The Gastro-Retention of a Multiparticulate Dosage Form

A Thesis submitted to The University of Manchester for the Degree of Doctor of Philosophy in the Faculty of Medical and Human Sciences

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ABSTRACT

Gastro-retentive dosage forms have the potential to improve drug bioavailability compared with that from many conventional available dosage forms and from modified release delivery systems. A floating dosage form based on freeze-dried calcium alginate beads has been developed. Riboflavin was selected as a model drug as it has a narrow absorption window within the small intestine.

Calcium alginate beads have been prepared and characterised. The calcium alginate beads were spherical in shape. The use of SEM and ESEM imaging methods showed the internal structure of the calcium alginate beads to consist of many cavities, thereby enabling floatation. The density of the calcium alginate beads was determined to be less than 1.000g.cm^{-3}. The buoyancy properties were investigated using resultant weight apparatus. Results showed that the calcium alginate beads remained buoyant in acidic media for a time period in excess of 13 hours.

An investigation of the mechanism by which the drug is released from the calcium alginate beads was undertaken. When calcium alginate beads were placed in aqueous media a gel barrier formed that could be identified by digital photography. It is likely that a combination of diffusion through the gel barrier and its erosion may lead to the drug release from the calcium alginate beads. The formulation of the calcium alginate beads was modified to include hydrophobic excipients.

In vitro assessments of the calcium alginate beads in physiologically relevant media were carried out in order to provide a predictive model of drug release rates prior to *in vivo* investigations. The results showed that riboflavin was released from the calcium alginate beads slowly in acidic media, representative of the stomach environment. In more alkali media that reflects the environment of the small intestine, the release of riboflavin was more rapid.

Two separate *in vivo* studies in volunteers were completed. An initial gamma scintigraphy study, investigated the behaviour of placebo calcium alginate beads administered under fasting conditions after administration of either water or a solution of citric acid. The subsequent study comprised a bioavailability study and gamma scintigraphy study. Calcium alginate beads containing riboflavin were administered under fasting or fed conditions. The results of the studies showed that citric acid has the potential to delay the gastric emptying of the calcium alginate beads. The administration of calcium alginate beads under fasting conditions with citric acid resulted in an amount of riboflavin absorbed that is close to or better than that achieved when the calcium alginate beads were administered under fed conditions with water.

The current work shows that calcium alginate beads have promise as a gastro-retentive dosage form. Further modifications to the calcium alginate bead formula will realise the ideal of the right amount of drug, that is delivered to the right place, at the right time.

DECLARATION

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I graduated from Aston University with a Pharmacy degree in 1999. After completing a pre-registration programme with Addenbrooke's NHS Trust (Cambridge) and SmithKline Beecham Pharmaceuticals (Harlow), I re-joined Addenbrooke's NHS Trust as a Resident Pharmacist. In 2001, I started at the University of Manchester to undertake a PhD with the collaboration of GlaxoSmithKline Pharmaceuticals under the supervision of Dr J T Fell, Professor J H Collett and Dr L G Martini (GlaxoSmithKline).

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When things go wrong
as they sometimes will,
when the road you're trudging
seems all uphill

When the funds are low
and the debts are high
and you want to smile
but you have to sigh,
when care is pressing you down a bit –
rest if you must but don't you quit.

Success is failure turned inside out –
the silver tint of the clouds of doubt,
and you never can tell
how close you are.
It may be near when it seems afar,
so stick to the fight when you're hardest hit....

It's when things seem worst
that you must not quit!

Author unknown

When you feel too old to do a thing – just do it!

For **all** my friends and members of my family who have helped and supported me during the good times and not so good times throughout the journey.

In memory of Mum.

I love you all.

LIST OF ABBREVIATIONS

ADP	Adenosine Diphosphate
ATP	Adenosine Triphosphate
CCK	Cholecystokinin
CLSM	Confocal Laser Scanning Microscopy
ESEM	Environmental Scanning Electron Microscopy
FAD	Flavin Adenine Dinucleotide
FDF	Floating Dosage Form
FDA	Food and Drug Administration
FMN	Flavin Mononucleotide
FRAP	Fluorescence Recovery After Photobleaching
GIP	Gastric Inhibitory Peptide
GRAS	Generally regarded as safe
HBS	Hydrodynamically Balanced System
HPLC	High Pressure Liquid Chromatography
HCl	Hydrochloric Acid
MMC	Migrating Motor or Myoelectric Complex
SEM	Scanning Electron Microscopy
UV	Ultraviolet

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ORIGIN AND SCOPE

Oral dosage forms currently account for the largest proportion of pharmaceuticals in use; not least because administration is both easy and convenient. However, the efficacy of some preparations is limited by variables such as the existence of a specific site for absorption within the gastrointestinal tract. Consequently, the majority of drugs administered do not realise the ideal of the required amount of drug in the right place at the right time. One possible method of achieving increased drug absorption from an oral preparation is by the production of an effective gastro-retentive dosage form.

When designing dosage forms, physiological and pharmaceutical limitations should be considered. Physiologically, gastrointestinal transit times range from minutes to hours depending on patient variables such as age[1], gender[1], posture[2] and disease state[3]. However, the largest contributor to the effective absorbance of substances from the gastrointestinal tract is whether the stomach is in the fed or fasted state. With regard to drugs, most absorption takes place in the small intestine. In the fed state the stomach and duodenum are responsible for gastric emptying by producing signals that result in long periods of irregular contractions[4]. Liquid, then food particles are orderly removed from the stomach after digestion[5]. In the fasted state gastric emptying is controlled by the migrating motor complex (MMC); a cycle that can be categorised into four parts. It is the third quarter of this cycle that consists of a brief period of large, powerful and regular contractions that empties the stomach of any remaining undigested material, including any residing dosage forms[6]. Therefore gastric emptying is the largest limiting physiological determinant to be considered when developing a modified release site-specific dosage form.

Pharmaceutically, the use of the oral route as a delivery system is currently inadequate for some drugs. For example, furosemide has only a narrow absorption window within the gastrointestinal tract, captopril is only stable at certain sites where gastrointestinal contents are of an optimal pH[7] and weak bases, such as chlordiazepoxide, are poorly soluble in intestinal fluids[8, 9].

Therapeutically, the following advantages are possible when a gastro-retentive dosage form has been developed:

- Improved patient compliance as only a single daily dose would be required instead of multiple doses throughout the day.
- Increased bioavailability.
- Reduced drug wastage due to rapid gastric emptying times.
- Increased solubility for poorly acid soluble drugs by extending their exposure time to the environmental conditions of the stomach. However, care should be exercised with regard to the choice of drug as some drugs may undergo degradation when exposed to the acidic nature of the stomach contents.
- The improved treatment of local conditions, for example *Helicobacter pylori*, which is the causative organism for chronic gastritis.

Initial investigations have confirmed that there is a need for an effective gastro-retentive dosage form. Current clinical practice is to use the drugs loperamide or guar gum to achieve prolonged gastro-retention[10]. However, gastro-retention is achieved as a side effect of the drugs and neither is particularly suitable for the purpose. Loperamide is an opiate derivative and can cause paralytic ileus in addition to other unwanted side effects such as nausea, vomiting, abdominal cramps, dizziness and drowsiness[11, 12]. Guar gum can cause abdominal distention and intestinal obstruction. In diabetic patients, hypoglycaemia can also result[13].

Many approaches have been made to develop dosage forms that can be retained in the stomach. These include large single dose units designed to be physically retained in the stomach and bio-adhesive systems that use polymers to adhere to the gastric mucosa[14]. Alternatively, some products have been designed to float on gastric contents. A hydrodynamically balanced system, (HBS), has been exploited commercially as Madopar CR® and a gas generating system are two approaches. An alternative approach showing particular promise is that of multiparticulate calcium alginate beads.

Alginates are versatile and non-toxic. They therefore make an ideal drug delivery system and previous studies have investigated the potential of calcium alginate beads as a novel gastro-retentive drug delivery system[8]. The calcium alginate beads were prepared by extruding a 2% w/v sodium alginate solution into a stirred solution of 0.02M calcium chloride. Sodium alginate is insoluble in calcium chloride and calcium alginate was then precipitated as gel beads. When freeze-dried, the calcium alginate beads measure 2 – 3mm in diameter and have a density lower than that of stomach contents and they therefore float on gastric contents. Initial *in vivo* studies with placebo calcium alginate beads have already shown that prolonged gastric emptying can be achieved when administered in the fed state[8].

Riboflavin has been selected as the model drug as it has a specific region for absorption in the gastrointestinal tract[15]. In addition, levels can be calculated by urinary analysis, eliminating the need for any invasive procedures.

Floating calcium alginate beads have already shown promise when dosed in the fed state[8]. However, if the emptying of dosage forms can be delayed in the fasted state, calcium alginate beads will provide an ideal novel drug delivery system.

CHAPTER 1 – INTRODUCTION

CHAPTER 1 – INTRODUCTION

Before attempting to design an oral dosage form, it is essential to have a full understanding of how the gastrointestinal tract functions. The first chapter has been designed in two parts. The first part will provide a physiological review of the gastrointestinal tract. The second half of the chapter will focus on approaches that have been made with the aim of prolonging the gastro-retention of a dosage form

1.1 GASTROINTESTINAL PHYSIOLOGY AND FUNCTION

1.1.1 SUMMARY OF BASIC STRUCTURE AND FUNCTION

In summary, the digestive or alimentary tract is of about 9m in length[16], comprises several organs and runs from the mouth to the anus. The whole system is responsible for four main objectives, namely:

- The movement of food along the tract via muscular contractions.
- The secretion of digestive juices and enzymes responsible for breaking down any digested material.
- The digestion or mechanical breakdown of complex foods ready for absorption.
- The absorption of nutrients and water from the digested products[1].

The structure of the gut is such that it has a highly muscular wall inter-layered with secretory glands and has a rich blood supply in order to achieve the above objectives[17]. A more detailed assessment of the gastrointestinal tract follows.

1.1.2 ANATOMY OF THE GASTROINTESTINAL SYSTEM

The digestive tract is shown in Figure 1.1. Each part is designed specifically to aid the digestive and absorptive process.

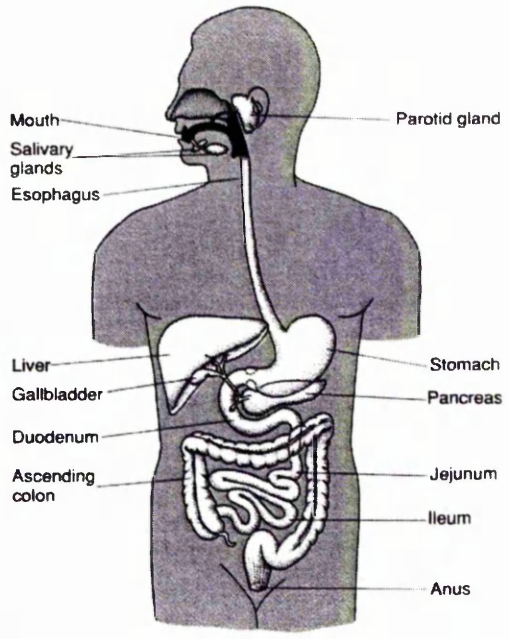


Figure 1.1 Diagram of the digestive tract[18].

1.1.2.1 Mouth to Oesophagus

The function of the mouth is to allow entry of the foods and the chewing action of the teeth enables the particle size to be reduced. In addition salivary glands, located in the buccal cavity secrete digestive enzymes which, when combined with the masticated food produce a thick mix. The mix is then released into the oesophagus via the oesophageal sphincter where peristaltic movements ensure delivery to the stomach, the process of which lasts approximately 4 to 8 seconds[19].

Virtually no absorption takes place in the mouth, except for a few drugs, e.g. glyceryl trinitrate, used in the treatment of angina.

1.1.2.2 The Stomach

The anatomy of the stomach is shown in Figure 1.2.

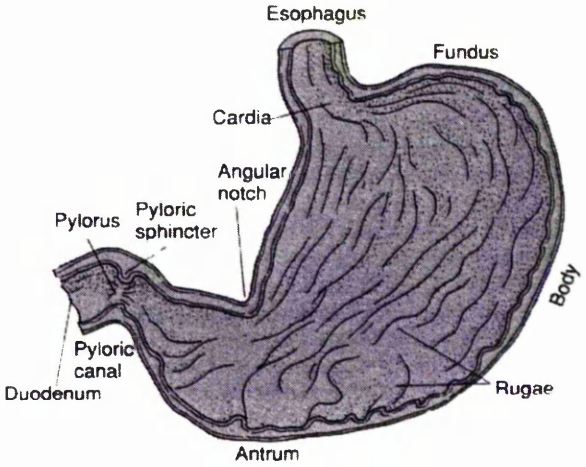


Figure 1.2 Anatomy of the stomach[17].

The stomach is J-shaped and can be divided into three principal regions, namely the fundus, the body and the antrum. Primarily the functions of the stomach are to store and further break down food ready for absorption[4]. The main absorptive site is the small intestine and this follows directly on from the stomach. Hence the process of breakdown may take many hours if a large meal has been ingested in order that maximum nutrients can be absorbed. When empty and at rest the stomach holds a nominal volume of about 50ml, but following the ingestion of food up to 1.5L can be accommodated[18].

The fundus is located at the top of the stomach and its function is to exert mild contractions on ingested contents to move them towards the antrum of the stomach. The body is the main part of the stomach and acts as a holding region for ingested contents. The antrum of the stomach can be seen to be distinct from the rest of the stomach. The muscle layers are thick and produce strong powerful contractions to mix the food with the gastric secretions before emptying the contents into the small intestine. Motility within the stomach can be divided into four distinct categories. They are:

1. **Filling**. When empty a residual volume of liquid remains in the stomach. When a meal is consumed, the volume increases but a process called receptive relaxation occurs. Receptive relaxation allows the stomach to

expand up to a certain point without being over-distended. Beyond the set point, pressure receptors initiate the emptying of the stomach[19].

2. **Storage**. Food is stored in the fundus until slow wave potentials sweep down the stomach length inducing peristaltic contractions and moving food towards the antrum[19].

3. **Mixing**. Once the food and liquid is mixed and broken down, a semi liquid mix known as chyme is produced. The chyme can then pass through the pyloric sphincter into the duodenum. However, only small volumes, (~30ml), can pass through at any one time[19]. The combination of the continuation of the peristaltic waves and the passage of only small amounts of chyme through the pyloric sphincter results in retrograde tumbling of the contents, known as retropulsion. The process ensures that effective mixing is maintained until emptying can be achieved[19].

4. **Emptying**. Gastric emptying is achieved by antral peristaltic contractions and the strength of the peristalsis determines how much chyme can pass through the pyloric sphincter. The overall mechanism of gastric emptying is affected by numerous factors that will be discussed later.

1.1.2.3 The Small Intestine

The structure of the small intestine is shown in Figure 1.3.

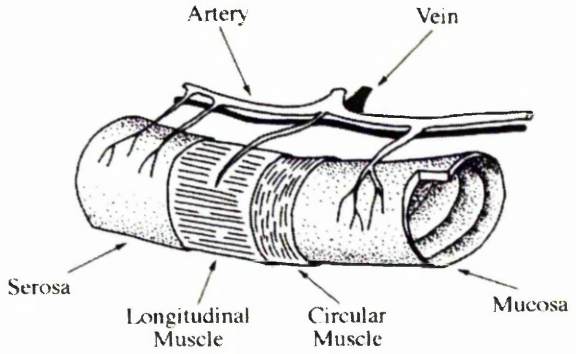


Figure 1.3 Structure of the small intestine[18].

The small intestine accounts for the longest part of the gastrointestinal tract, (GIT). It is about 6m in length and collectively consists of the duodenum, (8cm), the jejenum, (2.5m), and the ileum, (3.6m)[18]. The three regions do not differ anatomically but do have different functions. Overall, the small intestine is responsible for the absorption of substances from digested material and also carries out any final digestive processes. The small intestine is well suited for its functions of digestion and absorption. The muscular wall ensures that chyme can be further digested, mixed and passed along the length of the GIT. Movement along the small intestine is either by mixing contractions, (segmentation), or propulsive movements, (peristalsis)[4].

Segmentation occurs when localised contractions are present at interval of about 1cm along the length of the small intestine. Relaxed segments contract whilst contracted segments relax, the outcome of which ensures efficient mixing.

Although the contents are propelled slowly by segmentation, peristalsis is mostly responsible for the movement of materials along the length of the small intestine. The peristaltic waves are stronger in the proximal part of the small intestine but they weaken as they reach the terminal area. The consequence of such a process is optimal absorption of substances and nutrients as exposure to the absorptive site is at a maximum. Typically, contents make take an average of 4 hours to travel from the pylorus to the ileocaecal junction that joins the small and large intestines[4].

1.1.2.4 The Large Intestine (Colon)

The contents of the small intestine pass into the large intestine via the ileocaecal sphincter.

The structure of the large intestine is shown in Figure 1.4.

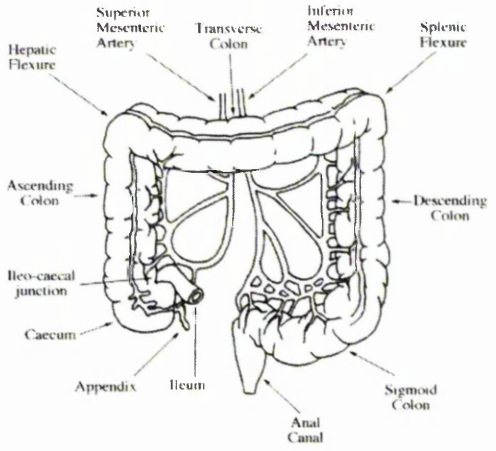


Figure 1.4 Structure of the large intestine[18].

The large intestine is much shorter than the small intestine with the total length approximating to 125cm. There are six main areas of the large intestine, namely the caecum, (8.5cm), the ascending colon, (20cm), the transverse colon, (45cm), the descending colon, (30cm), the sigmoid colon, (40cm) and the rectum and anus, (15cm)[18].

Figure 1.5 shows a more detailed structure of the colonic wall.

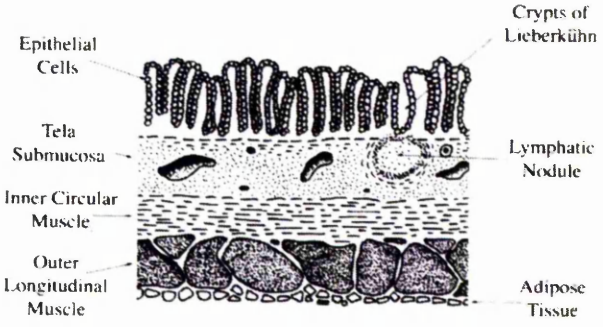


Figure 1.5 Detailed structure of the colonic wall[18].

Histologically, with respect to the small intestine both similarities and differences exist. The most notable difference is the lack of microvilli in the large intestine. Additionally, the areas do not contain as many folds and therefore the absorptive area is reduced. The structural difference occurs because no digestion takes place in the large intestine and consequently enzymes are not

secreted. The main function of the large intestine is to reabsorb water and some salts, since the absorption of all other substances has been completed in the small intestine[4].

Movement of substances within the large intestine are by haustral contractions. Haustral contractions are similar to the segmentation that occurs in the small intestine, but are much slower and this reflects the storage and absorptive functions of the large intestine. Mass movements also occur and these consist of large simultaneous contractions of the ascending and transverse colon, the purpose of which is to move the contents towards to rectum for defaecation. Finally, the defaecation reflex is initiated due to the pressure of the contents and the faeces are eliminated[4, 18].

1.1.3 REGULATION OF GASTROINTESTINAL FUNCTIONS: GASTRIC SECRETIONS

In addition to the muscular movements discussed, (Chapter 1, section 1.1.2.2), the whole process of digestion is controlled by a combination of gastric secretions and the intrinsic and extrinsic nervous systems.

In the stomach, the mucosal surfaces of the fundus and the antrum can be categorised into two distinct areas, known as the oxyntic and pyloric gland area respectively. Gastric pits are formed from the convoluted folds of the mucosa and the secretory cells are to be found lining the surface. The stomach produces approximately 2L of mucus per day either in the response to food or in the anticipation of food[17]. Figure 1.6 shows the arrangement of cells lining the stomach.

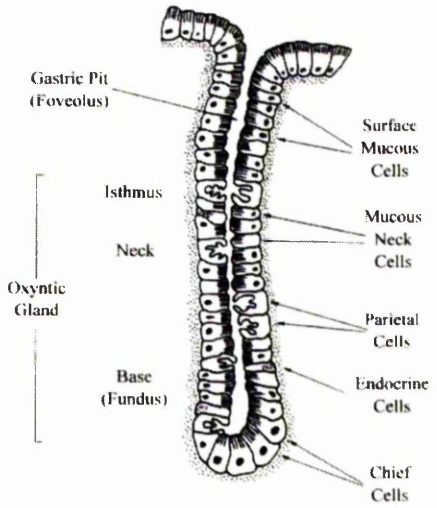


Figure 1.6 Diagram of cells lining the stomach[18].

The oxyntic glands of the oxyntic area produce several secretions. Mucus neck cells produce thin watery mucus, the function of which is to protect the mucosal linings from either self-digestion or acid damage. Further down the pit, chief cells secrete the major digestive secretion, pepsinogen. As pepsinogen, it is inactive but upon reaction with HCl, the molecule is converted to its active form, pepsin. Autocatalysis then produces more pepsin from other pepsinogen molecules. Pepsin is secreted in the inactive form as in the active form pepsin converts proteins to amino acids and peptides. Protein is the major component of most cells and if pepsin production is not regulated then self-digestion of the mucosa will result[17].

Parietal cells histologically are formed near or next to the chief cells. The parietal cells secrete HCl that has a pH of about 0.8. When coupled with secretions and gastric contents, the final pH is about 2.0.

HCl has no digestive function but it is essential for:

- The conversion of pepsinogen to pepsin.
- The maintenance of the acidic environment, essential for pepsin function.
- Killing any micro-organisms that have been ingested with the food.
- To initiate proteolysis[20].

Most secretions found in the small intestine originate from the pancreas. The exocrine glands of the stomach do however, secrete a few substances.

Mucus and bicarbonate ions are secreted by Brunner's Glands in the duodenal segment of the small intestine[18]. The primary function of the mucus is to protect the small intestine against digestive enzymes by forming a protective layer and a means of increasing the alkaline nature of the contents should any of them be particularly acidic[19].

The epithelial cells of the small intestine in themselves do not secrete enzymes into the lumen, but they contain enzymes to aid the digestion of substances whilst being absorbed across the epithelium[18, 19].

The secretions from the small intestine are in response to neural stimuli that in turn is directly proportional to the amount of chyme produced.

Secretions form the pancreas and liver also empty directly into the small intestine and an outline of their functions follows. Secretions from the pancreas amount to approximately 1L/day[18] and consist of a fluid part containing bicarbonate and an enzyme part. The enzyme portion continues the process of digestion whilst the large amount of bicarbonate secreted serves to reduce the acidic nature of the stomach contents in order that the intestinal wall is protected[18].

Enzymes secreted by the pancreas include the active enzymes pancreatic amylase and lipase which are responsible for carbohydrate and fat digestion respectively[19].

The pancreas also secretes the inactive proteolytic enzymes tryspinogen and chymotrypsinogen. Both of these require activation by the aforementioned enterokinase to prevent self-digestion of the small intestine[19].

The liver secretes about 600ml/day[18] of bile that is stored in the gall bladder until required. Bile is then emptied into the small intestine via the Sphincter of Oddi[19].

Bile is a mixture of many components. Briefly they are:

- Bilirubin, the breakdown product of haem from red blood cells. Here it is in the process of being eliminated from the body[19].
- Sodium, potassium, chloride and bicarbonate ions. Sodium and potassium ions aid the re-absorption of salts against electrochemical gradients whilst chloride and bicarbonate aid to reduce the acidity of the chyme.
- Cholesterol, from which is synthesized cholic and chenodeoxycholic acid. Further synthesis of cholic and chenodeoxycholic acid with glycine and taurine produces the bile salts. The main functions of the bile salts are to act as a surfactant with regards to the intestinal contents and to aid lipid absorption in the small intestine by producing micelles[18].

Gastrin, secretin, motilin, cholecystokinin, (CCK), and gastric inhibitory peptide, (GIP), are five main hormones that have an effect on the digestive system[4].

Gastrin is stimulated by protein in the stomach and secreted by G cells of the pyloric gland area. Gastrin causes the release of HCl and pepsinogen from the parietal and chief cells. The direct effect of gastrin is to enhance gastric motility, stimulate ileal motility and to induce colonic mass movements[20].

Secretin is produced by S cells of the duodenal mucosa from the endocrine cells in a response to the presence of acid in the duodenal lumen. The primary function of secretin is to inhibit gastric emptying and secretion, and to stimulate bicarbonate production from the pancreas and liver[4].

CCK is secreted by the duodenal and jejenal mucosa in response to the presence of fats and proteins. The effect is similar to that of secretin in that it inhibits gastric emptying and secretions. CCK also has a direct effect on the

Sphincter of Oddi causing its relaxation, so bile can be released to aid fat digestion[4].

Motilin is release into the duodenum and the jejenum and is a direct response to nervous stimulation. The only known function of motilin is to stimulate the MMC[17].

GIP inhibits acid secretion in a response to the presence of fat and glucose. It also stimulates insulin release.

1.1.4 REGULATION OF GASTROINTESTINAL FUNCTIONS: NEURAL CONTROL

The neural control of gastrointestinal functions can be further subdivided into the intrinsic and extrinsic nervous systems, both completing different functions.

1.1.4.1 THE INTRINSIC NERVOUS SYSTEM

Two main networks of nerves make up the intrinsic neural pathways. Running the whole length of the gut they are Auerbach's Plexus, located within the longitudinal and circular muscle, and Meissner's Plexus that is located within the submucosa. Meissner's plexus is shown in Figure 1.7.

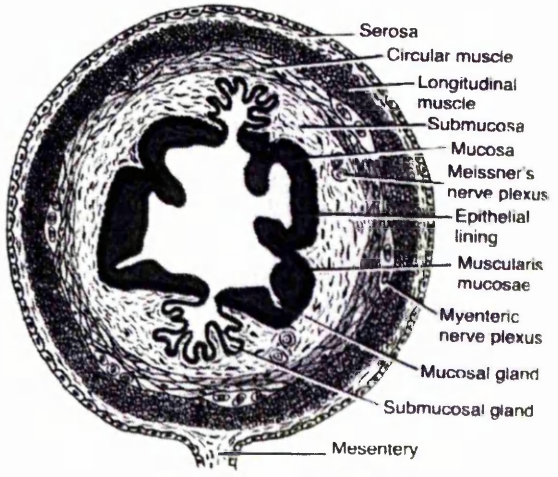


Figure 1.7 Cross section of gut showing Meissner's Plexus and the longitudinal muscle that contains Auerbach's Plexus[17].

