714	0.0304
19	68.29	68.25	68.27	6.70	17.264	6.486	2.944	2.9695	0.0255
20	67.88	67.94	67.91	6.51	17.249	6.387	2.959	2.9726	0.0136

time [h]	***146.75***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	69.19	69.10	69.15	6.81	16.552	6.629	3.0043	3.0333	0.0290
2	69.57	69.40	69.49	6.75	16.901	6.515	2.9546	2.9851	0.0305
3	69.98	69.92	69.95	6.96	16.734	6.805	2.9760	2.9777	0.0017
4	69.15	69.23	69.19	6.81	16.927	6.622	2.9620	2.9831	0.0211
5	66.75	66.76	66.76	6.39	17.602	6.138	2.9734	2.9901	0.0167
6	70.04	70.21	70.13	7.02	17.966	5.855	2.9740	2.9993	0.0253
7	68.65	68.71	68.68	6.75	17.019	6.584	2.9710	2.9984	0.0274
8	71.10	71.04	71.07	7.11	16.620	6.862	2.9771	3.0131	0.0360
9	69.69	69.67	69.68	6.88	16.672	6.669	2.9500	2.9796	0.0296
10	71.00	70.81	70.91	7.11	16.613	6.956	2.9551	2.9828	0.0277
11	70.34	70.27	70.31	6.98	16.662	6.821	2.9481	2.9811	0.0330
12	72.51	72.58	72.55	7.33	16.353	7.095	3.0043	3.0403	0.0360
13	69.79	69.80	69.80	6.83	16.843	6.643	2.9761	3.0117	0.0356
14	70.33	70.24	70.29	6.97	16.952	6.740	3.0047	3.0602	0.0555
15	71.36	71.54	71.45	7.13	16.599	6.922	2.9743	3.0042	0.0299
16	71.53	71.41	71.47	7.16	16.695	7.010	2.9554	2.9794	0.0240
17	71.84	71.99	71.92	7.19	16.679	7.109	2.9639	2.9915	0.0276
18	71.85	72.10	71.98	7.31	16.519	7.189	2.9742	2.9986	0.0244
19	71.14	70.92	71.03	7.16	16.838	6.912	2.9748	2.9976	0.0228
20	69.58	69.59	69.59	6.79	16.984	6.652	2.9713	2.9881	0.0168

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time [h] 157.75

Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	70.83	70.82	70.83	7.09	16.280	6.901	2.9760	2.9939	0.0179
2	70.88	70.95	70.92	6.99	16.676	6.740	2.9640	2.9813	0.0173
3	70.73	70.66	70.70	7.08	16.600	6.939	2.9439	2.9580	0.0141
4	70.09	70.08	70.09	6.95	16.805	6.744	2.9732	2.9814	0.0082
5	67.46	67.37	67.42	6.50	17.473	6.267	2.9759	2.9882	0.0123
6	71.72	71.61	71.67	7.27	16.692	7.129	2.9739	2.9923	0.0184
7	70.47	70.42	70.45	7.04	16.734	6.869	3.0042	3.0281	0.0239
8	72.91	72.89	72.90	7.41	16.334	7.148	2.9739	2.9937	0.0198
9	71.77	71.73	71.75	7.22	16.421	6.920	2.9486	2.9646	0.0160
10	72.43	72.64	72.54	7.38	16.392	7.177	2.9732	2.9861	0.0129
11	72.30	72.28	72.29	7.30	16.406	7.077	2.9558	2.9856	0.0298
12	74.70	74.57	74.64	7.67	16.406	7.042	2.9768	3.0092	0.0324
13	71.93	71.83	71.88	7.18	16.561	6.925	2.9737	3.0017	0.0280
14	72.17	72.12	72.15	7.27	16.651	7.041	3.0049	3.0423	0.0374
15	73.20	73.11	73.16	7.41	16.349	7.172	2.9588	2.9847	0.0259
16	73.12	73.26	73.19	7.45	16.434	7.271	2.9733	2.9898	0.0165
17	73.74	73.56	73.65	7.48	16.399	7.389	2.9755	2.9927	0.0172
18	73.59	73.97	73.78	7.61	16.238	7.470	2.9624	2.9791	0.0167
19	72.49	72.55	72.52	7.40	16.550	7.200	2.9563	2.9739	0.0176
20	70.71	70.51	70.61	6.96	16.856	6.780	2.9744	2.9807	0.0063

