DEVELOPMENT OF AN INTRARUMINAL CONTROLLED-RELEASE DEVICE

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

Doctor of Philosophy

at

The University of Waikato

by

BRADLEY JOHN MCLELLAN

THE UNIVERSITY OF
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Te Whare Wānanga o Waikato

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Abstract

Slow-release devices retained in the rumen, are a simple method for continuous administration of bioactives to ruminant animals. To satisfy regulatory requirements and avoid waste of bioactive due to under- or over-dosing, it is advantageous to have a constant and predictable release rate. Existing intraruminal controlled-release technologies cannot easily be adapted for different bioactives or rates of release and can be influenced by the variable physiological environment in the rumen. Some existing commercial products use the pressure generated by a hydrogen gas-producing cell to extrude fluids from a syringe-like device. This technology may provide advantages for ruminal controlled-release as the gas production rate is unaffected by environment in the rumen and can be easily adjusted using electrical resistance applied to the gas cell. This technology was adapted for use in the rumen in these studies.

Initial experiments identified the need for greater understanding of the rate that hydrogen is produced by the gas cell and the rate that gas diffuses through the barrel walls. Gas production rate was found to be inversely proportional to the resistance applied to the gas-producing cell. Factors affecting gas diffusion rate from the device were studied and a polymer was identified that reduced hydrogen diffusion to 5% of that for the initial components used. A relationship was developed to predict the release profile of a device. Controlled-release devices were constructed from selected materials. They released blank formulation at *in vitro* at a constant rate, which was within experimental variation of predicted values. Release rates from the devices used *in vivo* were slightly higher than predicted. The presence of rumen gases inside *in vivo* devices suggested that the difference may be due to inward diffusion of these gases; these may be eliminated by further study of barrel materials. Recommendations on the redesign of this technology for use as a generic intraruminal delivery system are given.

Acknowledgments

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…for Anna.

Contents

Figures

Tables

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A	cross-sectional area	cm^2
AU	absorbance unit	none
C	concentration	$kg \cdot m^{-3}$
D	diffusion coefficient	$m^2 \cdot s^{-1}$
D_L	change in volume due to diffusion through a barrel	$mL \cdot day^{-1}$
D_l	change in volume due to diffusion for l	$mL \cdot day^{-1}$
G	gas production rate	$mL \cdot day^{-1}$
I	current	A
J	flux	$kg \cdot m^{-2} \cdot s^{-1}$
l	plunger position	cm
L	total barrel length	cm
l_0	initial plunger position	cm
n	amount of a substance or number of replicates	moles or none
P	pressure	kPa
R	gas constant (8.314) or resistance	$J \cdot mol^{-1} \cdot K^{-1}$ or Ω
r_D	effective radius of diffusion	distance
r_i	internal radius	distance
r_e	external radius	distance
T	temperature	K
t	time	days
V	volume or voltage	L, mL or V
x	distance or displacement from l_0	m or cm

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Chapter 1: Introduction and literature review

1.1 Overview

The reticulorumen is a large compartment of the ruminant digestive tract where ingested feed is retained for microbial degradation of otherwise indigestible plant matter. This compartment is an ideal location to retain a device that can deliver bioactives directly into the digestive tract over long periods. There are several commercially available controlled-release devices that may be used to deliver materials via the reticulorumen. Release from these devices may be driven by chemical, physical and mechanical processes including diffusion, erosion and osmotic pressure.

Existing technologies for intraruminal controlled-release have various limitations. Many devices are dependent in some way on the rumen environment, which causes difficulties because the environment is variable. Devices are typically designed for a specific bioactive and may not be easily adapted to release a different material or at a different rate. For example, osmotic pump devices are limited to delivering highly potent bioactives because a large volume is required for the drive mechanism. Erosion-based devices can exhibit non-linear release or release rates depending on what the animal has been fed and are therefore better suited to bioactives that are effective over a wide range of concentrations.

The chemical and physical conditions of the rumen environment and its microbial activity make degradation a problem for many bioactives. Various formulations have been developed to shield bioactives from degradation. However, this can reduce the concentration of the active and its post-ruminal availability. The space that formulation, drive and retention elements require in existing intraruminal controlled-release technologies has prevented devices from carrying sufficient quantities of low-potency rumen-protected active for long-term sustained release.

To reduce the time-to-market of new bioactives and facilitate the long-term release of rumen-protected bioactives, a generic, high-capacity, intraruminal controlled-release technology compatible with a wide range of bioactives, independent of formulation or the rumen environment and capable of linear release at a rate that is easily adjusted is needed.

Small, commercially available, galvanic cells that emit hydrogen gas as they produce an electrical current are an alternative way of driving a controlled-release device. The gas produced can be trapped in a barrel and used to drive a plunger to extrude fluids. A device to slowly release lubricant into machinery and an infusion pump for human patients have used this technology commercially. It has also been used experimentally in an intravaginal controlled-release device for cattle. Adaptation of this technology for use in the rumen may provide advantages over other commercially-available controlled-release methods. The rate the cell produces gas is determined by the electrical current drawn by the gas cell; this is relatively independent of its environment. The current, and therefore the release rate, can be adjusted by altering the electrical resistance applied to the gas cell. The volume of the gas cell is less than 1 mL, yet it can produce about 160 mL of hydrogen therefore a large volume of formulation could be delivered from the device.

1.2 The role of controlled-release devices for ruminants

Ruminant animals provide important sources of meat, milk, wool and other products. These primary products contribute significantly to the national economy; New Zealand exported $12.6 billion worth of agricultural products in 2004, predominantly of ruminant origin (Ministry of Agriculture and Forestry 2005). Factors that limit production can therefore have significant economic effects and therefore ruminants are often supplemented with various bioactive substances for better health or production. Bioactives administered to cattle include nutrients such as copper, selenium and iodine (Vandamme and Ellis 2004), substances to treat or prevent diseases such as magnesium for grass staggers or antibiotics for mastitis (Holmes and Wilson 1987), hormones for the manipulation of reproduction (Rathbone et al. 1997) and various antiparasitic substances (Vandamme and Ellis 2004).

Many of these bioactives must be supplied to the animal frequently or continuously for maximum effect. Repeated administration, however, can be stressful to the animal, labour-intensive and in some situations, for example where animals are released to feed for long periods of time, impossible. The use of

controlled-release devices allows continuous administration of bioactives for long periods of time following a single application (Cardinal 2000; Vandamme and Ellis 2004). A wide range of controlled-release devices for cattle are currently available and use various mechanisms of delivery. These include external devices in the form of a collar, ear tag or tail tag (Miller 2000), or internal devices within the vagina (Rathbone et al. 1997), the teat (Gruet et al. 2001) or the reticulorumen (Cardinal 2000; Vandamme and Ellis 2004).

1.3 The Rumen

1.3.1 Physical characteristics

The digestive system of ruminant animals is characterised by specially adapted fore-stomachs known as the reticulum, the rumen and the omasum, which precede a "true stomach" known as the abomasum (Figure 1-1 and Figure 1-2). The first and largest chamber (around 7 L in sheep or 150 L in cattle, Hodgson and Brookes 1999) is the reticulorumen, often ambiguously abbreviated to "the rumen". This anaerobic chamber is comprised of the rumen and the reticulum, which are separated by the rumino-reticular fold. The rumen is comprised of various sacs, which are separated by folds known as pillars (Figure 1-1, Harfoot 1978a).

1.3.2 Rumination

Ingested feed is broken down physically by the process known as "rumination" or "chewing the cud" whereby digesta is mixed by the contractions of the rumen and boli are regurgitated for further mastication. Cows on pasture spend roughly equal amounts of time resting, eating and ruminating, which involves both the mixing of digesta in the reticulorumen and the regurgitation of boli for further mastication. The mixing cycle begins with contractions of the reticulum, which force most of its contents over the rumino-reticular fold. The pillars of the rumen then contract sequentially from front to back, mixing the digesta and bringing smaller particles to the top for passage to the omasum. If digesta is to be regurgitated, this occurs before the first contraction of the reticulum. A contraction of the reticulum raises the digesta level above the cardia, which

relaxes allowing digesta to enter the oesophagus. Peristaltic contractions of the oesophagus then carry a bolus of solid digesta to the mouth while allowing much of the associated liquid to fall back into the reticulorumen. After mastication, the regurgitated bolus is swallowed and another brought up before the next mixing cycle (Harfoot 1978a).

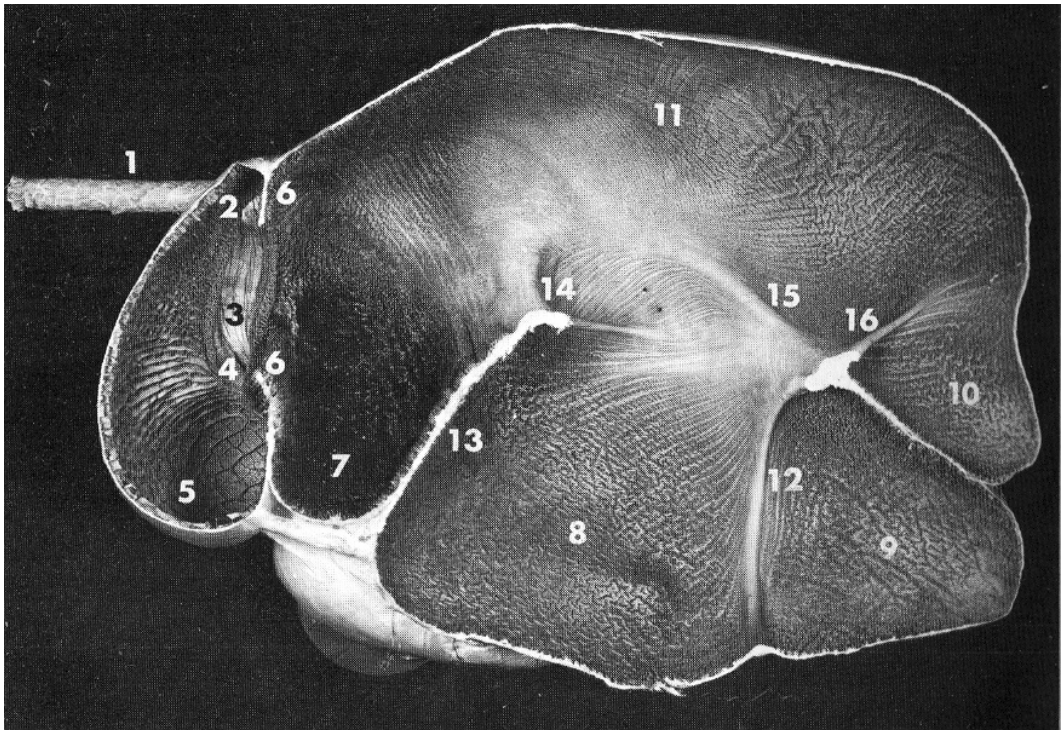


Figure 1-1: Cross-section of the rumen
1: Oesophagus. 2: Cardia. 3: Oesophageal groove. 4: Reticular-omasal opening. 5: Reticulum. 6: Rumino-reticular fold. 7: Anterior sac. 8: Ventral sack. 9: Ventral blind sack. 10: Dorsal blind sack. 11: Dorsal sack. 12: Ventral coronary pillar. 13: Anterior transverse fold. 14: Anterior transverse pillar. 15: Longitudinal pillar. 16: Dorsal coronary pillar. From Hungate 1966.

1.3.3 Microbial digestion

Physical degradation facilitates the enzymatic digestion of feed, including normally indigestible plant fibre, by microorganisms that break proteins and polysaccharides down to their constituent monomers. Sugar monomers are used for energy as they are converted to pyruvate by the glycolytic pathway. In the absence of oxygen, however, aerobic respiration cannot occur and pyruvate is instead converted to a variety of small carboxylic acids known as "volatile fatty acids" ("VFA"). Acetic, propionic and butyric acids are produced in varying proportions as well as small amounts of valeric acid and various branched fatty acids. Despite the production of these acids the pH in the rumen is typically

maintained between 5.8 and 6.8 by the secretion of large amounts of saliva (pH 8). Amino acids from digested proteins are deaminated and resynthesised therefore virtually all of the ruminant's protein requirements must therefore come from digestion of the microbes themselves (Harfoot 1978a). Rumen microbes also alter the lipid content of feed by hydrogenation of unsaturated lipids (Harfoot 1978b).

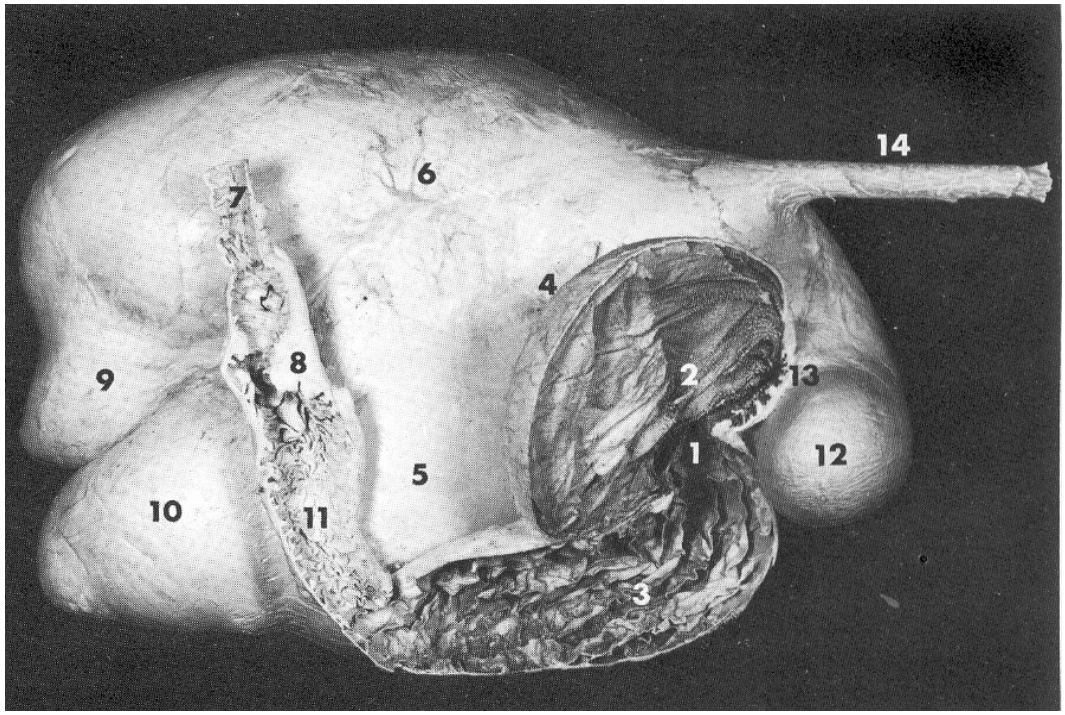


Figure 1-2: Cross-section of the omasum and abomasum
1: Omasal-abomasal orifice. 2: Omasum. 3: Abomasum. 4: Exterior of the omasum. 5: Ventral sack of the rumen. 6: Dorsal sack of the rumen. 7: Duodenum. 8: Pylorus. 9: Dorsal blind sack. 10: Ventral blind sack. 11: Pyloric portion of the abomasum. 12: Reticulum. 13: Vestibule of the omasum. 14: Oesophagus. From Hungate 1966.

1.3.4 Passage to the abomasum

Particles in the rumen that pass through the reticular-omasal opening to the omasum are generally less than ~2 mm for cattle or ~1 mm for sheep. In the folds of the omasum, water is removed and the VFAs are absorbed before the digesta passes through the omasal-abomasal orifice to the abomasum where it is finally subjected to the ruminant's own digestive enzymes (Harfoot 1978a).

1.4 Considerations for intraruminal controlled-release devices

1.4.1 Ruminal fistulation

Fistulated animals are an important tool in developing intraruminal devices and allow the devices to be tested *in situ* and accessed repeatedly without sacrificing the animal. The process of making a "fistula" or hole through to access the rumen of a live animal originated in the mid 19th century and, if properly performed, does not interfere with the consumption or digestion of food. A fistula is cut behind the left ribs, as high as possible to prevent loss of rumen contents. The fistula is fitted with a cannula (tube), which is sealed with a bung that can be removed to access the rumen. Fistulation has also been performed to allow access to most other sections of the digestive tract (Hungate 1966).

1.4.2 Rumination

Rumination has several important consequences for any device intended to operate in the rumen. The device must be robust enough to withstand the forces of rumination without alteration of its function and the device must have a means to prevent it from being regurgitated and expelled (Vandamme and Ellis 2004, Section 1.5.1). The mixing motion of rumination may be used as a driving force for controlled-release devices by erosion of a solid formulation (Section 1.5.2.1).

1.4.3 Variability of conditions

Physical and chemical conditions in the rumen can vary widely due to differences in the quantity of feed, the type of feed and regularity with which it is available in addition to differences in the microbial population of the gut. These factors vary between species and even within the same animal over time (Hungate 1966). This variability is of great importance in the design of intraruminal controlled-release devices if they are to function independently of the environment in the rumen.

Variation in pH is mainly due to differences in the rate microbes in the rumen produce VFA from the particular types of feed present and can reach 4.0 or 7.5 under extreme circumstances (Hungate 1966; Pell *et al.* 2000). The oxidation-reduction potential is typically around -350 mV (Harfoot 1978a) but can vary

from one part a rumen to another depending on exposure to ingested oxygen. This also varies quickly over time as the microbes in each litre of rumen fluid can consume 4 to 15-mL of oxygen per hour (Hungate 1966).

Of particular importance to this project are the gas composition and temperature in the rumen (Section 1.6.3). Carbon dioxide (CO_2), methane (CH_4) and nitrogen make up most of the gas present in the rumen in proportions of 65%, 27% and 7% respectively with the remainder being trace amounts of oxygen, hydrogen (H_2) and hydrogen sulphide (McArthur and Miltimore 1961). The proportions of these gases vary with changes in feed and the microbial population (Hungate 1966; Dempsey and Ellis 2003). When the rate of rumen gas production decreases after feeding, the rate of production of CO_2 decreases faster than that of CH_4. Also, the proportion of H_2 in the rumen is initially increased when an animal is fed after a long period of starvation (Hungate 1966). The temperature in the rumen is in the range of 38 to 42°C (Laby 1987c; Cardinal 2000).

1.4.4 Protection of bioactives in the rumen

Many bioactives that are commonly added to the diets of other domestic animals are not effective when given orally to ruminants due to microbial degradation in the rumen. Some well-documented examples include amino acids such as methionine and lysine, which are required by many high-producing ruminants in greater proportions than are present in the microbial protein available to them. Several techniques, including simple heat or chemical treatments and more complicated encapsulation (Wu and Papas 1997; Pell et al. 2000), have been used to protect both non-microbial protein sources and free amino acids in the rumen, thereby increasing post-ruminal supply of methionine.

Heat and chemical treatments usually protect by reducing protein solubility, though protection may not be efficient and protein absorption may be reduced. However, the low cost these techniques allow inefficiencies to be overcome by administering larger quantities of protected protein. A study comparing seven different chemical and heat treatments for protein found that each method tested protected different amino acids to varying degrees (Waltz and Stern 1989). Formaldehyde treatment was the most effective with only about 25% of protein

degraded after 24 hours in the rumen, however, formaldehyde is considered prohibitively toxic in many countries (Wu and Papas 1997). A large volume of bioactive would need to be contained in a controlled-release device for long-term delivery if it was protected by one of these techniques due to low protection efficiency and reduced availability of the active.

More sophisticated methods of rumen protection involve coating bioactive particles. These particles must be able to resist both microbial degradation and the physical stresses of rumination. These effects can be reduced by minimising the time the bioactive is retained in the rumen, which can be achieved through specific dimensions and density. Particles should be smooth, spherical, less than 2 mm in size and have a density between 1.2 and 1.5 $g \cdot cm^3$ to minimise rumen retention time. Higher-density particles may sink to the bottom of the reticulorumen and be retained longer while a lower-density particles are more likely to be regurgitated and chewed during rumination (Pell et al. 2000).

The most successful method of encapsulation involves coating particles of bioactive with smaller, inert particles, held together by a polymer, typically poly(2-vinylpyridine-co-styrene, 80:20), which is insoluble in the mild pH of the rumen but rapidly dissolves in the acid of the abomasum. This technique has been used commercially to protect methionine ("Smartamine®", Rhône Poulenc) as well as several other bioactives (Pell et al. 2000).

Methionine protected as Smartamine® degrades significantly more slowly than unprotected methionine when incubated with rumen fluid *in vitro* (Mbanzamihigo et al. 1997) and increases plasma methionine levels in cattle by more than 800% (Blum et al. 1999; Südekum et al. 2004). Although this is a good method of rumen protection, the expense of Smartamine® means that it is cheaper to supplement animals with a larger amount of unprotected methionine (Mbanzamihigo et al. 1997). However, this would be an effective method to protect a bioactive being administered by a controlled-release device because the protection is achieved from a coating that is only 15% by weight. Any bioactive of sufficiently high value to warrant protection by encapsulation could benefit from an accurate delivery device to maximise its efficiency.

1.5 Intraruminal controlled-release devices

The reticulorumen provides a site where controlled-release devices can be retained and release their payload directly into the digestive tract of the animal. Ideally, an intraruminal controlled-release device should release bioactive at a rate that is constant, adjustable and independent of the rumen environment. A constant release rate prevents wastage of bioactive through under- or over-dosing (Vandamme and Ellis 2004). Bioactives may require administration at different rates and to different sizes of animal so having an adaptable rate of release makes a device applicable to more bioactives and reduces the number of different delivery systems required. Independence from the rumen environment is especially important as the variable conditions in the rumen may otherwise cause the bioactive to be released at ineffective or hazardous levels. A large payload is also desirable to enable the delivery of protected bioactives and bioactives that require a high delivery rates and/or long exposure periods. Intraruminal controlled-release devices should be made from materials that are safe for the animal and, where applicable, subsequent human consumption. Finally, an intraruminal device should be retained in the rumen for the duration of its activity.

1.5.1 Retention

Devices are typically retained in the rumen by being either too heavy or too large to escape through the cardia or the reticular-omasal opening (Cardinal 2000; Vandamme and Ellis 2004). These methods have lead to research on gastroretentive dosage forms for humans (Klausner et al. 2003).

1.5.1.1 Density

Objects sufficiently dense to remain at the bottom of the rumen are less likely to be expelled because the cardia and reticular-omasal opening are both on the sides of the reticulorumen. This approach to retention was reported (Dewey et al. 1958) and later patented (Marston 1962) for long term delivery of cobalt to sheep using pellets made from clay and cobaltic oxide. These pellets had a density of 4.1 $g \cdot cm^{-3}$ and displayed 100% retention in a four month trial. It was suggested that a density of 3.5 to 5.5 $g \cdot cm^{-3}$ is sufficient for ruminal retention, depending on the size and weight of the bolus (though the largest bolus tested was only 51 mm long

with a 6-mm diameter), the type of animal and what that animal is fed. A 1-g bolus with a density of 4 $g \cdot cm^{-3}$ will be retained in sheep, whereas a bolus with this density must be at least 5 g to be retained in cattle. Sheep regurgitated boli with a density of 2.7 $g \cdot cm^{-3}$ two to three times faster when the animals were fed on long grass and lucerne than when they were fed finely-chopped oat-chaff (Marston 1962). Larger devices with densities of 1.8 and 1.9 $g \cdot cm^{-3}$ have since been shown to remain in the rumen for at least 30 and 34 days respectively (Byford et al. 1980; Riner et al. 1982).

Ruminal retention of controlled-release boli by density is best suited to bioactives that are sufficiently dense to remain in the rumen, metals for example, in erosion based devices. If used with other bioactive or device types, a high-density component must be included in the bolus, reducing the space available for bioactive and potentially remaining in the rumen for the lifetime of the animal. A non-toxic, high-density compound, typically barium sulphate, can be added to the formulation (Byford et al. 1980; Riner et al. 1982). Alternatively, a solid, high-density object can be incorporated into the bolus. Solid density elements must be selected carefully because materials dense enough to retain a large device are typically hard enough to damage machinery at the meat works. Some devices have overcome this issue using a patented technique to manufacture sintered iron density elements with a crush-strength comparable to that of bone (Peery and Eckenhoff 1993; Peery and Eckenhoff 1994).

The use of density for retaining the device used in this study would be inappropriate because a large volume of dense material would be required. The density of a gas cell-driven device decreases as its payload is extruded and is replaced with hydrogen. Once full of hydrogen, an object as dense as lead would need to occupy about 17% of the device's total volume to increase its density to over 2 $g \cdot cm^{-3}$. Devices in this study were retained, where necessary, by variable geometry (Section 1.5.1.2).

1.5.1.2 Variable geometry

A technique called "variable geometry" is another common method of retaining controlled-release devices in the rumen. This involves constraining the device so

it can pass through the oesophagus before it reverts, upon reaching the rumen, to a shape that is too large to fit through either the cardia or reticular-omasal opening (Laby 1974). Of the many variable geometry designs suggested (Figure 1-3), "wings" are the most common method of retaining a cylindrical device like that used in this study (Figure 1-4). "Wings" were based on the idea of a device shaped like an arrowhead with strips of flexible polymer that fold flat against the sides of a cylinder during dosing then spring out to 45° (Laby 1981). This design has since been further refined such that the angle between the wings and the barrel is nearly 90°, which reduces the frequency that the wings are bent into the dosing position by the motion of the rumen (Simpson and Gervais 1983).

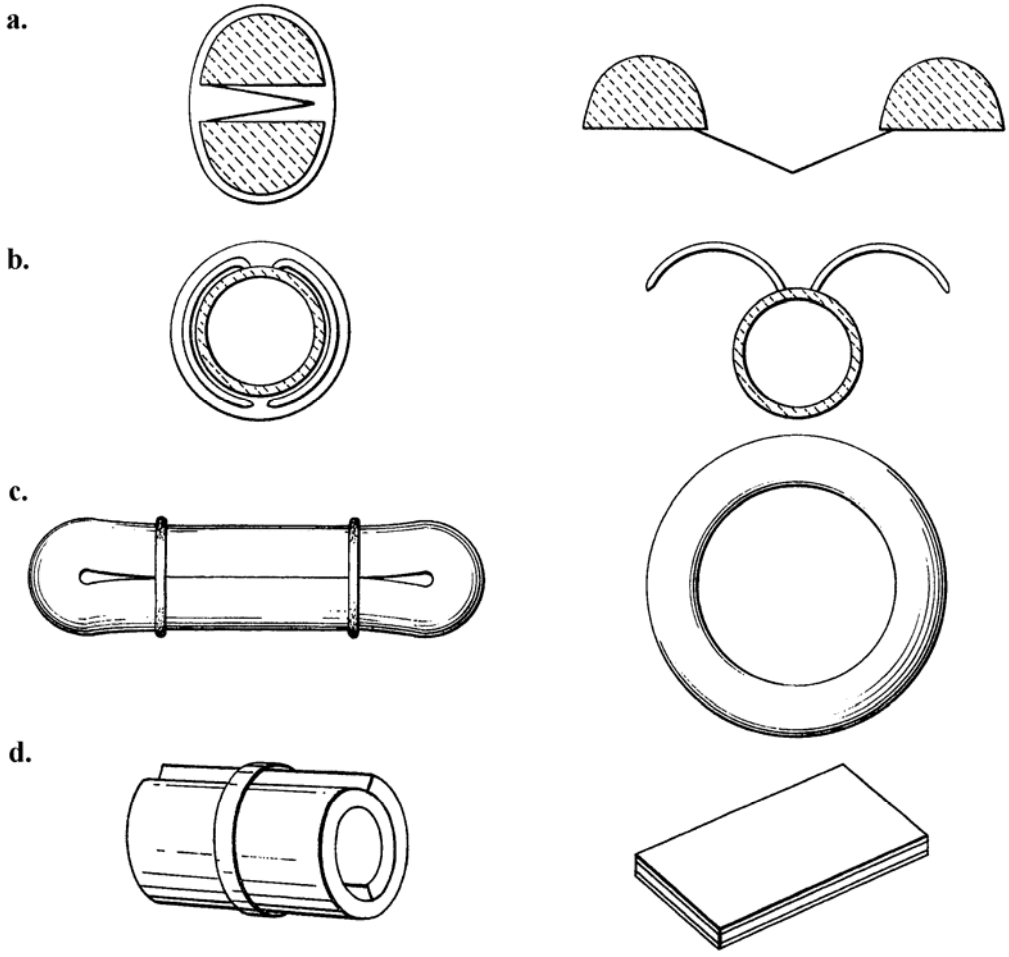


Figure 1-3: Some suggested device shapes for retention by variable geometry
Left column: constrained conformations. Right column: expanded conformations. (a, b and c from Laby 1974, d from Brewer and Griffin 1980)

Variable geometry potentially has an advantage over density for devices that are not naturally heavy. Elements of variable geometry are either incorporated into

the device design or applied externally rather than occupying space that could otherwise contain formulation.

A variable geometry device that does not take on its expanded conformation quickly can be regurgitated. This problem has been reduced as the various designs have evolved. The Paratect® flex, for example, is a flat sheet packaged as a rolled cylinder (Figure 1-3d). Several measures have been taken to ensure it reaches the rumen and is retained: the cylinder ends are blocked, which makes the device easier for the animal to swallow (Grimshaw and Weatherly 1991); the cylinder is constrained by a patented tape, which degrades quickly once dosed (Ranade and Curtis 1992; Ranade and Curtis 1994); and the outer side of the sheet is coated with an elastomer to aid unrolling (Curtiss and Lo 1989). After it is exhausted, this device degrades into pieces small enough to be regurgitated (Grimshaw and Weatherly 1991; Cardinal 2000), though this is theoretically possible for any variable geometry device.

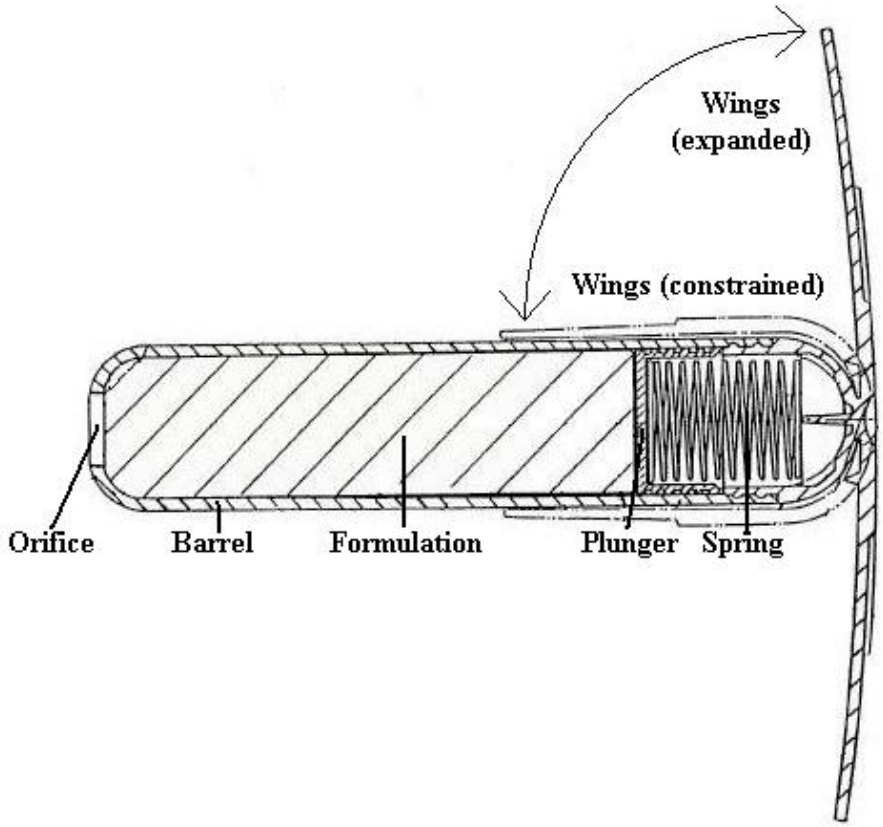


Figure 1-4: The Rumensin capsule
(Lowe and McArthur 1994; Lowe and McArthur 1996)

Winged devices may also have retention problems. Prolonged constraint may prevent wings unfolding properly or enable them bend into a "Y" position, which may allow regurgitation. Several modifications have been suggested to prevent this including various hinges, springs, struts and ridges (Simpson and Gervais 1983; Laby 1987a; Shepherd and Edwards 1989). Wings on the Rumensin capsule are supported by a dome shaped cap when constrained to prevent creasing and have ridges to aid unfolding and prevent folding past 90° (Simpson and Gervais 1983; Lowe and McArthur 1994; Lowe and McArthur 1996; Figure 1-4).

1.5.2 Release mechanisms

Several processes have been used to slowly release bioactive materials into the rumen. Mechanisms of some existing methods of intraruminal controlled-release are described here and linearity of release, independence from the rumen environment, adaptability and limitations are discussed.

1.5.2.1 Formulation

The simplest method of intraruminal controlled-release and the first to be developed is a solid bolus of formulation that is eroded by digesta motion in the rumen (Dewey et al. 1958; Marston 1962). Release rate from a simple eroding bolus decreases with time (Miller et al. 1977) making them more suitable for cheaper bioactives with wide therapeutic ranges such as mineral supplements. Several commercial devices, including the Rumensin capsule, have used a more complex approach to achieve linear release (Figure 1-4). This consists of a solid, waxy core of formulation contained in a cylindrical barrel and driven toward an orifice by a spring. When in the rumen, the formulation absorbs water, softens or swells, and is driven out through the orifice where it erodes (Laby 1981; Simpson and Gervais 1983; Vandamme and Ellis 2004). Some devices contain a stack of tablets instead because some bioactives can cause inconsistencies in the wax formulation, Elfazepam (Smith Kline Animal Health Products) and Diamphenethide (Wellcome Ltd.) for example (Laby 1987b).

Several factors determine the release rate from these spring-erosion type devices. All such devices are driven by the force of the spring and erosion of formulation from the orifice, though the release rate of devices containing tablets depends

more on their rate of gelling (Laby 1987b). A seal must be formed between the front of the formulation and the orifice to prevent digesta from entering the device. Pressure in the spring chamber decrease as the payload is released and quickly becomes the limiting factor of the release rate. This has been dealt with in two ways. Spring chambers of some devices are not sealed, allowing gas and digesta to enter. Instead, a seal must be formed between the plunger and the barrel to prevent digesta from eroding the back of the formulation (Lowe and McArthur 1994; Lowe and McArthur 1996). Alternatively, the chamber can remain sealed, making the rate that gases from the rumen diffuse into the spring chamber the rate limiting factor (Laby 1981; Laby and Kautzner 1986). This can initially elevate the release rate because rumen gases diffuse in much faster than air diffuses out, creating a surge of pressure (Laby 1981; Laby and Kautzner 1986).

Spring-erosion devices can carry a large payload and release it at a reasonably constant rate. However, because many factors can potentially affect the release rate, changes in rumen conditions are likely to affect its operation. The release rate of simple erosion devices depends on their location in the reticulorumen (Riner et al. 1982). This effect may also apply to spring-erosion devices. Changes in the proportions of gases in the rumen may affect release rate for sealed devices because different gases would diffuse into the device at different rates. The release rate from spring-erosion devices is typically adjusted by altering the formulation. A "trial and error" approach may be needed as release rate from a cast or tableted payload is affected by changes in type or concentration of bioactive (Laby 1987b).

If a spring-erosion device disintegrates, the spring and any remaining payload is released into the rumen, which could be fatal to the animal. Spring-erosion devices must be sufficiently robust to remain intact long after releasing their payload. The two body components of the Rumensin capsule are held together by interlocking grooves to prevent this (Lowe and McArthur 1994; Lowe and McArthur 1996).

1.5.2.2 Diffusion

Diffusion of a soluble substance from an insoluble, porous matrix can be used to slowly release bioactives in the rumen. The release rate from these devices typically decreases with time. However, a diffusion device in the form of a flat sheet can release at a near constant rate when coated with an impermeable layer and perforated with round holes (Cardinal 1986). This approach has been commercialised as Paratect® flex to deliver the anthelminthic morantel to cattle for 90 days (Figure 1-5).

The matrix is exposed to the solvent only at the inner surface of the perforations. A circular region depleted of bioactive forms around each perforation. As the radius of these depletion zones (and therefore the diffusion path) increases, bioactive diffuses out at a slower rate. This is offset by the increasing circumference of the depletion zones, which increases surface area of active exposed to the solvent. These two counteracting factors, after an initial burst, give a near zero-order release rate that can be adjusted easily by changing the number perforations in the sheet. The outer edges of this device do not need to be impermeably coated, although uncoated edges reduce linearity of release since the rate that the bioactive diffuses from the edges declines with time. Equations based on several parameters of the bioactive, the matrix, the solvent and the device have been derived that closely predict the release profile of the Paratect® flex (Boettner et al. 1988). As the release rate is dependant on bioactive solubility, diffusion-based devices are limited to bioactives that are at least slightly soluble in water (Cardinal 1986).

The release rate from the Paratect® flex shows an initial burst for several days zero-order release is attained. Release rate is then nearly constant, but begins to decrease after ~10 g of morantel has been released. This decline occurs when depletion zones around each perforation begin to overlap (Boettner et al. 1988). This limits this technology in its current to the delivery of relatively potent bioactives.

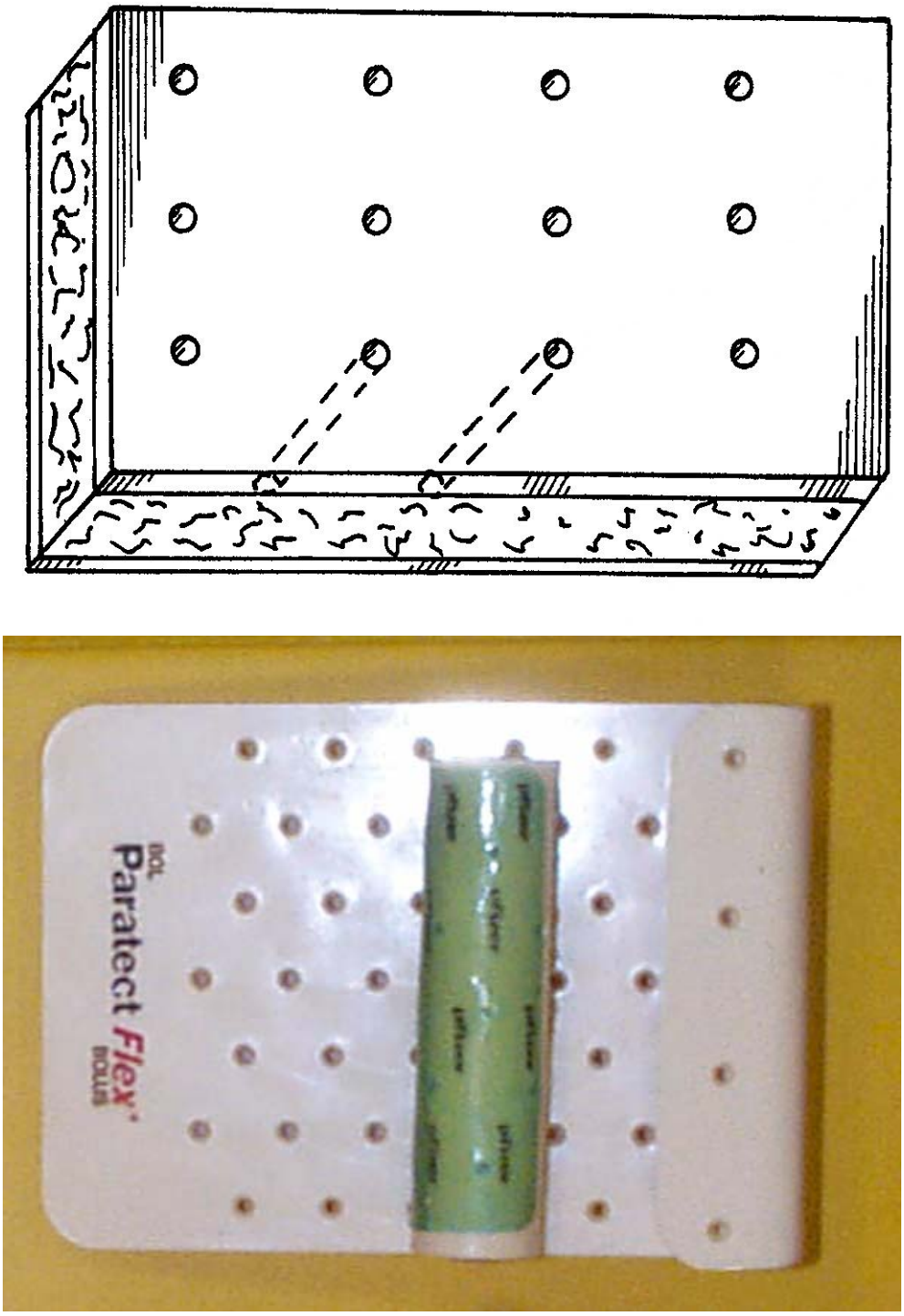


Figure 1-5: Matrix diffusion devices
Top: Diagram from patent Cardinal 1986. Bottom: The Paratect® flex.

1.5.2.3 Osmotic pump

The osmotic pump is one of the more complicated intraruminal controlled-release devices, but it is capable of zero-order delivery for long periods. This technology uses a material that absorbs water and swells to extrude formulation into the rumen. Water diffuses inward at a rate determined by the semi-permeable

material surrounding the absorbent material. This diffusion rate determines the release rate (Eckenhoff et al. 1986). A well-documented example is the Ivomec SR® bolus (Figure 1-6), which is retained in rumen by its density while releasing the antiparasitic Ivermectin to cattle 135 days.

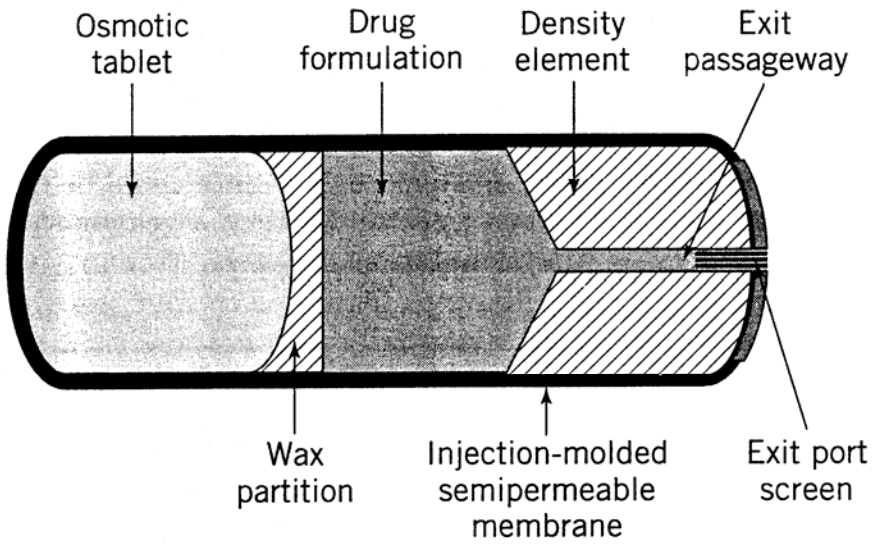


Figure 1-6: The Ivomec SR® bolus
(Cardinal and Witchey-Lakshmanan 1992)

The water uptake rate is determined by the osmotic gradient across the membrane. A tablet of sodium carbopol and sodium chloride generates an osmotic gradient of about 300 atm (~30400 kPa). This gradient decreases as the tablet absorbs water, decreasing the water diffusion rate through the membrane. This effect is offset by the swelling of the tablet, which increases the membrane area available for diffusion. These two counteracting factors provide a near constant release rate (Zingerman et al. 1992; Zingerman et al. 1997).

When developing the Ivomec SR® bolus, a highly variable release rate was observed *in vivo*. This was attributed to gas bubbles dissolved in rumen fluid entering the drug formulation (Zingerman et al. 1997). Increasing the pressure in the payload chamber by adding a screen to the orifice (Figure 1-6) and a thickener to the drug formulation produced a more constant release rate. However, due to this increased pressure, it took four weeks to reach steady-state release. This has been reduced to one to two weeks by adding a small amount of water to the

packaging so that pressure builds during storage (Wright et al. 1993a; Wright et al. 1993b; Wright et al. 1995).

The wax-based formulation the Ivomec SR® bolus delivers is solid below ~35°C, where it softens sufficiently for extrusion (Zingerman et al. 1997). The function of this device, therefore, depends on the temperature in the rumen not dropping below 35°C. This can occur (for example if the cow drinks a large amount of cold water), but only for persists for a short time.

Osmotic pump technology is restricted by the payload volume that can be loaded. The osmotic tablet, blank partition and density element occupy space within the Ivomec SR® bolus (though their proportions are somewhat exaggerated in Figure 1-6) leaving room for about 7.5 g of drug formulation, which is only 22% (by weight) Ivermectin (Zingerman et al. 1997). Thus, this form of osmotic pump is applicable for only the most potent bioactives as this ~1.7 g of bioactive must treat an animal weighing several hundred kilograms for more than four months.

Release rate is not easily adjustable as this requires changing the osmotic gradient or membrane permeability. Changing the concentration of the bioactive in the payload may be the simplest method of altering the rate of release, though this would be greatly limited in range by the device's low capacity and the viscosity and melting point requirements of the formulation.

1.6 A controlled-release device using the gas cell

This project investigated an intraruminal controlled-release technology that was driven by the gas-producing cell. Pressure generated by a gas cell was used to drive a plunger through a cylindrical barrel and extrude formulation. This concept had been used previously in several other applications (Section 1.6.2).

1.6.1 The gas-producing cell

The gas-producing cell (Figure 1-7) is a small galvanic cell that releases hydrogen gas through two small orifices when a electrical current is drawn (Winsel 1993). Hydrogen is produced from water in association with zinc oxidation at a rate dependant on the flow of electrons from the anode to the cathode (Gröning et al.

1999). Gas production is initiated by forming an electrical connection between the electrodes and the rate can be controlled by applying different resistances to the circuit. The gas cell will consume oxygen, if available, instead of producing hydrogen, though a "blue sticker" (not shown in Figure 1-7) covering the orifices prevents oxygen from entering (Winsel 1993). The gas cell can produce about 160 mL of hydrogen (gas-cell product information, Simatec Undated-a), presumably measured at room temperature and atmospheric pressure.

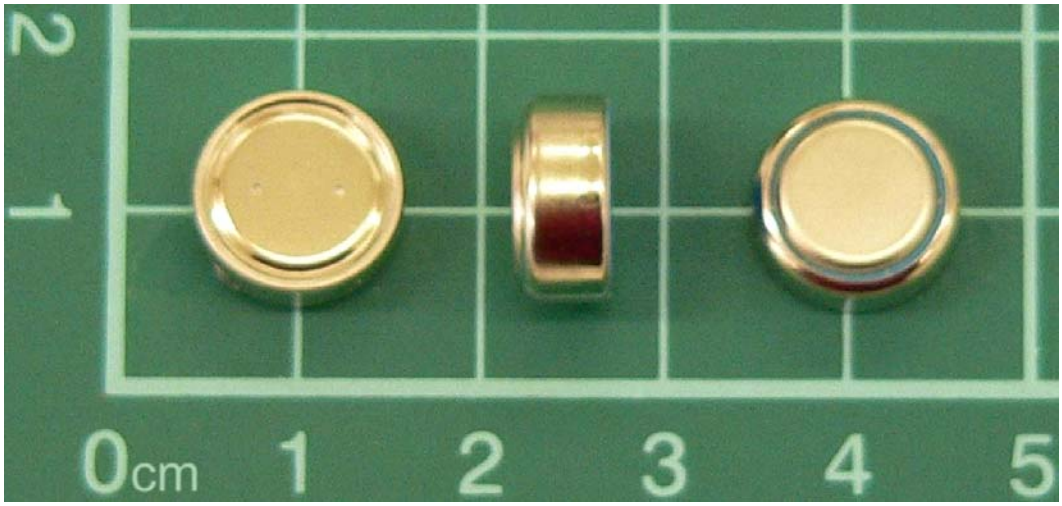


Figure 1-7: The gas-producing cell

1.6.2 Existing products that use the gas cell

The inventor of the gas cell suggested that its primary application was transporting liquids and there have been several attempts to create commercial products with this technology. The most successful of these, with over one million units sold annually, is the Simalube (Winsel and Sauer 2000; Simatec Undated-b), which is used to apply lubricant to machinery. This device is available in 30-, 60-, 125- or 250-mL versions and can be set to release over one to twelve months by adjusting a dial. The 125-mL Simalube uses two gas cells, which indicates that high pressure is required to extrude lubricant because each gas cell should produce about 160 mL of gas at atmospheric pressure. The Simalube can operate at temperatures between -20°C and 55°C, though tables are provided instructing the user in how to compensate the rate of release for different temperatures.

The Theratron infusion system (Fresenius, Figure 1-8) is another commercial application (Winsel and Sauer 2000). This is a syringe that automatically injects a patient with up to 60 mL of any chosen liquid.

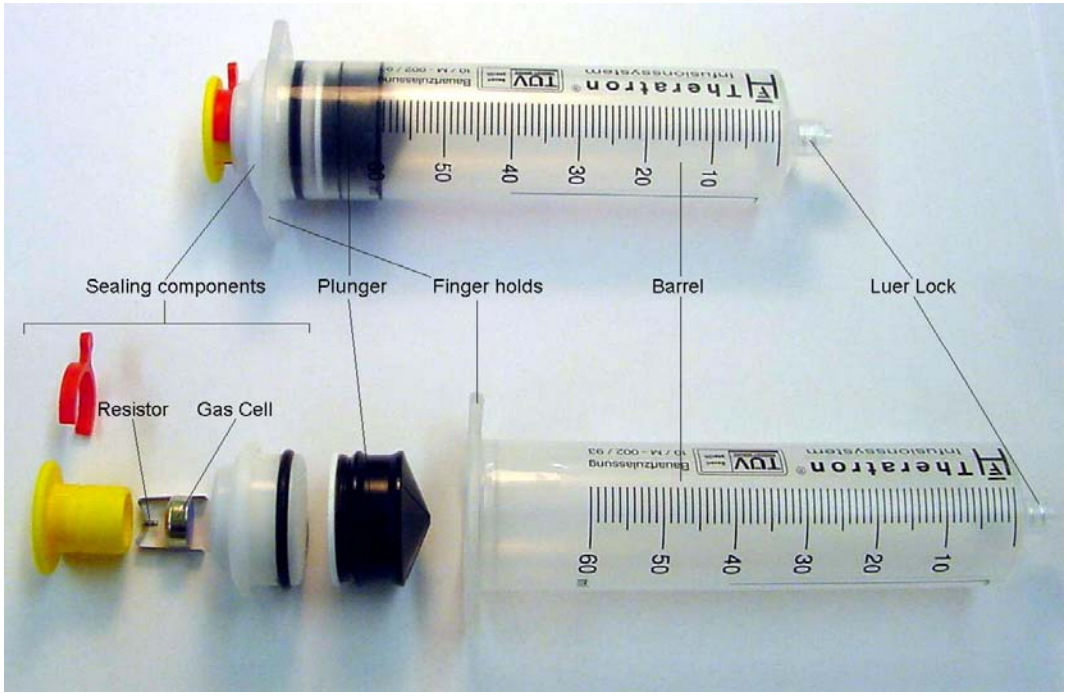


Figure 1-8: The Theratron

The sealing components contain a gas cell and a resistor, which contact after a button is pressed. This also seals the Theratron (Figure 1-9). If the gas cell "short circuits" (due to moisture for example), gas production rate may increase significantly giving a "dose dump". A flow limiter is provided to increase pressure causing the gas is released by the sealing components in the event of a dose dump. Theratrons are fitted with one of two resistors, which give a pumping rate of 2.5 or 0.5-mL per hour. Users are advised to expect a variation of +15% to -20% in this rate and a temperature compensation table is also provided (Theratron product information).

Several experimental gas cell driven controlled-release devices have been reported. A device that can give pulsed, transdermal drug delivery used a gas cell to empty blocks of liquid formulation separated by air blocks from a capillary (Gröning and Kuhland 1999). The same research group also used gas cells in a capsule to deliver pulses of a formulation that could be remotely controlled by a

computer via a magnetic switch (Gröning et al. 1999). An injection-moulded insert driven by a gas cell was used to deliver progesterone to the vagina of cattle for 8 days (Rathbone et al. 2002; Ismail 2006). This device 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.

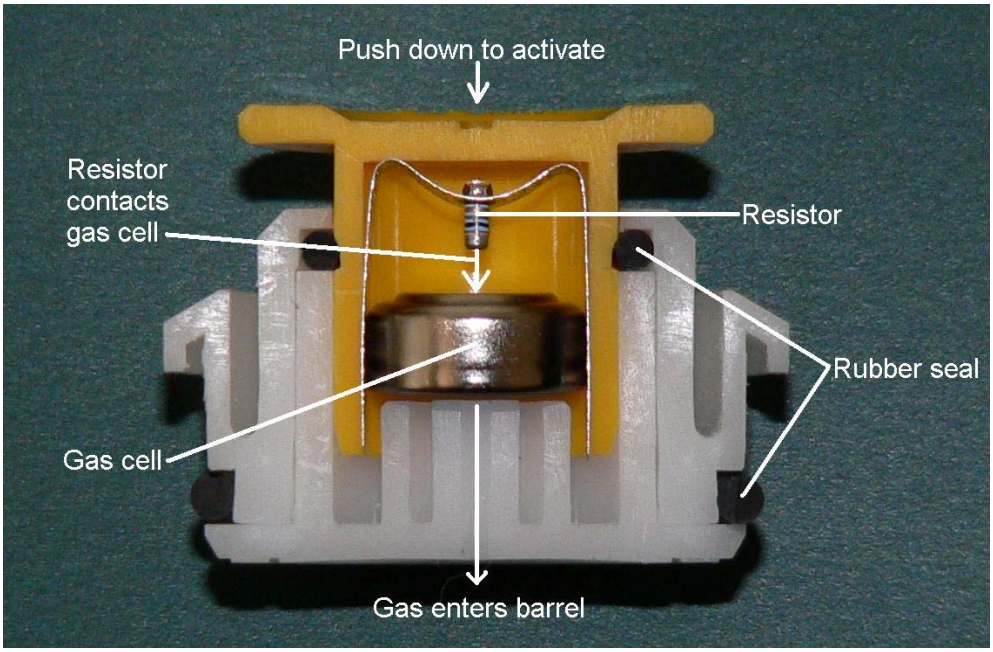


Figure 1-9: Cross-section of Theratron components containing a gas cell

1.6.3 Gas volume

Gas-driven devices rely on the payload being displaced by a volume of gas. Gas volume is related to the number of gas molecules, their temperature and pressure by the ideal gas law:

$$PV = nRT \qquad 1\text{-}1$$

where P is pressure (kPa), V is volume (L), n is the number of moles of gas, T is the temperature (K) and R is the gas constant (8.314 $J \cdot mol^{-1} \cdot K^{-1}$). Real gases will closely follow this equation when close to room temperature (25°C) and atmospheric pressure (101.325kPa, Atkins 1990). This has important consequences for a gas-driven device since gas volume, and therefore release rate, will depend on ambient temperature and pressure, both of which are reasonably constant in the rumen. The most relevant unit for measuring gas volume in this

study is millilitres (mL), at atmospheric pressure (101.325 kPa, 1 atm) and a convenient approximation of average rumen temperature (40°C, 313.15 K).

1.6.4 Gas diffusion

If two different gas mixtures are separated by a permeable material, gases will diffuse through the material until equilibrium is reached. This has consequences for a device that is driven by a gas cell. The gas inside will be primarily hydrogen, but the ambient gas environment will probably contain little hydrogen meaning that hydrogen will diffuse out and the ambient gases will diffuse in. The diffusion rate for each gas at steady-state can be described by Fick's first law (Martin et al. 1983; Atkins 1990):

$$J = -D\frac{dC}{dx} \qquad 1\text{-}2$$

where J is flux ($kg \cdot m^{-3} \cdot s^{-1}$), $\frac{dC}{dx}$ is the change in gas concentration (C, $kg \cdot m^{-2}$) within the permeable material with distance (x, m) and D is the diffusion coefficient ($m^2 \cdot s^{-1}$). The concentration of gas in the permeable material at the interface with the gas phase is proportional to its partial pressure and its solubility in the permeable material. At steady-state, the change in concentration is the difference between the gas concentration at the inner and outer surface of the permeable material (Middleman 1998). The volumetric diffusion rate is therefore directly proportional to the partial pressure difference and the surface area of the permeable material, and is inversely proportional to the thickness of the permeable material. The diffusion coefficient is influenced by the properties of the materials involved and can vary with temperature and concentration of the diffusing species.

There are several examples of gas diffusion being used beneficially in intraruminal controlled-release devices. It has been proposed that iodine could be encased in polyethylene and slowly released into the rumen as a gas by diffusion (Marston 1962). The observation that inward diffusion of gases gives an initial burst of pressure in sealed spring-erosion devices (Section 1.5.2.1) led to the suggestion that gas diffusion alone could drive an intraruminal controlled-release

device. A permanent partial-pressure gradient could be generated by containing a volatile substance in a material that is impermeable to that substance, but not the surrounding gases. The pressure generated by inward gas diffusion could be used to extrude bioactive into the rumen (Costigan et al. 1995).

The effect of gas diffusion on the device used in this study will more likely be detrimental. The rate of plunger movement in intravaginal gas cell controlled-release devices decreased with time, which was attributed gas loss by diffusion through the barrel wall (Ismail 2006). Changing the barrel material and applying a layer of aluminium tape reduced this to an acceptable level.

1.6.5 Advantages of a gas cell controlled-release device

A gas cell controlled-release device has advantages over existing technologies if a constant release rate can be achieved. The gas cell operates independently of its environment at an easily adjustable rate and occupies very little space, which allows a maximum payload volume.

This should be a safe delivery method if safety measures, like those used in the Theratron (Section 1.6.2) are incorporated. The gas cell would probably be expelled from the rumen if it were to escape the device and is too large to pass through the reticular omasal opening. Aluminium pellets with similar dimensions to a gas cell and a slightly lower density have been shown to be regurgitated by sheep at about 23% per month (Dewey et al. 1958). In the absence of a driving force, the payload should remain inside the barrel and not harm the animal.

The ability to monitor the progress of a device by measuring its weight or the displacement of the plunger is advantageous for the development an intraruminal delivery system. *In vivo* release from the Ivomec SR bolus was measured by fitting a container to the device to collect extruded formulation (Zingerman et al. 1992) while the Paratect flex must be completely dissolved and assayed for remaining bioactive (Boettner et al. 1988).

1.7 Aims

The aim of this project was to develop an intraruminal controlled-release device driven by hydrogen from a gas-producing cell with the following qualities:

- A constant release rate
- A release rate that is easily adjustable over days to months
- Independent of environmental factors
- The maximum amount of formulation for the volume of the device
- Safe for animals and subsequent human use

1.8 Research outline

Chapter 2 describes the initial experiments to test the hypothesis that gas-cell-driven controlled-release technology can be adapted for the bovine rumen. Devices based on earlier work were tested *in vitro* and *in vivo* in fistulated and intact cows to identify the challenges in adapting this technology for the rumen.

Chapter 3 describes the trials and development to increase the understanding of how the device functions. Methods were developed to measure gas production and diffusion through the barrel walls. An equation was derived to predict the combined effect of factors on device function.

Chapter 4 describes the effect of using various materials to construct the devices for *in vitro* and *in vivo* trials for up to 50 days.

The conclusions and recommendations are listed in Chapter 5.

Chapter 2: Exploratory experiments

A series of trials were undertaken to identify the challenges involved in adapting a gas cell driven controlled-release technology for use in the rumen. Devices were constructed from commercially-available components and tested both *in vitro* in a heated water bath and *in vivo* in the rumen of fistulated cattle.

2.1 Methods and materials

2.1.1 Trial 1

Devices were constructed in triplicate (Section 2.1.1.1) using barrels and plungers from 20-mL Omnifix syringes (B Braun, Germany) and the barrels, plungers and sealing components from 60-mL Theratrons (Theratron Economy 2.5-mL·hour^{-1}, Fresenius, Germany) and tested *in vivo* in the rumen of fistulated cattle. Formulations tested were water, oil & wax and 6% (w/v) HPMC in 70% (v/v) isopropanol. Gas cells were activated with MELF (metalised electrode face) resistors (MMA 0204 Professional MELF, Vishay Beyschlag, Germany) for use in Theratrons and standard resistors (0.25 W and 0.33 W carbon film resistor, Tyco Electronics, United Kingdom) for use in B Braun syringes. One-way valves and coatings were applied to some devices. The variables examined and the experimental design are summarised in Table 2-1.

Table 2-1: Device configurations tested in Trial 1

20-mL devices

Resistor (Ω)	One-way valve	Coating	Contents
1200	✔	✔	HPMC
1200	✔	✔	Oil/Wax
1200	✔	✔	Water
1200		✔	HPMC
1200		✔	Oil/Wax
2700	✔	✔	HPMC
2700	✔	✔	Oil/Wax
2700		✔	HPMC
2700		✔	Oil/Wax

60-mL devices

Resistor (Ω)	One-way valve	Coating	Contents
560	✔		HPMC
560	✔		Oil/Wax
560	✔		Water
560			HPMC
560			Oil/Wax
6800	✔		HPMC
6800	✔		Oil/Wax
6800	✔		Water
6800			HPMC
6800			Oil/Wax
6800	✔	✔	HPMC
6800	✔	✔	Oil/Wax
6800		✔	HPMC

2.1.1.1 Manufacture of devices

The oil & wax formulation was made by heating 1.8 L of sunflower oil (Sunfield Sunflower Oil, Tasti Products Limited, New Zealand) to about 55°C on a hotplate (CS76083V, Industrial Equipment and Control, Australia) while stirring with a magnetic flea. Then 110 g of beeswax (The Herbal Shop and Clinic, Hamilton, New Zealand) was added in small pieces (approximately 1 cm²). Once the wax had melted completely, the heat was turned off and stirring maintained as the mixture cooled.

To produce 2%, 5% and 6% (w/v) hydroxypropyl methylcellulose ("HPMC") (Metolose, Shin-Etsu, Japan), an appropriate weight of HPMC was added to 2.3 L of either MilliQ water or 70% (by volume) isopropanol (Labserv analytical grade propan-2-ol, Biolab, Australia) in water and stirred with an overhead stirrer (RZR 1, Heidolph, Germany) until dissolved. This solution was made to 2.5 L and mixed until homogenous. Before use, HPMC formulations were incubated at 40°C until all gas bubbles cleared.

To prepare the plungers, shafts of 20 mL B Braun syringes were cut off at the reinforcement ring, a few millimetres from the plunger (Figure 2-1a). The centre of the remaining shaft was then melted out with a heated metal rod. The four remaining shaft tabs (Figure 2-1b3) ensured the plunger remained aligned within the barrel while the gap in the centre allowed the gas cell to fit more closely behind the plunger.

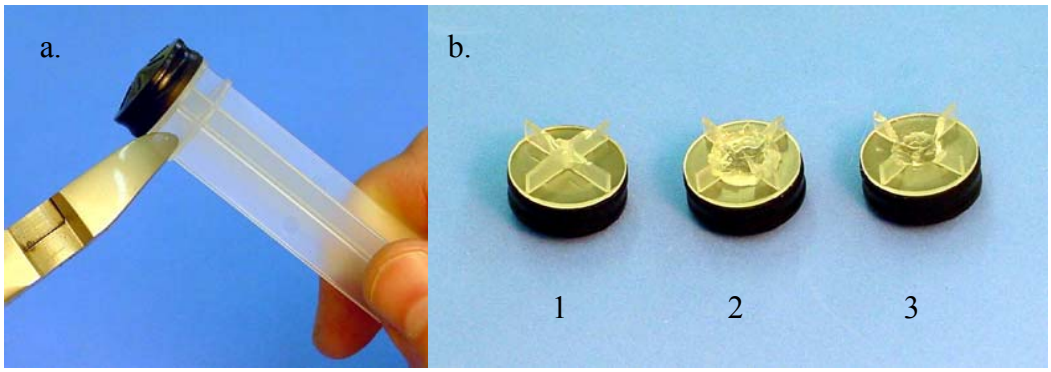


Figure 2-1: Preparation of B Braun plungers
Cutting of the shaft (a). Shaft remnants were melted (b2) and removed (b3).

The barrels of some devices were covered with aluminium tape (Sellotape, New Zealand) and polyethylene terephthalate (PET) shrink-wrap (PTLF470/10JCR and PTLF520/10JCR, Shrink Sleeve, England) to reduce gas diffusion. Two strips of aluminium tape were applied lengthwise along opposite sides of barrel, leaving two ~5-mm wide gaps to allow plunger position to be measured. The taped syringes were then covered with PET shrink-wrap to protect the aluminium tape from the rumen environment and further reduce gas diffusion. Devices were inserted into a barrel-length piece of PET shrink-wrap, which was then shrunk with a heat gun (Kx1682 2 Heat-In Line Heat Gun 1600 W, Black and Decker, England) set at its lowest temperature (approximately 140°C). Barrels were rotated to ensure even heating.

One-way valves were made by placing a 15-mm length of heat-shrink tube (XLP3BK or XLP3WH, Cabac, Australia) over the syringe orifice (Figure 2-2a). The tube was then shrunk with a heat gun (Figure 2-2b) at its highest temperature (approximately 560°C). The protruding heat-shrink tube was gently squeezed while still hot to flatten it (Figure 2-2c). The flattened section was cut off, leaving about 5 mm protruding from the end (Figure 2-2d). This was covered with either a 15-mm length of clear PVC tube stuck over the luer lock with superglue (Figure 2-2e) or a Theratron luer end-cap screwed into the luer lock and cut off just beyond the valve (Figure 2-2f). To check that air could pass through the valve, a plunger was inserted into the barrel to expel all the air from the syringe. The plunger was then retracted and held for approximately 5 s to check that air could not pass back through the valve. Valves that failed either test were discarded.

Each barrel was filled with the appropriate formulation, then the plunger was inserted and used to expel any trapped air from the orifice.

Gas cells (Varta 4690 11.6-mm by 5.4-mm gas-producing cell, Simatec, Switzerland) used in Theratron syringes were activated with Theratron sealing components using MELF resistors (Figure 1-8 and Figure 1-9). A narrow ring of rubber hose was used to hold a resistor in contact with the gas cell terminals in B Braun syringes. The blue sticker (Section 1.6.1) was first removed so the resistor contacted the top of the gas cell.

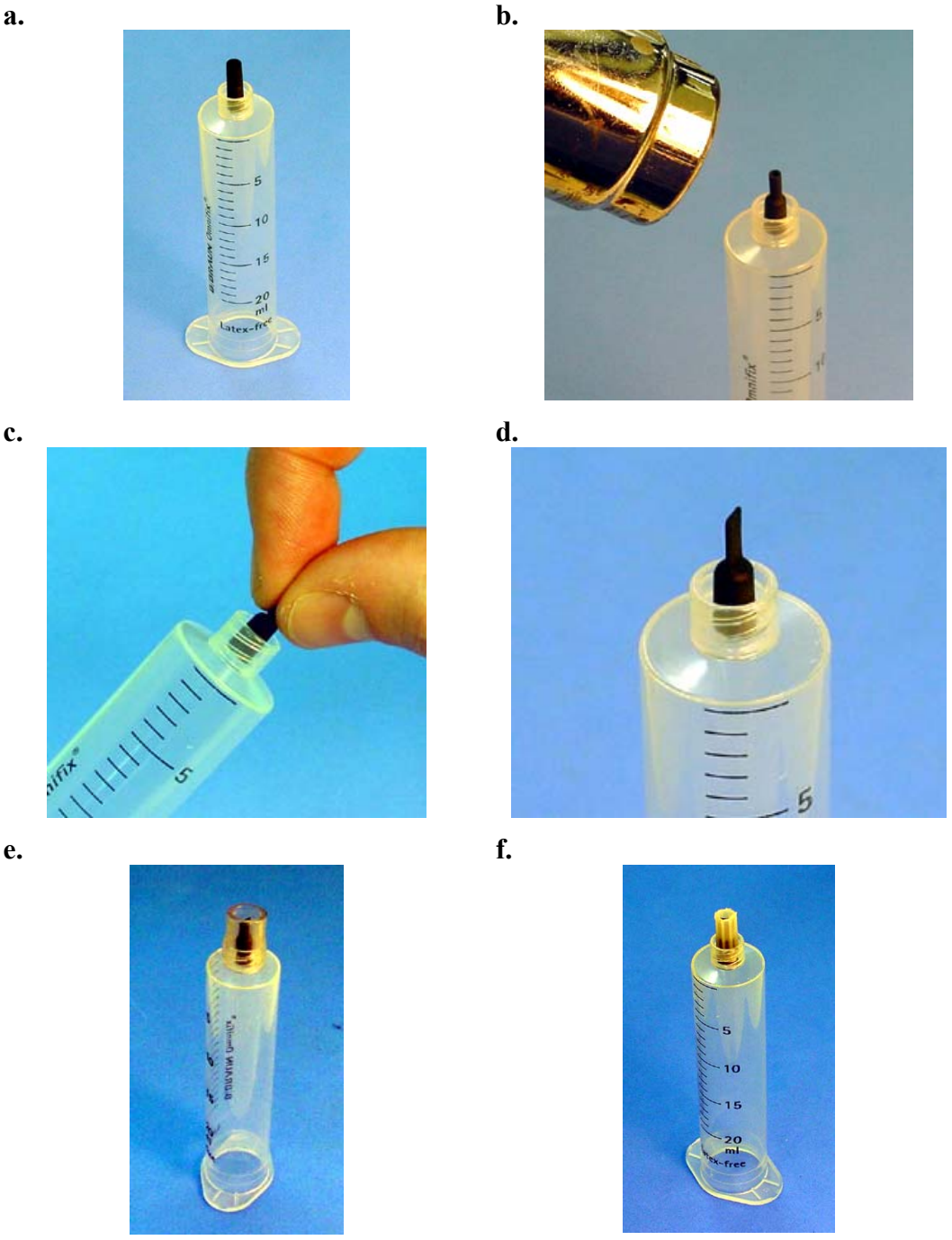


Figure 2-2: Construction of a one-way valve
a. Heat-shrink tube placed over the orifice. b. Heating the tube. c. Squeezing the tube. d. The completed valve. e. The valve protected with PVC tube. f. The valve protected with a Theratron luer end-cap.