Both intrinsic sets of nerves have completely different functions but work in tandem to control the functioning of the digestive tract.

Intrinsic nerves also release a variety of neurotransmitters that include acetylcholine, norepinephrine, adenosine triphosphate, serotonin, dopamine, cholecystokinin, substance P and somatostatin. The full functions of the above are not yet understood but some have been confirmed as having an effect on gastric motility, e.g. acetylcholine initiates gastric activity whilst norepinephrine inhibits gastric activity[4].

1.1.4.2 The Extrinsic Nervous System

The extrinsic nervous system consists of parasympathetic and sympathetic nerves from the autonomic nervous system.

Parasympathetic nerves originate in the brain and spinal cord. They consist of long ganglionic and short postganglionic fibres that innervate the gut by following the vagal pathway. Acetylcholine is released from the postganglionic nerve endings and the digestive system is then stimulated to produce secretions and enzymes and increase motility. Nerve impulses also react to receptors. For example, chemoreceptors are activated in response to tension receptors in the muscle layers and as a result the parasympathetic nervous system is stimulated[19].

Sympathetic nerves generally inhibit the activity of the GIT. The nerves originate in the thoracic and lumbar regions of the spinal cord and end with α receptors, (found in the sphincters) and β_2 receptors, (found in the organs). The fibres are short preganglionic fibres that release acetylcholine, (i.e. cholinergic fibres) and long postganglionic fibres that produce norepinephrine. The result of norepinephrine release is decreased motility and sphincter contraction that prevents further movement of stomach contents[19].

1.1.5 DIGESTION AND CELLULAR TRANSPORT OF SUBSTANCES IN THE GASTROINTESTINAL TRACT

Digestion is the chemical means by which food is broken down in preparation for absorption. Although the digestion of carbohydrates begins in the stomach, the main site of digestion is the small intestine[4].

Little absorption of substances takes place in the mouth, oesophagus and stomach. The small intestine is the major site of absorption for the products of digestion, whether nutritional or pharmaceutical.

The structure of the small intestine is specialised to absorb substances. The lining surface is folded to form microvilli, which increases the surface area from which to absorb nutrients. The structure of a microvilli is shown in Figure 1.8.

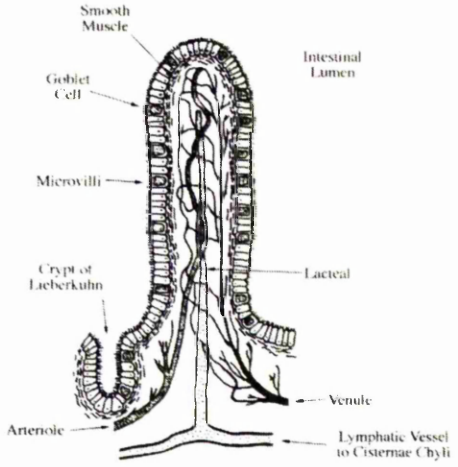


Figure 1.8 Structure of a microvilli[18].

The arrangement of microvilli to the basement membrane of the small intestine is shown in Figure 1.9.

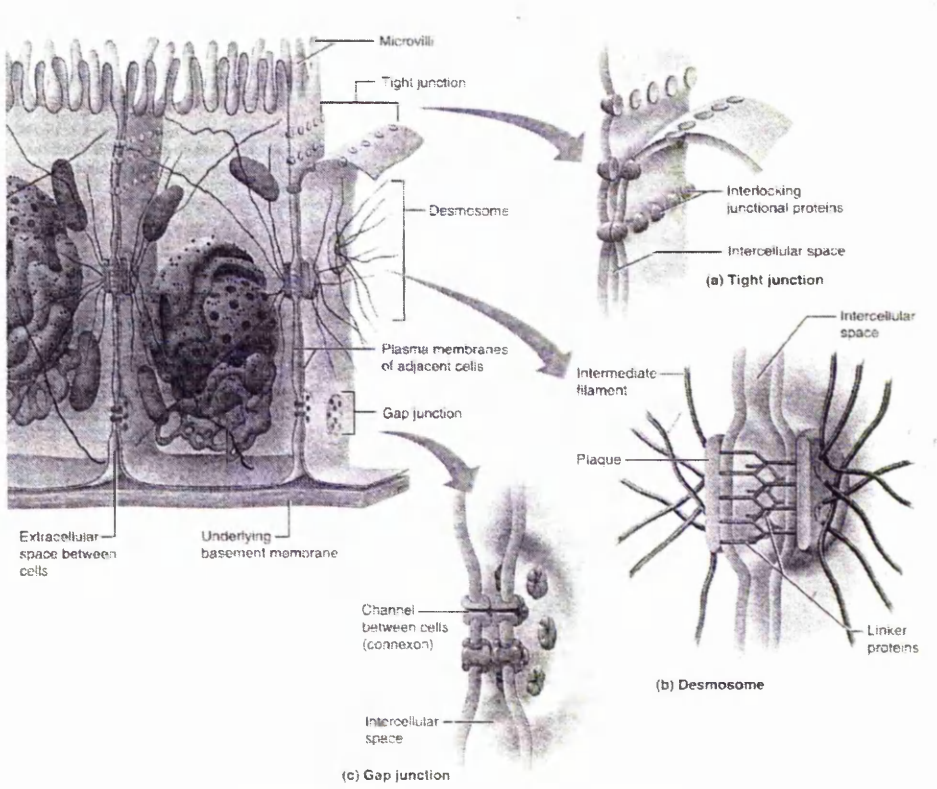


Figure 1.9 Basement membrane of the small intestine[21].

The arrangement of cells with microvilli to the basement membrane is such that adjacent cells are connected by junctions, which all serve different purposes. Tight junctions are designed to prevent substances from passing between cells. With specific regard to digestion, tight junctions prevent the entry of enzymes and micro-organisms into the circulatory system. Desmosomes are responsible for binding neighbouring cells. Typically they are found in muscular areas where it is likely that, under stress, the separation of cells could occur, thereby compromising the protective surface of the basement membrane. Gap junctions allow the selective passing of materials, such as sugars and ions, from one cell to another. They are normally found in electrically excitable tissue to ensure that the necessary muscle contractions occur[21].

The transport of substances through cells occurs either passively or actively.

1.1.5.1 PASSIVE TRANSPORT

Passive transport occurs either by diffusion or filtration and does not require an energy system to operate. Simple diffusion occurs when lipid soluble compounds such as oxygen, fat-soluble vitamins and some drugs, diffuse directly through the lipid bilayer of the membrane. Facilitated diffusion moves substance such as sugars and proteins across the lipid membrane through a channel protein or by the binding of the substance to a protein carrier. Finally, water moves across the membrane by osmosis[21].

Filtration also involves the movement of water through the membrane. However, it relies on a concentration gradient to move filtrate form high pressure areas to low pressure areas. The process is not selective and only molecules too large to pass between the cells are excluded from the process[21].

1.1.5.2 ACTIVE TRANSPORT

Active transport also moves substances across cells, but requires energy from ATP in order to complete the process. Endocytosis and exocytosis are the two main forms of vesicular transport requiring energy from ATP. With specific regard to digestion, endocytosis is responsible for absorbing dissolved solutes from the small intestine. Exocytosis is responsible for the secretion of mucus[21].

1.1.6 GASTRIC EMPTYING

1.1.6.1 GASTRIC EMPTYING IN THE FASTED AND FED STATE.

Stomach motility ensures that the emptying of contents into the small intestine following the ingestion of food is systematic. However, when the stomach is empty, the stomach enters a quiescent cycle or resting period. Gastric motility in the fed and fasting states are described further below.

Gastric motility in the fasted state

When all digested material has been removed from the stomach, an inter-digestive state or cycle of fasting motility exists. The cycle commonly known as the Migrating Myoelectric or Motor Complex, (MMC), serves to empty the

stomach of an indigestible materials and maintain the stomach in its normal resting phase.

The whole cycle lasts approximately 1½ hours[22], although inter-individual variation means that the cycle may be slightly longer or shorter. Classically there are four distinct phases to the cycle and these can be described as follows:

Phase I. A resting period lasting 40 – 60 minutes during which little or no motor activity takes place.

Phase II. Lasts 40 – 60 minutes during which time motor activity begins and increases so that contractions are produced.

Phase III. Also known as the 'housekeeper wave'. The phase is brief but powerful and regular contractions radiate from the site of origin in the stomach to the small intestine and caecum. Consequently the stomach is emptied of any remaining undigested material or dosage forms[6,18].

Phase IV. A period during which the contractions cease and activity returns to that as described in Phase I[6, 18].

Although the MMC is described as cyclical, if at any point food is ingested, then the inter-digestive phase stops and stomach activity reverts to the pattern described in the digestive phase[22].

The gastric emptying produced by the MMC is the largest limiting physiological determinant that must be overcome when developing a gastro-retentive dosage form[22]. Currently, any retention of dosage forms depends entirely on the time interval between phases I and III. When inter-individual variation is considered with other variables the possibility of retaining a drug to achieve increased bioavailability is very poor.

Gastric motility in the fed state

The ingestion of food leads to an interruption of the MMC. Activity is stimulated in the antrum, pylorus and duodenum the aim of which is to retard gastric emptying so it is slow and controlled. Hence contractions are continuous and irregular and in direct contrast to those in the fasted state.

Solids and liquids are emptied in an orderly manner from the stomach through the pylorus that acts as a filter. Liquids are initially filtered through followed by non-nutrient foods then nutrient foods.

Emptying of liquids

Water is a non-nutrient and a liquid. Hence it is the quickest substance to be emptied from the stomach. Generally for volumes ranging from 10ml to 1000ml the time taken for half the amount to empty from the stomach, ($t_{½}$), is about 10 minutes[23]. After water, isotonic liquids will then leave the stomach followed by hypotonic and hypertonic. The graduated response to the emptying of fluids is controlled by receptors in the stomach and duodenum that detect the osmolarity and chemical composition of the liquids. Results from studies demonstrate the difference in liquid composition with gastric emptying times. For example when the test solutions, glucose 0.8M and milk were given to volunteers, $t_{½}$ values of 7.5 minutes and 44.4 minutes were obtained respectively[5]. Following further studies it has been suggested that gastric emptying was delayed when fatty acids, e.g. oleic acid were added to test drinks[23].

Emptying of solids

The presence of solid food in the stomach initiates motor activity within the duodenum. The duodenal receptors are activated in response to the digested products of carbohydrates, fats and proteins, the density of which directly affects the delivery of the substances to the duodenum[18].

Carbohydrates of the same food group may also cause changes in gastric empting times. In an *in vivo* study comparing the gastric emptying of liquid after

subjects ate wholemeal and white bread, amounts of liquid leaving the stomach were found to be greater after eating white bread. The reason for the findings is that white bread stimulates gastric motility to a greater extent than brown bread and so allows a quicker passage of liquid through the pylorus[24].

Foods containing fats or fatty acids are reported to alter gastric emptying rates. The emptying of fats from the stomach is dependent upon the affinity of fat for calcium. Fats that contain 11 – 18 carbon atoms have a high affinity for calcium and the complexation of fat with the calcium atoms results in changes in duodenal tight junctions that in turn causes neural or hormonal feedback mechanisms to inhibit or stimulate duodenal motor activity[23]. In a study similar to that of the test drinks, results showed that foods with higher proportions of fat are emptied slower, e.g. mashed potato had a $t_{½}$ value of 60 minutes compared to that of beef-burgers that had a $t_{½}$ of 180 minutes[5], proving conclusively that foods with higher proportions of fat have slower gastric emptying times.

The effect of meal size on gastric emptying

Western diets usually consist of small regular meals eaten over a 24-hour period. The Western diet is in direct contrast to typical African diets where meals are usually large and eaten once over the same time period. Compared with small meals, larger meals require a greater time to be digested and the stomach remains in the digestive state for longer periods of time. However, once digestion has been completed the MMC is initiated and will continue until the presence of food interrupts the cycle. Therefore dosage forms administered to populations such as Africans are more likely to suffer from early gastric emptying as the MMC occurs more often. With regards to the Western population, the emptying of dosage forms from the stomach can be delayed by the frequent presence of food.

1.1.6.2 Other factors affecting gastric emptying

In addition to the presence or absence of food in the stomach, other physiological and extrinsic factors may also affect gastric emptying. A further explanation of such factors follows.

The effect of posture on gastric emptying

The presence or absence of food in the stomach is the greatest variable to be considered when studying gastric emptying. However the posture of the subject also has a direct effect on gastric emptying and therefore warrants special consideration.

The anatomy of the stomach, (section 1.1.2.2), suggests that differences in gastric emptying times and hence drug absorption differ markedly depending on whether the body is in the upright or supine position. In addition, if the subject is supine, gastric emptying may differ according to whether the subject is lying on the left or right hand side[25].

The transport of a dosage form from the mouth to the oesophagus can vary depending on whether the subject is in the upright or supine position. Although the dosage form may adhere to mucosal surfaces whether the subject is in the upright or supine position, the adherence of the dosage is more likely to occur when the subject is in the supine position. In addition to causing discomfort for the subject, the adherence of a dosage form to a mucosal surface may cause irritation and/or damage[2]. In order to prevent adherence of dosage forms to mucosal surfaces, dosage forms are often labelled with an instruction for the dosage form to be administered 'with a tumbler of water'. Studies have shown that when subjects are in the upright position, a quantity of 60ml of water is sufficient to prevent adherence of the dosage form to the mucosa. However, when the subject is in the supine position a volume of 240ml is required to ensure delivery of the dosage form to the stomach[2]. The choice of liquid to be administered with the dosage form also affects gastric emptying. Non-nutrient liquids, (e.g. water) empty from the stomach faster when the subject is in the supine position compared with being upright[18]. Complex solution, (e.g.

glucose), have shown no difference in the rate of gastric emptying regardless of the position of the subject[25].

When considering pharmaceutical preparations, raft forming alginate preparations empty from the stomach faster than a liquid meal when the subject lies on the left hand side compared to lying on their right hand side[2]. Physiologically a greater curvature of the stomach is maintained when subjects are positioned on the right hand side and therefore floating liquid formulations are retained in the stomach for longer periods of time[2].

In direct contrast to the raft formulation mentioned previously, studies investigating the effect of posture following the administration of a solid dosage form have also been performed. Results show that there is no difference in the gastric residence time of a conventional non-floating capsule dosage form of density 1.5 $g.cm^{-3}$ regardless of whether the subject was in the upright or supine position[1].

The aforementioned studies clearly have clearly defined the use of 'upright' or 'supine' subjects. It should be noted that often, by default, subjects adopt a semi-recumbent position, and studies conducted using such a position have shown good correlations between pharmacokinetic and scintigraphic results[25].

When developing a gastro-retentive dosage form, due consideration should be given to the fact that changes in posture can affect the gastric retention times of the dosage form. Therefore, if a once daily preparation is desired, administration of an oral dosage form prior to bedtime may not be advisable[26].

The effect of pH on gastric emptying

The pH of contents taken in through the mouth and travelling through the gastrointestinal tract will be different depending on the region of the gastrointestinal tract that the contents are in. The changes occur to ensure digestion and absorbance of nutrients whilst protecting the GIT from self-digestion.

Table 1.1 demonstrates the different areas of the gastrointestinal tract with their associated pH's and residence times.

Region	Length (m)	pH	Residence Time
Oesophagus	0.3	6.8	>30 seconds
Stomach	0.2	1.8 – 2.5	1 – 5 hours
Duodenum	0.3	5 – 6.5	>5 minutes
Jejenum	3	6.9	1 – 2 hours
Ileum	4	7.6	2 – 3 hours
Colon	1.5	5.5 – 7.8	15 – 48 hours

Table 1.1 Regions of the gastrointestinal tract with associated physiological variables[18].

The administration of some acids with food is thought to cause a delay in gastric emptying[27]. Normally, the ingestion of food results in a change in the pH of the stomach environment from approximately pH 2.0 to approximately pH 5.0, thus making the contents favourable for the further digestion of food and absorption of nutrients[27]. Maintaining the stomach contents at acidic pH is unfavourable for the small intestine. Therefore, a feedback loop ensures that gastric emptying is delayed until the pH of the contents is at an acceptable level for passage through the pyloric sphincter to the small intestine.

Factors that increase the rate of gastric emptying

Other common factors that increase the rate of gastric emptying, and their suggested mechanism are shown in Table 1.2.

Factor increasing gastric emptying	Reason for effect
Surgery	'Dose dumping' – due to osmotic insult and release of chemical following GI surgery[10].
Exercise	Mechanism not known[28].
Race – black population	Due to higher crude fibre content (maize) in diet compared to caucasian [29].

Table 1.2 Factors increasing the gastric emptying rate.

Factors decreasing the rate of gastric emptying

In addition to the factors considered above that increase the rate of gastric emptying, a decrease in gastric emptying can also be observed in certain circumstances. The circumstances and reason for effect are shown in Table 1.3.

Factor decreasing gastric emptying	Reason for effect
Stress	Activation of sympathetic nervous system[30].
Sex – female	Hormonal differences – mechanism, not known[31].
Age	Mechanism not known[1].
Alcohol	Possible sympathetic nervous system stimulation[32].
Circadian rhythm	Decrease shown in afternoon compared to morning. Mechanism not known[33].

Table 1.3 Factors decreasing the gastric emptying rate.

1.1.7 CONSIDERATIONS WHEN DESIGNING A GASTRO-RETENTIVE DOSAGE FORM

When designing a site-specific controlled release dosage form it is important to consider the environments of the gastrointestinal tract. It is also necessary to consider the drug intended for inclusion in such a dosage form as some drugs

will not be suited to the anticipated mode of delivery. Examples of drugs not suited to a gastro-retentive dosage form include:

- Non-steroidal anti-inflammatory drugs, (e.g. ibuprofen), that cause gastric irritation may lead to increased irritation, and the formation of ulcers or lesions.
- Drugs that are poorly soluble in the acidic environment of the stomach.
- Drugs that are well absorbed along the entire length of the intestine, (e.g. nitrates), and would therefore not benefit from being formulated as a gastro-retentive dosage form[34].

Many devices have been produced to overcome the variables discussed and the second part of the chapter will focus on such dosage forms.

1.2 GASTRO-RETENTIVE DOSAGE FORMS

Many dosage forms have been designed in order to prolong their residence time in the stomach and ultimately that of the selected drug. They include single unit systems, unfolding structures and floating dosage forms. These and other methods are described in the following sections.

1.2.1 PROPOSED METHODS TO PROLONG THE GASTRO-RETENTION OF A DOSAGE FORM

1.2.1.1 SINGLE UNIT SYSTEMS

Large single dosage forms, the hydrodynamically balanced system, and, more recently swelling systems are single unit dosage systems that have been designed to be retained in the stomach for prolonged periods of time. The systems mentioned are described in more detail below. However, the disadvantage of formulating a single unit system is that gastric emptying will be of an all or nothing nature.

Large single dosage forms

Large and irregular shaped dosage forms have been designed to be retained in the stomach. Specifically, they are intended to occlude the pyloric sphincter and to be released from the stomach during Phase III of the MMC when all other material has been emptied. However, as the diameter of the pyloric sphincter is 12.8 mm ± 0.7mm when open[35], the size of such dosage forms required to achieve gastro-retention is in excess of 15mm[34]. The disadvantages of such a large dosage form are that they are difficult to swallow[34] they may be likely to cause a permanent blockage at the pyloric sphincter[14] and they may also fragment earlier than anticipated thereby losing the gastro-retentive properties.

The Hydrodynamically Balanced System (HBS)

The HBS was developed by Sheth and Tossounian and is the only example of a single unit floating dosage form that has succeeded as a commercial application. The HBS, Figure 1.10, contains a mix of fillers, drug and hydrocolloids.

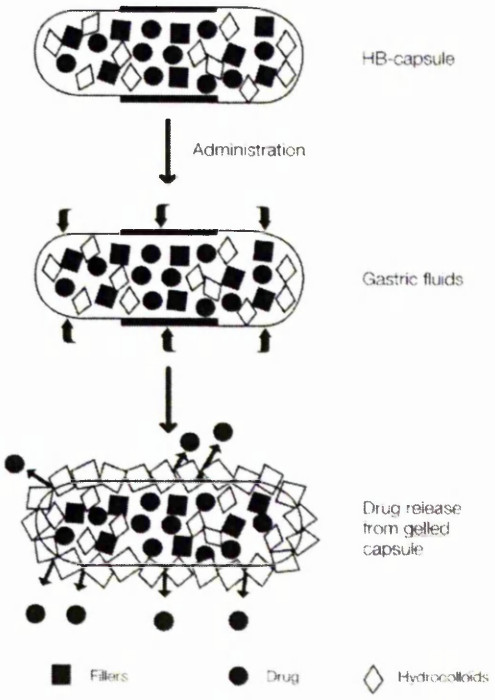


Figure 1.10 Schematic diagram of the HBS[36].

When in contact with gastric fluids, hydration of the polymer takes place and a gel barrier is formed around the dosage form. The drug is then released form the hydrophilic matrix by diffusion[36].

The HBS has been exploited commercially as Madopar™, used in the treatment of Parkinson's Disease. A controlled release preparation is ideal for it reduces the need for multiple tablets to be taken throughout the day and the on/off effect of traditional Madopar™ preparations can be avoided.

Swelling systems

Single unit gastro-retentive dosage forms based on hydrophilic polymers that swell in the acidic environment of the stomach to a size that occludes the pyloric sphincter are currently in development. The essential requirements for swelling systems are:

- The dosage form must swell prior to emptying. If Phase III of the MMC has been initiated the dosage form may be emptied before the dosage from is able to swell to a size that will not be emptied from the stomach.
- The increase in size must be large enough for the dosage from to be retained in the stomach.
- The drug delivery system must be biodegradable[3].

Recently, DepoMed Inc has filed a NDA for Glumetza™, (metformin)[37]. Although details of the dosage form and its precise mechanism of action do not appear in the literature, the drug is released from the dosage form by diffusion from a matrix allowing constant levels to be achieved over a 24 hour dosing period. A second drug, Proquin XR™ (ciprofloxacin), has completed Phase III clinical trials and a NDA is in the process of being completed. The solubility of ciprofloxacin in water is poor and drug release from the Proquin XR™ dosage form is achieved by erosion of the polymeric matrix[37].

1.2.1.2 EXPANDABLE GASTRO-RETENTIVE DOSAGE FORMS

Elegant dosage forms have been designed to expand or unfold in the stomach. The dosage form contains components that expand to irregular or geometric

shapes once the initial outer surface has been eroded by the acid contents of the stomach. The size of expansion or the irregular shape of the dosage form prevents its exit from the stomach to the small intestine by occlusion of the pylorus.

Unfolding structures

Figure 1.11 shows one example of an unfolding structure in the expanded state.

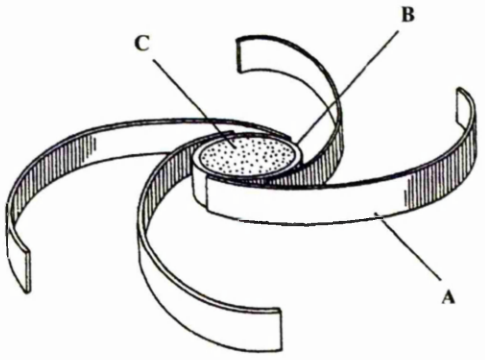


Figure 1.11 Unfolding structure designed to be retained in the stomach developed by Curatolo and Lo[38].

(A = retention arms, B = container for drug matrix, C = drug matrix)

Once swallowed, the flexible arms of the dosage form expand to a diameter of approximately 3cm. As the diameter of the dosage form is greater than that of the pylorus, delivery of the dosage form to the small intestine is prevented. The arms of the dosage form are designed to be flexible thereby preventing any damage to the mucosal surface of the stomach[38].

An alternative unfolding structure is shown in Figure 1.12

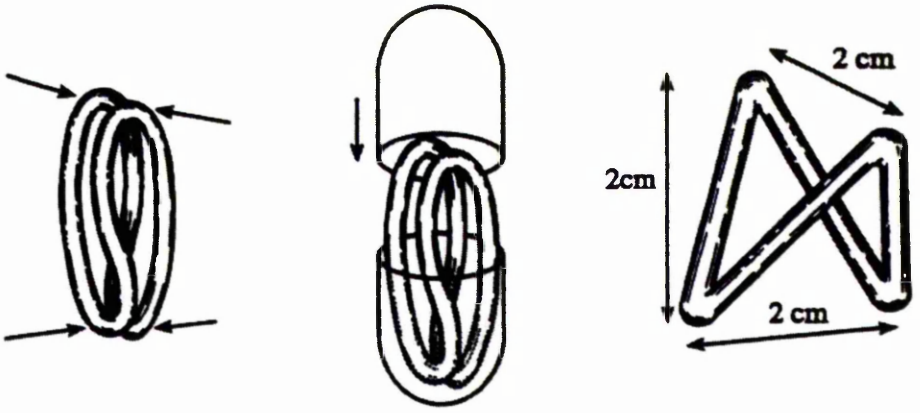


Figure 1.12 Unfolding drug delivery system developed by Caldwell et al[34].

The structure developed by Caldwell et al was designed to be retained in the stomach as the many geometric angles prevent an easy passage through the pyloric sphincter. However, the requirements of the drug delivery systems shown in Figures 1.11 and 1.12 are that they must not only be biodegradable, but their development and subsequent manufacture should be cost effective if a wide range of drugs are to be loaded into the systems.

Recently, Klausner et al have developed the unfolding structure shown in Figure 1.13.

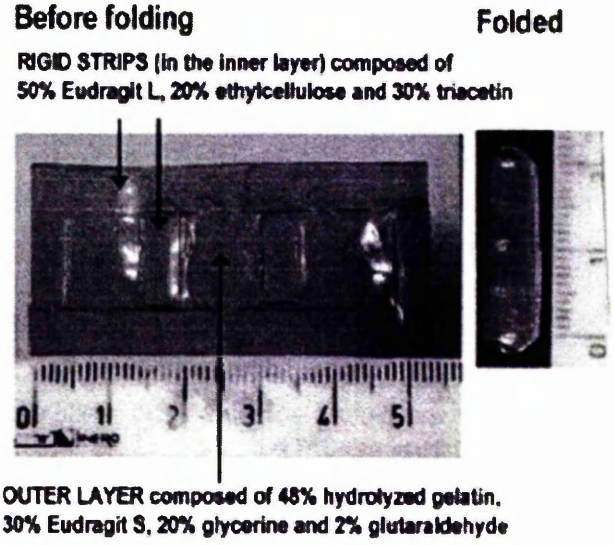


Figure 1.13 Unfolding drug delivery system developed by Klausner et al[39].