time [h] 171.75

Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	72.01	71.73	71.87	7.26	16.130	7.051	2.9743	2.9863	0.0120
2	72.10	71.94	72.02	7.17	16.510	6.906	2.9478	2.9565	0.0087
3	72.47	71.48	71.98	7.29	16.513	7.026	3.0040		
4	70.84	70.66	70.75	7.06	16.667	6.882	2.9718	2.9807	0.0089
5	67.99	68.05	68.02	6.60	17.374	6.366	2.9547		
6	73.44	73.40	73.42	7.56	16.416	7.405	2.9766	2.9946	0.0180
7	72.86	72.68	72.77	7.42	16.368	7.235	2.9492	2.9510	0.0018
8	74.93	75.01	74.97	7.75	16.018	7.464	2.9487	2.9801	0.0314
9	73.32	73.26	73.29	7.47	16.330	7.011	2.9473		
10	73.76	73.61	73.69	7.57	16.253	7.316	2.9742	2.9830	0.0088
11	75.46	75.61	75.54	7.84	15.861	7.622	3.0038	3.0707	0.0669
12	77.34	77.26	77.30	8.11	15.594	7.854	2.9497	3.0092	0.0595
13	74.58	74.80	74.69	7.64	16.088	7.398	2.9754	3.0457	0.0703
14	74.75	74.99	74.87	7.72	16.228	7.464	2.9758	3.0315	0.0557
15	75.38	75.36	75.37	7.77	16.005	7.516	2.9490	2.9941	0.0451
16	75.58	75.45	75.52	7.83	16.062	7.643	2.9732	2.9927	0.0195
17	76.34	76.23	76.29	7.91	15.993	7.795	2.9498	2.9740	0.0242
18	76.38	76.40	76.39	8.04	15.801	7.907	2.9735	2.9989	0.0254
19	75.37	75.26	75.32	7.87	16.110	7.640	2.9747	2.9993	0.0246
20	72.41	75.37	73.89	7.50	16.542	7.094	3.0044	3.0253	0.0209

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time [h] 182.75

Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1	73.22	72.87	73.05	7.46			2.9300	2.9902	0.0602
2	72.72	72.62	72.67	7.28	16.382	7.034	2.9625		
3	71.85	71.57	71.71	7.25	16.466	7.073	2.9464		
4	71.18	71.28	71.23	7.14	16.581	6.968	2.9744		
5	68.24	68.25	68.25	6.64	17.314	6.426	2.9712		
6	74.55	74.46	74.51	7.74	16.234	7.587	2.9757	2.9937	0.0180
7	74.15	74.13	74.14	7.65	16.116	7.487	3.0032	3.0152	0.0120
8	76.13	76.01	76.07	7.93	15.833	7.649	2.9738	2.9951	0.0213
9	74.66	74.55	74.61	7.69	15.971	7.370	2.9743	2.9986	0.0243
10	74.61	74.55	74.58	7.72	16.031	7.538	2.9739	2.9930	0.0191
11	76.48	76.61	76.55	8.00	15.716	7.767	2.9751	3.0208	0.0457
12	78.78	78.80	78.79	8.36	15.363	8.085	3.0062	3.0322	0.0260
13	76.42	76.38	76.40	7.92	15.772	7.714	2.9472	2.9926	0.0454
14	76.65	76.55	76.60	8.01	15.911	7.781	2.9735	3.0095	0.0360
15	77.49	77.67	77.58	8.14	15.618	7.903	2.9563	3.0124	0.0561
16	77.47	77.49	77.48	8.15	15.710	7.995	2.9743	2.9963	0.0220
17	78.39	78.37	78.38	8.26	15.658	8.130	2.9737	2.9882	0.0145
18	78.76	78.78	78.77	8.44	15.394	8.314	2.9555	2.9813	0.0258
19	77.43	77.44	77.44	8.22	15.767	7.983	2.9729	2.9971	0.0242
20	73.84	73.85	73.85	7.49	16.310	7.326	2.9643	2.9825	0.0182