Devices made from Theratron barrels were sealed by inserting Theratron sealing components containing the gas cell and resistor into the back of the loaded barrel and depressing the activation button (Figure 1-9). An activated gas cell was placed behind the plunger of devices made from B Braun syringes before a second

cut down plunger was inserted behind the gas cell to seal the device. A constriction in the barrel prevented pressure from forcing the second plunger out.

2.1.1.2 Experimental conditions

Activated devices were clipped to chain tethers (Figure 2-3) and inserted through the cannulae of ruminally-fistulated cattle. The chains were first covered with heat-shrink sleeve to prevent tangling in the rumen then joined end to end with split-rings and fishing swivels. An additional split-ring and swivel was used to attach a clip (Black New Ball-Bearing Swivel with Costlock Snap, Wilson, Korea) to one end of each length of chain. The devices were attached to the clips with a split ring attached to a hole drilled in a finger-hold (Figure 1-8) of each device. Tethers were anchored to the cannulae (Figure 2-4) with nylon fishing line (0.044 inch, Stren, Japan).

Figure 2-3: A chain tether

The ruminally-fistulated Friesian cows were kept at Dexcel Number 5 Dairy (Hamilton, New Zealand) and fed pasture supplemented with silage. All experiments involving animals were first approved by the AgResearch Animal Ethics Committee. Four or five devices were initially inserted into each cow but no cow had more than four devices after the short-term devices were removed. The weight and plunger position (Section 2.1.1.3) of short- and long-term devices were measured 11 and 29 times respectively during the trial.

Figure 2-4: Devices in a fistulated cow
Prototype devices tethered to a cannula. Photograph by Dr Craig Bunt (InterAg).

2.1.1.3 Sampling

The cannula was opened and all devices were removed from the rumen by gently pulling the tether. Each device was then unclipped from the tether and placed in a bucket of 40°C-water to prevent pressure changes due to cooling. Each device was removed from the bucket, dried with a paper towel, measured as described below, and then returned to the 40°C-water.

The plunger position was measured on opposite sides of the barrel using electronic callipers (CD-6″ CS, Mitutoyo, Japan or DSE digital callipers, Dick Smith Electronics, New Zealand). The average of these two values was used for further analysis to account for misalignment of the plunger. Weight of each device was measured using an electronic balance (CP3235 or PT1500, Sartorius, Germany). Devices were examined visually and any unusual observations were recorded. Particular attention was given to the state of the one-way valve and any contamination of the contents. After being measured, devices were reattached to the tether in random order and inserted into the rumen. The cannula plug was then replaced. Each device was out of the rumen for a maximum of 5 minutes.

2.1.2 Trial 2

A second experiment was performed *in vivo* to further investigate the data obtained in Trial 1 and to test new variables (Table 2-2). 60-mL Terumo syringes (Terumo, U.S.A.) were tested in addition to the syringes and formulations tested in Trial 1. Gas cells were activated using resistors of three different values for each syringe size. Devices were either tethered to the cannula (Section 2.1.1.2) or retained in the rumen by the variable geometry provided by retention wings from Rumensin capsules (Elanco, New Zealand). These wings were selected because the Rumensin capsule is a similar size to a 60-mL syringe and has wings that can be removed. Devices with retention wings were tested in fistulated and intact cattle. All devices were fitted with one-way valves.

Table 2-2: Device variables tested Trial 2

Fistulated

Resistor (Ω)	Tethered	Syringe	Contents	Replicates
3900		Terumo	Oil/Wax	4
3900		Theratron	Oil/Wax	5
6800		Terumo	Oil/Wax	4
6800		Theratron	Oil/Wax	5
1000	✔	B Braun	Water	3
4700	✔	B Braun	Water	3
10000	✔	B Braun	Water	3
1000	✔	B Braun	Oil/Wax	3
4700	✔	B Braun	Oil/Wax	3
10000	✔	B Braun	Oil/Wax	3
1000	✔	B Braun	HPMC	3
4700	✔	B Braun	HPMC	3
10000	✔	B Braun	HPMC	3
560	✔	Theratron	HPMC	3
3900	✔	Theratron	HPMC	3
6800	✔	Theratron	HPMC	3

Intact

Resistor (Ω)	Syringe	Recovery day	Replicates
3900	Terumo	19	4
3900	Terumo	39	3
3900	Theratron	19	4
3900	Theratron	39	3
6800	Terumo	39	3
6800	Terumo	82	4
6800	Theratron	39	3
6800	Theratron	82	4

2.1.2.1 Manufacture of devices

Devices were constructed using methods described in Section 2.1.1.1. Untethered devices were retained in the rumen by variable geometry. The syringe finger-holds were first removed with a lathe to prevent harm to the animal during dosing. Retention wings were removed from Rumensin capsules and cut down as shown

in Figure 2-5. The dome holding the wings was reduced to a diameter of 15 mm with a lathe and a 9-mm hole was drilled through its centre. The luer locks (Figure 1-8) of syringe devices were inserted into the 9-mm hole in the wings and held in place with superglue (Prism 406, Loctite, Australia) after treating both surfaces with polyolefin primer (770 Activator, Loctite, Australia).

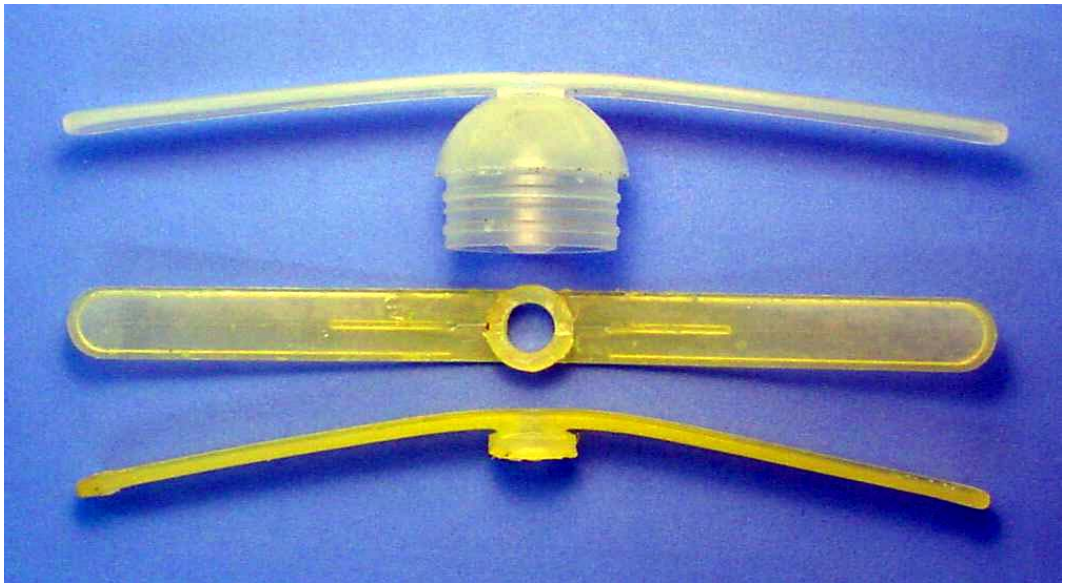


Figure 2-5: Retention wings from the Rumensin capsule
Top: Complete wings. Middle and bottom: Cut-down wings ready for attachment. The yellow colour is due to being in the rumen.

Several devices were regurgitated by the animals within the first few days of this trial when their wings became detached. This problem was overcome by reinforcing the wing attachment by winding cotton around the joint and covering the cotton with superglue. No further devices were regurgitated from fistulated cows. Whenever a device from an intact cow was regurgitated (and found), a new set of wings were attached using the modified wings and the device was reinserted. However, there was no way to confirm the presence of each device in an intact animal, so not all regurgitated devices were found and eight were missing at the end of trial. One device from an intact cow still had wings attached by the original method when it was recovered on day 82.

2.1.2.2 Experimental conditions

All cows were maintained as a single herd. A maximum of four devices were placed in the rumen of fistulated and intact cows and retrieved at appropriate times. The seven intact cows were dosed using a dosing gun.

2.1.2.3 Sampling

Devices were removed periodically from fistulated cows (Section 2.1.1.2). Short-term devices were retrieved and measured 10 times before being removed. Medium- and long-term devices were measured 21 times before being removed. Devices with retention wings were manually removed from the rumen, taking care not to damage the wings. Intact cows were slaughtered at the Ruakura Abattoir (Hamilton, New Zealand) for removal of devices after 19 (two cows), 30 (three cows) and 82 (two cows) days. Devices were stored in water at 40°C for up to 30 minutes before taking measurements.

2.1.3 Plunger swelling

Plungers from 60 mL Theratrons, 60 mL Terumo syringes and 20 mL B Braun syringes were subjected to a variety of liquids at 40°C in a stability cabinet (Digital series cooled incubator, Contherm Scientific Ltd., New Zealand) to assess the potential for interactions. Triplicate plungers were submerged in water, sunflower oil, isopropanol, fish oil (manufacturer unknown), silicone oil (collected from Rumensin capsules), hexaglycerol disterate & Span80. Control plungers were left in air.

2.1.4 *In vitro* trial

A full factorial experiment was done in triplicate *in vitro*. Devices were constructed as described in Section 2.1.1.1 with two release rates, (using the same resistor values as the 10- and 100-day devices in Trial 2), three barrel types (the same as Trial 2), two formulations (water and oil & wax) and two plunger types for 60-mL devices (Theratron plungers and "Elanco" plungers from Rumensin capsules). The combinations of variables evaluated are shown in Table 2-3.

2.1.4.1 Experimental conditions

Devices were kept in a 40°C temperature-controlled bath (Figure 2-6) made from a 1170-mm by 770-mm by 260-mm plastic tub reinforced with two double-lengths of corner-protector and covered with Perspex sheets. Water in the bath was heated and circulated with two water-bath heaters (Thermomix 1419, B Braun, West Germany) and two fish-tank pumps (Submersible pump, Resun,

China). A rack to hold devices in the bath was constructed by bolting lengths of corner-protector into a frame. Lead fishing sinkers were wired to the frame to add weight. Channels to hold the finger-holds of syringe barrels were made by stringing wire across the frame.

Table 2-3: Device configurations tested *in vitro*

Body	Plunger	Contents	Valve
Theratron	Elanco	Water	✔
Theratron	Elanco	Water	
Theratron	Elanco	Oil/Wax	✔
Theratron	Elanco	Oil/Wax	
Theratron	Theratron	Water	✔
Theratron	Theratron	Water	
Theratron	Theratron	Oil/Wax	✔
Theratron	Theratron	Oil/Wax	
Terumo	Elanco	Water	✔
Terumo	Elanco	Water	
Terumo	Elanco	Oil/Wax	✔
Terumo	Elanco	Oil/Wax	
Terumo	Theratron	Water	✔
Terumo	Theratron	Water	
Terumo	Theratron	Oil/Wax	✔
Terumo	Theratron	Oil/Wax	
Bbraun	Bbraun	Water	✔
Bbraun	Bbraun	Water	
Bbraun	Bbraun	Oil/Wax	✔
Bbraun	Bbraun	Oil/Wax	

2.1.4.2 Sampling

Devices were removed from the water bath, dried with a towel and then measured and weighed (Section 2.1.1.3). The method for measuring plunger position depended on the device (Figure 2-7) because Elanco plungers contact the barrel only at the trailing edge. Devices were returned to the bath within 2 minutes.

2.1.5 The influence of PET and aluminium coatings

The release profile of uncoated devices and devices coated with aluminium tape and/or PET shrink-wrap was tested. Triplicate 20-mL B Braun, 60-mL Theratron and 60-mL Terumo devices were constructed and filled with water as described in Section 2.1.1.1. Devices made from Terumo syringes were prepared, activated and sealed by the same methods as 20-mL B Braun syringes. One-way valves were not used. Gas cells for the 60-mL devices were activated with 560-Ω

resistors and 20-mL devices were activated with 1000-Ω resistors. After being activated, devices were put in the 40°C water bath and measurements were taken daily (Section 2.1.4.2).

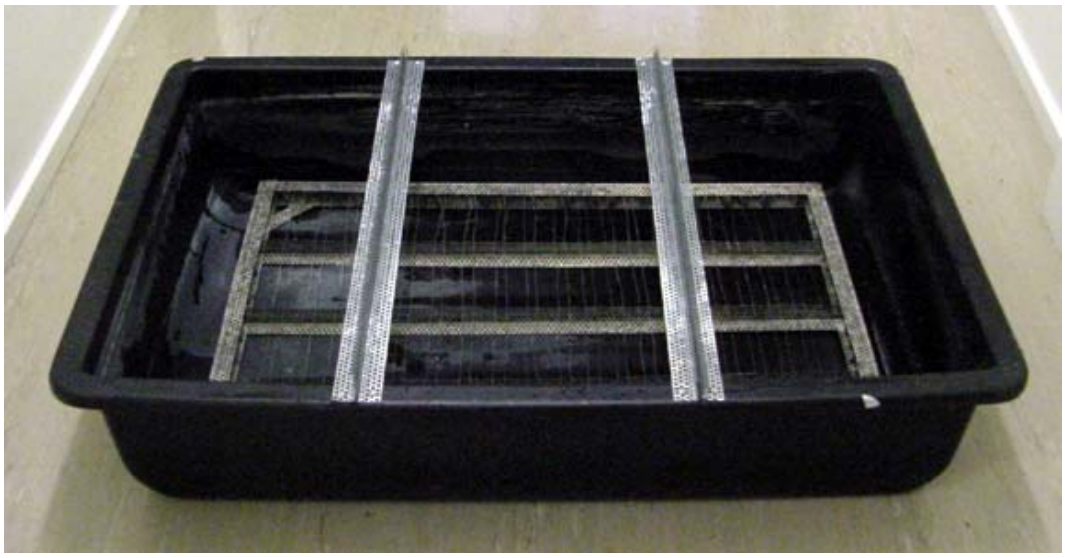


Figure 2-6: The *in vitro* test bath

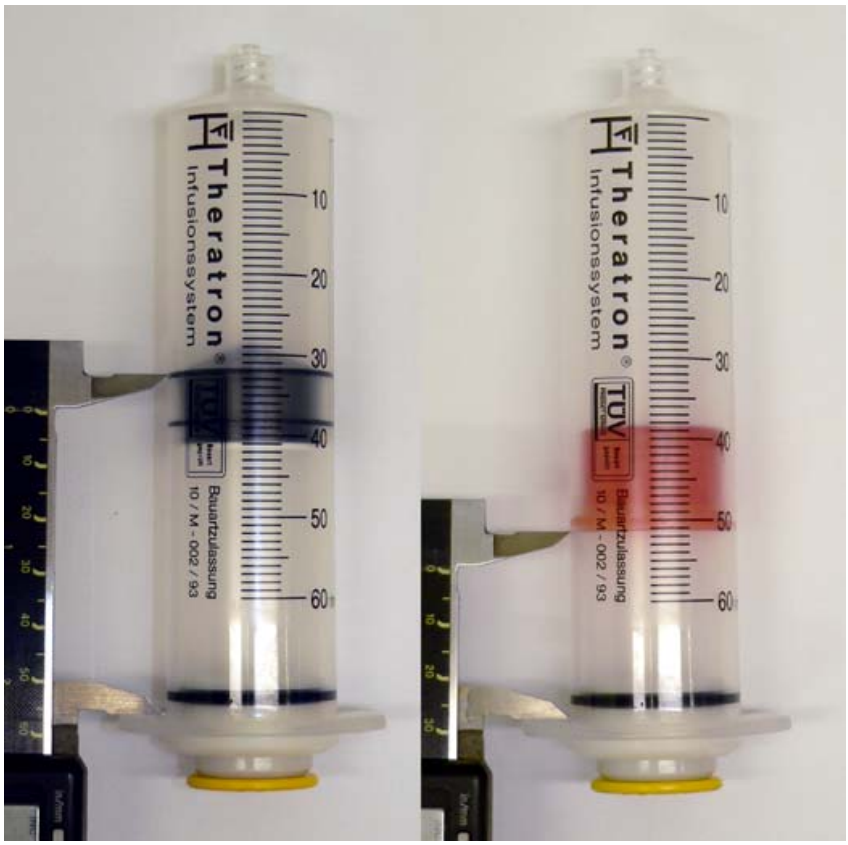


Figure 2-7: Measuring plunger position
Left: Theratron plunger. Right: Elanco plunger.

2.2 Results and discussion

Raw data are in Appendix A.

2.2.1 Trial 1

The primary aim of this project was to adapt the gas cell drive technology for use in the rumen of cattle. This *in vivo* experiment identified the challenges involved. Commercially-available syringes were used to make devices based on previous work on an intravaginal application of this technology (Ismail 2006).

Measurements were taken at the same time of day (starting at 8 am) that the devices were first administered. However, the order that the devices were measured was determined by the random order in which the animals arrived. The error for each time point was therefore approximately ± 1hour.

Average plunger position and weight for each configuration (Figure 2-8, Figure 2-9) were calculated from as few as two devices due to contamination (Section 2.2.1.3). Data from slow-releasing, 60-mL devices were very variable so raw data are presented. There was a direct correlation between plunger displacement and device weight except when digesta and rumen gas entered due to failure or absence of a one-way valve.

2.2.1.1 Release rate

To demonstrate the versatility of this technology, devices were set for fast (full payload over 10 days) or slow (100 days) release by using different resistors to activate the gas cells. The appropriate resistor was chosen using data from a previous calibration trial (C. Bunt, pers. comm.).

Devices with the fast gas production rate released at a constant rate and finished close to the intended time of ten days (Figure 2-8 and Figure 2-9). The exceptions were the devices with one-way valves containing oil & wax, which had a slower release rate than the other two formulations. This observation was supported by linear regression where possible (Table 2-4).

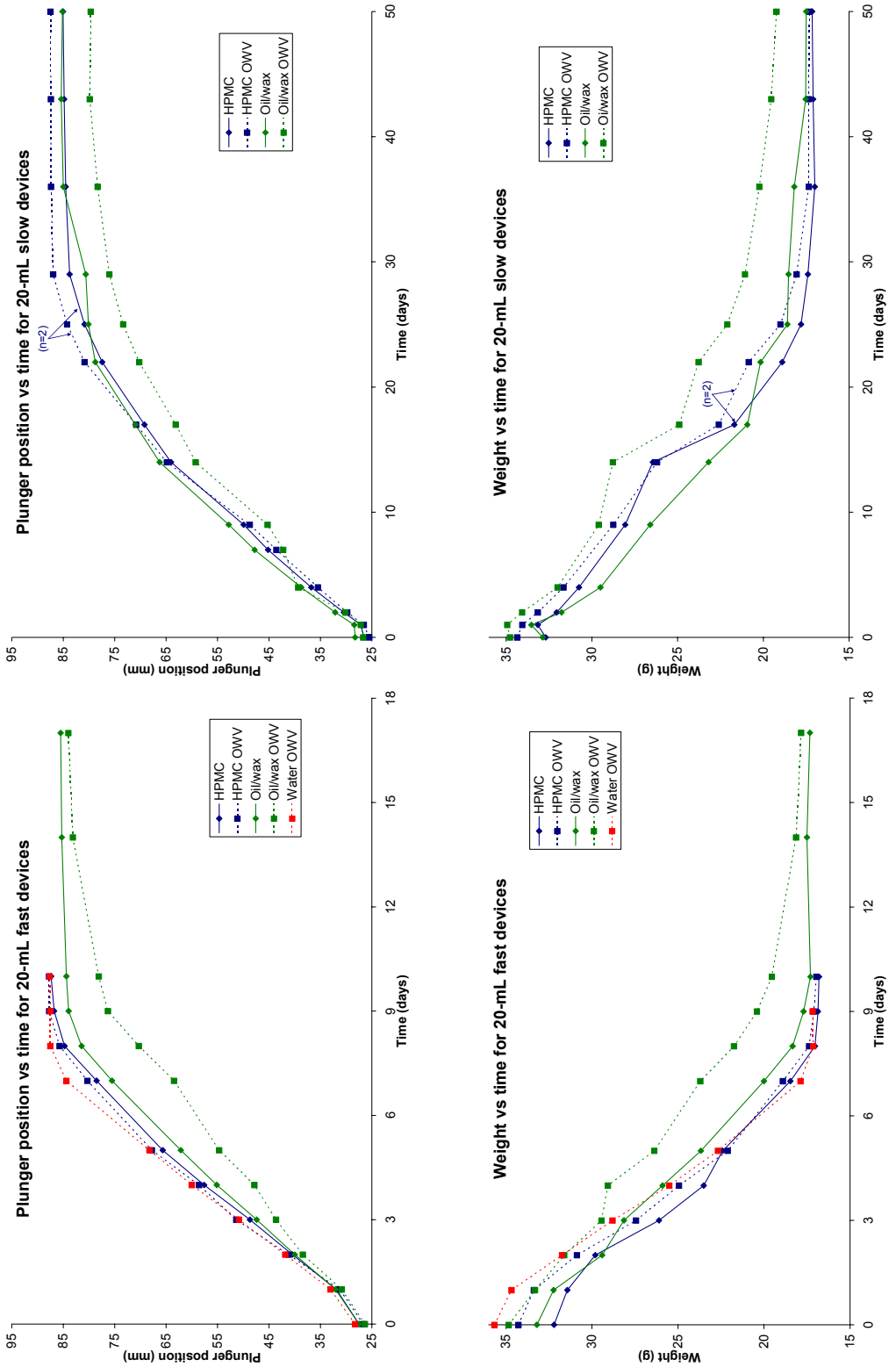


Figure 2-8: Effect of contents, one-way valve and release rate on the release profiles of 20-mL devices
n = 3 unless otherwise stated.

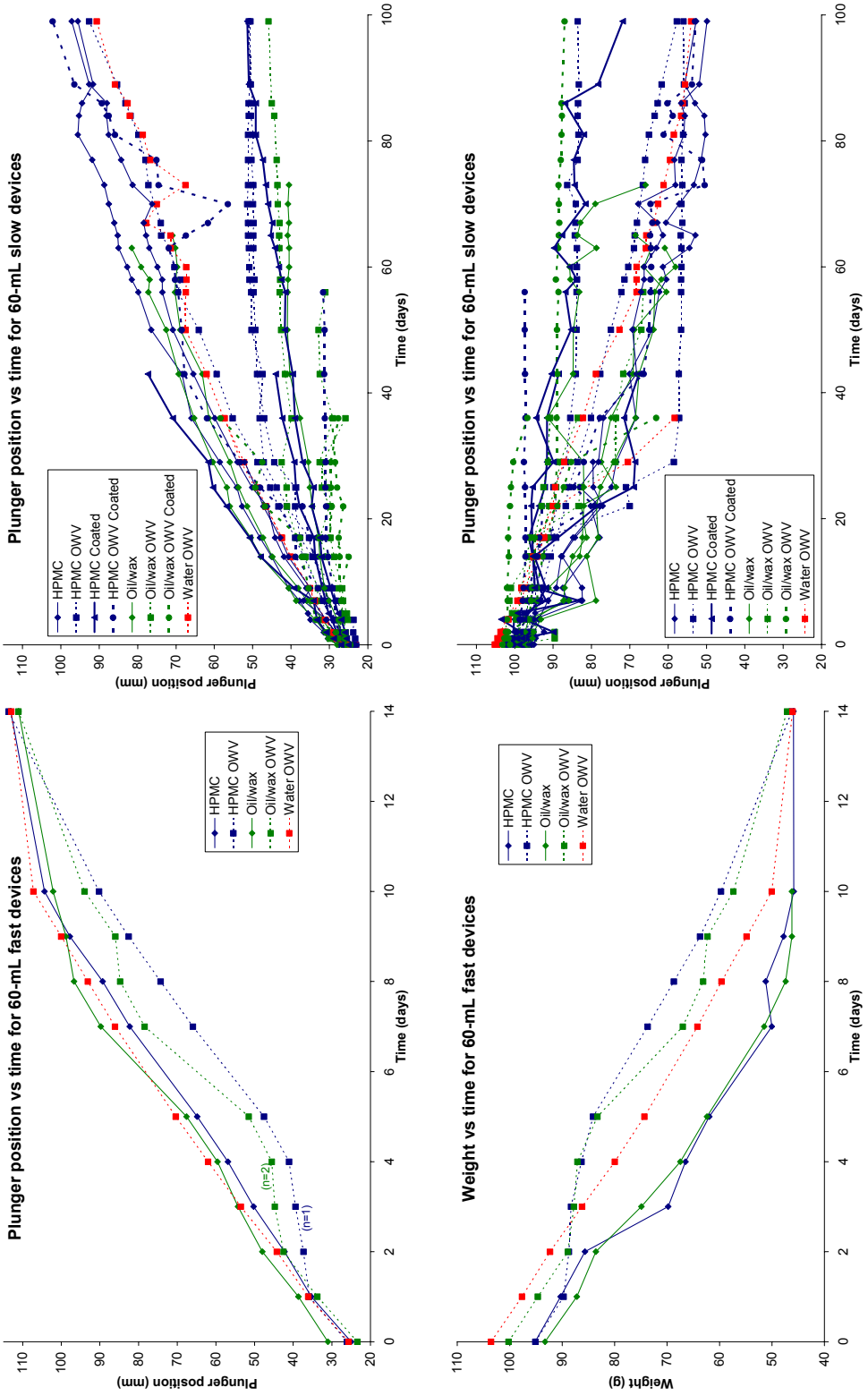


Figure 2-9: Effect of contents, one-way valve and release rate on the release profiles of 60-mL devices

n = 3 unless otherwise stated. n = 1 for all 60-mL, slow devices.

Table 2-4: Average release rate (mL·day^{-1})

		No one-way valve		One-way valve		
Barrel	**Rate**	*Oil/wax*	*HPMC*	*Water*	*Oil/wax*	*HPMC*
20 mL	**Fast**	2.31±0.10 (n=3)	2.60±0.03 (n=3)	2.64±0.06 (n=3)	1.68±0.25 (n=3)	2.74±0.65 (n=3)
20 mL	**Slow**	0.73±0.01 (n=2)	0.80±0.10 (n=2)		0.77±0.06 (n=3)	0.78±0.12 (n=3)
60 mL	**Fast**	6.45±0.11 (n=2)	5.94±0.11 (n=3)	5.49±0.29 (n=3)		
60 mL	**Slow**	0.82±0.05 (n=2)	0.56±0.04 (n=3)	0.58±0.04 (n=2)		0.50±0.09 (n=3)
60 mL coated	**Slow**		0.34±0.01 (n=2)			

Values are calculated by linear regression of plunger position data and converted to mL·day^{-1}. Devices without at least 5 consecutive linear data were omitted.

Slow, 20-mL devices reached their end point after 30 to 35 days, which is about one-third the intended time. Data from slow, 60-mL devices were highly variable and no plunger was within 10 mm of the barrel end by day 100. These results emphasised that gas production rate had not been specifically demonstrated and that the effect of electrical resistance on gas production rate was not well understood.

2.2.1.2 Barrel

Because of the variation in ruminant animal size (e.g., sheep, cattle, etc), different sized intraruminal controlled-release devices are required (typical dimensions of intraruminal devices are suggested in Laby 1987b and Edwards *et al.* 1989). Devices were made from either 20-mL B Braun syringes or 60-mL Theratron barrels to demonstrate that size variation can be easily accommodated with this technology. Barrel size did not appear to affect function as devices made from both barrel sizes operated at a near zero-order rate for the intended delivery time at the faster gas production rate. Devices with the lower gas production rate were not as successful. The initial release rate for 20-mL devices was greater than expected, but then declined (Figure 2-8). This greater release rate may have been due to the use of an incorrect resistor (Section 2.2.1.1), while the decreasing rate may be indicative of outward diffusion of the driving gas through the barrel walls.

2.2.1.3 Coating

Gas diffusion influences the release profile of gas cell controlled-release devices (Ismail 2006). Therefore, some devices were coated with aluminium tape, found previously to reduce gas diffusion to an acceptable level, and PET shrink-wrap.

As a commercial application of this technology, problems of gas diffusion may have been solved for the Theratron so coated and uncoated Theratron-based devices were made to test this. All B Braun devices were also coated.

The effect of PET & aluminium coating on the release profile of each slow, 60-mL device is depicted in Figure 2-10. These release profiles are too variable to determine if the presence of the coatings had any effect on the function of the device. The plungers of many devices moved initially but then slowed and stopped about half way down the barrel. This decrease also occurred for all slow releasing, 20-mL devices (all of which were coated), indicating that the presence of coating did not eliminate outward gas diffusion.

The amount of gas that escapes a device by diffusion is determined by the plunger position, as diffusion is proportional to surface area (Section 1.6.4). If the plunger reaches a position where outward diffusion of hydrogen is equal to the rate of gas production, the plunger will stop. This may have happened to several of the slow-releasing 60-mL devices, though it is unclear why some devices stopped and not others, independently of the coatings. These results indicate that adequate measures have probably not been taken to reduce hydrogen diffusion out of the Theratron. However, the Theratron barrel may allow sufficiently low gas diffusion for the 1- to 3-day use they were intended as the fast Theratron-based devices tested released linearly for 10 days.

Fast-releasing, 20-mL devices had a linear release profile, indicating that PET & aluminium coatings reduced gas diffusion through B Braun barrels to an insignificant rate relative to the gas production rate.

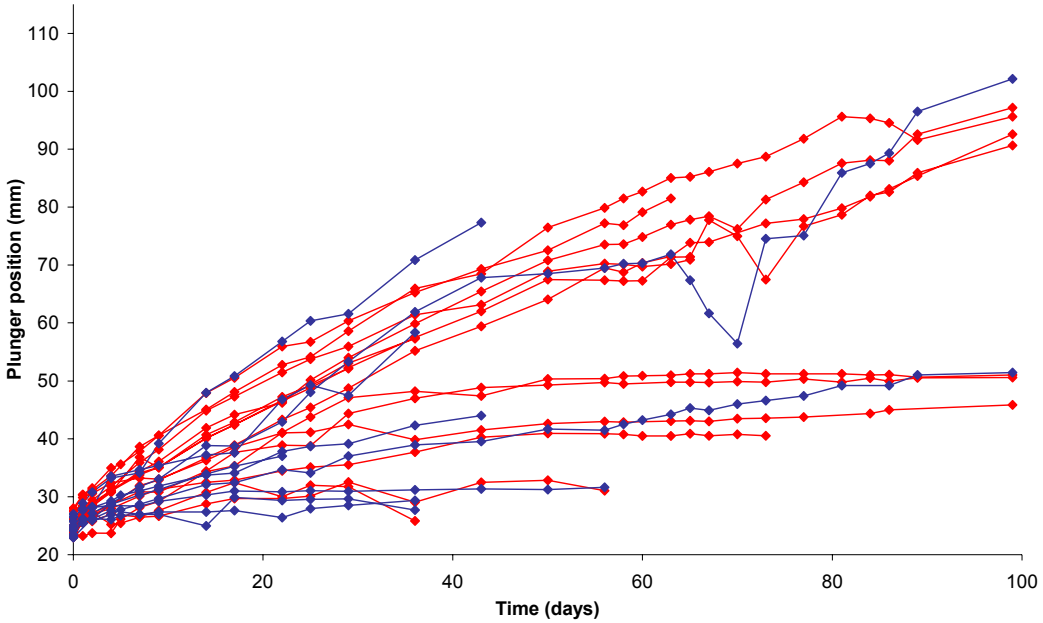


Figure 2-10: Effect of coatings on Theratron devices
× = coated devices, × = uncoated devices.

2.2.1.4 One-way valve

The need for a one-way valve to prevent digesta from entering this type of device has been identified previously (Costigan et al. 1995). Replicate devices with and without one-way valves (Section 2.1.1.1) were loaded with HPMC or oil & wax to test if viscosity or insolubility in water would prevent the formulation from becoming contaminated with digesta. All devices containing water were fitted with one-way valves.

The one-way valve had little effect on the release rate except for the fast-releasing, 20-mL devices containing oil & wax, which released significantly slower with a one-way valve (Table 2-4, further discussed in Section 2.2.3).

The PVC sheaths protecting the one-way valves (Figure 2-2e) were found to be ineffective early in the trial and many valves were lost. After three days all sheaths were replaced with Theratron luer end-caps (Figure 2-2f), which successfully held all one-way valves in place for the duration of the trial.

The fraction of devices contaminated with digesta is shown in Table 2-5 to demonstrate the efficacy of the one-way valves. The effects of contamination on device contents ranged from slight discolouration to total displacement. Digesta

entered all devices without one-way valves and 17 of the 39 devices with valves, though some of the latter may have been contaminated when their one-way valve was dislodged. Devices filled with oil & wax were the least likely to be contaminated, though this may have been because contamination was more difficult to detect in this opaque formulation. More slow devices appeared to be contaminated than fast, possibly due to longer exposure to the rumen environment.

Table 2-5: Effect of one-way valves, barrel type, release rate and contents on the number of devices contaminated with digesta
Data indicate number contaminated digesta from total number of devices tested.

		Fast Devices		Slow devices				Total	
		OWV	-	OWV	-	OWV	-	OWV	-
				Uncoated		Coated			
60 mL	Water	2/3	0/0	2/3	0/0	0/0	0/0	**4/6**	**0/0**
	Oil	0/3	3/3	2/3	0/0	1/3	3/3	**3/9**	**6/6**
	HPMC	1/3	3/3	3/3	3/3	1/3	3/3	**5/9**	**9/9**
20 mL	Water	3/3	0/0	0/0	0/0	0/0	0/0	**3/3**	**0/0**
	Oil	0/3	3/3	0/0	0/0	0/3	3/3	**0/6**	**6/6**
	HPMC	0/3	3/3	0/0	0/0	1/3	3/3	**1/6**	**6/6**
	Total	*6/18*	*12/12*	*7/9*	*3/3*	*3/12*	*12/12*	***16/39***	***27/27***

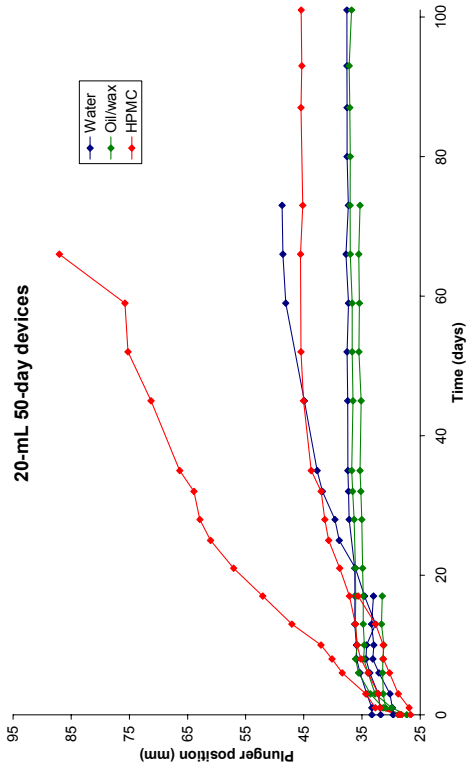
2.2.1.5 Contents

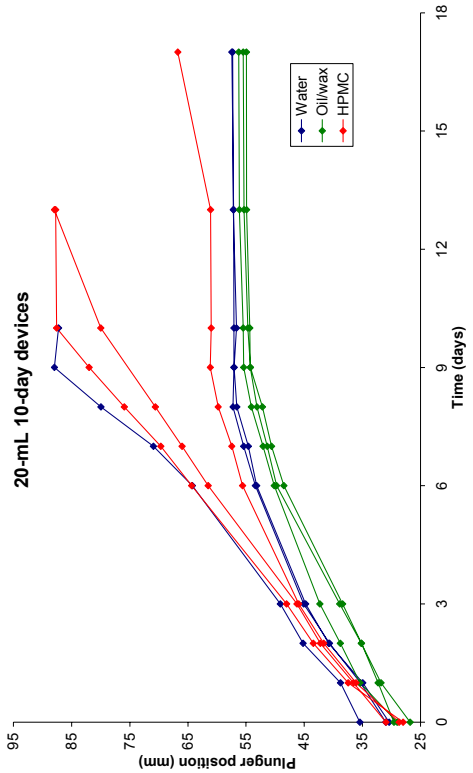
The average release rate from equivalent devices filled with distilled water and 6% HPMC were within experimental variation (Table 2-4). However, oil & wax was released more slowly from fast devices and was not completely expelled from some 20-mL devices because it had solidified. This could indicate that the oil & wax formulation had separated, which could explain its effect on release rate.

2.2.2 Trial 2

Devices made from commercially available components were tested *in vivo* again to further investigate the transition of this technology for use in the rumen. The function of devices in fistulated cows was compared to that of devices in intact cows. All devices were fitted with one-way valves due to the findings of Trial 1.

Results for this experiment were extremely variable as can be seen in Figure 2-11, Figure 2-12 and Figure 2-13 and only a five devices reached completion.





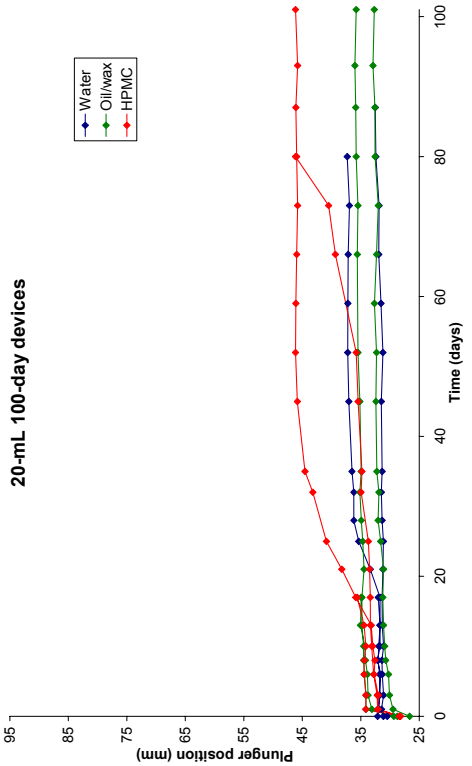


Figure 2-11: Effect of contents and release rate on plunger position of 20-mL devices (n=1)

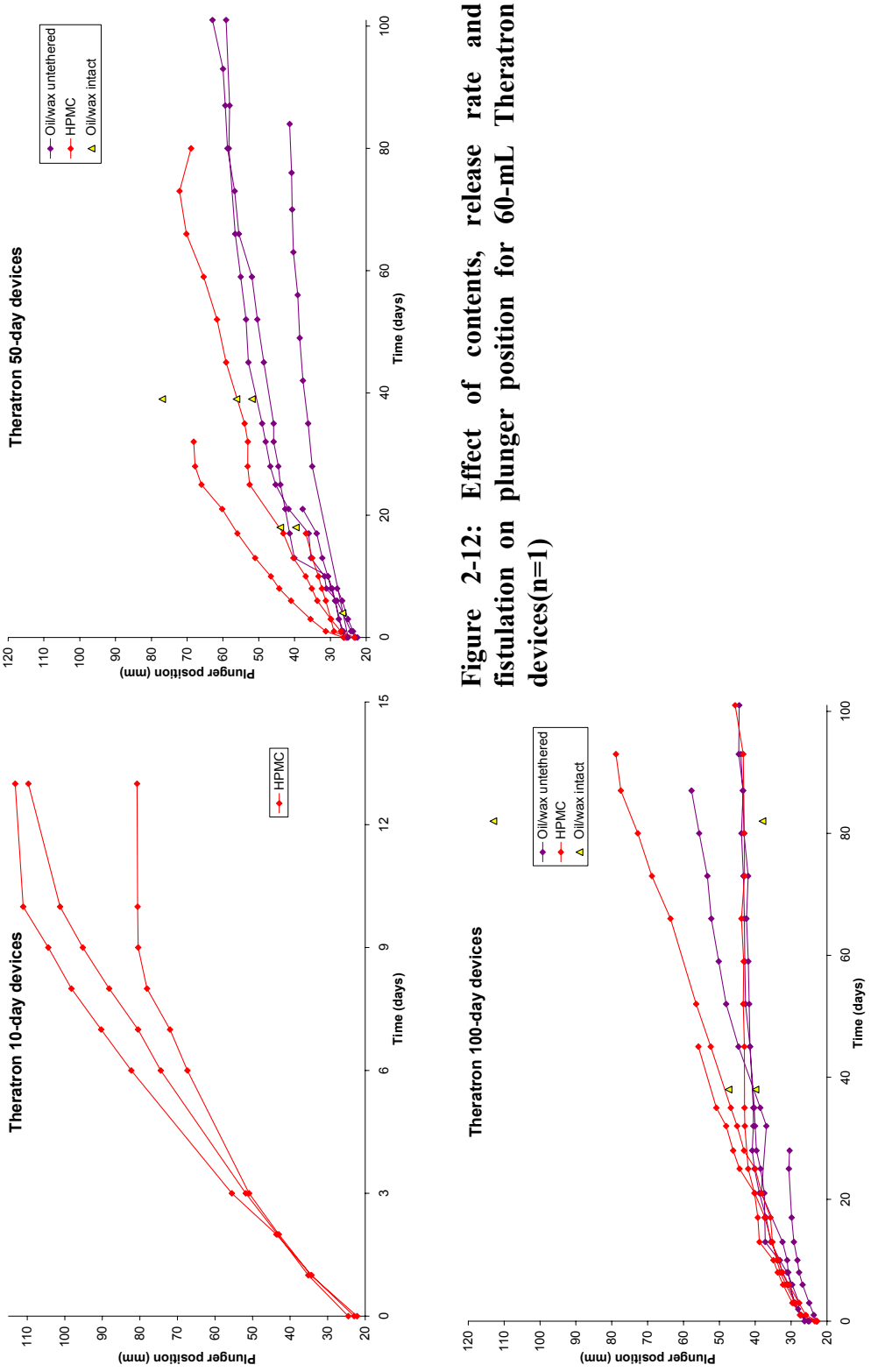


Figure 2-12: Effect of contents, release rate and fistulation on plunger position for 60-mL Theratron devices(n=1)

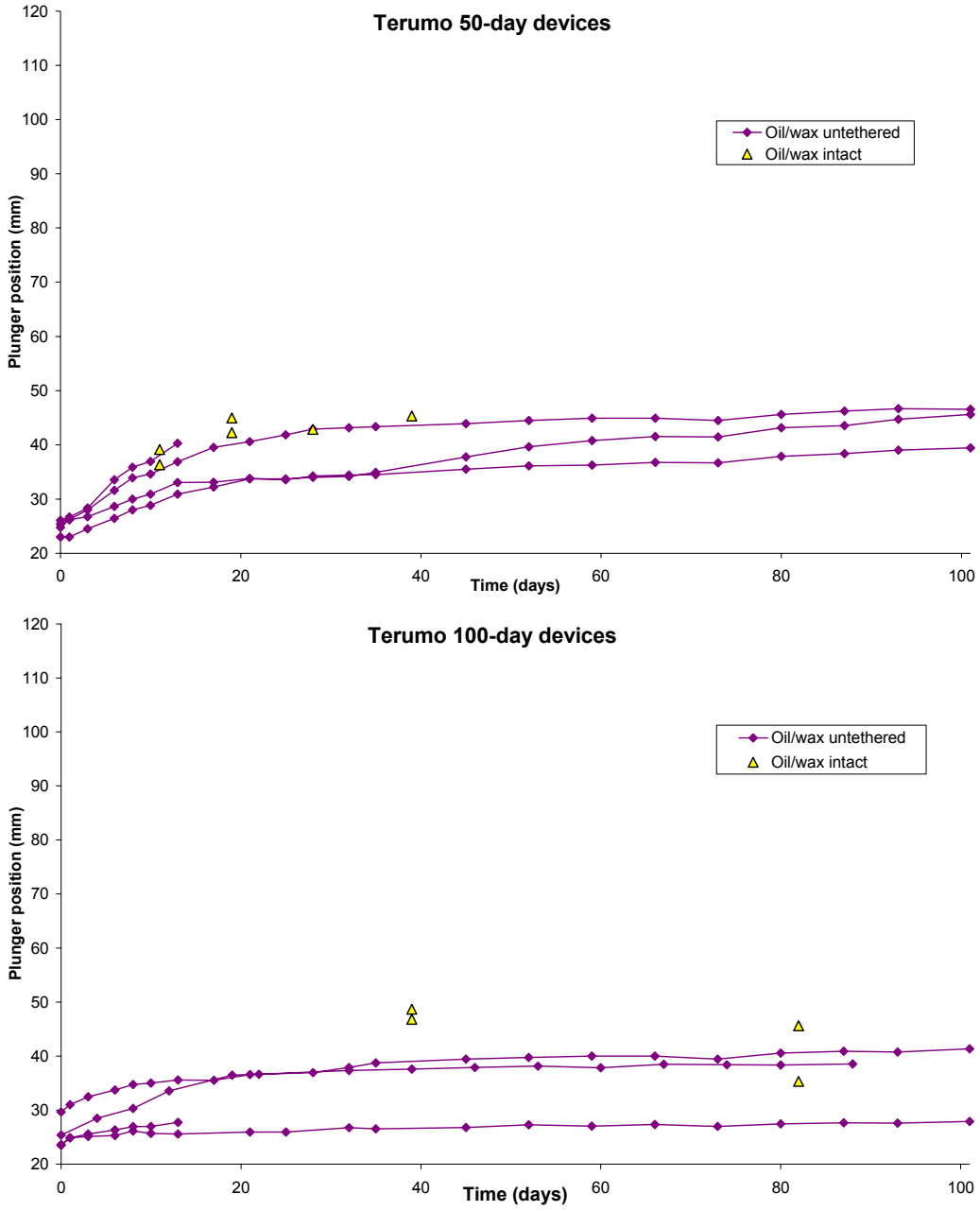


Figure 2-13: Effect of fistulation and release rate on plunger position for 60-mL Terumo devices (n=1)

2.2.2.1 Release rate

Devices were set to release at different rates by use of gas cells that were activated with different resistors. 50-day devices were tested in addition to 10- and 100-day devices as slower releasing devices behaved variably in Trial 1. Different resistances to those tested in Trial 1 were used with the intention of producing devices with lifetimes closer to the intended periods. The largest of these changes was for slow, 20-mL devices, where 10000-Ω resistors were used rather than the

2700-Ω resistors, with the intention of greatly decreasing the gas production rate. None of the 50- or 100-day devices reached completion and only three released more than half of their payload. This was consistent with a high rate of gas loss through diffusion compared with the rate of gas production.

2.2.2.2 Fistulated or intact cow

It is likely that gas leaking both into and out of the rumen through a fistula causes differences in gas composition and pressure in the rumen between fistulated and intact animals (Vandamme and Ellis 2004), which may influence the release profile of this type of device. Some 50- and 100-day devices were fitted with retention wings and tested in both fistulated and intact animals to test for any effect that this may have on their function.

Plunger position of devices in fistulated and intact cows indicated that device operation was independent of whether the cow was fistulated or intact (Figure 2-12 and Figure 2-13). However, no 50- or 100-day device came close to releasing its full payload so it cannot be inferred that properly functioning devices would do so independently of rumen fistulation.

It should be noted that it may be difficult to determine if devices are releasing at a lesser rate in intact cows than fistulated cows due to greater ambient pressure. Decrease in pressure when the rumen is cut open would cause a burst of release from the device before measurements could be taken.

2.2.2.3 Barrel

Results (Section 2.2.1.3) indicated that the Theratron has not been adapted to reduce gas diffusion. To confirm this, Theratron devices were tested alongside 60-mL Terumo syringes with similar dimensions (Figure 2-14 and Figure 2-15). No reliable rates of release were obtained from these results, although most of the plungers of Theratron devices progressed further than those of Terumo devices. Theratrons may therefore have slightly lower hydrogen permeability than Terumo syringes.

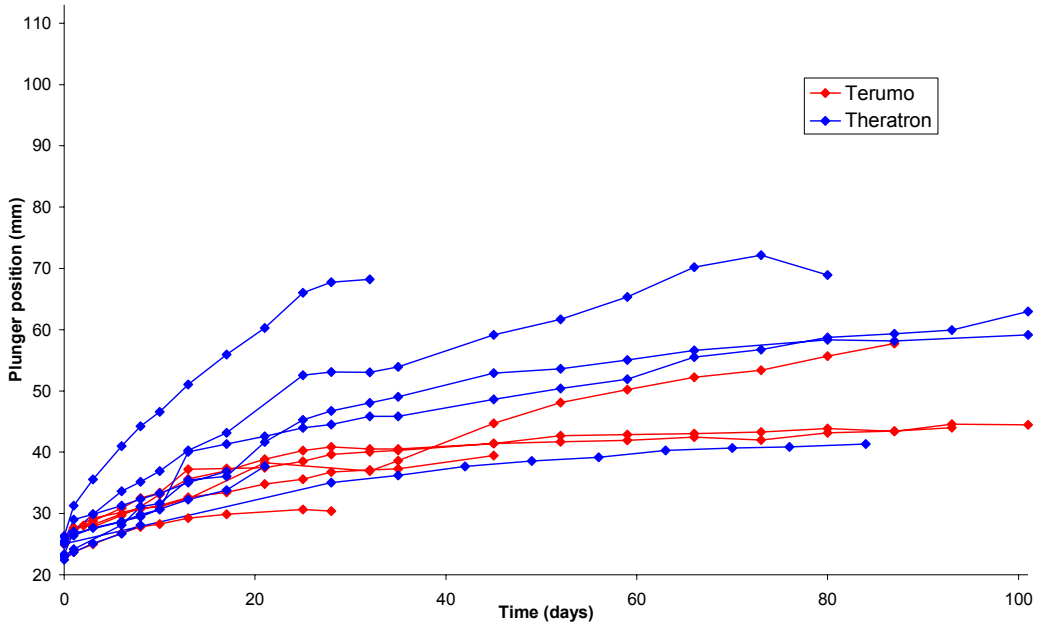


Figure 2-14: Effect of barrel type on plunger position of 60-mL, 50-day devices (n=1)

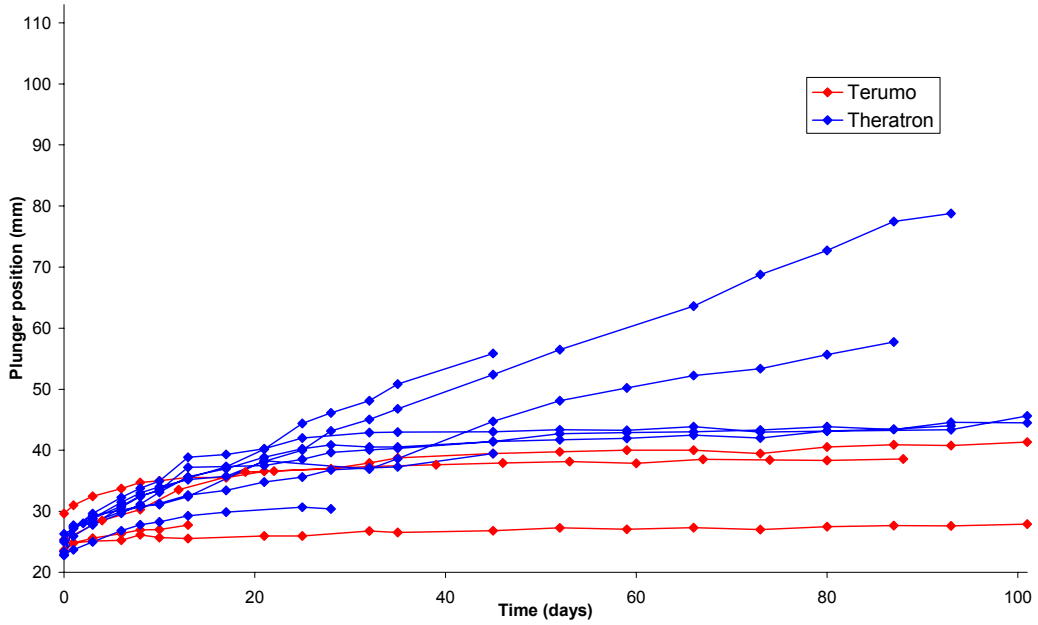


Figure 2-15: Effect of barrel type on plunger position of 60-mL, 100-day devices (n=1)

2.2.2.4 Coating

20-mL devices were not coated as a comparison to Trial 1 (in which all 20-mL devices were coated with PET and aluminium tape). The gradual decrease in the release rate of fast, 20-mL devices seen in this trial (Figure 2-11) was consistent

with outward diffusion of hydrogen which had been reduced by the PET & aluminium coatings used in Trial 1.

Only one plunger of a 50- or 100-day, 20-mL device moved more than 15 mm. This was probably due to a high rate of outward gas diffusion and the low rates of gas production as both device types were uncoated and used higher resistances than the equivalent 100-day devices in Trial 1.

2.2.2.5 One-way valve

All devices were fitted with one-way valves. Only 16 of the 54 devices became contaminated with digesta (Table 2-6), a lower proportion than the 17 of 39 that were contaminated in Trial 1 (Table 2-5). Contamination was due to the valves being ineffective rather than being temporarily absent except for one 100-day Theratron containing HPMC, which lost its valve. Data from this experiment therefore give a more accurate estimate of one-way valve efficacy than Trial 1. Devices containing water became contaminated at a slightly higher rate as might be expected of a lower viscosity payload.

Table 2-6: Effect of barrel type, release rate and contents on the number of devices contaminated with digesta

	Terumo			**Theratron**			**B Braun**			
	10-day	50-day	100-day	10-day	50-day	100-day	10-day	50-day	100-day	**Total**
Water	0/0	0/0	0/0	0/0	0/0	0/0	2/3	2/3	1/3	**5/9**
Oil/wax	0/0	0/4	1/4	0/0	3/5	1/5	0/3	0/3	1/3	**6/27**
HPMC	0/0	0/0	0/0	0/3	0/3	1/3	1/3	0/3	3/3	**5/18**
Total	**0/0**	**0/4**	**1/4**	**0/3**	**3/8**	**2/8**	**3/9**	**2/9**	**5/9**	**16/54**

Second number indicates total number of devices tested

2.2.2.6 Contents

When a device from an intact animal was disassembled, its Theratron plunger was found to be larger and heavier than an unused plunger. This plunger had been exposed to oil & wax in the device for 69 days. A further three devices from Trial 1 were then disassembled and it was found that a plunger, which had been exposed to oil & wax for 122 days had also swelled, but plungers from two devices containing 5% HPMC in isopropanol had not. This suggested that the observed swelling was caused by the oil & wax formulation.

2.2.3 Plunger swelling

Plungers from Theratrons, Terumo syringes and B Braun syringes were immersed in various liquids at 40°C. After 28 days, Theratron and B Braun plungers had swelled significantly if exposed to sunflower oil or fish oil (Figure 2-16). Theratron plungers eventually disintegrated in the latter (not shown). This demonstrates that plunger materials must be carefully selected when designing a generic delivery device.

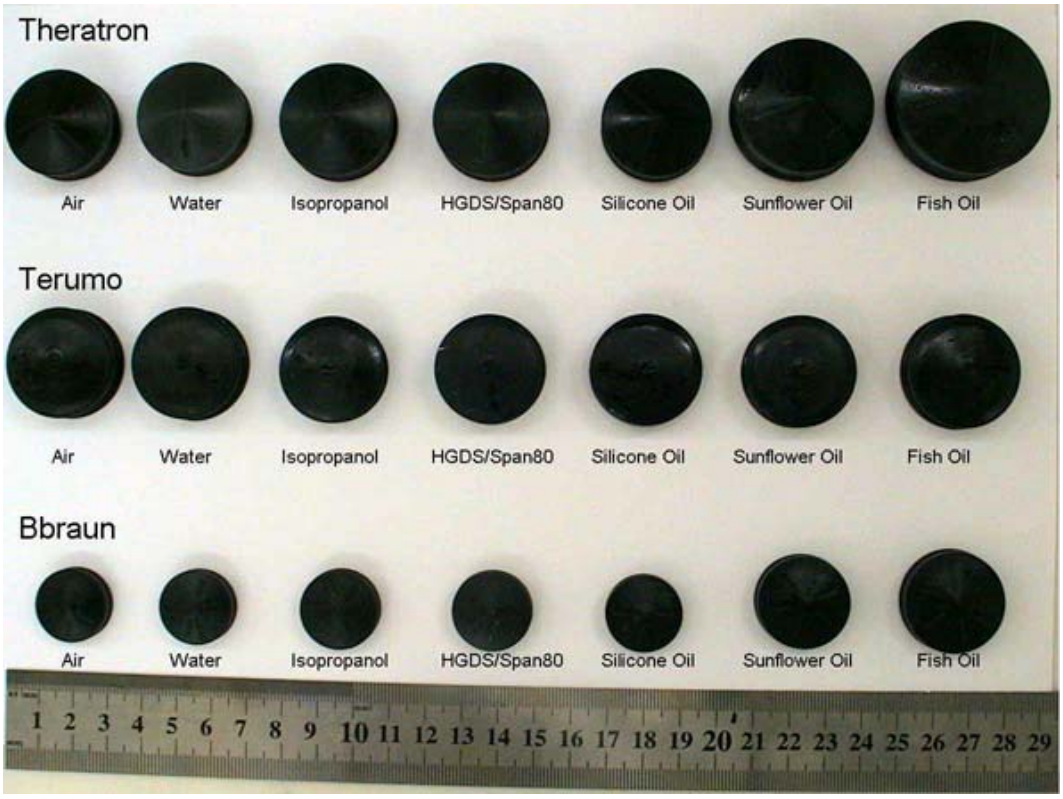


Figure 2-16: The effect of various solvents on each plunger type after 28 days

Plunger swelling probably increases frictional resistance to plunger movement. This would decrease release rate, potentially explaining the slower release rate of devices containing oil & wax in Trial 1 (Section 2.2.1.5). Increasing plunger friction provides an alternative explanation for the decreasing release rate observed in many devices, although release rate also decreased for devices with other formulations. The oil & wax formulation may also interact with other parts of the device such as the one-way valve.

2.2.4 *In vitro* trial

Experiments *in vivo* in Trials 1 and 2 had limited success, so similar devices were tested *in vitro* to identify problems due to the rumen environment as distinct from design problems with the device. In addition, an alternative plunger was tested, which may have reduced resistance to movement. More consistent data were generated *in vitro* than in the *in vivo* trials and only eight non-functioning devices had to be excluded from analysis of the 120 devices tested (Figure 2-19, Figure 2-17, Figure 2-18 and Figure 2-20).

2.2.4.1 Release rate

The 60-mL, fast releasing devices nearly completed expelling their contents in the planned 10 days but slower devices showed little or no plunger movement, which is consistent with a high gas diffusion rate relative to the gas production rate or increasing frictional resistance due to plunger swelling. These results support the lack of release observed for 100-day devices *in vivo* in Trial 1 (except for the 20-ml devices, which used different resistors). Small amounts hydrogen in the rumen (Section 1.3.3) would reduce outward gas diffusion slightly *in vivo*. However, this would not have been sufficient to cause the extent of plunger movement seen *in vivo*, which may be due to inward diffusion of rumen gases such as methane or carbon dioxide.

All fast devices showed some decrease in release rate. Average linear regressions were obtained for each device up to and including day 5 (Table 2-7). This was chosen as the time when the release rate of 60-mL devices was significantly different from the initial rate as average R^2 (excluding devices that did not release) decreased from 0.991 to 0.970 when data from the next sampling time were included. The 20-mL B Braun devices had a large decrease in plunger movement rate with plunger position, so linear regression was invalid. The large decrease is consistent with large gas losses due to diffusion.

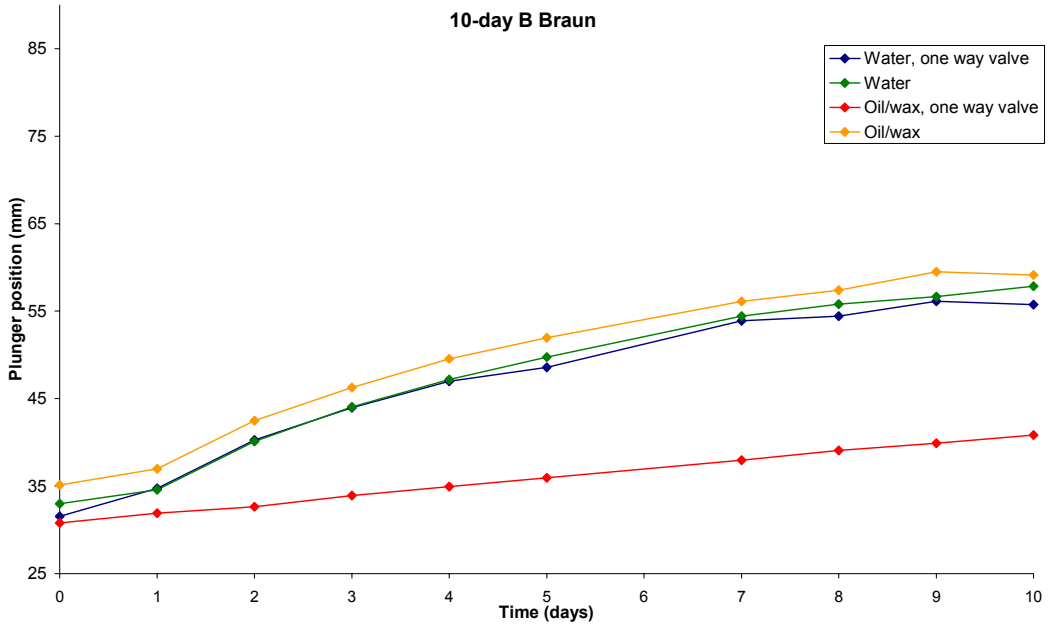


Figure 2-17: Effect of contents and one-way valve on average plunger position for 10-day, 20-mL devices (n=3)

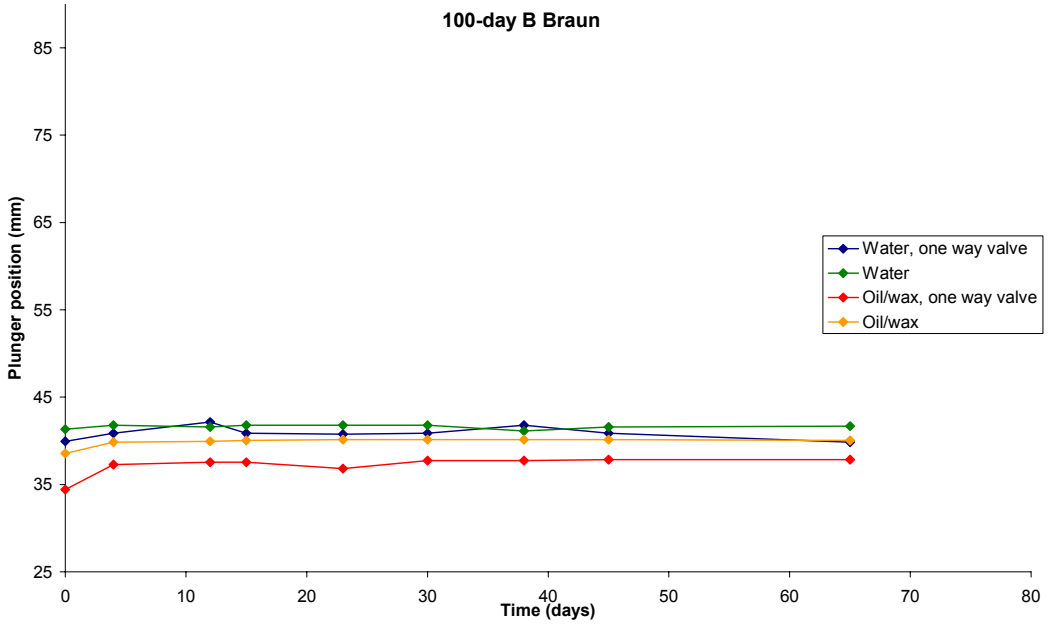


Figure 2-18: Effect of contents and one-way valve on average plunger position for 100-day, 20-mL devices (n=3)

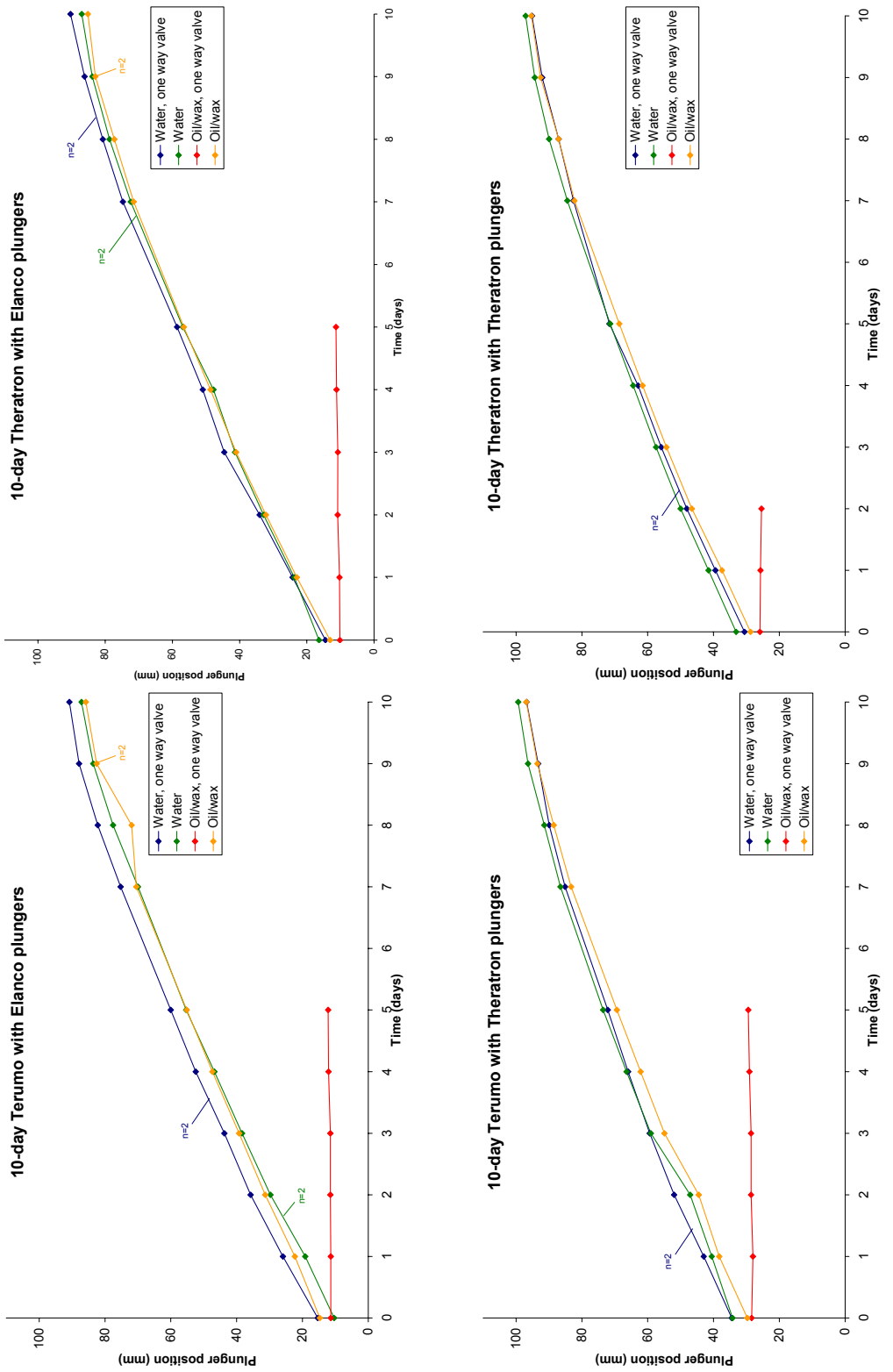


Figure 2-19: Effect of contents and one-way valve on average plunger position for 10-day, 60-mL devices (n=3 unless otherwise stated)

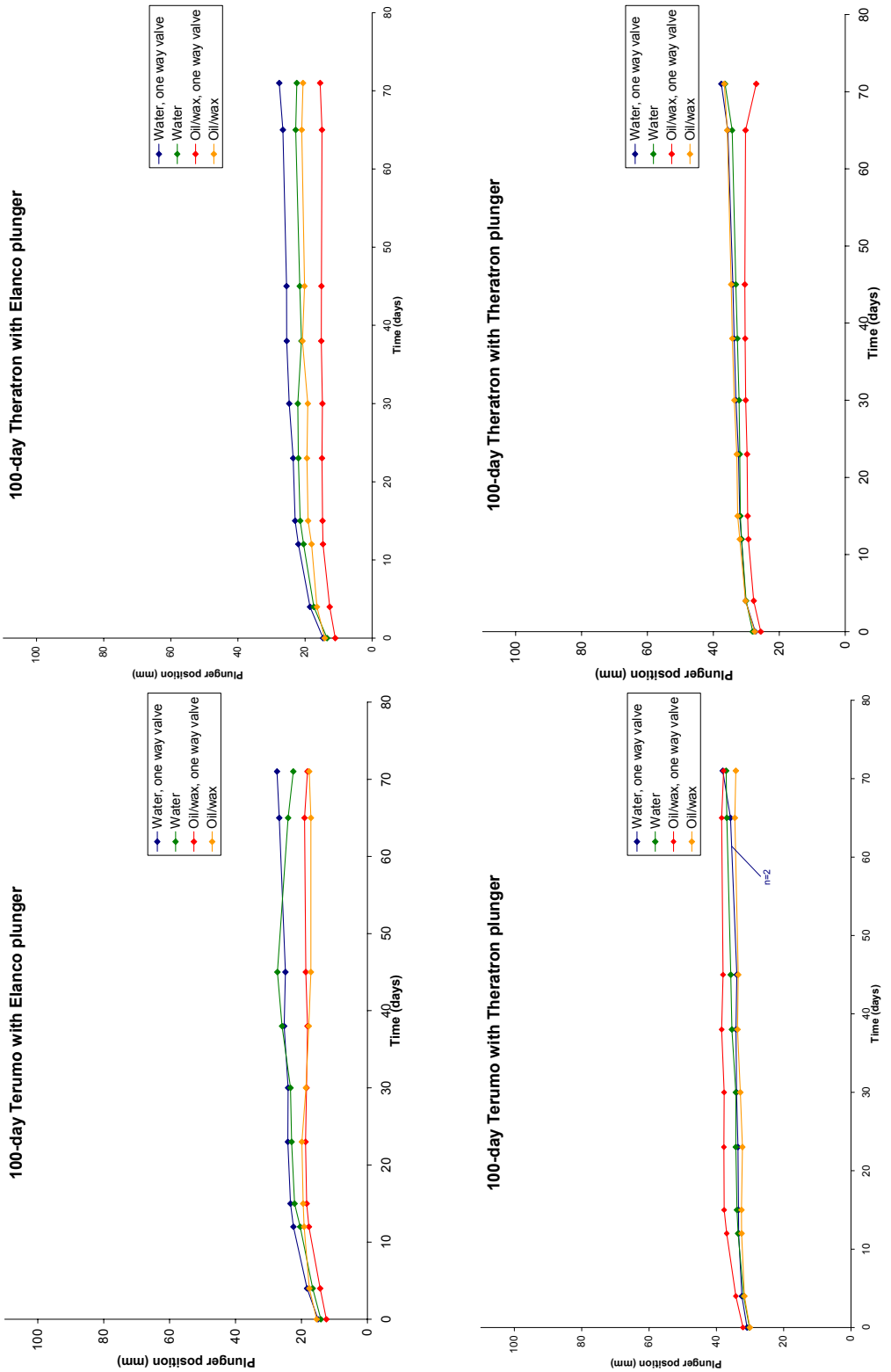


Figure 2-20: Effect of contents and one-way valve on average plunger position for 100-day, 60-mL devices (n=3 unless otherwise stated)

Table 2-7: Effect of contents, plunger type, barrel type and one-way valve on average release rate (mL·day^{-1}) for fast-releasing, 60-mL devices

Barrel / Plunger / Content / Orifice		-	One-way valve	-	One-way valve
		Water	Water	Oil/wax	Oil/wax
Theratron	Theratron	5.11±0.02 (n=3)	5.41±0.08 (n=2)	5.33±0.10 (n=3)	-0.15±0.31 (n=3)
Theratron	Elanco	5.56±0.24 (n=2)	5.57±0.57 (n=3)	5.80±0.07 (n=2)	0.17±0.02 (n=3)
Terumo	Theratron	5.56±0.46 (n=2)	5.13±0.16 (n=3)	5.44±0.22 (n=3)	0.17±0.11 (n=3)
Terumo	Elanco	6.04±0.28 (n=3)	6.03±0.05 (n=2)	5.54±0.34 (n=2)	0.11±0.08 (n=3)

Calculated release rates for data up to and including day 5.