The dosage form consists of layers of polymers and selected drug that is folded and encased in a gelatin capsule. Following disintegration of the capsule in gastrointestinal fluids, the dosage form unfolds within 15 minutes[40] and a rigid multilayer structure is produced. The rigidity of the structure ensures that it is retained within the stomach. Using the dosage form, levodopa was administered to dogs and results show that the dosage form was retained within the stomach for a minimum of 24 hours whilst therapeutic concentrations were maintained for 9 hours[40]. Subsequent administration of the dosage form to human volunteers achieved gastro-retention times in excess of 8 hours[39].

When considering the above drug delivery devices the requirements that apply to swelling systems also apply to the unfolding structures, namely that they must unfold prior to being expelled by Phase III of the MMC and the dosage form must be biodegradable. As stated above the dosage form developed by Klausner et al reaches its full size within 15-minutes, however expulsion of the dosage form may already have occurred within this time. With regard to the dosage form developed by Klausner et al, gastric retention times in excess of 24 hours were achieved in dogs. However, no information is available on the time taken for the dosage form to degrade and leave the stomach. If a once daily dosage regime is anticipated, administration of the dosage form on subsequent days may lead to blockage of the pyloric sphincter if the dosage form requires in excess of 24 hours to biodegrade. To date, none of the above drug delivery systems are in commercial production.

Superporous hydrogels

Superporous hydrogels are fast swelling drug delivery systems and as such are subject to the same requirements that apply to swelling systems, (section 1.2.1.1). Kos Pharmaceuticals ™ are current developing superporous hydrogels, (SPH). The SPH contains the components that form a hydrogel mixture, (i.e. monomer, cross linker, diluent and initiator), but in addition the SPH contains a foaming agent, a foaming acid and a foam stabiliser[41]. When sodium bicarbonate is added to the mixture, within 30 seconds the dosage form swells to its maximum size and produces interconnecting pores that with the effervescent excipients generate bubbles. Gastro-retention of the dosage form

therefore results. Once the required period of time of gastro-retention has elapsed, the dosage form biodegrades and is eliminated from the body. To date gastro-retention times in excess of 6 hours have been recorded using the SPH[41].

1.2.1.3. MUCOADHESIVES

Mucoadhesives are defined as the attachment and retention of substances onto a mucus site[42]. The whole of the gastrointestinal tract is lined with a layer of mucus, thereby making it an ideal target for a gastro-retentive system.

With the exception of the oesophagus, the mucus layer within the gastrointestinal tract is negatively charged[42]. The negative charge occurs as a result of the ester sulphate and sialic acid groups of the glycoproteins that occur within the mucus layer[42]. Hence, mucoadhesive drug delivery methods have been developed.

Colestyramine has recently been investigated for its mucoadhesive properties whilst chitosan and carbopol are two examples of polymers that have traditionally been used as mucoadhesives. Using chitosan and colestyramine as examples, the following section reviews the two polymers as possible mucoadhesives.

Colestyramine

Colestyramine is an ion exchange resin that is traditionally used in the management of hypercholesterolaemia, but literature studies suggest that colestyramine exhibits mucoadhesive properties and may be a useful component of a gastro-retentive dosage form. A study in fasted volunteers comparing the mucoadhesive properties of an ionic polymer and the cationic polymer colestyramine, found colestyramine to be an improved mucoadhesive. Historically, anionic polymers are considered to be better mucoadhesives when compared with cationic or neutral polymers, but colestyramine has a negative charge in an acidic pH that is typical of the stomach environment, and has demonstrated improved mucoadhesive properties[43]. The mucoadhesive

properties of colestyramine were further exploited[44]. Microcapsules containing sodium bicarbonate and colestyramine were developed. The design of the dosage form was such that the microcapsules would initially float on the stomach contents due to the production of the gas carbon dioxide from the reaction of sodium bicarbonate with stomach. Secondly, the presence of the colestyramine would adhere to the mucosal walls[44]. Although no *in vivo* studies were completed, it is possible that such a dosage form could be administered in the fed or fasted state.

The disadvantage of using colestyramine as a drug delivery system is that colestyramine impairs the absorption of the fat-soluble vitamins A, D, E and K, and therefore, if used long term, additional supplementation of the aforementioned vitamins may be required[45].

Chitosan

Chitosan, derived from chitin, is a polycationic polysaccharide[46] that has several features that make it suitable as a hydrophilic matrix in the preparation of controlled release dosage forms[47]. Chitosan is cheap, non-toxic, biodegradable and versatile. It can be combined with other polyanionic polymers such as alginates to control or alter drug release rates from matrix preparations[46]. However, the use of chitosan is limited because the mucoadhesive properties of chitosan are directly affected by the pH of the surface to which it is to adhere[42]. The pH of the environmental conditions affects the surface charges of both the mucus and polymers. Since the pH of the stomach contents changes from acidic to alkaline depending on whether the stomach is in the fed or fasted state, the degree of mucoadhesion of chitosan will also be affected. With the exception of high molecular weight chitosans, an assessment of other chitosans found their mucoadhesive properties to be poorer than polcarbopol when studied in physiological relevant media of pH 1.2 and pH 7.5[48].

Chitosan has been used to coat calcium alginate beads and the calcium alginate beads have shown greater mucoadhesion than non-coated calcium alginate beads when administered to pigs[49]. However, *in vivo* studies in humans using mucoadhesives as a drug delivery system have proved

inconclusive[14]. The disadvantages of using mucoadhesives are many and may include:

- The inability of the dosage form to adhere to the gastric mucosa. If the MMC is in Phase III, then the contractions may be so strong as to prevent adhesion to the wall of the stomach and the dosage form will be emptied from the stomach prematurely[50].
- The hyper secretion of mucus. The function of mucus is to provide a physiologically protective barrier for the stomach wall. The attachment of a dosage form to the mucus compromises the protection provided by the mucus. The body's response to such a situation is to produce increased quantities of mucus to assist in the clearing of the dosage form from the stomach and to replace the mucus removed with the dosage form[50]. The hyper secretion of mucus results in increased gastro intestinal transit, thereby making conditions unfavourable for further attachment of mucoadhesives[50].
- The lack of adherence of the dosage form to the mucosa as a result of a change in mucus viscosity or synthesis. Pharmaceutically, some drugs affect the synthesis of mucus. In particular, the broad-spectrum antibiotics, tetracycline is able to form a complex with the glycoproteins in the mucus resulting in increased mucus production. Increased mucus production increases the barrier across which the drug must diffuse and may in turn result in a rate-limiting barrier to absorption of the drug[42, 50].
- Irritation of the mucosal surface by the drug incorporated within the dosage form. The long-term administration of non-steroidal anti-inflammatory drugs, (NSAID's) such as aspirin and ibuprofen result in peptic ulceration if given on an empty stomach.
- Insufficient mucus production for adherence of the dosage form. Disease states, such as *H. pylori,* cause the erosion of the protective mucus layer of the stomach and in turn result in the production of peptic or duodenal ulcers. The hyposecretion of mucus prevents mucoadhesion and allows for the gastric emptying of the dosage form as a normal immediate release preparation[25].

- A tendency for the dosage form to stick to most surfaces where mucoadhesion does occur[51]. The surfaces may include the physiological mucosal surface, e.g. oesophagus, causing irritation and possible obstruction to the gelatin of the capsules in which they are prepared resulting in reduced bioavailability[18].

Although mucoadhesives have initially shown promise a novel drug delivery system, attachment to the specific site required is unreliable. In addition, as discussed, the formation of a dosage form that delivers the drug by mucoadhesion is not suitable for some drugs.

1.2.1.4 MAGNETIC STRUCTURES

Magnetic dosage forms have been developed using the principal of incorporating a magnet such as magnesium ferrite[34] into a tablet. An external magnet is then placed on the outside of the individual in order to site the dosage form within the stomach. Drawbacks of such systems include the application of a suitable external magnet[18], and also developing a method that will allow for the specific placement of the dosage form within then individual. Presently neither has been developed to a satisfactory standard[18].

1.2.1.5 HIGH DENSITY SYSTEMS

Conventional single unit dosage forms that are not designed to float have a density of approximately $1.5 g.cm^{-3}$ [52]. High-density systems of either a tablet or pellet formulation have been investigated as a gastro retentive dosage form. The dosage form is designed to have a density greater than $1.5 g.cm^{-3}$ [52] that sinks to the antrum of the stomach and remains there for an extended period of time. The resistance of the dosage form to antral contractions and the positioning of the dosage form deep within stomach folds achieved the prolongation of gastric emptying.

A study in human volunteers reported that pellets in excess of $2.6 g.cm^{-3}$ are required to prolong gastric retention[53]. The same study also reported that

densities greater than 2.6g.cm^{-3} may produce a corresponding increase in gastro-retentive times. The findings were further supported by Clarke et al[53] that concluded there was no difference in gastric emptying times when comparing the gastric emptying times of standard units, (1.18 – 1.40mm) of density 2.0g.cm^{-3} and 2.4g.cm^{-3} with a control of similar size and with a density of 1.5g.cm^{-3}.

Although Clarke et al[54] suggested that gastro-retention may be achieved using high-density systems, the method has disadvantages. Animal studies suggest that such dosage forms have the potential to be retained in the stomach for longer than intended[34] and, as with the outsize dosage forms, the potential for blockage at the pyloric sphincter is high and undesirable.

1.2.1.6 MISCELLANEOUS

Citric acid

Citric acid has been used in the current project as a possible method to prolong the gastro-retention of the calcium alginate bead dosage form. The reader should be directed to Chapter 5, section 5.2.1 for the theoretical explanation of using citric acid to prolong gastro-retention.

Magnesium chloride

Literature findings suggest that magnesium salts, particularly magnesium chloride and magnesium sulphate can delay gastric emptying. However, a recent study suggests that the dose required to obtain such an effect is approximately 800mg[55]. In addition the effect of administering high doses of magnesium chloride over prolonged periods of time has not been investigated. Pharmaceutically, magnesium sulphate is found in laxative preparations, and the consequent use of magnesium chloride to delay gastric emptying is questionable.

Passage delaying methods

Fatty acids have been suggested as a means to delay gastric emptying based on the synopsis that fats exhibit delayed gastric emptying physiologically.

However, studies in humans have shown no appreciable increase in delayed gastric emptying when using triethanolamine myristate[56]. The failure of such a system may be due to variable bile production, (required for fat breakdown), between subjects[55]. Passage delaying agents are also not ideal as they can cause spasticity or laziness of the intestine as an unwanted side effect[14].

1.2.1.7 FLOATING SYSTEMS

Various dosage forms designed to float on stomach contents have been developed and these are detailed below. The essential requirement for a floating dosage form is that the density must be less than that of gastric fluids.

Microballoons

The microballoon or hollow microsphere consists of an air filled cavity enclosed within a drug/polymer matrix. The advantages of the microspheres are that the amount and type of polymer can be varied to control drug release and a wide variety of drugs can be incorporated into the dosage form.

A study using microballoons incorporated with riboflavin and administered in fed and fasted volunteers achieved average gastro-retention times of 300 minutes and 60 minutes respectively[57]. A second *in vivo* study also concluded that the floating time of the microballoons was limited to about 1 hour in the fasted state[58]. In addition, following a meal of medium intake, (e.g. lunch), some microballoons failed to remain buoyant[58]. In order to be considered as a viable gastro-retentive dosage form, increased retention times must be achieved and the buoyancy of the microballoons must be maintained so as not to be emptied from the stomach during Phase III of the MMC.

Effervescent or gas forming agents

Floating systems have been produced from alginates. Additionally, carbon dioxide has been produced from a reaction between an acid and sodium bicarbonate to obtain buoyancy[59, 60]. Some systems produce the carbon dioxide when in contact with gastric contents[59] and some rely on the carbon dioxide being produced during the bead manufacture. In the latter case, the

carbon dioxide is trapped within the bead and the resulting beads are then freeze dried[60].

The effervescent system shown in Figure 1.14 consists of a conventional tablet formulation surrounded by an effervescent layer and a swelling membrane layer. The effervescing layer contains sodium bicarbonate and tartaric acid that when in contact with the acidic media of the stomach causes carbon dioxide to be formed. The carbon dioxide then allows the swelling layer to expand and the dosage form rises to the surface of the media and floats.

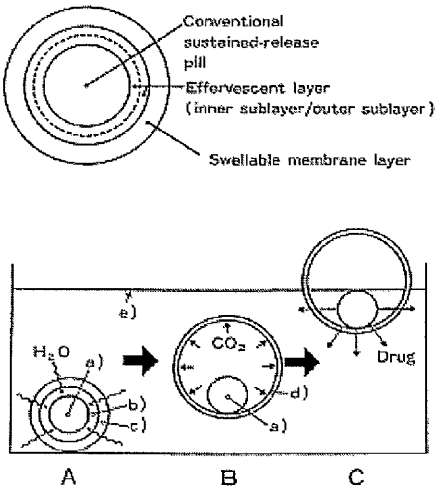


Figure 1.14 Structural characteristics and swelling mechanism of a gas generating system[59].

(A = water penetration, B = carbon dioxide production and floatation of dosage form, C = drug dissolution into aqueous media.

a = conventional SR tablet, b = effervescent layer, c = swelling layer, d = expanded swelling membrane layer, e = surface of aqueous media)

Although the effervescent systems have shown promise, there are some problems to be overcome. In the case of systems that rely on carbon dioxide production from a reaction of an acid such as citric acid, sodium bicarbonate and gastric fluids, the amount of carbon dioxide produced can vary between individuals. In addition, the production of carbon dioxide may cause discomfort

for the patient. Consequently the effectiveness of such a dosage form is questionable.

The method of incorporating carbon dioxide into beads and their subsequent freeze-drying requires further study and to date no *in vivo* studies have been carried out. *In vitro* studies have shown that the amount of carbon dioxide incorporated into the beads has an effect on drug release, strength and structure of the dosage form. Hence further studies are required to standardise the parameters[60].

1.2.1.8 CALCIUM ALGINATE BEADS

Freeze-dried calcium alginate beads are designed to float on the surface of stomach contents. They can therefore be categorised as a floating drug delivery system. However, since the current work is based wholly on floating calcium alginate beads, an individual section allocated to them is warranted. The production of alginate beads overcomes many of the difficulties associated with the above systems. Sodium alginate, from which the calcium alginate beads are produced), is also very versatile. It can be modified to accommodate other drug delivery vehicles, (e.g. liposomes)[61] and other excipients, (e.g. olive oil and calcium or sodium bicarbonate)[62]. The incorporation of olive oil with calcium alginate beads not only directly contributes to their buoyancy but also controls drug release rates, with increasing amounts of olive oil resulting in a corresponding decrease in drug release rate[62]. The inclusion of calcium or sodium bicarbonate within the calcium alginate beads produces carbon dioxide when in contact with stomach acid and therefore the buoyancy properties of the calcium alginate beads are improved[60].

The optimal conditions for producing the floating calcium alginate beads are discussed (Chapter 2, section 2.3.2.2). Calcium alginate is ideal as a drug delivery vehicle because in addition to the non-toxic properties, (Chapter 2, 2.2.1.5), once the drug has been exhausted from the dosage form, the calcium alginate beads are biodegradable.

Initial trials in human volunteers using gamma scintigraphy as the imaging method have shown that floating beads can be retained in the stomach for a period in excess of 6 hours in the fed state. In comparison non-floating beads have only been retained for about 1 hour[63]. Many drugs have been incorporated into the alginate beads including the anti-cancer agent 5-Flurouracill[64], nicotinic acid[65], paracetamol[66], and the broad-spectrum beta-lactam antibiotic amoxycillin[67].

1.3 AIMS AND OBJECTIVES

Calcium alginate beads have shown promise as a gastro-retentive dosage form. Nonetheless, in completed studies they have usually been administered with food. In common with other similar floating dosage forms, (e.g. microballoons), the administration of the dosage form in the fasted state usually results in their rapid expulsion from the stomach due to the action of the MMC.

The overall objective of the current project was to prolong the gastro-retention of calcium alginate beads, a floating multi-particulate dosage form. Of particular interest is the ability to prolong the gastro-retention of the dosage form when administered under fasting conditions. The presence of food has already been shown to prolong the gastro-retention of a dosage form in the stomach.

The individual aims of the project, that are discussed in the following chapters are:

- To modify the calcium alginate bead formula to allow for the inclusion of a model drug. Other excipients may also be incorporated into the formula in order that their effect may prolong gastric emptying still further.
- To carry out a full characterisation of the calcium alginate beads produced. The characterisation will provide information regarding the morphology of the calcium alginate beads, a knowledge of their buoyancy properties and an investigation into the mechanism of drug release from the calcium alginate beads.

- To investigate the *in vitro* release of a selected model drug from the calcium alginate beads by performing dissolution test in selected aqueous media that are designed to reflect changes in the gastrointestinal tract.
- To investigate the residence time of the calcium alginate beads in the stomach under different conditions of food intake. Of particular interest is the behaviour of the calcium alginate beads under fasted conditions. *In vivo* studies will determine the gastric emptying times of placebo and drug loaded floating calcium alginate beads, the potential of citric acid to delay the gastric emptying of calcium alginate beads, and the influence of prolonged gastro-retention on the bioavailability of a model drug from freeze-dried calcium alginate beads.

CHAPTER 2 – MATERIALS AND METHODS FOR CALCIUM ALGINATE BEAD PRODUCTION

CHAPTER 2 - MATERIALS AND METHODS FOR CALCIUM ALGINATE BEAD PRODUCTION

2.1 INTRODUCTION

Calcium alginate beads are formed as the result of the reaction between sodium alginate solution and calcium chloride solution[68]. The formula used to produce the calcium alginate beads was modified to allow for the inclusion of a model drug. Other excipients may also be added in order to alter the release rate of the model drug from the calcium alginate beads.

A description of the raw materials, used to produce the calcium alginate beads follows. The process used to produce the calcium alginate beads is also described. Using the aforementioned process, calcium alginate beads of different formulae were produced, the aim of which was to obtain a suitable formulation for use in the *in vivo* studies.

2.2 BEAD CONSTITUENTS

2.2.1 SODIUM ALGINATE

Sodium alginate is prepared commercially from the group of brown seaweeds known as phaeophyceae[61]. Algin, the raw material, is not found in any other plants and is extracted from the seaweed. The purification and subsequent production of sodium alginate is shown in Figure 2.1.

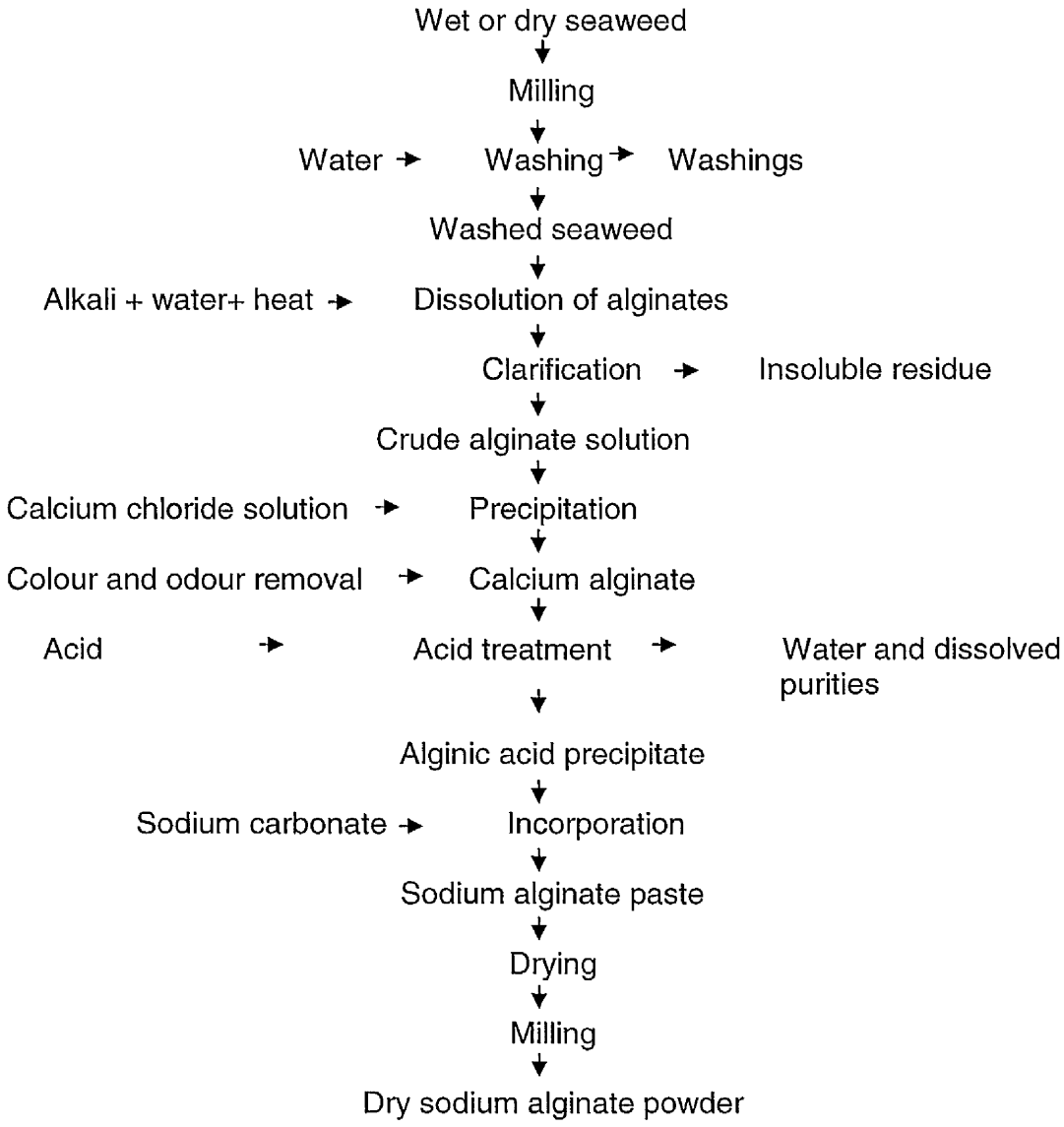


Figure 2.1 Manufacture of sodium alginate[69].

2.2.1.1 STRUCTURE

Sodium alginate is a linear co-polymer polysaccharide formed from the monosaccharides D-mannuronic (M) and L-guluronic (G) [70], the structure of which is shown in Figure 2.2.

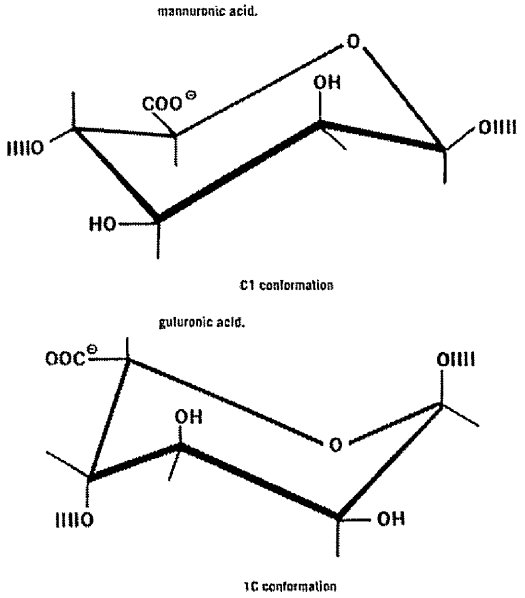


Figure 2.2 Structure of D-Mannuronic and L-Guluronic acid[69].

Each monosaccharide is joined by 1-4 glycosidic bonds, (as denoted by 01111 or 11110 above). The linking of the residues gives rise to homopolymeric sequences of solely M blocks or G blocks as well as a proportion that are alternating M and G blocks[69].

As a result of the equatorial conformation of the M units, the subsequent chain is flat and ribbon like in appearance. In direct contrast, the axial conformation of the G units produces a buckled ribbon like appearance[69]. Both M and G structures are shown in Figure 2.3.

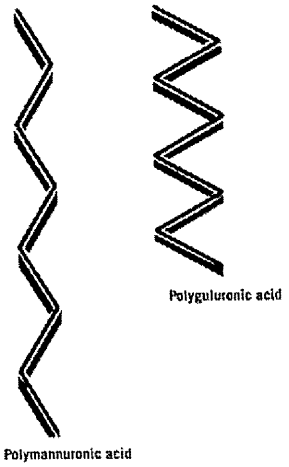


Figure 2.3 Chain appearance of polymannuronic, (M), and polyguluronic, (G), block residues of sodium alginate[69].

The number of M, G and M/G blocks depends on the species of brown seaweed from which the alginate is extracted; the direct consequence of which is a difference in properties of the particular alginate product. For example, changes in the number of M, G or M/G residues alters the molecular weight and hence the viscosity of the alginate[61]. For the purposes of producing alginate beads the higher viscosity grades of sodium alginate are used and these contain higher proportions of guluronic acid[69].

2.2.1.2 THE CHARACTERISATION OF SODIUM ALGINATE USING SCANNING ELECTRON MICROSCOPY

The surface morphology of sodium alginate was studied using scanning electron microscopy.

Sample preparation

A fine layer of raw material was placed on a sheet of filter paper. A double-sided carbon tab was attached to a 1cm aluminium stub using one self-adhesive face. The top self-adhesive face was then placed lightly on the filter paper, allowing the raw material to become attached.

Using an Emscope SC500, (Kent, England), the aluminium stubs with affixed samples were then sputter coated in argon for a period of 4 minutes at a voltage of 25 milliamps to give a final thickness of gold of 10 – 20nm. Samples were then viewed using a Cambridge 360 SEM, (Cambridge, England).

Sodium alginate is a coarse powder that is white to light yellowish-brown in colour, is tasteless and odourless. The sample, (Manugel GMB™), was obtained from ISP alginates[71]. A typical SEM of sodium alginate is shown in Figure 2.4.