time [h] 197.75

Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1									
2									
3									
4									
5									
6	75.18	75.11	75.15	7.85	16.120	7.701			
7	75.08	75.16	75.12	7.81	15.952	7.651			
8	76.62	76.44	76.53	8.01	15.715	7.767			
9	75.53	75.41	75.47	7.83	15.865	7.476			
10	75.43	75.14	75.29	7.84	15.941	7.628			
11	80.23	80.17	80.20	8.61	15.100	8.383	2.9636		
12	80.90	81.04	80.97	8.72	15.012	8.436	2.9566		
13	79.25	79.26	79.26	8.39	15.308	8.178	2.9503		
14	79.05	78.89	78.97	8.40	15.530	8.162	2.9771		
15	80.03	79.92	79.98	8.53	15.253	8.268	2.9660		
16	79.95	80.37	80.16	8.59	15.310	8.395	2.9511		
17	80.86	81.16	80.86	8.67	15.247	8.541	3.0028		
18	81.27	81.37	81.32	8.86	15.020	8.688	3.0057		
19	80.10	80.22	80.16	8.66	15.357	8.393	3.0032		
20	76.48	76.33	76.41	7.91	15.907	7.729	2.9557		

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time [h] 208.25

Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11	80.59	80.36	80.48	8.65			2.9566	3.0583	
12	81.04	81.28	81.16	8.75			2.9570	3.0172	
13	79.66	79.38	79.52	8.44			2.9541	3.0137	
14	79.69	79.56	79.63	8.51			2.9639	3.0314	
15	80.43	80.53	80.48	8.62			2.9755	3.0164	
16	80.89	80.76	80.83	8.70			2.9757	2.9710	
17	81.74	81.53	81.64	8.80			2.9749	3.0265	
18	82.03	82.30	82.17	9.00			2.9510	3.0247	
19	80.75	80.99	80.87	8.78			2.9733	3.0243	
20	77.26	77.21	77.24	8.05			2.9744	2.9774	

time [h] 218.25

Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11	80.48	80.30	80.39	8.64	14.939	8.544			
12	81.39	81.59	81.49	8.80	14.872	8.576			
13	80.21	80.01	80.11	8.53	15.153	8.333			
14	80.19	80.20	80.20	8.60	15.301	8.391			
15	80.93	80.88	80.91	8.69	15.087	8.434			
16	81.34	81.62	81.48	8.81	15.095	8.610			
17	82.16	82.00	82.08	8.87	15.035	8.753			
18	82.18	82.36	82.27	9.01	14.803	8.905			
19	81.66	81.63	81.65	8.91	15.150	8.600			
20	78.37	78.59	78.48	8.25	15.548	8.088			

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time [h]	***227.25***								
Device	side 1	side 2	average	volume expelled [ml]	weight of the device [g]	total weight loss of the device [g]	weight empty sample vial [g]	weight sample vial with dried formulation [g]	weight dry matter [g]
1									
2									
3									
4									
5									
6									
7									
8									
9									
10									
11	80.70	80.54	80.62	8.68					
12	81.51	81.63	81.57	8.82					
13	80.51	80.48	80.50	8.60					
14	80.68	80.61	80.65	8.67					
15	81.00	81.04	81.02	8.71					
16	81.85	82.10	81.98	8.89					
17	82.14	82.23	82.19	8.89					
18	82.33	82.28	82.31	9.02					
19	81.90	82.01	81.96	8.96					
20	79.27	79.13	79.20	8.37					