Theratrons with one-way valves containing water had similar *in vivo* (Table 2-4) and *in vitro* (Table 2-7) release rates while those containing oil & wax without one-way valves released significantly faster *in vivo*. It is possible that neither of these observations is significant as all but one of the corresponding devices was contaminated with digesta *in vivo*.

2.2.4.2 Barrel

60-mL devices were made from Theratron and Terumo barrels with the intention of identifying any differences in the permeability of these barrels to hydrogen gas. Equivalent fast-releasing Theratron and Terumo devices without one-way valves had similar release rates (Table 2-7). Small differences were expected as the Terumo barrels are about 3% wider than the Theratrons, but used the same plungers. This would have caused Terumo devices to have slightly less friction between barrel and plunger and require slightly more gas volume per mm of plunger movement than equivalent Theratron devices. However, these factors should counteract each other and are probably insignificant.

The release profiles for the two barrel types were very similar (Figure 2-21) suggesting that both barrels were similarly affected by gas diffusion.

2.2.4.3 One-way valve

One-way valve effectiveness is difficult to measure *in vitro* because there is no digesta that can enter the barrel. However, any differences in release rate or

profile can be attributed to one-way valves rather than the digesta present in devices without one-way valves.

One-way valves had no significant effect on release rate from devices containing water (Table 2-7) except for Theratron devices with Theratron plungers, which had faster release rates if there was a one-way valve. Devices containing oil & wax, however, released only a few millilitres when a one-way valve was present. This was not noted in the *in vivo* trials, although two of the four 10-day devices in Trial 1 with no plunger movement had one-way valves and contained oil & wax. The observation that oil & wax can swell plungers was discussed in Section 2.2.2.4. This formulation may also interact with the heat-shrink tube used to make the one-way valves, causing them to either seal or become too rigid to open.

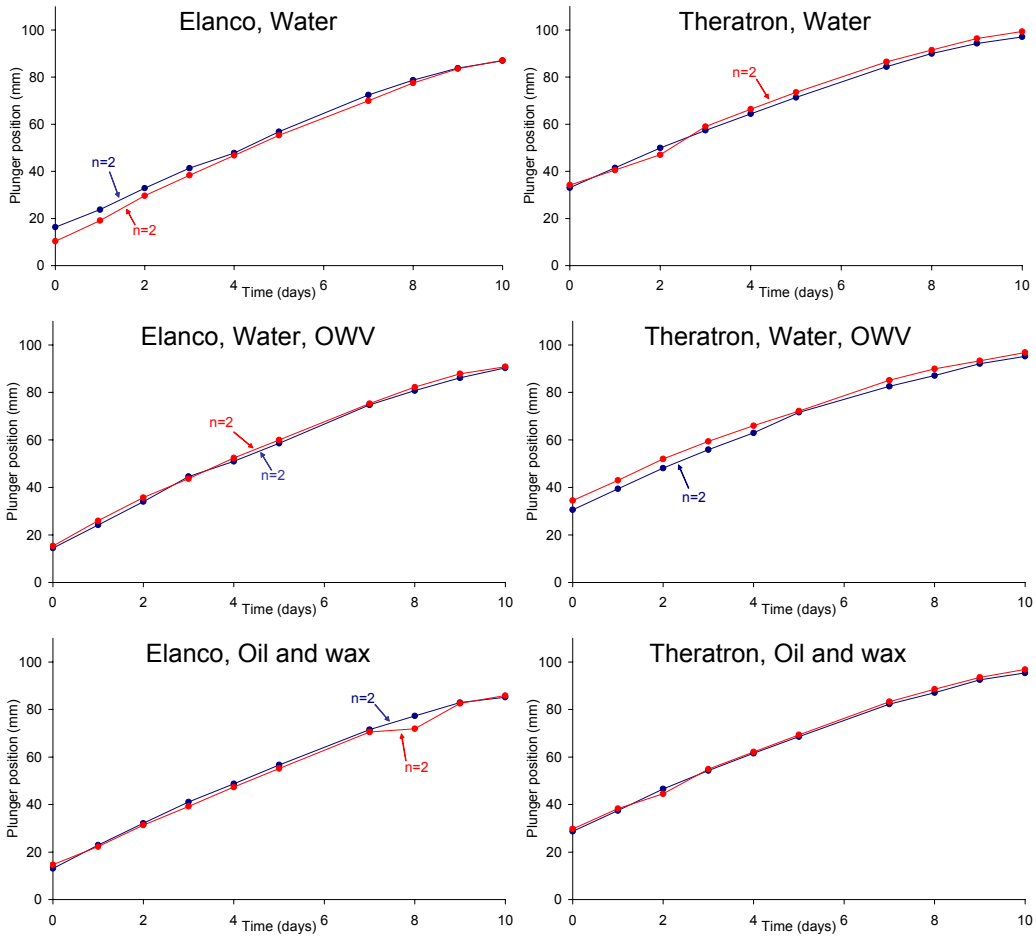


Figure 2-21: Effect of plunger type, barrel type, contents and one-way valve on average plunger position for 10-day 60-mL devices
●=Terumo barrel, ●=Theratron barrel. Devices with one-way valves containing oil & wax were omitted as their plungers did not move. n=3 unless otherwise stated.

2.2.4.4 Contents

The oil & wax in many devices separated into a transparent layer that resembled the sunflower oil and an opaque layer (Figure 2-22). This may have been caused by being stationary at 40°C for a long time. The solid wax seen in devices *in vivo* (Section 2.2.1.5) may be evidence that separation can occur even when the device is constantly in motion. Oil-based formulations were not used in subsequent experiments because they interacted with plungers and one-way valves. Identifying suitable materials that allow delivery of oil-based formulations using this technology requires further study.

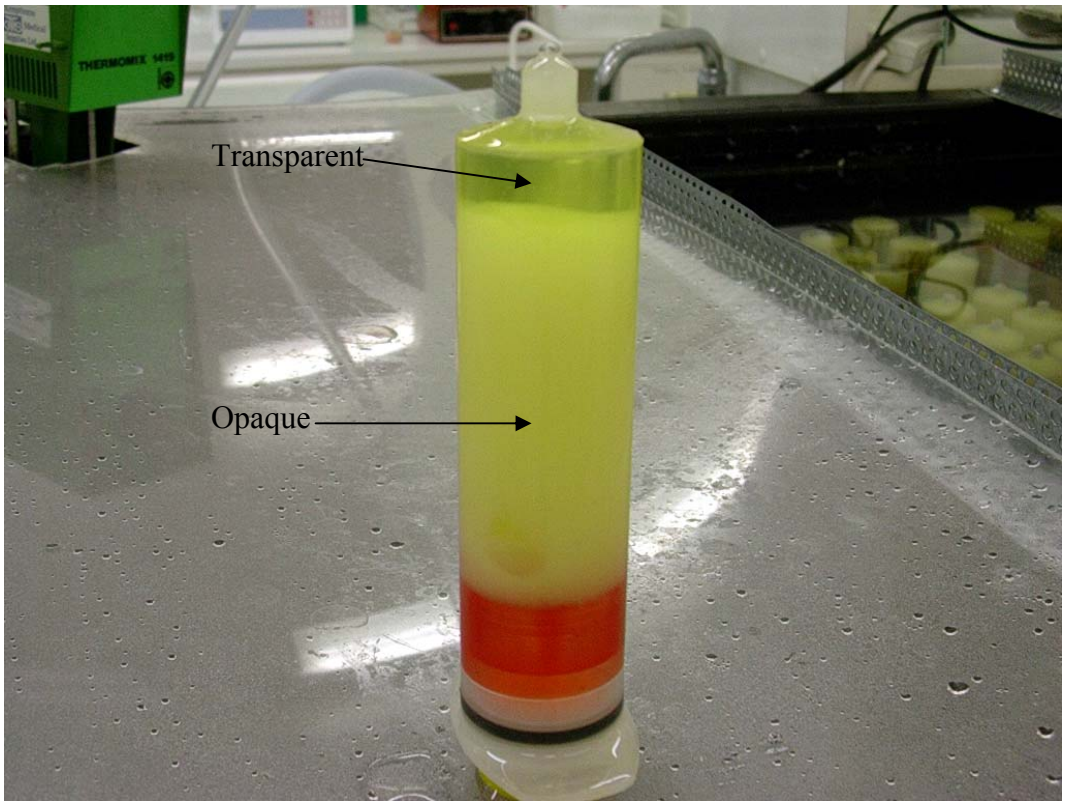


Figure 2-22: Separation of oil & wax formulation

2.2.4.5 Plunger

The 60-mL devices were assembled with either Theratron plungers or "Elanco" plungers from Rumensin capsules. The looser fitting Elanco plungers were tested in this experiment as a possible lower-friction alternative to the tight-fitting rubber plungers. The Elanco plungers yielded a slightly greater release rate than the Theratron plungers (Table 2-7), probably because the plunger of the former has less friction. The average position of Elanco plungers at day zero was 14.0 ± 2.5

mm compared with 31.7 ± 3.3 mm for the Theratron plungers. With a consistently greater barrel surface area exposed to the gas, devices with Theratron plungers would have been subject to a greater rate of gas diffusion, which may have caused this apparent difference in rate. Plunger position measurements were taken to the leading edge of Theratron plungers (Figure 2-7), though the barrel is only exposed to the gas as far as the trailing edge, ~10.5 mm less than the measured plunger position. The initial barrel length exposed in devices with Theratron plungers was therefore 21.2 ± 3.3 mm. This is very similar to the initial position of the Elanco plunger, though it may still have contributed to the difference in rate.

The plungers in eight devices (excluding those with the oil formulation and one-way valves) either did not move at all, did not move for several days, or began to move then stopped. Six of the devices that either failed or partially failed had Elanco plungers, indicating that a better sealing plunger may be necessary.

2.2.5 Effect of PET and aluminium coatings

In vivo results indicated that gas diffusion is a problem and that coating a device with aluminium tape and PET shrink-wrap may reduce gas loss by diffusion (Section 2.2.2.4). Decreasing release rates (thought to indicate gas diffusion) were also observed for uncoated devices *in vitro* (Section 2.2.4), but coated devices were not tested for comparison. To demonstrate the effect of these coatings, uncoated devices and those coated with PET shrink-wrap and/or metal tape were tested *in vitro*.

All coatings caused an increased and more constant release rate than uncoated devices (Figure 2-23, Figure 2-24, Figure 2-25 and Table 2-8). This supports the hypothesis that these coatings reduce gas diffusion. There was a delay before the plunger of Terumo and B Braun syringes moved, which may be because their larger initial gas volume needed longer to be pressurised. All data were supported by weight measurements (not shown).

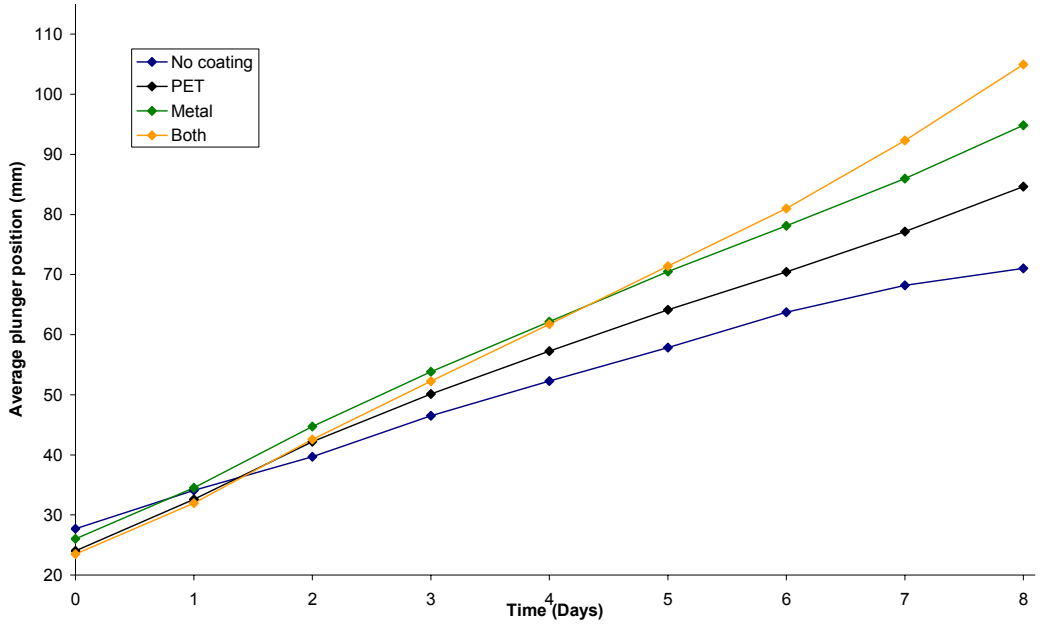


Figure 2-23: Effect of coatings on average plunger position with time for Theratron devices (n=3)

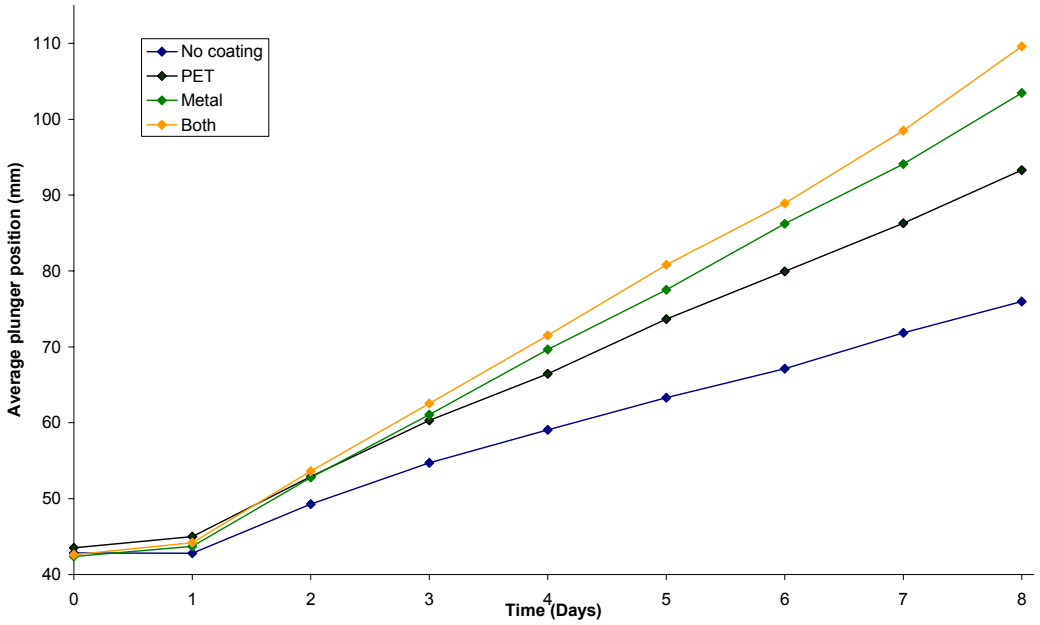


Figure 2-24: Effect of coatings on average plunger position with time for Terumo devices (n=3)

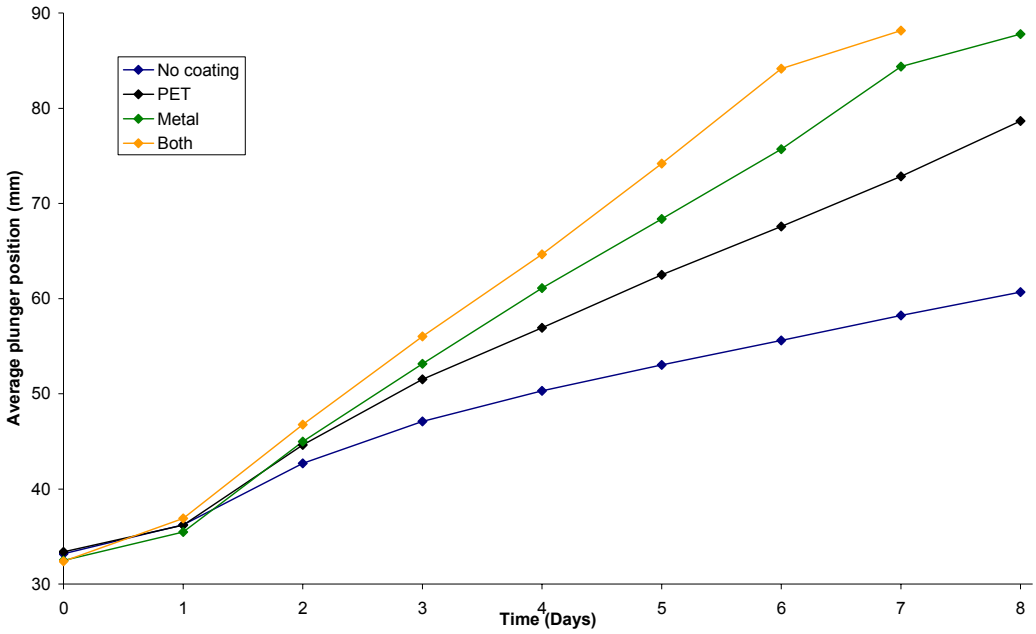


Figure 2-25: Effect of coatings on average plunger position with time for B Braun devices (n=3)

Table 2-8: Effect of barrel type and coatings on release rate (mL·day^{-1})

	No coating	PET	Aluminium	Both
Theratron	4.34±0.48 (n=2)	5.06±0.30 (n=3)	5.81±0.34 (n=2)	6.83±0.06 (n=2)
Terumo	3.07±0.06 (n=2)	4.54±0.17 (n=3)	5.63±0.52 (n=3)	6.12±0.13 (n=2)
B Braun	-	1.80±0.05 (n=3)	2.45±0.17 (n=3)	2.87±0.16 (n=3)

Release rate of uncoated, B Braun devices decreased too rapidly to obtain valid linear regression.

2.3 Conclusions

Devices tested in this chapter displayed a number of limitations and a high degree of variability. The use of untested components in the harsh rumen environment may have caused this. Gas production by the gas cell and gas diffusion, both in and out of the devices, were identified as important factors in device function and neither were well understood. It was concluded that gas production and diffusion need to be studied further to advance the understanding of this technology.

Chapter 3: Principles of device function

Experiments were performed to quantify and control gas production rate by gas cells and the rate of gas loss due to diffusion through the device barrels. These studies involved measuring changes in gas volume, which were monitored by recording the water level in glass tubes. It was thought that studying these factors further would help explain results obtained from experiments discussed in Chapter 2.

3.1 Methods and materials

3.1.1 Gas production rate

The effect of a variety of resistors on the rate gas cells produce gas was determined. This involved periodically measuring water level in graduated cylinders (which had been displaced by the evolved gas), ambient temperature and ambient pressure. The ideal gas law was used to calculate the gas present (Appendix B.2) and a linear regression was performed to calculate gas production rate. This experiment was done at room temperature and at 40°C using three different types of collection cylinders.

3.1.1.1 Warm room

The warm room was a sealed, insulated room that was heated to 40°C. Temperature was logged (Diligence EV N2003 temperature logger, Comark, Korea), within one decimal place every minute for seven days to check stability. Approximately 60 heating cycles took place per day. The maximum temperature was 41.1°C, the minimum was 38.8°C and average was 39.7°C with a coefficient of variation of 1.0% (Appendix B.2).

3.1.1.2 50-mL burette method

Approximately 4-cm long sections were cut from the orifice end of Theratron syringes. The orifice of each syringe section was drilled wider before it was attached with hot-melt glue (Bostik New Zealand Ltd) to the open end of a 50-mL burette to form a funnel (Figure 3-1c). Insulated wires were soldered to the terminals of each gas cell. The gas cells were protected from water by placing

them in the end of cut-down commercial syringes (Figure 3-2). A protected gas cell was held in each syringe funnel with hot glue (Figure 3-1d).

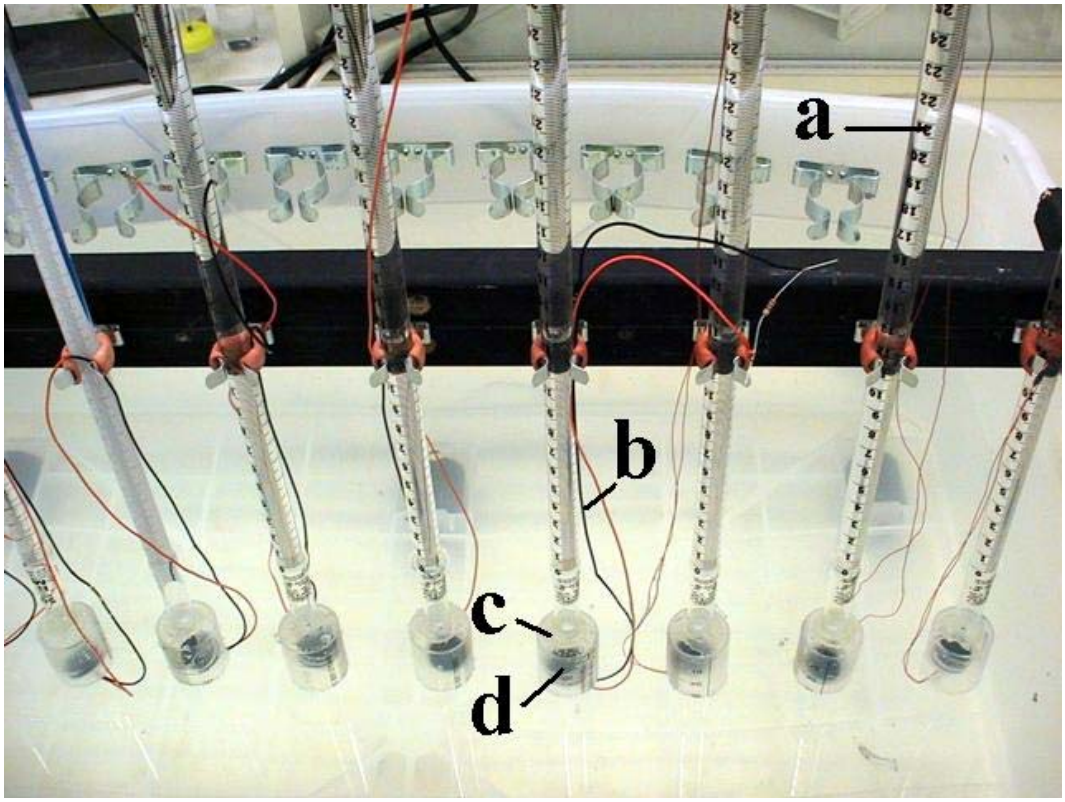


Figure 3-1: 50-mL burette method at room temperature
a = burettes for collection of gas. b = wires soldered to gas cell. c = funnel made from end of 60-mL syringe. d = gas cell, protected as shown in Figure 3-2.

Each apparatus was suspended in a water bath with the zero of the scale at water level. Resistors were connected to wires from the gas cell (Figure 3-1b). The gas produced was collected in the burettes and the volume measured periodically. When the water was nearly completely displaced, the burette was refilled by withdrawing gas through the tap and the displaced volume was added to subsequent volume calculations.

Gas cells with 0- (no resistor), 27-, 56-, 150-, 220-, 390-, 470-, 560-, 750-, 910-, 1200- and 1800-Ω resistors were tested in triplicate at room temperature and 9 replicates of 0 Ω (no resistor) were tested at 40°C.

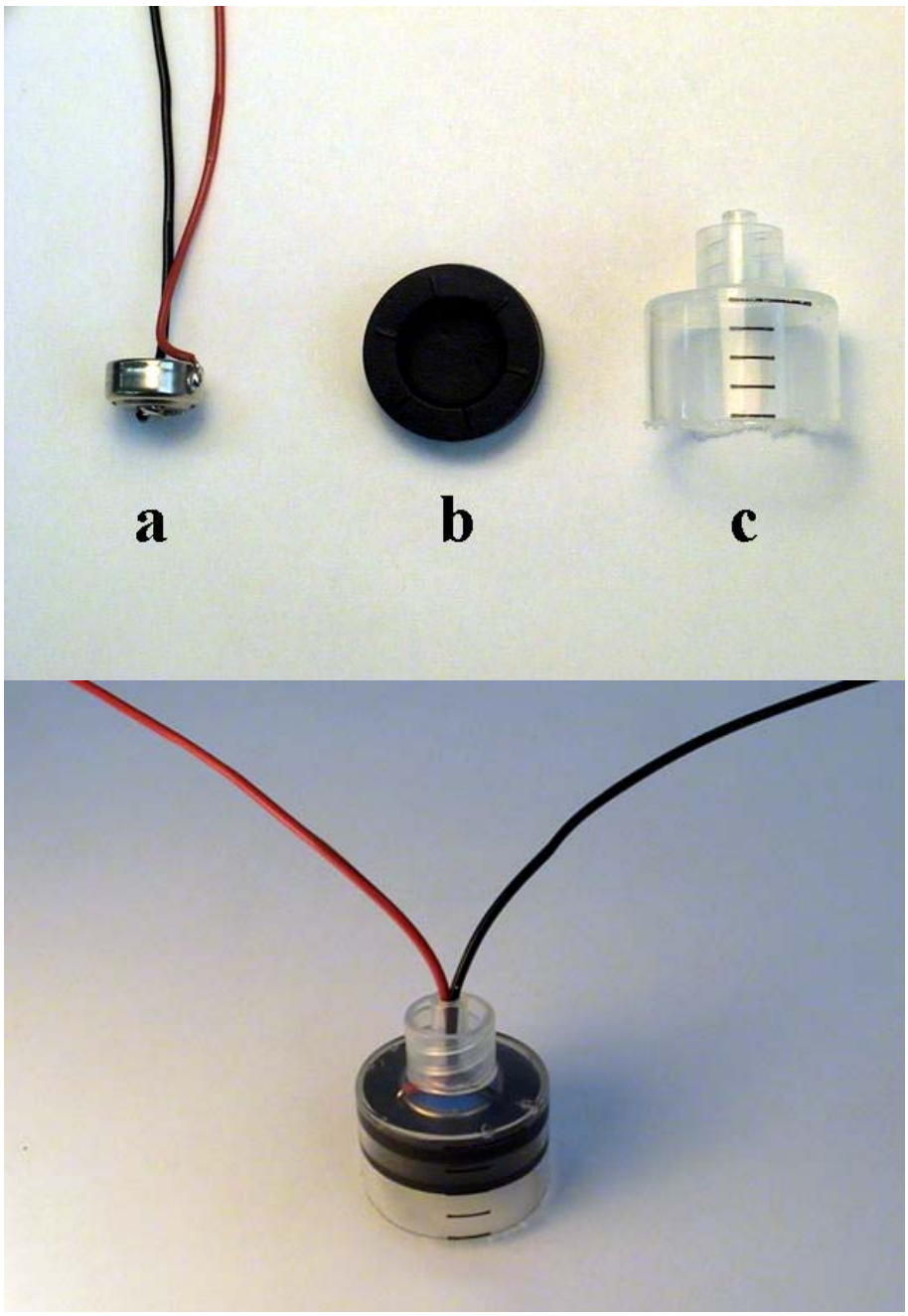


Figure 3-2: Gas cell housing for underwater gas production.
Top: a gas cell with soldered wires (a) and a plunger (b) and tip (c) from a 20-mL B Braun syringe. Bottom: a gas cell housed in the syringe tip, allowing wires and gas out while keeping gas cell dry.

3.1.1.3 Large cylinder method

Gas collection cylinders were made by cutting 14-mm outer diameter, 1.5-mm wall thickness glass tubing into 750-mm lengths and flaring one end to ~30 mm. Measuring tape was attached to each tube. After setting up the cylinders and gas cells (Section 3.1.1.2) at 40°C (Figure 3-3), the cylinders were filled with water and the open end sealed with a rubber bung, which could be removed to refill the

tubes. Five replicates of 270-, 330-, 390-, 470-, 560-, 820-, 1200-, 1500-, 2200-, 2700-, 3300-, 5600- and 8200-Ω resistors were tested.

This experiment was repeated with white petroleum jelly (Vaseline, Rexona, New Zealand) on the negative terminal of each gas cell to prevent moisture from causing corrosion and current leakage.

Figure 3-3: Large cylinder set up for measuring gas production rate

3.1.1.4 5-mL burette method

Gas cells were activated by soldering a resistor between the terminals (Figure 3-4, left). Six 5-mL burettes were suspended vertically with open taps below the water level of a bath in the 40°C room. A rubber bung with a gas cell attached was inserted into the funnel of each burette so that the gas cell was suspended in the funnel and the water level in the tube was in the desired region of the scale. The water level in each burette was recorded periodically.

Six replicates of 270-, 330-, 390-, 560-, 820-, 1200-, 3900-, 4700- and 5600-Ω resistors were tested using this method. Spray-on rubber (Undercoat, Dupli-Color Products Company, United States of America) was applied to the negative terminal of gas cells that had been activated with 3900-, 4700- or 5600-Ω resistors (Figure 3-4, right) to prevent damage caused by the presence of water vapour.

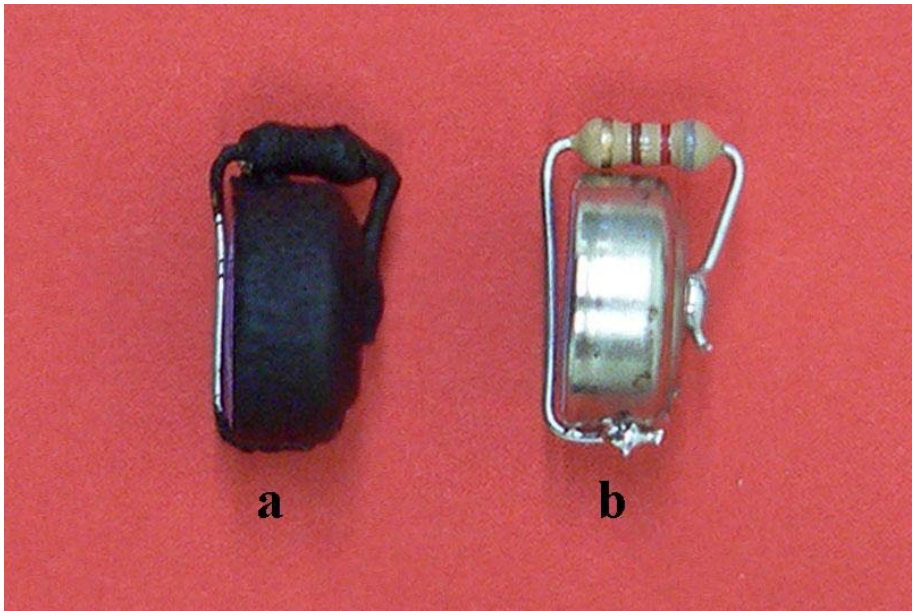


Figure 3-4: Activated gas cells with (a) and without (b) waterproof coating

3.1.2 Gas diffusion rate

The rate of gas loss due to diffusion through various device barrels was measured by monitoring the water level in a graduated glass tube protruding from the bottom of a device barrel containing either H_2 or CO_2 gas.

3.1.2.1 Measurement tubes

Glass tubing (outer diameter 11 mm, wall-thickness 1.5 mm) was cut into 500-mm lengths and fused with 35-mm long pieces of narrower tube (outer diameter 8 mm, wall thickness 1 mm) to form a constriction. A measuring scale was attached to each tube. Each tube was calibrated by adding known volumes of water from a 50-mL burette and recording the water level. Volume (mL) was obtained by dividing the scale reading in centimetres by 1.99 $cm \cdot mL^{-1}$ (Appendix B.2.4, Table B-4).

3.1.2.2 Barrels

The three commercial syringes (see Chapter 2) and the injection-moulded barrels of gas cell driven intravaginal devices made from low-density polyethylene Alkatuf 820 (LDPE), high-density polyethylene (HDPE), high molecular weight high-density polyethylene LM6007 (HMWHDPE), polyethylene terephthalate glycol (PETG), polybutylene terephthalate Valox 350U (PBT), Nylon 66 A3K (Nylon), Super-tough nylon (ST Nylon) and Acetal Delrin 1260 (polyoxymethylene, POM) were tested. Glass syringes (Eterna-matic 50 mL, Sanitex, Switzerland) were also tested as a negative control. Before being used, the orifices of all barrels were sealed. Commercial syringes were sealed with a Theratron luer lock end-cap. Wings were removed from injection-moulded barrels and the three exposed openings were sealed with hot glue.

3.1.2.3 Assembly

Barrels and measurement tubes were assembled as shown in Figure 3-5. A rubber bung with an appropriately sized hole was inserted into the open end of each barrel (Figure 3-5b) and then a measurement tube was inserted into each bung. Each assembly was suspended over a bath of water in the 40°C room so the zero mark of the scale was at the water level. Each tube was filled with water by drawing all of the air out through a tube. The gas being tested was then pumped into each barrel until all water was displaced. Once gas temperature had equilibrated, gas volume in each tube was adjusted so the water level was within the scale. Hydrogen (B.O.C., New Zealand) and carbon dioxide (Air Liquide, New Zealand) were each tested twice.

Because carbon dioxide quickly dissolved in the water, 2.25 $mol \cdot L^{-1}$ sodium chloride (Pams Products, New Zealand) was added to the water and a foam float was placed in each tube (Figure 3-5d) to reduce the surface area of water exposed to the gas. No foam floats were used during the second run with carbon dioxide, although the water contained NaCl.

Figure 3-5: Measuring of gas diffusion
a = device barrel. b = rubber bung. c = graduated tube. d = foam float.

3.1.2.4 Sampling

The water level inside each tube, ambient temperature and ambient pressure were recorded periodically. The first time hydrogen was tested, temperature was not recorded, but assumed to be 40°C. Temperature was recorded only once during each sampling time during the first experiment with carbon dioxide. In the second trial for both gases, temperature was recorded initially, then again after every 5 tubes were measured. The appropriate temperature for each measurement was calculated by interpolation. Pressure, volume and temperature in each tube were used to calculate the absolute gas volume.

3.1.2.5 Raw data conversion

The height of water in each tube was converted to gas volume, mL at 40°C and 1 atm (Section 1.6.3; see Appendix B.2). The rate of change in volume due to diffusion was then calculated by linear regression.

3.1.3 Predicting plunger position with time

3.1.3.1 Finite difference approximation

The release profile of a device operating at atmospheric pressure with no gas diffusion can be calculated from the gas production rate and barrel dimensions. Compensation for pressure can be incorporated by adjusting the gas production rate using Boyle's law (discussed in Section 3.2.3.2). Compensating for gas diffusion, however, is more complicated.

The surface area of barrel material exposed to the driving gas is determined by the barrel length behind the plunger, l in cm. The diffusion rate for any value of l (D_l, mL·day^{-1}) through a barrel with a total length of L (cm) is a proportion of the diffusion rate through the entire barrel (D_L, mL·day^{-1}):

$$D_l = \frac{D_L l}{L} \qquad 3\text{-}1$$

The change in volume behind the plunger (V, mL) with change in time (t, days) at any plunger position is equal to the rate of gas production (G, mL·day^{-1}) minus the rate of gas diffusion at that plunger position:

$$\frac{dV}{dt} = G - D_l \qquad 3\text{-}2$$

Thus, the rate of plunger movement for a constant gas production rate is dependant on its position in the barrel. The rate of change in volume with change in time at any plunger position can be calculated by Equation 3-2 and used to approximate the plunger position some time later. The shorter this period of time is, the more accurate the approximation will be. A spreadsheet was created to calculate the plunger position in this way at incrementally increasing points in time (example shown in Figure 3-6).

Gas production in mL/day:	10.4
Barrel length cm:	11.6
Full barrel gas diffusion in mL/day:	8.2
Diameter in mm:	29
Starting position in mm:	0
Operating pressure in atm:	1.09
Calculations per day:	1000

Time days	Plunger position cm	Volume ml@atm	Volume ml@ pressure	Proportion of barrel exposed	Diffusion rate mL/day
0	*0*	0	0	0	0
0.001	*0.0014445*	0.0104	0.0095413	0.0001245	0.0010211
0.002	*0.0028889*	0.020799	0.0190816	0.000249	0.0020421
0.003	*0.0043331*	0.0311969	0.028621	0.0003735	0.0030631
0.004	*0.0057772*	0.0415939	0.0381595	0.000498	0.0040839
0.005	*0.0072211*	0.0519898	0.0476971	0.0006225	0.0051046
0.006	*0.0086649*	0.0623847	0.0572337	0.000747	0.0061252

Figure 3-6: First iterations of plunger position spreadsheet

3.1.3.2 Analytical solution

Substituting Equation 3-1 into Equation 3-2 gives an expression of change in volume with time at plunger position l:

$$\frac{dV}{dt} = G - \frac{D_L l}{L} \qquad 3\text{-}3$$

The volume behind the plunger in a cylindrical barrel is equal to its cross-sectional area (A, cm^2) multiplied by its length (l, cm):

$$V = Al \qquad 3\text{-}4$$

Substituting Equation 3-4 into Equation 3-3 gives an expression of change in plunger position with time:

$$\frac{dl}{dt} = \frac{G}{A} - \frac{D_L l}{AL} \qquad 3\text{-}5$$

When a device is initiated, the plunger position may not be zero. To allow for this, l can be divided into plunger position at time zero (a constant, l_0), and plunger displacement from l_0 (x, cm):

$$l = x + l_0 \qquad 3\text{-}6$$

Substituting Equation 3-6 into Equation 3-5, rearranging, integrating and simplifying gives plunger position as a function of time:

$$l = \frac{GL}{D_L} - \left(\frac{GL}{D_L} - l_0\right) e^{-\frac{D_L t}{AL}} \qquad 3\text{-}7$$

3.2 Results and discussion

Data from this chapter are in Appendix B.

3.2.1 Gas production rate

Gas production by the gas cell is the most important factor determining the release rate from a device. However, this had not been measured directly. Gas produced by gas cells was collected periodically to study rate and profile of gas production. The relationship between resistance and gas production rate was established at room temperature and compared with subsequent experiments at 40°C. The profile of the full duration of gas production was then studied and a third method was used to accurately measure the rate of hydrogen production and oxygen consumption at 40°C.

3.2.1.1 50-mL burette method·

Data collected from the 50-mL burettes at room temperature (Section 3.1.1.1) indicated there was an inverse relationship between gas production rate and electrical resistance (Table 3-1). Theory indicates that gas production rate is directly proportional to current. It should therefore be directly proportional the reciprocal of the resistance, also known as "conductance" (Lyons 1967). Data obtained show a direct relationship between gas production rate and conductance ($R^2 = 0.996$) for conductance ≤4.5 mS (i.e. resistance ≥220 Ω) (Figure 3-7).

Table 3-1: Effect of resistance on gas production rate (mL·day^{-1} at 40°C, 1 atm)

Resistance (Ω)	50-mL burette method	Large tube method	5-mL burette method
27	69.9±3.6 (n=3)		
56	45.2±1 (n=3)		
150	19±1.7 (n=3)		
220	15.1±0.5 (n=2)		
270		14.5±0.8 (n=5)	13.3±0.4 (n=6)
330		11.7±0.3 (n=4)	10.4±0.5 (n=4)
390	9.35±0.07 (n=3)	9.40±0.33 (n=4)	9.51±0.22 (n=6)
470	7.51±0.44 (n=3)	8.06±0.19 (n=4)	
560	6.45±0.12 (n=2)	6.94±0.16 (n=4)	6.87±0.28 (n=5)
750	4.77±0.04 (n=3)		
820		4.77±0.46 (n=5)	4.84±0.12 (n=5)
910	3.98±0.07 (n=3)		
1200	3.05±0.02 (n=2)	3.14±0.26 (n=5)	3.34±0.09 (n=6)
1500		2.32±0.16 (n=5)	
1800	1.93±0.02 (n=2)		
2200	1.65±0.04 (n=3)	1.53±0.17 (n=5)	
2700		1.26±0.13	
3300	0.84±0.14 (n=3)	1.22±0.10 (n=5)	
3900			1.11±0.04 (n=6)
4700			0.87±0.04 (n=6)
5600		0.38±0.03 (n=5)	0.75±0.04 (n=6)
8200		0.21±0.07 (n=5)	

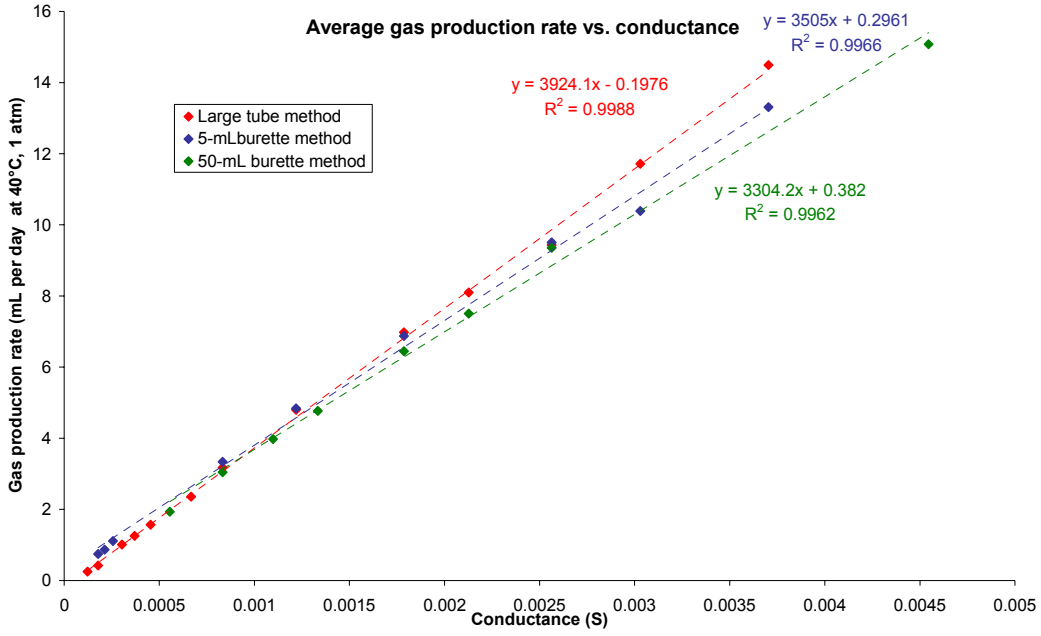


Figure 3-7: Effect of conductance on gas production rate

The reciprocal of gas production rate was plotted against resistance (Figure 3-8) to better examine the effect of higher resistance on gas production rate as errors among the larger values may become insignificant when the reciprocal is calculated. Excluding resistances below 220 Ω, a linear relationship ($R^2 = 0.999$) between resistance and rate^{-1} was observed when resistance was ≤2200 Ω.

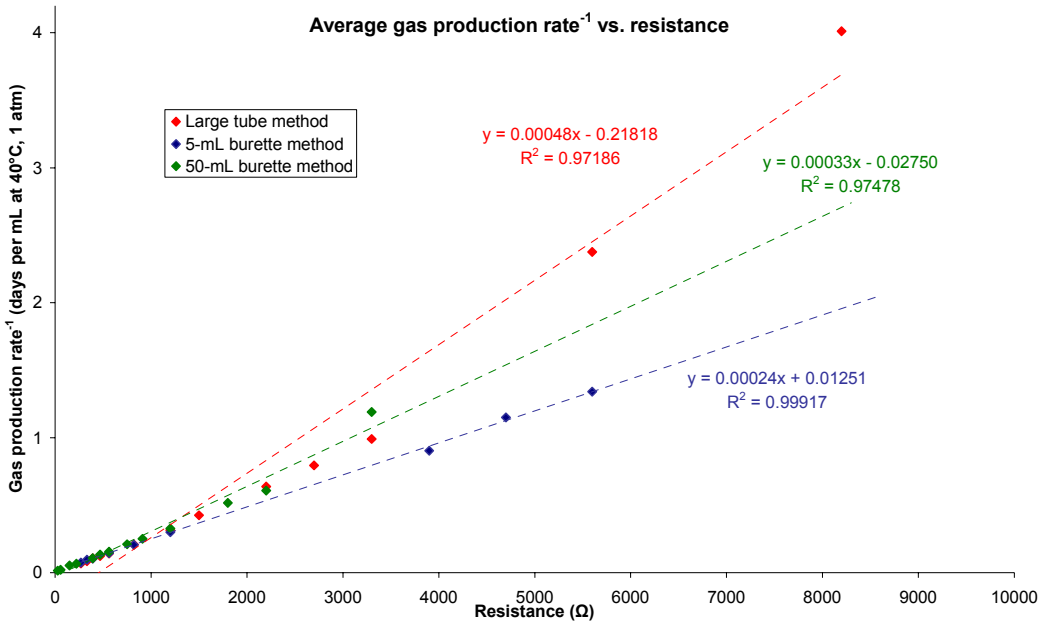


Figure 3-8: Effect of resistance on gas production rate^{-1}

The ambient pressure was assumed to be atmospheric for volume calculations so variation in ambient pressure may have caused inaccuracies. The measured temperature was between 15°C to 25°C but would have been lower overnight. Temperatures as low as 1.8°C were recorded near these experiments. Fluctuations in temperature were compensated for in volume calculations but gas production rate may also be affected by temperature. For example, the resistance of the resistors and the reaction rate in the gas cell could be affected by temperature, which would affect the gas production rate. Only nine gas cells could be tested simultaneously because it was expensive to have more burettes. Therefore, resistances were tested sequentially over three months, so they were tested under different ambient temperatures and pressures. Accuracy of temperature and volume were 0.5°C and 100 µL respectively. Two other methods to measure gas production rate were developed to increase accuracy and overcome ambient temperature and pressure variation.

The rate of gas production with no external resistance was tested by this method at 40°C. Gas production began to slow after approximately 5 hours, when 80 to 90 mL had been produced (Figure 3-9), and continued to do so until the average total volume of 161 ± 13 mL (40°C, 1 atm) was reached about 7 days later. Gas production rate of the three other resistances that were still being measured when 80 mL of gas had been produced did not decrease, suggesting that this may not occur for all resistances. Average gas production rate over the first 5 hours was 424 ± 24 mL·day^{-1} (40°C, 1 atm), which was almost twice the gas production rate from a gas cell with no external resistance at room temperature (241 mL·day^{-1} at 40°C, 1 atm). This suggests that the gas production rate and at room temperature may be different to that at typical temperatures in the rumen.

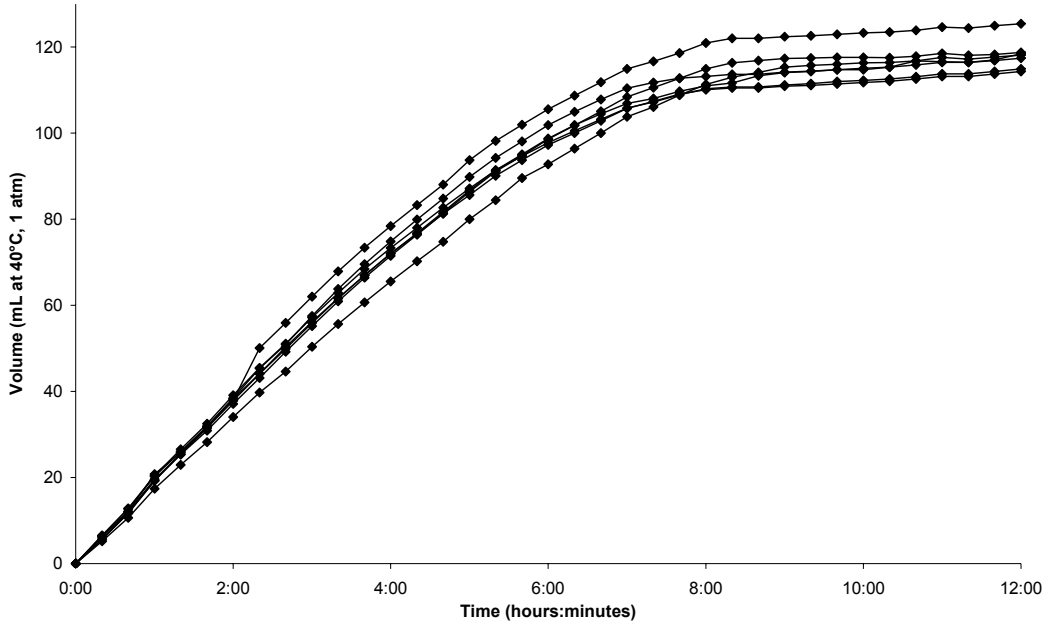


Figure 3-9: Gas production profiles gas cells with no resistor (0 Ω) at 40°C, first 12 hours

3.2.1.2 Large cylinder method

To overcome limitations of the 50-mL burette method, gas production rate was measured by collecting gas in large cylinders in the 40°C room. These cylinders were cheap to manufacture and therefore up to fifty gas cells could be tested simultaneously. A digital thermometer and an electronic weather station were used to provide more accurate ambient temperature and pressure data. Temperature was recorded only once during each sampling, immediately before recording the water level inside the first tube. Later experiments indicated that the

temperature may have changed slightly during each sampling time due to the heating cycle of the warm room (Section 3.1).

Early in the first attempt at this experiment, approximately half the gas cells had a greatly accelerated rate of gas production consistent with a "short circuit" of the gas cell (data not shown). This was probably caused by water vapour as this high failure rate was not observed at room temperature where less vapour would have been present. Petroleum jelly was smeared on the back of each gas cell to protect the terminals in further trials and only seven out of sixty five gas cells failed in this way, although some corrosion still occurred (Figure 3-10). Five gas cells stopped producing gas much earlier than others with the same resistor. This was later found to be due to the wires corroding or the gas cell chamber dislodging from the collection tube.

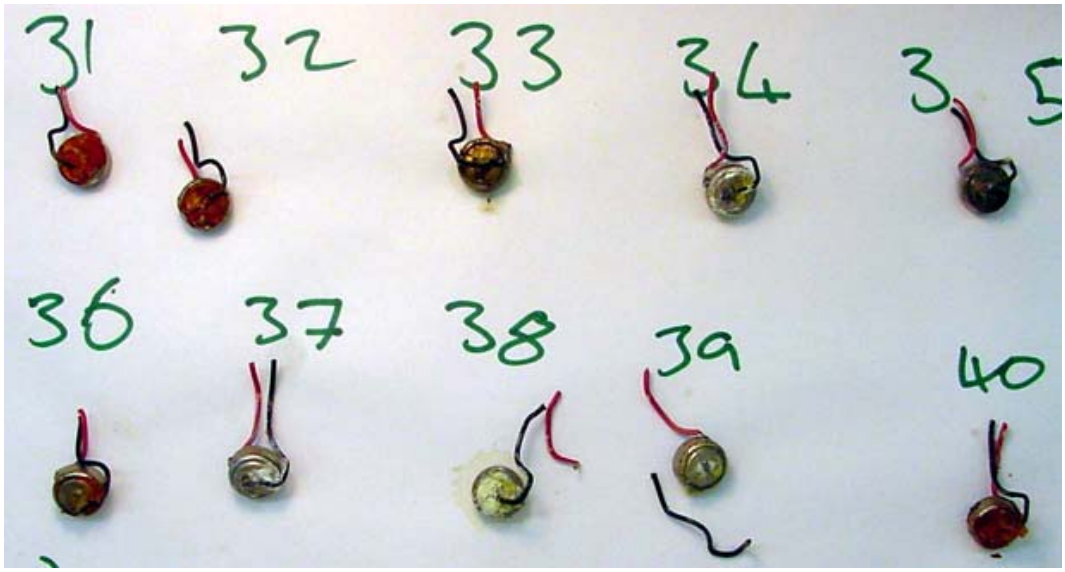


Figure 3-10: Condition of gas cells after 292 days

The mean gas production profiles (Figure 3-11) show three important things; gas was produced linearly until the maximum total volume was reached; gas volume slowly decreased once gas production ceased; and the maximum volume of gas was different for each resistance.

All gas cells tested stopped producing gas at a discreet time. This was in contrast to the decreasing gas production rate observed when gas cells were tested with no external resistance (Figure 3-9). After excluding data from gas cells with irregular

gas production rates (due presumably to moisture damage), gas production for gas cells that reached a discreet endpoint was linear (R^2 = 0.979). A controlled release device with a gas cell could therefore use its full gas production capacity to release payload at a constant rate.

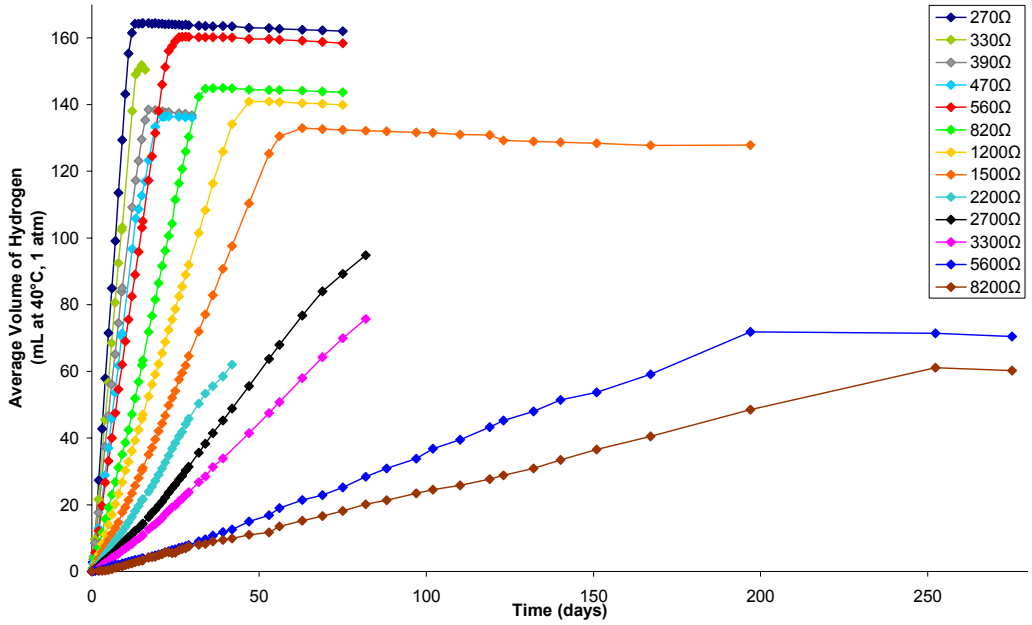


Figure 3-11: Effect of resistance on gas production rate

The decrease in gas volume once gas production ceased was consistent with hydrogen being absorbed by the water. The hydrogen in each tube would be in contact with the same surface area of water under the same environmental conditions and should therefore have been dissolved at the same rate. Average gas loss of 0.037 ± 0.016 mL·day^{-1}(40°C, 1 atm) was calculated by linear regression of gas volume in 21 tubes containing gas cells that had stopped producing gas. Hydrogen dissolution rate for the 50-mL burette method is unknown and may have varied with ambient temperature, further reducing accuracy of data obtained by this method.

Hydrogen dissolution only partly contributes to the decrease in total gas volume with increasing resistance. For example, if two gas cells that produce the same volume of gas were activated with different resistors simultaneously and tested under conditions that gave the same hydrogen dissolution rate, the slower gas cell should produce a lower maximum volume than the faster gas cell. If hydrogen

dissolution was the only cause of the reduced volume, the volume produced by the faster gas cell would equal the volume produced by the slower gas cell by the time the slower gas cell finished producing gas. This did not occur (Figure 3-11) so other factors must be involved.

The total gas volume decreased with resistance and/or time (Figure 3-12, Figure 3-13 respectively). The environmental conditions used in this experiment may have damaged the gas cells (Figure 3-10 for example) and limited total gas volume produced. Three resistances (330, 390 and 470 Ω) tested at a different time to the other ten had maximum volumes that differed from that of similar resistances tested previously, which supports this theory as conditions may have differed slightly when the experiment was repeated. A decrease in total gas volume produced at slower gas production rates would limit the use of this type of device and requires further investigation.

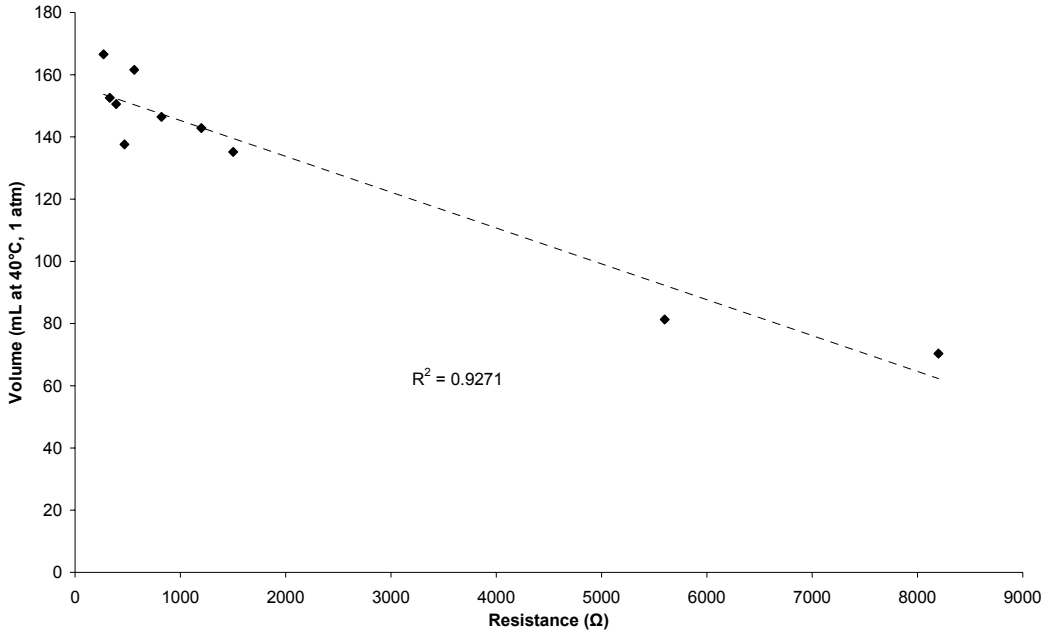


Figure 3-12: Relationship between resistance and average maximum gas volume plus estimated gas loss

The decrease in gas volume produced (thought to be due to hydrogen dissolution) was subtracted from all calculated gas production rates. The rates obtained were usually within one standard deviation of those observed at room temperature, indicating that temperature had little or no effect on gas production. There was a

direct relationship ($R^2 = 0.999$) between conductance and gas production rate up to 3.7 mS (270 Ω), with no resistances tested beyond this value (Figure 3-8). There was also a direct relationship ($R^2 = 0.999$) between resistance and reciprocal gas production rate to 3300 Ω, indicating this method is valid over a wider range of gas production rates than the 50-mL burette method (Section 3.2.1.1).

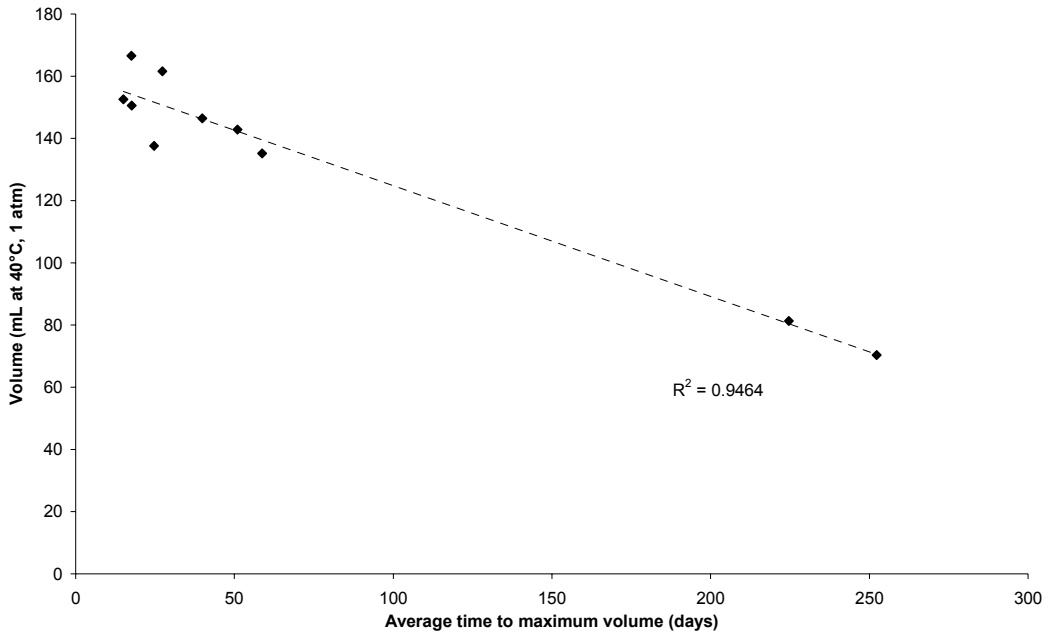


Figure 3-13: Relationship between operating time and average maximum gas volume plus estimated gas loss

3.2.1.3 5-mL burette method

To generate more accurate rate measurements, gas was collected in 5-mL burettes. Gas cells were suspended in a funnel at the top of each burette and were not directly exposed to water and its potentially damaging effects. Recording ambient temperature immediately before taking each volume measurement increased accuracy. This method was also used to observe the consumption of oxygen by the gas cell.

In the first experiment (270 Ω), burettes were filled above the scale to allow the largest possible gas volume change to be measured. Gas volume initially decreased before increasing and did not reach the scale for several hours. To better observe this decrease in gas volume, burettes were filled to about middle of

the scale when testing 560-Ω resistors. Gas volume decreased for between 3.5 and 4.5 hours before increasing (Figure 3-14). This decrease was 19.3 ± 0.9% of the initial air volume, which is close to the approximate oxygen content of air (20.9%) suggesting that oxygen was consumed before hydrogen was produced. One of the functions of the blue sticker covering the gas-releasing holes in the gas cell is to prevent oxygen entering the gas cell (Winsel 1993). The decrease in volume was only observed if the sticker had been removed, suggesting that it should not be removed before use.

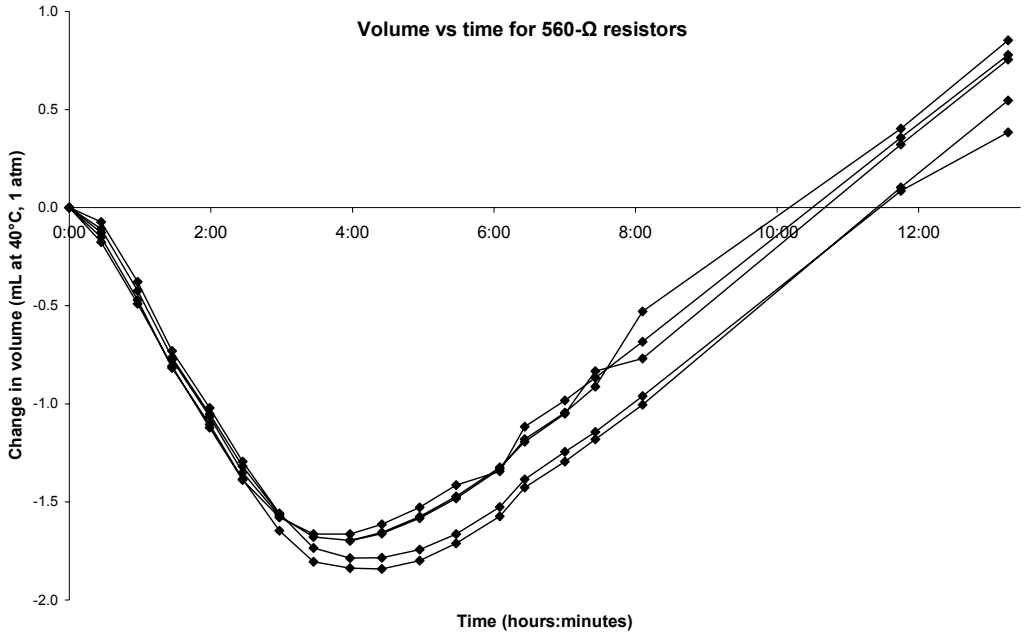


Figure 3-14: Change in volume, indicating oxygen consumption and hydrogen production in 5-mL burettes

When calculating the average gas production rate for each resistance (Table 3-1), hydrogen dissolution rate was assumed to be negligible as surface area of water exposed to the hydrogen in a 5-mL burette is about 5% of that in the larger cylinders. There was a direct relationship between gas production rate and conductance ($R^2 = 0.997$) or resistance ($R^2 = 0.999$) over the entire range of resistances tested (Figure 3-8). As the most accurate of the three methods, values calculated by this method were used as expected values for subsequent experiments. The resistance required for a gas production rate that had not been determined experimentally could be calculated using Equation 3-8 for lower gas production rates and Equation 3-9 for higher gas production rates.

$$G^{-1} = 0.000237R + 0.0125 \quad \text{3-8}$$

$$G = 3500R^{-1} + 0.296 \quad \text{3-9}$$

3.2.2 Rate of gas diffusion

The gas production rate from the gas cell could now be accurately controlled (Section 3.2.1). For that control to extend to the rate of extrusion from a device, all of the gas produced must drive the plunger. Trials had shown that the gas diffusion properties of a barrel affected the release profile from a device (Section 2.2.5). Applying coatings is not an ideal method for reducing gas diffusion as errors in application or damage to the coating in the rumen will cause variability. Barrels were made from several different polymers using the same mould. The hydrogen and carbon dioxide gas diffusion properties of several materials were investigated and an appropriate material was chosen for further experiments. Typical results are shown in Figure 3-15.