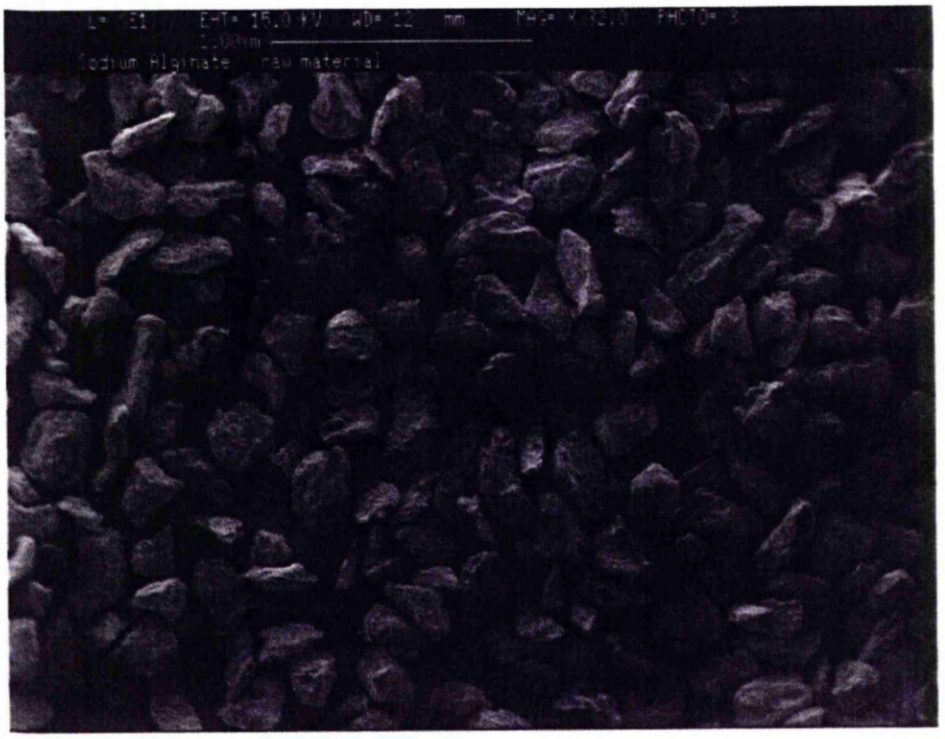


Figure 2.4 SEM appearance of sodium alginate.

Sodium alginate has a rough granular appearance with the large proportion of granules exhibiting uniformity in size. Irregularly shaped or sized granules may require additional milling or sieving in order to achieve elegant solutions. The apparent uniformity of the granules indicates that no pre-treatment of the raw sodium alginate is necessary.

2.2.1.3 PROPERTIES

pH (1% w/v solution)[71]	neutral
Viscosity (1 % w/v solution)[71]	110 - 270 m Pa.s (cP)
Solubility in water[71]	Soluble. Forms viscous solutions. Pastes are formed at concentrations over 5%.
Stability[71]	Stable

2.2.1.4 GELATION OF SODIUM ALGINATE

The production of calcium alginate beads occurs because sodium alginate is insoluble in solutions containing calcium salts. Hence an insoluble precipitate or gel is produced. The reaction can be represented by Equation 2.1.

Equation 2.1

(NaAlg = sodium alginate, Ca = calcium, CaAlg = calcium alginate, Na = sodium)

$$2NaAlg + Ca^{2+} \longrightarrow 2CaAlg + 2Na$$

The different shapes of the M and G block residues determine their relevant functions. As a result of their different shapes, the functions are also different[69].

The mechanism of gelation predominantly concerns the di-axial conformation of the G blocks with calcium ions. The G block conformation is rigid, and, due to the buckled nature electronegative cavities exist which can be occupied by divalent calcium ions[72], as shown in Figure 2.5 below.

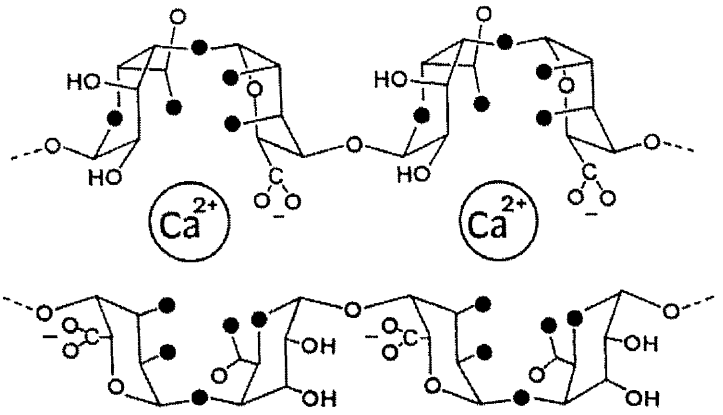


Figure 2.5 Binding of calcium ions with G block residues of sodium alginate[63].

The function of the M block residues is to aid solubilisation. The di-equatorial conformation determines that there is an absence of suitable sites for the interstitial binding of calcium ions as in the G blocks. However, when very high concentrations of calcium were used, there was evidence demonstrated of binding to M blocks, although further studies showed that this binding was weak, non-specific and non-co-operative[69].

Based on the above findings, Grant et al proposed the 'egg box' model of calcium alginate, Figure 2.6[73].

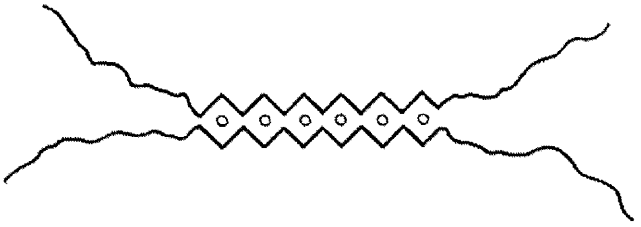


Figure 2.6 The 'egg box' model of calcium alginate[69].

2.2.1.5 TOXICOLOGY

Sodium alginate has been subjected to extensive tests to determine toxicological parameters. In animal studies that included mice, rats, guinea pigs and dogs no mortalities, toxicities, harmful effects or irritations were reported. Literature sources state that no adverse effects were apparent when doses in

the range of 175 - 200mg/kg/day were administered to human volunteers for periods in excess of 21 days. Hence, sodium alginate is regarded as non-toxic and non-irritant[74].

Sodium alginate is listed in FDA, (Food and Drug Administration), regulations as a substance that is GRAS, (generally regarded as safe). It is licensed in the U.K. for use in non-parenteral medications and is approved as a food additive, (E401), by the European Union[74].

2.2.1.6 USES OF SODIUM ALGINATE

The properties of alginates have made them ideal for many purposes. Alginates have been used as thickening agents in cosmetics, general colloidal applications such as stabilising solids in fruit drinks and in the preparation of films and fibres[74].

They have also been widely used within the pharmaceutical industry. When used in solid dosage forms they have been used as a binder in tablets and as a disintegrant in capsules[74]. In line with their applications in the cosmetic and food industry they have also been used as suspending agents in creams and as stabilising agents in oil in water emulsions. Surgical dressings have also been produced with sodium alginate where it acts as a haemostatic agent[75].

Sodium alginate has also been used to produce novel drug delivery systems and to date pilocarpine has been used in such a way. On contact with the lachrymal fluids a gel is formed which provides 24-hour delivery of the drug[76].

With specific regard to oral delivery a number of drugs have been incorporated into calcium alginate beads. Such drugs include ampicillin[77], ascorbic acid[78], insulin[79], ibuprofen[80], naproxen[81], sulphamethoxazole[82] and paracetamol[66].

2.2.2 RIBOFLAVIN

Riboflavin, also known as Vitamin B2[83], is a water soluble vitamin found in a variety of nutritional sources including yeast, milk, green leafy vegetables, heart, liver and kidney. Commercially it is manufactured synthetically.

2.2.2.1 STRUCTURE

The structure of riboflavin or 7,8-dimethyl-10-(D-ribo-2,3,4,5-tetrahydroxypentyl) isoalloxazine is shown in Figure 2.7(a)[84]. The structure of riboflavin-5'-phosphate is shown in Figure 2.7(b).

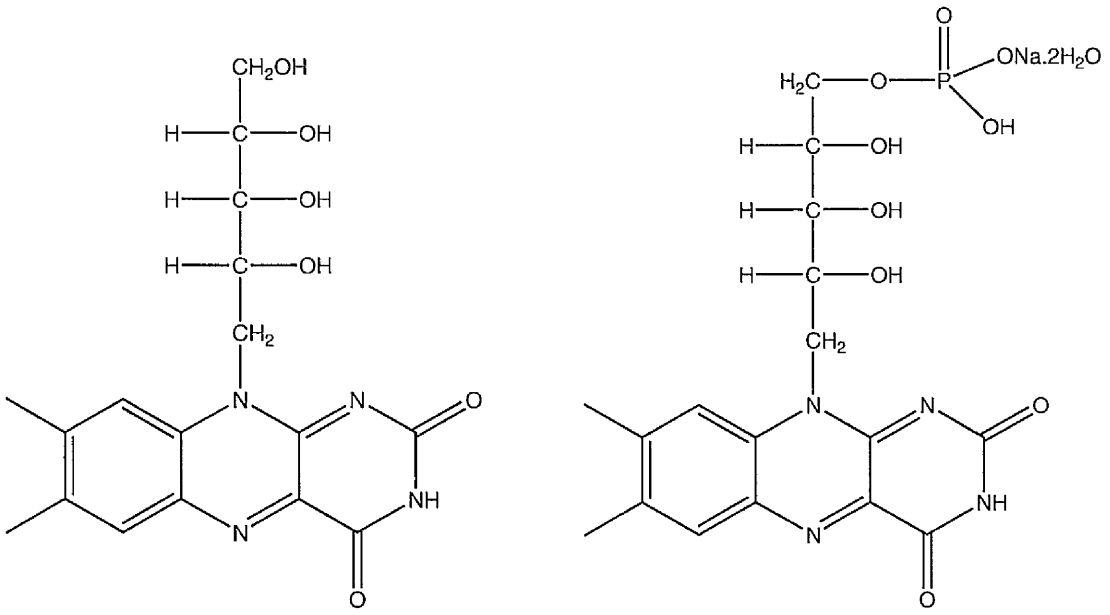


Figure 2.7(a) Structure of riboflavin[84], (b) riboflavin-5'-phosphate.

2.2.2.2 APPEARANCE

Riboflavin is a yellow or orange crystalline powder[85]. It has a slight yeast like odour and a bitter taste. The appearance of riboflavin as viewed by SEM is shown in Figure 2.8. Samples of riboflavin were prepared as for sodium alginate, (Chapter 2, section 2.2.1.2) and viewed using Cambridge 360 SEM, (Cambridge, England).

Figure 2.8 SEM appearance of riboflavin

2.2.2.3 PROPERTIES

Molecular Weight[86]	376.4
Empirical Formula[86]	$C_{17}H_{20}0_6$
Dissociation Constant - pKa's at 20°C[86]	1.9, 10.2
Solubility in water[86]	1 in 3000 - 20000. The variability in the values is due to differences in internal crystal structure.
Solubility in alcohol[86]	<1 in 10000
Stability[86]	Degrades in solution in the presence of light.

2.2.2.4 TOXICOLOGY

Riboflavin has a LD_{50} value of 340 - 560 $mg.kg^{-1}$, and following studies in dogs is considered non-toxic and non-teratogenic[84].

2.2.2.5 USES OF RIBOFLAVIN

The uses of riboflavin extend from those that are non-pharmaceutical through to physiological uses.

As a non-pharmaceutical agent, riboflavin has been widely used in the food industry as a colouring agent[87].

Therapeutically, riboflavin has been used to correct riboflavin deficiency, usually as part of a multi B vitamin preparation[87].

Physiologically, riboflavin is required to release the energy from food for metabolic reactions[87].

Use in correcting vitamin deficiency

Riboflavin can be used to correct deficiency of the vitamin. However, riboflavin deficiency alone is rare[84]; more commonly it is likely to be part of a multiple vitamin B group deficiency, which in turn is due to inadequate dietary intake. Consequently it is difficult to determine the extent of any deficiency but estimates can be calculated by performing enzyme assays. Such assays are preferred in comparison to obtaining direct riboflavin levels, as these are not considered to be accurate enough to be of diagnostic value. Assay results that present with an excretion rate of less than 50mg/day are said to be characteristic of riboflavin deficiency, (arabinoflavinosis)[84]. Typical signs and symptoms are cheilosis, angular stomatitis, keratitis, surface lesions of the genitalia, seborrhoeic dermatitis, normocytic anaemia and itching or burning of the eyes.

Therapeutic doses in the range of 2 - 10mg/day are required to correct any deficiency and once plasma concentrations are back within the suggested range, intake values should follow those recommended. Intake is normally related to diet and figures suggest that 0.6mg riboflavin should be obtained from 1000 kCal of food[84]. Hence, based on a normal adult, quantities should be in the range of 1.4mg for males and 1.2mg for females[88].

Members of the population that may warrant additional supplementation include those who are pregnant and those suffering with hepatitis or cirrhosis of the liver. In the former population class, increased levels of riboflavin are required to prevent skeletal foetal abnormalities. In the latter situation the liver loses the ability to store the small amount of the vitamin that would normally be found in non-cirrhotic or non-hepatitis patients.

Use in physiological reactions

Riboflavin in the circulation is extensively bound to plasma proteins. It is widely distributed within the body, with only small amounts being stored in the liver, spleen, kidneys and heart muscle. Any excess riboflavin is excreted in the urine unchanged[89].

Riboflavin is essential in order to utilise energy from food intake. Specifically the coenzymes, FAD, (flavine adenine dinucleotide), and FMN, (flavin mononucleotide), are responsible for metabolic reactions within the respiratory chain. However, in order for this to occur, the initial requirement is for riboflavin to be converted to its active co-enzymes FMN and FAD. The reaction sequences shown in Figure 2.9 are enzyme catalysed by flavokinase and are dependent on riboflavin being specifically absorbed in the proximal part of the gastrointestinal tract.

Riboflavin + ATP⟶ FMN + ADP

FMN + ATP⟶ FAD + PP

Figure 2.9 Conversion of riboflavin to the co-enzymes FMN and FAD[90].

Use as a model drug to study gastric emptying

Riboflavin has been identified from literature sources as a suitable model drug for use in a study of gastric emptying. The main reason for using riboflavin is that it has a specific absorption site within the proximal part of the gastro-intestinal tract for absorption and this is confirmed by several findings [90,91].

Levy and Jusko have shown that when riboflavin is given with food there is a linear relationship between the dose of riboflavin administered and the amount of riboflavin recovered in the urine[92]. However, when given on an empty stomach the amount of riboflavin recovered decreases with increasing dose. From their data, they concluded that there was a limited capacity for riboflavin absorption. In later studies that compared the fasted and fed state, the process was shown to be saturable following the administration of large doses[90]. The saturation process was confirmed by figures that initially demonstrated a decrease in the excretion rate of riboflavin when given on empty stomach, but following a meal the excretion rate increased. It was concluded that the absorption mechanism was located solely or mainly in the proximal region of the gastro-intestinal tract. However, the effect was not due to delayed gastric emptying but instead due to re-absorption in the small intestine that in turn was as a result of the presence of food stimulating bile[89].

The specific absorption site of riboflavin within the gastro-intestinal tract makes it an ideal model drug to use in experiments to monitor gastric emptying. As riboflavin is excreted renally, urine collection and subsequent analysis by HPLC will make for an ideal assay method to calculate the amount of riboflavin absorbed. In addition, the method is non-invasive and therefore acceptable to volunteers taking part in the study.

2.2.3 CITRIC ACID

Citric acid is a naturally occurring product in many plant species, including lemon juice where levels are in the region of 5-8%[74]. Citric acid may also be produced commercially by re-crystallising the fermented products of molasses that are produced by the mycobacterium, *Aspergillus niger*[74].

2.2.3.1 STRUCTURE

Citric acid or 2-hydroxypropane-1,2,3-propanetricarboxylic acid monohydrate is a carboxylic acid, the structure of which is shown in Figure 2.10.

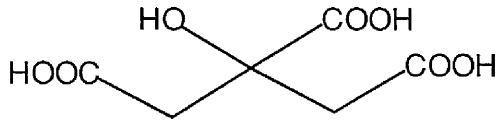


Figure 2.10 Structure of citric acid[87].

2.2.3.2 Appearance

Citric acid is a white odourless crystalline powder with a tart acid taste[87].

2.2.3.3 Properties

Molecular Weight[86]	210.14
Empirical Formula[86]	$C_6H_8O_7.H_2O$
Acidity[86]	pH 2.2 (1.0% w/v aqueous solution)
Dissociation Constant - pKa's at 25°C[86]	3.128, 4.761, 6.396.
Solubility in ethanol 95%[86]	1 in 1.5 parts
Solubility in water[86]	1 in <1 part

2.2.3.4 Toxicology

Citric acid is regarded as non-toxic when used as an excipient as amounts are too small to be considered harmful. The oral LD_{50} values for the mouse and rat are 5.04g.kg^{-1} and 6.73g.kg^{-1} respectively[74].

Citric acid is considered harmful when amounts are taken in excess and in such cases may lead to erosion of the teeth[74].

Citric acid should be used with caution in renal patients. The co-administration of citric acid with aluminium containing compounds leads to increased absorption of aluminium and potentially harmful serum levels[74].

2.2.3.5 Uses of citric acid

Traditionally citric acid has been used in the food industry as an acidity regulator and also as a flavour enhancer[86].

Pharmaceutically the main uses of citric acid are as a flavouring agent and as an excipient with sodium bicarbonate in the production of effervescent granules and tablets[86].

Therapeutically, citric acid has been used to dissolve renal calculi but more recently it has been suggested as a viable alternative to fatty meals for diagnosing the presence of *Helicobacter pylori,* the causative organism of chronic gastritis[93].

The diagnosis of Helicobacter pylori (H. pylori)

The diagnosis of *H. pylori is* carried out by measuring the amount of carbon dioxide, (CO_2), produced when a patient exhales[93]. The procedure utilises urea that is radio-labelled with ^{13}C. When the urea reacts with the enzyme urease, produced by the organism, CO_2 is produced. In *H.pylori* positive patients, CO_2 levels are far in excess of negative *H.pylori* patients[93]. If gastric emptying is retarded then increased reaction times are possible between the radio-labelled agent and the enzyme resulting in increased CO_2 recovery and an improvement in the diagnosis of *H.pylori*[94].

Previous studies have shown that low concentrations of citric acid in the region of $1g.200ml^{-1}$ are sufficient for use in the diagnostic process are equally as effective as the fatty acid meals traditionally used[94]. In addition it is also thought possible that using citric acid solution has the advantage of enhancing the intragastric distribution of urea[40]. The addition of sweeteners make for a more palatable solution[95] and patient preference for such solutions over the fatty acid meals has been established.

The use of citric acid to retard gastric emptying

The use of citric acid in the diagnosis of *H. pylori* represents a new possible commercial medical application of the substance. However, the suggestion that citric acid may be used as an excipient to retard gastric emptying has been established for many years[96]. The mechanism by which a delay in gastric emptying is achieved has been discussed, (Chapter 5, section 5.2.1).

2.2.4 MAGNESIUM STEARATE

2.2.4.1 APPEARANCE

Magnesium stearate is a fine white powder of low bulk density. It has a slight odour of stearic acid[74].

2.2.4.2 PROPERTIES

Molecular weight[97]	591.34
Empirical formula[97]	$[CH_3(CH_2)_{16}COO]_2Mg$

2.2.4.3 TOXICOLOGY

Magnesium stearate is listed in the FDA, (Food and Drug Administration), regulations as a substance that is GRAS, (Generally Regarded as Safe). Magnesium stearate is considered to be non-toxic, although the ingestion of excess quantities may induce diarrhoea or cause mucosal irritation[97].

2.2.4.4 USES OF MAGNESIUM STEARATE

Within the pharmaceutical industry, magnesium stearate is used as a lubricant in the manufacture of tablets and capsules. Typical amounts used are in the range of 0.25 – 0.5% w/w[74].

Magnesium stearate is hydrophobic. Magnesium stearate has previously been used in the preparation of a gastric floating drug delivery system, and showed improved buoyancy properties[98]. Magnesium stearate has been incorporated into the current calcium alginate bead formula in order to obtain improved buoyancy of the calcium alginate beads, thus exploiting the hydrophobic property.

2.3 PRODUCTION OF FLOATING CALCIUM ALGINATE BEADS

2.3.1 BACKGROUND

Initial work in 1998 resulted in the production of a floating dosage form[67] followed by some *in vivo/in vitro* studies. The floating dosage forms developed were calcium alginate beads. The formation of the calcium alginate beads was achieved by allowing droplets of sodium alginate to pass into a solution of calcium chloride[68]. The resulting gel beads were freeze-dried and as their density is lower than that of gastric contents, they therefore float. The aim was to produce the calcium alginate beads with modified formulae so that they may subsequently be used for *in vitro* and *in vivo* studies.

2.3.2 EXPERIMENTAL SECTION

2.3.2.1 MATERIALS

Sodium alginate as Manugel GMB (ISP Alginates, Surrey, England), citric acid (BDH Chemicals, Poole, England), calcium chloride (BDH Chemicals, Poole, England), riboflavin (Merck, Darmstadt, Germany), magnesium stearate (BDH Chemicals, Poole, England), ethanol GPR grade, (BDH Chemicals, Poole, England), and liquid nitrogen (BOC, Manchester, England) were used as received. The solvent used in all cases was singly distilled water.

2.3.2.2 PRODUCTION OF CALCIUM ALGINATE BEADS

Process parameters

The method of producing calcium alginate beads was based on the methods previously determined[63, 68]. The process parameters are as follows:

Sodium alginate concentration	2% w/w
Sodium alginate flow rate	1.54 ml.min^{-1}
Needle gauge	21 G
Sodium alginate dropping height	20 cm
Calcium chloride concentration	0.02M
Sodium alginate:calcium chloride volume ratio	1:2.5
Curing time	30 minutes
Temperature	25°C
Freezing method	Liquid Nitrogen
Drying method	Freeze-drying

The preparation of the calcium chloride solution is described in Appendix I. Sodium alginate solutions were prepared the day before bead production and were allowed to stand overnight at room temperature to de-aerate.

Figure 2.11 is a schematic representation of bead formation.

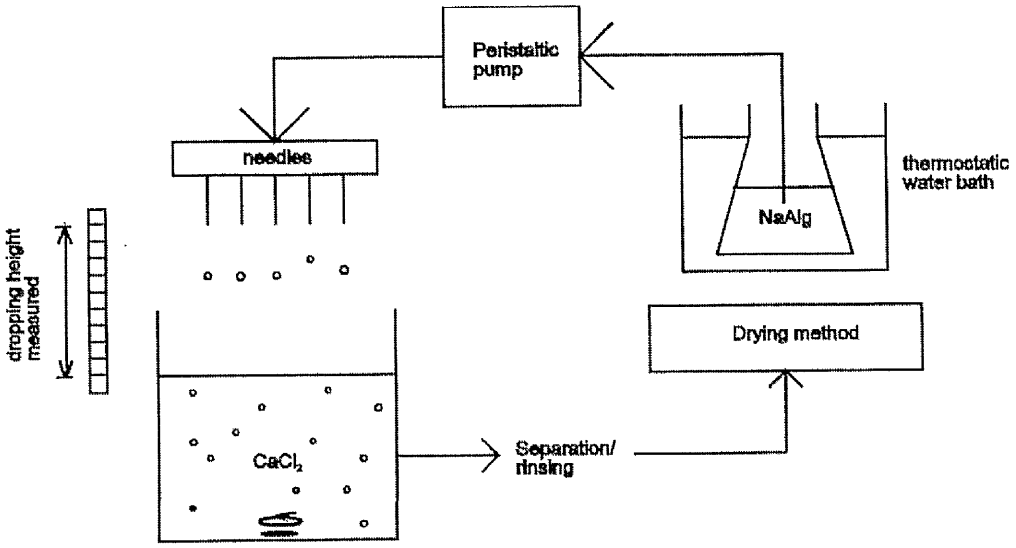


Figure 2.11 Schematic diagram of laboratory production of calcium alginate beads[63].

The sodium alginate solution was extruded through 21G needles using a peristaltic pump, (Watson-Marlow 202U, Cornwall, England) into stirred calcium chloride at a flow rate of 1.54ml.min^{-1}. Prior to extruding the sodium alginate solution into the calcium chloride for the purposes of calcium alginate bead

production, sodium alginate solution was extruded through the peristaltic pump into a measuring cylinder for 1 minute and the volume measured. When an amount of 1.54ml occupied a measuring cylinder, the pump speed was determined to be at the correct position and the drop volume was constant. Calcium alginate beads were then produced using the remainder of the sodium alginate solution. Once all the sodium alginate had been used the calcium alginate beads remained in the stirred calcium chloride 30 minutes for gelation to occur, (a process known as curing). The resulting calcium alginate beads were then separated and rinsed with 3 x 100ml aliquots of singly distilled water. They were then rapidly snap-frozen with liquid nitrogen before drying using an Edwards Modulo freeze-drier, (West Sussex, England), with bell jar attachment. Temperature and pressures used dry the calcium alginate beads were -40°C and 80Nm^{-2} respectively.

Formulation parameters

Incorporation of sodium alginate into solution.

In order to obtain a smooth solution, the sodium alginate was added to three quarters of the final volume of water and mixed using a Heidolph RZR1 shear mixer, (Schwabach, Germany), for four minutes at 75% of maximum speed after which a smooth homogenous mix was obtained. The remaining solution was then made up to volume with water and mixed for a further minute.

Incorporation of riboflavin into sodium alginate solution.

Riboflavin is poorly soluble in water. Therefore to allow for the incorporation into the aqueous sodium alginate mix, the required amount of riboflavin was dispersed in 0.25ml ethanol GPR grade. The sodium alginate was then prepared using three quarters of the total volume of water. The suspension of riboflavin in ethanol was then added to the sodium alginate solution and mixed for two minutes. The riboflavin/sodium alginate solution was then made up to volume to give a final concentration of 2% w/v sodium alginate.

Incorporation of citric acid into sodium alginate solution.

Citric acid is freely soluble in water; the solubility in sodium alginate is unknown. Therefore citric acid was initially dissolved in the volume of water that would be

used to prepare the sodium alginate. The sodium alginate was then incorporated as above.

Literature sources suggest concentrations of $1g.200ml^{-1}$ may retard gastric emptying[94]. Consequently, the concentrations of citric acid used were 0% w/v, 0.5% w/v, 0.75% w/v and 1 % w/v.

Incorporation of magnesium stearate into sodium alginate solution.
The sodium alginate solution was then prepared using three quarters of the total volume of water, as described. The required amount of magnesium stearate was weighed, added to the sodium alginate solution and mixed using a Heidolph RZR1 shear mixer, (Schwabach, Germany), at 75% of maximum speed until all magnesium stearate had been incorporated into the solution. The sodium alginate/magnesium stearate solution was then made up to volume to give a final concentration of 2% w/v sodium alginate.

2.4 RESULTS AND DISCUSSION

2.4.1 FORMULATION MODIFICATIONS

Dissolution of sodium alginate

Incorporation of sodium alginate into the water in the manner mentioned above produced a smooth solution. After overnight standing the solution was free from any air bubbles that may otherwise affect the final beads. The method of mixing was therefore deemed sufficient and reproducible. All future sodium alginate solutions would be prepared in the same manner.

Incorporation of riboflavin into sodium alginate solution

Samples of calcium alginate beads containing riboflavin have been produced. The amounts of riboflavin used resulted in final concentrations of 0.00% w/v, 0.03% w/v, 0.06% w/v and 0.12% w/v of the sodium alginate solution. The calcium alginate beads containing the lowest amount of riboflavin were light in colour. They were thought to contain insufficient amounts of riboflavin for the *in vivo* studies but would be useful to use to characterise the calcium alginate

beads. Calcium alginate beads containing the largest amount of riboflavin would also be used for *in vitro* work only. When producing the solution, it was noted that the solution appeared to have lighter and darker areas, indicating that the solution may have been saturated with riboflavin. Using such a solution may produce calcium alginate beads containing different amounts of riboflavin, thereby making the results of some experimental procedures inaccurate.

Incorporation of citric acid into sodium alginate solution

When citric acid was used at concentrations 0.05% w/v and 0.75% w/v citric acid, calcium alginate beads were produced that were visually comparable with calcium alginate beads that contained no citric acid. When citric acid was incorporated at a concentration of 1% w/v sodium alginate solution, and the resulting mix extruded into calcium chloride solution, gel beads failed to form and the calcium alginate resembled that of fine feather like flakes. The fine feather like flakes observed when using citric acid 1% w/v solution are likely to be insoluble particles of alginic acid. When concentrations of 0.5% w/v and 0.75% w/v citric acid are used, the pH will remain sufficiently high to avoid precipitation of alginic acid. When, the sodium alginate is dispersed in a 1% w/v citric acid solution, the pH is reduced and alginic acid is precipitated[100]. Therefore concentrations of 1% w/v citric acid solution would not be studied any further. The calcium alginate beads containing 0% w/v citric acid solution would be considered for *in* vivo studies whilst the calcium alginate beads containing 0.5% w/v and 0.75% w/v citric acid would be utilised for *in vitro* studies.

Incorporation of magnesium stearate into sodium alginate solution

Although magnesium stearate is hydrophobic, when mixed at a sufficiently high speed as described, magnesium stearate was incorporated into the sodium alginate solution. The visual appearance of the solution was that of a smooth homogenous mix with fine particles of magnesium stearate.

Production of calcium alginate beads

Calcium alginate floating beads of different formulae have been prepared using the method described, (Chapter 2, section 2.3.2.2). Placebo calcium alginate beads have been produced as a control for the all other calcium alginate beads

with different formulae. In addition, placebo calcium alginate beads containing no citric acid would be used for the *in vivo* studies. Calcium alginate beads containing different concentrations of riboflavin were produced to ascertain the most suitable concentration of riboflavin to use for the *in vivo* studies. Although, the experimental work has showed that a concentration of 0.06% w/v riboflavin was best suited for the purpose of performing *in vivo* studies, calcium alginate beads containing concentrations of 0.03% w/v and 0.12% w/v riboflavin would provide valuable comparable data for characterisation and *in vitro* dissolution experiments.

The purpose and method of incorporating magnesium stearate within the calcium alginate beads has been stated, (Chapter 2, section 2.3.2.2). However, when the magnesium stearate/sodium alginate solution was used to prepare the calcium alginate beads, it was noted that the particles of magnesium stearate within the sodium alginate solution frequently blocked the cannula. Therefore, although further use of magnesium stearate within the calcium alginate bead formula should not be discouraged, it may be necessary to consider reducing the particle size of magnesium stearate to avoid process problems when producing the calcium alginate beads.

Since the effect of citric acid on riboflavin is not known, calcium alginate beads containing citric acid would not be used for *in vivo* studies. However, calcium alginate beads containing citric acid would be used for *in vitro* studies and therefore provide valuable comparative data for calcium alginate beads not containing citric acid.

Table 2.1 shows a summary of the calcium alginate beads produced for the current investigation.

Sample	Riboflavin concentration (%) (w/v)	Citric acid concentration (%) (w/v)	Magnesium stearate (%) (w/v)
1a	0.00	0.00	0.00
1b	0.00	0.50	0.00
1c	0.00	0.75	0.00
2a	0.03	0.00	0.00
2b	0.03	0.50	0.00
2c	0.03	0.75	0.00
3a	0.06	0.00	0.00
3b	0.06	0.50	0.00
3c	0.06	0.75	0.00
4a	0.12	0.00	0.00
4b	0.12	0.50	0.00
4c	0.12	0.75	0.00
With magnesium stearate	0.00	0.00	0.65

Table 2.1 Final formulae of calcium alginate beads.

In summary, formulae 1a and 3a will be used for both *in vivo* and *in vitro* studies. All remaining formulations will be used for *in vitro* studies only.

2.5 CONCLUSION

Freeze-dried calcium alginate beads, suitable as a multiple unit drug delivery system have been produced. The initial formula has been modified to include a model drug, riboflavin. Citric acid and magnesium stearate have also been incorporated into the calcium alginate beads. Overall, the method has proved to be robust and re-producible. However, if magnesium stearate is to be incorporated in future batches of calcium alginate beads, consideration should be given to using magnesium stearate with a reduced particle size. The

problems of blocked cannulas, as experienced in the current work can be avoided.

The physical properties of the calcium alginate beads will be assessed. Using various methods, the characterisation will provide an understanding of the structure, floating ability and method of drug release from the calcium alginate beads.

CHAPTER 3 – THE CHARACTERISATION OF CALCIUM ALGINATE BEADS

CHAPTER 3 – THE CHARACTERISATION OF CALCIUM ALGINATE BEADS

3.1 INTRODUCTION

The aim of performing an extensive characterisation of the calcium alginate beads was to obtain information regarding the structure, floating ability and changes that occur when the dosage form is placed in aqueous media.

Methods for the assessments of the weight, diameter, density, resultant weight and SEM have been described previously[63]. The inclusions of the aforementioned techniques were also necessary to compare the calcium alginate bead samples produced for the current studies with that described in the literature.

The calcium alginate beads produced from the formulation containing magnesium stearate were characterised as far as diameter, mass, density, SEM, X-ray microanalysis and digital photography only. Further evaluation was beyond the scope of this work.

3.2 EXPERIMENTAL SECTION

3.2.1. PHYSICAL PARAMETERS

3.2.1.1 DIAMETER

The diameter of the calcium alginate beads was measured using a Moore and Wright 0 – 25mm micrometer, (Sheffield, England) with measuring limits of ±10%. A sample, (n = 10), of placebo beads, riboflavin loaded beads and placebo beads containing magnesium stearate were measured. Measurements for each sample were repeated twice. Mean diameters and standard deviations were recorded, Appendix II.

3.2.1.2 WEIGHT

The weights of the calcium alginate beads were obtained using a Mettler AC 100 balance (Zurich, Switzerland). A sample (n = 30), of placebo, riboflavin loaded beads and placebo beads containing magnesium stearate was weighed. Measurements for each sample were repeated twice. Mean weights and standard deviations were recorded, Appendix II.

3.2.1.3 DENSITY

The buoyancy properties of placebo and riboflavin loaded calcium alginate beads were determined using resultant weight measurements, (Chapter 3, section 3.2.3.1). In addition the densities of the calcium alginate beads were also derived mathematically and experimentally.

Mathematical determination

Mathematically, the figures for the density of the calcium alginate beads were obtained using Equation 3.1 to calculate the volume of a sphere, and Equation 3.2 to calculate the density of the calcium alginate beads.

Equation 3.1

$$V = \frac{4}{3}\pi r^3$$

where v = volume of a bead

r = radius of a bead

Equation 3.2

$$D = \frac{m}{v}$$

where D = density of a bead

m = mass of a bead

v = volume of a bead

Experimental determination

The AccuPyc® 1330 helium pycnometer, (Norcross, USA), with a 1cc sample cup was used to determine the density of a sample of calcium alginate beads.

The pycnometer measures the pressure changes of helium in a calibrated sample volume. The sample chamber is filled with helium and once filled, the gas is discharged into a second empty chamber. The changes in pressure in the calibrated sample volume allow density values to be calculated. Samples must be dry as the presence of water vapour affects measurements. The freeze-dried calcium alginate beads were therefore suitable for determining the density using the pycnometer. For each sample of calcium alginate beads, duplicate measurements were performed.

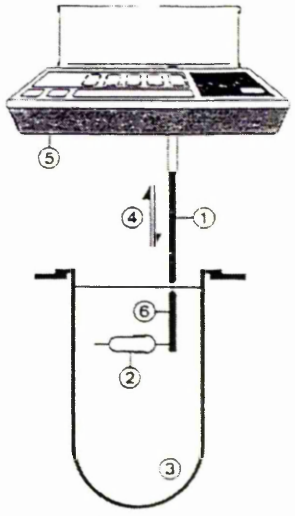
3.2.2 METHODS

3.2.2.1 DETERMINATION OF BUOYANCY PROPERTIES OF CALCIUM ALGINATE BEADS USING RESULTANT WEIGHT APPARATUS

Initial buoyancy tests were performed by placing 20 beads within a flask to which 25ml of media was added. The flasks were then stoppered and shaken for 5 minutes using a bottle shaker. Observations of the number of beads that remained floating were made at specific time intervals, and overall they were left for 24 hours. Although it is possible to verify that the calcium alginate beads are buoyant, using such a method, the test is inadequate. Using such a system provides no information about the kinetics of the floating dosage form, (FDF), or how the behaviour of the dosage form changes once immersed in different media. Of particular relevance is the consideration of the behaviour of the calcium alginate beads in acidic media that is reflective of stomach environment. Resultant weight is a term used to describe the buoyancy properties of a FDF. The resultant weight system was developed by Timmermans and Moës[100] and considers the vertical and gravitational forces exerted by the FDF and hence provides a quantitative measurement of the floating ability of a FDF over the required study period. Resultant weight measurements will determine the buoyancy properties of the calcium alginate beads once they are placed in an acidic media that reflects the environmental conditions of the stomach.

Apparatus and theoretical measurement considerations

The resultant weight apparatus is shown in Figure 3.1.



1. Linear force transmitter device
2. Test object
3. Fluid medium
4. Electromagnetic measuring module
5. Weighing balance
6. Attachment to hold object in place

Figure 3.1 Resultant weight apparatus[101].

The resultant weight apparatus is essentially an "inverted balance". The resultant weight measuring system is composed of an electronic top loader balance and a cylindrical Teflon / stainless-steel shaft, rigidly connected in a vertical position underneath the pan of the balance, Figure 3.2.

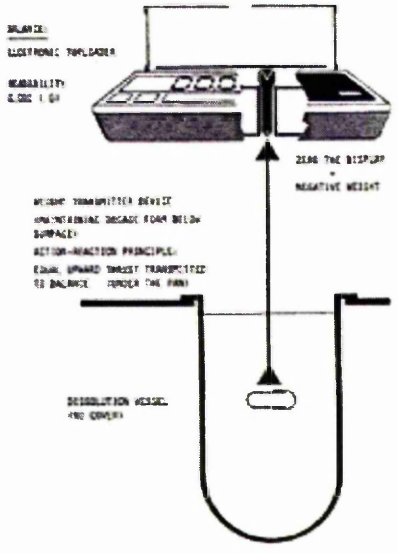


Figure 3.2 Resultant weight apparatus showing attachment of balance to shaft[102].

The lower extremity of the shaft is ended by an 8 mesh closed cylindrical holder that is capable of maintaining a dosage form immersed in the liquid medium of a dissolution vessel. The liquid medium is thermostatically controlled. The weight transmitter device (cylindrical holder), transmits the upward thrust developed by the dosage form (calcium alginate beads) to the measuring system of the balance. A laboratory set up of the apparatus is shown in Figure 3.3.

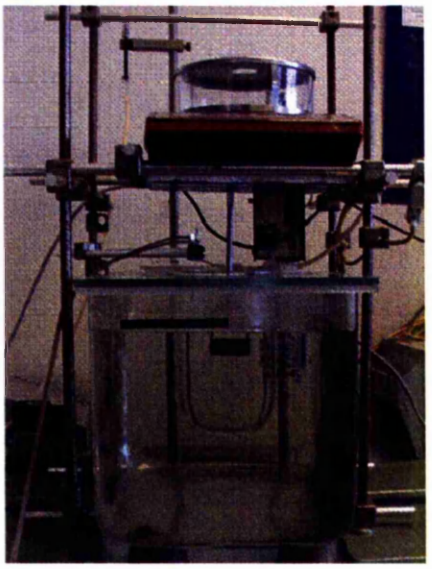


Figure 3.3 Laboratory set up of resultant weight apparatus[102].

The resultant weight measurement is based on the following considerations. An incompressible fluid will exert a buoyant force, F_{buoy}, perpendicular to the surface, S, of an immersed object, (2). The buoyant force, F_{buoy}, is the sum of (a) the downward force, FB, acting at a depth of hB and a pressure equal to pB and (b) the upward force, FA, acting at a depth of hA and a pressure of pA. The aforementioned forces are shown diagrammatically in Figure 3.2.

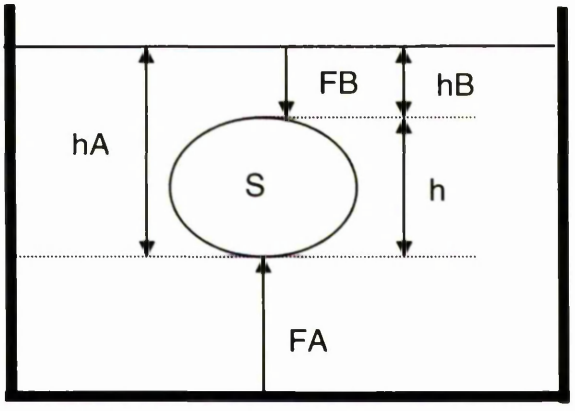


Figure 3.4 Diagrammatical illustrations of forces acting on a solid[101].

Thus, mathematically, the forces can be represented as Equation 3.3[103].

Equation 3.3

$$F_{buoy} = FA - FB = pA\ S - pB\ S = df\ g\ hA\ S - df\ g\ hB\ S$$

Where df = density of the fluid

g = acceleration due to gravity

F_{buoy} will always be a positive integer and directed vertically upwards. Since all forces exerted by the fluid on the other faces of the immersed object can be paired in opposite directions, the resultant force will be equal to zero[103].

Considering that the depth difference of the fluid equals the object height, (h), and that the volume of the solid, V, can be expressed as S h, Equation 3.3 can be re-written as Equation 3.4[101].

Equation 3.4

$$F_{buoy} = df\ g\ S\ (hA - hB)$$

$$F_{buoy} = df\ g\ V$$

The downward gravity force on the weight of the object of the calcium alginate beads, F_{grav}, must also be considered, as shown in Figure 3.3.

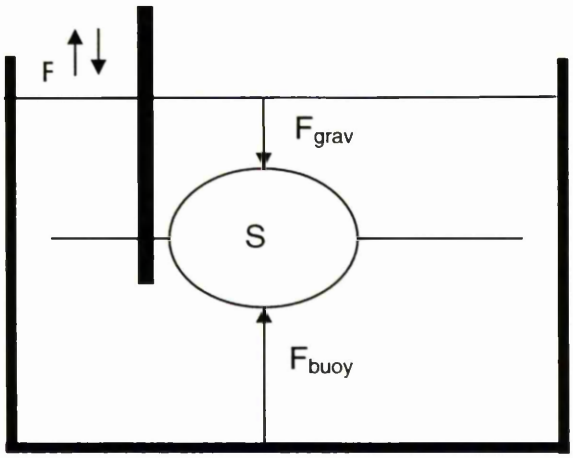


Figure 3.5 Representation of the downward forces acting on a submerged object[101].

Taking into account F_{grav}, the total force, F, can be written as Equation 3.5[103].

Equation 3.5

$$F = F_{buoy} - F_{grav}$$

And $F = df\ g\ V - ds\ g\ V$

So $F = (df - ds)\ g\ V$

Where ds = the density of the solid.

If ds<df, F is positive, has an upward direction and the object floats, albeit with part of its volume submerged.

If ds>df, F is negative, has a downward direction and the object sinks.

If ds = df, the resultant of all vectoral forces is zero and the object is in equilibrium.

If the calcium alginate beads are buoyant they will therefore rise to the surface of the liquid if not contained. When placed in a suitable container such as a cage, if the calcium alginate beads have a density less than that of the medium, they will exert an upward force on the container. The upward force exerted by the calcium alginate beads will diminish over time if:

1. The calcium alginate beads erode over time.
2. The calcium alginate beads take up sufficient amounts of medium such that F_{grav} is grater than F_{buoy}. A negative resultant weight will therefore be recorded and the calcium alginate beads will sink.

In order to accurately measure the either the upward or downward forces exerted on an object, the object must be fully submerged. The force transmitter device is connected to both the electromagnetic measuring module of a balance and the test material. Thus the object is not only submerged in the medium but the force required to maintain submersion of the test material is also measured. The value is expressed as weight units, (mg), hence the term resultant weight is

derived. The magnitude and direction of the resultant weight is equal to that of the total force, F[103].

Sample preparation

For the purposes of characterisation, all calcium alginate beads were produced from solutions containing 2% w/v sodium alginate. The calcium alginate beads loaded with the model drug were produced from a sodium alginate solution containing riboflavin 0.06% w/v. For the formulation containing magnesium stearate, magnesium stearate was incorporated into the sodium alginate solution to give a final concentration of 0.65% w/v magnesium stearate.

On the day of the experiment a volume of 1200ml of media was allowed to equilibrate to room temperature and added to the test vessel. The media used to assess the resultant weight of the calcium alginate beads was freshly prepared and consisted of 0.1M HCl/0.05% w/w Tween 80 adjusted to pH 1.2.
A mass of calcium alginate beads, (approximately 100mg), was taken from the bulk sample to be analysed. They were then placed in a mesh cage. Both the cage and calcium alginate bead sample were weighed and the measurement recorded. Following immersion in the media, successive resultant weight measurements were taken at 1-minute intervals throughout the test period and subtracted from the initial resultant value.

3.2.2.2 Scanning Electron Microscopy (SEM)

The external and internal morphology of the freeze-dried calcium alginate beads were studied using scanning electron microscopy, (SEM).

Sample Preparation

Whole or half calcium alginate bead samples were fixed to 1cm aluminium stubs using double-sided self-adhesive carbon tabs. A number (n = 4) of samples were prepared in order to eliminate artefacts and so obtain consistency of results. Using an Emscope SC500, (Kent, England), the aluminium stubs with affixed samples were then sputter coated in argon for a period of 4 minutes at a voltage of 25 milliamps to give a final thickness of gold of 10 – 20nm.

Samples were then viewed using a Cambridge 360 SEM, (Cambridge, England).

3.2.2.3 X-RAY MICROANALYSIS

X-ray microanalysis is a technique used to determine the presence of specific elements within a sample. Elements are identified as follows. A high energy electron beam is directed at the sample to be analysed and as a result electron(s) from the outer shells of the sample material are removed. Electrons from the electron beam replace the removed electrons, but subsequent surplus energy produces X-rays. The surplus energy is specific to the atom from which it came and hence identification of the element is possible.

The aim of performing X-ray microanalysis on the calcium alginate beads was to determine the presence of riboflavin within the beads. If successful, X-ray mapping could then be used to determine the distribution of riboflavin within the calcium alginate beads. Since X-ray microanalysis requires that the compound present must contain elements containing an atomic number less than carbon, calcium alginate beads were prepared using riboflavin-5'-phosphate. Amounts of riboflavin-5'-phosphate used to prepare the calcium alginate beads were identical to the previously prepared riboflavin loaded calcium alginate beads. It was anticipated that the presence of phosphorous within the calcium alginate beads would be detected by X-ray microanalysis.

Apparatus

A Cambridge 360 SEM fitted with a Link AN10000, (Manchester, England), system was used to view the calcium alginate beads.

Sample preparation

Whole or half calcium alginate bead samples were fixed to 1cm aluminium stubs using double-sided self-adhesive carbon tabs. A number (n = 4) of samples were prepared in order to eliminate artefacts and so obtain consistency of results. Using a Biorad E6200 turbo carbon coater, (Hemel Hempstead, England), the aluminium stubs were coated for 2.5 seconds with evaporated

carbon from a 1.5 – 2mm carbon rope under a pressure of 10^{-1} – 10^{-2} millibar and at a voltage of 20 amps. Samples were then analysed using a Cambridge 360 SEM, (Cambridge, England).

3.2.2.4 Environmental scanning electron microscopy (ESEM)

ESEM is an imaging method that can be used to view samples in the natural or wet state. A Philips XL30 ESEM-FEG, (Eindhoven, The Netherlands), was used to view dry calcium alginate beads and calcium alginate beads after immersion in aqueous media.

Sample preparation

Three calcium alginate beads from each sample were prepared as follows. The calcium alginate beads were attached to a platform with Tissue tac®. The sample was then frozen by dipping into liquid nitrogen slush under vacuum and drawn into the microscope chamber to prevent any condensation of the water vapour. The bead was then sliced in situ with a surgical steel scalpel blade and the sample heated to -60°C. The sliced surface was then re-frozen in the cryo-transfer stage and sputter coated with gold to a thickness of 50Å.

Calcium alginate beads prepared in the wet state were subject to the following additional preparation prior to attachment to the platform with Tissue tac®. A Caleva USP XXII dissolution apparatus, (Heusenstamm, Germany), parameters as section 4.2.4, was used to circulate a sample, (n = 10), of placebo calcium alginate beads in Sørensen's citrate buffer, pH 3.0, (Appendix I), for 45 minutes prior to observation by ESEM.

3.2.2.5 Digital photography

A Caleva USP XXII dissolution, (parameters as section 4.2.4), apparatus, was used to circulate a sample, (n = 10), of placebo calcium alginate beads in singly distilled lab water for 48 hours prior to observation by digital photography. The calcium alginate beads were then removed from the media and placed in a petri dish with a nominal amount of media to prevent dehydration of the sample. A

Fuji S1 digital camera, (Tokyo, Japan), with Meiji light microscope, (Tokyo, Japan), attachment and 10x focus, was then used to view and photograph the calcium alginate beads.

3.2.2.6 CONFOCAL LASER SCANNING MICROSCOPY

The diffusion of riboflavin from the riboflavin loaded calcium alginate beads have been measured using confocal-FRAP, (confocal-fluorescence after photobleaching). Using fluorescence, areas of the calcium alginate beads can be illuminated. The fluorophores simply shuttle between the ground state and excited state, as the fluorophores return to the ground state fluorescence is emitted. After the initial fluorescent measurement, the beam power is increased 100 times and focused to a smaller area (bleach area). The illuminated bleach area is photolysed and the fluorophores irreversibly loose their ability to fluoresce, and are converted to a non-fluorescent product. The recovery of fluorescence in the bleach area is monitored. Consequently the diffusion of riboflavin can be measured and provide information regarding the movement of riboflavin throughout the bead. The confocal aperture of the CLSM eliminates photons from the out of focus regions, enabling the diffusion of a fluorescent marker across a thick transparent object such as a calcium alginate bead to be measured.

Sample preparation and analysis

The diffusion measurement can be derived from the following equations.

Equation 3.6 is derived from methods determined by Axelrod et al[105] and focuses on a 2D measurement of the square area measuring 50 pixels by 50 pixels that is scanned by the CLSM.

The time to diffuse out of the bleach area, τ, can be expressed as Equation 3.6.

Equation 3.6

$$\tau = \frac{a^2}{4D}$$

Where τ = time taken for the riboflavin to diffuse out of the bleach area.

a = radius of the bleach

D = the diffusion rate of riboflavin.

Furthermore, the decrease in bleach intensity, r(t), was derived by Kubitscheck et al[106] and measures the relative change in the signal over time. The decrease in bleach intensity, r(t), can be expressed as Equation 3.7.

Equation 3.7

$$r(t) = 1 - \frac{Ii(t) - Ii(t_0)}{Ii(t_{-1}) - Ii(t_0)} = \exp\left(-\frac{2}{1 + 2\tau/t}\right)$$

With t is time after bleach, t_0 is bleach time, and t_{-1} is before the bleach, I_i is the average intensity measured at the different times in the bleach area.

Equation 3.7 is re-arranged to give Equation 3.8.

Equation 3.8

$$\ln\left(1 - \frac{Ii(t) - Ii(t_0)}{Ii(t_{-1}) - Ii(t_0)}\right) = -\frac{2}{1 + 2\tau/t}$$

Re-arranging Equation 3.8 for 2τ gives Equation 3.9.

Equation 3.9

$$2\tau = \left(\frac{-2}{\ln\left(1 - \frac{Ii(t) - Ii(t_0)}{Ii(t_{-1}) - Ii(t_0)}\right)} - 1\right) t$$

Replacing 2τ with Equation 3.6 gives Equation 3.10.

Equation 3.10

$$\frac{a^2}{2D} = \left(\frac{-2}{\ln\left(1 - \frac{Ii(t) - Ii(t_0)}{Ii(t_{-1}) - Ii(t_0)}\right)} - 1 \right) t$$

Therefore *Dt* can be expressed as Equation 3.11.

Equation 3.11

$$\frac{a^2}{2}\left(\frac{\ln\left(1 - \frac{Ii(t) - Ii(t_0)}{Ii(t_{-1}) - Ii(t_0)}\right)}{-2 - \ln\left(1 - \frac{Ii(t) - Ii(t_0)}{Ii(t_{-1}) - Ii(t_0)}\right)} \right) = Dt$$

Linear regression analysis of plots of Equation 3.11 against time allows the calculation of the translational diffusion *D*. The measurements are not influenced by the rotational diffusion and only the translational diffusion can be measured by this method. Equation 3.11 can be plotted as in Figure 3.6, modified, the slope of which determines the diffusion of riboflavin from the calcium alginate beads expressed as $cm.s^{-1}$.

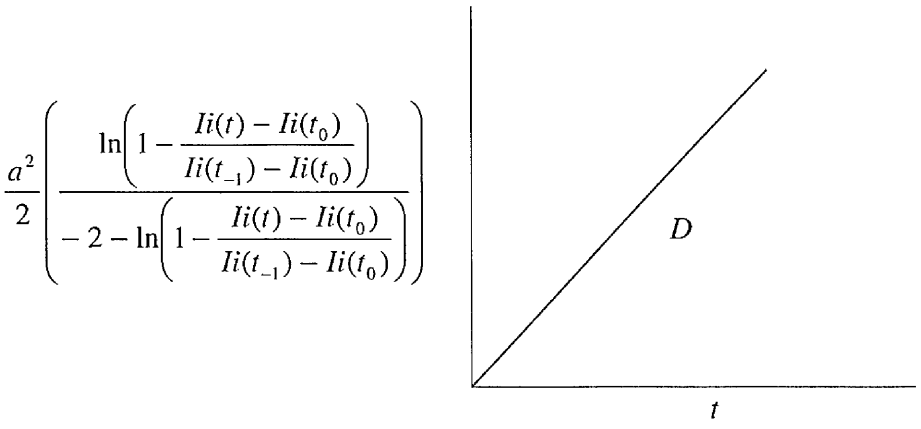


Figure 3.6 Plot of Equation 3.11 to determine the rate of riboflavin diffusion from the calcium alginate beads.