Testing of the other major rumen gases may provide additional useful information. However, methane was not tested because of safety concerns. Additionally, the high nitrogen concentration in air would decrease the concentration gradient so the nitrogen diffusion rate was not investigated.

During initial experiments with hydrogen, the temperature of the warm room was not measured and was assumed in calculations to be a constant 40°C. In this experiment, rates of volume change due to diffusion were different from those measured in later experiments (Table 3-2), possibly because there was no compensation for temperature changes. Temperature was measured once per sampling when carbon dioxide was first tested. Significant temperature changes may have occurred between the recording of the first temperature and when the last burette was measured so a single measurement would be insufficient to compensate for temperature fluctuations. Multiple temperature measurements were taken at each sampling (Section 3.1.2.4) in subsequent experiments. An accurate temperature reading is important for this experiment as each scale division is equal to approximately 50 μL in a total gas volume of up to 100 mL.

Under typical conditions a temperature change of 1°C would cause the water level to change by more than 6 of these scale divisions.

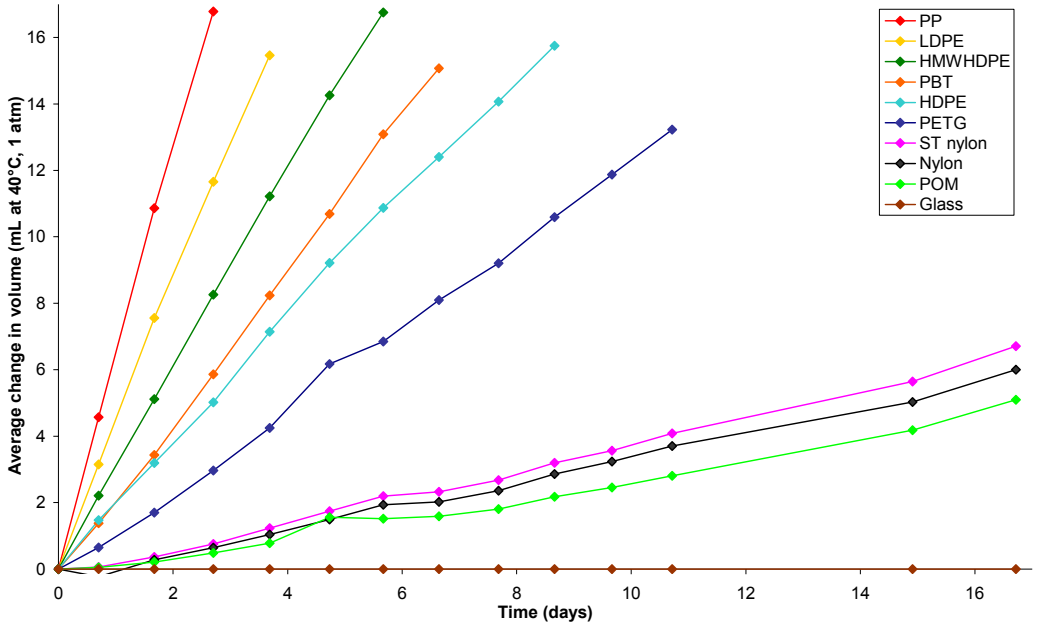


Figure 3-15: Average change in gas volume with time for barrels filled with hydrogen

Table 3-2: Rate of change in volume due to gas diffusion

Barrel	Hydrogen		Carbon Dioxide	
	Temperature not recorded	Temperature recorded	Temperature recorded once	Temperature recorded
PETG	1.62±0.04 (n=5)	1.25±0.02 (n=4)	1.03±0.15 (n=5)	0.61±0.09 (n=4)
Polypropylene	6.78±0.13 (n=5)	6.23±0.04 (n=4)	6.26±0.54 (n=4)	5.01±0.15 (n=4)
1.1-mm POM	0.38±0.01 (n=5)	0.27±0.03 (n=9)	1.98±0.16 (n=5)	1.33±0.33 (n=4)
1.8-mm POM		0.11±0.02 (n=5)		
LDPE		4.20±0.12 (n=4)		7.98±0.26 (n=4)
HDPE	2.11±0.06 (n=5)	1.83±0.08 (n=4)	3.19±0.15 (n=5)	2.67±0.20 (n=4)
HMWHDPE	3.36±0.15 (n=5)	2.97±0.04 (n=4)	5.38±0.22 (n=5)	4.06±0.24 (n=4)
ST Nylon		0.40±0.01 (n=4)		0.36±0.15 (n=4)
Nylon	0.45±0.02 (n=5)	0.37±0.15 (n=4)	0.83±0.13 (n=4)	0.41±0.04 (n=4)
PBT	2.94±0.07 (n=5)	2.31±0.02 (n=4)	2.00±0.12 (n=5)	1.00±0.14 (n=4)
Theratron	1.20±0.26 (n=5)	7.83±0.15 (n=5)	6.57±0.08 (n=3)	6.57±0.08 (n=3)
Terumo	7.61±0.27 (n=4)	7.05±0.08 (n=5)	7.61±0.77 (n=4)	5.55±0.77 (n=4)
B Braun	5.98±0.07 (n=4)	5.89±0.08 (n=5)	5.66±0.16 (n=4)	5.66±0.16 (n=4)
Glass	0.00±0.05 (n=3)	0.15±0.04 (n=6)	2.23±0.03 (n=3)	0.95±0.15 (n=3)

Values are expressed in $mL \cdot day^{-1}$(40°C, 1 atm) ± standard deviation. Values for all materials except glass were adjusted by subtracting the average change in volume for glass at each time point.

Glass syringes filled with carbon dioxide had a greater rate of volume change than those filled with hydrogen, which indicates that some carbon dioxide was still dissolving in the water.

Materials with low hydrogen permeability did not necessarily have low carbon dioxide permeability. The data indicate that either type of nylon would be suitable for barrel material because they have low permeability to both hydrogen and carbon dioxide. However, the friction welding technique used to seal these barrels cannot be used with nylon and therefore POM was chosen for further experiments as it has a low hydrogen permeability.

3.2.2.1 Effect of barrel thickness

POM barrels manufactured with a 64% greater wall thickness lost ~59% less hydrogen (Figure 3-16 and Table 3-2). A decrease of ~39% per surface area was expected as flux is inversely proportional to thickness (Section 1.6.4). The effective surface area of diffusion for a cylinder can be calculated from the effective radius of diffusion (r_D):

$$r_D = \frac{r_e - r_i}{\ln(r_e / r_i)}$$ **Equation 3-10**

where r_e is the external radius of the cylinder and r_i is the internal radius (Kjeldsen 1993). This increase in wall thickness would therefore have increased the surface area of diffusion by about 3%.

3.2.2.2 Measured diffusion rates

The environment within each barrel was initially composed entirely of hydrogen or carbon dioxide, creating a concentration gradient between the interior and exterior of the barrel walls. The external environment contains many gases, all with a concentration gradient between the exterior and interior. Therefore, results obtained from these experiments do not give absolute hydrogen or carbon dioxide diffusion rate, but the sum of all gas diffusion in both directions. These values are more relevant to the function of a gas-driven device than individual coefficients of diffusion, which would need to be known for all the gases involved to calculate the rate of change in total gas volume due to diffusion. As the magnitude of inward diffusion is unknown, apparent low hydrogen or carbon dioxide permeability could also be explained by the barrel material having a high permeability to environmental gases. Different rates of inward diffusion for

different gases may mean that the results of this experiment would have been different if conducted in an environment of rumen gas.

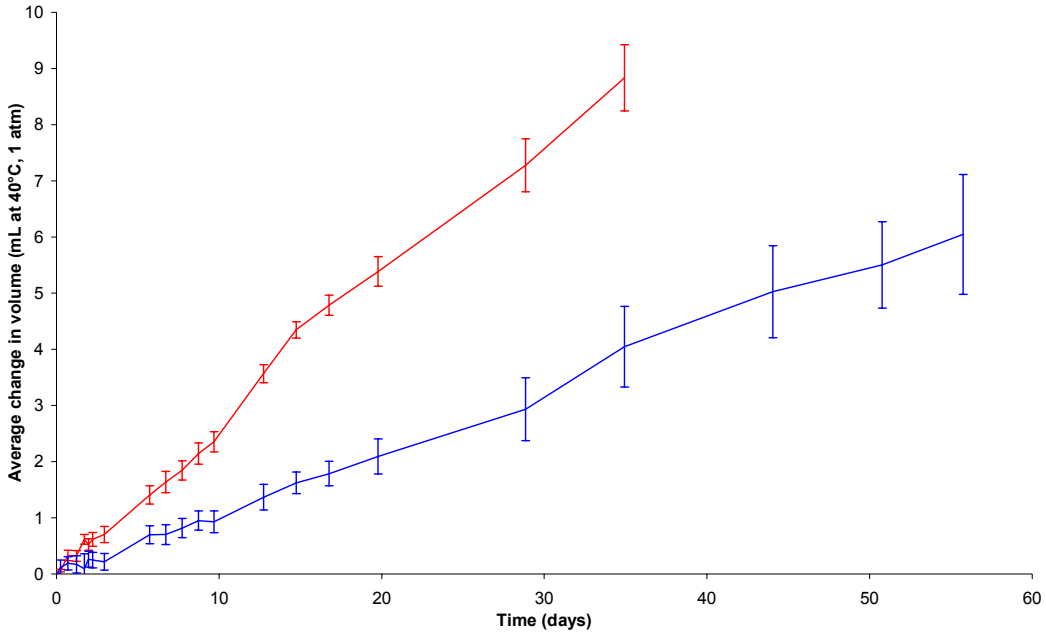


Figure 3-16: Effect of POM wall thickness on average change in gas volume
— = 1.1-mm wall. — = 1.8-mm wall. Error bars show standard deviation (n=5).

The reported solubility values and diffusion coefficients (Table 3-3) were used to calculate the theoretical, steady-state rates of gas loss by diffusion for HDPE and LDPE barrels filled with hydrogen and carbon dioxide (Table 3-4). Calculated values of these loses were 50% to 90% of those observed, although the values in Table 3-3 are for unspecified grades of HDPE and LDPE at 25°C.

Published values of POM permeability to oxygen, nitrogen and carbon dioxide are 0.09, 0.024 and 0.45 barrer (1×10^{-10} mL·cm·s^{-1}·cm^{-2}·cmHg^{-1} at 0°C, 1 bar) respectively (Kjeldsen 1993). Thus, POM barrel filled with carbon dioxide with 1.1-mm walls should lose 0.39 mL·day^{-1} (40°C, 1atm) by diffusion. This is about 30% of the value obtained experimentally. However, the type of POM used to determine the published permeability was not specified.

3.2.2.3 Inward diffusion

Hydrogen and carbon dioxide diffusion outwards occurred at a greater rate than the sum of any inward gas diffusion. If atmospheric gases diffuse into the barrels,

hydrogen or carbon dioxide within would be diluted, decreasing the partial pressure. This would result in a gradual decrease in the rate of inward and outward diffusion. Thus, the net diffusion rate would gradually decrease during this experiment.

Table 3-3: Published solubilities and diffusion coefficients of selected gases
(Composite-Agency Undated)

Polymer	Gas	Diffusion coefficient $m^2 \cdot s^{-1}$	Solubility $m^3 \cdot m^{-3} \cdot bar^{-1}$ (25°C, 1 bar)
HDPE	H_2	2.6×10^{-11}	0.073
	N_2	9.3×10^{-12}	0.025
	O_2	1.7×10^{-11}	0.046
	CO_2	1.2×10^{-11}	0.29
LDPE	H_2	4.7×10^{-11}	0.16
	N_2	3.2×10^{-11}	0.021
	O_2	4.6×10^{-11}	0.05
	CO_2	3.7×10^{-11}	0.25

Table 3-4: Measured and predicted diffusion rates of gases through HDPE and LDPE barrels ($mL \cdot day^{-1}$ and 40°C, 1atm)

	High-density polyethylene			Low-density polyethylene		
Contents	**Gas**	**Calculated**	**Measured**	**Gas**	**Calculated**	**Measured**
hydrogen	H_2	1.14		H_2	4.54	
	N_2	-0.11		N_2	-0.32	
	O_2	-0.11		O_2	-0.32	
	CO_2	-0.0008		CO_2	-0.0021	
	Total	**0.93**	**1.83±0.08**	***Total***	**3.90**	**4.20±0.12**
carbon dioxide	CO_2	2.10		CO_2	5.58	
	N_2	-0.11		N_2	-0.32	
	O_2	-0.11		O_2	-0.32	
	Total	**1.88**	**2.67±0.20**	***Total***	**4.94**	**7.98±0.26**

To investigate if this happened, residual plots for the average rate of volume change were generated. Only data from experiments with multiple temperature measurements and for barrels with at least 10 recorded data points, to ensure that the regression is accurate, are shown (Figure 3-17) to ensure that linear regressions were valid. These graphs show the difference between the observed

average change in gas volume and the expected value for that time, based on linear regression of the entire data set. Data for hydrogen fall close to the regression line for all barrel materials, indicating that the gas diffusion rate did not decrease over time. For carbon dioxide, however, the points change from below the regression line to above it then back again. This indicates decreasing net diffusion as would be expected if an external gas were diffusing into the barrel. There is no reason for inward air diffusion to occur at different rates, depending on the non-air gas in the barrel. It is possible that these fluctuations were caused by some environmental factor, humidity for example, and that the pattern seen for carbon dioxide has occurred by chance. The residual plots of all barrel types were comparable to those of HDPE, which should allow about 0.2 $mL \cdot day^{-1}$ (40°C, 1 atm) of inward diffusion (Table 3-4). At this rate, the partial pressure of the internal gas (and therefore the rate of diffusion) would have been reduced by <10% during the hydrogen experiment and <5% during the carbon dioxide experiment.

3.2.3 Predicting plunger position

Gas production and diffusion are the main factors in determining the release profile from this device. Plunger position at any time can be predicted mathematically when these values are known. A method was developed to calculate the plunger position at many incrementally increasing time points (Section 3-9). Equation 3-7 was developed to overcome the limitations of this method.

Finite difference approximation does not give an exact prediction because the diffusion value used in each iteration is true only at the time of the previous iteration. The value generated by this process approaches the true theoretical value of plunger position as iterations used to generate the value approaches infinity. Plunger position predicted by iterative calculation approached the value predicted by Equation 3-7 as iterations per day increased (Figure 3-18), indicating that the two methods agree. For simplicity, Equation 3-7 was used in all subsequent calculations.

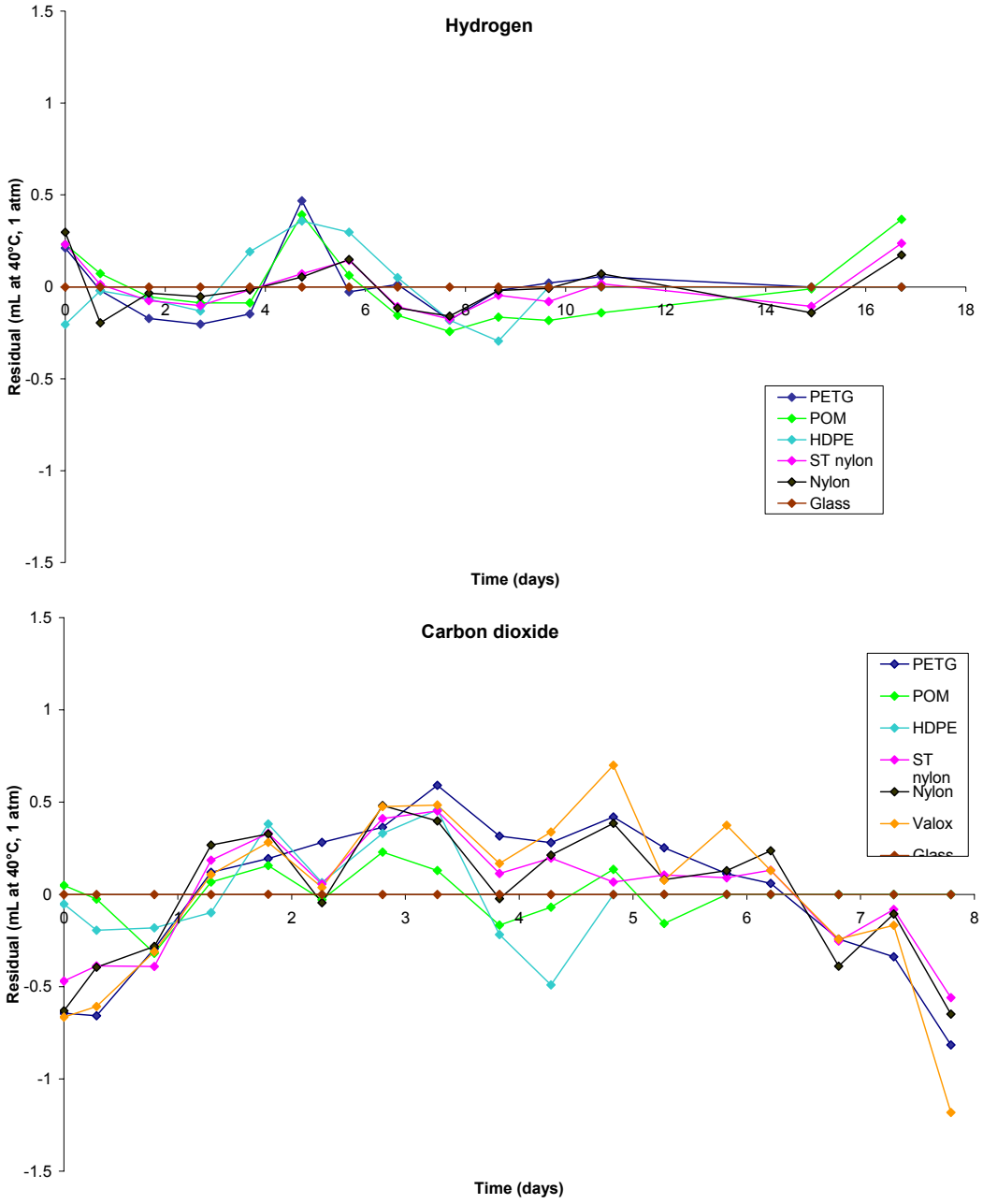


Figure 3-17: Residual plot indicating difference between regression line and actual average change in gas volume

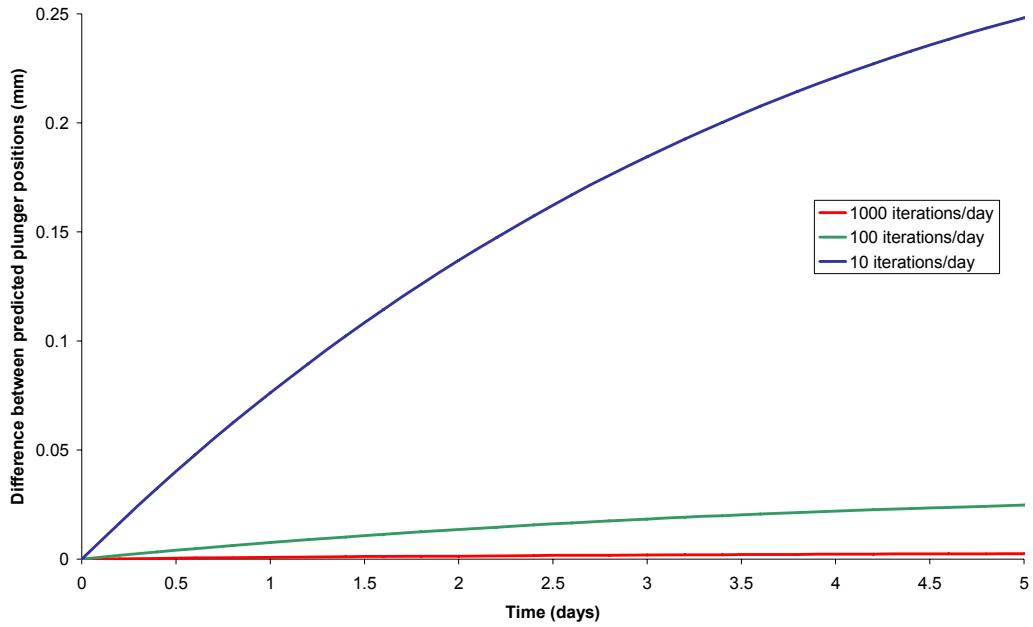


Figure 3-18: Effect of number of iterations on the difference between values predicted by finite difference approximation and Equation 3-7
Difference between the predicted plunger position for a Theratron with a 330Ω resistor at 40° and 1 atm.

3.2.3.1 Assumptions

Equation 3-7 gives plunger position as a function of time with the following assumptions:

- *l*, as calculated from Equation 3-7, represents the length of barrel exposed to the hydrogen. Although this is not necessarily equal to plunger position (for example, the Theratron plunger in Figure 2-7), it can be corrected by adding the distance between the measurement point and the point where the plunger seals with the barrel.

- Gas diffusion rate is directly proportional to surface area of exposed barrel material. The barrel must be made of a homogenous material, with walls of even thickness and a cavity of constant diameter.

- Gas diffusion rate per surface area is constant with time. This may not be true if inward diffusion of another gas causes the partial pressure gradient controlling outward hydrogen diffusion to decrease. The magnitude of inward gas diffusion is unknown, however Equation 3-7 will hold true if

this steady-state is reached rapidly or inward diffusion is too slow to be significant over the device's lifetime.

- Any gas initially in the device is assumed to be of the same composition and pressure as will be in the device at all times during its operation. In practice, this will probably be air at atmospheric pressure. In this case the hydrogen partial pressure gradient will initially be much lower than it was under the conditions that D_L was measured. The resulting decrease in diffusion rate will only be as significant as the initial diffusion rate because contaminating gases will be quickly diluted with hydrogen. If pressure required to drive the plunger is greater than the starting pressure in the gas chamber, there will be an initial "lag" while gas pressure builds. Length of this period is related to initial volume and pressure required. For these reasons, initial volume should be minimised.

- Equation 3-7 is undefined when A, L or D_L is equal to zero. In practice, this is not an issue as barrel length or cross-sectional area cannot be zero and plunger position can be predicted easily in a system where no gas diffusion occurs.

3.2.3.2 Compensating for pressure and temperature

The term G in Equation 3-7 refers to the rate of gas production. This was measured in $mL \cdot day^{-1}$ at 40°C and 1 atm. Inside the device, however, conditions may differ from these.

Pressure will probably be greater than atmospheric as there will be some resistance to plunger movement. The diffusion rate of any gas is proportional to its partial pressure gradient. Therefore, a device operating at 2 atm would lose twice as much gas (moles) by diffusion as a device operating at 1 atm. However, gas lost from the device at higher pressure would occupy the same volume as that lost from the lower pressure device. Therefore, D_L should remain constant at any pressure.

An increased pressure will decrease the volumetric gas production rate (G), which can be adjusted using Boyle's law:

$$P_1V_1 = P_2V_2 \qquad 3\text{-}11$$

and gas production rate can be adjusted for temperature changes with Gay-Lussac's law:

$$\frac{V_1}{V_2} = \frac{T_1}{T_2} \qquad 3\text{-}12$$

3.2.3.3 Effect of gas production rate

The predicted release profiles (Equation 3-7) of Theratron devices driven by various gas production rates allowing for the measured amount of gas diffusion are shown in Figure 3-19.

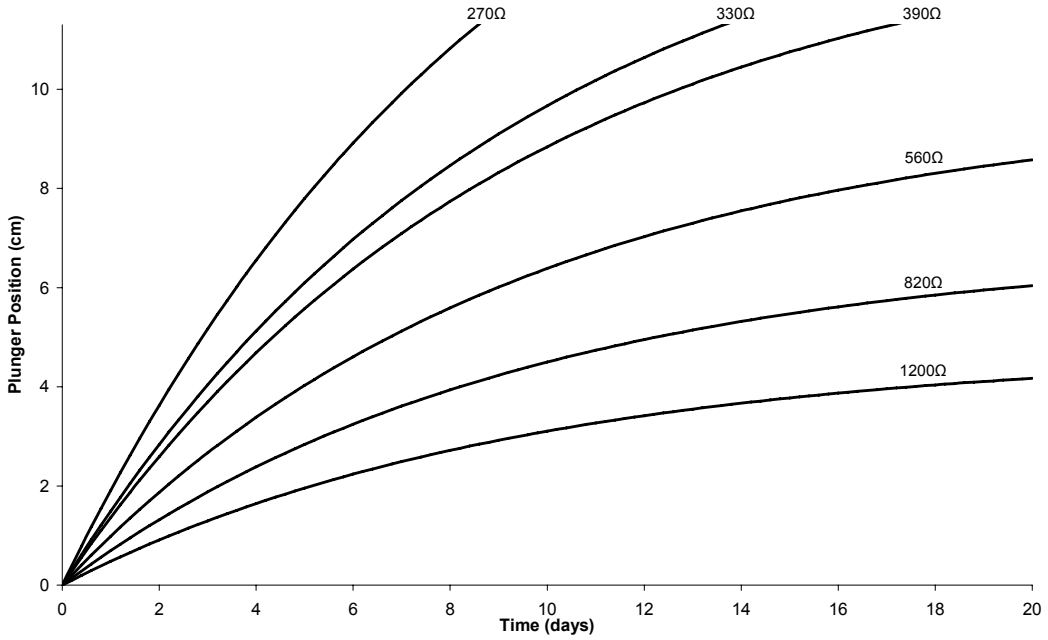


Figure 3-19: Effect of resistor on theoretical plunger position for Theratrons

The release profile deviates further from linearity as gas production rate decreases and approaches the rate of gas loss. When gas loss rate through the full length of a barrel exceeds gas production rate, the plunger will not reach the end of the barrel. Plunger movement will gradually decrease as plunger position tends to an asymptote where gas loss equals gas production. The point where this occurs can be calculated by rearranging Equation 3-1 given that $G = D_l$ at the asymptote:

$$l_{\lim} = \frac{GL}{D_L} \qquad 3\text{-}13$$

If the plunger was in a position beyond the asymptote, it would move backward.

3.2.3.4 Effect of gas diffusion rate

The predicted release profiles for devices made from barrels of various materials are shown for different gas production rates in Figure 3-20 and Figure 3-21. Control of gas diffusion is more important at lower gas production rates, as are necessary for longer acting devices.

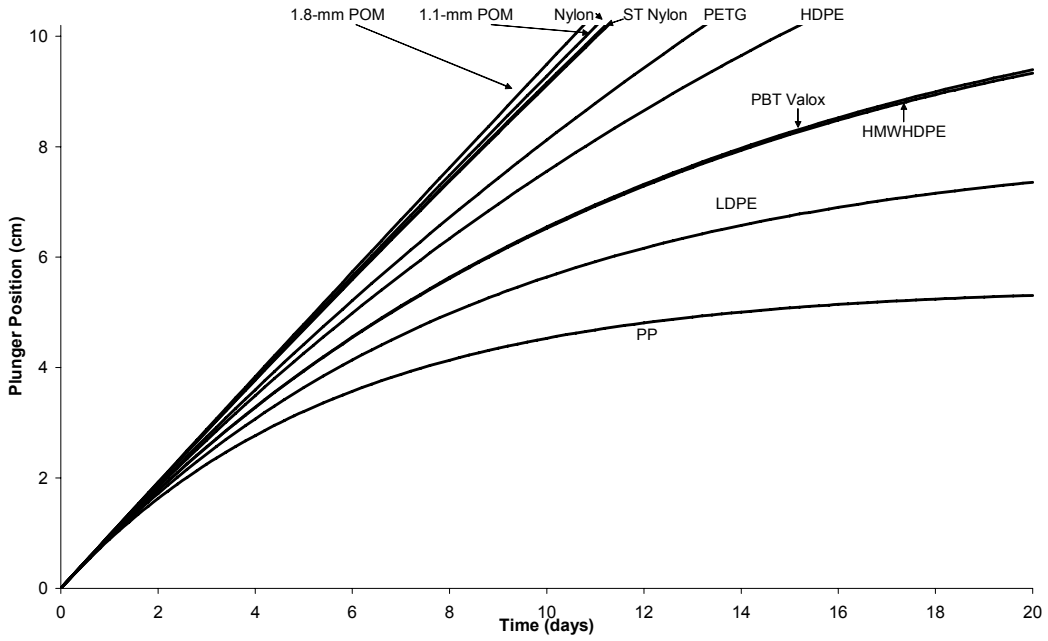


Figure 3-20: Effect of barrel material on theoretical plunger position for 3.34 mL·day^{-1} (40°C, 1 atm)

Figure 3-21 shows the predicted plunger position with time for a gas production rate of 0.4 mL·day^{-1}(40°C, 1 atm) as could be generated using a resistor of approximately 10500 Ω. Under these conditions, even 1.8-mm thick POM barrels are affected by gas diffusion with the 0.11 mL·day^{-1} (40°C, 1 atm) full-barrel diffusion rate having a significantly different release profile than the equivalent barrel with no diffusion.

Gas diffusion could theoretically be reduced to allow a longer duration of delivery by using thicker barrel walls and/or a less permeable material. Gas diffusion

through glass or metal barrels, while inappropriate for this application, should be negligible. Because of the components available, devices with a target period no longer 50 days were used in subsequent experiments.

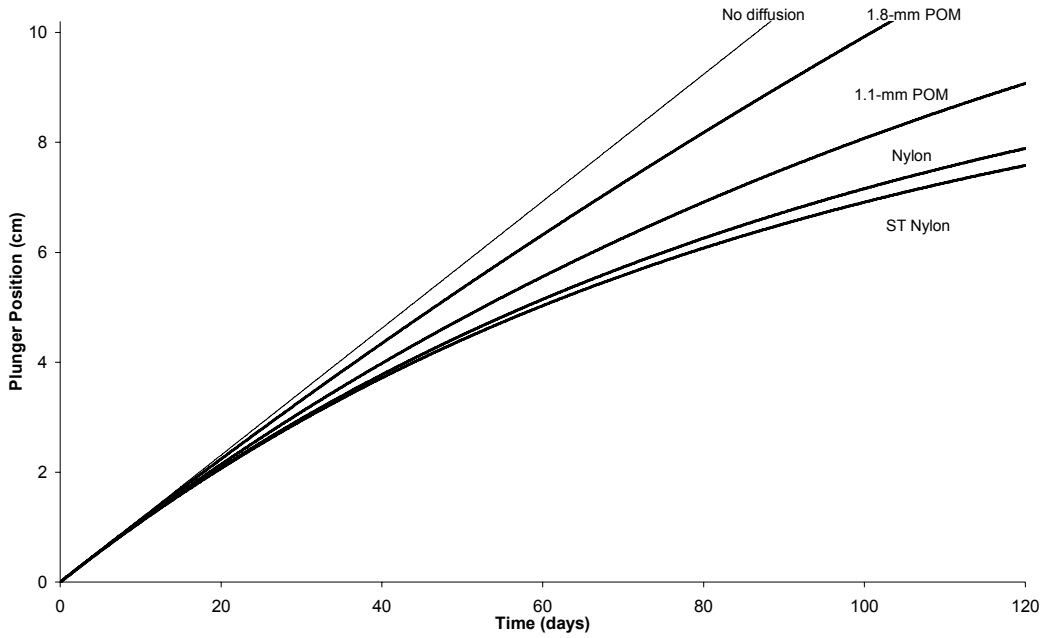


Figure 3-21: Effect of barrel material on theoretical plunger position for 0.4 mL·day^{-1} (40°C, 1 atm)

3.2.3.5 Theoretical profiles of devices from Chapter 2

With an increased theoretical understanding of the function of this device, previous data were reassessed. The release profiles calculated using Equation 3-7 were compared with results from Chapter 2. Predicted release profiles were approximations as the operating pressure was unknown and not compensated for.

The predicted release profiles of all short-term devices were similar to data observed *in vitro* although the observed plunger position was always less than what was predicted. A small increase in operating pressure of 0.1 to 0.2 atm, which would be expected due to resistance to plunger movement, may account for this difference. Theoretical release profiles for all non-coated, 50- and 100-day devices (Figure 3-22) show that plungers should not have moved more than a few millimetres from their starting position and some should have moved backwards (this was prevented by the presence of the gas cell). *In vivo* examples where slower devices released more than half their payload were consistent with inward

diffusion of rumen gases. Inward diffusion may also explain why 10-day Theratron devices in Trial 1 released linearly while both the predicted profile and *in vitro* data had decreasing release rates. Theoretical profiles could not be generated for coated barrels as D_L was unknown.

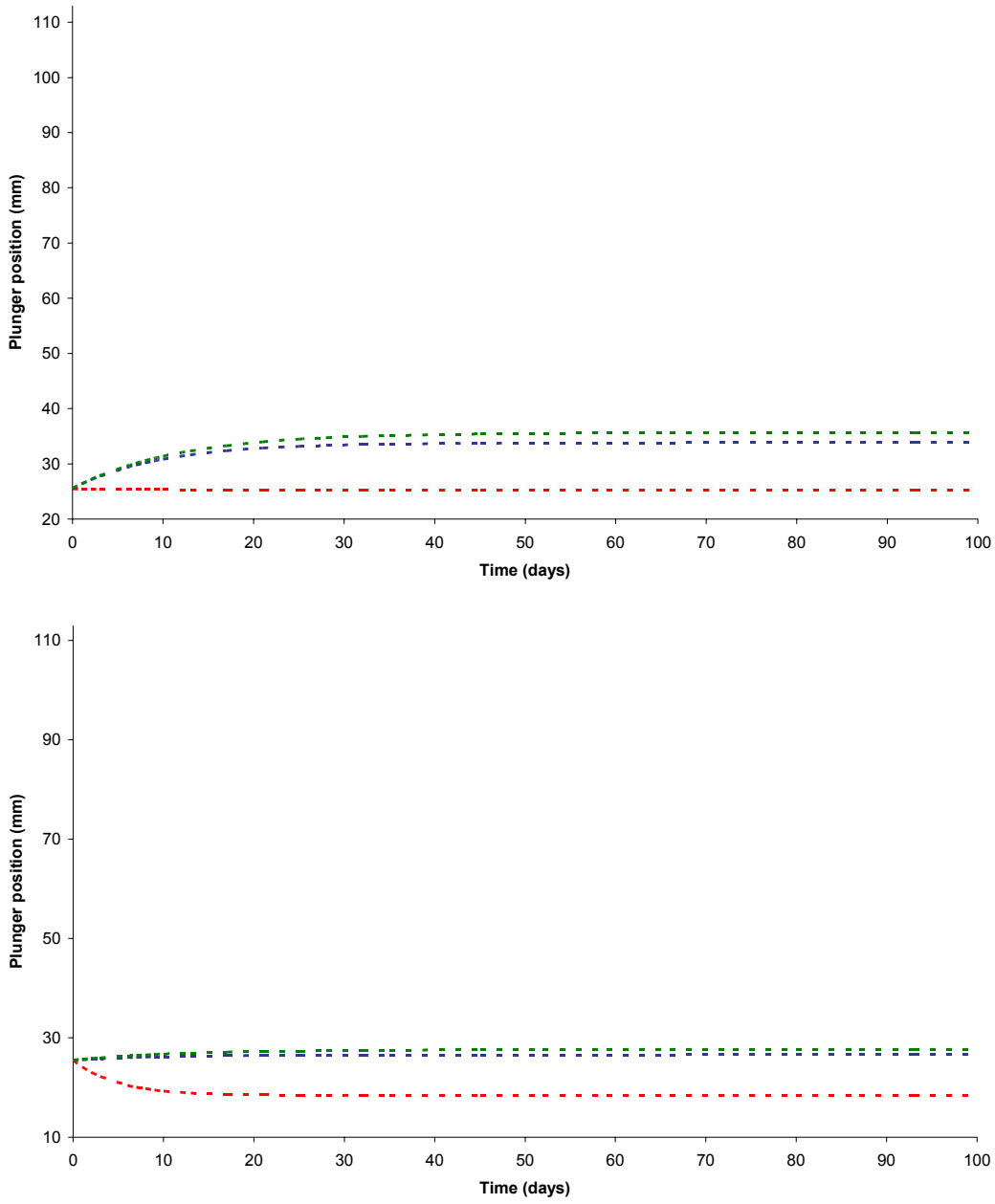


Figure 3-22: Theoretical release profiles for 50- (top) and 100-day devices (bottom) used in Trial 2
--- = B Braun. --- = Theratron. --- = Terumo.

3.3 Conclusions

Experiments in this chapter generated sufficient data on gas production rate and diffusion to predict theoretical release profiles of devices operating in air. However, it was unclear if these predictions were relevant to devices operating in an atmosphere of rumen gas. Behaviour of many devices tested in Chapter 2 that were assumed to have failed was shown to be consistent with the theory developed in this Chapter. Devices made from materials identified in this chapter should have sufficiently low hydrogen permeability to give a constant release rate over extended periods.

Chapter 4: Application

Devices were tested both *in vivo* and *in vitro* to demonstrate that the findings of Chapter 3 provided sufficient information to make a device that releases linearly at the desired rate. Devices were tested *in vitro* and *in vivo* at two different release rates. *In vitro* devices were incubated at 40°C in air as submerging devices in water may alter the gas diffusion rate.

4.1 Methods and materials

4.1.1 Effect of water on polyoxymethylene

Five POM barrels were left in 40°C water for 322 days to check for water absorption. Barrels were then wiped dry and further dried at 40°C for 10 minutes before being weighed to 3 decimal places.

4.1.2 Measurement of operating pressure

The gas pressure inside devices of each type to be used in these studies was measured using 0 to 6.9-kPa (0-1 p.s.i.) and 0 to 34.5-kPa (0-5 p.s.i.) Differential Piezo-Resistive Pressure Transducers (supplied by RS New Zealand) powered by a Digital Display 0-30 V 2.5 A Lab Power Supply (Dick Smith Electronics, New Zealand). Output was recorded with a Pico ADC-100 voltage logger (Pico Technology Limited, United Kingdom). A pressure sensor was inserted into a hole drilled in the top of a POM screw-cap and sealed in place with hot glue. Devices to be monitored were screwed onto this cap and held under 40°C water such that only the pressure transducer protruded.

4.1.3 Testing of devices *in vitro* and *in vivo*

Devices were constructed with the following variables as listed in Table 4-1. Devices were made from 1.8-mm POM barrels (7 replicates) with either POM "Hard" plungers, or Santoprene Thermoplastic Rubber 75 Shore A Hardness "Soft" plungers with a POM component to maintain form (Figure 4-1). Devices were filled with either 2% or 5% HPMC (Section 2.1.1.1). HDPE barrels (1.1-mm wall thickness) with soft plungers were tested for comparison (8 replicates). Devices were driven by gas cells activated with either 1200 or 5600-Ω resistors. Devices were tested both *in vitro* in the 40^{o}C room (Section 3.1) and *in vivo* in the

rumen of fistulated cattle (Section 2.1.1.2). Nylon barrels (1.1-mm wall thickness) with soft plungers, 2% HPMC, 1200-Ω resistors and sealed with rubber bungs were tested *in vivo* alongside the slower POM and HDPE devices.

Table 4-1: Device configurations used for *in vitro* and *in vivo* experiments

Barrel	Resistor (Ω)	Plunger	%HPMC
1.8-mm POM	1200	Hard	2%
1.8-mm POM	1200	Hard	5%
1.8-mm POM	1200	Soft	2%
1.8-mm POM	1200	Soft	5%
1.8-mm POM	5600	Hard	2%
1.8-mm POM	5600	Hard	5%
1.8-mm POM	5600	Soft	2%
1.8-mm POM	5600	Soft	5%
HDPE	1200	Soft	2%
HDPE	5600	Soft	2%

4.1.3.1 Manufacture of devices

The wings of the injection-moulded barrels were designed for intravaginal retention and may harm the animal if used in the rumen. Before use, the wings were removed from the barrel using a hacksaw. This left three open orifices in the end of the barrel; the two smaller orifices were sealed with hot glue and the larger orifice was used to attach a one-way valve.

To attach the one-way valves, the thread was removed from the valves (check-valve w/female inlet and male luer lock, Qosina, U.S.A, Figure 4-2) by melting with a soldering iron. The valves were coated with hot glue and inserted to the large orifice.

The orifice was sealed by screwing an end cap onto the luer lock of the valve. The formulation to be tested was then poured in through the large open end of the barrel and the plunger was inserted and used to expel any trapped air. Lubricant (Hydra-Slip silicone lubricant, Lo-Chlor Chemicals, Australia) was applied to plungers of the *in vitro* 50-day devices to prevent failures due to gas leaking past the plungers.

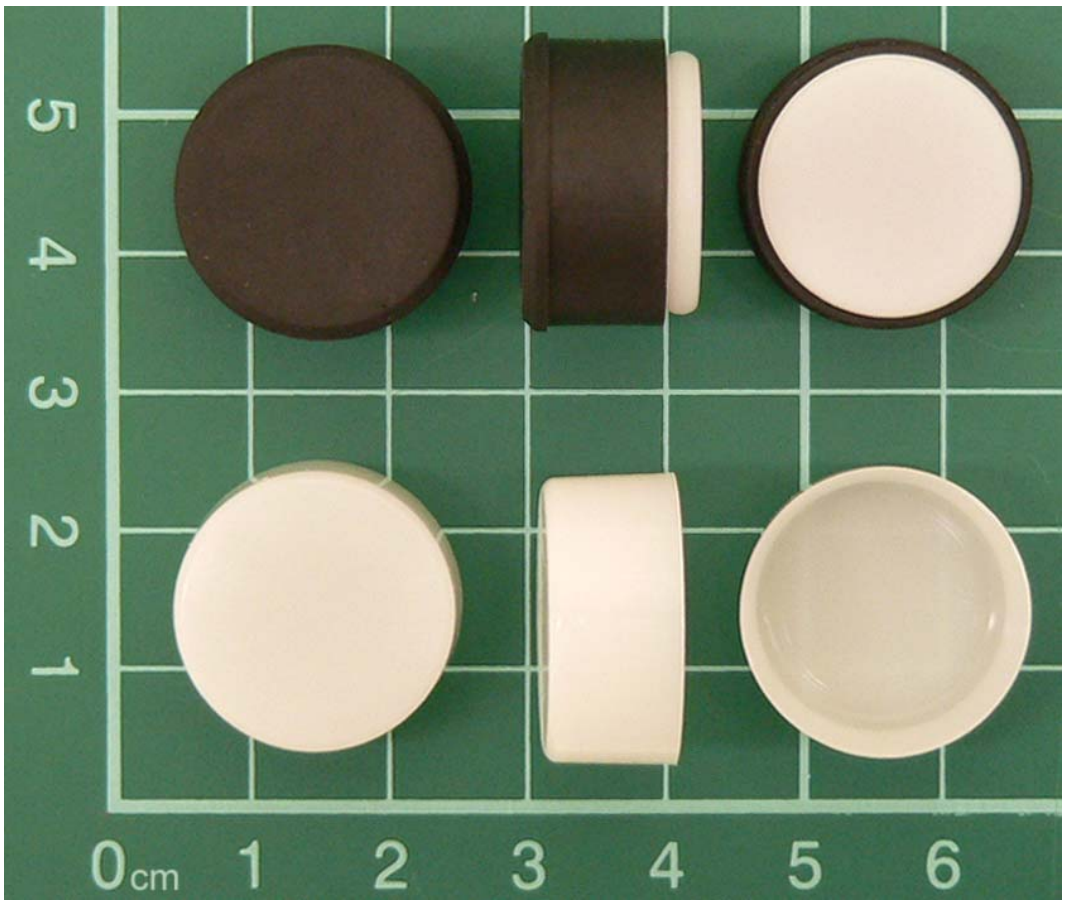


Figure 4-1: Plungers used in injection moulded barrels
Top: Soft plungers, Bottom: Hard plungers.

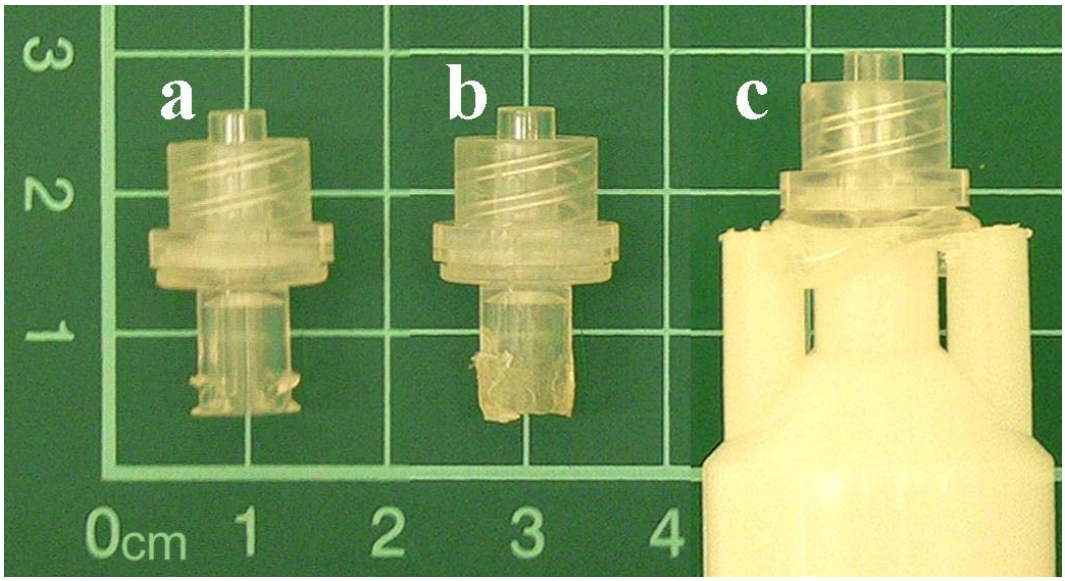


Figure 4-2: The Qosina one-way valve
One-way valve initially (a), with thread removed (b) and attached to a device (c).

Gas cells were activated by soldering a resistor between the terminals without removing the blue sticker. Gas cells were placed on a piece of masking tape, with

the blue sticker side down, and then spray-on rubber was applied to the terminals of each gas cell (Figure 3-4) to prevent moisture damage.

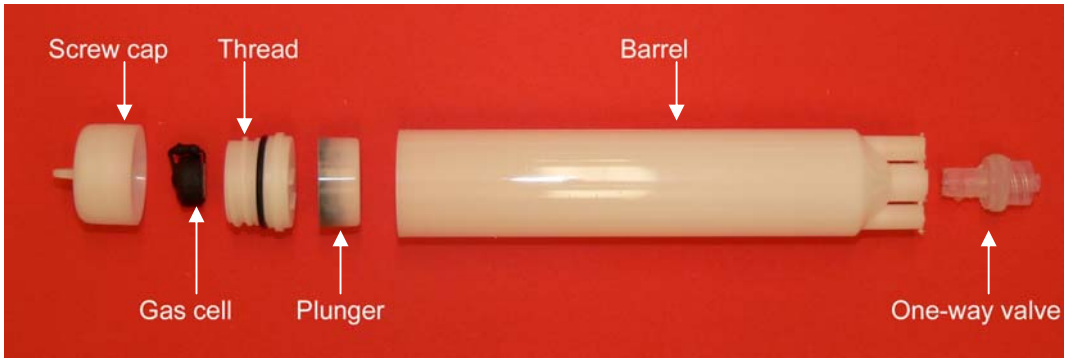


Figure 4-3: Components of an injection moulded device

A thread was friction welded to each barrel and devices were then sealed by screwing a cap onto the thread (Figure 4-3). Excess lubricant was removed with 10% toluene in ethanol before spin welding. Each loaded barrel was placed in the friction welder (Figure 4-4). A thread component was placed on the bit, lowered onto the barrel and spun for 2 to 3 s without any additional force. A 1250-g weight was applied (at a set distance from the fulcrum) and spinning continued until the lip on the thread component was level with the barrel end (~5 s). An O-ring was then placed below the thread to form a seal between the thread and the cap. The friction weld was tested by sealing the device onto "the leak tester" (Figure 4-5) and pressurising it to ~200 kPa with a bicycle pump. The pressurised weld was held under water for 20 s to check for leaks. An activated gas cell was placed in the thread component before screwing on the sealing cap.

4.1.3.2 Experimental conditions

The first devices tested *in vitro* were placed in trays with the orifice submerged in shallow water with the barrel resting on the side of the tray. After the first run for 50-day devices *in vitro*, devices were held in a test-tube rack so their orifices pointed down into shallow water. Devices tested *in vivo* were clipped to a tether through a hole drilled in the tab of the screw-cap and inserted through the cannulae of ruminally fistulated cattle (Section 2.1.1.2). Three or four devices were inserted in each cow.

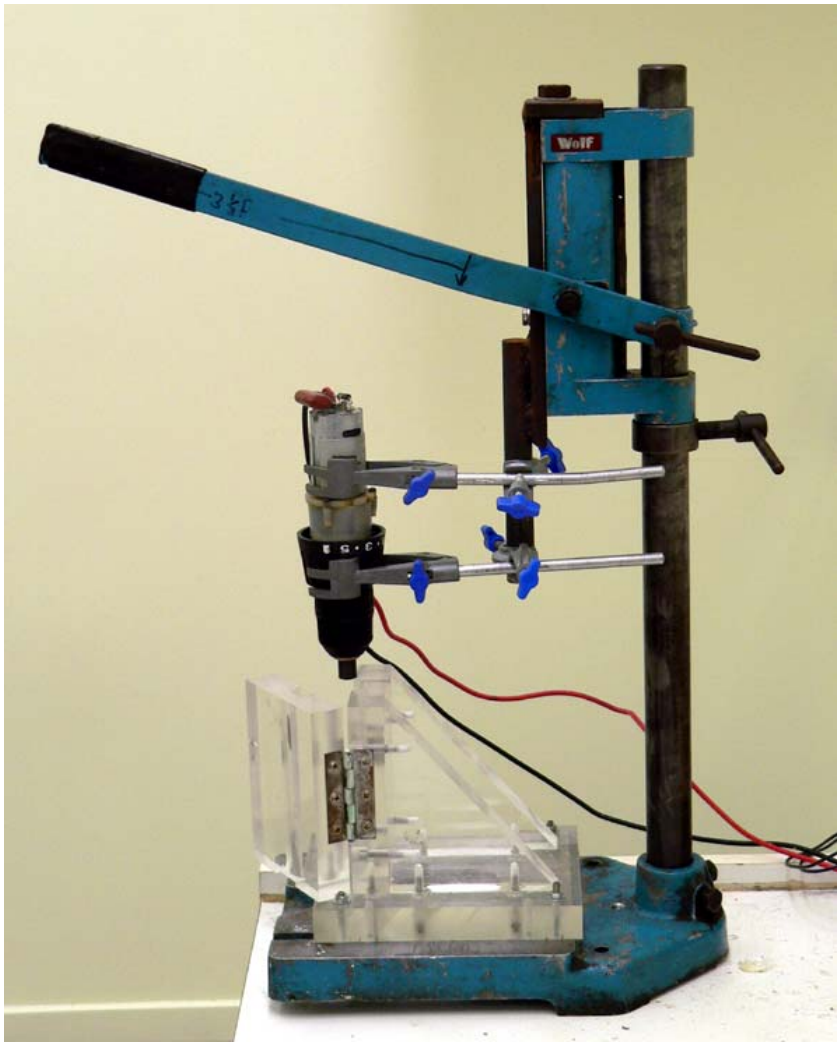


Figure 4-4: Spin welder

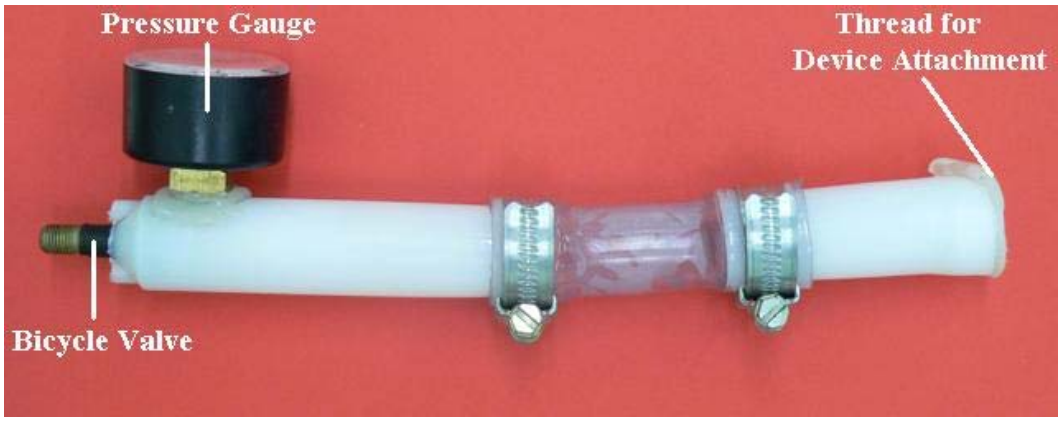


Figure 4-5: Leak tester

4.1.3.3 Sampling

Sampling of devices *in vivo* was conducted as described in Section 2.1.1.3. Plunger position and weight *in vitro* were measured without removing the devices from the 40°C room. Plunger position of devices with soft plungers was measured from the front of the plunger while that of hard plungers was measured from the back (similar to Figure 2-7).

4.1.3.4 Gas analysis

Once spent, each device was removed from the rumen and within 5 hours a gas sample taken and analysed by gas chromatography. A 10-mL syringe was fitted with a needle, flushed with helium to remove air and then used to withdraw a sample (minimum 6 mL) from a device. The HDPE devices were sampled by inserting the needle directly through the barrel wall. This method could not be used to obtain gas samples through the thicker and harder walls of the POM devices so samples were taken by removing the one-way valve and inserting the needle through the plunger. Devices with hard plungers could not be sampled because the needle could not penetrate the plunger. Gas samples were injected immediately into a Perkin Elmer, AutoSystem XL gas chromatograph with an Altech, Hayesep Q – mesh 80/100 column and a VICI 10–port injection valve with 1-mL sample loop, at an oven temperature of 40°C with a helium carrier at 20 mL·minute^{-1}. The sample loop was flushed with helium to remove all other gas before injecting a sample of at least 3 mL. Gases were detected with a thermal conductivity detector. Peak areas were calculated using TurboChrom 6.1.1.0.0:K20 and compared with standards of known concentration. Each sample was injected a second time with the signal reversed to allow detection of hydrogen.

4.2 Results and discussion

Data generated in this chapter can be found in Appendix C.

4.2.1 Selection of components

Thickened POM barrels, chosen for their low hydrogen permeability (Section 3.2.2), were compared to HDPE barrels. "Soft" santoprene plungers, made with

the same tool as plungers used in the intravaginal device (Ismail 2006), and "hard" plungers, made from POM in a new design based on plungers from the Rumensin capsule (Figure 4-1) were tested in the POM barrels.

Two concentrations of HPMC were used to investigate the effects that payload viscosity may have on device function. To ensure that water in the formulation and the environment was not absorbed by the POM barrels and plungers, six POM barrels were submerged in 40°C water for 11 months (Section 4.1.1). Over this time the barrel weight increased by an average of 0.4 ± 0.05%. This should not interfere significantly with device function assuming that any associated change in size was of similar proportions. Any material that has been shown to be compatible could be delivered using this device. Barrels and plunger could also be manufactured from different polymers to accommodate different materials.

The barrel diameter needed to be measured accurately to calculate the cross-sectional area for use in Equation 3-7. Six POM barrels were measured in two perpendicular directions at both ends and in centre of the barrel by Millennium Plastics (Hamilton, New Zealand). The average internal diameter was 20.52 ± 0.02 mm. The average internal diameter of the open end of 10 HDPE barrels measured in two perpendicular directions with electronic callipers was 20.70 ± 0.02 mm. Because of their larger diameter, hard plungers could not be used in HDPE barrels.

Equation 3-7 predicts that 1200- and 5600-Ω resistors would give gas production rates that should release the contents of the POM devices over 8 and 43 days respectively. The same resistors were also used in HDPE barrels. Predicted profiles indicated that the release rate from these devices should decrease significantly with increasing plunger displacement due to the higher hydrogen permeability of HDPE.

The operating pressure of a device depends on the ambient pressure. The pressure difference between the gas chamber and the environment is therefore more important than absolute pressure. Gas pressure within each configuration was monitored using differential pressure transducers (Section 4.1.2). Three or four devices of each configuration were tested while being driven by a "short-

circuited" gas cell (i.e. terminals connected with solder, no external resistance). The operating pressure decreased slightly with plunger position in devices with soft plungers (Figure 4-6) and increased slightly in devices with hard plungers. An additional device of each configuration that had first been equilibrated to 40°C revealed similar pressure profiles to the unequilibrated devices, indicating that variation in pressure was not due to thermal expansion of the plunger and barrel when temperature was increased to 40°C.

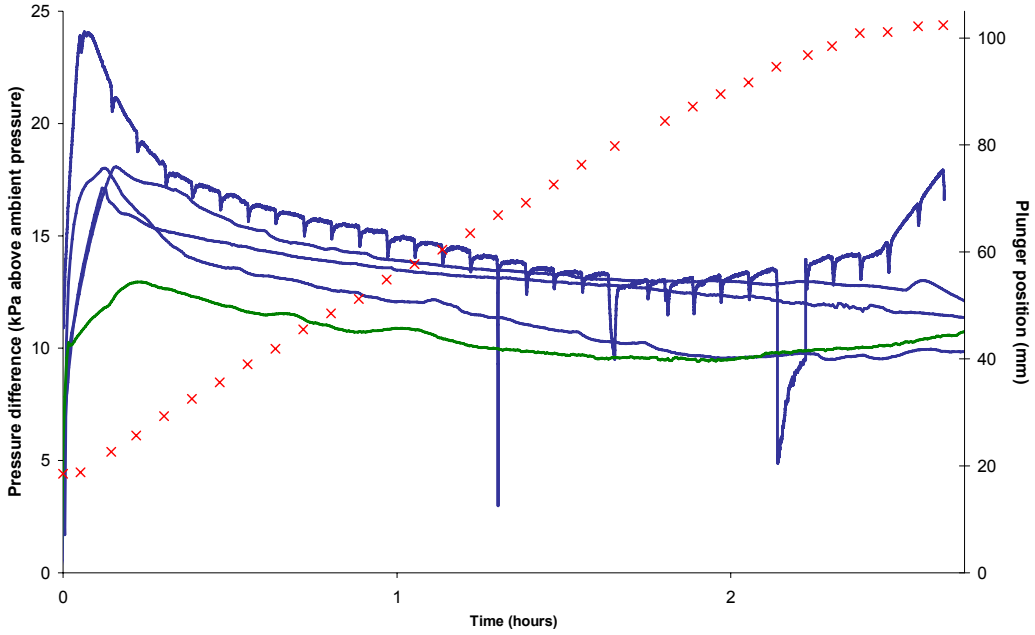


Figure 4-6: Operating pressure of a POM device with soft plunger containing 2% HPMC (n=1)

— = Pressure. — = Pressure for device that was first equilibrated to 40°C. × = Plunger position. Brief periods of reduced pressure are due to temperature changes when the device was withdrawn from the 40°C water to measure plunger position.

One device was tested with a gas cell using a 1200-Ω resistor to confirm that the pressures measured at the accelerated gas production rate were comparable to those of slower devices. Data obtained agreed well with data of equivalent devices with short-circuited gas cells.

A pressure in the mid range was selected for adjusting gas production rate (Section 3.2.3.2) when predicting the release profile for each device configuration (Table 4-2) and added to the ambient pressure (assumed to be standard atmospheric pressure - 101.325 kPa) to calculate the total pressure. For example, 14 kPa was chosen for data shown in Figure 4-6, although the actual operating

pressure was generally in the range of 10 to 18 kPa (equivalent to around ±3% variation in total pressure). If the average pressure inside the rumen is known, the actual pressure could be added to values in Table 4-2 instead.

Table 4-2: Operating pressure (kPa) for device configurations tested

		POM		PE
	HPMC	2%	5%	2%
Plunger	Hard	2	10	-
	Soft	14	14	6

4.2.2 Device stability

Commercial one-way valves were fitted to all devices. About 28% of the devices tested became visually contaminated with digesta (Table 4-3), which is comparable the 30% that were contaminated in Trial 2 (Table 2-6). There are many types of one-way valve available commercially. Those used here, selected for their low cost and opening pressure may not be the most suitable.

More slow-releasing devices became contaminated than fast, which could be attributed to longer exposure to the rumen. A greater number of devices filled with the less viscous formulation or with hard plungers were contaminated than the devices with more viscous formulation and soft plungers. These two factors decrease resistance to plunger movement meaning that a lesser force is required to drive digesta into the device.

Many contaminated devices with soft plungers continued to release whereas those with hard plungers typically stopped functioning. This was expected as the soft plungers form a gas-tight seal with the barrel while the hard plungers require the formulation to be present, which could be disrupted by bubbles.

Several other types of failure were also observed, both *in vitro* (Table 4-4) and *in vivo* (Table 4-5). Plunger movement rate in some devices suddenly accelerated, releasing their entire remaining payload between sampling times. This was probably due to moisture causing the gas cell to short-circuit. Several devices failed to start releasing or stopped mid-barrel. This may have caused by a leak in the gas chamber that was not detected during leak testing or had formed after testing. Splits were observed in the spin-welded joint of some HDPE barrels that

had stopped releasing. Another reason may be that damage to the gas cell or resistor stopped gas production.

Table 4-3: Devices contaminated *in vivo*

			Barrel	POM		HDPE	
				27%		*31%*	
			HPMC	**2%**	**5%**	**2%**	
Gas rate		*Plunger*		*40%*	*14%*	*31%*	**Hard**
Fast	*18%*	**Hard**	*20%*	4/9	0/8	1/8	***34%***
		Soft	*14%*	1/7	1/7	-	
Slow	*39%*	**Hard**	*50%*	4/7	3/7	4/8	**Soft**
		Soft	*21%*	3/7	0/7	-	***18%***

Total ***28%***

Table 4-4: Failures *in vitro*

			Barrel	POM		HDPE	
				47%		*44%*	
			HPMC	**2%**	**5%**	**2%**	
Gas rate		*Plunger*		*37%*	*59%*	*44%*	**Hard**
Fast	*28%*	**Hard**	*36%*	1/9	3/8	5/8	***40%***
		Soft	*14%*	1/7	1/7	-	
Slow	*67%*	**Hard**	*45%*	2/7	6/7	2/8	**Soft**
		Soft	*100%*	7/7	7/7	-	***57%***

Total ***47%***

Results for slow, 50-day devices were taken in the third trial when lubricant had been applied to the plungers

Table 4-5: Non-contamination failures *in vivo*

			Barrel	POM		HDPE	
				15%		*6%*	
			HPMC	**2%**	**5%**	**2%**	
Gas rate		*Plunger*		*7%*	*24%*	*6%*	**Hard**
Fast	*13%*	**Hard**	*16%*	1/9	3/8	0/8	***17%***
		Soft	*7%*	1/7	0/7	-	
Slow	*14%*	**Hard**	*18%*	0/7	3/7	1/8	**Soft**
		Soft	*7%*	0/7	1/7	-	***7%***

Total ***13%***

The plunger of every 50-day POM device tested *in vitro* moved less than 30 mm (less than one third the length of the barrel). Gas then began to leak past the plunger. In these circumstances, the gas continued to extrude the contents because the device was inverted (Figure 4-7). This also occurred when the experiment was repeated. Only when lubricant had been applied to the plungers (third experiment) did any POM device release more than a few millilitres. In the third experiment, only six devices released their full payload, though most

functioned properly for at least two weeks. Release rates were calculated on data generated during this time.

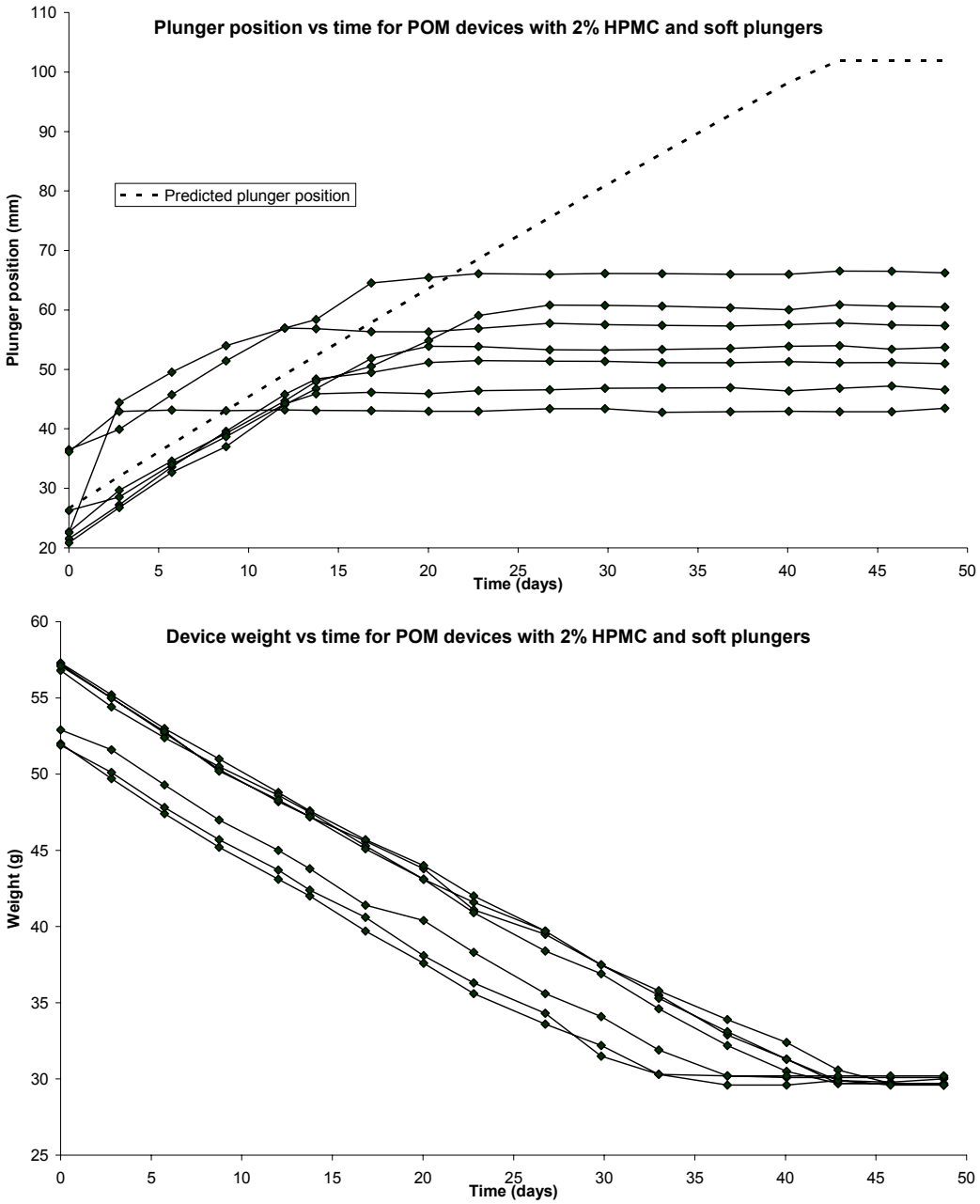


Figure 4-7: Effect of gas leaking past the plunger on plunger position and device weight
Plungers stopped moving (top) and gas leaked around the plungers and continued to drive extrusion causing device weight to continue to decrease (bottom).

Five of the six devices that released their entire payload contained 2% HPMC and all had hard plungers. This was unexpected as it was thought that the tighter fitting (soft) plunger and more viscous formulation (5% HPMC) would be less likely to allow gas to pass the plunger. This failure mode was not observed *in*

vivo, although gas in the formulation may have caused immediate contamination of the formulation with digesta and therefore gone undetected. Gas probably leaks past the plunger when a channel is formed. The constant motion in the rumen may cause the formulation to block these channels, allowing the device to continue releasing.

Non-contamination-related failures were less common *in vivo*, although many of these events may have caused contamination and therefore gone unnoticed.

Intraruminal devices must be sufficiently robust to withstand the harsh conditions in the rumen. It would advantageous if *in vitro* data from this technology accurately represented the *in vivo* function of the device. The components available currently limit both of these aspects; although there is no evidence to suggest either would be unattainable if a more robust one-way valve and better designed plunger were used.

Both soft, sealing plungers (Theratron) and looser-fitting, hard plungers (Simalube) have been used commercially. Each may be better suited to specific situations. Sealing plungers, like that of the Theratron, may be better for low viscosity formulations because they have higher frictional resistance and can seal independently. The soft plungers tested in this study absorbed some lipophilic substances, which could alter their mechanical properties. Hard plungers, like that of the Simalube, appear to be compatible with lipophilic formulations but aqueous formulations may pass the incomplete seal and damage the gas cell. As the gas-tight seal around hard plungers is dependent on the formulation so they may be limited to delivering more viscous formulations. A plunger consisting of a viscous, waxy substance compressed between two plates by a spring (Figure 4-8) has been proposed for erosion-based intraruminal devices (Laby and Kautzner 1986). This assembly could potentially be used as a gas-tight seal with low frictional resistance plunger for a gas cell driven device.

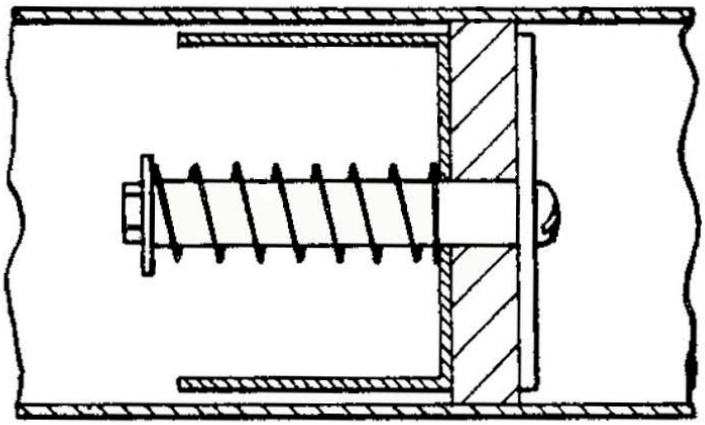


Figure 4-8: Wax and spring plunger
(Laby and Kautzner 1986**)**

4.2.3 Release profile

Two different types of barrel were used. Those made from POM with thicker walls should be sufficiently impermeable to hydrogen to allow a near constant release rate at either of the gas production rates used. Devices with 2% HPMC and hard plungers were the only configuration of POM barrels without a large number of failures *in vitro* with the slower gas production rate (Figure 4-9). Release rate from these devices was constant with R^2 all above 0.997 with the exception of the slow devices *in vivo* ($R^2 = 0.985$, one of only two configurations of POM devices with R^2 below 0.994). Devices tested *in vitro* closely followed the release profiles predicted by Equation 3-7. These results demonstrated that this technology can be used to release formulations at a constant, adjustable rate, both *in vitro* and *in vivo*.

HDPE devices *in vitro* with the faster rate of gas production displayed a decreasing release rate with a profile that also closely followed that predicted by Equation 3-7 (Figure 4-10). The actual release rate tended to be faster than the predicted for devices with the slower gas production rate, although the values were typically within experimental variation. The volume of air present in these devices initially may also have altered the rate of gas loss through diffusion (Section 3.2.3.1).

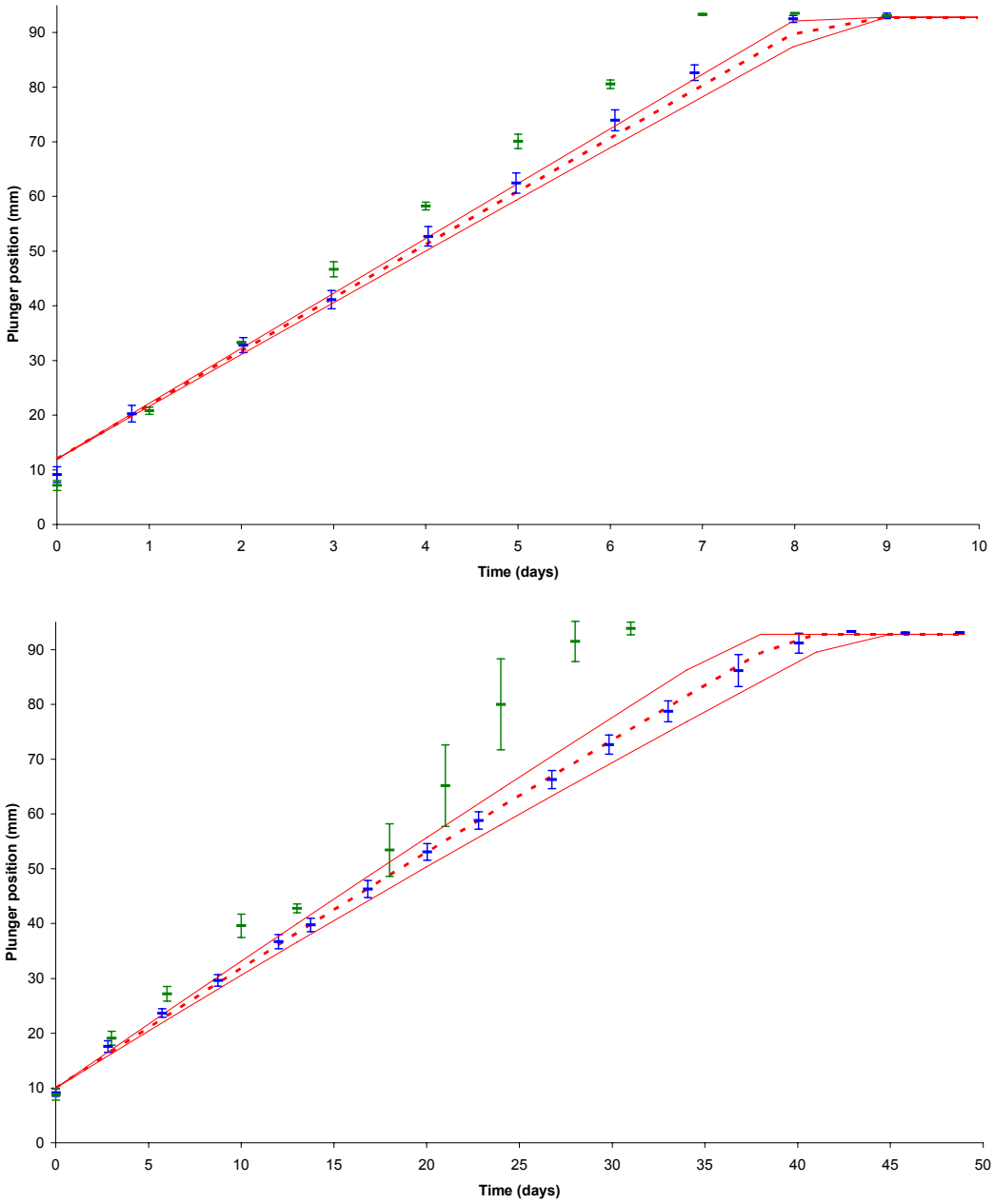


Figure 4-9: Effect of gas production rate and environment on plunger position for POM devices with hard plungers and 2% HPMC
Average release profile ± standard deviation of devices not listed as failures in Table 4-4. — = *in vitro* — = *in vivo* - - - = predicted — = predicted range including experimental error in G and D_L. Top: Fast gas production. Bottom: Slow gas production.

The release rate of *in vitro* POM devices was within experimental variation of the predicted rate for slow devices and up to 13% faster for fast devices (Table 4-6). *In vivo* devices, however, these devices released significantly faster than the rate of gas production. Differences *in vivo* were most likely due to differences in D_L caused by the different gas environment in the rumen. The release profiles were consistent with a D_L value of about -1 mL·day^{-1} (40°C, 1 atm) for the faster

releasing devices and -0.5 mL·day^{-1} (40°C, 1 atm) for the slower devices. This suggests that gases from the rumen may have diffused into the device at a greater rate than hydrogen diffused out of the device.

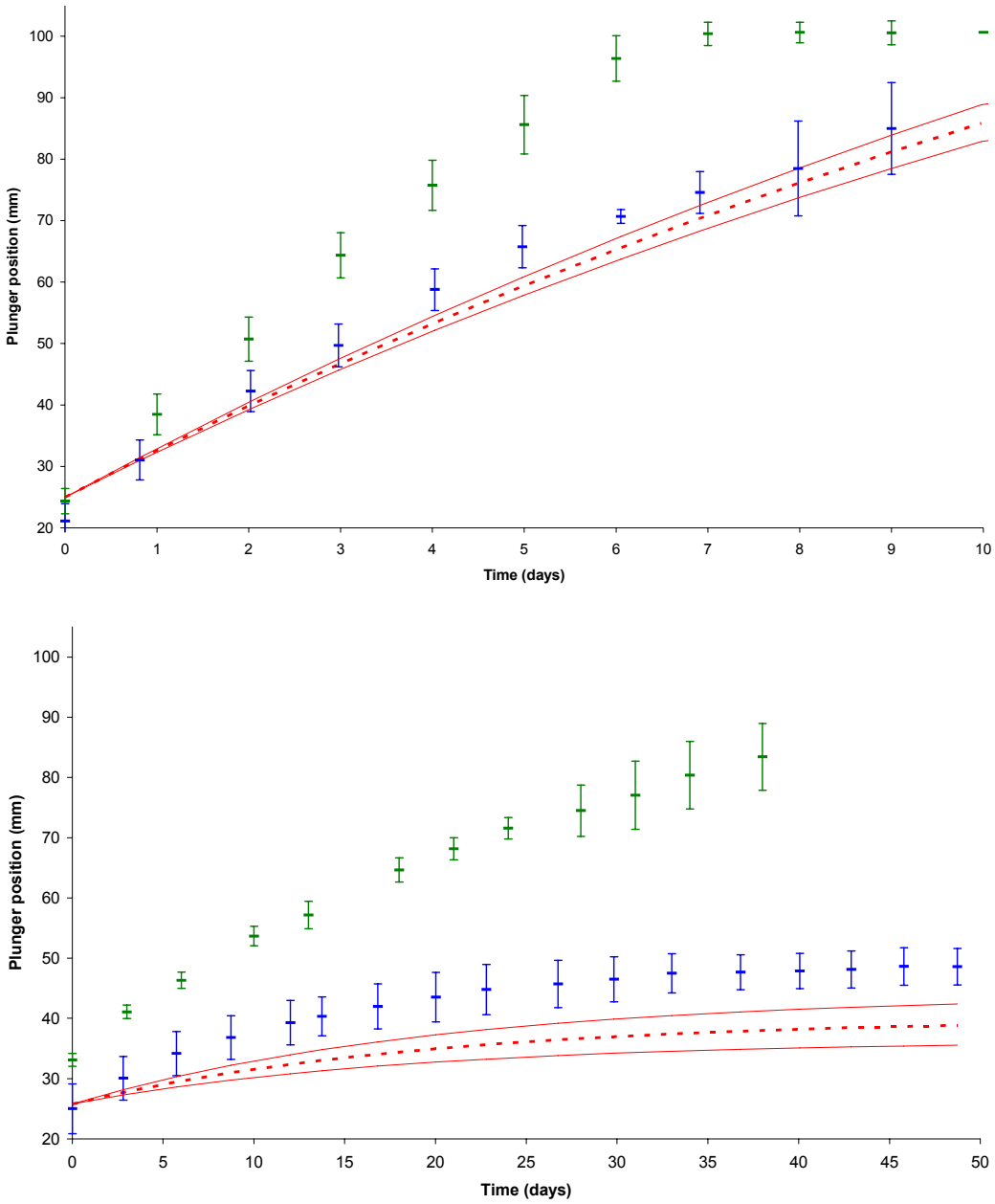


Figure 4-10: Effect of gas production rate and environment on plunger position for HDPE devices
Average release profile ± standard deviation of devices not listed as failures in Table 4-4. — = *in vitro* — = *in vivo* - - - = predicted — = predicted range including experimental error in G and D_L. Top: Fast gas production. Bottom: Slow gas production.

The release rate of devices with HDPE barrels (Figure 4-10) decreased too rapidly for valid linear regression. Release profiles were consistent with D_L values of

about -2 mL·day^{-1} (40°C, 1atm) for the faster releasing devices and 0 mL·day^{-1} (40°C, 1atm) for slower devices. Equation 3-7 is not valid when both inward and outward diffusion occur at significant rates (Section 3.2.3.1), which may explain the difference in apparent D_L between fast and slow release profiles.

Table 4-6: Effect of contents, plunger and environment on the rate of release from fast releasing POM devices (mL·day^{-1})

HPMC	Plunger	*In vivo*		*In vitro*	
		Observed	Expected	Observed	Expected
2%	*Hard*	4.10±0.20 (n=4)	3.22	3.45±0.03 (n=6)	3.21
2%	*Soft*	3.60±0.25 (n=6)	2.87	3.19±0.07 (n=7)	2.87
5%	*Hard*	3.98±0.08 (n=8)	2.98	3.37±0.02 (n=4)	2.99
5%	*Soft*	3.65±0.13 (n=7)	2.87	3.00±0.14 (n=6)	2.87

Table 4-7: Effect of contents, plunger and environment on the rate of release from slow releasing POM devices (mL·day^{-1})

HPMC	Plunger	*In vivo*		*In vitro*	
		Observed	Expected	Observed	Expected
2%	*Hard*	1.03±0.15 (n=7)	0.69	0.68±0.03 (n=5)	0.68
2%	*Soft*	0.77±0.11 (n=7)	0.60	0.58±0.05 (n=5)	0.63
5%	*Hard*	0.89±0.08 (n=7)	0.64	0.69±0.04 (n=4)	0.65
5%	*Soft*	0.87±0.08 (n=7)	0.60	0.58±0.03 (n=7)	0.61

4.2.4 Gas analysis

It was difficult to obtain gas samples from the devices, and samples could not be obtained from devices with hard plungers. Due to contamination with air, oxygen and nitrogen detected are not included in the data presented (Table 4-8). Carbon dioxide was not detected in a sample of air so all carbon dioxide detected in devices was assumed to be from the rumen. Devices with HDPE barrels contained carbon dioxide and methane in proportions comparable to that expected in rumen gas (Section 1.3.1), though methane was present in reduced quantities in POM devices. This suggests that devices made from POM devices might release at different rates in animals producing different proportions of carbon dioxide and methane. Rumen gases made up a greater proportion of the total gas in the slower releasing devices and devices with HDPE barrels. Slower devices could be expected to contain more rumen gases as they were exposed to the rumen for longer. The thickened POM barrels appear to be less permeable to both gases

than HDPE. Hydrogen was the only non-air gas detected in samples obtained from fast *in vitro* devices.

The data demonstrate that the difference in release rate between *in vitro* and *in vivo* devices could be reduced by using a barrel material with low carbon dioxide, methane and hydrogen permeability. The release rate may then be independent of gas composition in the rumen.

Table 4-8: Effect of barrel material and device duration on non-air gas content

Barrel	Duration	Methane	Carbon dioxide	Hydrogen
POM	*10 days*	1.7±1.8% (n=6)	12.1±6.2% (n=6)	86.1±7.2% (n=6)
	50 days	2.3±0.1% (n=4)	35.8±2.0% (n=4)	61.9±1.9% (n=4)
HDPE	*10 days*	12.4±6.3% (n=5)	25.4±11.0% (n=5)	62.2±7.2% (n=5)
	50 days	31.3±12.5% (n=8)	56.1±13.1% (n=8)	12.6±21.2% (n=8)

4.2.5 Nylon barrels

The inward diffusion of rumen gases, primarily carbon dioxide, at a greater rate than air appears to be the only difference in release profiles of *in vitro* and *in vivo* POM devices. Nylon appears to have a low permeability to both hydrogen and carbon dioxide (Table 3-2) but was not used in further trials because it could not be spin-welded. Nylon could be a suitable barrel material if it also has low methane permeability. When it was found that carbon dioxide had diffused into fast-releasing, POM devices *in vivo*, fast-releasing nylon devices with soft plungers were assembled, sealed with rubber bungs (instead of spin welding), and tested *in vivo* with the slower devices. The internal diameter of the nylon barrels (20.74 ± 0.02 mm) was too large for the soft plungers and large amounts of formulation leaked into the gas chamber before the devices were recovered for the first measurements 4 days later.

4.3 Conclusions

Devices tested in this chapter demonstrated that the gas cell technology is potentially capable of long-term, linear release, independent of the rumen environment. The release profiles from *in vitro* devices were very similar to those predicted by Equation 3-7. If diffusion of environmental gases could also be controlled, devices would more closely follow the predicted profiles. A more

reliable plunger may increase reproducibility, which was low both *in vitro* and *in vivo*.

Additional study of the operating pressure in devices may produce useful data, particularly at slower rates of gas production. This was not attempted due to the time involved as the available equipment could monitor only one device at a time.

Delivering a model bioactive would have given extra evidence of the effectiveness of this device for ruminal controlled-release. Preliminary experiments were performed with phlorizin, a potential model bioactive. However, phlorizin did not produce the expected physiological response when dosed directly to the rumen (Appendix D).

Chapter 5: Conclusions and recommendations

5.1 Conclusions

The aim of this project was to develop an intraruminal controlled-release device driven by a gas-producing cell that could operate independently of environmental factors at a constant and adjustable rate. Gas production and diffusion were studied and devices were tested *in vitro* and *in vivo* with the following key findings:

5.1.1 Commercial components

- Gas loss by diffusion limits the duration that devices using Theratron, Terumo and B Braun barrels release linearly.
- Coating barrels with PET shrink-wrap and aluminium tape reduces gas loss by diffusion.
- Theratron, Terumo and B Braun plungers swell when exposed to oils.

5.1.2 Principles of the gas-cell driven controlled release device

- There is an inverse relationship between gas production rate from gas cells and electrical resistance. This is approximately:

  $$G = 3500R^{-1} + 0.296$$

  where G is gas production rate and R is resistance.

- Gas loss rate due to diffusion through hydrogen- or carbon dioxide-filled barrels made from nine different materials was lowest from POM and nylon barrels respectively.
- A model for plunger position with time for any gas production rate, which incorporates gas loss due to diffusion, was developed (Equation 3-7):

  $$l = \frac{GL}{D_L} - \left(\frac{GL}{D_L} - l_0\right)e^{-\frac{D_L t}{AL}}$$

where l is plunger position, t is time, G is gas production rate, L is barrel length, A is cross-sectional area and D_L is gas diffusion rate through the entire barrel.

5.1.3 Capabilities of the gas-cell driven controlled release device

- Devices releasing liquids into the rumen require a one-way valve to protect unreleased contents from contamination with digesta.
- *In vitro* release profiles of fast and slow releasing POM devices and fast releasing HDPE devices fitted the model within experimental variation.
- POM devices released their contents at a constant rate *in vitro* and *in vivo* at both gas production rates tested.
- Inward diffusion of rumen gases increased release rates *in vivo*.

5.2 Recommendations

Recommendations for designing components for a gas cell driven, generic intraruminal controlled-release device are shown in Figure 5-1.

A cylindrical device barrel with a larger diameter will have a lower ratio of surface area to volume and will be less affected by gas diffusion. The maximum diameter for intraruminal devices for cattle is about 40 mm (Laby 1987b). Barrel walls should be at least 4 mm thick to minimise gas diffusion, giving an internal diameter of about 32 mm. The largest capacity barrel possible will allow long-term or high-volume delivery. At the recommended maximum length for an intraruminal device (200 mm; Laby 1987b), a 32-mm internal diameter device has a total volume similar to the maximum volume of hydrogen produced by a gas cell. A more appropriate plunger travel distance is about 150 mm, giving a volume of about 120 mL to be delivered at an operating pressure of up to 30 kPa above atmospheric pressure. A shorter device or multiple gas cells would be needed for more viscous formulations.

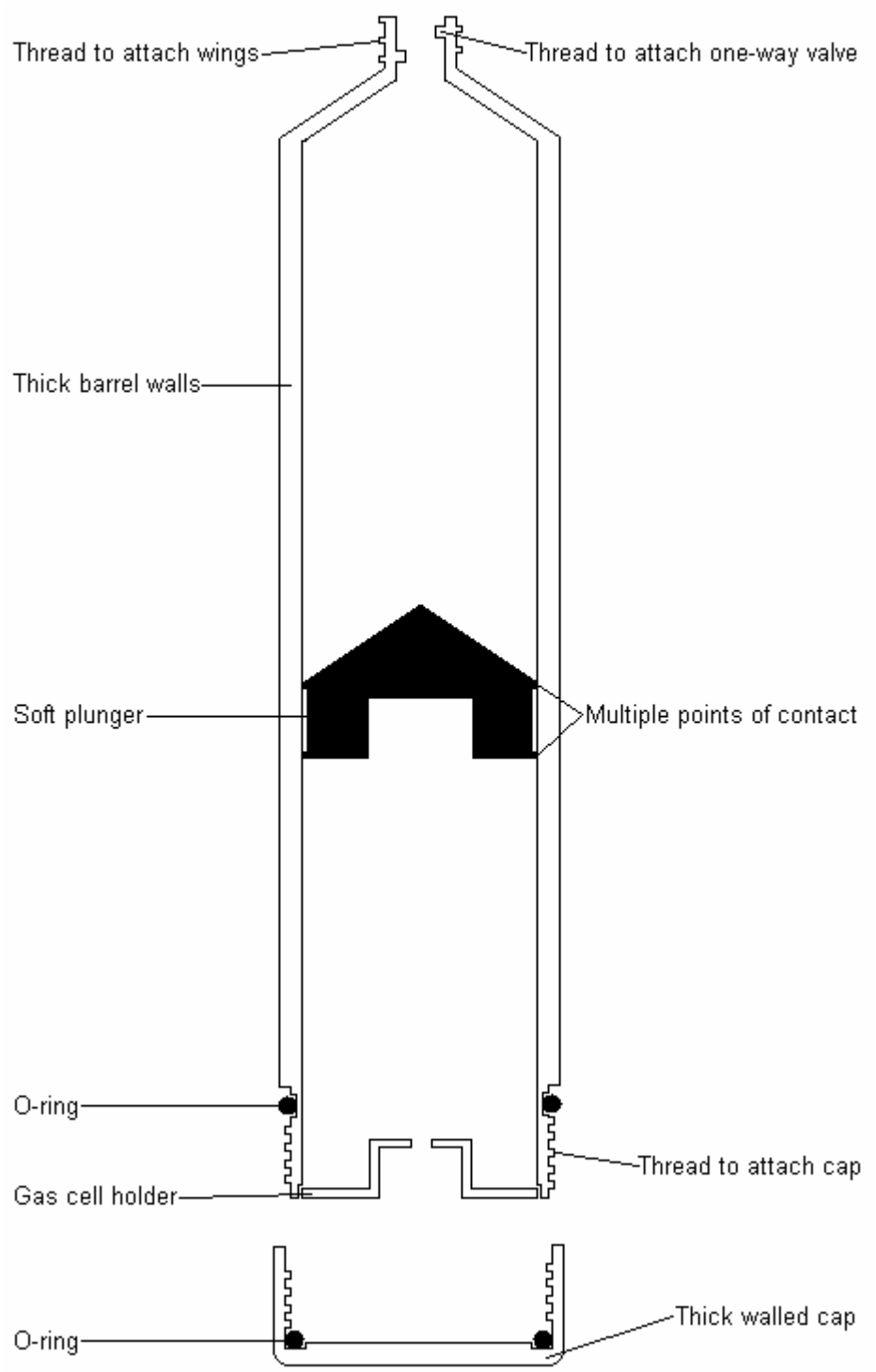


Figure 5-1: Cross-section of a suggested design for a device for use in cattle

A reliable method of sealing the device is required. This could be achieved by putting a thread onto the barrel that allows a cap to be screwed on. This cap should be made from the same material as the barrel and have the same wall thickness, though it may need to be thinner around the circumference in order to

fit over the barrel without exceeding 40 mm in diameter. Using an O-ring on either side of the thread would reduce gas loss through the thinner walls. A component could be placed on the end of the thread before the cap is applied to prevent movement of the gas cell. The act of screwing on a cap could be coupled to activating the gas cell, in a manner similar to activating a Theratron device. To prevent damage to the gas cell, a water-proof coating may need to be applied to the terminals and resistor. Wires protruding from this coating could be connected to complete the circuit and initiate gas production.

The device should have an orifice at one end with a way of attaching a commercially-available one-way valve such as a luer lock. An appropriate valve is needed and different valves could be used for different applications. A one-way valve could be built into the orifice in a final product.

The device could be retained in the rumen with wings, similar to a Rumensin capsule. A method to attach separately-moulded wings may be advantageous and allow the barrel and wings to be made from different materials.

Devices should be made from material with low permeability to hydrogen, carbon dioxide, methane and possibly nitrogen and water. Polyoxymethylene appears to be sufficiently impermeable to hydrogen and possibly methane. Increasing the barrel wall thickness will reduce permeation, but reduce payload capacity. Nylon has low hydrogen and carbon dioxide permeability and may be a better alternative if it is also sufficiently impermeable to methane. The barrel could be coated in a second material with low permeability to specific gases if a material with all the required properties cannot be identified.

If a device of these dimensions was made from POM, its D_L value would be similar to the existing 1.8-mm POM barrels. Releasing a payload over 100 days would require resistance of about 3300 Ω. The release profile would be proportionally similar to that of 1.8-mm POM (Figure 3-21).

Designing an ideal plunger requires further study. Several different plunger materials may be required to overcome the effect of individual formulations on plunger dimensions. A suitable starting point is a soft plunger similar to that of

the Theratron. The plunger should contact the barrel at more than one point but the contact area should be minimal to ensure a complete seal with minimum frictional resistance.

A device designed to these specifications should be able to deliver any payload at the desired linear rate in the rumen of cattle or, with suitably adjusted dimensions, to any other ruminant. If the operating pressure and temperature are identified and diffusion of all relevant gases minimised, this system could be adapted for any environment where a gas cell can function.

The main advantage of this device over existing technologies is that the release rate can be easily adjusted. Being able to release any bioactive at any rate with existing components would reduce the time it takes a product to reach the market. Once perfected for linear release, this device could be electronically controlled to release in a predetermined way or in response to a condition detected by sensors on the device. The cost of manufacturing a gas cell driven device may make it more suited for high-value bioactives requiring accuracy and versatility or as a research tool for developing new bioactives.

Chapter 6: References

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APPENDICES

Contents of appendices

Appendix A:Data from Chapter 2

A.1 Trial 1

A.1.1 Configuration of individual devices

Device	Resistor (Ω)	Orifice	Coating	Syringe	Contents
1	1200	Valve	✔	B Braun	HPMC
2	1200	Valve	✔	B Braun	HPMC
3	1200	Valve	✔	B Braun	HPMC
4	1200	Valve	✔	B Braun	Oil/Wax
5	1200	Valve	✔	B Braun	Oil/Wax
6	1200	Valve	✔	B Braun	Oil/Wax
7	1200	Valve	✔	B Braun	Water
8	1200	Valve	✔	B Braun	Water
9	1200	Valve	✔	B Braun	Water
10	1200	-	✔	B Braun	HPMC
11	1200	-	✔	B Braun	HPMC
12	1200	-	✔	B Braun	HPMC
13	1200	-	✔	B Braun	Oil/Wax
14	1200	-	✔	B Braun	Oil/Wax
15	1200	-	✔	B Braun	Oil/Wax
16	560	Valve	✔	Theratron	HPMC
17	560	Valve	✔	Theratron	HPMC
18	560	Valve	✔	Theratron	HPMC
19	560	Valve	✔	Theratron	Oil/Wax
20	560	Valve	✔	Theratron	Oil/Wax
21	560	Valve	✔	Theratron	Oil/Wax
22	560	Valve	✔	Theratron	Water
23	560	Valve	✔	Theratron	Water
24	560	Valve	✔	Theratron	Water
25	560	-	✔	Theratron	HPMC
26	560	-	✔	Theratron	HPMC
27	560	-	✔	Theratron	HPMC
28	560	-	✔	Theratron	Oil/Wax
29	560	-	✔	Theratron	Oil/Wax
30	560	-	✔	Theratron	Oil/Wax
31	2700	Valve		B Braun	HPMC
32	2700	Valve		B Braun	HPMC
33	2700	Valve		B Braun	HPMC
34	2700	Valve		B Braun	Oil/Wax
35	2700	Valve		B Braun	Oil/Wax
36	2700	Valve		B Braun	Oil/Wax
37	2700	-		B Braun	HPMC
38	2700	-		B Braun	HPMC
39	2700	-		B Braun	HPMC
40	2700	-		B Braun	Oil/Wax
41	2700	-		B Braun	Oil/Wax
42	2700	-		B Braun	Oil/Wax
43	6800	Valve		Theratron	HPMC
44	6800	Valve		Theratron	HPMC
45	6800	Valve		Theratron	HPMC
46	6800	Valve		Theratron	Oil/Wax
47	6800	Valve		Theratron	Oil/Wax
48	6800	Valve		Theratron	Oil/Wax
49	6800	Valve		Theratron	Water
50	6800	Valve		Theratron	Water
51	6800	Valve		Theratron	Water
52	6800	-		Theratron	HPMC
53	6800	-		Theratron	HPMC
54	6800	-		Theratron	HPMC
55	6800	-		Theratron	Oil/Wax
56	6800	-		Theratron	Oil/Wax
57	6800	-		Theratron	Oil/Wax
58	6800	Valve	✔	Theratron	HPMC
59	6800	Valve	✔	Theratron	HPMC
60	6800	Valve	✔	Theratron	HPMC
61	6800	Valve	✔	Theratron	Oil/Wax
62	6800	Valve	✔	Theratron	Oil/Wax
63	6800	Valve	✔	Theratron	Oil/Wax
64	6800	-	✔	Theratron	HPMC
65	6800	-	✔	Theratron	HPMC
66	6800	-	✔	Theratron	HPMC

A.1.2 Plunger position in mm of 10-day devices

	Day											
Device	**0**	**1**	**2**	**3**	**4**	**5**	**7**	**8**	**9**	**10**	**14**	**17**
1	27.5	30.6	41.1	48.4	55.7	59.8	74.8	86.9	87.8	87.7		
2	25.5	28.7	37.6	43.7	53.6	63.3	78.4	82.4	87.9	87.9		
3	27.0	34.4	44.9	62.0	66.6	80.2	87.7	87.9	87.6			
4	27.0	31.8	38.4	47.3	52.4	58.1	69.1	75.6	83.9	81.2	84.2	84.7
5	25.5	30.4	38.1	41.0	44.9	54.7	62.4	71.3	76.5	79.2	84.1	84.8
6	26.5	30.4	38.8	42.6	46.3	51.2	58.8	64.0	68.6	73.9	81.2	82.6
7	28.3	33.6	42.0	51.6	61.1	69.4	85.7	87.6	87.4			
8	27.5	32.0	41.5	50.8	59.7	67.8	83.9	87.4	87.6			
9	29.0	33.6	42.0	50.2	59.2	67.5	83.6	87.6	87.6	87.7		
10	28.0	32.6	40.1	49.5	57.8	66.4	80.3	87.8	87.7	87.5		
11	27.0	31.8	41.2	48.2	57.9	65.2	78.4	84.1	87.1	87.4		
12	27.5	31.4	40.1	48.5	57.2	65.5	76.9	82.3	85.7	87.2		
13	27.0	32.2	40.4	47.8	55.3	62.7	76.5	83.4	85.2	58.4	85.9	86.2
14	27.0	31.2	38.4	45.4	53.1	59.7	72.8	79.1	83.7	84.5	85.3	85.7
15	28.5	32.9	41.3	49.1	57.1	64.1	77.3	81.9	83.1	84.3	84.8	84.7
16	26.0	35.9	37.3	39.4	41.1	47.6	66.0	74.3	82.6	90.2	113.6	
17	28.0	32.3	33.8	34.6	34.3	34.5	35.5	35.3	35.5	35.7	40.2	41.3
18	23.0	25.8	26.3	26.7	27.7	27.5	28.9	28.7	92.3	29.7	30.7	31.6
19	22.8	31.4	39.9	42.1	42.7	44.4	79.4	86.4	87.7	93.3	111.0	
20	24.0	36.1	45.1	47.4	48.4	58.6	77.5	83.2	84.3	94.7	111.2	
21	23.3	24.8	26.1	26.9	27.9	27.6	28.0	28.1	28.3	23.8	29.3	29.7
22	25.0	35.5	43.4	52.4	60.2	67.1	82.4	89.2	95.3	109.5	113.1	
23	25.3	36.7	45.3	55.4	64.1	73.3	89.0	95.8	102.5	102.4	112.7	
24	26.8	36.0	44.0	53.0	61.8	70.7	87.0	94.5	102.2	109.7	113.0	
25	25.0	35.5	45.2	56.7	65.7	75.0	92.5	98.5	106.6	111.9	112.5	
26	25.0	34.6	44.2	54.7	63.9	72.1	88.6	95.1	104.3	110.9	113.1	
27	24.5	35.4	44.8	55.2	65.1	74.1	90.9	96.8	104.7	112.2		
28	29.0	40.4	50.0	60.3	70.2	80.8	98.9	107.1	111.4	112.5		
29	33.0	46.7	57.0	67.4	77.2	86.6	103.4	110.4	111.8	108.0		
30	25.0	26.3	27.0	27.5	28.5	28.7	29.4	29.3	30.0	30.5	33.1	34.3

A.1.3 Weight in g 10-day devices

	Day											
Device	**0**	**1**	**2**	**3**	**4**	**5**	**7**	**8**	**9**	**10**	**14**	**17**
1	34.0	33.7	31.7	27.3	25.9	23.8	20.2	17.1	17.3	17.1		
2	34.4	33.7	30.9	30.0	26.4	23.6	19.5	18.1	17.0	16.8		
3	34.4	32.7	30.0	25.0	22.5	18.9	17.0	16.9	17.0			
4	34.8	32.0	31.8	28.7	26.8	25.3	22.3	20.3	19.4	18.9	18.2	17.5
5	35.2	34.3	31.4	29.8	31.9	26.6	23.8	21.3	19.5	19.0	17.9	17.7
6	34.5	33.6	31.6	29.8	28.5	27.2	25.0	23.6	22.3	20.7	18.3	18.3
7	35.7	34.8	31.7	28.1	25.0	22.0	17.3	17.1	17.3			
8	35.8	34.8	31.7	29.0	25.5	23.0	18.1	16.9	16.8			
9	35.5	34.4	31.8	29.3	26.0	23.0	18.2	17.4	17.4	15.2		
10	32.5	32.0	29.8	27.1	25.6	22.9	18.6	16.8	16.9	16.9		
11	32.0	32.0	29.5	26.4	22.7	21.4	18.2	17.1	16.8	16.7		
12	32.1	30.3	30.1	24.8	22.2	22.9	18.6	17.2	16.9	16.8		
13	33.3	32.5	29.7	28.0	25.9	23.8	19.9	17.7	17.3	17.0	17.8	17.3
14	33.5	32.4	28.9	28.4	26.5	24.3	21.0	19.2	17.9	17.4	17.5	17.3
15	32.8	31.8	29.6	28.0	25.3	22.9	19.1	18.1	17.9	17.5	17.2	17.4
16	95.1	89.7	88.7	88.3	86.3	84.1	73.7	68.7	63.7	59.7	46.2	
17	96.4	94.3	93.4	91.5	91.0	90.8	90.9	90.9	90.8	90.0	88.8	87.7
18	98.4	96.2	95.7	94.7	93.7	93.5	94.6	94.0	92.8	94.2	83.6	81.5
19	100.5	95.6	90.4	86.1	88.6	87.8	66.3	61.6	61.3	57.6	47.0	
20	99.9	93.7	87.4	89.4	85.5	78.7	67.7	64.6	63.3	57.1	47.2	
21	99.5	99.6	99.0	98.0	96.8	97.1	96.9	97.0	96.7	96.8	96.7	96.4
22	104.1	98.0	93.0	87.3	81.5	76.4	66.8	62.4	58.0	48.7	46.4	
23	103.8	97.4	92.0	85.3	78.5	72.5	62.5	58.1	53.3	53.1	46.7	
24	102.7	97.5	92.0	86.0	79.9	74.0	63.3	58.3	53.2	48.3	45.0	
25	95.3	89.3	86.9	81.5	74.6	57.8	51.0	47.0	47.9	45.8	45.9	
26	95.0	91.3	84.4	58.1	58.4	66.1	49.1	55.4	47.7	45.9	45.9	
27	94.4	92.2	86.9	81.9	75.1	69.4	58.5	49.2	48.1	46.2		
28	95.0	88.7	87.7	76.5	67.9	63.5	51.5	47.7	46.0	46.0		
29	91.5	85.7	79.5	73.3	67.0	61.3	51.5	47.1	46.4	46.4		
30	97.6	97.6	97.6	95.6	80.8	54.6	73.5	80.3	84.5	96.7	90.4	90.0

A.1.4 Plunger position in mm of 100-day devices

	Day														
Device	**0**	**1**	**2**	**4**	**5**	**7**	**9**	**14**	**17**	**22**	**25**	**29**	**36**	**43**	**50**
31	25.5	27.3	31.4			43.7	49.5	68.7	75.0	84.6	86.3	86.5	86.9	87.3	87.5
32	25.0	26.2	29.0	34.7		43.4	48.0	61.1	66.6	77.1	82.2	87.5	87.8	87.5	87.5
33	26.0	26.3	29.0	36.3											
34	26.0	26.6	29.6	47.9		43.0	45.6	57.3	60.2	70.0	74.5	78.5	80.7	81.7	
35	27.0	27.9	30.1	35.2		41.8	46.1	59.4	63.7	70.6	73.8	75.5	77.7	79.7	80.7
36	27.0	27.0	30.8	34.9		41.9	44.2	61.1	65.5	70.2	71.8	74.1	76.5	78.1	78.6
37	27.0	27.2	31.2	38.4		48.3	52.9	67.1	71.7	79.4	83.2	85.2	85.2	86.1	86.8
38	27.8	28.0	31.1	37.7		46.6	51.9	66.3	71.3	79.6	83.0	86.4	87.2	87.4	87.4
39	28.0	28.1	31.0	36.9		43.9	47.4	57.3	61.5	66.5	69.7	73.4	75.6	76.0	76.0
40	29.0	27.1	30.5	36.6		44.5	48.7	60.5	65.2	71.5	73.7	74.7	84.2	84.5	84.5
41	26.8	29.4	34.3	40.3		49.9	56.1	72.0	76.0	84.4	85.8	84.7	85.9	86.7	85.9
42	29.0	28.9	31.7	39.4		49.1	53.7	66.4	71.8	80.5	80.9	82.5	85.0	85.0	85.1
43	23.0	25.7	26.5	28.6		32.0	32.9	36.7	38.9	43.3	45.4	48.8	55.2	59.4	64.1
44	23.5	23.3	23.8	23.7	26.1	28.2	29.2	34.3	35.3	41.1	43.8	47.1	48.2	47.4	50.3
45	24.0	26.5	27.2	28.7		30.9	30.8	34.4	37.7	38.9	38.8	44.4	47.0	48.9	49.3
46	23.8	28.5	27.3	25.2	25.4	26.5	26.7	28.8	29.7	29.7	30.1	32.6	29.1	32.5	32.9
47	26.3	29.9	30.6	32.4		33.2	33.0	36.3	38.6	41.1	41.2	42.5	39.9	41.6	42.6
48	23.5	25.6	25.8	27.0		26.5	27.6	30.8	32.5	30.0	32.0	31.8	25.8		
49	23.3	26.8	28.8	31.0		33.8	35.1	40.1	42.4	46.7		53.1	57.3		
50	25.0	28.1	28.9	31.2		34.1	35.1	40.2	42.4	46.5	48.8	52.3	57.5	62.0	67.5
51	25.0	26.9	28.6	113.1											
52	27.5	30.4	31.5	35.0	35.6	37.9	40.6	45.1	48.1	52.8	54.2	58.6	66.0	68.5	76.5
53	23.5	26.5	28.3	30.9		34.4	36.1	41.9	44.2	46.2	50.2	54.0	59.9	65.5	70.8
54	24.5	26.7	28.5	30.8		36.8	35.0	40.7	42.9	47.3	49.2	52.1			
55	26.0	28.2	29.5	31.8		35.9	38.2	44.9	47.3	51.5	53.8	56.0	61.4	63.2	68.9
56	24.0	26.7	27.1	28.0		30.1	31.3	32.5	32.9	34.5	35.1	35.5	37.7	40.3	41.0
57	28.0	30.3	31.2	33.6		38.7	40.6	47.9	50.5	56.0	56.8	60.4	65.3	69.3	72.6
58	23.0		26.3	28.7		30.3	31.3	33.9	35.3	37.0					
59	23.0	26.2	28.2	28.5		28.4	29.2	30.3	31.0	30.9	31.1	31.0	31.2	31.4	31.3
60	23.5	26.0	27.2	28.9		31.8	33.0	38.9	38.8	42.9	48.1	53.3	61.9	67.8	68.5
61	24.2	25.5	26.2	26.1	26.8	27.0	27.0	25.0	29.9	29.4	29.6	29.7	27.7		
62	24.5	26.0	26.2	27.6		27.0	27.4	27.4	27.6	26.4	28.0	28.5	29.4		
63	25.8	27.7	28.3	33.5	42.7	34.7	35.4	37.2	37.6	46.8	49.3	47.5	58.4		
64	26.5	28.0	28.1	29.3	30.3	31.0	31.9	33.7	34.1	37.8	38.7	39.2	42.3	44.0	
65	25.0	25.5	25.9	26.9	27.8	28.7	29.7	32.1	32.5	34.7	34.1	37.0	39.0	39.6	41.7
66	27.0	28.9	30.8	33.2		34.1	39.2	48.0	50.8	56.8	60.4	61.6	70.9	77.3	

Continued next page…

	Day													
Device	**56**	**58**	**60**	**63**	**65**	**67**	**70**	**73**	**77**	**81**	**84**	**86**	**89**	**99**
31														
32														
33														
34														
35														
36														
37														
38														
39														
40														
41														
42														
43	69.5	68.8	70.4	71.5	73.9	74.0		77.2	77.9	79.8	81.8	83.2	85.4	92.6
44	50.4	50.8	50.9	51.0	51.2	51.2	51.4	51.2	51.2	51.2	51.0	51.1	50.6	51.0
45	49.8	49.5	45.6	49.8	49.8	49.8	49.9	49.8	50.3	49.8	50.5	50.0	50.5	50.6
46	31.1													
47	43.0	42.9	31.9	43.1	43.1	43.0	43.5	43.6	43.8	54.4	44.4	45.0	92.5	45.9
48														
49														
50	67.4	67.3	67.3	71.4	71.4	77.8	75.0	67.5	76.7	78.7	82.0	82.6	85.9	90.7
51														
52	79.9	81.5	82.7	85.0	85.2	86.1	87.5	88.7	91.8	95.6	95.3	94.5	91.6	95.6
53	73.6	73.6	74.9	77.0	77.8	78.4	76.2	81.3	84.3	87.6	88.1	88.0	92.6	97.2
54														
55	70.3	70.1	69.7	70.2	70.9									
56	40.9	40.8	40.5	40.5	40.9	40.6	40.8	40.6						
57	77.2	76.9	79.1	81.5										
58														
59	31.7													
60	69.5	70.2	70.3	71.9	67.4	61.7	56.5	74.6	75.1	85.9	87.5	89.3	96.5	102.1
61														
62														
63														
64														
65	41.5	42.5	43.2	44.2	45.3	44.9	46.0	46.6	47.4	49.2		49.2	51.0	51.4
66														

A.1.5 Weight in g of 100-day devices

	Day														
Device	**0**	**1**	**2**	**4**	**5**	**7**	**9**	**14**	**17**	**22**	**25**	**29**	**36**	**43**	**50**
31	33.9	33.2	32.3	30.8		28.5	24.6	21	18.9	17.8	17.4	17.4	17.5	17.4	17.4
32	34.8	34.9	34	32.5		29	27.8	24.2	22.8	20.2	18.7	17.3	17.2	17.2	17.2
33	34.2	34.5	33.3	31.8		28.1									
34	35.1	35.2	34.6	31.9		29.4	28.7	25.8	24.6	22.8	20.8	19.5	18.9	18.7	
35	34.5	34.7	34.1	32.6		29.8	28.6	25	23.7	21.8	21	20.4	19.7	19.3	19
36	34.7	34.9	33.5	31.5		29.6	29	23.9	23	21.7	21.4	20.8	20	19.7	19.6
37	32.6	33.1	32	30.8		28	26	20.9	18.6	17.6	17.3	17.1	17.4	17.3	17.1
38	32.8	33.2	32.1	30.7		28.1	26.9	22.5	19.2	18	17.5	16.9	16.8	17	16.9
39	32.8	33.1	32.5	30.9		17.4	23.1	23.1	18.3	21.3	20.8	19.8	18.7	19.8	19.3
40	32.5	34	30.3	31		27.9	22.8	21.9	21.3	20.1	20.1	19.2	17.4	17.5	17.5
41	33.4	32.8	32.5	27.4		24.4	20.9	19.5	18.9	17.4	17.5	17.5	17.4	17.4	17.4
42	32.7	33.8	32.5	30.1		27.5	25.9	21.4	20.3	18.3	18	17.9	17.8	17.6	17.8
43	97.9	97.5	97	96		93.4	92.8	90.6	89.2	86.7	85.9	83.7	80.1	77.7	74.9
44	97.5	99	98.8	99.3	97.3	95.8	95.1	95.1	92	70	71	58.5	57.1	57.2	56.6
45	96.8	97.7	95.2	96.3		94.5	94.5	92.2	90.7	89	89.9	86.6	85.5	84.1	83.9
46	99.1	89.6	89.6	99.6	99.3	98.4	98.2	96.8	95.9	83.4	82.8	73.7	73.6	71.7	67
47	96.4	96	95.7	95		94.1	94.2	92.7	91.2	89.5	89.5	88.9	89.1	88.9	89
48	99.1	100	99.4	97		96.6	96.6	95.9	92.6	91	89.2	88.2	83.6		
49	104.9	103.7	102.6	101.6		98.4	97.2	94.1	92.3	89		70.5	58.3		
50	105.2	103.9	103.7	102.1		99.3	98.3	95.2	93.6	90	89.5	87.1	82.2	78.8	72.7
51	104.5	104.5	103.2												
52	95	94.6	92.4	95.2	94.7	86.3	87.5	70	77.9	80.2	74.8	73.5	68.4	70.1	64.1
53	96.2	96.5	96.4	93.8		91.3	89.2	87.9	84.8	78	83.1	78.2	76.9	68.2	69.3
54	95.6	95.8	96.2	95		82.4	82.5	87.7	84.3	77.1	79.5	79.6			
55	97	97.6	95.8	95.1		87.3	87	84.9	81.3	82.7	73.6	77.5	74.9	69.2	68.8
56	98.5	98	97.3	96.4		85.9	91.2	83.1	82.4	82	82	82.1	91.4	84.8	84.7
57	96	95.4	95.1	93.2		78.8	61.4	81.2	78.3	78.7	77.7	74.2	68.5	67.6	63.8
58	101.3	99.7	99	97		95.6	95.3	93.9	93.1	92.8					
59	100.4	100.4	99.5	98.7		97.9	97.7	97.2	97.2	87.1	97.3	97.6	97.2	97.3	97.4
60	102	100.8	99.7	98.1		96.5	96.2	94.1	93.6	79.2	84.6	82	77.8	66.4	64.9
61	102.6	102.5	102.4	102	100.7	101.4	101	95.8	57.2	93	92.5	91.3	90.8		
62	103.2	102.7	102.3	102		101.8	102	101.5	101.7	101.2	101	100.4	96.7		
63	101.2	101.6	101.7	96.3	29.3	95.7	95.5	94.8	94.8	88.9	87.2	85.3	63.1		
64	96.4	98.5	100.4	100.2	97.5	91.8	93.7	59.8	96.3	93.7	92.2	91.6	91.6	88.6	
65	98.9	99.6	98.6	103.5	99.2	83.3	94.8	95.3	53.9	95.7	95.4	89.9	94.4	90.4	85.3
66	96.1	98.1	89.9	97.6		93.7	91.9	94.9	67.6	78.4	69	68.6	71.5	67.6	

Continued next page…

	Day													
Device	**56**	**58**	**60**	**63**	**65**	**67**	**70**	**73**	**77**	**81**	**84**	**86**	**89**	**99**
31														
32														
33														
34														
35														
36														
37														
38														
39														
40														
41														
42														
43	72.2	71.4	70.4	68.9	68.7	68.1		66.6	66	65	63.5	62.7	61.7	57.7
44	56.6	56.6	56.5	56.5	56.7	56.3	56.5	56.3	56.5	56.1	56.1	55.7	55.9	56
45	83.9	83.8	83.8	83.8	84.3	84.2	84.1	86.3	83.6	83.4	83.6	83.5	83.3	83.6
46	66.5													
47	88.6	89.3	32.5	88.7	88.5	88.4	88.5	88.6	88	87.8	87.7	87.8	53.5	87
48														
49														
50	68.2	68.2	68.2	65.8	65.7	63.7	62.6	61.2	59.5	58.5	56.6	56.2	55.6	54
51														
52	62.3	60.5	61.4	54.5	52.9	60.6	57.1	53.3	51.4	50.3	50.6	53	55.7	52.7
53	67.1	66.3	66.3	63.1	61.4	62.8	67.8	58.1	58.4	55.9	55.7	56.6	51.9	49.9
54														
55	60.5	97.8	66.4	64.4	68.5									
56	83.2	85.5	85.7	78.7	83.7	82.9	79	65.9						
57	63.5	61.8	58.1	60.9										
58														
59	97.4													
60	64.6	64.6	64.4	64.5	64.7	64.1	64.6	50.5	51.1	61.2	58.8	60.2	53.8	53
61														
62														
63														
64														
65	86.8	83.9	85.6	89.8	87.7	67.8	81.6	84.4	84.6	82	0	86.9	78.3	71.9
66														

A.2 Trial 2

A.2.1 Configuration of individual devices

Device	Resistor	Tethered	Syringe	Contents
201	3900Ω		Terumo	Oil/Wax
202	3900Ω		Terumo	Oil/Wax
203	3900Ω		Terumo	Oil/Wax
204	3900Ω		Terumo	Oil/Wax
208	3900Ω		Theratron	Oil/Wax
209	3900Ω		Theratron	Oil/Wax
210	3900Ω		Theratron	Oil/Wax
211	3900Ω		Theratron	Oil/Wax
216	6800Ω		Terumo	Oil/Wax
217	6800Ω		Terumo	Oil/Wax
218	6800Ω		Terumo	Oil/Wax
221	6800Ω		Terumo	Oil/Wax
222	6800Ω		Theratron	Oil/Wax
223	6800Ω		Theratron	Oil/Wax
224	6800Ω		Theratron	Oil/Wax
225	6800Ω		Theratron	Oil/Wax
228	6800Ω		Theratron	Oil/Wax
229	1000Ω	✔	B Braun	H2O
230	1000Ω	✔	B Braun	H2O
231	1000Ω	✔	B Braun	H2O
232	4700Ω	✔	B Braun	H2O
233	4700Ω	✔	B Braun	H2O
234	4700Ω	✔	B Braun	H2O
235	10000Ω	✔	B Braun	H2O
236	10000Ω	✔	B Braun	H2O
237	10000Ω	✔	B Braun	H2O
238	1000Ω	✔	B Braun	Oil/Wax

Device	Resistor	Tethered	Syringe	Contents
239	1000Ω	✔	B Braun	Oil/Wax
240	1000Ω	✔	B Braun	Oil/Wax
241	4700Ω	✔	B Braun	Oil/Wax
242	4700Ω	✔	B Braun	Oil/Wax
243	4700Ω	✔	B Braun	Oil/Wax
244	10000Ω	✔	B Braun	Oil/Wax
245	10000Ω	✔	B Braun	Oil/Wax
246	10000Ω	✔	B Braun	Oil/Wax
247	560Ω	✔	Theratron	HPMC
248	560Ω	✔	Theratron	HPMC
249	560Ω	✔	Theratron	HPMC
250	3900Ω	✔	Theratron	HPMC
251	3900Ω	✔	Theratron	HPMC
252	3900Ω	✔	Theratron	HPMC
253	6800Ω	✔	Theratron	HPMC
254	6800Ω	✔	Theratron	HPMC
255	6800Ω	✔	Theratron	HPMC
256	1000Ω	✔	B Braun	HPMC
257	1000Ω	✔	B Braun	HPMC
258	1000Ω	✔	B Braun	HPMC
259	4700Ω	✔	B Braun	HPMC
260	4700Ω	✔	B Braun	HPMC
261	4700Ω	✔	B Braun	HPMC
262	10000Ω	✔	B Braun	HPMC
263	10000Ω	✔	B Braun	HPMC
264	10000Ω	✔	B Braun	HPMC

A.2.2 10-day devices by plunger position in mm

	Day										
Device	**0**	**1**	**2**	**3**	**6**	**7**	**8**	**9**	**10**	**13**	**17**
229	35.5	38.8	45.2	49.1	64.3	71.0	80.0	88.0	87.3		
230	31.0	35.4	40.7	45.1	53.4	55.4	57.3	57.1	57.1	57.2	57.5
231	30.4	35.0	40.8	44.8	53.2	54.6	56.6	57.1	56.7	57.2	57.3
238	29.6	32.3	35.2	38.4	48.5	50.7	52.2	54.3	54.7	55.4	55.5
239	26.8	31.8	35.2	38.9	49.8	51.4	53.1	54.3	54.4	54.9	55.0
240	29.0	35.4	38.8	42.3	50.2	52.1	54.1	55.4	55.5	56.2	56.3
247	22.2	34.5	43.0	50.9	67.4	72.0	78.2	80.5	80.6	80.8	80.7
248	24.5	35.2	43.4	51.8	74.5	80.5	88.2	95.3	101.3	109.7	
249	23.0	34.3	43.7	55.6	82.3	90.3	98.3	104.4	111.1	113.2	
256	31.0	36.6	42.3	46.3	61.5	66.0	70.6		80.0	88.0	
257	28.7	36.1	41.7	46.0	55.6	57.5	59.8	61.2	61.0	61.1	66.8
258	28.0	37.5	43.5	48.1	64.4	69.7	76.0	82.0	87.6	87.8	

A.2.3 10-day devices by weight in g

	Day										
Device	**0**	**1**	**2**	**3**	**6**	**7**	**8**	**9**	**10**	**13**	**17**
229	30.7	29.6	27.5	26.4	21.5	19.5	16.7	14.5	14.5		
230	32.0	30.7	29.0	27.9	25.2	24.6	24.2	24.0	23.9	24.0	24.1
231	32.0	30.8	29.0	27.8	25.2	24.7	24.2	23.9	24.0	23.8	23.2
238	31.0	30.3	29.5	28.7	25.7	25.2	24.7	24.1	24.0	23.7	23.8
239	31.9	30.5	29.6	28.4	25.4	25.0	24.4	24.0	24.0	24.0	23.8
240	31.2	29.4	28.3	27.4	25.3	24.6	24.3	23.8	23.8	23.7	23.7
247	98.3	92.1	87.0	82.7	72.8	70.4	67.0	65.5	65.3	65.5	
248	97.0	91.1	86.7	81.9	68.8	65.4	60.8	56.9	52.9	48.5	
249	97.7	91.8	86.4	79.4	64.0	59.4	54.7	51.3	47.7	46.0	
256	30.3	29.1	27.4	26.7	22.6	21.2	20.0		17.0	15.3	
257	30.5	28.4	26.8	25.8	23.2	22.7	22.2	21.6	21.4	21.5	19.8
258	31.0	28.5	26.5	25.5	21.2	19.8	18.0	16.1	14.7	14.7	

A.2.4 50-day devices by plunger position in mm

Device	Day 0	1	3	6	8	10	13	17	21	25	28	32	35	45	52	59	66	73	80	87	93	101
232	31.8	31.9	32.2	33.7	34.3	34.2	32.7	34.6	36.2	38.9	39.7	41.8	42.7	44.9		48.1	48.6	48.8	43.4			
233	33.3	33.3	34.2	35.4	35.9	36.0	36.3	36.2	36.3	31.9	37.2	37.3	37.4	37.5	37.6	37.3	37.8	37.4	37.6	37.6	37.6	37.6
234	29.7	29.8	30.2	32.1	33.2	33.0	33.4	33.1														
241	28.2	31.0	31.4	31.5	31.4	31.3	31.7	31.5														
242	28.8	31.4	33.6	35.6	36.1	35.8	36.2	36.1	36.2	31.6	36.4	36.6	36.8	36.6	36.7	36.7	37.0	37.1	37.0	37.1	37.2	36.8
243	27.3	29.9	32.8	34.0	34.7	34.6	34.8	34.8	34.9	33.4	35.1	35.2	35.4	35.2	35.5	35.5	35.6	35.4				
250	26.3	31.3	35.6	41.0	44.3	46.6	51.1	56.0	60.3	66.0	67.8	68.2										
251	25.5	29.0	29.9	31.3	32.4	33.3	35.1	36.9														
252	23.4	27.1	29.9	33.7	35.2	36.9	40.3	43.2	61.8	52.6	53.1	53.1	54.0	59.2	61.7	65.4	70.2	72.2	68.9			
259	28.3	31.9	32.2	34.0	35.2	35.9	36.2	37.2	38.9	40.8	41.4	42.0	43.8	45.1	45.5		45.6	45.2	40.4	45.5	45.4	45.5
260	26.7	26.9	28.8	30.3	31.3	31.4	32.7	35.7														
261	28.7	32.7	34.4	38.4	40.2	42.1	47.1	52.1	57.1	61.1	62.9	63.9	66.4	71.3	75.3	75.8	87.1					
201	26.1	26.7	28.4	33.6	35.9	37.0	40.3															
202	23.0	23.0	24.5	26.5	28.0	28.9	30.9	32.2	33.7	33.7	34.0	34.2	34.9	37.8	39.7	40.8	41.6	41.5	43.2	43.6	44.7	45.6
203	25.4	26.2	26.8	28.7	30.0	30.9	33.1	33.1	33.8	33.6	34.3	34.4	34.5	35.5	36.1	36.3	36.8	36.7	37.9	38.4	39.1	39.5
204	24.8	26.3	28.0	31.6	33.9	34.6	36.9	39.6	40.6	41.9	42.9	43.2	43.4	43.9	44.5	44.9	44.9	44.5	45.6	46.2	46.7	46.6
208	25.4	26.4	27.7	28.7	29.5	30.7	32.3	33.8	37.7	24.6												
209	26.2	26.8		28.7	29.8	30.7	40.1	41.4	42.6	44.0	44.6	45.9	45.9	48.6	50.4	51.9	55.6	56.8	58.7	59.4	60.0	63.0
210	22.5	23.7	25.1	26.7	28.1	38.5	24.9															
211	23.0	24.2		28.2	31.1	31.7	35.5	36.1	41.7	45.3	46.8	48.1	49.1	52.9	53.6	55.1	56.6	51.7	58.4	58.2		59.2

A.2.5 50-day devices by weight in g

Device	Day 0	1	3	6	8	10	13	17	21	25	28	32	35	45	52	59	66	73	80	87	93	101
232	31.5	31.6	31.7	31.2	31	30.8	30.7	30.7	29.8	28	27.7	26.8	42.8	25.2		16.7	18.1	17.2	17.1			
233	31	31.3	31.3	30.6	30.4	30.2	30.3	30	30.1	28.7	28.4	27.2	22.4	22.8	19.3	16.4	16.1	15.9	16.3	16.4	16.2	16.2
234	32.3	32.3	32.2	31.8	31.5	31.4	31.3	31														
241	31.5	30.6	30.7	30.7	30.6	30.7	30.7	30.5														
242	31.3	30.6	30.3	29.6	29.4	29.3	29	26.3	25.5	24.6	24.4	24	23.6	22.8	22.2	21.6	21.2	20.8	20.8	21.2	20.9	20.9
243	31.6	30.9	30.2	29.6	29.5	29.2	28.7	28.2	27.5	26.7	26.5	25.5	25	23.8	23.6	23.3	22.5	21.4				
250	95.7	93.4	91.2	88.3	86.5	84.5	82		77	73.1	71.7	71.1										
251	94.9	94.3	93.9	93.8	93.2	92.7	91.5	90.9														
252	97.9	96.3	94.6	92.6	91.9	90.7	88.9		84.8	81.4	81.4	81.4	81.9	77.9	76.6	74.4	72	69.7	73.3			
259	31	29.9	30	29.5	29.2	28.9	28.8	28.5	27.9	27.3	27.2	27.1	26.8	26.7	26.1		25.8	24.6	21	24.8	24.2	23.8
260	30.9	30.5	30.5	29.9	30.1	29.2	28.5	27.6														
261	31.4	29.9	29.7	28.6	28.1	27.4	26.3	24.5	23.2	21.7	21	20.6	20.3	18.6	17.3	17.2	15.7					
201	101.1	100.4	99.8	96.7	95.4	94.7	88.0															
202	102.6	102.8	102.3	101.1	100.1	100.6	99.4	97.4	97.7	97.4	97.0	96.5	94.9	94.3	93.6	93.3	92.6	92.7	92.0	92.0	91.7	
203	101.3	100.8	101.0	100.0	99.4	98.5	98.0	97.2	95.6	97.4	97.6	97.5	97.2	97.0	97.0	96.6	97.1	96.4	96.2	96.1	96.1	
204	101.7	101.1	100.1	98.2	97.0	96.1	95.3	93.2	92.0	91.6	91.6	91.5	91.4	91.2	91.0	91.0	91.4	91.0	91.1	91.1	90.3	
208	101.6	101.3	100.8	100.7	100.4	99.5	98.8	94.8	91.6													
209	101.2	101.1		101.9	100.7	100.4	94.0	97.2	91.7	91.3	90.5	90.5	89.7	88.6	87.8	85.9	85.2	84.3	83.4	83.4	82.7	
210	103.7	102.4	102.4	101.2	100.7	100.3	102.5															
211	103.2	102.8	102.5	100.7	99.0	98.4	97.4	91.7	89.5	88.1	87.6	87.1	86.3	85.6	84.7	84.2	84.0	83.1	83.1		83.1	

A.2.6 100-day devices by plunger position in mm

Device	Day 0	1	3	6	8	10	13	17	21	25	28	32	35	45	52	59	66	73	80	87	93	101
235	32.1	32.1	31.8	31.8	32.1	32.0	31.7	31.7	33.4	35.4	36.2	36.2	36.5	37.1	37.3	37.2	37.2	37.0	37.3	34.8		
236	31.2	31.5	31.1	31.3	31.4	31.1	31.3	31.4	31.2	31.2	31.4	31.5	31.4	31.5	31.2	31.6	32.0	31.9	32.4	32.5		
237	30.5	32.0	42.6	31.6	32.2	31.7	31.7	32.0														
244	28.8	34.1	34.1	34.4	34.5	34.5	34.8	34.8	34.5	34.7	34.9	35.2	35.0	35.2	35.5		35.6	35.5	35.8	35.9	36.0	35.8
245	26.7	29.5	30.1	30.3	30.8	30.9	31.1	31.3	31.1	31.6	32.1	31.9	32.3	32.4	32.3	32.7	32.3	32.0	32.6	32.6	32.9	32.7
246	29.4	33.1	33.8	33.9	34.2	34.3	35.1	35.0														
253	22.8	25.9	27.9	30.7	32.4	33.6	35.5	37.3	40.2	44.4	46.2	48.1	50.9	55.9								
254	22.9	27.5	28.9	31.5	33.0	34.0	35.2	35.8	38.2	40.0	43.2	45.1	46.8	52.4	56.5		63.6	68.8	72.8	77.5	78.8	73.4
255	23.4	27.2	29.7	32.3	33.8	35.0	38.9	39.3	40.3	42.0		42.9	43.0	43.1	43.4	43.3	43.9	43.0	43.1		43.4	45.6
262	28.3	34.2	34.1	34.5	34.4	34.1	34.5	35.6														
263	28.2	32.1	32.2	32.8	34.3	33.2	33.4	33.4	33.5	33.7	38.6	35.0	34.8	35.5	35.8		39.4	40.5	46.2			
264	28.4	32.1	32.0	32.8	32.6	33.0	33.2	35.9	38.3	40.9		43.2	44.6	45.9	46.2	46.1	46.0	45.8	46.0	46.1	45.8	46.2
216	23.6	24.9	25.6	26.4	27.0	27.0	27.8															
217	29.7	31.0	32.5	33.7	34.7	35.0	35.6	35.5	36.6	31.7	37.0	37.9	38.8	39.5	39.8	40.0	40.0	39.5	40.6	40.9	40.8	41.4
218	23.5	24.9	25.1	25.3	26.2	25.7	25.6	30.8	26.0	26.0		26.8	26.6	26.8	27.3	27.1	27.4	27.0	27.5	27.7	27.6	27.9
221							25.4	28.5	30.3	33.6		36.5	36.6	37.4	37.6	37.9	38.2	37.9	38.5	38.4	38.4	38.6
222	26.3	0.0	27.8	29.7	31.2	33.1	35.7	37.0	38.9	40.3	40.9	40.6	40.6	41.5	42.7	42.9	43.1	43.3	43.9	43.4	44.6	44.5
223	23.0	23.7	25.0	26.8	27.8	28.3	29.3	29.9														
224	25.3	27.5	29.3	33.7	30.8	31.1	32.4	18.4	38.3	30.7	30.4	36.9	38.6	44.7	48.1	50.2	52.3	53.4	55.7	57.8		
225	25.0	27.4	28.8	30.9	32.5	33.4	37.2	37.4	37.5	38.5	39.7	40.1	40.3	41.5	41.7	42.0	42.5	42.0	43.2	43.5	44.0	
228		27.7	28.2	30.0	30.8	31.3	32.7	33.5	34.8	35.6	36.8	37.1	37.3	39.5								

A.2.7 100-day devices by weight in g

Device	Day 0	1	3	6	8	10	13	17	21	25	28	32	35	45	52	59	66	73	80	87	93	101
235	31.3	31.3	31.7	31.5	31.5	31.3	31.3	31.1	31	27.9	28.6	25.1	23.5	22.8	15.3	15.3	15.5	15.2	15.5	15.5		
236	31.8	31.9	31.9	31.8	31.8	31.8	31.9	31.7	31.8	31.1	31	30.2	39.3	29	29	29.1	27.9	21.7	18.9	20.3		
237	32	31.8	88.8	31.7	31.6	31.7	31.7	31.8														
244	31.1	29.8	30.1	29.8	29.6	29.7	29.1	28.4	28.5	27.9	28	27.7	27.5	26.6	26.1		25.7	25.1	25.5	25.4	25.5	25.4
245	32.1	31.2	31.2	30.9	30.9	30.8	30.8	30.8	30.4	30.4	30.3	30.4	30	29.8	29.7	29.9	29.1	27.6	28	27.7	27.7	27.7
246	31.3	30.4	31.2	30.1	30	29.8	29.9	29.9														
253	98.1	96.4	95.3	94	93.9	92.3	91.4	90.5	88.6	86.2	85.1	84.1	82.5	79.5								
254	97.9	95.1	94.7	93.3	92.8	91.9	91.5	91.2	89.9	88.8	86.7	85.2	85	81.5	79.6		75.9	71.5	70	67.6	65.9	65.3
255	98.4	96.4	95	93.7	92.5	91.9	88.4	88.6	88.5	88.5		88.4	88.3	88.6	88.7	88.7	88.6	88.5	88.5	88.5	88.5	88.6
262	30.9	29.1	29.2	29	29	28.6	28.5	27.7														
263	30.8	29.6	30.7	29.5	31	29.4	29	28.3	28	26.9	26.5	26.2	25.5	25.4	25.3		24.3	24.6	25.1			
264	30.8	29.8	29.8	29.7	29.3	29	28.6	27	26.9	25.9		24.4	23.4	20.8	15.5	17.4	26	24.6	21.8	15.9	25.4	25.2
216	102.7	101.3	101.1	100.7	100.2	100.0	100.3															
217	98.6	97.8	97.8	96.7	96.1	96.0	95.8		95.0	95.6	95.3	94.8	94.1	94.1	93.9	93.8	93.9	93.6	93.5	93.5	93.5	93.6
218	102.1	101.9	102.0	102.1	101.8	101.6	102.0		101.4	101.4		101.9	101.4	101.5	101.7	101.3	101.6	101.5	101.6	101.5	101.6	101.5
227												94.9	94.5	91.6	94.5	94.4	94.5	94.4	94.4	94.3	94.1	94.5
222	100.0	0.0	100.2	99.5	98.9	97.4	91.6	95.7	94.6	93.0	92.9	93.1	92.9	92.9	92.4	92.2	92.1	91.7	91.9	92.1	91.8	91.9
223	103.1	102.8	102.3	101.2	100.6	100.3	99.9															
224	100.1	99.9	100.0	92.6	99.6	99.1	98.6		94.3	79.3	64.1	64.2	63.9	64.1	63.8	63.9	64.0	64.1	63.9	63.9	63.9	
225	100.8	100.6	99.5	98.6	97.7	96.7	94.6		95.4	94.2	93.8	93.4	93.3	93.0	92.6	92.4	92.5	92.5	92.4	91.8	91.6	91.4
228		100.6	100.3	99.4	99.2	99.0	98.1	97.4	97.2	96.0	95.9	95.6	95.2	94.4								

A.3 In vitro *release studies*

A.3.1 10-day devices by plunger position in mm

Shaded values were converted from the units of the scale on the outside of the syringe.