Samples of calcium alginate beads were prepared in the following way. A calcium alginate bead loaded with riboflavin (excitation 480nm, emission

565nm) was placed in a cavity microscope slide and 2-3 drops of singly distilled lab water was added to the well. The slide was then placed under a confocal microscope and diffusion images taken at 5 seconds intervals until 25 images were acquired. The data were than analysed by the method presented in the previous section.

3.3 RESULTS AND DISCUSSION

3.3.1 DIAMETER

The average diameters of samples of the calcium alginate beads, (n = 10), are shown in Figure 3.7, with error bars representing the standard deviations of the measurements within a calcium alginate bead sample. Values for the individual diameters of the calcium alginate beads for the different formulations are shown in Appendix II.

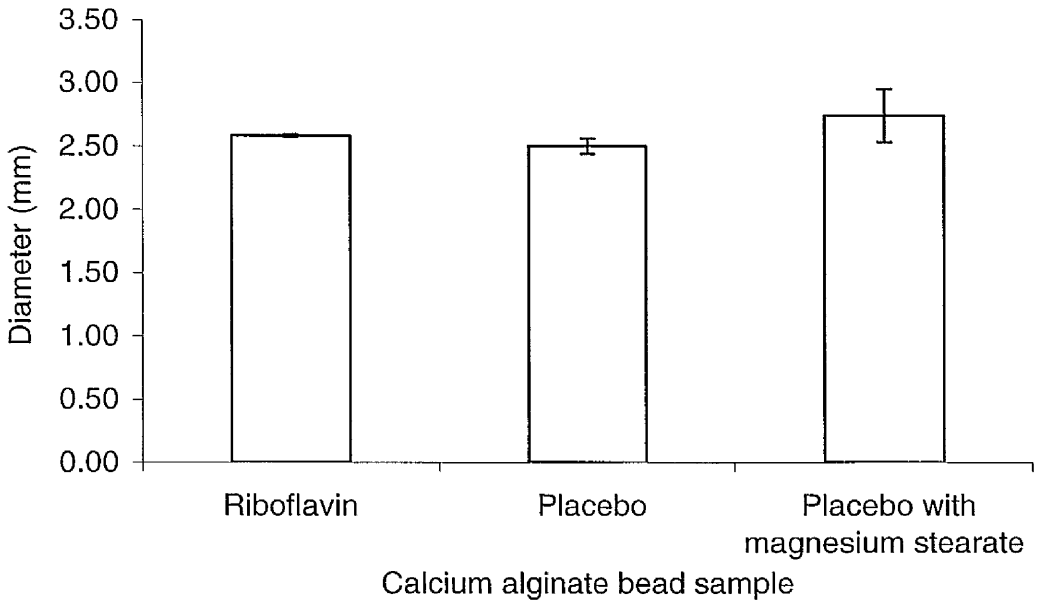


Figure 3.7 Bead diameters (n = 10) from beads containing riboflavin, placebo beads and placebo beads containing magnesium stearate.
(Error bars represent the standard deviation of the diameter results)

The diameter of the calcium alginate beads varied according to the formulation. The results show that placebo beads containing magnesium stearate showed an increase in diameter of 8.5% when compared with those containing riboflavin. The calcium alginate beads containing riboflavin were in turn 16.3% larger than the placebo calcium alginate beads.

The diameters of the placebo calcium alginate beads compare well to those obtained previously which are in the order of 3mm[63]. The change in diameter of the calcium alginate beads produced for the current studies is minimal and not expected to affect the behaviour of the calcium alginate beads. The effect of changing process parameters such as needle gauge size, the temperature of the sodium alginate solution and the volume ratio of sodium alginate solution:curing solution have been investigated previously and have been shown to have an effect on the size or shape of the calcium alginate beads. With specific regard to diameter measurements, changes in the diameter of calcium alginate beads can be achieved by using different gauge needles[67]. Decreasing the needle gauge results in an increased droplet size of the extruded sodium alginate solution. Hence the diameter of the calcium alginate beads is increased. Similarly, a decrease in temperature of the sodium alginate solution results in an increase in the viscosity of the sodium alginate solution. As a consequence, the diameter of calcium alginate beads formed from sodium alginate solutions maintained at lower temperatures increases when compared with calcium alginate beads formed from sodium alginate solutions that were maintained at higher temperatures[107]. Therefore, for the current study, process parameters were kept constant, (Chapter 2, section 2.3.2.2.)

3.3.2 WEIGHT

The average weights of the calcium alginate beads, (n = 30), are shown in Figure 3.8, with error bars representing the standard deviations of the measurements within a calcium alginate bead sample. Individual figures for the weights of calcium alginate beads of different formulations are shown in Appendix II

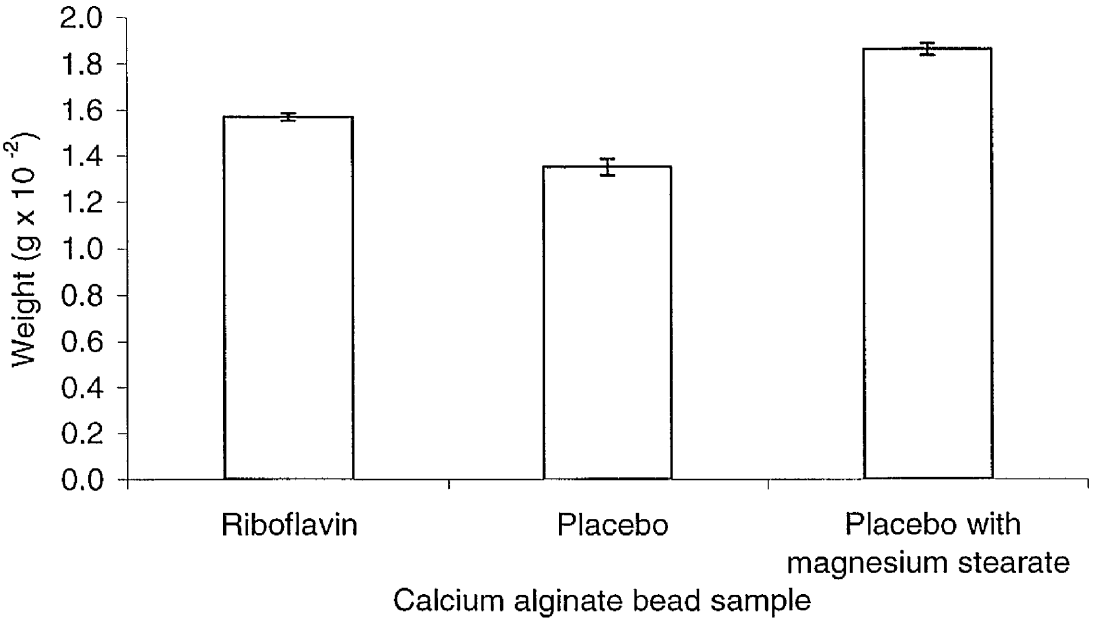


Figure 3.8 Weight of samples of calcium alginate beads, (n = 30), containing riboflavin, placebo beads and placebo beads containing magnesium stearate.

(Error bars represent the standard deviation of the sample weights)

The weight of the calcium alginate beads depended on the formulation. Placebo calcium alginate beads containing magnesium stearate had an increased mass of 6.2% when compared to calcium alginate beads containing riboflavin that in turn had a mass 3.2% greater than the placebo calcium alginate beads.

The consequence of using smaller gauge needles is a relative increase in weight of the beads by 100%. However, the increased mass did not impair the flotation or buoyancy of the calcium alginate beads, confirmation of which is shown using the resultant weight apparatus (Chapter 3, section 3.3.4)

As discussed, (Chapter 3, section 3.3.1), changes in process parameters have been shown to affect the size and formation of the calcium alginate beads. The same applies to the weight of the beads. Changes in the temperature of the sodium alginate solution are known to result in a change of the viscosity of the

sodium alginate solution[68]. An increase in temperature of 10°C of the sodium alginate solution showed that calcium alginate bead weight increased by 5.26%[63]; the change being due to a corresponding decrease in sodium alginate solution viscosity thus increasing the flow rate and hence droplet size. Hence, process parameters were kept constant, (Chapter 2, section 2.3.2.2).

3.3.3 DENSITY

3.3.3.1 MATHEMATICAL DETERMINATION

Using Equation 3.1 (Chapter 3, section 3.2.1.3), the volume of placebo, riboflavin loaded calcium alginate beads and placebo calcium alginate beads containing magnesium stearate has been calculated, Table 3.1.

Calcium alginate bead sample	**Calcium alginate bead radii (cm) (calculated from Appendix II)**	**Calcium alginate bead volume (cm^3)**
Placebo	0.125	8.2×10^{-3}
Placebo containing riboflavin	0.129	8.9×10^{-3}
Placebo containing magnesium stearate	0.137	1.1×10^{-2}

Table 3.1 Calculation of calcium alginate bead volumes for various formulations.

Using the bead volume obtained from Equation 3.1, the density of the bead was obtained using Equation 3.2, (Chapter 3, section 3.2.1.3). The results are shown in Table 3.2.

Calcium alginate bead sample	**Mass of calcium alginate bead (g) (calculated from Appendix II)**	**Calcium alginate bead volume (cm^3) (from Table 3.1)**	**Density ($g.cm^{-3}$)**
Placebo	4.5×10^{-4}	8.2×10^{-3}	5.0×10^{-2}
Placebo containing riboflavin	5.2×10^{-4}	8.9×10^{-3}	5.0×10^{-2}
Placebo containing magnesium stearate	6.2×10^{-4}	1.0×10^{-2}	6.0×10^{-2}

Table 3.2 Calculation of calcium alginate beads densities for various formulations.

The mathematically calculated densities of the three samples of calcium alginate beads are similar and are all less than $1 g.cm^{-3}$. The results therefore suggest that the calcium alginate beads should float when placed in aqueous media. Experimentally, the calcium alginate beads float, as shown by the resultant weight results, (Chapter 3, section 3.3.4)

3.3.3.2 Experimental Determination

Figure 3.9 shows the density measurements for three samples of calcium alginate beads as measured by the pycnometer.

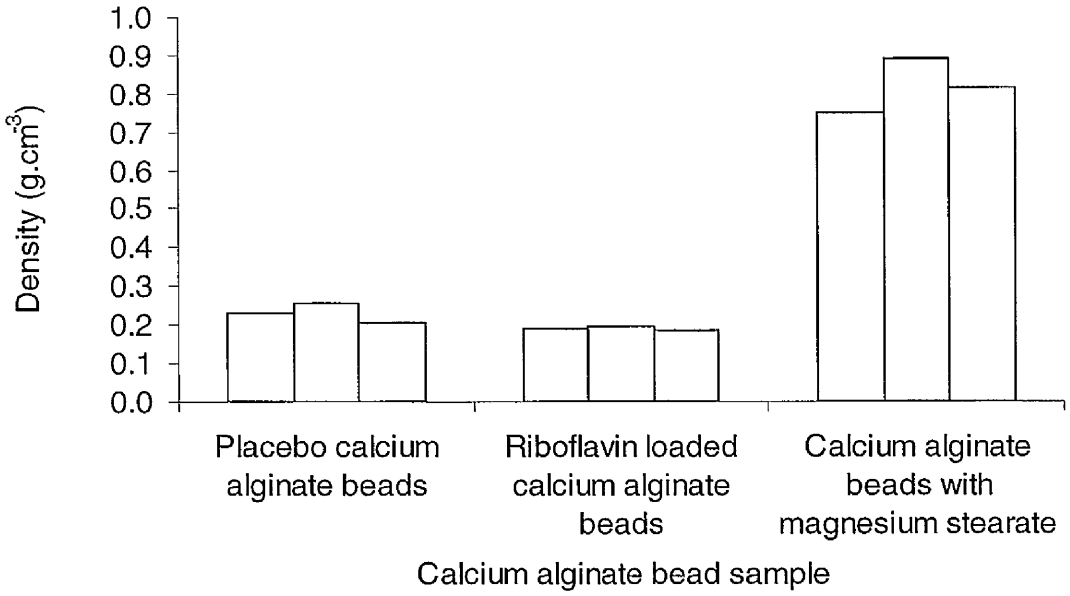


Figure 3.97 Average densities of samples of calcium alginate beads measured by pycnometer.

Density results obtained using the pycnometer for the placebo calcium alginate beads are approximately 0.2g.cm^{-3}. As calcium alginate beads have not been previously prepared with either riboflavin or magnesium stearate, no comparable figures are available. However, similar dosage forms such as hollow microspheres that are also designed to float on stomach contents have showed comparable density values[108]. Figure 3.5 shows that variability occurs between and within samples of calcium alginate beads when measuring the density using the pycnometer. However, the following points should be considered when using a pycnometer to measure the densities of the calcium alginate beads.

- When using the pycnometer to obtain density measurements, the manufacturer recommended that a volume equivalent to the provided standard should be used when taking measurements. Considering the nature of the calcium alginate beads, such a volume is difficult to obtain, and was borne out by error readings generated by the equipment when attempting to obtain measurements.
- When the calcium alginate beads are placed in the sample cup, as a result of the spherical shape and relatively large size of the calcium

alginate beads compared to the diameter of the 1cc sample cup, void volumes presented within the sample cup. Such volumes will have a direct effect on the results obtained as the equipment calculates the density measurements based on pressure changes in a standard volume.

- When passing a gas under pressure into a closed chamber containing fragile calcium alginate beads, the calcium alginate beads have the potential to become damaged easily. Hence damage to the outer surfaces of the calcium alginate beads will affect the pressure changes within the standard volume, thereby making results inaccurate.
- Since the calcium alginate beads are porous, it is it is possible that the helium used to make the measurements penetrates into the calcium alginate beads, thereby affecting the measurement.

Considering the results obtained and possible sources of error, the method of measuring densities of calcium alginate beads using a pycnometer has demonstrated that the results may only be considered as approximate values. The method therefore is not recommended for obtaining further measurements.

3.3.4 RESULTANT WEIGHT

The resultant weight measurements of the placebo and riboflavin loaded calcium alginate beads are shown in Figures 3.10 to 3.11.

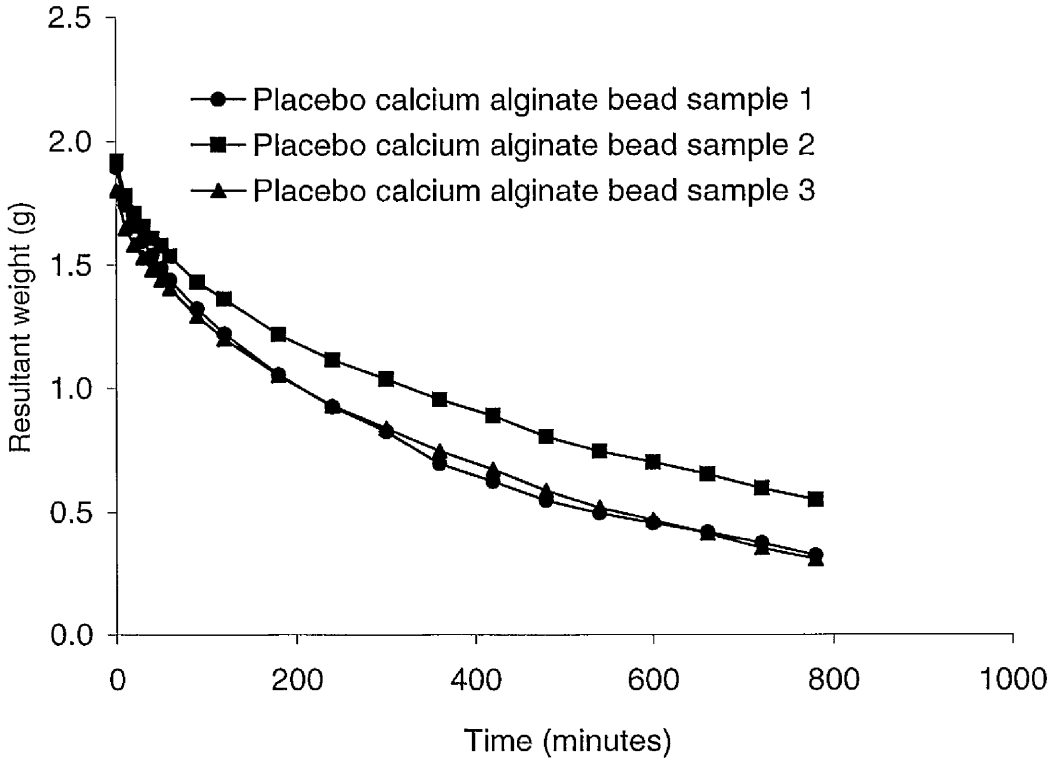


Figure 3.10 Resultant weight measurements for placebo calcium alginate beads.

The results above show a positive resultant weight value over the study period indicating that the calcium alginate beads continued to float for the specified time period. Statistically, (Standard deviation, $p = < 0.05$), there is no difference between the three sets of measurements demonstrating that consistency has been achieved throughout the experiment.

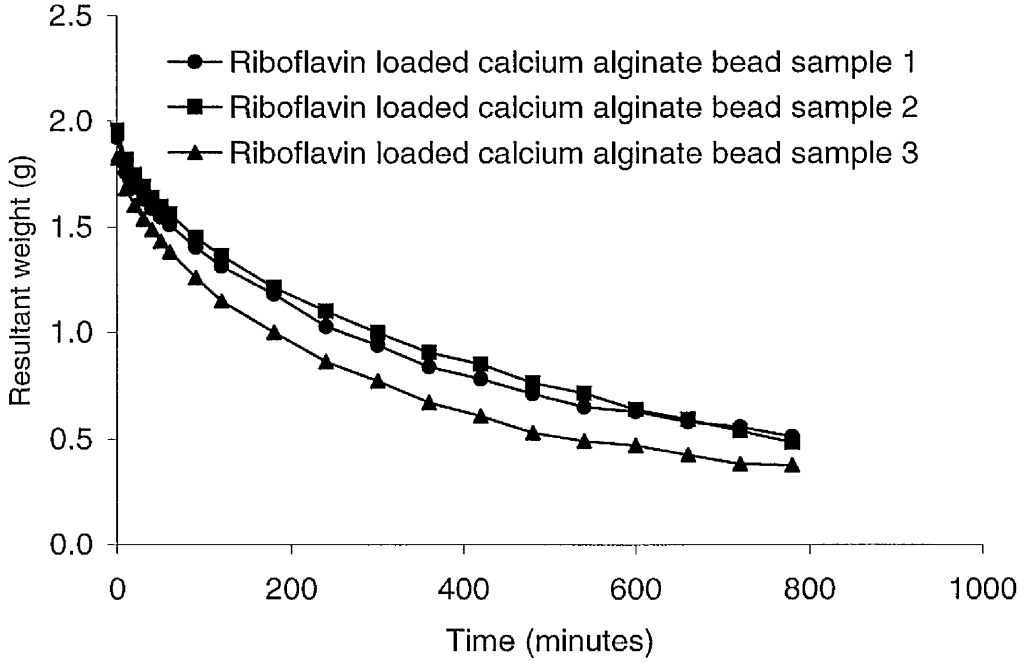


Figure 3.11 Resultant weight measurements for riboflavin loaded calcium alginate beads.

The results for the riboflavin loaded calcium alginate beads, Figure 3.11, are similar to those obtained for the placebo calcium alginate beads, showing that a positive resultant weight value was obtained for the study period and hence demonstrating that the riboflavin loaded calcium alginate beads float. Statistically, (Standard deviation $p = < 0.05$) as with the placebo calcium alginate beads there is no difference between the three measurements.

Figure 3.12 shows the mean resultant weight values for both the placebo and riboflavin loaded calcium alginate beads.

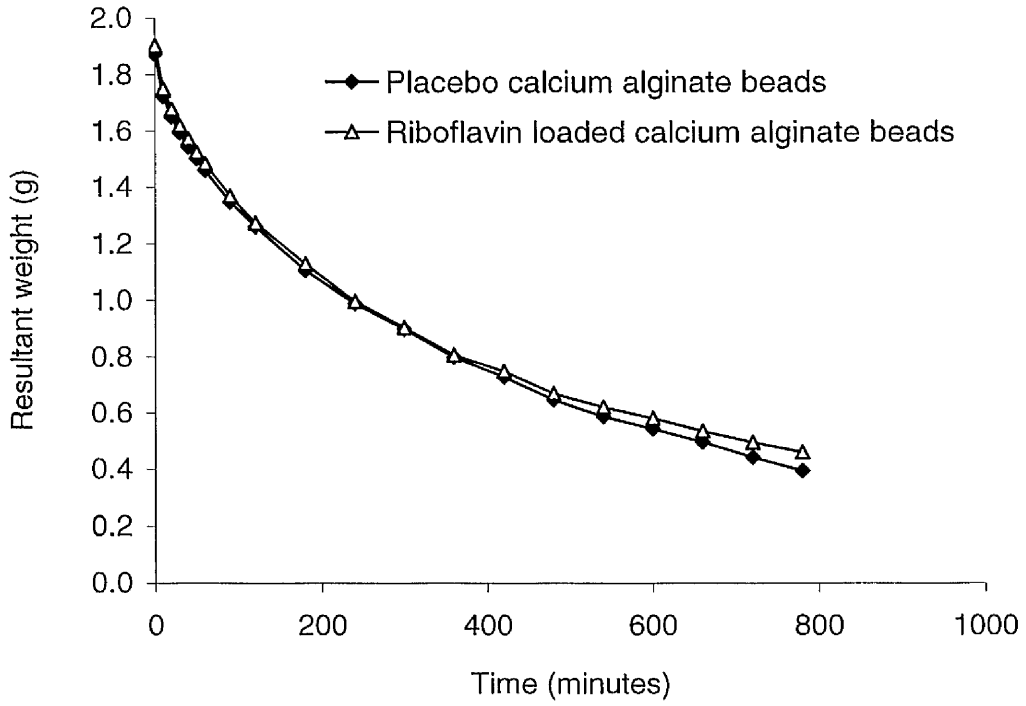


Figure 3.12 Mean resultant weight values for placebo and riboflavin loaded calcium alginate beads.

The difference in resultant weight values between the placebo and riboflavin loaded formulations of calcium alginate beads is not significant and hence the buoyancy or floating ability of the beads has not been affected.

3.3.5 SCANNING ELECTRON MICROSCOPY (SEM)

The SEM's of a whole and half calcium alginate beads are shown in Figures 3.13 to 3.18. The calcium alginate beads viewed by SEM are placebo, placebo containing riboflavin and placebo containing magnesium stearate.

Overall, the shape of the calcium alginate beads that were produced was spherical, regardless of formulation. They also had a spongy texture that was expected from the freeze-drying process. The internal morphology of the calcium alginate beads clearly shows the numerous cavities that form as a result of the freeze-drying process, regardless of formulation. The cavities are unique to the floating calcium alginate beads and hence enable floatation.

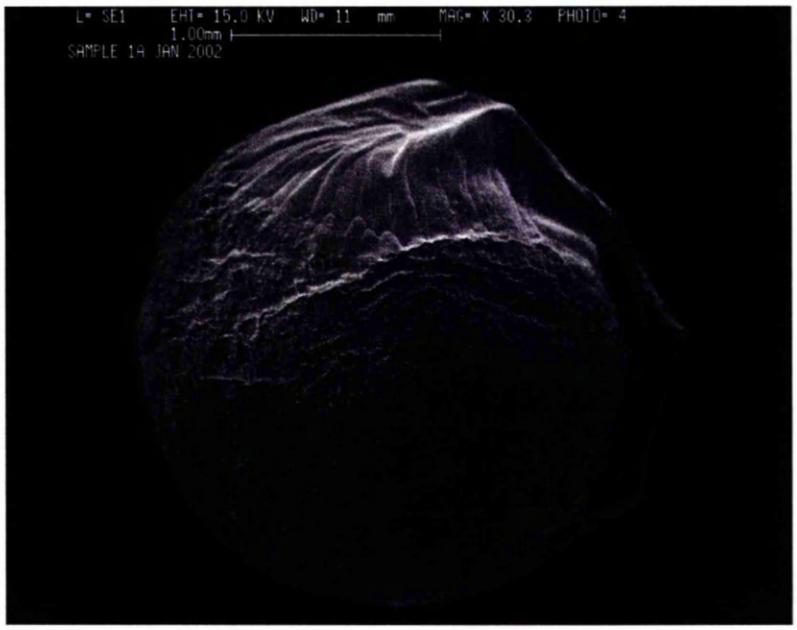


Figure 3.13 SEM of whole placebo calcium alginate bead showing external morphology *(x 30.3).*

The outer surface is textured and slightly contoured. Figure 3.14 shows the cavities present within the calcium alginate beads; however, they are not uniform in shape size or distribution.

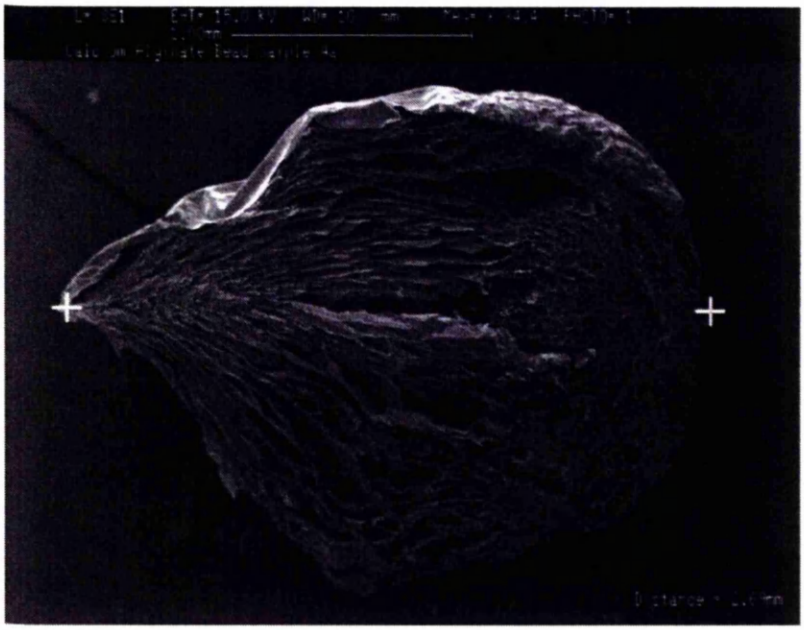


Figure 3.14 SEM of cross section of placebo calcium alginate bead showing internal morphology *(x 34.4).*

Figure 3.15 SEM of whole calcium alginate bead containing riboflavin showing external morphology *(x 32.0).*

The SEM of the whole calcium alginate bead containing riboflavin, Figure 3.15, shows a definite contouring of the surface of the calcium alginate bead that may be due to the inclusion of riboflavin within the calcium alginate bead formula. All samples prepared with riboflavin showed a similar morphology. Furthermore, the cavities in Figure 3.16 show a better definition that may also result from the addition of riboflavin.