				Day									
Body	***Plunger***	***Contents***	***Valve***	**0**	**1**	**2**	**3**	**4**	**5**	**7**	**8**	**9**	**10**
Theratron	*Elanco*	*Water*	✔	16.4	25.7	35.7	44.6	52.5	60.2	76.6	82.5	88.2	91.3
				12.5	22.7	32.3	26.0	49.4	57.0	72.9	79.0	84.1	89.4
				14.2	23.9	33.2	23.2	45.0	52.1	38.6	44.9	41.9	37.5
Theratron	*Elanco*	*Water*		14.7	22.3	31.4	40.8	48.9	56.9	72.4	78.7	83.8	87.0
				18.0	25.3	34.4	41.9	46.7	44.6	41.0	38.9	36.5	33.5
				13.2	14.5	14.8	14.4	14.4	14.8	15.1	15.3	15.2	14.7
Theratron	*Elanco*	*Oil/Wax*	✔	9.2	9.5	9.8	9.9	10.3	10.3				
				9.8	9.3	9.9	10.1	10.5	10.7				
				11.4	11.9	12.7	12.3	12.6	13.0				
Theratron	*Elanco*	*Oil/Wax*		13.4	14.8	14.7	14.9	14.5	14.6	15.2	15.4	15.1	14.7
				12.7	23.6	31.5	40.5	48.2	56.4	71.8	78.0	83.5	85.0
				13.5	22.3	32.7	41.6	49.2	56.9	71.2	76.6	82.3	85.3
Theratron	*Theratron*	*Water*	✔	30.2	39.5	48.5	56.6	63.6	70.2	83.5	88.0	93.0	96.2
				30.9	39.3	47.7	55.2	62.3	73.0	81.6	86.1	91.2	94.2
				30.9	37.2	42.6	48.0	50.9	56.1	58.2	58.1	56.3	53.1
Theratron	*Theratron*	*Water*		33.0	41.3	49.7	57.6	64.3	70.9	83.8	89.5	94.0	97.0
				33.0	41.6	50.1	57.8	64.8	71.4	84.1	89.4	93.4	95.8
				33.2	41.6	50.1	57.0	64.2	71.8	85.3	91.0	95.5	98.5
Theratron	*Theratron*	*Oil/Wax*	✔	25.4	25.4	25.7							
				26.8	26.6	25.3							
				25.4	25.1	25.3							
Theratron	*Theratron*	*Oil/Wax*		28.1	36.8	46.1	54.2	61.2	68.5	82.3	87.9	92.8	95.7
				29.9	38.7	47.7	55.4	62.8	70.0	83.7	87.9	94.3	97.2
				28.2	36.8	45.9	53.4	60.6	67.2	80.8	85.5	90.4	93.3
Terumo	*Elanco*	*Water*	✔	14.8	24.9	34.9	42.8	51.5	59.0	74.2	81.2	86.5	89.7
				15.9	27.0	36.5	44.6	53.3	61.0	76.3	83.2	89.2	91.9
				16.1	18.2	18.3	18.7	18.5	18.4	19.2	19.1	18.9	19.6
Terumo	*Elanco*	*Water*		10.7	20.1	30.1	37.7	46.0	54.5	68.3	75.7	81.6	85.4
				13.6	23.9	33.3	40.9	49.7	56.9	67.5	68.6	68.7	61.7
				10.0	18.2	29.3	39.0	47.5	56.1	71.6	79.4	85.5	88.8

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A.3.2 10-day devices by weight in g

				Day									
Body	***Plunger***	***Contents***	***Valve***	***0***	***1***	***2***	***3***	***4***	***5***	***7***	***8***	***9***	***10***
Terumo	*Elanco*	*Oil/Wax*	✔	11.3	11.4	11.9	11.6	12.1	13.0				
				11.5	11.1	11.0	11.6	12.2	11.6				
					11.7	11.6	11.2	11.8	11.9				
Terumo	*Elanco*	*Oil/Wax*		12.1	17.0	26.2	34.0	42.3	50.0	65.8	72.2	77.7	81.7
				15.5	15.4	15.3	14.9	15.0	15.0	15.0	15.8	15.2	15.6
				17.3	27.6	36.4	44.5	52.4	60.3	75.2	71.6	87.4	90.0
Terumo	*Theratron*	*Water*	✔	35.7	43.9	52.6	59.8	66.8	71.5	84.9	89.3	92.4	94.9
				33.5	42.0	51.0	58.2	64.8	71.7	84.5	89.9	92.4	98.1
				34.3	43.0	52.4	60.1	66.3	73.1	85.9	90.5	95.1	97.4
Terumo	*Theratron*	*Water*		35.1	35.4	35.8	34.8	34.8	36.0	52.9	60.2	65.9	69.2
				28.9	37.3	46.6	53.2	60.6	67.5	80.3	85.3	90.5	93.3
				39.5	43.8	47.6	64.9	72.2	79.6	92.7	97.6	102.2	105.4
Terumo	*Theratron*	*Oil/Wax*	✔	27.6	27.6	28.3	28.4	29.4	29.4				
				29.0	28.0	28.6	28.4	28.6	29.4				
				28.6	28.5	28.9	29.0	29.3	29.6				
Terumo	*Theratron*	*Oil/Wax*		26.4	34.8	47.0	51.4	58.6	65.8	79.8	85.0	90.3	93.5
				32.3	40.8	42.4	58.2	65.5	72.9	87.1	92.5	97.3	100.8
				30.5	39.2	44.1	55.1	62.3	69.4	83.0	88.2	93.0	96.3
Bbraun	*Bbraun*	*Water*	✔	31.4	33.7	39.2	42.8	45.8	48.3	52.4	53.0	54.1	54.6
				31.2	36.4	41.9	45.8	48.8	48.6	55.7	56.8	58.2	58.8
				32.0	34.2	39.7	43.3	46.3	48.8	53.5	53.5	70.1	53.8
Bbraun	*Bbraun*	*Water*		33.1	34.8	40.3	44.4	47.7	50.2	55.2	56.3	57.7	58.5
			✔	33.4	34.8	40.6	44.4	47.7	50.5	54.9	56.3	57.4	58.5
				32.5	34.2	39.4	43.3	46.1	48.6	53.2	54.9	54.9	56.6
Bbraun	*Bbraun*	*Oil/Wax*		31.4	33.4	33.9	35.0	36.4	37.0	38.9	39.7	40.6	41.7
				29.5	30.9	32.0	33.4	34.2	35.6	37.5	38.9	39.7	40.8
				31.4	31.4	32.0	33.4	34.2	35.3	37.5	38.6	39.4	40.0
Bbraun	*Bbraun*	*Oil/Wax*		35.6	36.7	42.2	45.5	48.8	51.3	55.7	56.8	61.0	58.8
				34.2	37.0	42.8	46.6	49.9	52.1	56.3	57.7	58.8	59.3
				35.6	37.2	42.5	46.6	49.9	52.4	56.3	57.7	58.8	59.3

				Day									
Body	***Plunger***	***Contents***	***Valve***	**0**	**1**	**2**	**3**	**4**	**5**	**7**	**8**	**9**	**10**
Theratron	*Elanco*	*Water*	✔	86.1	80.0	74.2	68.3	62.9	58.4	48.1	43.4	39.9	37.9
				88.7	81.6	76.2	70.3	64.9	60.4	49.5	45.4	42.0	39.7
				87.8	81.4	76.0	72.4	67.8	63.4	65.6	65.8	66.5	66.9
Theratron	*Elanco*	*Water*		86.6	82.1	76.7	70.5	65.1	60.1	49.8	45.8	42.4	40.2
				86.7	80.0	74.6	69.8	66.3	66.7	68.3	68.4	68.8	69.2
				88.2	87.1	87.6	87.3	87.0	87.0	86.8	86.7	86.7	86.6
Theratron	*Elanco*	*Oil/Wax*	✔	86.0	86.1	86.4	85.9	85.8	85.8				
				85.9	86.1	86.3	85.8	85.6	85.6				
				84.6	84.7	84.9	84.4	84.5	84.3				
Theratron	*Elanco*	*Oil/Wax*		83.0	82.5	82.5	82.1	82.5	82.7	82.2	82.3	82.3	82.3
				83.7	78.3	72.6	67.1	62.5	58.0	48.5	45.0	41.8	39.8
				83.6	77.8	72.3	67.0	62.2	57.7	49.2	46.0	42.7	40.6
Theratron	*Theratron*	*Water*	✔	98.5	93.0	87.3	82.1	77.7	73.4	64.5	61.3	58.1	56.1
				98.0	92.7	87.5	82.5	78.1	74.1	65.4	62.4	59.3	57.1
				98.8	94.5	91.4	87.6	85.8	82.8	81.3	81.6	82.6	84.4
Theratron	*Theratron*	*Water*		96.4	91.4	86.0	80.9	76.5	72.1	63.5	60.4	57.1	55.2
				96.5	91.4	86.0	80.9	76.3	72.4	63.8	60.6	57.7	55.8
				96.2	91.5	85.8	81.0	76.6	71.6	62.7	59.6	56.3	54.2
Theratron	*Theratron*	*Oil/Wax*	✔	97.7	98.2	98.4							
				97.4	97.8	97.6							
				97.6	98.3	98.0							
Theratron	*Theratron*	*Oil/Wax*		95.6	90.5	84.9	80.1	75.7	71.5	63.2	60.3	57.1	55.1
				94.7	89.4	84.3	79.3	75.0	70.8	62.6	59.4	56.6	54.4
				95.9	90.4	85.3	80.5	76.3	72.3	64.4	61.3	58.7	56.8
Terumo	*Elanco*	*Water*	✔	86.8	80.0	73.7	67.8	62.5	57.2	46.6	42.6	39.1	36.5
				84.1	79.1	73.0	67.3	61.8	56.6	46.1	41.5	38.0	35.5
				86.5	84.5	84.5	84.6	84.7	84.6	84.4	84.7	84.0	84.5
Terumo	*Elanco*	*Water*		89.6	82.8	76.9	70.8	65.4	60.0	49.9	45.8	42.1	39.2
				86.5	80.6	74.4	68.4	63.3	57.9	50.0	50.0	49.9	49.6
				89.4	82.8	76.6	70.6	65.1	59.8	48.7	44.2	41.0	38.3

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				Day									
Body	***Plunger***	***Contents***	***Valve***	***0***	***1***	***2***	***3***	***4***	***5***	***7***	***8***	***9***	***10***
Terumo	*Elanco*	*Oil/Wax*	✔	84.7	84.6	84.4	84.2	84.3	83.9				
				84.9	84.8	84.8	84.6	84.3	84.3				
				83.8	84.0	84.1	84.0	84.0	84.0				
Terumo	*Elanco*	*Oil/Wax*		81.6	80.7	74.6	69.4	64.6	59.7	50.3	46.6	43.5	40.8
				82.2	81.9	82.0	81.9	81.9	82.0	81.7	81.7	81.7	81.6
				80.0	74.7	68.6	62.8	58.0	53.3	43.8	40.0	37.1	35.3
Terumo	*Theratron*	*Water*	✔	95.4	89.8	84.3	79.0	74.4	71.4	62.7	60.0	57.5	56.2
				97.3	91.2	85.6	80.3	75.7	71.4	62.7	59.2	56.2	54.1
				96.0	91.0	84.1	79.0	74.8	70.3	61.7	58.7	55.8	54.2
Terumo	*Theratron*	*Water*		95.6	95.3	95.5	95.2	95.3	95.0	83.7	79.1	75.2	73.1
				96.0	94.4	88.3	83.0	78.8	74.0	65.3	62.0	58.8	56.9
				91.9	86.4	80.9	75.6	70.7	66.3	57.3	54.0	50.8	49.1
Terumo	*Theratron*	*Oil/Wax*	✔	96.5	96.6	96.6	96.3	96.1	96.2				
				96.2	96.3	96.4	96.4	96.0	96.0				
				96.6	96.6	96.6	96.3	96.2	96.2				
Terumo	*Theratron*	*Oil/Wax*		95.9	91.0	85.5	80.8	76.4	72.1	63.7	60.4	57.2	55.3
				92.5	87.9	82.1	77.2	72.7	68.5	59.7	56.4	53.4	51.6
				93.7	88.5	83.2	78.5	74.2	70.0	61.7	58.5	55.5	53.6
Bbraun	*Bbraun*	*Water*	✔	31.2	30.5	28.7	27.4	26.5	25.7	24.5	24.1	23.7	23.4
				30.5	29.6	27.9	26.4	25.4	24.8	23.3	22.8	22.4	22.2
				31.2	30.6	28.6	27.3	26.4	25.6	24.0	23.9	24.0	23.9
Bbraun	*Bbraun*	*Water*		30.4	29.9	28.1	26.2	25.7	24.8	23.2	22.5	22.2	21.9
			✔	30.3	29.6	27.9	26.4	25.4	24.5	22.9	22.3	22.0	21.8
				30.4	30.6	28.3	26.8	25.9	25.2	23.6	22.9	22.5	22.5
Bbraun	*Bbraun*	*Oil/Wax*		29.8	29.2	29.1	28.8	28.6	28.3	27.7	27.4	27.0	26.8
				30.3	30.1	29.7	29.4	29.2	28.9	28.0	27.7	27.4	27.1
				30.0	30.0	29.7	29.4	29.3	29.0	28.2	27.8	27.6	27.3
Bbraun	*Bbraun*	*Oil/Wax*		28.2	28.1	26.5	25.2	24.4	23.5	22.0	21.6	21.4	21.1
				28.9	27.8	26.2	24.8	24.0	23.2	21.7	21.3	21.0	20.7
				28.3	27.6	26.0	24.7	23.9	23.1	21.6	21.2	20.9	20.7

A.3.3 100-day devices by plunger position in mm

				Day									
Body	***Plunger***	***Contents***	***Valve***	**0**	**4**	**12**	**15**	**23**	**30**	**38**	**45**	**65**	**71**
Theratron	*Elanco*	*Water*	✔	16.2	19.7	23.7	24.4	25.1	25.2	25.6	25.7	27.4	28.1
				12.7	16.8	20.0	20.9	21.3	23.4	24.0	23.9	23.4	24.8
				15.0	19.0	22.3	23.6	24.3	25.6	26.7	26.9	29.0	30.1
Theratron	*Elanco*	*Water*		12.7	16.5	19.4	20.6	21.2	21.7	16.9	17.9	20.1	22.3
				14.7	18.8	22.3	22.9	23.4	22.4	22.0	22.6	22.0	23.3
				12.9	16.9	19.7	20.8	21.4	22.4	24.3	24.2	26.2	21.8
Theratron	*Elanco*	*Oil/Wax*	✔	10.7	11.4	12.7	12.4	12.8	12.2	12.8	12.7	12.7	13.1
				10.5	12.6	14.9	14.9	15.1	15.2	15.5	15.5	15.6	16.6
				11.8	14.1	16.5	17.0	16.9	17.1	17.3	17.1	16.6	16.8
Theratron	*Elanco*	*Oil/Wax*		13.8	17.5	20.3	21.2	21.4	20.9	22.2	22.0	22.4	21.6
				13.9	14.0	13.1	14.7	14.9	14.4	16.6	16.3	17.9	18.6
				14.9	17.8	20.8	21.5	22.1	22.2	23.6	22.3	22.7	21.6
Theratron	*Theratron*	*Water*	✔	27.8	31.2	32.6	33.4	33.6	34.2	34.8	35.2	35.8	38.0
				27.6	29.8	30.5	30.8	31.4	31.9	32.6	32.8	34.5	36.3
				26.5	29.5	31.4	31.8	32.2	33.3	33.7	34.3	36.5	38.5
Theratron	*Theratron*	*Water*		28.4	30.5	31.5	32.0	31.9	31.8	32.0	32.1	33.1	35.5
				28.0	30.4	31.8	32.1	32.3	32.8	33.5	33.9	35.0	36.8
				27.8	29.3	31.0	31.3	31.7	32.0	32.7	33.5	34.9	37.1
Theratron	*Theratron*	*Oil/Wax*	✔	25.3	27.4	29.8	30.3	30.5	30.5	30.9	31.2	30.1	
				26.8	28.7	28.9	29.0	29.3	30.1	30.1	30.2		24.1
				24.8	27.1	29.4	29.5	29.5	29.9	30.0	30.0	30.4	29.9
Theratron	*Theratron*	*Oil/Wax*		27.0	29.6	30.9	31.8	32.2	33.0	34.0	34.2	35.1	35.8
				27.5	30.3	32.2	32.5	32.8	33.7	34.1	34.7	36.1	37.5
				27.8	30.8	32.8	33.6	33.7	34.2	34.7	34.9	36.2	36.8
Terumo	*Elanco*	*Water*	✔	15.2	18.4	22.8	23.2	23.8	23.9	25.1	24.9	28.6	28.7
				14.5	17.9	22.4	23.5	24.7	23.0	24.3	25.0	24.0	25.4
				15.1	18.6	22.1	23.2	23.9	25.2	26.3	24.7	27.5	28.1
Terumo	*Elanco*	*Water*		14.6	18.7	22.3	24.2	24.3	24.1	26.5	27.0	21.8	19.0
				15.3	17.0	21.3	22.2	23.3	23.1	25.4	25.4	26.3	25.9
				12.4	14.1	17.5	19.8	21.4	22.8	25.7	29.5	56.6	71.8

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Body	Plunger	Contents	Valve	Day 0	4	12	15	23	30	38	45	65	71
Terumo	*Elanco*	*Oil/Wax*	✔	12.8	15.2	17.5	18.2	18.2	18.4	17.4	18.5	19.0	17.8
				12.2	13.8	18.0	18.6	19.0	18.2	18.2	18.3	19.0	18.6
				12.4	13.9	17.7	18.4	19.0	18.8	18.9	19.3	19.2	18.1
Terumo	*Elanco*	*Oil/Wax*		15.1	16.5	15.2	14.7	14.1	12.4	12.3	10.6	9.8	9.1
				16.5	19.3	21.9	22.6	23.4	22.0	23.3	23.7	24.5	26.2
				14.6	17.0	20.4	21.1	22.1	21.5	94.1			
Terumo	*Theratron*	*Water*	✔	29.9	31.6	33.2	33.5	33.8	34.6	34.7	34.5	34.7	35.7
				30.6	32.9	34.2	34.6	35.1	35.8	35.9	35.8	36.9	40.5
				32.1	32.8	32.8	32.2	31.9	31.6	31.8	31.5	61.4	61.3
Terumo	*Theratron*	*Water*		31.0	32.8	34.7	34.8	34.9	56.0	35.8	35.9	37.9	50.2
				31.6	33.6	35.3	35.7	113.1					
				29.3	31.0	32.4	33.0	33.5	34.2	35.0	35.5	35.8	37.1
Terumo	*Theratron*	*Oil/Wax*	✔	30.5	33.4	35.8	36.5	36.6	36.7	37.0	37.3	37.3	36.5
				29.8	31.5	33.3	33.8	34.0	34.2	35.0	34.5	34.6	34.5
				36.0	37.8	41.7	42.8	42.6	42.2	43.2	42.3	43.4	42.6
Terumo	*Theratron*	*Oil/Wax*		30.2	30.8	30.9	30.6	29.1	28.9	29.3	29.1	29.2	28.7
				31.5	33.5	34.4	34.5	35.0	35.9	36.7	36.4	38.3	37.8
				28.5	30.6	32.2	32.5	32.7	33.8	35.2	35.0	36.0	36.2
Bbraun	*Bbraun*	*Water*	✔	17.1	16.8	14.8	16.8	16.8	16.8	15.8	16.8	17.8	
				16.7	16.2	16.8	16.2	16.3	16.2	16.2	16.2	16.3	
				15.3	15.1	15.1	15.1	15.1	15.1	15.1	15.1	15.1	
Bbraun	*Bbraun*	*Water*		14.3	14.3	14.3	14.3	14.2	14.2	14.8	14.3	14.3	
			✔	16.1	15.7	15.8	15.7	15.8	15.8	15.8	15.8	15.8	
				17.2	17.1	17.2	17.1	17.1	17.1	17.2	17.2	17.1	
Bbraun	*Bbraun*	*Oil/Wax*		18.1	17.3	17.2	17.1	17.1	17.0	17.0	17.0	17.0	
				18.4	18.2	18.1	18.1	19.0	18.0	18.0	18.0	18.0	
				18.6	16.5	16.4	16.5	16.4	16.5	16.5	16.4	16.4	
Bbraun	*Bbraun*	*Oil/Wax*		16.8	16.2	16.2	16.1	16.1	16.1	16.1	16.1	16.1	
				17.0	16.2	16.2	16.2	16.1	16.1	16.1	16.1	16.1	
				16.8	16.8	16.7	16.7	16.7	16.7	16.7	16.7	16.8	

A.3.4 100-day devices by weight in g

				Day								
Body	***Plunger***	***Contents***	***Valve***	**0**	**4**	**12**	**15**	**23**	**30**	**38**	**45**	**65**
Theratron	*Elanco*	*Water*	✔	86.6	84.0	82.0	81.9	81.6	81.2	81.5	81.5	80.7
				88.7	86.3	84.2	83.9	83.8	82.7	82.3	82.5	82.9
				87.0	84.5	82.4	81.9	81.7	80.9	80.6	80.6	79.5
Theratron	*Elanco*	*Water*		88.4	86.0	84.1	83.5	83.4	82.9	85.6	85.9	85.0
				86.7	84.2	82.5	82.1	81.8	82.0	82.9	83.0	83.8
				88.1	86.1	84.2	83.6	83.4	82.4	81.8	81.8	80.6
Theratron	*Elanco*	*Oil/Wax*	✔	85.9	85.2	84.6	84.7	84.9	85.0	85.1	85.4	85.6
				85.3	84.5	83.1	83.1	83.1	83.3	83.3	83.6	83.7
				84.0	83.6	82.0	81.8	81.9	82.0	82.2	82.5	82.7
Theratron	*Elanco*	*Oil/Wax*		83.2	81.1	79.2	78.7	78.9	78.6	78.8	78.7	78.7
				82.6	82.5	82.6	82.6	82.8	82.7	82.4	82.3	81.8
				83.2	80.9	79.2	78.7	78.7	78.1	78.0	78.3	78.6
Theratron	*Theratron*	*Water*	✔	100.3	98.5	97.8	97.7	97.7	97.4	97.4	97.3	97.0
				100.9	99.5	99.3	99.4	99.1	98.8	98.7	98.6	97.9
				101.6	99.7	98.9	98.8	98.7	98.2	98.1	98.0	96.7
Theratron	*Theratron*	*Water*		99.8	98.4	98.1	98.0	98.2	98.3	98.4	98.6	98.4
				100.0	98.7	97.8	97.6	97.8	97.6	97.4	97.1	97.0
				102.0	98.9	98.2	97.8	97.9	98.0	97.7	97.6	96.7
Theratron	*Theratron*	*Oil/Wax*	✔	98.7	97.7	96.4	96.7	96.3	96.1	96.1	96.1	95.8
				96.1	95.5	95.1	95.3	95.3	95.3	95.4	95.6	
				97.3	95.8	95.5	95.4	95.5	95.6	95.7	96.0	96.1
Theratron	*Theratron*	*Oil/Wax*		96.3	94.3	93.6	93.3	93.1	92.8	92.6	92.9	92.6
				96.1	93.7	92.8	92.7	92.8	92.5	92.5	92.3	91.7
				95.7	93.4	92.4	92.2	92.3	92.1	92.0	92.2	91.5
Terumo	*Elanco*	*Water*	✔	87.2	84.4	82.6	82.5	82.3	82.2	81.8	81.9	80.0
				87.6	84.9	82.7	82.2	81.9	83.1	82.8	82.0	83.4
				86.9	84.1	82.1	81.7	81.4	80.7	80.0	81.4	79.9
Terumo	*Elanco*	*Water*		86.7	83.6	82.3	82.0	82.0	81.5	80.8	80.3	84.1
				86.2	84.6	82.7	82.3	82.0	81.4	80.7	80.3	79.9
				87.2	87.0	85.1	84.8	84.1	83.4	82.6	79.3	61.3

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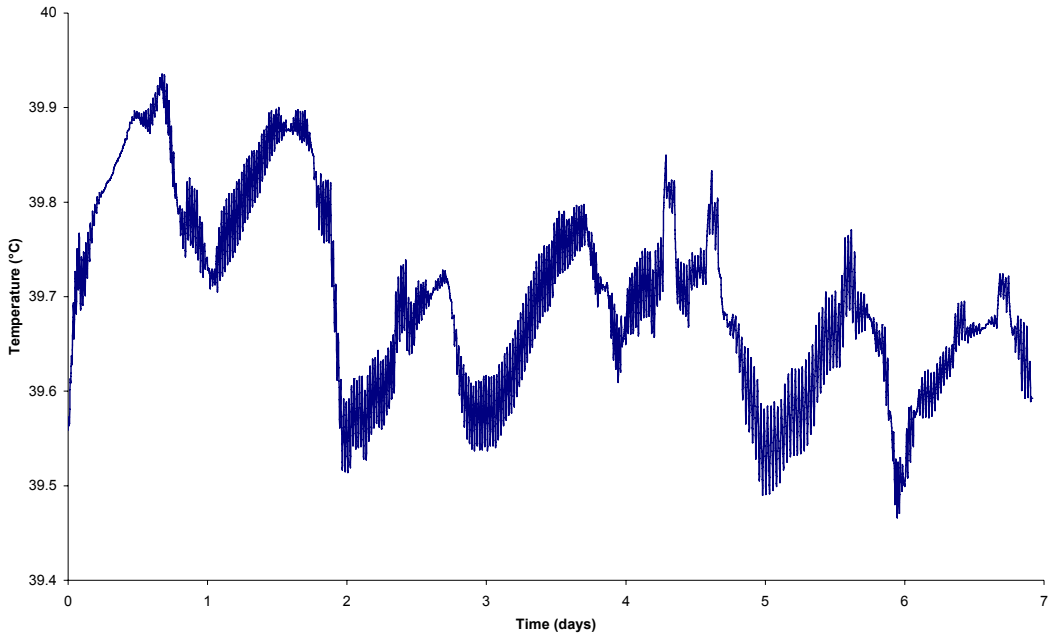
Body	Plunger	Contents	Valve	Day 0	4	12	15	23	30	38	45	65
Terumo	*Elanco*	*Oil/Wax*	✔	83.5	82.6	81.0	80.7	80.6	80.7	81.0	81.1	81.3
				84.4	83.1	81.2	81.1	81.0	81.0	81.2	81.4	81.4
				84.1	83.0	81.2	80.8	80.5	80.6	80.7	80.9	81.2
Terumo	*Elanco*	*Oil/Wax*		82.0	81.2	81.9	82.4	83.0	81.2	84.7	85.4	87.0
				82.0	79.6	78.1	77.9	77.4	77.1	76.9	77.0	76.8
				82.4	80.1	78.2	78.1	77.8	77.8	35.3		
Terumo	*Theratron*	*Water*	✔	99.0	97.8	97.4	97.2	97.5	96.9	97.1	97.2	97.5
				98.2	96.6	96.1	96.1	96.2	96.0	96.2	96.4	96.0
				97.6	97.0	97.3	97.6	98.4	98.7	99.2	99.4	79.6
Terumo	*Theratron*	*Water*		98.0	96.4	95.7	95.9	96.1	95.8	95.7	95.8	94.8
				97.2	95.8	95.2	95.1	43.8				
				99.2	97.7	97.1	97.1	97.0	96.5	96.4	96.4	96.4
Terumo	*Theratron*	*Oil/Wax*	✔	95.2	93.7	92.3	92.1	92.1	92.1	92.2	92.4	92.8
				94.9	93.5	92.4	92.4	92.3	92.3	92.5	92.5	92.9
				92.7	91.0	89.1	88.8	88.7	88.7	88.9	89.1	89.4
Terumo	*Theratron*	*Oil/Wax*		94.5	93.4	94.0	94.3	94.8	95.4	95.6	95.9	96.7
				93.2	91.6	91.2	91.2	91.0	90.8	90.7	90.9	90.5
				95.2	93.3	92.3	92.4	92.3	92.0	91.5	91.8	91.8
Bbraun	*Bbraun*	*Water*	✔	37.9	38.8	44.3	38.8	38.8	38.8	41.5	38.8	36.0
				39.0	40.4	38.8	40.4	40.1	40.4	40.4	40.4	40.1
				42.9	43.4	43.4	43.4	43.4	43.4	43.4	43.4	43.4
Bbraun	*Bbraun*	*Water*		45.7	45.7	45.7	45.7	45.9	45.9	44.3	45.7	45.7
				40.7	41.8	41.5	41.8	41.5	41.5	41.5	41.5	41.5
				37.7	37.9	37.7	37.9	37.9	37.9	37.7	37.7	37.9
Bbraun	*Bbraun*	*Oil/Wax*	✔	35.2	37.4	37.7	37.9	37.9	38.2	38.2	38.2	38.2
				34.3	34.9	35.2	35.2	32.7	35.4	35.4	35.4	35.4
				33.8	39.6	39.9	39.6	39.9	39.6	39.6	39.9	39.9
Bbraun	*Bbraun*	*Oil/Wax*		38.8	40.4	40.4	40.7	40.7	40.7	40.7	40.7	40.7
				38.2	40.4	40.4	40.4	40.7	40.7	40.7	40.7	40.7
				38.8	38.8	39.0	39.0	39.0	39.0	39.0	39.0	38.8

Appendix B: Data from Chapter 3

B.1 Temperature data from the warm room



B.2 Treatment of raw data

Raw data generated in Sections 3.1.1 and 3.1.2 were volume or height of water, depending on measurement tube used. The height was either read from the scale or, where the scale indicated volume, calculated from the scale reading using a conversion factor (determined by measuring the scale with digital callipers). Pressure due to head of water was calculated by multiplying height of water by 0.098 kPa·cm^{-1}. This was then subtracted from the ambient pressure measured with a weather station (BA116, Oregon Scientific, China), to give the total pressure. Volume was calculated by adding volume within the scale (read from the scale or calculated from height of water) to the known volume above the scale. Temperature was measured in degrees Celsius and converted to Kelvin by adding 273.15. The gas in moles, along with known conditions, was converted to gas volume (mL at 40°C, 1 atm) using the ideal gas law:

$$V_{(40°C,1atm)} = \frac{nR313.15K}{101.325kPa} \cdot 1000$$

B.2.1 50mL burette method

Height of water was measured from the scale at each sampling time together with the conversion, mm·mL^{-1} of scale, as measured with digital callipers (Table B-1).

Table B-1: Measurements of 50-mL burettes

Burette	1	2	3	4	5	6	7	8	9	10	11	12
mm/mL	11.6	11.6	11.0	11.5	11.6	10.3	10.7	11.6	11.3	10.7	10.2	10.3
Volume above scale (mL)	2.5	2.8	2.8	3.5	3.7	4.2	3.5	3.6	4.5	3.4	3.0	3.6

B.2.2 Large cylinder method

Water height at each sampling time was converted to volume with the calibration factor 1.059 cm·mL^{-1}, determined by adding known volumes of water to five tubes with a burette and recording height from the scale (Table B2).

Table B-2: Relationship between scale reading and volume for five measurement tubes

1		2		3		4		5		
mℓ	cm	mℓ	cm	mℓ	cm	mℓ	cm	mℓ	cm	
0.0	73.4	*0.0*	143.2	*0.0*	72.8	*0.0*	143.3	*5.5*	72.8	
4.6	68.7	*8.0*	134.8	*10.5*	61.9	*6.5*	136.7	*15.1*	62.7	
13.6	59.7	*19.1*	123.6	*21.5*	50.2	*14.5*	128.2	*22.2*	55.2	
19.7	52.5	*28.8*	112.9	*29.8*	41.5	*32.2*	109.3	*33.0*	43.3	
33.7	37.5	*38.6*	102.5	*36.1*	34.8	*36.8*	104.6	*40.3*	36.0	
41.6	29.2	*46.6*	94.6	*41.4*	29.2	*42.8*	98.1	*45.8*	30.1	
50.4	20.0	*52.5*	87.9			*47.8*	92.9	*51.9*	23.7	
59.2	10.3	*60.0*	80.0	*54.1*	15.7	*59.2*	80.7	*62.5*	12.4	**Average**
Slope (cm/mℓ)	**1.068**		**1.053**		**1.056**		**1.059**		**1.060**	**1.059**
R squared	**1.000**		**1.000**		**1.000**		**1.000**		**1.000**	

B.2.3 5mL burette method

Water height was calculated from distance above the water level and distance per mL scale on the burette. Total volume was calculated by adding volume from the scale to volume above the scale and subtracting 1 mL (approximate volume of a gas cell with resistor and hot glue). These three values were measured for each burette (Table B3). The resulting gas volumes were subtracted from the first on-scale measurements to give change in gas volume for each sampling time.

Table B-3: Measurements of 5-mL burettes

Burette	1	2	3	4	5	6
Volume above scale (mℓ)	6.7	7.0	7.0	6.0	7.4	6.9
Distance from scale to water level (cm)	5.7	5.0	5.7	5.7	5.7	5.7
cm/mℓ	6.58	6.82	6.83	7.15	6.70	7.38

B.2.4 Gas diffusion

Gas volume above the scale was 70 mL for 60-mL commercial syringes, 26 mL for 20-mL commercial syringes, 31 mL for injection-moulded barrels and 75, 76 or 77 mL for the three glass syringes (depending on how far in each bung could be pushed). The volume within the scale was calculated by dividing the reading by a conversion factor and subtracting the total volume of the scale. The conversion factor of 1.99±0.02 $cm \cdot mL^{-1}$ was obtained by adding known volumes of water to each of the fifty tubes with a burette (Table B4).

The average total change in volume for glass syringes at each time was subtracted from change in volume in every device at that time to compensate for gas that may be entering or leaving by any route other than through the wall of the barrel.

Table B-4: Relationship between scale reading and volume for all measuring tubes

	1		2		3		4		5		6	
	mL	cm	mL	cm	mL	cm	mL	cm	mL	cm	mL	cm
	0.0	45.1	*0.0*	44.5	*0.0*	45.8	*0.0*	44.2	*0.0*	46.6	*0.0*	43.1
	5.0	35.3	*5.0*	34.8	*5.0*	36.1	*5.0*	34.3	*5.0*	36.8	*5.0*	33.2
	7.0	31.4	*7.0*	30.8	*7.0*	32.2	*7.0*	30.3	*7.0*	32.8	*7.0*	29.1
	10.0	25.5	*10.0*	24.6	*10.0*	26.1	*10.0*	24.3	*10.0*	26.9	*10.0*	23.2
	15.0	15.5	*15.0*	14.9	*15.0*	16.1	*15.0*	14.4	*15.0*	17.0	*15.0*	13.2
Slope (cm/mL)		**1.97**		**1.98**		**1.98**		**1.99**		**1.97**		**1.99**
R squared		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**

	7		8		9		10		11		12	
	mL	cm	mL	cm	mL	cm	mL	cm	mL	cm	mL	cm
	15.1	42.0	*14.7*	45.8	*30.1*	44.5	*22.5*	41.4	*20.6*	43.3	*23.4*	40.1
	16.7	39.8	*18.3*	38.2	*33.1*	38.7	*25.3*	35.9	*23.5*	37.7	*26.0*	35.3
	21.4	30.7	*21.8*	31.2	*37.6*	30.1	*28.4*	29.3	*26.5*	31.9	*28.6*	30.1
	23.5	25.1	*25.5*	23.7	*41.8*	21.0	*32.1*	22.5	*29.0*	26.8	*31.5*	24.1
	26.5	19.4	*27.8*	19.1	*44.7*	15.2	*34.2*	18.2	*31.8*	21.4	*33.9*	19.3
	29.2	13.9	*30.1*	14.8	*46.5*	11.7	*36.7*	13.1	*36.5*	12.2	*36.7*	13.9
Slope (cm/mL)		**2.03**		**2.02**		**2.01**		**1.99**		**1.96**		**1.98**
R squared		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**

	13		14		15		16		17		18	
	mL	cm	mL	cm	mL	cm	mL	cm	mL	cm	mL	cm
	16.4	42.9	*30.0*	43.1	*19.4*	39.1	*32.1*	40.0	*29.3*	41.0	*18.5*	38.2
	18.5	38.7	*32.1*	39.3	*21.7*	34.5	*34.5*	35.3	*32.8*	34.2	*20.7*	34.3
	23.1	29.6	*36.2*	31.3	*24.7*	28.6	*36.5*	31.1	*34.2*	31.4	*23.0*	29.4
	24.9	26.0	*40.3*	22.9	*27.8*	22.4	*38.0*	28.1	*38.4*	23.0	*25.7*	24.3
	27.3	21.3	*42.4*	18.8	*29.5*	18.8	*40.9*	22.2	*40.4*	19.1	*27.5*	20.6
	30.0	15.9	*44.1*	15.5	*32.1*	13.8	*43.7*	16.8	*43.9*	12.0	*29.8*	16.0
Slope (cm/mL)		**1.98**		**1.97**		**1.99**		**2.01**		**1.99**		**1.98**
R squared		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**

	19		20		21		22		23		24	
	mL	cm	mL	cm	mL	cm	mL	cm	mL	cm	mL	cm
	29.8	37.6	*22.5*	42.7	*27.8*	43.9	*31.2*	44.8	*22.5*	44.5	*27.9*	41.5
	31.4	34.0	*25.4*	37.2	*30.5*	38.7	*34.4*	38.6	*26.3*	37.1	*31.0*	35.7
	34.2	28.6	*28.4*	31.2	*32.2*	35.7	*37.9*	31.8	*29.4*	31.1	*34.4*	28.6
	36.7	23.6	*31.8*	24.2	*36.1*	27.8	*40.9*	25.7	*32.0*	25.9	*36.9*	23.5
	38.8	19.6	*34.9*	18.4	*38.6*	22.6	*45.0*	17.6	*35.0*	20.0	*39.6*	18.1
	42.8	11.7	*37.1*	14.0	*41.3*	17.4	*48.0*	11.7	*38.1*	13.9	*42.5*	12.3
Slope (cm/mL)		**1.98**		**1.97**		**1.97**		**1.97**		**1.96**		**2.01**
R squared		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**

	25		26		27		28		29		30	
	mL	cm	mL	cm	mL	cm	mL	cm	mL	cm	mL	cm
	29.6	44.4	*27.3*	44.0	*18.7*	45.6	*25.1*	45.0	*25.1*	44.7	*19.9*	41.0
	32.8	38.2	*30.1*	38.6	*21.8*	39.6	*30.0*	35.6	*27.9*	39.4	*23.4*	34.3
	35.5	32.3	*33.0*	33.1	*26.3*	30.9	*33.2*	29.2	*31.6*	31.5	*26.0*	29.1
	38.3	27.0	*35.7*	27.6	*29.4*	24.5	*36.3*	22.8	*34.8*	25.3	*29.8*	21.5
	40.6	22.4	*39.0*	21.2	*31.9*	19.8	*38.1*	19.2	*37.5*	19.8	*31.5*	18.0
	43.4	16.7	*41.8*	15.5	*35.2*	13.3	*41.2*	13.2	*39.7*	15.6	*33.9*	13.2
Slope (cm/mL)		**2.01**		**1.96**		**1.96**		**1.99**		**2.01**		**1.99**
R squared		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**

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	31		**32**		**33**		**34**		**35**		**36**	
	mL	**cm**	**mL**	**cm**	**mL**	**cm**	**mL**	**cm**	**mL**	**cm**	**mL**	**cm**
	17.7	41.0	*23.7*	40.3	*28.3*	43.0	*31.6*	43.8	*23.5*	41.4	*32.3*	41.2
	21.1	34.6	*26.8*	34.4	*31.1*	37.8	*34.4*	38.7	*26.6*	35.5	*35.7*	34.5
	25.7	25.4	*29.4*	30.0	*34.4*	31.6	*37.7*	32.1	*29.5*	30.0	*38.5*	29.0
	28.6	19.7	*33.0*	21.8	*36.7*	26.9	*39.9*	29.6	*33.8*	21.2	*41.8*	22.2
	30.7	15.6	*35.8*	16.0	*39.4*	21.7	*44.6*	18.4	*36.5*	15.9	*44.2*	17.4
	33.5	10.2	*38.1*	11.4	*43.0*	14.7	*47.7*	12.4	*39.0*	11.0	*47.2*	11.4
Slope (cm/mL)		**1.96**		**2.03**		**1.93**		**1.96**		**1.97**		**2.00**
R squared		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**

	37		**38**		**39**		**40**		**41**		**42**	
	mL	**cm**	**mL**	**cm**	**mL**	**cm**	**mL**	**cm**	**mL**	**cm**	**mL**	**cm**
	20.9	39.5	*26.5*	39.5	*23.8*	41.3	*24.1*	43.6	*21.2*	41.5	*28.1*	41.5
	25.2	31.1	*29.7*	33.3	*27.3*	35.8	*27.8*	36.5	*24.7*	35.1	*31.0*	35.9
	26.6	28.1	*33.4*	26.0	*30.2*	29.8	*31.6*	29.0	*28.2*	28.0	*33.5*	31.0
	29.3	22.6	*35.7*	21.5	*33.4*	23.2	*34.0*	24.1	*30.6*	23.2	*37.0*	23.9
	31.3	18.5	*38.2*	16.4	*36.2*	17.8	*36.8*	18.6	*33.1*	18.2	*39.0*	19.9
	34.4	12.3	*41.0*	10.9	*38.3*	13.7	*39.6*	13.2	*35.9*	12.6	*41.9*	14.2
Slope (cm/mL)		**2.02**		**1.98**		**1.94**		**1.97**		**1.98**		**1.99**
R squared		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**

	43		**44**		**45**		**46**		**47**		**48**	
	mL	**cm**	**mL**	**cm**	**mL**	**cm**	**mL**	**cm**	**mL**	**cm**	**mL**	**cm**
	23.8	40.5	*31.3*	37.4	*29.1*	43.6	*24.3*	43.0	*23.0*	42.6	*24.9*	44.4
	27.1	34.3	*34.0*	32.1	*33.0*	36.1	*28.0*	35.5	*25.7*	37.4	*28.4*	37.4
	29.7	28.6	*36.0*	28.1	*35.7*	30.6	*31.8*	27.7	*28.0*	32.7	*31.8*	30.7
	32.7	21.8	*37.7*	24.5	*38.3*	25.4	*35.2*	20.9	*31.5*	25.6	*34.8*	24.7
	34.1	18.4	*40.2*	19.6	*41.3*	19.5	*37.4*	16.4	*35.5*	17.6	*37.6*	19.2
	37.9	12.4	*43.4*	13.2	*44.6*	13.0	*40.2*	10.9	*38.8*	10.9	*40.9*	12.6
Slope (cm/mL)		**2.06**		**2.01**		**1.98**		**2.02**		**2.01**		**1.99**
R squared		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**		**1.00**

	49		**50**			
	mL	**cm**	**mL**	**cm**		
	26.7	43.5	*27.7*	42.8		
	30.3	36.8	*31.2*	36.3		
	33.4	30.5	*34.4*	29.8		
	35.3	26.7	*37.3*	23.9		
	39.0	19.4	*40.7*	17.0		
	42.6	12.3	*43.2*	12.1	**Average**	**Standard deviation**
Slope (cm/mL)		**1.97**		**1.99**	**1.99**	**0.02**
R squared		**1.00**		**1.00**		

B.3 Final data for Chapter 3 in mL (40°C, 1atm)

B.3.1 50-mL burette method

B.3.1.1 0Ω

Time (H:M)	Burette 1	2	3
0:00:00	0.0	0.0	0.0
0:28:00	4.8	4.8	5.0
1:21:00	13.6	13.5	14.7
2:09:00	21.7	21.4	22.2
2:29:00	25.8	25.5	27.8
2:59:00	30.1	29.6	32.1
3:29:00	34.9	34.4	37.2
4:24:00	42.9	42.3	45.8

B.3.1.2 27Ω

Time (H:M)	Burette 4	5	6
0:00:00	0.0	0.0	0.0
0:28:00	0.9	0.9	1.1
1:21:00	3.9	3.5	4.1
2:09:00	6.7	5.9	6.8
2:29:00	8.1	7.2	8.2
2:59:00	9.5	8.4	9.6
3:29:00	11.1	9.8	11.3
4:24:00	14.0	12.5	14.0
9:21:00	28.8	25.8	27.9
21:11:00	61.5	57.7	64.8
21:56:00	63.7	59.8	66.9
22:26:00	65.1	61.5	68.5
23:23:00	68.4	64.3	71.5
24:04:00	70.8	66.5	73.8
25:09:00	74.3	69.7	77.2
26:05:00	77.2	72.5	80.2
26:22:00	78.1	73.2	81.1

B.3.1.3 56Ω

Time (H:M)	Burette 7	8	9
0:00:00	0.0	0.0	0.0
0:28:00	0.2	0.2	0.0
1:21:00	2.0	2.0	0.2
2:09:00	3.7	3.7	3.0
2:29:00	4.6	4.5	3.9
2:59:00	5.5	5.4	4.7
3:29:00	6.5	6.3	5.7
4:24:00	8.2	8.0	6.0
9:21:00	16.4	16.0	16.7
21:11:00	39.0	38.7	39.7
21:56:00	40.5	40.0	41.0
22:26:00	41.4	41.0	42.0
23:23:00	43.3	42.8	43.8
24:04:00	44.8	44.2	45.4
25:09:00	46.8	46.4	47.5
26:05:00	48.7	48.2	49.3
26:22:00	49.1	48.7	49.8

B.3.1.4 150Ω

Time (days)	Burette 1	2	3
0.00	0.0	0.0	0.0
0.19	3.2	4.0	3.7
0.85	15.4	15.5	17.5
1.01	18.6	18.6	21.2
1.11	20.5	20.6	23.5
2.09	38.5	38.4	43.9
3.24	58.8	59.3	67.4
3.87	70.6	70.8	81.2
4.87	88.9	88.6	102.4
4.99	91.2	91.1	105.0
5.86	107.0	106.0	
5.95	108.6	107.4	
6.18	112.7	111.2	
6.91	124.2	122.4	
7.00	125.5	122.7	
7.90	127.1	128.5	

B.3.1.5 220Ω

Time (days)	Burette 4	5	6
0.00	0.0	0.0	0.0
0.19	2.1	2.5	1.1
0.85	11.4	12.3	3.6
1.01	13.8	14.8	4.3
1.11	15.2	16.4	4.7
2.09	29.6	31.6	9.1
3.24	46.1	48.7	14.3
3.87	55.4	58.5	17.4
4.87	70.3	74.2	22.4
4.99	72.2	76.3	23.2
5.86	85.0	88.5	27.8
5.95	86.5	91.1	28.3
6.18	89.9	94.8	29.6
6.91	102.0	106.4	33.5
7.00	102.6	107.0	34.0
7.90	115.7	121.8	38.8

B.3.1.6 390Ω

Time (days)	Burette 7	8	9
0.00	0.0	0.0	0.0
0.19	1.2	1.3	1.3
0.85	7.1	7.2	7.1
1.01	8.7	8.8	8.6
1.11	9.7	10.8	9.2
2.09	18.6	18.9	18.6
3.24	29.0	29.5	29.1
3.87	34.8	35.5	34.9
4.87	44.3	45.0	44.3
4.99	45.4	46.3	45.7
5.86	53.6	53.5	53.8
5.95	54.5	55.5	54.8
6.18	56.6	57.8	57.0
6.91	63.6	64.9	64.0
7.00	64.7	65.9	65.1
7.90	73.0	74.5	73.4

B.3.1.7 470Ω

Time (days)	Burette 10	11	12
0.00	0.0	0.0	0.0
0.19	0.2	0.9	0.9
0.85	5.4	5.4	5.7
1.01	6.6	6.9	6.5
1.11	7.5	7.3	7.7
2.09	15.3	14.2	15.0
3.24	24.1	21.5	23.5
3.87	29.0	26.1	28.4
4.87	37.1	33.6	36.1
4.99	38.2	34.6	37.2
5.86	45.1	41.0	43.8
5.95	46.0	41.8	44.6
6.18	47.9	41.8	46.4
6.91	84.9	41.8	52.0
7.00	95.6	41.8	52.9
7.90		48.6	59.9

B.3.1.8 560Ω

Time (days)	Burette 1	2	3
0.00	0.0	0.0	0.0
0.71	4.1	2.3	2.8
0.91	5.5	3.2	4.2
1.68	10.1	5.9	8.9
1.88	11.6	6.8	10.4
2.84	17.7	10.4	16.4
3.86	24.7	11.0	23.3
4.70	29.4	11.1	29.1
4.87	31.6	11.2	30.4
5.71	36.5	11.0	35.5
5.94	38.1	11.1	36.9
6.69	42.2	10.8	41.2
6.79	43.2	10.9	42.2
7.69	48.2	10.8	47.5
7.91	49.8	10.9	49.2
8.70	52.6	10.8	53.9
9.73	56.0	11.0	61.8
10.77	58.4	11.3	69.9
11.68	59.3	10.9	75.0
12.66	60.4	11.3	81.8

B.3.1.9 750Ω

	Burette		
Time (days)	**4**	**5**	**6**
0.00	0.0	0.0	0.0
0.71	2.6	2.8	2.9
0.91	3.9	3.8	3.6
1.68	7.4	7.5	7.2
1.88	8.3	8.5	8.3
2.84	12.8	12.7	12.7
3.86	17.7	17.7	17.9
4.70	22.0	21.8	22.2
4.87	23.0	22.6	23.3
5.71	26.7	26.4	26.8
5.94	27.8	26.7	28.1
6.69	30.8	30.5	31.1
6.79	31.6	31.4	31.7
7.69	35.5	35.4	35.6
7.91	36.9	36.7	36.9
8.70	40.3	40.2	40.2
9.73	46.3	46.1	45.7
10.77	52.0	51.9	45.8
11.68	55.5	55.4	49.7
12.66	59.5	59.3	53.1
13.68	66.0	65.7	53.4

B.3.1.10 910Ω

	Burette		
Time (days)	**7**	**8**	**9**
0.00	0.0	0.0	0.0
0.71	2.2	2.2	2.2
0.91	3.0	3.1	3.1
1.68	5.9	5.9	6.0
1.88	6.8	6.9	6.9
2.84	10.4	10.4	10.4
3.86	14.5	14.5	14.8
4.70	18.1	18.2	18.2
4.87	18.7	18.8	18.9
5.71	21.6	22.0	21.9
5.94	22.5	23.1	22.9
6.69	25.0	25.6	25.5
6.79	25.7	26.2	26.1
7.69	28.9	29.6	29.4
7.91	30.0	30.6	30.6
8.70	32.7	33.3	33.4
9.73	39.5	40.5	40.6
10.77	44.0	45.3	45.4
11.68	43.7	45.3	45.3
12.66	47.6	49.4	49.3

B.3.1.11 1200Ω

	Burette		
Time (days)	**10**	**11**	**12**
0.00	0.0	0.0	0.0
0.71	1.3	1.7	1.6
0.91	1.4	2.3	2.2
1.68	1.6	4.7	3.7
1.88	2.2	5.4	5.0
2.84		8.2	7.9
3.86		11.3	10.8
4.70		14.1	13.5
4.87		14.8	14.1
5.71		17.2	16.3
5.94		18.0	17.0
6.69		20.0	18.9
6.79		20.5	19.6
7.69		23.2	22.0
7.91		24.0	22.9
8.70		26.3	25.0
9.73		55.3	30.4
10.77			34.2
11.68			34.0
12.66			37.1

B.3.1.12 1800Ω

	Burette		
Time (days)	**1**	**2**	**3**
0.00	0.0	0.0	0.0
0.72	0.9	0.0	0.9
1.83	3.0	0.0	2.9
2.79	4.6	1.0	4.5
3.89	6.7	3.2	6.5
4.71	8.2	4.6	8.1
5.75	10.2	6.6	10.0
6.78	12.2	8.4	12.0
7.81	14.4	10.4	14.1
8.92	16.4	12.3	16.0
11.73	21.7	15.9	21.4
13.77	25.5	20.5	25.3
14.85	27.8	21.4	27.6
15.74	29.8	21.4	29.7
18.78	36.0	21.5	36.2
19.72	37.7	21.5	37.5
20.80	40.0	21.4	39.7
21.74	42.4	21.7	41.8
22.77	43.9		43.6
24.76			47.2
25.75			48.0
26.89			52.0

B.3.1.13 2200Ω

Time (days)	Burette 7	8	9
0.00	0.0	50.0	50.0
0.72	0.3	49.8	49.6
1.83	1.9	48.0	47.8
2.79	3.3	46.7	46.6
3.89	5.0	44.9	45.0
4.71	6.2	43.6	43.8
5.75	8.3	42.0	42.3
6.78	9.5	40.4	40.9
7.81	10.7	38.8	39.4
8.92	12.3	37.2	37.9
11.73	16.5	32.8	34.0
13.77	19.9		31.1
14.85	21.9		28.9
15.74	23.5		27.9
18.78	28.6		23.1
19.72	30.1		21.8
20.80	31.9		19.8
21.74	33.8		17.9
22.77	35.1		16.9
24.76	38.0		13.9
25.75	39.5		12.6
26.89	42.2		10.4
39.92	64.8		29.4
43.84	71.7		23.0
46.74	76.5		19.0
47.81	77.8		17.5
53.98	88.6		7.8

B.3.1.14 3300Ω

Time (days)	Burette 10	11	12
0.00	0.0	0.0	0.0
0.72	0.3	0.2	0.2
1.83	1.5	1.3	1.5
2.79	2.3	1.8	2.3
3.89	4.1	1.8	3.1
4.71	4.2	2.1	3.6
5.75	5.1	2.8	3.9
6.78	6.1	2.9	4.4
7.81	7.0	3.5	5.1
8.92	7.9	4.3	6.0
11.73	10.4	6.1	8.5
13.77	12.3	8.7	10.6
14.85	13.7	10.5	10.8
15.74	14.7	11.9	11.4
18.78	17.7	16.5	14.3
19.72	18.6	18.6	15.3
20.80	19.7	30.1	15.6
21.74	20.9		15.7
22.77	21.7		15.5
24.76	23.4		16.2
25.75	24.3		16.9
26.89	26.1		17.8

B.3.1.15 0Ω 40°C

Time (hours)	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**
0:00:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0:20:00	6.4	6.5	0.0	6.2	6.6	5.7	5.6	5.2	0.0
0:40:00	12.1	12.7	0.0	12.1	12.9	11.7	11.7	10.6	0.0
1:00:00	19.6	20.3	0.0	20.8	20.4	19.3	19.2	17.4	0.0
1:20:00	25.4	26.1	0.0	25.9	26.5	25.4	25.7	22.9	0.0
1:40:00	30.9	31.9	0.0	31.7	32.5	31.5	31.9	28.2	0.0
2:00:00	37.2	38.3		37.9	39.1	37.9	38.5	34.1	0.0
2:20:00	43.1	44.2		44.1	45.5		45.4	39.7	0.0
2:40:00	49.3	50.3		49.9	51.1		50.9	44.6	
3:00:00	55.2	56.2		55.9	57.2		57.6	50.4	
3:20:00	61.0	61.7		61.6	62.9		63.8	55.7	
3:40:00	66.4	67.0		66.9	68.4		69.6	60.7	
4:00:00	71.5	72.1		72.0	73.4		74.8	65.6	
4:20:00	76.4	76.6		76.8	78.0		79.9	70.2	
4:40:00	81.3	81.3		81.7	82.7		84.8	74.8	
5:00:00	86.4	85.7		86.7	87.2		89.9	80.0	
5:20:00	91.1	90.1		91.4	91.4		94.3	84.5	
5:40:00	94.7	93.7		95.1	94.6		98.1	89.6	
6:00:00	98.6	97.3		98.7	97.8		101.9	92.8	
6:20:00	101.8	100.0		101.9	100.6		105.0	96.4	
6:40:00	105.1	102.9		104.5	103.2		107.9	100.1	
7:00:00	108.5	105.7		106.9	105.9		110.4	103.8	
7:20:00	110.6	107.4		108.1	107.2		111.8	106.1	
7:40:00	112.7	109.1		109.8	108.9		112.8	108.8	
8:00:00	115.0	110.1		111.0	110.3		113.2	111.3	
8:20:00	116.4	110.5		111.7	110.7		113.7	113.0	
8:40:00	116.9	110.5		113.4	110.7		113.7	114.1	
9:00:00	117.4	110.9		114.1	111.2		114.2	115.4	
9:20:00	117.5	111.1		114.4	111.5		114.4	115.8	
9:40:00	117.6	111.5		114.7	112.0		114.8	116.0	
10:00:00	117.6	111.8		115.0	112.3		114.8	116.3	
10:20:00	117.5	112.1		115.3	112.6		115.4	116.4	
10:40:00	117.9	112.6		116.8	113.1		115.9	116.8	
11:00:00	118.6	113.2		116.6	113.8		116.5	117.6	
11:20:00	118.1	113.2		116.5	113.8		116.5	117.2	
11:40:00	118.3	113.7		116.9	114.3		117.0	117.7	
12:00:00	118.8	114.3		117.4	114.9		118.5	118.1	
25:34:00	129.9	129.3		126.1	130.0		131.3	130.9	
26:50:00	130.8	130.4		126.5	131.1		132.3	131.8	
28:04:00	131.1	131.3		126.8	132.2		133.3	132.9	
29:12:00	131.9	132.2		127.0	133.2		134.0	133.8	
30:12:00	132.1	132.9		127.2	134.0		134.7	134.4	
31:51:00	132.0	133.9		127.2	135.2		135.7	135.4	
33:00:00	133.1	135.2		127.5	136.5		136.9	135.6	
34:09:00	133.0	137.1		127.4	137.8		138.9	138.7	
49:44:00	133.0	143.5		127.3	146.4		145.8	146.8	
52:54:00	133.0	143.9		127.4	148.2		147.4	147.8	
74:14:00	135.4	151.8		132.0	155.9		154.6	157.7	
129:29:00	137.6	161.1		142.0	164.5		164.3	165.2	
145:47:00	137.6	162.3		143.7	165.6		165.4	165.2	
153:35:00	137.6	163.0		144.5	166.2		165.9	165.2	
170:01:00	137.6	163.5		145.6	167.0		166.6	165.3	
177:32:00	137.6	163.7		145.9	167.2		166.8	165.3	
194:01:00	137.6	163.9		146.6	167.7		167.0	165.3	
201:08:00				146.8	167.8		167.1		
218:06:00				146.9	167.9		167.1		

B.3.2 Large cylinder method

B.3.2.1 270Ω

Time (days)	1	2	3	4	5
0.0	0.0	0.0	0.0	0.0	0.0
0.2	3.1	2.8	2.3	2.9	2.8
1.0	13.5	8.8	2.5	5.5	12.9
2.0	28.8	10.3	25.0	6.2	28.2
3.1	44.2	10.5	40.2	6.3	43.9
4.1	58.8	11.1	54.8	6.4	60.0
5.0	72.3	11.9	68.5	8.6	73.7
6.0	87.8	19.5	77.2	14.0	89.8
7.0	102.7	14.2	88.4	14.9	106.1
8.0	117.3	19.7	101.6	15.1	121.9
9.1	132.4	35.8	116.7	31.3	139.0
10.1	145.2	51.5	130.3	45.9	154.0
11.0	155.6	66.0	143.0	56.9	167.4
12.0	156.7	80.9	156.3	56.9	171.5
13.0	156.4	91.7	164.6	71.6	171.6
14.0	156.5	93.2	164.7	87.0	171.6
15.0	156.4	93.5	165.0	101.3	171.6
15.2	156.6	93.9	165.1	105.2	171.6
17.0	156.4	94.4	165.1	130.6	171.7
18.0	156.1	95.3	165.0	144.8	171.6
19.0	156.5	96.8	165.1	158.2	171.6
20.0	156.2	98.5	165.0	169.7	171.6
21.0	156.2	100.8	165.0	169.9	171.6
22.0	155.7	102.8	164.8	170.0	171.6
23.0	156.0	105.8	164.9	170.2	171.6
24.0	155.8	108.2	164.8	170.2	171.6
25.0	155.5	108.4	164.7	170.1	171.6
26.1	155.7		164.7	170.2	171.6
27.0	155.4		164.7	170.1	171.5
28.0	155.6		164.7	170.1	171.5
29.0	155.4		164.6	170.1	171.5
32.0	155.0		164.5	169.9	171.4
34.0	154.9		164.4	169.7	171.4
36.2	154.8		164.3	169.8	171.4
39.2	154.9		164.3	169.6	171.5
42.0	154.6		164.2	169.5	171.5
47.0	154.0		163.8	169.1	171.4
53.0	153.8		163.6	168.8	171.4
56.1	153.3		163.5	168.5	171.3
63.0	152.9		163.2	168.2	171.2
69.0	152.6		162.9	167.9	171.4
75.1	152.1		162.6	167.5	171.2

B.3.2.2 330Ω

Time (days)	1	2	3	4	5
0.0	0.0	0.0	0.0	0.0	0.0
0.4	1.9	4.4	4.5	3.8	4.5
0.9	3.9	9.5	9.8	6.8	9.9
2.0	6.7	21.0	22.0	14.3	21.7
3.9	15.0	44.6	45.9	26.1	45.9
4.9	20.4	56.1	56.6	28.2	57.5
6.0	25.5	67.7	68.1	31.7	69.7
6.9	30.6	79.6	80.1	34.4	82.1
7.9	31.4	91.8	90.6	38.7	95.2
9.0	34.0	103.9	94.8	43.5	108.4
9.1	34.3	104.6	95.7	44.3	109.5
12.1	47.9	137.8	130.5	78.8	145.9
13.2	54.5	146.2	143.0	90.9	157.9
14.0	59.2	147.1	145.8		158.3
15.0	59.9	151.9	145.5	91.2	158.2
15.9	74.2	147.2	145.9	92.0	158.3

B.3.2.3 390Ω

Time (days)	1	2	3	4	5
0.0	0.0	0.0	0.0	0.0	0.0
0.4	1.0	3.9	3.6	3.8	3.4
0.9	2.5	7.8	9.9	8.0	7.9
2.0	2.7	14.3	17.5	17.7	17.5
3.9	5.6	25.2	37.2	37.5	37.5
4.9	8.2	32.4	46.2	46.8	47.2
6.0	8.7	41.1	55.7	55.7	57.0
6.9	9.8	48.4	64.4	64.1	66.9
7.9	11.5	58.7	73.4	72.9	77.1
9.0	14.0	69.3	82.8	81.7	87.1
9.1	14.4	70.7	83.9	82.9	88.1
12.1	26.1	101.6	109.0	111.0	107.6
13.2	26.3	113.1	119.8	121.6	110.5
14.0	26.2	120.2	128.0	129.2	112.1
15.0	26.1	127.8	136.9	137.6	114.2
15.9	31.0	135.4	144.3	145.4	116.4
16.9	31.7	144.4	147.6	151.3	116.6
19.0	31.5	150.8	147.3	151.0	116.6
21.2	31.4	150.5	147.0	150.6	116.2
21.9	31.2	150.3	146.7	150.3	116.0
23.0	31.3	150.2	146.6	150.3	116.4
26.1	31.2	150.2	146.3	149.9	116.0
28.0	31.4	150.0	146.1	149.6	115.9
30.0	30.8	149.4	145.8	149.3	115.4

B.3.2.4 470Ω

Time (days)	1	2	3	4	5
0.0	0.0	0.0	0.0	0.0	0.0
0.4	2.8	0.8	3.7	2.2	1.5
0.9	6.7	3.5	6.5	4.8	3.6
2.0	14.5	11.2	14.6	12.8	7.8
3.9	30.4	27.4	29.7	29.5	14.9
4.9	37.9	35.3	38.3	37.4	18.4
6.0	46.6	43.9	47.1	46.5	20.7
6.9	54.4	51.4	55.0	54.4	23.0
7.9	54.5	58.7	63.6	63.0	25.2
9.0	54.6	67.2	72.3	72.2	28.0
9.1	54.6	67.9	73.3	73.1	28.2
12.1	59.4	93.0	98.2	99.0	40.0
13.2	62.1	102.2	107.0	108.3	40.5
14.0	68.9	105.8	107.1	112.9	40.3
15.0	77.1	110.2	107.1	120.9	40.4
15.9	85.8	114.8	107.6	128.8	40.6
16.9		120.8	112.0	136.9	40.7
19.0		129.9	122.4	147.7	40.7
21.2		132.1	127.2	149.6	41.3
21.9		132.8	127.0	149.7	41.2
23.0		132.9	126.9	149.6	40.3
26.1		132.8	126.8	149.6	41.2
28.0		132.6	126.7	149.4	41.1
30.0		132.2	126.3	149.8	40.8

B.3.2.5 560Ω

Time (days)	**1**	**2**	**3**	**4**	**5**
0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.9	0.8	1.0	0.5	1.3
1.0	3.7	5.4	5.6	5.6	6.1
2.0	3.9	12.2	12.2	12.4	12.0
3.1	4.1	19.4	19.2	19.6	20.4
4.1	3.7	27.0	26.1	26.2	27.3
5.0	4.6	33.1	32.7	32.8	33.9
6.0	5.0	39.2	40.0	39.7	41.2
7.0	5.1	47.3	47.3	46.9	48.6
8.0	5.1	54.5	54.5	53.7	55.7
9.1	5.6	62.0	62.0	61.0	63.2
10.1	6.0	69.1	69.0	67.7	70.1
11.0	12.9	75.7	75.5	74.2	76.9
12.0	13.1	82.4	82.6	81.0	84.1
13.0	13.5	89.7	86.7	87.9	91.3
14.0	13.6	96.9	93.3	94.7	98.5
15.0	13.6	104.1	101.1	101.4	105.6
15.2	13.6	106.1	103.0	103.3	107.8
17.0	13.7	117.8	116.4	114.7	120.2
18.0	13.5	125.1	124.3	121.2	127.3
19.0	13.6	131.6	132.1	127.6	134.4
20.0	13.5	137.8	139.7	133.8	141.1
21.0	13.5	147.9	147.8	140.0	148.1
22.0	13.4	150.5	154.9	145.7	153.8
23.0	13.7	157.5	162.0	150.1	154.7
24.0	13.6	163.1	162.5	150.2	154.4
25.0	13.4		162.8	149.8	154.0
26.1	13.4		162.8	150.1	154.2
27.0	13.4		162.8	150.0	154.1
28.0	13.4		162.8	150.3	154.3
29.0	13.4		162.7	149.9	154.1
32.0	13.3		162.7	149.8	153.8
34.0	13.2		162.5	149.9	153.7
36.2	13.2		162.5	149.8	153.8
39.2	13.6		162.4	149.9	153.8
42.0	13.2		162.4	149.9	153.8
47.0	14.2		162.1	149.3	153.0
53.0	12.7		162.3	149.2	153.0
56.1	12.6		161.8	149.2	153.0
63.0	12.5		161.6	148.8	152.6
69.0	12.2		161.4	148.3	152.0
75.1	11.9		161.1	147.8	151.6

B.3.2.6 820Ω

Time (days)	**1**	**2**	**3**	**4**	**5**
0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.3	0.5	0.7	1.6	0.6
1.0	2.8	3.8	4.0	3.9	3.8
2.0	4.9	8.4	8.6	2.8	8.6
3.1	9.7	13.2	13.4	8.3	11.5
4.1	13.9	17.9	18.2	8.8	18.1
5.0	18.0	22.4	22.6	9.3	22.6
6.0	22.3	27.2	27.6	9.6	27.4
7.0	26.7	32.1	32.4	10.3	32.4
8.0	30.9	37.0	37.2	13.3	37.1
9.1	35.6	42.1	42.5	18.7	37.3
10.1	39.8	46.3	47.3	23.6	37.4
11.0	43.7	51.8	52.3	28.4	37.2
12.0	48.1	56.2	56.9	33.6	41.8
13.0	52.3	60.6	61.8	39.0	46.2
14.0	55.3	65.5	66.9	44.5	50.7
15.0	56.6	70.1	72.1	50.1	55.2
15.2	57.0	71.5	73.6	51.8	56.5
17.0	59.6	79.5	82.3	61.2	64.3
18.0	61.0	84.1	87.4	66.6	68.5
19.0	63.1	88.6	92.7	72.1	73.0
20.0	64.4	93.2	97.6	77.5	77.6
21.0	66.6	98.0	102.9	83.3	82.2
22.0	67.8	102.3	107.6	88.6	86.2
23.0	69.2	106.7	112.9	94.1	88.8
24.0	70.6	110.0	118.0	99.9	89.3
25.0	72.3	113.6	123.3	105.2	103.8
26.1	75.1	117.6	128.2	111.3	108.6
27.0	77.1	121.3	132.6	116.0	113.0
28.0	81.4	125.9	137.9	121.8	118.3
29.0	85.8	129.8	142.4	126.9	122.1
32.0	93.0	141.4	150.2	142.1	135.6
34.0	93.0	146.8	150.2	143.4	138.7
36.2	93.1	146.9	150.2	143.4	138.8
39.2	93.1	147.1	150.3	143.4	138.9
42.0	93.1	147.0	150.3	143.5	138.8
47.0	92.6	146.4	149.5	143.5	138.3
53.0	92.5	146.4	149.5	143.4	138.2
56.1	92.4	146.3	149.5	143.4	138.3
63.0	92.3	146.2	149.1	143.4	137.9
69.0	92.1	145.9	148.8	143.4	137.5
75.1	91.4	145.5	148.5	143.4	137.3