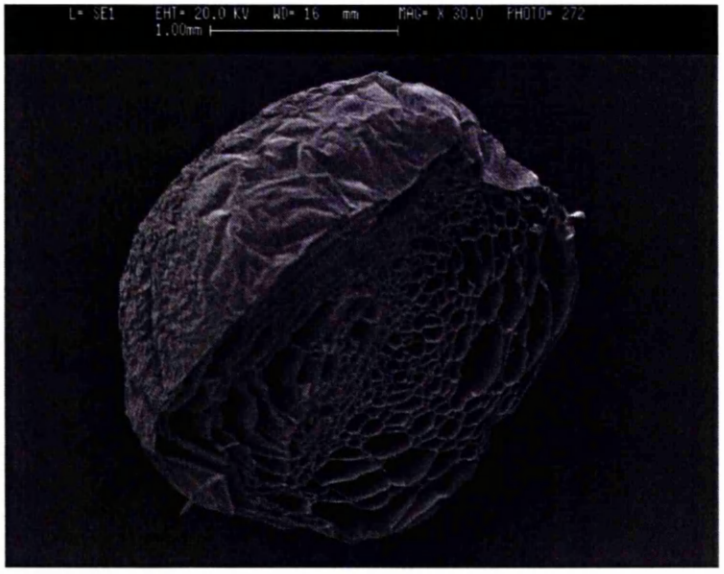


Figure 3.16 SEM of cross section of calcium alginate bead containing riboflavin showing internal morphology *(x 30.0).*

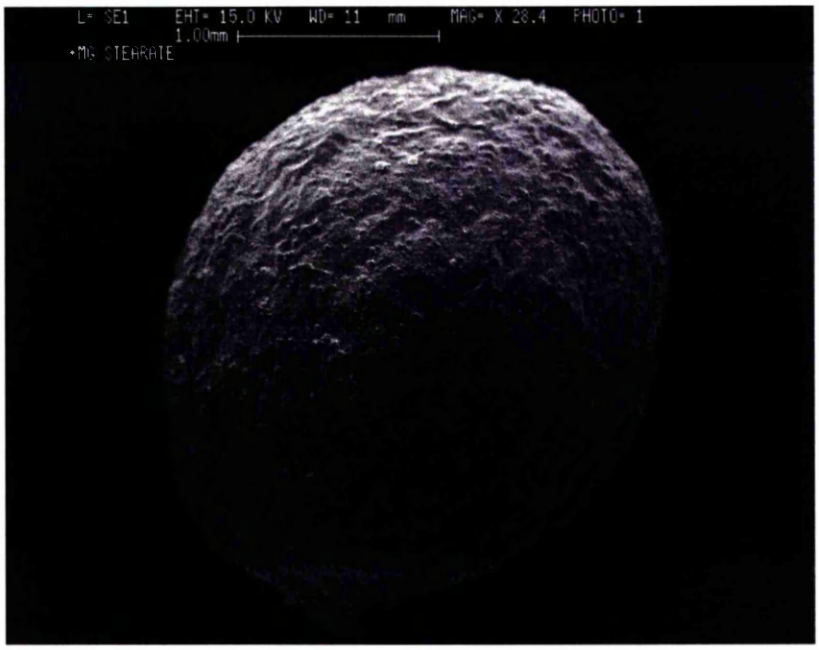


Figure 3.17 SEM of cross section placebo calcium alginate bead containing magnesium stearate showing external morphology *(x 28.4).*

Figure 3.18 shows that the integrity of the calcium alginate bead has not been compromised by the addition of magnesium stearate to the formula, although the outer surface is less contoured

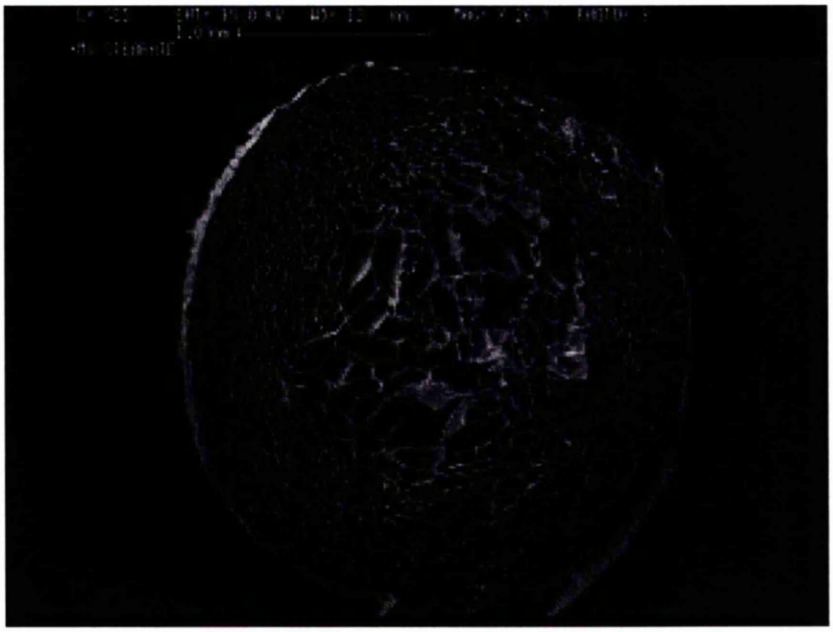


Figure 3.18 SEM of cross section calcium alginate bead containing magnesium stearate showing internal morphology *(x 26.7).*

The cross section of the calcium alginate bead in Figure 3.18 demonstrates that the inclusion of magnesium stearate within the calcium alginate bead formula may produce more consistently and more uniformly shaped cavities within the calcium alginate beads.

3.3.6 X-RAY MICROANALYSIS

The results from the X-ray microanalysis are described below. Placebo calcium alginate beads were analysed in addition to calcium alginate beads containing riboflavin and magnesium stearate. The analysis of placebo calcium alginate beads acted as a control for the riboflavin and magnesium stearate containing calcium alginate beads.

When reviewing the X-ray microanalysis plots, peak sizes can be compared on a 1:1 basis. Therefore peaks of equivalent sizes demonstrate that equivalent amounts of atoms were present in the samples analysed. In all the figures, two calcium peaks are visible. The smaller of the two peaks is an 'escape peak' and indicates that when the electron beam was directed at the sample, electrons were removed from inner and outer shells.

For each analysis, two plots are presented. The smaller of the two plots is drawn on a different scale, and may give additional information on the sample scanned.

3.3.6.1 X-RAY DATA RESULTS OF PLACEBO CALCIUM ALGINATE BEADS

Figures 3.19 and 3.20 show the X-ray microanalysis data results of placebo calcium alginate beads.

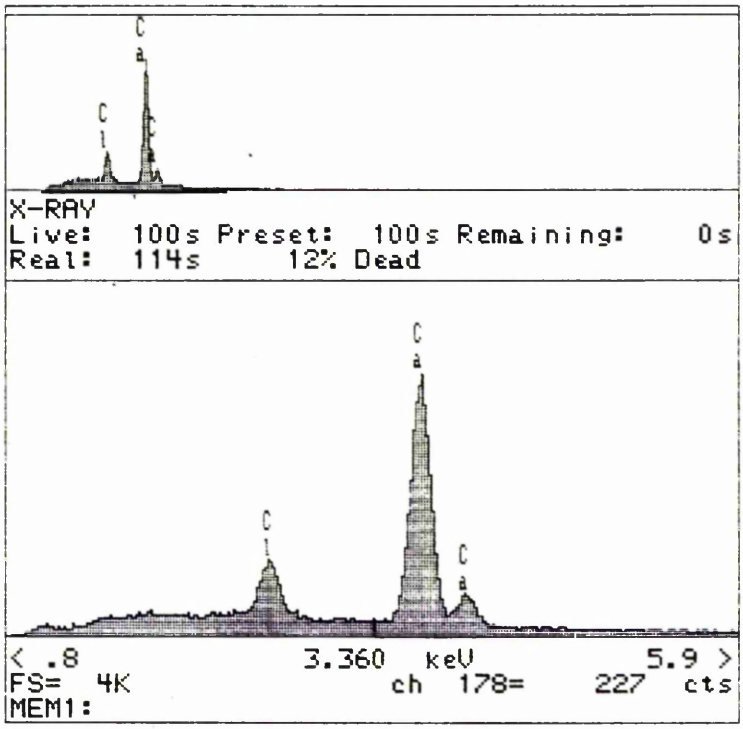


Figure 3.19 X-ray microanalysis data result of a placebo calcium alginate whole bead.

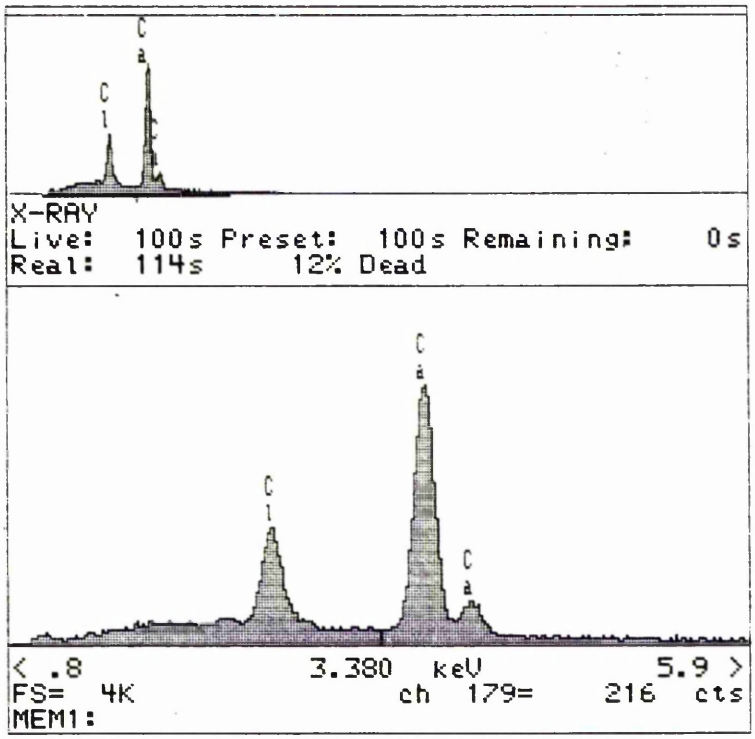


Figure 3.20 X-ray microanalysis data result of a cross section of a placebo calcium alginate bead.

Both data results show the appearance of only two elements within the placebo calcium alginate beads, namely calcium and chlorine. The presence of both elements was expected

The presence of calcium within the samples was expected as calcium alginate beads are formed by dropping sodium alginate into calcium chloride solution, 0.02M. Hence, calcium is a main constituent of the calcium alginate beads.

The presence of chlorine was also expected as calcium chloride solution 0.02M is used to cure the calcium alginate beads and therefore chlorine will also be present in large amounts.

3.3.6.2 X-RAY ANALYSIS DATA OF PLACEBO CALCIUM ALGINATE BEADS CONTAINING MAGNESIUM STEARATE

Figures 3.21 and 3.22 shows the surface of a whole calcium alginate bead and the section of a calcium alginate bead containing magnesium stearate as viewed by X-ray microanalysis.

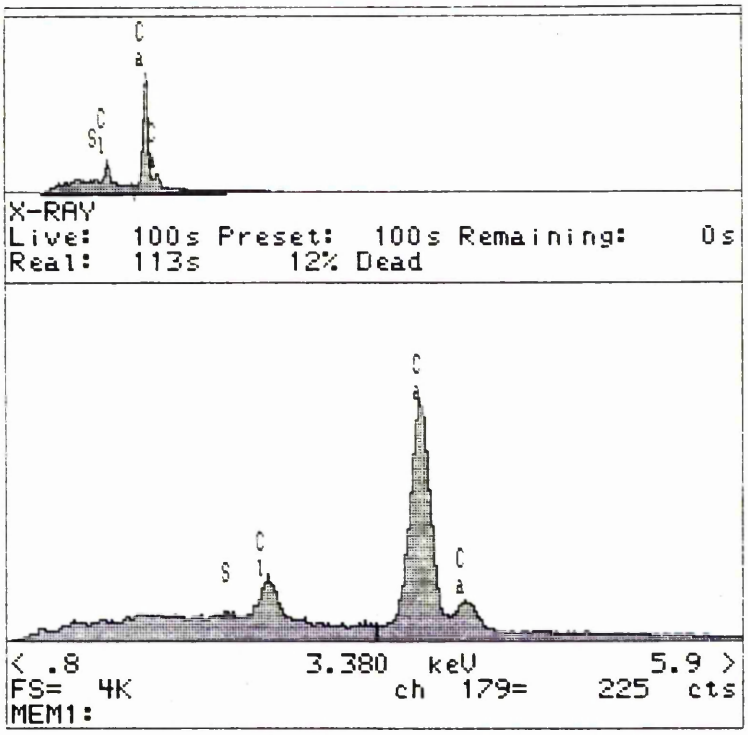


Figure 3.21 X-ray microanalysis data results of a whole placebo calcium alginate bead containing magnesium stearate.

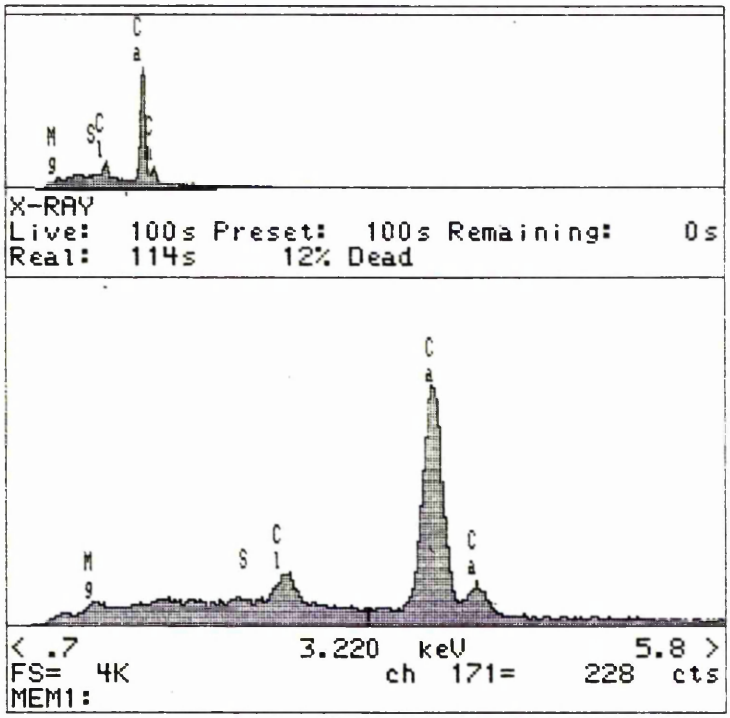


Figure 3.22 X-ray microanalysis data results of the cross section of a placebo calcium alginate bead containing magnesium stearate.

As discussed in section 3.3.6.1, both calcium and chlorine are shown to be present in the calcium alginate beads. Both elements are also present in equivalent amounts.

The presence of magnesium within the calcium alginate bead has also been shown. Visually, the presence of magnesium can be seen on the outer surface of the calcium alginate bead. However, the X-ray microanalysis plots suggest that not only is magnesium present in small quantities but it is also only occurs within the calcium alginate bead. The results may indicate that during curing of the calcium alginate beads, magnesium stearate was washed from the outer surface of the calcium alginate bead.

The X-ray microanalysis results of the whole calcium alginate bead containing magnesium stearate, and of the cross section of the calcium alginate bead containing magnesium stearate show that sulphur is present within the sample, albeit in small amounts. The presence of sulphur may be attributed to the magnesium stearate. Magnesium stearate contains trace amounts of sulphur[109] and consequently is depicted as a trace amount on the X-ray microanalysis spectra.

3.3.6.3 X-RAY ANALYSIS DATA OF CALCIUM ALGINATE BEADS CONTAINING RIBOFLAVIN-5'-PHOSPHATE

Figures 3.23 and 3.24 show the X-ray microanalysis data results of calcium alginate beads containing riboflavin-5'-phosphate.

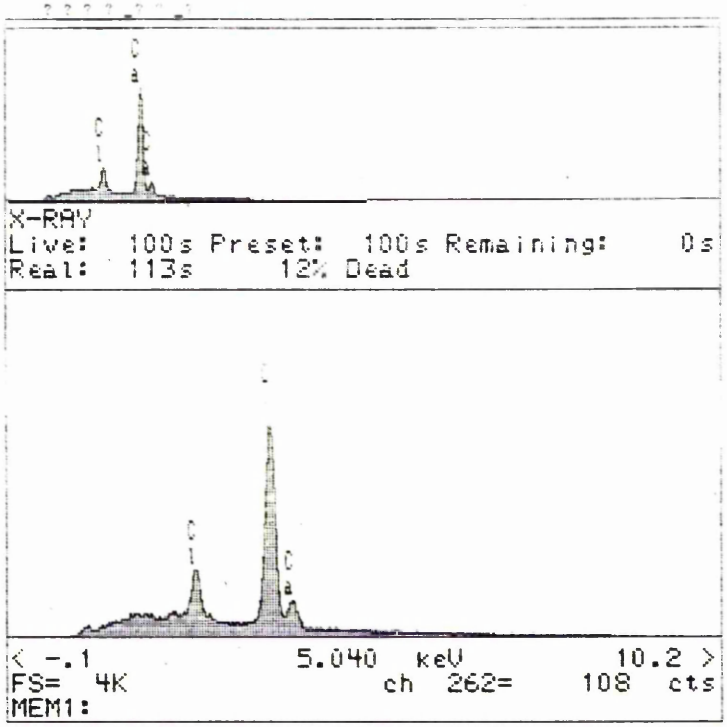


Figure 3.23 X-ray microanalysis data results of a whole calcium alginate bead containing riboflavin-5'-phosphate.

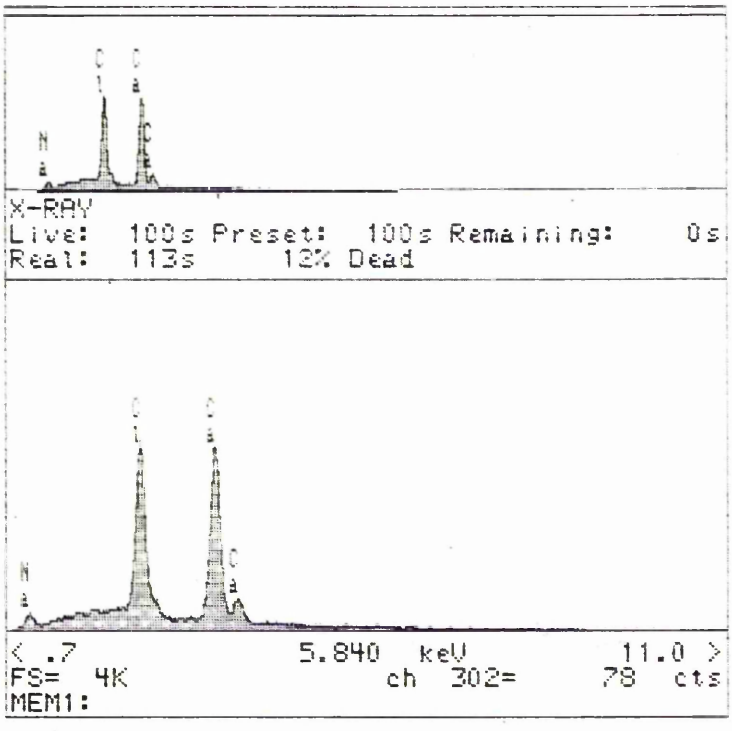


Figure 3.24 X-ray microanalysis data results of a cross section of a calcium alginate bead containing riboflavin-5'-phosphate.

The X-ray microanalysis results for the whole calcium alginate beads containing riboflavin-5'-phosphate, Figure 3.23, shows the presence of chlorine and calcium. In addition, the data results for the cross section, Figure 3.24, shows the presence of sodium. Neither the whole nor the cross section results show the presence of any phosphate. Further evaluation of the results indicates the following:

Calcium

The peak sizes of calcium within the spectra of the whole and cross section of the calcium alginate beads containing riboflavin-5'-phosphate are similar, thereby suggesting that calcium is present in similar quantities.

Chlorine

The X-ray microanalysis results for the cross section of the calcium alginate bead containing riboflavin-5'-phosphate shows an increased quantity of chlorine present in the sample when compared to the whole calcium alginate bead. The difference in respective heights of the chlorine peaks and hence the amount of chlorine present may be attributed to the washing process during the production of the calcium alginate beads. Following the curing process, the calcium alginate beads are washed with 3 x 100ml aliquots of glass distilled water. The process of washing may be responsible for 'washing off' some of the chlorine. Hence, the removal of the chlorine will occur on the outer surface of the calcium alginate bead and not on the interior surfaces.

Sodium

The presence of sodium is expected and predictable as the calcium alginate beads are produced from sodium alginate. The majority of sodium from the sodium alginate is displaced by calcium, but small amounts of sodium remain, hence the relatively small size of the sodium peak.

Phosphorus

Neither X–ray microanalysis spectra shows the presence of phosphorus, despite the preparation of calcium alginate beads with riboflavin-5'-phosphate. The riboflavin-5'-phosphate molecule is shown in Figure 2.7(b). Only a small part of the molecule is phosphorus and therefore the absence of phosphorus in

the X-ray microanalysis results may be attributed to insufficient amounts of phosphorus in the calcium alginate bead. Riboflavin-5'-phosphate was added so that the sodium alginate/riboflavin-5'-phosphate solution from which the beads were produced contained a final amount of 0.06% w/v riboflavin-5'-phosphate. In order to study the presence of phosphorus further using X-ray microanalysis producing calcium alginate beads with increased amounts of riboflavin-5'-phosphate may confirm the presence of phosphorus atoms. Alternatively, the preparation of dilutions of riboflavin-5'-phosphate in sodium alginate and subsequent drying of the solution onto stubs may determine the minimum detectable concentration of riboflavin-5'-phosphate by X-ray microanalysis.

3.3.7 ENVIRONMENTAL SCANNING ELECTRON MICROSCOPY (ESEM)

A cross section of a dry calcium alginate placebo bead as viewed by ESEM is shown in Figure 3.25.

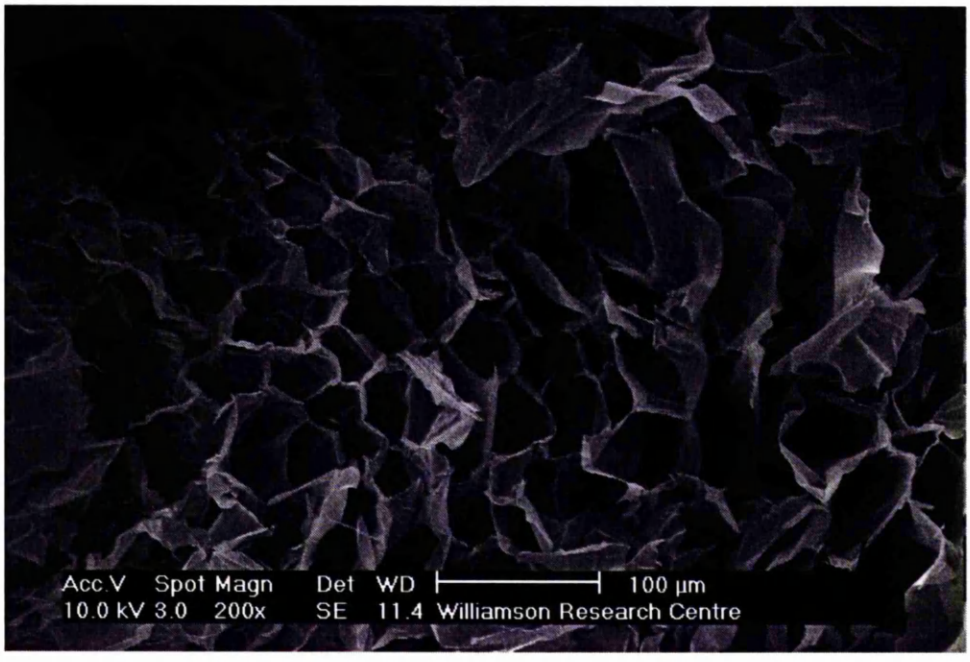


Figure 3.25 Cross section of a dry placebo calcium alginate bead as viewed by ESEM *(x 200).*

Although comparison of images obtained by ESEM and SEM is sometimes difficult, since the sample preparation and equipment used to view the samples for both techniques are different, the ESEM image clearly shows the cavities within the calcium alginate beads. Such cavities compare well to those viewed by SEM.

Figures 3.26 and 3.27 shows ESEM images detailing the morphology of a calcium alginate bead after immersion in Sørensen's citrate buffer, pH 3.0.