B.3.2.7 1200Ω

Time (days)	**1**	**2**	**3**	**4**	**5**
0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.2	0.1	-0.5	0.3	0.5
1.0	2.4	2.2	1.6	2.3	2.6
2.0	5.5	4.9	4.5	5.2	5.5
3.1	8.5	7.8	7.6	8.3	8.6
4.1	11.5	11.2	10.5	11.1	11.5
5.0	14.3	13.6	13.4	14.3	14.2
6.0	17.5	24.8	16.3	17.2	17.2
7.0	20.6	44.2	19.6	20.3	20.5
8.0	23.7		22.5	23.0	23.5
9.1	27.0		26.0	26.4	27.5
10.1	30.1		30.8	29.3	30.9
11.0	33.2		31.8	32.1	34.3
12.0	36.5		34.9	35.3	37.8
13.0	39.8		37.8	38.2	41.5
14.0	43.1		41.0	41.1	44.9
15.0	46.7		43.6	44.1	48.6
15.2	47.9		45.2	45.4	49.8
17.0	53.5		50.3	50.5	55.5
18.0	57.1		53.4	53.5	60.5
19.0	60.4		56.3	56.3	63.3
20.0	63.8		59.0	59.3	66.8
21.0	67.3		62.1	62.3	70.1
22.0	70.9		65.4	65.4	73.7
23.0	74.6		68.7	68.7	77.7
24.0	77.9		71.7	71.7	81.0
25.0	81.2		74.8	74.5	84.3
26.1	85.1		78.5	77.9	88.4
27.0	88.2		81.4	80.7	91.3
28.0	91.9		84.9	84.0	95.1
29.0	94.7		87.8	86.8	98.5
32.0	105.2		97.1	95.5	108.4
34.0	111.5		103.1	103.4	115.3
36.2	118.0			108.0	122.8
39.2	128.0			116.7	133.0
42.0	137.0			124.5	140.8
47.0	146.4			133.5	143.0
53.0	146.4			133.5	142.7
56.1	146.1			133.5	142.6
63.0	145.9			133.0	142.3
69.0	145.7			132.9	142.0
75.1	145.3			132.6	141.8

B.3.2.8 1500Ω

Time (days)	**1**	**2**	**3**	**4**	**5**
0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.2	0.3	0.0	0.3	0.0
1.0	1.7	2.0	1.4	1.8	1.9
2.0	3.9	4.1	3.2	3.9	4.3
3.1	6.2	6.1	4.0	6.0	6.6
4.1	8.4	8.2	4.2	7.9	9.2
5.0	10.4	10.6	4.8	9.6	11.2
6.0	12.8	12.2	5.5	11.9	13.9
7.0	15.1	14.1	5.5	14.0	16.2
8.0	17.3	15.9	6.1	16.0	18.5
9.1	22.7	18.0	8.2	18.3	21.2
10.1	22.2	19.9	10.3	20.3	23.5
11.0	24.3	21.8	12.5	22.2	25.9
12.0	26.5	23.7	14.6	24.1	28.6
13.0	28.9	25.8	16.9	26.1	31.1
14.0	31.2	27.9	19.1	28.1	33.7
15.0	33.5	30.0	21.5	30.6	36.0
15.2	34.3	30.7	22.1	31.3	36.9
17.0	38.3	34.4	26.2	35.2	41.2
18.0	40.4	36.6	28.3	37.2	43.3
19.0	42.8	39.1	30.5	39.5	45.9
20.0	45.0	42.1	33.1	41.6	48.4
21.0	47.3	44.0	35.7	43.9	51.0
22.0	49.4	46.4	38.1	45.8	53.6
23.0	52.4	49.6	41.0	48.8	57.1
24.0	54.4	51.8	43.7	51.0	59.5
25.0	56.2	53.8	45.9	53.0	61.8
26.1	61.9	56.7	48.8	55.7	64.6
27.0	63.8	58.5	50.8	57.4	67.1
28.0	66.3	59.5	53.6	59.8	69.9
29.0	68.5	63.6	55.9	62.2	72.7
32.0	75.2	71.5	62.1	69.3	81.7
34.0	79.8	76.7	66.7	74.6	87.8
36.2	85.0	82.5	72.3	79.7	94.8
39.2	91.8	90.6	80.0	87.1	104.3
42.0	96.8	98.5	86.9	93.5	112.3
47.0	109.0	112.4	98.4	105.4	126.8
53.0	120.6	127.9	114.0		138.7
56.1	125.5	136.3	121.6		138.7
63.0	125.4	136.4	131.3		138.4
69.0	125.3	136.1	131.2		137.9
75.1	125.1	136.0	131.0		137.6
82.0	124.9	135.6	130.7		137.3
88.2	124.5	135.5	130.5		137.4
97.0	124.6	135.2	130.1		136.7
102.0	124.5	135.3	130.0		136.6
110.2	124.2	134.6	129.5		135.9
119.0	124.2	134.4	129.4		135.4
123.1	124.1	134.4	129.2		
132.1	123.8	134.2	129.0		
140.2	123.8	133.7	128.7		
151.0	123.5	133.3	128.3		
167.1	123.2	132.3	127.7		
197.0	123.3	130.6	129.7		

B.3.2.9 2200Ω

Time (days)	1	2	3	4	5
0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.0	0.1	-0.1	0.1	0.1
1.0	1.0	1.1	0.7	1.2	0.7
2.0	2.5	2.4	2.2	2.4	2.3
3.1	3.9	3.8	3.6	3.8	4.0
4.1	5.3	5.2	5.1	5.2	5.5
5.0	6.4	6.2	6.5	6.2	6.8
6.0	7.9	7.4	7.9	7.6	8.2
7.0	9.4	9.1	9.6	9.0	9.5
8.0	10.0	11.6	10.9	10.3	10.9
9.1	10.3	18.8	12.8	12.0	12.6
10.1	11.6	34.3	14.5	13.2	14.0
11.0	13.4	58.1	16.2	14.4	15.3
12.0	15.3		17.6	16.0	16.6
13.0	17.2		19.6	17.2	18.1
14.0	19.2		21.1	18.6	19.5
15.0	21.3		23.0	20.0	20.9
15.2	21.8		23.5	20.1	21.4
17.0	25.5		26.4	22.5	21.3
18.0	27.6		28.1	23.9	22.2
19.0	29.5		29.5	25.3	24.6
20.0	31.5		30.8	26.9	26.6
21.0	33.9		32.2	28.5	29.0
22.0	36.0		33.9	30.0	31.0
23.0	37.8		35.9	32.0	33.2
24.0	40.7		37.6	33.8	35.3
25.0	42.1		39.3	35.5	37.0
26.1	44.4		40.8	37.6	39.1
27.0	45.8		42.3	38.9	40.7
28.0	48.1		43.9	41.3	43.1
29.0	49.7		45.3	43.2	44.9
32.0	54.5		47.6	48.8	50.4
34.0	58.1		51.3	52.4	51.5
36.2			55.0	56.5	55.2
39.2			59.1	61.2	55.3
42.0			64.5	66.4	55.2
47.0			67.9	73.6	55.4
53.0			67.7	81.6	55.3
56.1			67.9	82.0	55.2
63.0			78.0	81.8	55.3
69.0			86.8	89.9	55.2
75.1			96.2	89.8	55.2
82.0			106.5	89.6	55.2
88.2			113.5	89.6	
97.0			116.6	85.1	55.2
102.0			117.4	88.9	55.1
110.2			116.4	88.0	55.1
119.0			125.8	92.7	55.3
123.1			126.4	97.6	55.3
132.1			127.8	99.1	55.3
140.2			127.4	107.2	55.2
151.0			127.1	110.3	55.2
167.1			126.4	106.4	55.1
197.0			125.1	105.3	55.1

B.3.2.10 2700Ω

Time (days)	1	2	3	4	5
0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.0	0.0	0.0	0.0	0.0
1.0	0.5	0.7	1.3	0.6	0.7
2.0	1.4	2.2	1.5	1.7	1.6
3.1	2.4	2.9	2.6	2.7	2.6
4.1	3.2	3.8	3.4	3.6	3.4
5.0	3.9	4.7	4.2	4.4	4.2
6.0	4.6	5.7	5.3	5.5	5.2
7.0	5.3	6.9	6.2	6.5	6.2
8.0	6.0	8.0	7.2	7.8	7.2
9.1	6.9	9.3	8.3	9.1	8.4
10.1	7.4	10.4	9.2	10.2	9.5
11.0	8.3	11.6	9.7	11.4	10.6
12.0	9.1	12.7	10.0	12.5	11.7
13.0	10.1	14.0	10.0	13.7	13.2
14.0	10.8	15.2	10.1	14.9	14.1
15.0	11.8	16.3	10.6	16.1	15.3
15.2	12.1	16.9	10.7	16.5	15.8
17.0	13.8	18.8	12.2	18.6	17.8
18.0	15.0	20.1	13.2	20.4	19.0
19.0	16.1	21.1	14.1	21.2	19.9
20.0	17.3	22.3	15.2	22.5	21.1
21.0	18.5	23.6	16.2	23.8	22.3
22.0	20.0	24.8	17.2	25.2	23.7
23.0	21.4	26.3	18.6	26.8	25.0
24.0	22.8	27.6	19.7	28.3	26.2
25.0	23.9	29.0	20.9	29.4	27.0
26.1	25.3	30.0	22.4	31.3	28.8
27.0	26.3	31.2	23.4	32.6	29.8
28.0	28.0	32.7	25.0	34.4	31.2
29.0	29.2	33.7	26.3	35.7	32.2
32.0	33.7	37.4	30.6	40.3	36.1
34.0	36.1	39.9	33.0	43.1	39.2
36.2	39.2	42.8	36.3	47.1	42.1
39.2	42.7	45.8	40.2	51.7	45.7
42.0	46.1	49.6	43.8	55.9	49.1
47.0	53.2	55.6	50.3	63.3	55.6
53.0	61.8	63.3	58.5	72.0	63.3
56.1	66.2	67.3	62.7	76.3	67.5
63.0	76.0	76.3	72.1	85.3	74.2
69.0	83.4	83.2	79.7	93.4	80.3
75.1	86.4	90.7	87.4	101.4	80.1
82.0	93.8	98.9	91.1	110.2	80.0

B.3.2.11 3300Ω

Time (days)	1	2	3	4	5
0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.0	-0.1	-0.1	0.0	-0.1
1.0	0.3	0.5	0.3	0.2	0.4
2.0	0.9	1.2	1.1	1.0	0.9
3.1	1.8	2.1	1.7	1.6	2.1
4.1	2.6	2.7	2.3	2.5	2.7
5.0	3.4	3.4	3.3	3.1	3.2
6.0	4.8	4.1	3.5	3.8	4.1
7.0	5.0	4.9	4.2	4.6	5.0
8.0	5.8	5.6	4.8	5.2	5.4
9.1	6.7	6.3	5.7	5.9	6.3
10.1	7.4	7.0	6.1	6.6	7.1
11.0	8.3	7.7	6.7	7.2	7.6
12.0	9.1	8.5	7.4	8.3	8.3
13.0	10.0	9.5	8.2	9.4	9.2
14.0	10.6	10.2	8.9	10.2	9.9
15.0	11.5	11.6	9.9	10.9	10.7
15.2	11.8	11.3	10.3	11.3	10.7
17.0	13.4	12.6	11.9	12.8	12.6
18.0	14.1	13.8	12.9	13.9	13.4
19.0	14.8	14.4	13.9	14.5	14.0
20.0	15.6	15.4	14.6	15.4	14.8
21.0	16.7	16.2	15.8	16.2	15.9
22.0	17.5	17.1	16.9	17.2	17.3
23.0	18.5	18.2	18.0	18.2	18.3
24.0	19.3	19.2	19.1	19.3	19.4
25.0	19.8	19.8	19.9	20.1	19.8
26.1	21.0	21.0	21.2	21.0	21.1
27.0	21.6	21.8	22.1	21.8	21.8
28.0	22.9	23.2	23.1	22.6	22.8
29.0	23.6	24.1	24.0	23.6	23.7
32.0	26.2	26.9	27.1	26.2	27.1
34.0	27.8	28.5	28.9	27.8	28.7
36.2	30.5	31.2	31.9	30.3	31.5
39.2	32.9	33.9	34.5	32.8	34.2
42.0				34.9	
47.0	40.4	39.9	42.4	34.8	41.6
53.0	46.6	43.9	48.7	34.6	47.3
56.1	49.8	46.2	52.1	34.6	50.4
63.0	57.3	51.4	59.3	34.3	57.4
69.0	66.8	51.6	62.6	33.9	63.4
75.1	73.2	52.1	66.6	33.9	70.2
82.0	76.2	54.4	72.9	33.7	78.1
88.2	79.3				84.8
97.0	115.8				94.6
102.0	132.4				99.5
110.2	132.4				106.7
119.0	132.2				
123.1	132.6				
132.1	131.5				
140.2	131.3				
151.0	130.9				
167.1	130.2				
197.0	126.8				

B.3.2.12 5600Ω

Time (days)	1	2	3	4	5
0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.0	0.0	0.0	-0.1	0.1
1.0	0.1	0.5	0.1	-0.1	0.3
2.0	0.5	0.7	-0.3	1.1	0.6
3.1	0.9	1.1	0.4	0.6	0.8
4.1	1.0	1.1	-0.2	1.0	1.2
5.0	1.3	1.3	0.9	1.1	1.6
6.0	1.7	1.7	1.3	1.5	1.8
7.0	1.8	2.0	1.7	1.8	2.6
8.0	1.8	2.3	1.9	2.3	2.6
9.1	2.2	3.1	2.3	2.6	2.7
10.1	2.3	3.8	2.6	3.0	2.9
11.0	2.6	4.3	2.9	3.3	3.2
12.0	2.9	5.0	3.3	3.8	3.2
13.0	3.1	5.4	3.8	4.2	3.5
14.0	3.3	5.6	3.9	4.5	3.6
15.0	4.1	6.1	4.1	5.0	3.8
15.2	3.6	6.1	4.3	5.0	3.9
17.0	4.0	6.8	4.5	5.8	4.2
18.0	4.2	7.1	4.9	6.2	4.5
19.0	4.4	7.3	5.4	6.4	4.7
20.0	4.6	7.8	5.7	6.8	4.9
21.0	5.0	8.2	6.0	7.1	5.1
22.0	5.5	8.6	6.3	7.6	5.5
23.0	5.7	9.2	6.8	7.9	5.8
24.0	5.9	9.5	7.2	8.7	6.2
25.0	6.2	9.9	7.4	9.0	6.3
26.1	6.6	10.4	7.7	9.5	6.8
27.0	6.8	10.7	8.0	10.0	7.0
28.0	7.1	11.3	8.4	10.4	7.3
29.0	7.4	11.6	8.7	10.8	7.6
32.0	8.3	12.6	10.0	12.2	8.6
34.0	8.9	13.6	10.6	13.1	9.2
36.2	10.0	14.9	11.9	14.3	10.3
39.2	10.9	16.4	13.4	15.7	11.1
42.0	11.9	17.7	14.0	16.8	11.9
47.0	13.7	19.2	17.3	18.5	13.8
53.0	15.7	20.0	18.8	19.8	16.1
56.1	19.6	20.8	20.0	19.9	17.3
63.0	19.5	21.6	22.9	20.7	21.8
69.0	21.4	22.3	25.3	20.7	21.9
75.1	23.6	23.3	27.9	20.9	24.3
82.0	26.5	24.4	31.2	21.1	27.4
88.2	28.7	25.2	34.1	20.9	30.0
97.0	30.2	26.9	37.5	20.9	33.8
102.0	33.9	27.5	39.7	21.1	36.8
110.2	36.3	29.2	42.7	20.6	39.4
119.0	39.8	31.7	46.8	20.6	43.1
123.1	41.7	32.1	48.4	20.6	45.6
132.1	44.1	32.7	52.0	20.1	47.8
140.2	47.3	33.2	55.7	20.1	51.3
151.0	51.5	33.9		19.9	55.8
167.1	56.4	33.4		18.9	61.7
197.0	71.0	32.5		17.5	72.6
252.3	67.7	28.3		14.1	75.1
275.2	67.1	26.7		13.2	73.8

B.3.2.13 8200Ω

Time (days)	1	2	3	4	5
0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.1	-0.8	0.1	0.0	-0.1
1.0	0.1	-0.8	0.0	0.0	0.0
2.0	0.3	-0.5	0.1	0.9	-0.1
3.1	0.4	-0.4	0.3	0.5	0.3
4.1	0.6	-0.2	0.4	0.4	0.4
5.0	0.8	0.0	0.5	0.5	0.6
6.0	1.1	0.3	0.9	0.9	0.8
7.0	1.1	0.6	0.9	1.5	1.1
8.0	1.6	0.9	13.4	1.3	1.2
9.1	1.8	1.3	18.7	1.7	1.4
10.1	2.0	1.7	27.8	1.8	1.7
11.0	2.4	2.1	36.4	2.0	1.9
12.0	2.4	2.4		2.4	2.0
13.0	2.8	3.0		2.6	2.2
14.0	3.1	3.2		2.7	2.5
15.0	3.3	3.6		3.0	2.5
15.2	3.3	3.7		3.1	2.5
17.0	3.9	5.2		3.4	3.2
18.0	4.3	4.8		3.7	3.3
19.0	4.3	4.9		4.0	3.3
20.0	4.8	5.5		4.3	3.5
21.0	5.1	5.8		4.5	3.8
22.0	5.4	6.2		5.7	4.1
23.0	5.6	6.5		5.1	4.3
24.0	5.9	5.2		5.3	4.7
25.0	6.0	5.2		5.6	4.7
26.1	6.3	7.3		5.5	4.3
27.0	6.5	7.5		6.2	5.2
28.0	6.8	7.7		6.4	5.5
29.0	8.0	7.6		6.8	5.7
32.0	8.1	8.3		7.7	6.5
34.0	8.6	7.8		8.3	7.0
36.2	9.4	8.5		9.2	7.6
39.2	10.1	8.2		10.1	7.8
42.0	10.4	8.5		10.8	7.7
47.0	12.0	8.8		12.2	7.6
53.0	13.7	9.5		12.0	7.6
56.1	14.6	10.1		15.9	7.5
63.0	16.6	10.9		18.1	18.8
69.0	17.8	12.0		20.0	
75.1	19.4	13.0		22.0	
82.0	21.3	14.8		24.4	
88.2	22.4	15.6		26.1	
97.0	24.0	17.3		28.9	
102.0	24.8	18.2		30.4	
110.2	26.1	19.1		32.2	
119.0	27.1	20.8		35.2	
123.1	28.8	21.2		36.4	
132.1	30.6	23.0		39.1	
140.2	33.1	25.2		42.1	
151.0	36.5	27.7		45.6	
167.1	38.9	32.0		50.6	
197.0	47.3	39.7		58.6	
252.3	56.7	40.6		85.9	
275.2	56.4	39.8		84.5	

B.3.3 5-mL burette method

B.3.3.1 270Ω

Time (H:M)	1	2	3	4	5	6
0:00				0.00		
0:24				0.25	0.00	0.00
0:40	0.00			0.38	0.14	0.14
1:01	0.24		0.00	0.60	0.35	0.36
1:20	0.42	0.00	0.19	0.78	0.52	0.54
1:40	0.59	0.08	0.36	0.95	0.80	0.70
2:00	0.76	0.35	0.52	1.12	0.85	0.86
2:20	0.95	0.53	0.68	1.29	1.13	1.06
2:40	1.19	0.77	0.90	1.50	1.23	1.22
3:00	1.36	0.94	1.08	1.67	1.41	1.43
3:20	1.54	1.10	1.19	1.83	1.56	1.59
3:40	1.71	1.26	1.40	2.00	1.72	1.76
4:00	1.96	1.50	1.63	2.22	1.94	1.98
4:20	2.15	1.71	1.83	2.42	2.13	2.17
4:39	2.33	1.88	2.00	2.59	2.30	2.34
5:00	2.51	2.06	2.18	2.77	2.47	2.51

B.3.3.2 330Ω

Time (H:M)	1	2	3	4	5	6
0:00	0.00	0.00			0.00	
0:37	-0.61	-0.60	0.00	0.00	-0.50	0.00
1:14	-1.07	-0.99	-0.38	-0.38	-0.83	-0.39
1:41	-1.32	-1.17	-0.66	-0.57	-0.98	-0.48
2:05	-1.39	-1.26	-0.71	-0.60	-0.98	-0.48
2:20	-1.44	-1.29	-0.73	-0.61	-0.98	-0.48
2:35	-1.46	-1.28	-0.76	-0.59	-0.98	-0.49
2:50	-1.44	-1.24	-0.74	-0.53	-0.93	-0.45
3:05	-1.40	-1.17	-0.67	-0.44	-0.87	-0.36
3:20	-1.34	-1.09	-0.63	-0.39	-0.78	-0.29
3:35	-1.29	-1.04	-0.60	-0.30	-0.72	-0.22
3:50	-1.23	-0.98	-0.52	-0.25	-0.65	-0.11
4:06	-1.12	-0.87	-0.39	-0.13	-0.53	-0.05
4:20	-1.03	-0.75	-0.30	0.00	-0.41	0.11
4:35	-0.91	-0.64	-0.20	0.12	-0.31	0.21
5:05	-0.68	-0.42	0.01		-0.08	
5:20	-0.56	-0.28	0.13			
5:35	-0.46	-0.18	0.20			
5:50	-0.38	-0.09	0.29			
6:05	-0.29	-0.01	0.41			

B.3.3.3 560Ω

Time (H:M)	1	2	3	4	5	6
0:00	0.00	0.00	0.00	0.00	0.00	0.00
0:27	0.60	-0.13	-0.15	-0.18	-0.07	-0.11
0:58	0.69	-0.65	-0.47	-0.49	-0.38	-0.42
1:27	0.66	-0.77	-0.82	-0.81	-0.73	-0.76
1:59	0.70	-1.07	-1.10	-1.12	-1.02	-1.05
2:27	0.66	-1.35	-1.38	-1.39	-1.29	-1.32
2:58	0.74	-1.57	-1.65	-1.58	-1.56	-1.56
3:27	0.86	-1.68	-1.80	-1.66	-1.73	-1.48
3:58	0.85	-1.70	-1.84	-1.66	-1.79	-1.70
4:25	0.66	-1.66	-1.84	-1.61	-1.78	-1.66
4:57	0.72	-1.57	-1.80	-1.53	-1.74	-1.58
5:28	0.78	-1.47	-1.71	-1.41	-1.66	-1.48
6:05	0.91	-1.32	-1.57	-1.34	-1.53	-1.33
6:26	1.04	-1.19	-1.43	-1.12	-1.38	-1.18
7:00	1.14	-1.05	-1.29	-0.98	-1.24	-1.04
7:26	1.23	-0.83	-1.18	-0.87	-1.14	-0.91
8:06	1.40	-0.77	-1.01	-0.68	-0.96	-0.53
11:45		0.32	0.10	0.36	0.09	0.40
13:16		0.76	0.55	0.78	0.38	0.85

B.3.3.4 820Ω

Time (H:M)	1	2	3	4	5	6
0:00	0.00		0.00	0.00	0.00	0.00
0:31	0.12		0.12	0.12	0.13	0.13
1:00	0.19		0.18	0.20	0.22	0.20
1:32	0.29		0.30	0.30	0.30	0.31
2:01	0.37		0.38	0.39	0.42	0.40
2:32	0.47		0.51	0.50	0.50	0.51
3:07	0.61		0.63	0.61	0.66	0.64
3:32	0.67		0.70	0.66	0.70	0.70
4:00	0.81		0.82	0.79	0.84	0.82
4:31	0.87		0.90	0.86	0.91	0.91
5:46	1.12		1.16	1.11	1.18	1.18
6:13	1.19		1.22	1.18	1.27	1.24
6:42	1.33		1.37	1.31	1.39	1.38
7:09	1.39		1.43	1.36	1.46	1.45
7:38	1.51		1.55	1.48	1.59	1.58
8:39	1.73		1.76	1.70	1.80	1.79
9:46	1.94		1.99	1.91	2.02	2.02

B.3.3.5 1200Ω

Time (days)	1	2	3	4	5	6
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.02	0.07	0.06	0.08	0.07	0.08	0.08
0.04	0.10	0.08	0.12	0.16	0.12	0.13
0.06	0.06	0.05	0.07	0.11	0.10	0.06
0.08	0.18	0.15	0.20	0.21	0.19	0.19
0.13	0.33	0.28	0.30	0.35	0.30	0.32
0.16	0.45	0.41	0.40	0.49	0.46	0.47
0.21	0.57	0.52	0.52	0.59	0.59	0.60
0.25	0.68	0.65	0.61	0.71	0.71	0.70
0.29	0.83	0.79	0.76	0.85	0.88	0.84
0.34	1.00	0.96	0.92	1.00	1.04	1.00
0.38	1.15	1.11	1.07	1.17	1.17	1.19
0.42	1.24	1.21	1.16	1.28	1.28	1.25
0.45	1.42	1.39	1.51	1.32	1.47	1.44
0.57	1.80	1.78	1.80	1.55	1.84	1.85
1.00	3.40	3.23	3.46	3.46	3.02	3.46
1.14	3.80	3.86	3.89	3.88	3.86	3.90
1.24		4.27				

B.3.3.7 5600Ω

Time (days)	1	2	3	4	5	6
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.07	0.76	0.08	0.11	0.84	0.10	0.02
0.16	0.77	0.09	0.12	0.87	0.10	0.06
0.22	0.77	0.09	0.12	0.93	0.10	0.03
0.35	0.77	0.12	0.14	0.88	0.12	0.05
1.05	1.26	0.64	0.65	1.41	0.63	0.60
1.12	1.34	0.72	0.71	1.47	0.68	0.66
1.19	1.40	0.78	0.77	1.53	0.75	0.72
1.36	1.53	0.90	0.90	1.67	0.89	0.87
1.96	2.07	1.44	1.43	2.20	1.36	1.42
2.08	2.22	1.57	1.56	2.34	1.48	1.56
2.17	2.31	1.67	1.65	2.43	1.56	1.66
3.04	3.05	2.40	2.37	3.18	2.21	2.41
3.13	3.16	2.50	2.47	3.28	2.31	2.52
3.23	3.22	2.55	2.51	3.34	2.36	2.57
3.34	3.31	2.65	2.61	3.44	2.44	2.66
3.50	3.50	2.82	2.78	3.62	2.60	2.85
4.09	4.08	3.34	3.29	4.23	3.09	3.39

B.3.3.6 3900Ω

Time (days)	1	2	3	4	5	6
0.00	0.00	0.00	0.00	0.00	0.00	0.00
0.05	0.14	0.11	0.15	0.19	0.15	0.23
0.09	0.14	0.11	0.15	0.19	0.15	0.21
0.78	0.65	0.63	0.63	0.67	0.62	0.71
0.92	0.80	0.77	0.78	0.83	0.76	0.87
0.98	0.83	0.81	0.82	0.86	0.80	0.90
1.02	0.92	0.88	0.90	0.94	0.87	0.99
1.06	0.92	0.88	0.91	0.95	0.88	1.00
1.11	0.98	0.95	0.99	1.01	0.94	1.05
1.73	1.66	1.60	1.68	1.66	1.59	1.76
1.89	1.82	1.89	1.82	1.88	1.76	1.93
1.98	1.91	1.97	1.93	1.97	1.85	2.04
2.02	2.04	2.10	2.05	2.10	1.94	2.14
2.09	2.06	2.11	2.08	2.11	1.98	2.18
2.25	2.24	2.30	2.27	2.31	2.16	2.34
2.76	2.81	2.87	2.86	2.91	2.71	2.86
3.01	3.07	3.15	3.13	3.18	2.97	3.09
3.10	3.17	3.25	3.24	3.29	3.06	3.20
3.77	3.95	4.04	4.02		3.81	3.94
4.05	4.19				4.02	4.15

B.3.4 Gas diffusion

B.3.4.1 Hydrogen without temperature measurements

Time (days)	PETG				Nylon				HMWPE				PBT Valox				POM			
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1	1.1	0.6	0.5	0.6	0.6	0.4	0.4	0.4	0.7	0.7	0.8	0.6	0.7	0.6	0.5	0.5	0.1	0.2	0.2	0.1
0.9	2.4	1.8	1.8	1.9	2.0	1.0	0.8	0.8	0.8	3.6	3.3	3.4	3.2	3.4	3.4	2.8	2.9	2.8	2.7	0.4	0.4	0.6	0.6	0.5	0.4
1.0	3.6	3.0	2.9	2.9	3.0	1.9	1.7	1.6	1.6	1.5	4.7	4.5	4.5	4.1	4.2	3.5	3.4	3.3	3.3	2.9	1.0	1.2	1.2	1.0	0.9
1.4	3.3	2.8	2.8	2.8	2.9	1.3	1.1	1.2	1.1	1.1	5.5	5.0	5.2	4.8	5.1	4.3	4.3	4.3	4.2	4.1	0.5	0.7	0.7	0.6	0.5
1.9	4.2	3.7	3.8	3.8	3.8	1.9	1.6	1.8	1.8	0.0	6.8	6.3	6.5	5.9	6.5	5.4	5.4	5.4	5.3	5.2	0.5	0.7	0.8	0.7	0.4
2.4	4.5	0.0	3.8	4.3	4.4	1.6	1.3	1.4	1.3	1.2	8.9	8.1	8.3	7.6	8.4	7.1	7.0	7.0	6.9	6.8	0.8	1.0	1.0	0.9	0.7
2.9	6.6	6.0	6.3	6.2	6.2	2.7	2.6	2.7	2.5	2.5	11.6	10.7	11.0	10.1	10.8	9.4	9.3	9.2	9.0	8.9	0.5	1.7	1.7	1.7	1.5
3.3	6.4	5.9	6.1	6.1	6.1	2.0	2.0	2.1	2.0	1.7	12.5	11.6	11.8	10.9	11.8	10.2	10.1	10.6	9.8	9.6	1.4	1.5	1.5	1.4	1.4
3.9	7.3	6.8	7.1	7.0	7.1	2.6	2.3	2.5	2.3	2.2	14.4	13.3	13.6	12.7	13.6	11.9	11.7	11.6	11.3	11.2	1.7	1.9	1.8	1.8	1.6
4.9	8.9	8.4	8.8	8.7	8.8	3.0	2.7	3.0	2.7	2.6	17.6	16.3	16.6	15.4	16.4	14.7	14.5	14.3	13.9	13.8	2.0	2.2	2.1	2.1	2.0
5.0	9.8	9.3	9.7	9.6	9.7	3.8	3.4	3.6	3.4	3.0	18.2	17.2	17.5	16.2	17.5	15.4	15.2	15.0	14.6	14.4	2.7	2.4	2.4	2.4	2.3
5.4	9.9	9.4	9.8	9.8	10.0	3.4	3.1	3.3	3.1	3.0	19.3	17.9	18.3	17.0		16.2	16.0	15.9	15.4	15.2	2.3	2.4	2.3	2.3	2.3
5.9	11.2	10.7	11.1	11.1	11.1	4.2	3.9	4.2	3.9	3.8						18.2	17.8	17.7	17.3	17.1	3.3	3.3	3.2	3.2	3.1
6.9	11.9	11.4	12.1	11.9	12.0	3.9	3.6	3.9	3.6	3.5											3.1	3.0	2.9	2.9	2.9
8.0	14.2	12.8	13.1	13.4	13.5	4.2	3.9	4.3	3.9	3.8											3.1	3.1	3.0	3.1	3.0
8.9	15.0	14.5	15.2	15.2	15.4	5.0	4.7	4.8	4.7	4.6											3.8	3.7	3.6	3.7	3.6
9.3	15.4	14.9	15.7	15.6	15.6	5.0	4.6	5.0	4.7	4.6											3.8	3.7	3.7	3.7	3.7
9.9	16.3	15.8	16.7	16.6	16.5	5.3	4.9	5.4	4.9	4.9											4.0	4.0	3.9	3.9	3.9
10.9	17.5					5.5	5.1	5.6	5.1	5.1											4.2	4.3	4.1	4.1	4.1
11.9						6.1	5.7	6.2	5.7	5.7											4.6	4.9	4.7	4.6	4.5
Rate (ml/day)	1.6	1.6	1.6	1.6	1.6	0.46	0.44	0.48	0.44	0.46	3.6	3.3	3.4	3.1	3.4	3.0	3.0	3.0	2.9	2.9	0.40	0.38	0.37	0.38	0.38

Time (days)	HDPE				PP				B Braun				Terumo				Theratron				Glass			
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.1	0.4	0.4	0.3	0.3	0.4	1.0	0.9	0.9	0.9	0.6	0.7	0.7	0.7	0.7	0.9	0.4	0.9	0.7	1.0	0.9	1.0	0.9	0.0	0.0	0.0	0.0
0.9	2.1	2.1	2.2	2.2	1.8	6.5	6.0	6.2	6.2	6.1	5.5	5.6	5.6	5.5	6.7	6.5	6.7	6.3	7.2	7.1	7.4	7.0	0.1	-0.1	0.0	0.0
1.0	2.9	2.7	2.7	2.6	2.4	7.6	7.2	7.4	7.4	7.2	6.4	6.4	6.4	6.2	7.3	7.6	7.7	8.0	8.3	8.2	8.5	8.0	0.0	0.0	0.0	-0.1
1.4	3.2	3.1	3.2	3.2	3.3	9.9	9.3	9.4	9.6	9.5	8.4	8.5	8.5	8.3	10.5	10.3	10.5	10.9	11.3	11.1	11.6	10.9	0.0	0.0	0.0	0.0
1.9	4.1	4.0	4.3	4.1	4.3	13.3	12.7	12.8	13.0	12.9	11.3	11.4	11.3	11.0	13.3	14.2	14.5	15.0	15.4	15.1	15.8	14.9	0.0	0.0	0.0	0.0
2.4	5.2	5.0	5.3	5.1	5.3	16.4	15.6	15.8	16.0	16.1	14.1	14.3	14.2	13.9	17.6	17.4	18.0	18.7		18.6		18.6	0.1	0.0	-0.1	-0.1
2.9	6.9	6.7	7.0	6.7	6.9		19.2				17.4	17.6	17.4	17.1									0.1	0.0	-0.1	-0.1
3.3	7.5	7.3	7.6	7.4	7.6																		0.1	0.0	-0.1	-0.1
3.9	8.8	8.5	8.9	8.7	8.9																		0.1	0.0	-0.1	-0.1
4.9	10.8	10.5	11.0	10.7	10.9																		0.0	0.0	0.0	0.0
5.0	11.3	10.9	11.4	11.1	11.3																		-0.5	0.3	0.2	0.2
5.4	12.0	11.6	12.2	11.8	12.0																		-0.5	0.2	0.3	0.3
5.9	13.5	13.0	13.5	13.2	13.3																		-0.5	0.2	0.3	0.3
6.9	14.9	14.3	15.2	14.6	14.8																		-0.5	0.2	0.3	0.3
8.0	16.6	16.1	16.9	16.3	16.5																		-0.5	0.2	0.3	0.3
8.9		17.9		18.1																			-0.4	0.2	0.2	0.2
9.3																							-0.5	0.2	0.3	0.3
9.9																							-0.4	0.2	0.2	0.2
10.9																							-0.4	0.3	0.2	0.2
11.9																							-0.5	0.3	0.2	0.2
Rate (ml/day)	2.1	2.0	2.2	2.1	2.1	6.9	6.6	6.7	6.8	6.9	6.0	6.1	6.0	5.9	7.3	7.5	7.6	8.0	8.2	7.9	8.4	7.9	-0.06	0.03	0.03	0.03

B.3.4.2 Carbon dioxide with only one temperature measurement

Time (days)	PETG					Nylon					HMWHDPE					PBTValox					POM				
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.7	1.1	0.9	2.0	1.4	1.1	0.7	1.6	1.2	1.2		3.2	3.6	4.2	3.6	3.7	2.0	2.6	1.7	2.4	2.7	2.3	1.7	1.9	2.2	2.0
0.9	1.6	1.6	2.6	1.9	1.5	1.0	1.9	1.5	1.3		4.6	4.8	5.2	4.6	4.8	3.0	2.5	2.7	2.9	3.0	2.9	2.3	2.4	2.8	2.6
1.2	2.6	2.7	4.0	3.1	2.5	2.1	3.3	2.7	2.6		6.7	6.8	7.3	6.3	6.7	4.2	3.8	4.0	4.1	3.9	4.0	3.2	3.4	3.5	3.4
1.8	3.0	3.2	4.6	3.6	3.1	2.3	3.6	3.1	3.0		9.2	9.6	9.8	8.7	9.4	5.2	4.9	4.9	5.3	5.8	5.2	4.4	4.5	5.4	4.5
2.0	3.8	4.0	5.4	4.5	3.9	3.3	4.3	3.7	3.7		10.8	11.1	11.3	10.2	11.0	5.9	5.7	5.6	6.1	6.5	6.0	5.1	5.2	6.1	5.1
2.8	3.6	4.2	5.4	4.4	3.8	2.8	4.2	3.5	4.1							6.4	6.3	6.1	6.6	7.1	6.7	5.7	5.9	6.9	5.9
3.2	4.0	4.7	5.9	4.9	4.2	3.1	4.7	4.0	4.1							7.1	7.1	6.8	7.4	7.9	7.5	6.3	6.5	7.5	6.5
3.8	3.9	4.7	5.9	4.9	4.2	2.9	4.6	3.8	3.7							7.5	7.7	7.4	7.9	8.5	8.4	7.1	7.3	8.4	7.5
4.7	4.1	5.0	6.3	5.3	4.6	3.2	5.0	4.2	4.3																
Rate (ml/day)	0.8	1.1	1.2	1.1	0.9	0.65	0.97	0.83	0.87	5.4	5.5	5.5	5.0	5.4	1.9	1.9	1.9	1.9	2.0	2.2	2.1	1.8	1.9	2.2	1.9

Time (days)	HDPE					PP					B Braun					Terumo					Theratron				Glass
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0				0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.7	2.5	2.7	3.3	2.7	2.8	4.6	5.0				4.7	4.4	4.3	4.6	4.5	3.4	4.4	4.6	5.0		5.1	5.0	-0.1	-0.1	0.2
0.9	3.2	3.4	4.1	3.5	3.6	5.7	6.4				5.8	5.3	5.4	5.7	5.7	4.5	5.6	5.9	6.8		6.5	6.4	0.1	0.0	-0.1
1.2	4.3	4.6	5.2	4.6	4.6	7.2	7.8				7.3	7.0	7.0	7.1	7.1	5.4	6.8	7.2	8.3		8.1	8.0	0.0	-0.3	0.3
1.8	6.2	6.4	7.3	6.7	6.7	10.6	11.6				10.7	10.2	10.0	10.3	11.4	8.2	10.1	10.6	11.9		11.9	11.6	0.0	-0.3	0.2
2.0	7.1	7.3	8.2	7.6	7.7	11.9					11.9	11.4	11.2	11.5	12.6	9.0	11.3	12.0	13.5		13.4	13.2	0.0	-0.2	0.2
2.8	8.6	8.9	9.9	9.4	9.3																		0.0	-0.2	0.1
3.2	9.6	9.8	10.9	10.5	10.3																		0.1	-0.2	0.2
3.8																							0.1	-0.3	0.2
4.7																							0.1	-0.2	0.1
Rate (ml/day)	3.0	3.1	3.4	3.3	3.2	5.9	6.5	5.8	6.9		5.9	5.6	5.5	5.6	6.3	4.5	5.6	5.9	6.6		6.6	6.5	0.0	0.0	0.0

B.3.4.3 Hydrogen

Time (days)	PETG				Polypropylene				POM				LDPE			
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.7	0.7	0.6	0.7	0.6	4.5	4.6	4.7	4.5	0.0	0.1	0.0	0.1	3.0	3.3	3.2	3.1
1.7	1.7	1.6	1.8	1.7	10.6	11.1	10.7	11.1	0.2	0.2	0.2	0.3	7.6	7.4	8.0	7.2
2.7	3.0	2.9	3.1	2.9	16.8	16.8	16.8	16.8	0.4	0.5	0.5	0.6	12.0	11.7	11.6	11.3
3.7	4.3	4.1	4.4	4.2					0.6	0.8	0.8	0.9	16.0	15.4	15.5	15.0
4.7	6.2	6.1	6.4	6.0					1.5	1.6	1.6	1.6				
5.7	6.8	6.7	7.1	6.8					1.6	1.5	1.5	1.5				
6.6	8.1	8.0	8.4	8.0					1.5	1.5	1.6	1.7				
7.7	9.2	9.0	9.5	9.1					1.8	1.8	1.8	1.9				
8.7	10.6	10.4	10.9	10.5					2.1	2.1	2.3	2.2				
9.7	11.8	11.7	12.2	11.8					2.3	2.3	2.6	2.5				
10.7	13.1	13.0	13.6	13.1					2.7	2.6	3.1	2.8				
14.9									4.0	3.5	4.5	4.7				
16.7									4.9	4.2	5.2	6.1				

Time (days)	HDPE				HMWHDPE				ST Nylon				Nylon			
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.7	1.6	1.4	1.4	1.4	2.2	2.2	2.3	2.2	0.1	0.1	0.0	0.1	0.1	0.0	-0.4	-0.6
1.7	3.0	3.3	3.2	3.2	5.1	5.1	5.1	5.1	0.4	0.4	0.2	0.5	0.3	0.2	0.4	0.2
2.7	4.9	5.3	4.7	5.2	8.3	8.2	8.2	8.4	0.8	0.8	0.6	0.8	0.6	0.6	0.8	0.6
3.7	6.7	7.4	7.2	7.2	11.3	11.1	11.1	11.4	1.3	1.2	1.1	1.3	1.0	0.9	1.2	1.0
4.7	8.7	9.6	9.3	9.3	14.3	14.2	14.1	14.4	1.8	1.8	1.5	1.8	1.4	1.3	1.7	1.5
5.7	10.3	11.3	10.9	11.0	16.8	16.7	16.4	17.0	2.3	2.2	2.1	2.3	1.8	1.7	2.2	2.0
6.6	11.8	12.8	12.5	12.5					2.4	2.3	2.2	2.5	1.9	1.9	2.2	2.1
7.7	13.2	14.6	14.2	14.3					2.7	2.6	2.5	2.8	2.2	2.2	2.6	2.5
8.7	14.8	16.4	15.9	16.0					3.3	3.2	3.0	3.3	2.7	2.6	3.1	3.0
9.7									3.6	3.5	3.4	3.7	3.1	2.9	3.5	3.4
10.7									4.1	4.0	3.9	4.4	3.5	3.4	4.0	3.9
14.9									5.8	5.7	5.4	5.7	4.6	4.7	5.5	5.3
16.7									6.9	6.8	6.5	6.7	5.6	5.6	6.6	6.3

Time (days)	PBT				Glass		
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.7	1.3	1.4	1.4	1.4	0.0	0.0	0.0
1.7	3.4	3.4	3.5	3.5	0.1	0.0	-0.1
2.7	5.7	5.8	6.0	5.9	0.1	0.0	-0.1
3.7	8.1	8.2	8.3	8.3	0.1	0.1	-0.2
4.7	10.5	10.7	10.8	10.8	0.1	0.1	-0.2
5.7	12.8	13.1	13.3	13.2	0.1	0.1	-0.2
6.6	15.0	15.0	15.1	15.2	0.0	0.2	-0.2
7.7					0.0	0.2	-0.2
8.7					0.0	0.3	-0.2
9.7					0.0	0.3	-0.3
10.7					0.0	0.4	-0.4
14.9					0.0	0.4	-0.4
16.7					0.0	0.5	-0.4

Time (days)	Theratron					Terumo					B Braun				
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.2	1.8	1.8	2.0	1.9	1.9	1.7	1.6	1.7	1.6	1.5	1.5	1.5	1.5	1.5	1.5
0.7	6.5	6.3	6.5	6.3	6.3	5.6	5.6	5.8	5.7	5.7	4.5	4.5	4.6	4.5	4.5
1.2	10.0	9.7	10.0	9.7	9.8	8.6	8.7	8.8	8.9	8.8	7.4	7.3	7.5	7.5	7.3
1.7	14.2	13.8	14.1	13.8	12.6	12.3	12.4	12.6	12.7	12.6	10.3	10.2	10.5	10.4	10.3
2.0	15.9	15.5	15.9	15.5	15.5	13.8	13.9	14.1	14.2	13.8	11.8	11.6	11.9	11.8	11.7
2.2	17.8	17.3	17.8	17.4	17.3	15.5	15.6	15.8	15.9	15.7	13.2	13.0	13.3	13.3	13.0

Time (days)	POM					Thicker POM					Glass		
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.2	0.0	0.0	0.0	0.1	0.0	0.0	0.0	0.3	0.2	0.1	0.0	0.0	0.0
0.7	0.4	0.4	0.4	0.3	0.2	0.1	0.1	0.4	0.2	0.1	0.1	0.0	-0.1
1.2	0.4	0.3	0.4	0.4	0.2	0.0	0.0	0.4	0.3	0.2	0.0	0.0	0.0
1.7	0.7	0.6	0.6	0.7	0.5	-0.3	0.1	0.3	0.2	0.1	0.1	0.0	-0.1
2.0	0.6	0.5	0.6	0.6	0.4	0.1	0.1	0.5	0.3	0.2	0.1	0.0	-0.1
2.2	0.7	0.6	0.6	0.7	0.4	0.1	0.1	0.4	0.4	0.2	0.1	0.0	-0.1
2.9	0.8	0.7	0.7	0.8	0.5	0.0	0.1	0.4	0.3	0.2	0.1	0.1	-0.1
5.7	1.5	1.3	1.4	1.6	1.2	0.4	0.7	0.8	0.8	0.8	0.1	0.1	-0.1
6.7	1.8	1.6	1.6	1.8	1.4	0.4	0.8	0.8	0.8	0.8	0.1	0.1	-0.2
7.7	2.0	1.8	1.9	2.0	1.6	0.5	0.9	0.9	0.9	0.8	0.2	0.1	-0.3
8.7	2.3	2.1	2.2	2.3	1.8	0.6	1.0	1.1	1.0	1.0	0.1	0.2	-0.3
9.7	2.5	2.4	2.5	2.4	2.0	0.6	1.0	1.1	1.0	1.0	0.1	0.2	-0.3
12.7	3.8	3.4	3.6	3.4	3.5	1.0	1.4	1.5	1.4	1.6	0.0	0.3	-0.4
14.7	4.6	4.1	4.3	4.3	4.3	1.3	1.7	1.8	1.7	1.6	0.1	0.4	-0.5
16.8	5.0	4.5	4.7	4.9	4.8	1.4	1.8	1.9	1.9	1.8	0.0	0.6	-0.5
19.8	5.6	5.0	5.3	5.5	5.6	1.6	2.1	2.2	2.5	2.1	0.0	0.6	-0.6
28.9	7.5	6.5	7.1	7.4	7.8	2.1	2.8	2.8	3.6	3.3	-0.1	1.1	-1.0
34.9	9.1	8.0	8.6	9.0	9.5	2.9	4.1	4.0	4.8	4.5	-0.3	1.4	-1.1
44.1		9.5				3.7	5.0	5.0	5.8	5.6	-0.3	1.8	-1.5
50.8						4.2	5.5	5.5	6.1	6.2	-0.2	2.0	-1.7
55.7						4.3	6.1	6.1	6.9	6.9	-0.3	2.2	-1.9

B.3.4.4 Carbon dioxide

Time (days)	PETG				Polypropylene				POM				LDPE			
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.3	0.1	0.2	0.2	0.2	1.2	1.3	1.3	1.6	0.3	0.1	0.5	0.4	1.6	1.9	2.2	1.5
0.8	0.7	0.9	0.8	0.9	4.0	4.5	3.9	4.4	0.3	0.0	1.3	1.2	6.5	6.2	5.9	6.0
1.3	1.5	1.2	1.6	1.9	6.9	6.8	6.7	6.8	1.1	1.3	2.5	2.2	10.5	10.0	10.1	10.1
1.8	1.6	2.0	1.9	2.2	9.2	9.2	9.0	9.7	2.1	1.8	3.3	3.1				
2.3	2.0	2.3	2.2	2.7	11.3	11.2	11.0	11.8	2.4	2.1	3.8	3.7				
2.8	2.3	2.7	2.6	3.3	13.9	13.8	13.6		3.1	3.0	4.9	4.8				
3.3	2.8	3.2	3.2	3.8					3.6	3.6	5.5	5.4				
3.8	2.8	3.2	3.2	3.9					3.8	3.9	6.2	6.0				
4.3	3.1	3.6	3.6	3.8					4.3	4.6	7.1	6.9				
4.8	3.4	3.8	4.0	4.8					5.1	5.4	8.1	7.9				
5.3	3.5	4.0	4.0	4.9					5.3	5.6	8.5	8.4				
5.8	3.6	4.2	4.2	5.2					5.9	6.2						
6.2	3.8	4.3	4.4	5.3					6.1	6.5						
6.8	3.8	4.4	4.5	5.4					6.5	6.9						
7.3	4.1	4.6	4.7	5.6												
7.8	3.8	4.4	4.5	5.5												

Time (days)	**HDPE**				**HMWHDPE**				**ST Nylon**				**Nylon**			
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.3	0.5	0.7	0.6	0.5	1.2	1.0	1.0	1.2	0.1	0.2	0.4	0.1	0.1	0.7	0.3	0.3
0.8	1.7	2.2	2.0	1.6	3.3	3.0	3.1	3.6	0.2	0.5	0.8	0.0	0.5	1.1	0.5	0.5
1.3	0.0	2.5	3.7	3.4	5.5	5.4	5.4	6.1	1.5	1.1	1.4	0.6	1.2	1.8	1.3	1.4
1.8	4.4	5.6	5.0	4.8	7.3	7.4	7.3	8.3	2.0	1.2	1.6	0.9	1.5	2.0	1.4	1.9
2.3	5.2	5.4	6.9	5.6	8.8	8.9	8.8	9.8	2.0	1.0	1.6	0.8	1.0	1.9	1.4	1.8
2.8	6.2	8.4	7.9	7.2	11.0	11.4	11.1	12.2	2.6	1.6	2.1	1.2	2.0	2.5	2.0	2.5
3.3	8.0	9.5	9.1	8.5	12.6		12.7		3.1	1.7	2.3	1.3	2.2	2.6	2.0	2.6
3.8	8.8		10.1	9.4					3.1	1.5	2.2	1.1	1.8	2.5	1.9	2.5
4.3	10.0			10.6					3.4	1.7	2.4	1.3	2.4	2.9	2.2	2.9
4.8									1.7	2.7	1.9	2.8	2.8	3.2	2.6	3.3
5.3									3.9	1.8	2.7	1.5	2.8	3.1	2.4	3.2
5.8									4.1	2.0	2.9	1.6	3.1	3.4	2.7	3.5
6.2									4.2	2.6	2.9	1.7	3.1	3.4	3.7	3.5
6.8									4.2	2.0	2.9	1.6	3.0	3.3	2.5	3.3
7.3									4.6	2.3	3.2	1.9	3.5	3.8	3.0	3.8
7.8									4.3	1.9	3.0	1.6	3.2	3.4	2.6	3.5

Time (days)	**PBT**				**Glass**		
0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
0.3	0.4	0.4	0.1	0.4	-0.1	0.2	-0.2
0.8	1.1	1.1	0.9	1.2	0.0	0.2	-0.2
1.3	2.1	1.9	1.7	2.2	0.0	0.3	-0.4
1.8	2.8	2.5	2.3	3.0	0.1	0.4	-0.6
2.3	3.1	2.7	2.4	3.1	0.3	0.4	-0.8
2.8	4.1	3.6	3.3	4.1	0.2	0.4	-0.7
3.3	4.6	3.9	3.7	4.7	0.3	0.5	-0.8
3.8	4.9	4.1	3.9	4.9	0.4	0.6	-1.1
4.3	5.5	4.6	4.5	5.5	0.4	0.6	-1.0
4.8	6.3	5.3	5.2	6.8	0.4	0.5	-0.9
5.3	6.5	5.3	5.1	5.9	0.4	0.7	-1.1
5.8	7.2	5.9	5.8	7.2	0.4	0.7	-1.1
6.2	7.3	6.0	5.9	7.2	0.6	0.7	-1.3
6.8	7.6	6.2	6.1	7.4	0.5	0.9	-1.4
7.3	8.4	6.8	6.8		0.5	0.9	-1.4
7.8		6.8	6.8		0.5	0.9	-1.5

Appendix C: Data from Chapter 4

C.1 Diameter of 1.8-mm POM barrels

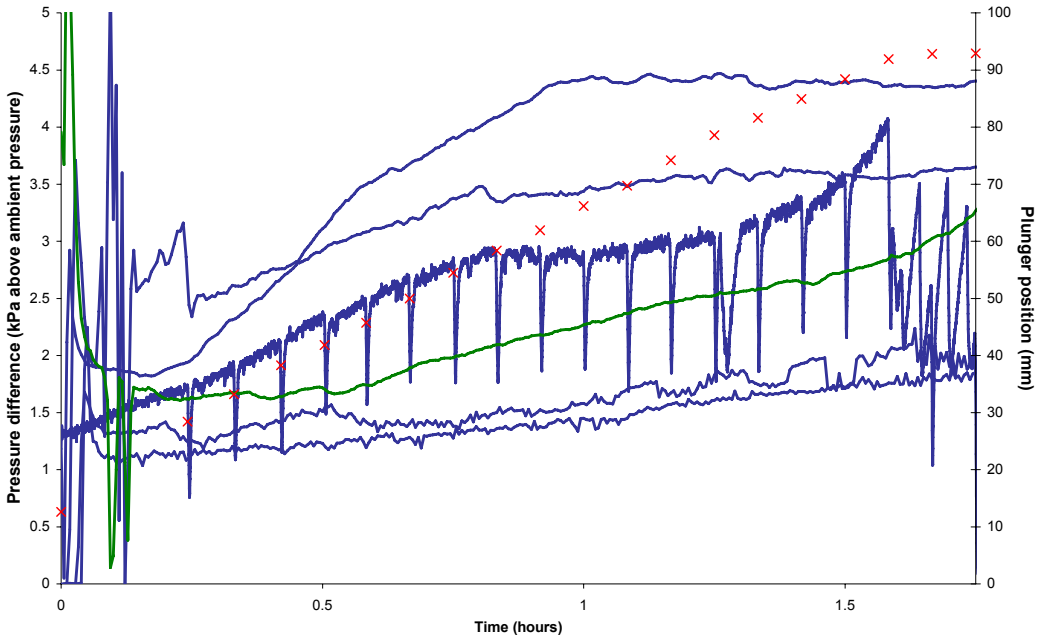
Measurements are shown in mm.

Position of measurement:	Wings end		Middle		Open end		
Direction of measurement:	Perpendicular to partline	Partline	Perpendicular to partline	Partline	Perpendicular to partline	Partline	Average ± standard deviation
Barrel 1	20.52	20.50	20.55	20.54	20.55	20.53	*20.52±0.02*
Barrel 2	20.49	20.50	20.53	20.53	20.52	20.55	*20.52±0.02*
Barrel 3	20.50	20.53	20.53	20.52	20.52	20.53	*20.52±0.01*
Barrel 4	20.54	20.51	20.55	20.51	20.53	20.55	*20.52±0.02*
Barrel 5	20.53	20.52	20.54	20.52	20.49	20.48	*20.51±0.02*
Average ± standard deviation	*20.52±0.02*	*20.51±0.01*	*20.54±0.01*	*20.52±0.01*	*20.52±0.02*	*20.53±0.03*	***20.52±0.02***

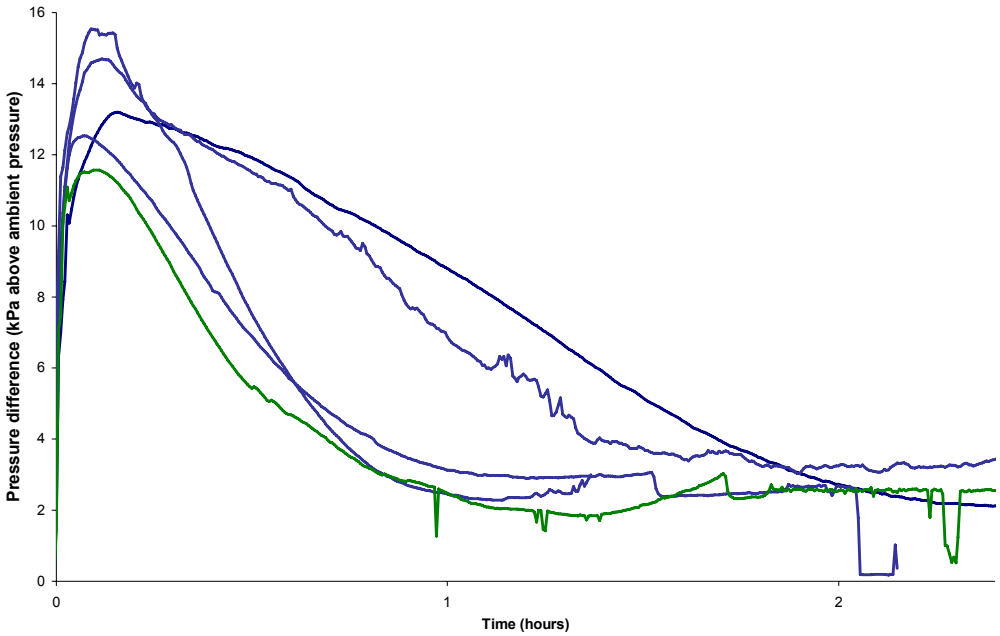
C.2 Gas pressure

Plunger position is shown as red points. Data from devices that were first equilibrated to 40°C are shown in green.

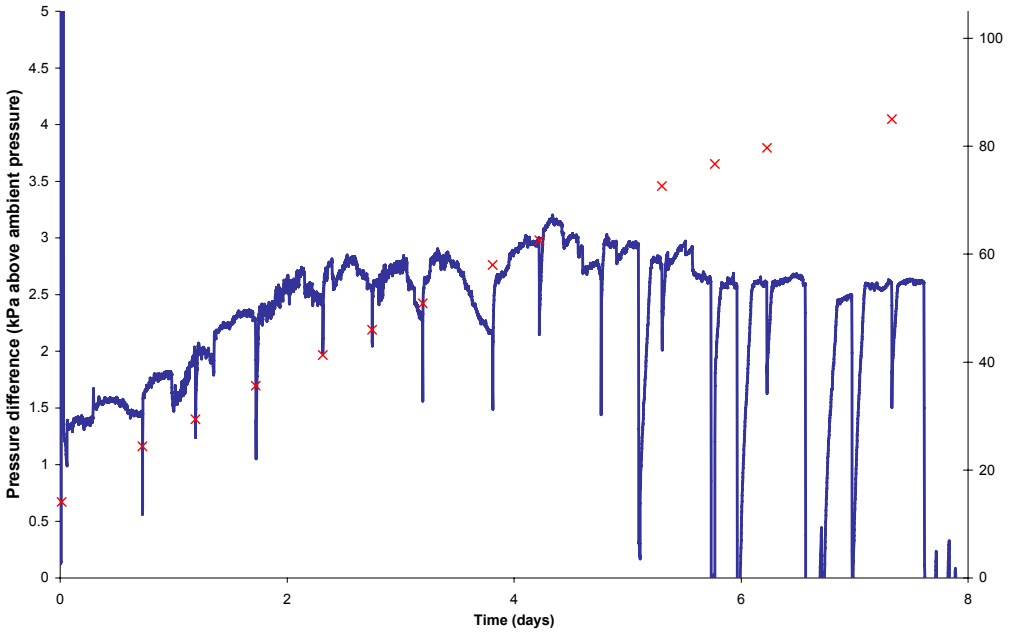
C.2.1 POM devices with 2% HPMC and hard plungers



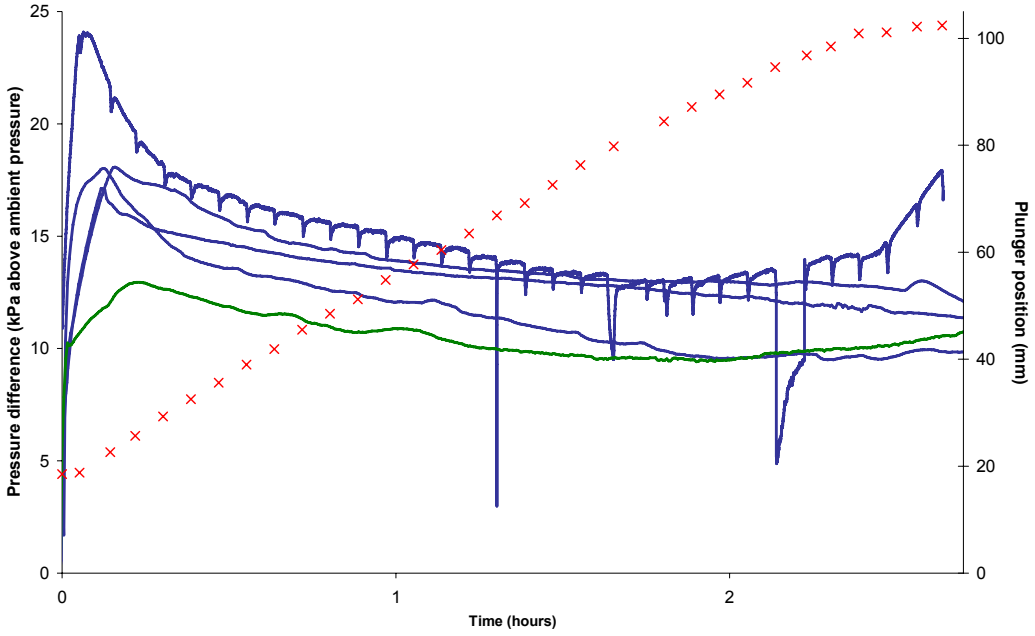
C.2.2 PE devices with 2% HPMC and soft plungers



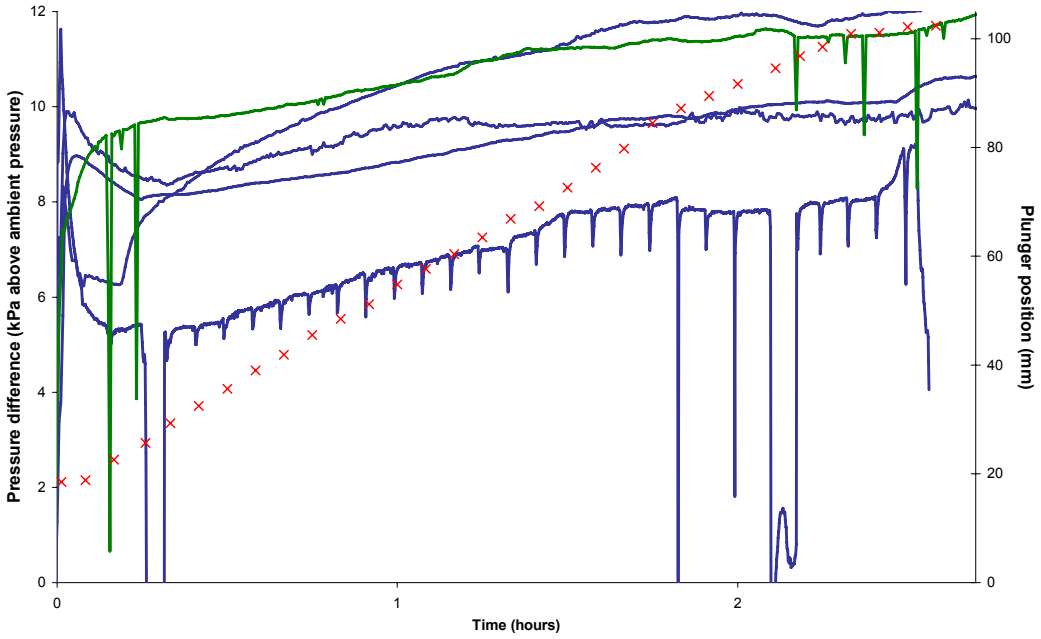
C.2.3 POM device with 2% HPMC, hard plunger, 1200-Ω resistor



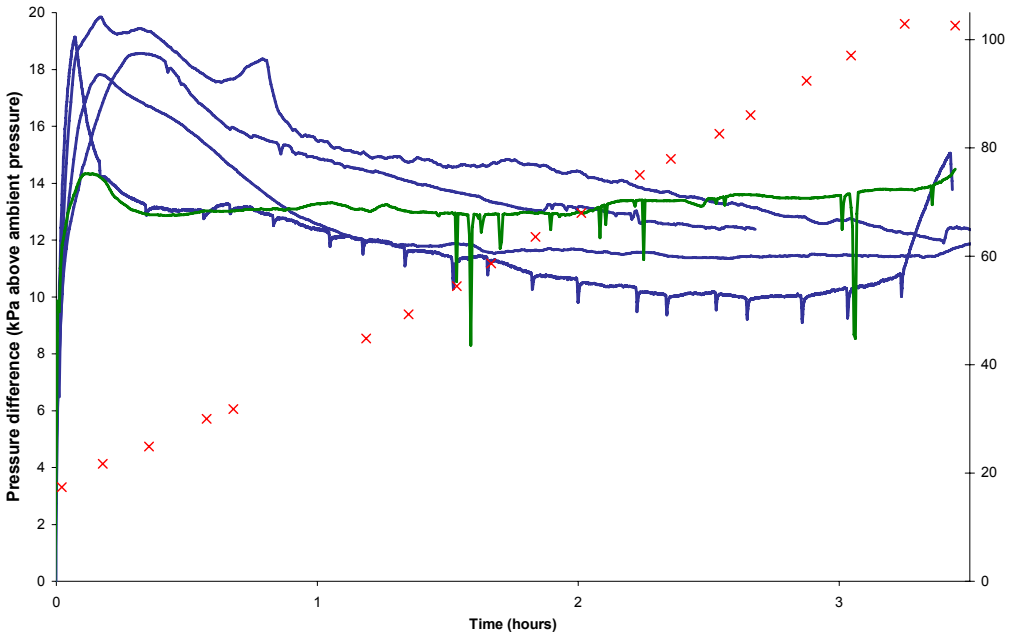
C.2.4 POM devices with 2% HPMC and soft plungers



C.2.5 POM devices with 2% HPMC and hard plungers



C.2.6 POM devices with 5% HPMC and soft plungers



C.3 10-day devices in vitro

C.3.1 Plunger position in mm

POM barrels with 2% HPMC and Hard plungers

	Day															Slope
Replicate	**0.0**	**0.8**	**2.0**	**3.0**	**4.0**	**5.0**	**6.1**	**6.9**	**8.0**	**9.0**	**10.0**	**11.0**	**11.9**	**15.8**	**17.6**	**(mm/day)**
1	10.3	21.2	32.8	41.1	53.2	63.1	74.2	83.1	93.1	93.2						10.4
2	9.9	20.8	33.7	0.0	53.8	63.4	75.3	83.5	92.6	92.2						10.5
3	8.2	19.0	31.6	40.0	50.6	61.2	71.9	81.5	92.0	92.9						10.4
4	6.7	17.8	30.8	39.0	50.4	59.3	71.3	80.3	91.5	93.7						10.4
5	10.4	21.8	34.3	42.8	53.8	63.6	75.4	83.5	92.8	93.4						10.4
6	9.8	21.6	33.6	51.5	71.7	91.4	93.4	93.0	93.0	93.2						0.0
7	9.5	21.1	33.7	42.9	54.5	64.3	75.6	84.0	93.1	93.0						10.6
Predicted	12.0	20.0	31.9	41.2	51.4	60.7	71.1	79.4	89.7	92.8	92.8	92.8	92.8	92.8	92.8	

POM barrels with 2% HPMC and Soft plungers

	Day															Slope
Replicate	**0.0**	**0.8**	**2.0**	**3.0**	**4.0**	**5.0**	**6.1**	**6.9**	**8.0**	**9.0**	**10.0**	**11.0**	**11.9**	**15.8**	**17.6**	**(mm/day)**
1	16.9	25.5	36.7	45.0	56.8	65.8	76.4	80.4	92.4	101.6	101.5					9.4
2	17.5	26.2	37.4	45.5	56.8	66.5	75.5	84.1	93.3	101.6	102.0					9.5
3	17.2	25.5	37.4	45.9	58.0	66.9	78.2	86.9	96.9	101.5	101.9					10.0
4	16.3	25.1	36.2	44.3	56.6	65.3	76.0	84.4	91.7	101.3	101.8					9.6
5	16.9	25.1	36.9	45.1	56.2	66.1	70.7	85.9	95.2	101.2	101.5					9.7
6	16.8	25.0	0.0	45.3	56.6	66.1	76.3	85.1	95.2	101.1	101.7					9.8
7	17.8	26.1	0.0	45.6	56.8	65.7	75.5	75.9	76.1	102.2	101.7					9.5
Predicted	17.0	24.2	34.8	43.2	52.3	60.6	69.9	77.3	86.5	95.3	102.0	102.0	102.0	102.0	102.0	

POM barrels with 5% HPMC and Hard plungers

	Day															Slope
Replicate	**0.0**	**0.8**	**2.0**	**3.0**	**4.0**	**5.0**	**6.1**	**6.9**	**8.0**	**9.0**	**10.0**	**11.0**	**11.9**	**15.8**	**17.6**	**(mm/day)**
1	7.9	18.8	31.8	37.4	51.5	61.0	71.7	78.3	78.4	79.0	78.8	78.9	79.9			10.2
2	7.4	18.5	30.7	36.5	49.9	60.4	71.0	79.3	81.8	82.9	82.6	82.3	83.2			10.3
3	7.7	18.4	30.7	36.6	49.8	60.4	70.4	78.5	82.0	82.4	82.9	82.8	83.2			10.1
4	5.1	5.1	5.5	5.2	5.6	5.1	5.4	4.8	4.5	4.9	4.5	4.8	4.4			
5	7.4	18.7	30.2	36.6	50.5	60.1	41.4	74.5	85.8	87.7	87.5	87.9	87.9			10.3
6	7.4	18.5	30.8	39.0	41.3	41.7	41.1	41.5	41.5	41.7	40.9	40.9	41.3			
7	6.1	6.2	5.9	9.1	22.1	31.4	35.5	34.8	35.2	35.4	34.3	34.4	35.2			

POM barrels with 5% HPMC and Soft plungers

	Day															Slope
Replicate	**0.0**	**0.8**	**2.0**	**3.0**	**4.0**	**5.0**	**6.1**	**6.9**	**8.0**	**9.0**	**10.0**	**11.0**	**11.9**	**15.8**	**17.6**	**(mm/day)**
1	17.6	27.0	38.0	45.9	57.7	64.9	71.4	76.6	85.1	95.6	101.4					8.4
2	17.0	25.0	35.5	42.9	53.9	61.2	70.5	78.6	88.3	99.5	101.7					9.0
3	17.0	17.3	17.6	17.5	17.7	18.0	17.7	17.4	18.3	18.0	18.0					
4	16.8	25.2	35.9	43.7	54.7	62.7	72.5	80.2	90.6	101.8	101.9					9.3
5	16.5	23.9	34.6	41.6	53.2	61.7	70.8	78.7	89.4	94.9	94.8	102.0				8.9
6	17.0	24.8	35.4	44.5	52.6	63.9	75.2	83.1	94.6	101.4	101.4					9.6
7	17.6	25.6	37.4	45.0	56.8	65.6	75.0	83.7	93.8	101.2	101.1					9.4
Predicted	17.0	24.2	34.8	43.2	52.3	60.6	69.9	77.3	86.5	95.3	102.0	102.0	102.0	102.0	102.0	

HDPE barrels with 2% HPMC and Soft plungers

	Day															Slope
Replicate	**0.0**	**0.8**	**2.0**	**3.0**	**4.0**	**5.0**	**6.1**	**6.9**	**8.0**	**9.0**	**10.0**	**11.0**	**11.9**	**15.8**	**17.6**	**(mm/day)**
1	19.9	19.9	20.0	19.8	20.2	20.2	20.5	20.1	20.2	19.9	14.9	19.8	19.6	20.3		
2	20.6	29.8	40.8	48.4	57.0	64.2	72.0	77.3	84.0	90.8	90.4	95.8	96.7	96.7		
3	24.2	34.8	46.1	53.7	62.7	69.7	70.0	70.8	69.7	76.6	82.6	88.3	87.1	87.4	86.8	
4	18.6	28.7	40.0	47.1	56.7	63.4	70.1	75.8	81.9	87.7	93.3	97.3	99.7	101.5		
5	23.9	23.7	23.9	24.2	24.1	23.9	24.0	23.9	23.5	23.3	23.8	24.2	23.6	23.5		
6	25.4	36.6	48.4	55.6	65.3	72.6	79.8	79.6	88.2	93.9	94.8	94.9	95.3	95.4		
7	25.9	37.2	48.3	55.8	65.2	65.7	66.1	65.6	65.3	72.8	73.2	77.4	77.7	77.2	77.2	
8	25.9	25.7	25.8	26.1	26.1	26.0	26.1	25.7	25.5	25.7	25.5	25.6	25.2	25.9		
Predicted	25.0	31.2	40.0	46.5	53.4	59.3	65.6	70.4	76.1	81.2	85.8	90.3	94.6	102.0	102.0	

C.3.2 Weight in g

POM barrels with 2% HPMC and Hard plungers

	Day														
Replicate	**0.0**	**0.8**	**2.0**	**3.0**	**4.0**	**5.0**	**6.1**	**6.9**	**8.0**	**9.0**	**10.0**	**11.0**	**11.9**	**15.8**	**17.6**
1	57.5	54.0	49.9	0.0	43.2	40.0	36.7	33.4	29.9	28.6					
2	57.4	54.0	49.3	0.0	42.9	39.5	35.8	33.3	30.0	28.8					
3	58.0	54.7	50.0	47.4	43.0	40.2	36.5	33.6	30.0	29.7					
4	58.0	54.7	50.5	47.4	43.9	40.9	39.3	33.8	30.2	29.5					
5	57.2	53.6	49.5	46.4	42.5	39.4	35.4	32.4	29.4	28.8					
6	56.8	53.1	48.7	43.1	36.3	30.1	29.1	28.9	28.7	28.8					
7	57.4	53.9	49.5	46.4	42.8	39.2	35.5	32.5	29.7	28.8					

POM barrels with 2% HPMC and Soft plungers

	Day														
Replicate	**0.0**	**0.8**	**2.0**	**3.0**	**4.0**	**5.0**	**6.1**	**6.9**	**8.0**	**9.0**	**10.0**	**11.0**	**11.9**	**15.8**	**17.6**
1	57.7	54.4	50.3	47.6	45.0	42.1	38.2	36.8	32.9	30.0	29.7				
2	58.3	55.2	51.6	48.8	44.8	42.0	38.8	36.0	32.9	30.0	29.6				
3	58.1	54.8	50.7	47.7	44.6	41.6	38.2	35.1	31.8	30.4	30.2				
4	57.5	54.0	50.1	47.3	45.4	42.3	38.9	35.8	33.5	30.2	29.7				
5	57.3	54.1	50.3	47.3	44.2	40.8	37.6	34.8	33.5	30.1	30.1				
6	57.8	54.7		48.1	45.5	42.3	38.8	36.0	31.3	30.5	29.9				
7	57.8	54.4		48.4	45.0	41.5	38.0	38.0	38.8	29.9	29.6				

POM barrels with 5% HPMC and Hard plungers

	Day														
Replicate	**0.0**	**0.8**	**2.0**	**3.0**	**4.0**	**5.0**	**6.1**	**6.9**	**8.0**	**9.0**	**10.0**	**11.0**	**11.9**	**15.8**	**17.6**
1	58.1	55.0	50.8	48.3	44.0	40.5	39.2	34.4	30.8	30.9	30.6	30.7	29.6		
2	58.1	55.3	51.1	48.8	44.3	40.7	37.0	34.4	31.0	30.8	30.7	30.8	29.4		
3	58.3	54.9	50.7	48.4	44.3	40.8	37.2	34.4	31.2	30.4	30.2	30.2	30.5		
4	58.5	58.3	58.4	58.2	58.1	57.9	58.0	58.0	57.9	57.7	57.7	57.6	58.0		
5	58.6	55.4	51.1	48.9	44.0	41.0	37.1	34.7	30.9	29.8	29.8	29.8	29.4		
6	58.6	55.1	50.8	47.8	43.9	40.6	37.2	35.8	35.5	35.1	35.3	35.3	33.0		
7	57.9	54.3	50.0	47.5	43.3	40.0	39.2	36.8	36.7	36.5	36.4	36.9	34.4		

POM barrels with 5% HPMC and Soft plungers

	Day														
Replicate	**0.0**	**0.8**	**2.0**	**3.0**	**4.0**	**5.0**	**6.1**	**6.9**	**8.0**	**9.0**	**10.0**	**11.0**	**11.9**	**15.8**	**17.6**
1	58.1	55.0	51.0	48.4	44.6	41.6	38.3	35.2	32.7	30.5	29.8				
2	58.5	55.3	51.7	48.8	45.0	42.2	39.0	36.2	33.0	30.5	30.1				
3	57.9	57.4	57.8	57.5	57.3	57.2	57.1	57.2	57.1	57.0	57.2				
4	58.6	55.7	52.0	49.2	45.5	42.5	39.3	36.4	33.1	30.1	29.9				
5	58.0	55.2	51.6	49.2	45.1	41.9	39.1	36.1	32.0	31.0	30.9	29.8			
6	58.2	55.2	51.8	48.8	45.5	41.8	38.0	35.1	32.0	30.2	30.1				
7	58.6	55.6	51.7	49.1	45.0	41.7	38.6	35.8	32.6	30.0	29.7				

HDPE barrels with 2% HPMC and Soft plungers

	Day														
Replicate	**0.0**	**0.8**	**2.0**	**3.0**	**4.0**	**5.0**	**6.1**	**6.9**	**8.0**	**9.0**	**10.0**	**11.0**	**11.9**	**15.8**	**17.6**
1	44.1	43.8	43.8	43.6	43.6	43.5	43.4	43.6	43.5	43.4	43.6	43.6	43.6	43.8	
2	43.5	39.9	36.2	33.6	32.2	30.2	28.0	26.0	24.0	21.6	20.0	18.3	17.6	17.1	
3	43.8	40.3	36.3	33.8	31.0	28.5	26.4	25.2	23.8	23.7	21.3	20.0	18.9	17.5	19.9
4	44.1	40.3	36.3	33.9	30.6	30.0	28.5	26.7	25.2	23.1	21.2	19.6	18.8	17.3	
5	43.9	43.8	43.7	43.6	43.4	43.6	43.5	43.5	43.5	43.7	43.8	43.4	43.6	43.6	
6	43.2	39.2	35.3	32.8	30.2	28.1	25.6	24.4	21.6	20.3	20.1	20.1	20.2	20.3	
7	43.1	39.2	35.3	33.1	30.3	27.9	25.6	24.0	22.9	21.7	20.9	20.6	18.9	17.3	17.3
8	43.2	43.4	43.1	43.0	42.8	42.8	42.7	42.8	42.7	42.8	42.8	42.8	42.8		

C.4 50-day devices in vitro *attempt three with lubricant*

C.4.1 Plunger position in mm

POM barrels with 2% HPMC and Hard plungers

Replicate \ Day	0.0	2.8	5.7	8.7	12.0	13.7	16.8	20.0	22.8	26.8	29.8	33.0	36.8	40.1	42.9	45.8	48.7	Rate (mm/day)
1	25.9	33.7	40.1	44.9	50.7	53.9	61.2	69.0	74.9	83.7	93.7	93.0	93.3	93.5	93.5	93.5	93.1	2.17
2	9.0	17.1	23.3	29.1	35.7	39.0	45.1	52.2	57.7	65.4	72.0	77.5	83.9	88.9	93.2	93.0	93.2	1.99
3	9.6	19.0	24.1	29.2	36.6	39.2	45.8	52.8	58.5	66.1	72.2	78.5	85.8	91.7	93.3	92.8	93.2	2.01
4	8.9	10.6	10.4	10.6	10.8	10.7	10.7	10.7	10.9	10.9	10.9	10.7	10.8	10.4	10.4	10.9	10.4	
5	8.2	8.7	8.3	8.5	0.0	8.2	8.7	8.5	8.2	8.5	8.4	8.3	8.3	8.4	8.5	8.3	8.4	
6	9.8	17.8	24.5	31.2	38.6	41.6	48.6	55.3	61.2	68.7	75.2	81.5	90.4	93.4	93.4	93.1	93.0	2.14
7	8.3	16.5	22.8	29.0	36.1	39.3	45.7	52.1	58.0	65.0	71.3	77.5	84.7	90.8	93.4	93.3	93.2	2.02
Predicted	11.4	17.5	23.8	30.2	37.1	40.8	47.2	53.8	59.4	67.4	73.5	79.8	87.2	92.8	92.8	92.8	92.8	2.04

POM barrels with 2% HPMC and Soft plungers

Replicate \ Day	0.0	2.8	5.7	8.7	12.0	13.7	16.8	20.0	22.8	26.8	29.8	33.0	36.8	40.1	42.9	45.8	48.7	Rate (mm/day)
1	21.6	27.3	33.6	39.6	45.8	48.4	49.5	51.2	51.5	51.4	51.4	51.1	51.1	51.3	51.1	51.2	51.0	1.98
2	20.9	26.8	32.7	37.0	44.1	46.8	51.9	53.9	53.9	53.3	53.3	53.4	53.6	53.9	54.0	53.4	53.7	1.84
3	22.7	29.7	34.6	39.3	44.7	48.0	50.6	54.9	59.1	60.8	60.8	60.7	60.4	60.1	60.9	60.7	60.5	1.77
4	36.5	40.0	45.8	51.5	57.0	58.4	64.6	65.5	66.1	66.0	66.2	66.1	66.0	66.0	66.6	66.5	66.3	1.69
5	26.3	28.6	34.0	38.7	44.2	45.9	46.2	46.0	46.5	46.6	46.9	46.9	47.0	46.4	46.9	47.2	46.6	1.54
6	36.2	43.0	43.2	43.1	43.2	43.1	43.1	43.0	43.0	43.4	43.4	42.8	42.9	43.0	42.9	42.9	43.5	
7	22.6	44.5	49.6	54.0	57.0	56.9	56.4	56.3	56.9	57.8	57.6	57.4	57.3	57.6	57.8	57.5	57.4	
Predicted	26.7	32.0	37.5	43.1	49.1	52.3	57.9	63.6	68.5	75.5	80.8	86.3	92.8	98.2	102.0	102.0	102.0	1.81

POM barrels with 5% HPMC and Hard plungers

Replicate \ Day	0.0	2.8	5.7	8.7	12.0	13.7	16.8	20.0	22.8	26.8	29.8	33.0	36.8	40.1	42.9	45.8	48.7	Rate (mm/day)
1	11.6	19.9	26.5	31.1	31.4	31.4	31.2	31.2	31.1	31.5	31.1	31.0	31.4	31.0	31.3	31.2	30.9	2.23
2	20.2	25.4	27.6	34.0	35.3	35.2	34.9	35.3	35.4	35.1	35.4	35.2	35.2	35.4	35.7	35.3	35.1	
3	9.8	18.1	24.4	30.4	36.0	40.1	46.1	53.2	58.8	56.7	72.4	78.8	85.7	91.6	93.7	93.6	93.6	2.08
4	7.5	8.3	8.0	8.3	8.3	8.5	8.8	8.0	8.6	8.0	8.4	8.0	7.9	8.0	8.8	8.2	8.3	
5	23.7	31.0	37.4	40.5	44.4	48.4	55.5	60.1	61.3	60.3	60.7	60.4	60.3	60.5	60.2	60.5	60.5	1.94
6	11.2	19.2	25.5	31.8	38.9	42.3	48.4	54.8	58.7	58.9	59.0	58.5	58.9	58.8	58.6	58.7	58.6	2.08
7	9.5	11.2	11.0	11.5	11.4	11.4	11.3	11.2	11.3	11.0	11.0	11.0	11.2	11.0	11.1	10.9	11.1	
Predicted	14.0	19.6	25.4	31.4	37.7	41.1	47.0	53.1	58.3	65.7	71.4	77.1	84.0	89.8	92.8	92.8	92.8	1.89

POM barrels with 5% HPMC and Soft plungers

Day

Replicate	**0.0**	**2.8**	**5.7**	**8.7**	**12.0**	**13.7**	**16.8**	**20.0**	**22.8**	**26.8**	**29.8**	**33.0**	**36.8**	**40.1**	**42.9**	**45.8**	**48.7**	**Rate (mm/day)**
1	34.5	39.6	45.3	50.1	55.4	58.8	64.2	69.0	74.4	77.9	82.3	87.5	90.0	94.6	94.1	94.7	94.4	1.60
2	34.0	37.9	43.3	47.8	53.3	56.2	64.3	66.3	72.8	82.6	82.8	83.7	83.6	83.6	83.5	83.9	83.4	1.74
3	36.0	40.6	46.4	51.2	58.3	60.3	60.5	60.8	61.1	61.0	61.1	61.5	60.5	61.1	61.2	61.4	61.1	1.81
4	41.0	45.8	51.6	55.9	62.3	65.1	71.3	75.8	81.1	83.5	83.1	83.2	82.9	82.9	82.8	83.1	82.4	1.76
5	37.0	42.8	48.6	53.0	53.8	54.0	53.9	53.4	53.9	53.1	53.6	53.4	53.1	53.5	53.3	53.4	53.2	1.84
6	33.9	38.6	44.0	48.2	55.1	57.4	58.4	58.5	58.4	58.1	57.9	58.3	58.2	58.3	57.8	58.2	57.7	1.72
7	38.9	44.0	49.7	55.1	60.0	60.4	60.9	60.9	60.9	60.9	60.9	60.0	60.6	60.7	60.8	60.7	60.8	1.78
Predicted	36.5	41.7	47.1	52.6	58.5	61.6	67.1	72.8	77.6	84.5	89.7	95.1	101.4	102.0	102.0	102.0	102.0	1.79

HDPE barrels with 2% HPMC and Soft plungers

Day

Replicate	**0.0**	**2.8**	**5.7**	**8.7**	**12.0**	**13.7**	**16.8**	**20.0**	**22.8**	**26.8**	**29.8**	**33.0**	**36.8**	**40.1**	**42.9**	**45.8**	**48.7**
1	24.6	31.5	35.8	39.6	42.1	42.3	45.0	46.3	47.5	47.8	48.3	48.5	48.4	48.7	48.5	50.2	48.7
2	24.9	31.4	36.8	39.8	43.4	43.5	45.6	47.7	49.1	49.8	50.3	50.2	50.0	50.5	50.1	50.2	50.0
3	24.7	31.2	35.7	38.1	41.1	42.7	44.4	46.1	47.6	49.2	50.2	51.1	51.0	51.2	52.3	52.6	52.9
4	32.8	34.4	37.0	38.3	39.1	40.3	40.6	42.0	42.8	42.9	44.3	45.3	44.9	45.4	46.4	46.7	47.6
5	25.0	25.1	24.9	25.1	24.7	24.2	24.7	24.5	24.7	24.7	24.3	23.8	24.2	24.1	24.5	24.1	24.3
6	22.2	27.9	32.2	34.7	36.9	38.5	40.5	42.7	44.1	45.2	45.2	47.6	48.1	48.0	48.3	48.4	48.8
7	41.1	48.6	52.2	54.2	55.4	55.2	55.2	55.3									
8	21.0	24.0	27.6	30.5	33.4	34.8	35.9	36.5	37.8	39.7	40.8	42.3	43.6	43.7	43.3	43.7	43.6
Predicted	27.0	28.7	30.2	31.5	32.8	33.4	34.3	35.1	35.6	36.3	36.8	37.2	37.6	37.9	38.1	38.3	38.5

C.4.2 Weight in g

POM barrels with 2% HPMC and Hard plungers

Day

Replicate	0.0	2.8	5.7	8.7	12.0	13.7	16.8	20.0	22.8	26.8	29.8	33.0	36.8	40.1	42.9	45.8	48.7
1	52.2	50.0	47.9	45.9	44.1	43.1	40.4	38.1	36.1	33.4	29.9	29.0	28.8	28.9	28.9	29.0	29.1
2	57.9	55.6	53.3	51.2	49.2	48.1	46.0	43.8	41.8	39.6	37.0	35.3	33.2	31.6	30.1	28.9	28.8
3	57.8	55.3	53.0	51.0	49.0	47.9	45.6	43.5	41.5	39.3	37.0	35.1	32.4	30.7	29.5	29.0	28.9
4	58.0	55.6	53.2	51.0	48.9	47.8	45.5	43.3	41.4	39.3	37.1	35.2	32.8	31.0	29.3	29.0	28.9
5	58.0	55.7	53.4	51.1	0.0	47.9	45.8	43.6	41.1	40.3	38.6	37.1	35.2	33.9	31.8	30.3	29.0
6	57.8	53.3	53.0	50.6	48.4	47.3	45.0	42.7	40.7	39.4	36.2	34.3	31.7	30.8	29.6	29.6	29.4
7	58.2	55.7	53.3	51.1	49.2	48.0	45.8	43.6	41.7	39.9	37.4	35.4	32.8	31.0	30.1	28.9	28.9

POM barrels with 2% HPMC and Soft plungers

Day

Replicate	0.0	2.8	5.7	8.7	12.0	13.7	16.8	20.0	22.8	26.8	29.8	33.0	36.8	40.1	42.9	45.8	48.7
1	57.2	55.0	52.7	50.3	48.2	47.2	45.6	43.8	41.1	39.5	37.5	35.5	32.9	31.3	29.9	29.8	30.0
2	57.3	55.2	53.0	51.0	48.8	47.6	45.7	44.0	42.0	39.7	37.5	35.8	33.9	32.4	30.6	29.7	29.6
3	56.8	54.4	52.4	50.5	48.6	47.5	45.3	43.1	41.6	39.7	34.1	35.3	33.1	31.3	29.7	29.7	29.6
4	52.9	51.6	49.3	47.0	45.0	43.8	41.4	40.4	38.3	35.6	34.1	31.9	30.2	30.1	30.1	30.1	30.1
5	57.1	55.0	52.8	50.2	48.3	47.2	45.1	43.1	40.9	38.4	36.9	34.6	32.2	30.5	29.7	29.7	29.7
6	52.0	49.7	47.4	45.2	43.1	42.0	39.7	37.6	35.6	33.6	32.2	30.3	29.6	29.6	29.9	29.6	29.6
7	51.9	50.1	47.8	45.7	43.7	42.4	40.6	38.1	36.3	34.3	31.5	30.3	30.2	30.2	30.2	30.2	30.2

POM barrels with 5% HPMC and Hard plungers

Day

Replicate	0.0	2.8	5.7	8.7	12.0	13.7	16.8	20.0	22.8	26.8	29.8	33.0	36.8	40.1	42.9	45.8	48.7
1	57.1	54.7	52.6	50.4	48.2	47.2	45.2	43.0	41.0	38.8	36.9	34.9	32.7	31.0	29.1	28.9	28.9
2	54.3	53.6	52.1	49.8	47.3	46.2	43.7	41.5	39.7	37.3	35.3	33.2	31.1	29.1	28.8	28.8	28.9
3	57.1	55.5	53.2	51.1	49.0	48.0	45.6	43.4	41.5	39.2	37.1	35.1	32.5	30.7	29.1	28.9	28.8
4	58.2	55.9	52.4	51.3	49.3	48.0	45.7	43.4	41.5	39.1	37.2	35.0	32.8	31.0	29.3	29.0	28.9
5	53.1	51.0	48.9	47.4	46.5	45.1	42.7	40.2	38.1	35.8	33.8	31.7	29.2	29.0	28.8	28.8	28.8
6	57.5	55.2	52.8	50.6	48.5	43.4	45.1	43.1	41.2	38.9	36.8	34.6	32.3	30.7	28.1	29.0	28.9
7	57.8	55.4	53.0	50.8	48.9	47.6	45.3	43.2	41.2	39.0	37.1	35.1	32.8	31.0	29.3	29.1	29.0

POM barrels with 5% HPMC and Soft plungers

Day

Replicate	**0.0**	**2.8**	**5.7**	**8.7**	**12.0**	**13.7**	**16.8**	**20.0**	**22.8**	**26.8**	**29.8**	**33.0**	**36.8**	**40.1**	**42.9**	**45.8**	**48.7**
1	52.7	50.9	48.9	47.1	45.2	44.2	42.0	40.4	38.5	39.3	35.1	34.0	32.9	31.3	30.9	29.7	29.2
2	53.5	52.0	50.1	48.2	46.2	45.3	42.6	41.4	39.1	35.4	35.1	33.7	30.7	29.9	29.9	29.9	29.9
3	53.0	51.4	49.2	47.1	44.9	44.1	41.9	40.5	38.4	36.1	34.2	32.0	30.0	29.8	29.7	29.8	29.8
4	51.0	49.3	47.2	45.4	43.2	42.2	39.8	38.1	36.1	34.5	32.3	30.3	29.4	29.4	29.7	29.3	29.4
5	51.9	49.7	47.6	45.6	44.8	43.7	41.2	39.1	37.4	35.1	33.2	31.2	29.8	29.8	29.7	29.7	29.7
6	54.1	52.4	50.4	48.7	46.5	45.3	42.9	40.7	39.0	36.7	34.8	32.8	30.5	30.2	30.2	30.2	30.1
7	51.0	50.1	47.9	45.8	43.7	42.6	40.6	38.6	37.1	34.8	32.6	29.9	29.8	29.7	29.7	29.8	29.8

HDPE barrels with 2% HPMC and Soft plungers

Day

Replicate	**0.0**	**2.8**	**5.7**	**8.7**	**12.0**	**13.7**	**16.8**	**20.0**	**22.8**	**26.8**	**29.8**	**33.0**	**36.8**	**40.1**	**42.9**	**45.8**	**48.7**
1	44.0	41.6	40.0	39.7	37.6	37.3	36.1	35.8	34.9	34.5	33.9	33.5	33.1	32.7	32.4	31.5	31.4
2	41.9	41.7	40.0	38.8	37.7	37.2	36.4	35.6	35.0	34.7	34.6	34.6	34.6	34.7	34.6	34.6	34.5
3	43.4	41.3	39.6	38.4	37.4	36.7	36.0	35.2	34.5	34.2	33.7	33.2	32.7	32.6	32.1	31.3	31.2
4	39.3	37.4	35.9	34.7	34.0	33.5	32.5	31.8	31.2	30.8	30.4	29.8	29.2	29.2	28.9	28.5	27.8
5	44.2	44.2	44.1	43.9	44.0	44.2	44.0	44.0	43.9	44.1	43.9	43.8	43.9	44.0	43.8	43.8	43.7
6	44.7	42.5	41.0	39.8	38.9	38.5	37.3	36.6	36.1	35.9	35.4	34.7	34.5	34.7	34.4	33.6	33.1
7	36.5	33.8	32.3	31.4	30.4	30.0	29.1	28.3									
8	45.6	44.2	42.7	41.5	40.7	40.0	39.2	38.8	38.2	37.8	37.1	36.4	36.1	36.2	36.2	36.1	36.0

C.5 10-day devices in vivo

C.5.1 Plunger position in mm

POM barrels with 2% HPMC and Hard plungers

	Day												
Replicate	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Slope (mm/day)**
1	7.8	21.3	33.4	45.7	57.8	69.2	80.0	93.4	93.5	93.0	93.2	91.4	12.4
2	9.3	23.6	35.4	46.6	56.1	58.9	59.9	60.7	72.9	84.7			11.7
3	6.6	20.1	38.7	44.8	56.4	58.9	68.3	77.3	84.2	76.1	76.3	75.9	12.4
4	7.3	15.4	29.1	31.7	32.6	32.5	32.0	32.1	31.3	31.5	30.6	30.8	
5	6.5	20.4	33.2	47.7	58.8	71.0	81.1	93.2	93.5	93.2			13.2
6	4.1	11.4	22.8	31.6	44.4	44.7	44.3	41.4	41.1	41.1	40.1	39.8	
7	9.7	24.2	31.5	37.5	42.0	45.0	47.7	47.9	41.1	49.3	49.7	49.0	
Predicted	7.3	17.2	27.0	36.8	46.6	56.3	66.0	75.7	85.3	92.8	92.8	92.8	9.8

POM barrels with 2% HPMC and Soft plungers

	Day												
Replicate	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Slope (mm/day)**
1	19.5	29.5	39.9	50.1	61.1	72.0	84.4	96.6	101.3	101.8	102.3	101.7	11.0
2	17.6	29.4	39.6	50.0	61.1	72.4	84.8	95.2	101.8	101.9	102.4	101.7	11.1
3	17.8	27.7	38.1	101.6									
4	16.3	26.9	37.8	50.0	62.8	74.8	86.1	98.9	101.7	101.7	102.1	101.7	11.9
5	22.2	33.5	43.7	54.7	65.8	76.2	88.3	99.6	101.7	101.6	102.2	101.8	11.0
6	16.9	27.3	38.1	48.9	59.4	70.7	83.2	94.0	101.9	102.4	102.1	101.9	11.0
7	16.2	24.4	34.5	46.5	52.6	60.5	73.4	83.4	93.2	101.2	102.2	102.3	9.5
Predicted	18.0	26.8	35.6	44.4	53.1	61.8	70.4	79.0	87.6	96.2	102.0	102.0	8.7

POM barrels with 5% HPMC and Hard plungers

	Day												
Replicate	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Slope (mm/day)**
1	6.5	19.6	31.4	42.3	54.4	66.1	80.5	92.6	92.7	93.4	93.7	93.3	12.2
2	7.2	19.4	30.6	42.8	55.5	66.3	76.5	81.8	93.2				11.9
3	5.7	5.7	5.7	12.9	25.2	37.3	49.5	62.3	73.6				12.2
4	7.5	22.4	34.0	46.2	58.5	70.3	83.9	93.7	93.7	92.4	93.6	93.4	12.3
5	6.4	20.2	31.5	43.1	54.6	67.1	79.0	91.4	93.9	0.0	0.0	0.0	12.0
6	7.8	20.5	32.9	46.2	57.2	69.1	79.2	85.6	93.5	93.2	93.1	92.3	12.0
7	4.3	4.4	4.3	16.8	28.2	40.4	53.3	65.8	77.8	90.4	93.3	93.5	12.3
8	7.9	10.5	17.0	28.3	40.2	52.0	65.2	77.3	89.5				11.6
Predicted	6.5	15.6	24.7	33.8	42.9	51.9	61.0	69.9	78.9	87.8	92.8	92.8	9.1

POM barrels with 5% HPMC and Soft plungers

	Day												Slope
Replicate	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**(mm/day)**
1	17.9	27.3	38.2	50.8	62.1	74.4	86.0	98.3	101.6	101.8	101.7	101.8	11.6
2	18.0	28.7	40.1	50.5	61.1	71.9	81.8	91.8	101.4	101.8	101.9	102.0	10.6
3	18.7	28.4	38.9	49.9	60.6	70.7	82.4	92.8	101.4	101.9	102.0	102.1	
4	16.9	26.9	37.2	48.7	59.1	70.2	83.4	94.0	101.6	102.3	101.9	101.8	11.1
5	18.4	29.5	40.3	50.7	61.9	72.0	83.5	94.9	100.8	102.2	102.1	102.0	10.9
6	17.6	29.3	39.5	51.0	62.2	74.2	86.5	98.3	101.1	101.4	101.9	102.1	11.5
7	17.7	30.2	40.0	51.6	61.8	73.0	85.8	97.0	100.4	99.9	101.9	102.0	11.2
Predicted	17.9	26.7	35.4	44.2	52.9	61.6	70.2	78.9	87.5	96.0	102.0	102.0	8.7

HDPE barrels with 2% HPMC and Soft plungers

	Day												Slope
Replicate	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**(mm/day)**
1	20.8	32.8	44.6	58.3	69.7	81.4	93.2	101.3	101.3	101.4	101.8	101.6	12.1
2	26.0	40.9	54.6	68.4	81.0	85.3	85.3	87.2	100.4				13.8
3	25.3	40.9	52.7	65.3	76.2	87.5	98.1	97.0	97.7				12.0
4	22.1	33.8	44.6	46.9	49.9	57.1	65.7	74.1	80.2	90.6	98.4	100.8	
5	26.1	40.8	53.7	68.2	80.4	91.5	101.2	101.5	102.0	101.5	101.1	101.3	12.6
6	24.6	39.2	51.2	65.5	78.2	87.7	97.4	101.4	101.0	101.3	101.3	101.2	12.2
7	23.1	36.3	48.9	55.5	56.2	63.0	68.0	84.2	86.9	83.6	86.3	64.7	
8	25.0	38.8	51.5	64.8	74.5	80.2	92.2	101.0	101.3				12.5
Predicted	24.1	31.8	39.0	45.9	52.5	58.7	64.6	70.2	75.6	80.6	85.4	90.0	7.1

C.5.2 Weight in g

POM barrels with 2% HPMC and Hard plungers

	Day											
Replicate	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	58.0	53.8	49.9	45.6	41.7	37.9	34.9	31.0	29.9	29.4	30.4	30.7
2	57.3	53.0	50.2	46.7	43.2	40.4	38.4	36.3				
3	58.3	54.1	50.4	46.1	42.2	41.2	38.4	34.9	29.8	29.3	29.5	28.9
4	58.0	54.9	49.9	47.0	49.5	49.5	48.8	47.6	46.9	45.7	44.8	43.4
5	58.4	54.0	50.4	46.5	42.3	38.2	34.4	29.1				
6	58.5	54.5	52.2	48.9	44.7	43.9	48.4	48.8	45.7	42.8	41.8	40.9
7	57.6	54.2	50.4	48.5	47.3	46.1	45.3	45.7	45.7	45.4	45.4	45.4

POM barrels with 2% HPMC and Hard plungers

	Day											
Replicate	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	58.0	53.8	49.9	45.6	41.7	37.9	34.9	31.0	29.9	29.4	30.4	30.7
2	57.3	53.0	50.2	46.7	43.2	40.4	38.4	36.3				
3	58.3	54.1	50.4	46.1	42.2	41.2	38.4	34.9	29.8	29.3	29.5	28.9
4	58.0	54.9	49.9	47.0	49.5	49.5	48.8	47.6	46.9	45.7	44.8	43.4
5	58.4	54.0	50.4	46.5	42.3	38.2	34.4	29.1				
6	58.5	54.5	52.2	48.9	44.7	43.9	48.4	48.8	45.7	42.8	41.8	40.9
7	57.6	54.2	50.4	48.5	47.3	46.1	45.3	45.7	45.7	45.4	45.4	45.4

POM barrels with 5% HPMC and Hard plungers

	Day											
Replicate	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	58.7	54.8	50.9	47.0	43.2	39.0	34.4	30.3	29.6	29.1	28.7	29.0
2	58.0	54.4	51.0	47.0	43.0	39.3						
3	58.7	55.8	52.9	49.9	48.7	46.4						
4	58.2	53.8	49.8	45.7	41.6	37.6	33.2	29.6	29.3	30.3	29.1	29.0
5	58.0	54.1	50.6	46.6	42.8	38.7	34.7	30.6	29.3			
6	57.7	54.0	50.1	46.0	42.1	38.1	34.9	32.8	29.7	29.3	29.1	29.9
7	58.5	55.6	53.8	50.5	48.2	45.7	41.8	38.6	35.2	31.0	29.7	29.3
8	58.2	55.2	52.5	49.2	47.2	42.9	39.5	35.4	31.5			

POM barrels with 5% HPMC and Hard plungers

	Day											
Replicate	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	58.7	54.8	50.9	47.0	43.2	39.0	34.4	30.3	29.6	29.1	28.7	29.0
2	58.0	54.4	51.0	47.0	43.0	39.3						
3	58.7	55.8	52.9	49.9	48.7	46.4						
4	58.2	53.8	49.8	45.7	41.6	37.6	33.2	29.6	29.3	30.3	29.1	29.0
5	58.0	54.1	50.6	46.6	42.8	38.7	34.7	30.6	29.3			
6	57.7	54.0	50.1	46.0	42.1	38.1	34.9	32.8	29.7	29.3	29.1	29.9
7	58.5	55.6	53.8	50.5	48.2	45.7	41.8	38.6	35.2	31.0	29.7	29.3
8	58.2	55.2	52.5	49.2	47.2	42.9	39.5	35.4	31.5			

HDPE barrels with 2% HPMC and Soft plungers

	Day											
Replicate	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
1	54.0	41.0	37.2	32.8	28.8	24.8	20.4	18.1	17.8	17.0	17.0	17.0
2	43.6	38.4	33.9	29.2	25.0	23.5	23.5	20.4	17.3			
3	43.6	38.4	34.6	30.3	0.0	0.0	0.0	0.0	0.0			
4	43.6	39.3	36.1	35.1	34.6	32.2	29.8	27.4	25.3	21.9	19.3	18.4
5	43.8	38.6	34.3	29.4	25.4	21.5	18.3	17.1	17.0	17.0	17.0	17.1
6	43.8	54.9	35.1	30.2	26.2	22.8	19.5	18.2	18.0	17.2	17.2	17.2
7	44.4	39.4	35.6	33.1	33.1	30.9	28.1	22.0	21.8	18.8	26.4	20.7
8	43.1	38.4	34.8	29.6	26.3	22.5	18.7	18.0	17.2			

C.6 50-day devices in vivo

C.6.1 Plunger position in mm

POM barrels with 2% HPMC and Hard plungers

	Day												
Replicate	**0**	**3**	**6**	**10**	**13**	**18**	**21**	**24**	**28**	**31**	**34**	**38**	**Slope (mm/day)**
1	7.0	16.8	23.2	36.6	37.1	45.5	54.1	55.3	56.2	57.0	57.6	58.6	2.9
2	8.2	18.9	25.9	37.5	42.4	54.9	63.6	77.2	93.9	95.2			2.9
3	7.1	17.9	26.5	37.3	37.3	38.5	39.3	42.0	47.9	47.4	47.5	47.2	3.0
4	7.8	18.7	29.3	34.2	39.7	39.5	40.2	38.6	39.7	39.5	39.4	38.4	2.7
5	8.2	18.0	27.1	39.6	42.3	48.1	58.7	73.5	87.3	93.4			3.1
6	10.0	20.4	28.6	41.8	43.7	57.3	73.3	89.4	93.4	93.0			3.1
7	10.2	21.5	32.1	51.6	54.6	58.0	60.3	75.6	80.8	84.9	86.6	90.6	4.1
Predicted	10.0	16.6	23.2	31.9	38.3	46.7	53.0	61.3	67.4	75.5	81.5	89.4	

POM barrels with 2% HPMC and Soft plungers

	Day												Slope
Replicate	**0**	**3**	**6**	**10**	**13**	**18**	**21**	**24**	**28**	**31**	**34**	**38**	**(mm/day)**
1	20.3	28.4	34.3	43.4	45.8	52.0	57.0	64.7	70.8	77.2	81.1	84.1	1.7
2	19.7	28.8	35.8	46.9	54.0	67.9	73.2	82.3	90.9	100.8			2.6
3	20.7	29.4	36.6	47.3									2.6
4	20.4	28.7	35.8	46.6	54.0	67.4	67.4	86.1	96.5	101.4			2.6
5	21.3	29.5	36.2	47.6	48.4	66.1	66.1	78.4	88.3	90.6	91.1	90.6	2.3
6	21.0	26.6	32.3	41.7	47.8	58.2	64.7	71.3	78.3	83.0	83.9	82.7	2.1
7	19.4	28.1	35.5	46.7	53.8	65.3	71.9	80.7	87.7	91.8	96.8	97.9	2.4
Predicted	20.4	26.2	32.0	39.6	45.2	52.7	58.2	65.5	70.8	78.0	83.2	90.2	

POM barrels with 5% HPMC and Hard plungers

	Day												Slope
Replicate	**0**	**3**	**6**	**10**	**13**	**18**	**21**	**24**	**28**	**31**	**34**	**38**	**(mm/day)**
1	8.8	15.1	22.2	33.2	34.4	48.7							2.5
2	8.8	10.3	11.6	23.3	32.6	47.8	40.8	42.0	42.0				3.0
3	8.0	13.0	21.1	33.2	40.5	55.1	63.5	74.7	80.6				2.7
4	8.7	9.2	11.1	21.6	25.1	36.6	43.2	54.5	24.6	25.2	24.1	24.0	2.3
5	9.6	16.1	25.8	37.9	40.7	52.4	52.9	37.2	38.2	38.2	40.4	37.4	2.9
6	9.9	11.8	13.2	26.0	34.3	41.8	50.6	61.6	73.4	81.9	89.3	93.4	2.7
7	9.0	10.4	15.5	27.8	28.2	38.6	47.4	59.3	69.7	77.2	85.9	93.3	2.8
Predicted	8.9	12.5	18.0	30.0	34.3	46.0	53.8	65.2	74.6	82.9	89.6	93.4	

POM barrels with 5% HPMC and Soft plungers

	Day												Slope
Replicate	**0**	**3**	**6**	**10**	**13**	**18**	**21**	**24**	**28**	**31**	**34**	**38**	**(mm/day)**
1	16.4	23.3	31.1	40.3	45.7	54.2	54.2	73.3	84.4	91.9	98.3	101.5	2.3
2	17.8	25.1	32.6	44.1	51.8	66.2	66.2	86.5	97.9	101.3			2.8
3	18.0	25.0	31.8	41.8	52.9	61.5	61.5	70.3	91.0	101.2			2.5
4	17.0	24.3	31.0	42.0	49.2	63.2	71.6	81.7	87.4	88.9	88.9	88.9	2.7
5	18.0	26.3	33.4	44.7	52.0	66.7	75.3	87.0	99.6	101.3			2.9
6	21.5	30.0	37.9	50.6	58.3	59.4	64.7	72.4	81.6	88.3	96.8	101.4	2.9
7	18.9	26.5	33.1	43.5	49.5	52.4	53.7	58.5	62.6	94.0	101.6	101.4	2.4
Predicted	18.2	24.1	29.8	37.5	43.1	50.6	56.1	63.4	68.9	76.0	81.3	88.2	

HDPE barrels with 2% HPMC and Soft plungers

	Day												Slope
Replicate	**0**	**3**	**6**	**10**	**13**	**18**	**21**	**24**	**28**	**31**	**34**	**38**	**(mm/day)**
1	25.5	38.3	49.0	52.3	63.5	79.9	90.5	98.5	99.5	100.2			
2	30.7	45.0	55.3	63.9	52.6	53.0	53.4	54.0	55.4	57.6			
3	23.7	30.6	40.9	55.5	70.8	101.3	101.2	101.3	101.3	101.0			
4	25.9	40.4	49.6	67.1	76.0	88.0	95.6	100.4	101.4	101.7			
5	25.3	36.2	47.9	59.3	59.2	67.0	69.8	71.4	72.7	83.3			
6	24.7	38.3	50.2	66.1	72.7	80.0	80.0	80.9	82.6	83.3			
7	25.3	38.2	49.1	63.3	68.9	67.7	67.7	72.5	72.9	73.0			
8	25.6	41.3	52.8	69.2	78.6	91.9	91.9	101.4	101.3	101.2			
Predicted	25.8	27.8	29.6	31.5	32.8	34.1	35.0	35.9	36.5	37.1	37.6	38.0	

C.6.2 Weight in g

POM barrels with 2% HPMC and Hard plungers
Day

Replicate	0	3	6	10	13	18	21	24	28	31	34	38
1	57.9	56.2	55.9	54.6	54.7	54.6	52.3	48.0	44.6	40.8	39.4	35.8
2	57.8	54.5	51.8	48.8	47.3	43.0	40.2	35.8	30.4	29.7		
3	58.1	55.2	53.2	52.0	51.9	52.0	52.1	49.6	41.4	37.2	36.4	34.6
4	57.7	54.7	52.3	49.8	48.2	48.2	48.1	48.0	48.2	46.8	45.4	41.4
5	57.7	54.7	52.0	48.1	47.1	45.4	42.0	37.3	32.8	29.9		
6	57.2	54.3	51.7	47.3	46.9	0.0	40.2	35.8	32.2	30.0		
7	57.1	53.9	51.3	48.9	48.6	48.8	48.6	45.2	40.8	36.8	35.6	35.4

POM barrels with 2% HPMC and Soft plungers
Day

Replicate	0	3	6	10	13	18	21	24	28	31	34	38
1	57.0	54.8	53.1	50.6	49.5	47.4	45.7	43.2	40.7	38.7	37.4	35.8
2	57.0	54.2	51.9	48.2	46.2	41.4	39.8	37.1	34.2	31.2		
3	56.7	54.3	52.8	48.2	45.7	41.8	41.8	35.6	32.5	30.0		
4	56.9	54.2	51.9	48.7	46.0	41.8	41.8	35.7	32.2			
5	57.1	54.6	52.6	48.9	47.9	42.5	42.5	36.9	35.2	34.5	34.4	34.4
6	57.2	55.9	54.2	50.9	48.4	44.3	42.7	40.6	38.5	36.8	36.8	36.8
7	56.8	55.3	52.8	48.9	46.7	42.7	40.6	37.7	35.4	33.9	32.2	32.0

POM barrels with 5% HPMC and Hard plungers
Day

Replicate	0	3	6	10	13	18	21	24	28	31	34	38
1	57.9	55.5	53.1	50.0	49.6	45.0						
2	57.8	54.7	53.1	50.0	47.8	45.8	45.8	46.0	45.9	45.9	45.8	44.6
3	58.2	55.9	53.7	50.1	47.8	43.3	40.3	36.9	34.6	31.8	29.8	29.2
4	57.9	55.5	53.6	50.9	50.0	46.6	45.0	42.7	44.3	44.4	44.4	44.1
5	57.4	55.1	52.0	48.1	46.3	40.9	37.6	37.0	33.5	32.4	31.8	30.6
6	57.5	55.0	54.6	50.9	49.2	46.6	44.3	40.7	36.8	34.3	32.0	29.4
7	57.9	55.1	53.1	49.3	49.1	46.8	44.9	41.7	38.6	36.0	33.0	30.4

POM barrels with 5% HPMC and Soft plungers
Day

Replicate	0	3	6	10	13	18	21	24	28	31	34	38
1	58.6	56.4	53.9	50.5	48.5	46.0	46.0	40.2	36.6	34.0	31.6	30.6
2	58.2	55.8	53.5	50.0	47.4	42.6	42.6	35.7	31.9	30.6		
3	57.7	55.4	53.4	50.0	47.0	43.6	43.6	37.6	34.3	31.2		
4	57.9	55.8	53.8	50.5	47.9	43.7	40.9	37.4	35.9	35.0	35.0	35.0
5	58.0	56.0	53.4	49.7	47.2	42.2	39.3	35.3	31.5	30.6		
6	57.1	54.2	51.8	47.7	44.9	44.5	43.0	39.8	37.2	34.6	32.2	30.4
7	58.3	56.0	54.0	50.4	48.4	47.4	46.9	45.3	44.0	33.5	29.8	30.0

HDPE barrels with 2% HPMC and Soft plungers
Day

Replicate	0	3	6	10	13	18	21	24	28	31	34	38
1	43.3	39.0	35.8	34.8	31.2	25.6	22.0	19.4	18.8	18.8		
2	42.1	37.7	33.8	30.5	23.0	23.1	23.1	17.6	18.0	17.9		
3	43.9	40.5	37.2	32.8	28.0	18.0	18.1	17.9	17.9	17.7		
4	43.7	38.8	36.0	30.6	27.5	23.2	20.5	19.0	17.4	18.3		
5	43.6	39.2	36.1	31.3	27.3	18.4	17.9	17.3	17.6	25.1		
6	43.9	39.7	35.7	30.4	27.2	25.6	25.6	25.6	25.1	25.1		
7	43.5	39.5	36.0	31.0	28.8	28.2	28.2	18.6	18.4	18.4		
8	43.8	38.8	34.8	29.2	26.3	21.5	23.5	18.1	19.6	18.3		

C.7 Composition of non-air gas in devices

C.7.1 10-day *in vivo*

	Methane	Carbon Dioxide	Hydrogen
POM barrels with 2% HPMC and Soft plungers	1.0%	15.8%	83.2%
	2.9%	17.3%	79.7%
	4.9%	15.0%	80.1%
POM barrels with 5% HPMC and Soft plungers	0.4%	11.9%	87.7%
	0.5%	12.5%	86.9%
	0.6%	0.2%	99.2%
HDPE barrels with 2% HPMC and Soft plungers	6.3%	31.1%	62.7%
	11.2%	37.7%	51.1%
	20.1%	8.8%	71.0%
	17.6%	20.9%	61.4%
	7.0%	28.3%	64.7%

C.7.2 50-day *in vivo*

	Methane	Carbon Dioxide	Hydrogen
POM barrels with 2% HPMC and Soft plungers	2.4%	36.6%	61.0%
POM barrels with 5% HPMC and Soft plungers	2.1%	37.7%	60.2%
	2.3%	33.1%	64.6%
	2.4%	35.9%	61.7%
HDPE barrels with 2% HPMC and Soft plungers	19.5%	61.9%	18.5%
	42.1%	57.9%	0.0%
	14.2%	25.3%	60.4%
	17.8%	61.3%	20.9%
	37.9%	61.0%	1.1%
	44.8%	55.2%	0.0%
	43.5%	56.5%	0.0%
	30.8%	69.2%	0.0%

Appendix D: A model bioactive

D.1 Phlorizin

Phlorizin (glucose, 1-[2-(beta.D-glucopyranosyloxy)-4, 6-dihydroxyphenyl]-3-(4-hydroxyphenyl)-1-propanone) was considered as a model bioactive in this study (Figure C-1). It generates the physiological response of reduced blood glucose concentration and increased glucose excretion in urine (Ehrenkranz et al. 2005). Both of these responses have been observed in cattle when phlorizin was administered by subcutaneous injection (Veenhuizen et al. 1988; Bradford and Allen 2005; Meier et al. 2005). Use of phlorizin as a research tool to simulate hypoglycaemia in cattle is being investigated (Meier et al. 2005). An intraruminal controlled-release device would provide a convenient administration method.

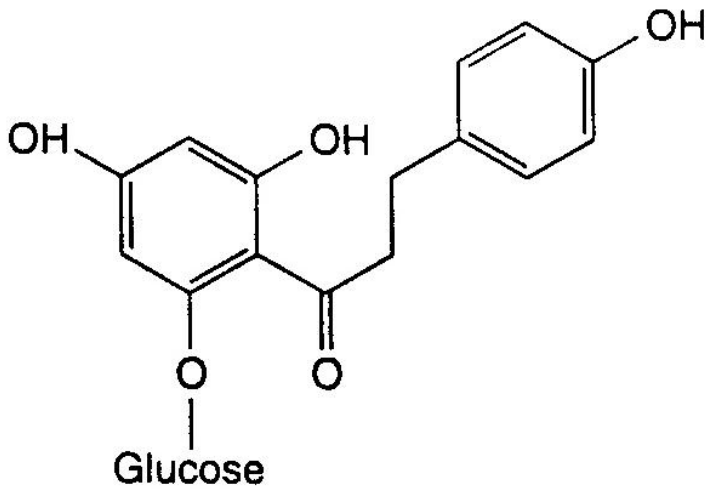


Figure D-1: Chemical structure of phlorizin

D.2 Response from phlorizin in the rumen

Phlorizin was administered directly to the rumen of fistulated cattle to test the physiological response. Two cows were given phlorizin twice daily at 200 and 400 g·day^{-1} of 60% phlorizin (120 and 240 g·day^{-1} of phlorizin) for seven days. This was much greater than the 2 to 8 g·day^{-1} previously administered subcutaneously (Veenhuizen et al. 1988; Amaral-Phillips et al. 1993; Bradford and Allen 2005; Meier et al. 2005) as some degradation in the rumen was anticipated. Only two animals were used to comply with ethical approval.

Glucose concentration in the blood and urine of both cows was measured twice daily and showed no significant change during the trial, indicating that phlorizin either did not produce a physiological response when delivered to the rumen or is extensively degraded. Experiments were performed to investigate the stability of phlorizin in the rumen.

D.3 Stability of phlorizin

D.3.1 Materials

Calcium chloride anhydrous (source unknown), carbon dioxide (Air Liquide, New Zealand), citric acid (AnalaR, BDH, England), cysteine hydrochloride (source unknown), disodium hydrogen phosphate (Analytical grade, Labserv), hydrochloric acid (36%, Ajax Finechem, Australia), magnesium chloride anhydrous (source unknown), methanol (Methyl Alcohol Anhydrous, Mallinckrodt Baker inc, U.S.A.), milli-q water, 95% phlorizin (source unknown), phloretin (124K7042, Sigma-Aldrich inc., U.S.A.), phlorizin (95% and 60%, source unknown), potassium chloride (reagent grade, Scharlau Chemie, Spain), sodium azide (Source unknown), sodium bicarbonate (Labserv), sodium chloride (source unknown), sodium hydroxide (AnalaR, BDH, England), sodium sulphide (source unknown), tris(hydroxymethyl)aminomethane (Tris(base), Ultrapure Bioreagent, BioSpectra, USA).

D.3.2 Equipment

Column (Symmetry Shield™ RP_{18} 5-µm 6.6 × 250 mm, Waters, Ireland), filters (GV 0.22µm, Millipore, Ireland), HPLC (ÄKTAbasic 10, Amersham Biosciences, Sweden), mincer R70 (Compact meat mincer, Krefft GmbH, Germany), pH meter (pH 510, Eutech Instruments, Singapore), syringe (30 mL and 1 mL Terumo, U.S.A.), syringe filters (Millex-GS 33-mm, MCE and PVDF, Millipore, Ireland), water bath (GLS400 and OLS200, Grant Instruments, England).

D.3.3 HPLC method

An HPLC method based on that of Escarpa and Gonzalez (1998) was developed to quantify phlorizin. All solvents and samples were filtered prior to use and pumped at 1 mL minute^{-1}. The column was first equilibrated with 1 column volume of 5% methanol, 95% water. Sample was loaded onto the column by pumping 2 mL solvent through a 100µL sample loop. Unbound sample was then washed through with an additional column volume before the proportion of methanol was increased to 55% where it remained for 1 mL before being linearly increased to 75% over three column volumes. Methanol concentration was then increased to 100% for 2.5 column volumes. Phlorizin was detected by absorbance at 280 nm.

A typical chromatogram for a 0.5 g·L^{-1} phlorizin in water (Figure D-2) shows a small peak at about 19.5 min, which is probably phlorizin without the glucose moiety (also known as phloretin). Pure phloretin produced a similar peak.

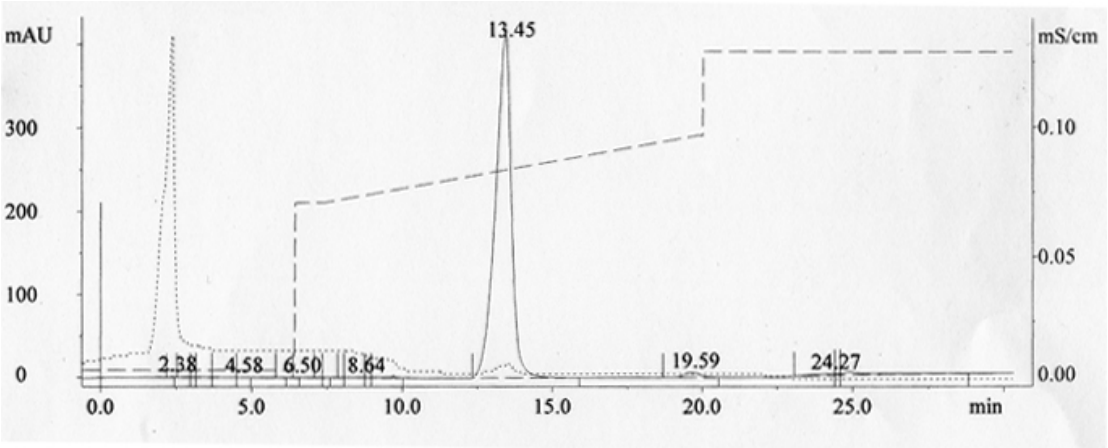


Figure D-2: Typical chromatogram for phlorizin

Results (Figure D-3) showed a linear relationship (R^2=0.99996) between log peak area and log concentration of phlorizin between 10^{-4} and 1 g·L^{-1}. Samples were tested in increasing phlorizin concentrations as residual phlorizin from each run eluted in the subsequent run. Phlorizin was dissolved in 50% water, 50% methanol at concentrations greater than 1 g·L^{-1} (points shown in green) due to the poor solubility of phlorizin in water

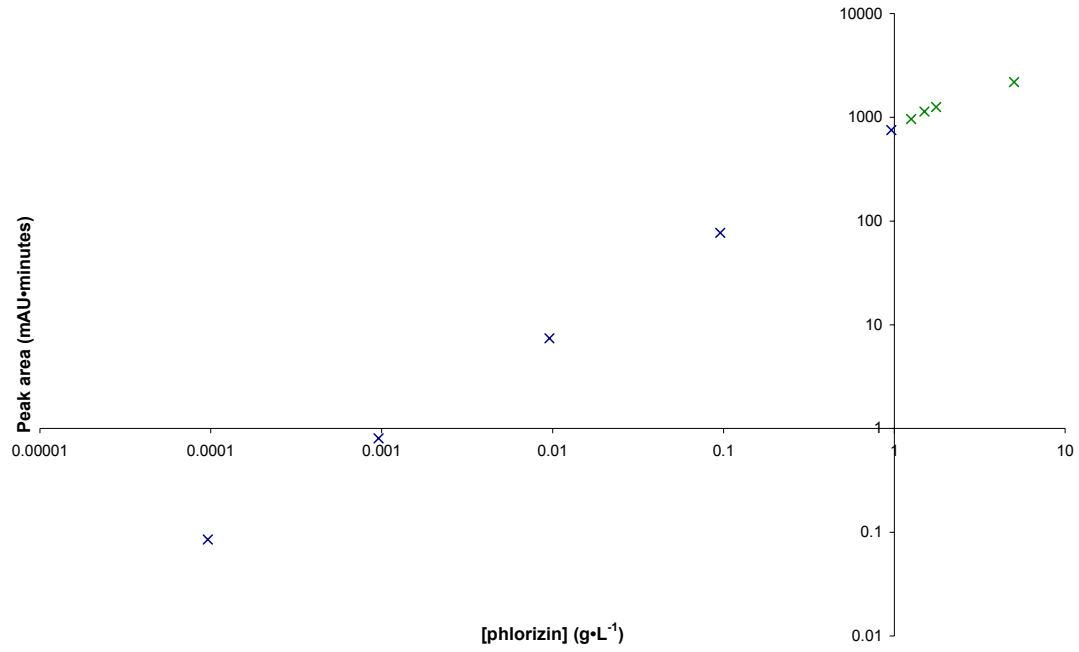


Figure D-3: Log of phlorizin concentration vs. log of peak area

D.3.4 Stability in rumen fluid *in vitro*

Phlorizin was incubated with rumen fluid *in vitro* to determine its stability under the approximate conditions of the rumen.

D.3.4.1 McDougall's buffer

$NaHCO_3$	9.8 $g \cdot L^{-1}$
$Na_2HPO_4.12H_2O$	9.3 $g \cdot L^{-1}$
NaCl	0.47 $g \cdot L^{-1}$
KCl	0.57 $g \cdot L^{-1}$
$CaCl_2$ anhydrous	0.04 $g \cdot L^{-1}$
$MgCl_2$ anhydrous	0.06$g \cdot L^{-1}$

95% phlorizin was added to a concentration of 0.083 or 0.021 $g \cdot L^{-1}$. McDougall's buffer was saturated with carbon dioxide before use.

D.3.4.2 Reducing agent

Cysteine hydrochloride	0.315 g
Water	48 mL
1 $mol \cdot L^{-1}$ Sodium hydroxide	2 mL

Sodium sulphide 0.315 g

Reducing agent was made immediately before use.

D.3.4.3 Rumen fluid incubation

Duplicate incubations were done with final phlorizin concentrations of 0.0161g·L^{-1} and 0.0645g·L^{-1} in separate bottles for each sample. 50-mL Schott bottles were fitted with bicycle valves to allow gases to escape. 2.5 g of minced, frozen lucerne was placed in each incubation bottle and incubated at 39°C for 1 hour. 12 mL of McDougall's buffer, 0.5 mL of reducing agent and 3 mL of fresh, strained rumen fluid was added to each bottle, which was then incubated at 39°C in a water bath and shaken at 90 oscillations per minute. Samples were removed after 0, 1, 2, 4, 6, 8, 10, 12 and 24 hours and stored at -18°C. Control samples without rumen fluid or reducing agent were frozen at time zero.

Phlorizin was detected when diluted in McDougall's buffer (Appendix D.3.3). However, phlorizin was not detected in either the high phlorizin, time zero sample or the high phlorizin with no rumen fluid or reducing agent, control sample. This indicates that phlorizin may have been adsorbed to the lucerne or degraded very rapidly. No other samples were analysed.

D.3.5 pH stability

The pH stability of phlorizin was tested to determine if pH values in the ruminant digestive tract could cause degradation. Sodium azide was added to each buffer to give a final concentration of 0.05% to prevent microbial growth.

D.3.5.1 Stock solutions

0.8 mol·L^{-1} hydrochloric acid
0.4 mol·L^{-1} potassium chloride
0.2 mol·L^{-1} citric acid
0.4 mol·L^{-1} disodium hydrogen phosphate
0.2 mol·L^{-1} sodium hydroxide
0.2 mol·L^{-1} tris(hydroxymethyl)aminomethane
0.05 mol·L^{-1} sodium bicarbonate
1 g·L^{-1} phlorizin

D.3.5.2 4x Buffers

pH	mL stock /100mL	mL stock /100mL	pH adjusted with	Final pH at 4x	Final pH at 1x	Neutralised pH
1	6.25mL KCl	48.5mL HCl	Nothing	1.04	1.56	6.61
3	39.8mL citric acid	10.2mL Na_2HPO_4	NaOH	3.01	3.00	6.72
5	24.3mL citric acid	25.7mL Na_2HPO_4	NaOH		5.11	6.84
9	50mL tris	5mL NaOH	HCl	9.03	8.41	7.50
11	50mL $NaHCO_3$	10.7mL NaOH	$NaHCO_3$	11.01	10.58	7.36

D.3.5.3 20x Buffer

pH	mL stock /100mL	mL stock /100mL	pH adjusted with	Final pH at 4x	Final pH at 1x	Neutralised pH
1	65mL citric acid	12.21g Na_2HPO_4	HCl	7.06	7.22	7.19

D.3.5.4 Incubation

All reagents were first equilibrated to 40°C. 37.5 mL of 1 $g \cdot L^{-1}$ phlorizin was then added to 12.5 mL of each 4x buffer and to 12.5 mL of 20x pH7 buffers that had first been diluted to 4x concentration. All buffered phlorizin solutions were incubated in a covered, 40^{o}C water bath to maintain temperature and exclude light. A 500-µL sample at each pH was taken periodically and put into a 1.2-mL vial. 125 µL of 20x pH7 buffer was added to neutralise pH before samples were frozen for later analysis.

Although the results were very variable, phlorizin appeared to degrade slowly at pH1 and more quickly at pH11 (Figure D-4). Degradation at pH5 and 7, the approximate range of pH in the rumen, occurred only very slowly

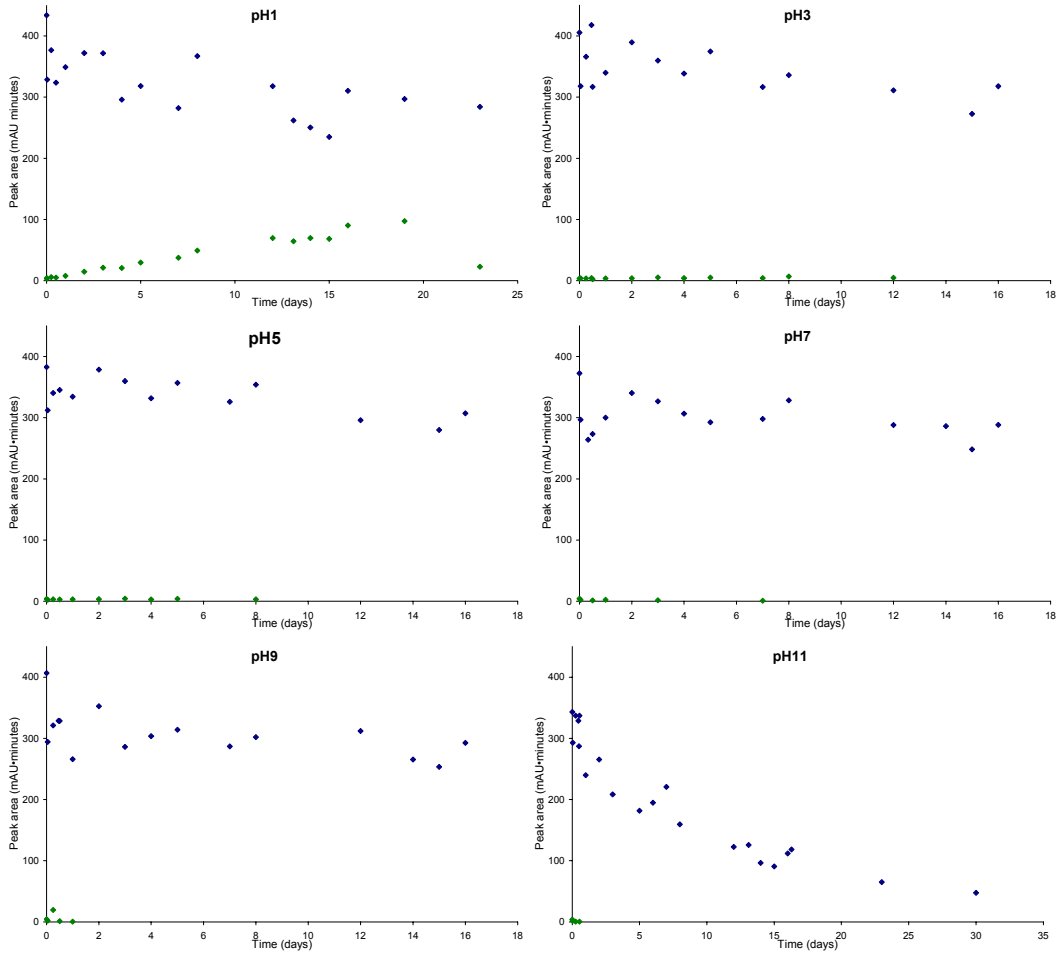


Figure D-4: Effect of pH and time on peak area of phlorizin and phloretin
× = phlorizin. × = phloretin.

Degradation rate at pH 1 was slow so phlorizin should not degrade significantly in the <2 hours digesta typically spends in the abomasum. The gradual decrease phlorizin concentration at pH 1 seemed to coincide with appearance of a peak thought to represent phloretin and indicates removal of the glucose moiety from phlorizin.

Degradation was much faster at pH 11. However, phlorizin would not encounter alkaline conditions this extreme *in vivo*. The degradation was accompanied by appearance of several peaks for unknown break-down products, most of which eluted with the unbound sample.

D.3.5.5 Source of variability

To determine if freezing samples before analysis caused the variability in data, phlorizin was again incubated at 40°C in pH 1, 7 and 11 buffer solutions (Appendix D.3.5.4). The process was staged so each sample could be neutralised

and analysed immediately, before the next sample was taken. Data (Figure D-5) were still as variable as those from frozen samples, indicating that freezing did not cause variability.

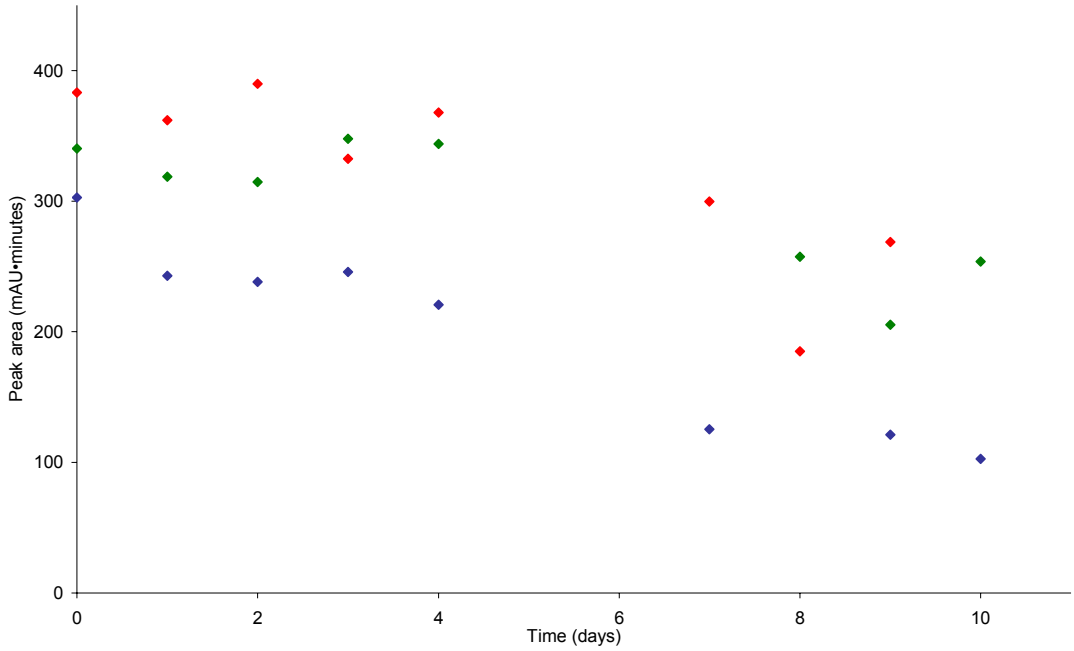


Figure D-5: Effect of pH and time on peak area of phlorizin (samples not frozen)
× = pH1. × = pH7. × = pH11.

To further investigate the cause of the variability, a phlorizin standard was analysed eleven times and results showed a coefficient of variation in peak area of 1.3%. A phlorizin sample that had been incubated at pH11 was then analysed. Three blank runs and another four samples of phlorizin standard were then analysed before data within the range of prior samples incubated at pH 11 was obtained. This indicates that a component in one or more of the buffers may be causing the variability.