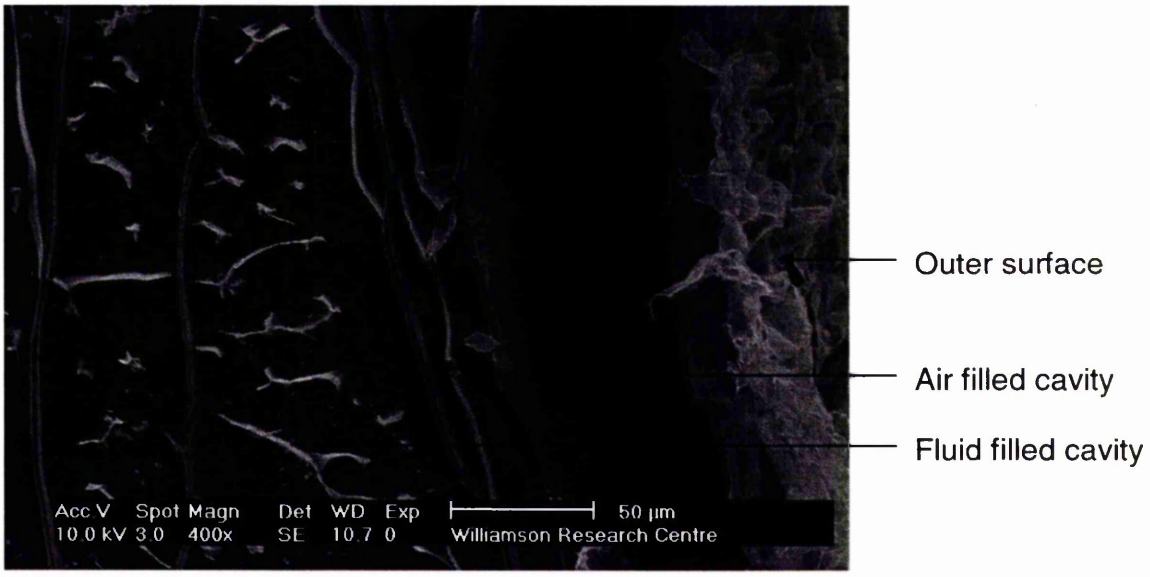


Figure 3.26 ESEM image of placebo calcium alginate bead after immersion in aqueous media *(x 400).*

The outer surface of the calcium alginate bead is detailed on the right of the image in Figure 3.26. However, in contrast to the outer surfaces viewed by SEM, the surface is not contoured, but has a textured appearance. The textured appearance may occur as a result of immersion in aqueous media and subsequent swelling or erosion of the surface of the calcium alginate bead. Conversely, the appearance may be an artefact of ice crystals and occurs as a result of the cryo or freezing process used during preparation of the calcium alginate beads for ESEM imaging.

Figure 3.26 may also provide some detail as to the processes that occur when the calcium alginate beads are immersed in aqueous media. Adjacent to the

outer surface of the calcium alginate bead, two cavities are visible, both of which appear to be air filled. Moving towards the centre of the calcium alginate bead, the cavities appear to contain some aqueous media. Figure 3.27 also confirms such a finding.

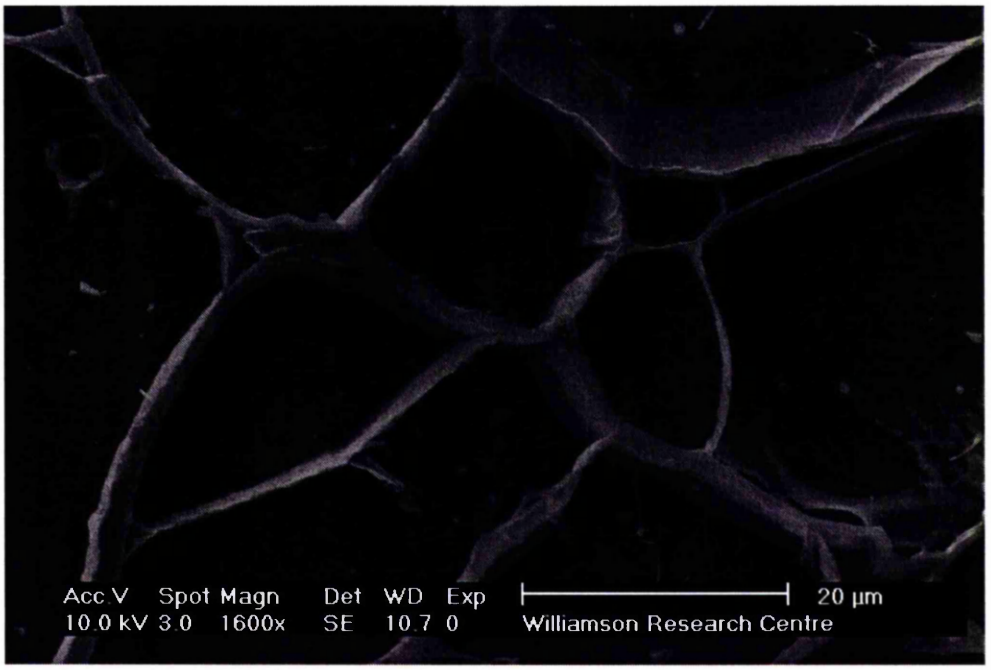


Figure 3.27 ESEM cross-section image of placebo calcium alginate bead after immersion in aqueous media *(x 1600).*

Figure 3.27 shows at a higher magnification the cavities within the calcium alginate beads, and confirms that a change has occurred to the internal morphology on immersion of the calcium alginate beads in aqueous media. Although Figure 3.27 shows the cavities to be partially filled with fluid, further evaluation by ESEM of calcium alginate beads after circulation in selected media is required. Additional assessment would confirm whether the calcium alginate beads fill with media and the subsequent changes that occur in the physico-chemical properties of the calcium alginate beads.

3.3.8 DIGITAL PHOTOGRAPHY

A digital camera image of a placebo calcium alginate bead after circulation in aqueous media is shown in Figure 3.28.

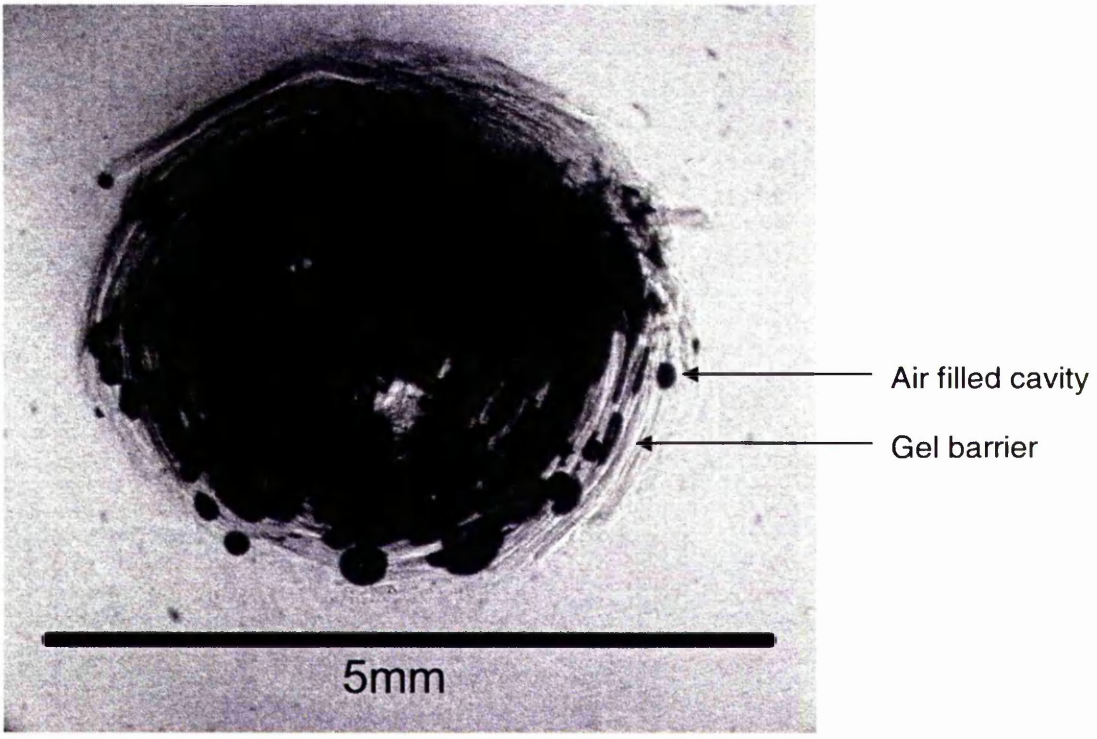


Figure 3.28 Digital camera image of placebo calcium alginate bead after immersion in aqueous media.

Freeze-dried calcium alginate beads consist of many air filled cavities that are formed as part of the freeze-drying process. Such cavities are shown in Figure 3.27 as dark areas. Figure 3.28 image shows the change in the outer surface of the calcium alginate bead and may be attributed to the development of a gel barrier around the calcium alginate bead, the formation of which is due to the hydration of the calcium alginate bead following immersion in glass distilled water. The hydration process is confirmed by the resultant weight results, (Chapter 3, section 3.3.4), that show an immediate decrease in resultant weight due to the uptake of liquid when the samples are initially placed in the media.

When considering the average diameter of calcium alginate beads in the dry state, a subsequent increase in the size of the calcium alginate bead by approximately 80% is evident when the calcium alginate beads were placed in aqueous media.

Figure 3.29 shows a placebo calcium alginate bead containing magnesium stearate that had also been immersed in aqueous media.

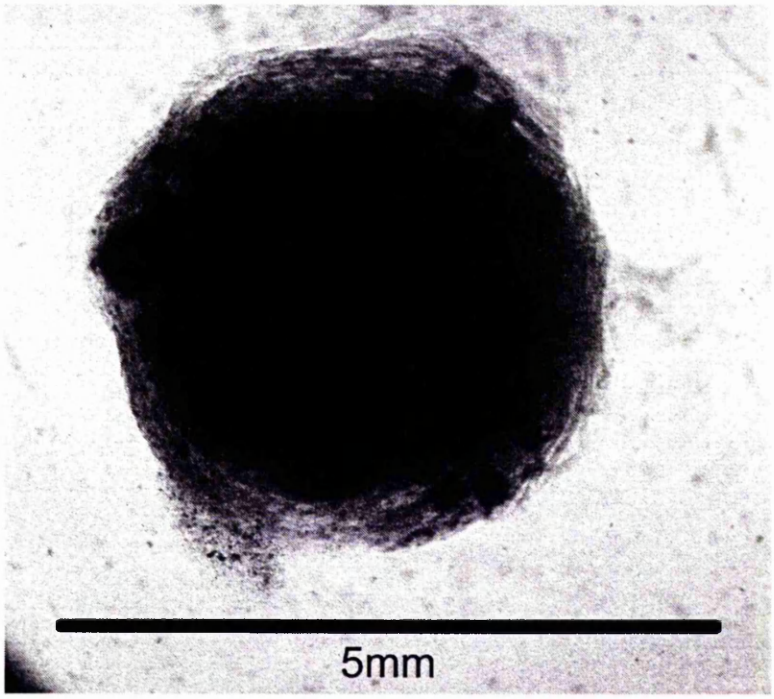


Figure 3.29 Digital camera image of placebo calcium alginate bead containing magnesium stearate after immersion in aqueous media.

The image shown in Figure 3.29 is similar to that of Figure 3.28. However, the presence of a gel barrier is not as clear as Figure 3.28 and may be due to the magnesium stearate within the bead occluding the barrier. The presence of magnesium stearate was confirmed by X-ray microanalysis and may result in the grainy appearance of the image.

Evaluation of the swelling or erosion characteristics of calcium alginate beads as not been previously investigated. However, the hydration[110], swelling[111], and

formation of gas bubbles[112] within polymer matrices such as hydroxypropylmethylcellulose, (HPMC), have been well documented. HPMC, therefore, serves as an ideal model polymer for which to initially consider the assessment and resultant method of drug release from the calcium alginate beads.

As confirmed by the resultant weight results, (Chapter 3, section 3.3.4), hydration of the calcium alginate beads occurs when the calcium alginate beads are placed in aqueous media. Digital camera images have also showed the formation of a gel barrier following immersion in aqueous media. Both aforementioned processes will have a direct effect on the rate of drug release from the calcium alginate beads. The calcium alginate beads are essentially hydrogel matrices throughout which the rate of drug release is complex and it is likely that any drug release will occur by more than one method.

Literature studies confirm that a swellable dosage form is likely to release the drug over a period of time by various interactions, as shown in Figure 3.30.

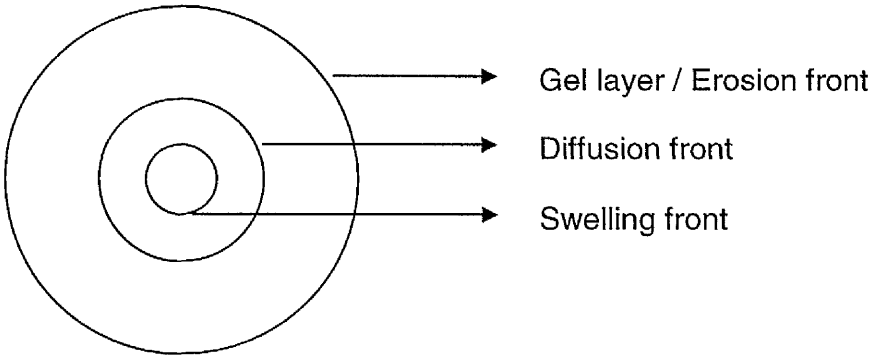


Figure 3.30 Diagram to detail mechanisms of drug release from a hydrogel matix[113].

The following mechanisms of drug release can be identified from a hydrogel matrix. The eroding or matrix-solvent front allows direct release of drug into the solvent. The diffusion front allows the drug to diffuse through the matrix for subsequent release into the solvent and the swelling front forms a glassy-rubbery region at the centre of the matrix. One or more of the mechanisms may occur at any one time, but a combination of all three mechanisms result in the

linear release of drug from the matrix. However, the thickness of the gel layer that varies over time as a result of the aforementioned mechanisms must also be considered.

The evaluation of drug release from the calcium alginate beads, with particular regard to the above points, is considered further when reviewing the results for the *in vitro* dissolution studies, (Chapter 4, section 4.3.4).

3.3.9 CONFOCAL LASER SCANNING MICROSCOPY-FRAP

Confocal-FRAP has been used to measure the diffusion of riboflavin from the calcium alginate bead to the aqueous media and also within the calcium alginate bead matrix. The results are shown in Figure 3.31

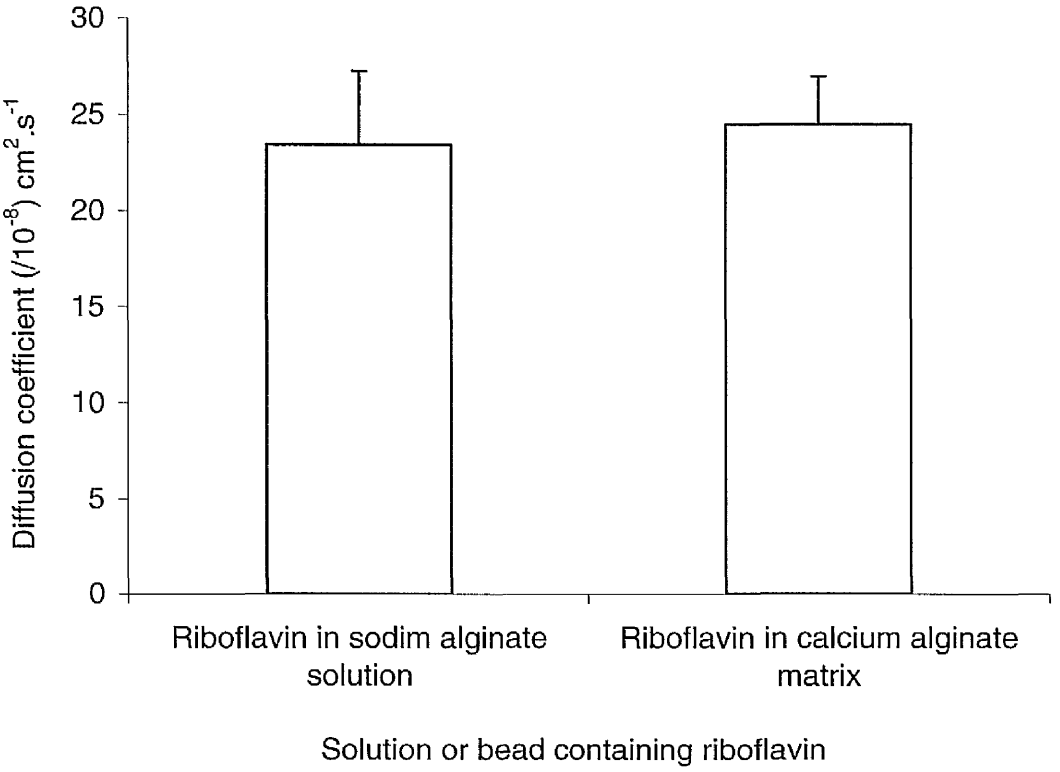


Figure 3.31 Diffusion coefficients of riboflavin through a sodium alginate solution or through a calcium alginate bead matrix.

Figure 3.31 shows that the diffusion coefficients of riboflavin from the calcium alginate bead into the aqueous media is similar to the diffusion of riboflavin molecules within the matrix. The results indicate that when the calcium alginate

beads are place in aqueous media the movement of riboflavin molecules out of the matrix occurs rapidly. The rapid movement of molecules from the dosage form means that the drug is unlikely to remain in the dosage form for long enough to demonstrate prolonged gastro-retention.

Although further evaluation of the diffusion of riboflavin from calcium alginate beads was beyond the scope of this work, further investigations are required to determine the reason for the rapid movement of riboflavin from the calcium alginate beads. Initial investigations may centre on the respective molecular sizes of riboflavin and calcium alginate, based on the results from the confocal-FRAP. If riboflavin presents with a molecular size that is much smaller than that of pores of the calcium alginate beads, then rapid diffusion of the riboflavin through the matrix into solution occurs. In order to prevent the rapid diffusion of riboflavin into solution, it may be necessary to consider the incorporation of a polymer to retard riboflavin release.

3.4 CONCLUSION

The characterisation of calcium alginate beads with different formulations has been investigated. The mean weight for a sample of calcium alginate beads, (n = 30), was 13.5mg ± 0.3mg for placebo calcium alginate beads, 15.7mg ± 0.1mg for riboflavin loaded calcium alginate beads and 18.6mg ± 0.2mg for placebo calcium alginate beads containing magnesium stearate. The diameter of a sample of calcium alginate beads, (n = 10), was 2.5mm ± 0.6mm for placebo calcium alginate beads, 2.58mm ± 0. 1mm for riboflavin loaded calcium alginate beads and 2.74mm ±0.2mm for calcium alginate beads containing magnesium stearate.

When considering buoyancy characteristics, resultant weight was the preferred method for assessing the floating ability of the calcium alginate beads. In the current study all calcium alginate beads, regardless of formulation, demonstrated buoyancy properties. Density measurements obtained by mathematical and experimental methods provided further evidence of the

floating ability of the calcium alginate beads. When comparing the mathematical and experimental methods of obtaining density measurements, the mathematical methods were shown to produce more reliable results.

SEM and ESEM have provided information regarding the internal and external morphology of both dry and wet calcium alginate beads. ESEM has provided initial investigations into the behaviour of placebo calcium alginate beads when immersed in aqueous media. Further evaluation of differing formulations of calcium alginate beads is required as is their behaviour in a range of media designed to reflect pH changes along the gastrointestinal tract.

Using the technique of X-ray microanalysis proved inconclusive with regards to determining the presence of riboflavin by detecting phosphate within riboflavin-5'-phosphate. Further investigations are required to determine if the amount of riboflavin-5'-phosphate was incorporated into the calcium alginate beads in sufficient quantities to allow for detection by X-ray microanalysis, or, whether the phosphate group of riboflavin-5'-phosphate is binding to another molecule within the calcium alginate beads thus preventing its detection by X-ray microanalysis.

Confocal laser scanning microscopy-FRAP has confirmed the rapid release of the model drug from the calcium alginate matrix, but further investigations are required to determine if the same occurs in other aqueous media. Although the calcium alginate bead formula has been modified to allow for the inclusion of magnesium stearate, the formula should be further modified to allow for the inclusion of the model drug. The assessment of calcium alginate beads containing magnesium stearate and the model drug using confocal laser scanning microscopy-FRAP would be useful, since the hydrophobic nature of magnesium stearate may reduce the hydration rate of the calcium alginate beads. Therefore, the drug release rate from the calcium alginate bead may also be reduced. The modification of the calcium alginate bead formula to allow for the inclusion of a polymer may also delay the drug release from the matrix.

Digital photography has confirmed that when calcium alginate beads are placed in aqueous media, a gel barrier is formed from the interaction of the calcium alginate bead surface with the media. Additional studies surrounding the gel barrier with specific regard to formation, degradation over time and drug passage across diffusion, swelling and eroding fronts will provide more information regarding the physico-chemical aspects of the calcium alginate beads.

The characterisation of the calcium alginate beads has resulted in obtaining an understanding of the properties of the dosage form that can be considered in additional studies.

CHAPTER 4 – THE *IN VITRO* RELEASE OF RIBOFLAVIN FROM CALCIUM ALGINATE BEADS

CHAPTER 4 – THE *IN VITRO* RELEASE OF RIBOFLAVIN FROM CALCIUM ALGINATE BEADS

4.1 INTRODUCTION

The aim of the *in vitro* work was to investigate the release of riboflavin from the calcium alginate beads in a range of media, designed to reflect the pH changes throughout the gastrointestinal tract. It was anticipated that the profile produced would also provide information that would predict how the calcium alginate beads would behave *in vivo*.

4.2 MATERIALS AND METHODS

4.2.1 MATERIALS

Dihydrogen potassium phosphate GPR (BDH Chemicals Poole, England), disodium phosphate dihydrate GPR, (BDH Chemicals Poole, England), concentrated HCl S.G. 1.16, (BDH Chemicals Poole, England), glacial acetic acid (>99%), (BDH Chemicals Poole, England), sodium acetate dihydrate GPR, (BDH Chemicals Poole, England), sodium hydroxide GPR, (BDH Chemicals Poole, England) were used as received. The solvent used in all cases was glass distilled water

4.2.2 METHODS – RIBOFLAVIN ASSAY

4.2.2.1 DETERMINATION OF WAVELENGTH OF MAXIMUM ABSORPTION FOR RIBOFLAVIN

A Cecil CE 2041 scanning UV spectrophotometer, (Cambridge, England) fitted with quartz 10mm cells and bandwidth 5nm was used to determine the major λ max of riboflavin in a range of aqueous solvents, namely 0.1M HCl, pH 1.2, Sørensen's phosphate buffer, pH 7.4, acetate buffer, pH 5.0, sodium chloride 0.9% and calcium chloride 0.02M. The buffers and methods of preparation are shown in Appendix I.

4.2.2.2 SOLUBILITY DETERMINATION OF RIBOFLAVIN IN 0.1M HYDROCHLORIC ACID

Amounts of riboflavin, sufficient to give theoretical concentrations ranging from 0.5mg.25ml^{-1} to 5mg.25ml^{-1}, were added to 0.1M HCl, (Appendix I). The solutions were shaken at 37°C in a Grant GS50, (Cambridge, England), thermostatically controlled water bath at 35 shakes/minute throughout the study period. Samples were taken at weekly intervals for four weeks and diluted 0.3ml:2.7ml with HCl. A Cecil CE 1020 UV spectrophotometer, (Cambridge, England) operating at a wavelength of 267nm and using a quartz 10mm cell was used to analyse the samples.

4.2.2.3 DETERMINATION OF STABILITY OF RIBOFLAVIN IN SOLUTION

Riboflavin has been documented to degrade in the presence of light[86]. The stability of riboflavin in solution was therefore investigated, using UV spectrophotometry.

UV spectrophotometry

The stability of riboflavin in solution using UV spectrophotometry was assessed in the following way. Since riboflavin dissolves more readily in an aqueous sodium chloride solution than water, the analysis was performed using solutions of riboflavin in 0.9% w/v sodium chloride. Two duplicate sets of riboflavin solutions concentrations 20mcg.ml^{-1}, 10mcg.ml^{-1} and 4mcg.ml^{-1} were prepared. One set was covered with aluminium foil whilst the other set was left uncovered, in daylight. Both sets were then allowed to stand at room temperature for 98 hours. A Cecil CE 1020 spectrophotometer, (Cambridge, England) operating at 267nm with 10mm quartz cells was used to analyse 3ml aliquots from both sets at time-points of 0, 2, 19, 26, 91 and 98 hours. The maximum time-point of 98 hours had been selected as a reasonable time within which degradation may occur.

4.2.3 DETERMINATION OF DRUG LOADING OF CALCIUM ALGINATE BEADS

4.2.3.1. DRUG LOADING DETERMINATION

Specified quantities of calcium alginate beads were taken from samples of calcium alginate beads containing different amounts of riboflavin, weighed and placed in a volumetric flask. Sørensen's phosphate buffer, pH 7.4, (Appendix I) was added to the flask to a volume of 25ml. The flasks were stoppered, covered with aluminium foil to prevent photolytic degradation and then shaken for 105 minutes using a Griffin flask shaker, (London, England), at maximum speed until all the beads had dissolved. The resulting solutions were centrifuged for 30 minutes at 4000 rpm, (Sanyo MSE Centaur 2, Watford, UK), model. All supernatant was removed and the remaining pellet was made up to 100ml with Sørensen's phosphate buffer, pH 7.4. Samples were assayed at 267nm using a Cecil CE 1020 spectrophotometer, (Cambridge, England), using quartz 10mm cells. Drug loading was calculated according to Equation 4.1.

Equation 4.1

$$\text{Drug loading (\%)} = \frac{\text{Actual concentration obtained (mcg.ml}^{-1}\text{)} \times 100}{\text{Theoretical concentration (mcg.ml}^{-1}\text{)}}$$

Each drug loading assessment was completed in triplicate.

4.2.3.2 DETERMINATION OF DRUG LOSS DURING PRODUCTION OF CALCIUM ALGINATE BEADS

Calcium alginate beads were prepared according to the methods detailed in Chapter 2, (section 2.3.2.2). However, during the curing process, drug loss from the calcium alginate beads was evident as demonstrated by the yellow colour of the curing solution. In addition, the storing solution was also noted to be yellow in colour, since if the calcium alginate beads required storing prior to freezing with liquid nitrogen, they were stored in glass distilled water. The observation suggested that riboflavin had leached out from the calcium alginate beads

during storage. The determination of the magnitude of the loss of riboflavin from both the curing and storing solutions was essential for the accuracy of further studies.

Calcium alginate beads are cured in 0.02M calcium chloride solution. Therefore in order to calculate amount of riboflavin lost during the curing process, standard samples of riboflavin in 0.02M calcium chloride solution were prepared. A Cecil CE 1020 UV spectrophotometer, (Cambridge, England) operating at a wavelength of 267nm and using a quartz 10mm cell was used to analyse the samples. Each analysis was carried out in triplicate. A calibration curve of UV absorbance of the riboflavin solution versus the concentration of the riboflavin solution was constructed, Appendix III).

The curing solutions used in the production of the calcium alginate beads were retained. They were assayed by UV spectrophotometry to determine the loss of riboflavin from the calcium alginate beads into the curing solution. Drug concentrations were calculated using the appropriate calibration curve, (Appendix III). The amount of drug loss was then calculated according to the Equation 4.2.

Equation 4.2

$$\text{Drug loss (\%)} = \left[\frac{C_{rib}\,(\text{mcg.ml}^{-1}) - C_{cs}\,(\text{mcg.ml}^{-1})}{C_{rib}\,(\text{mcg.ml}^{-1})} \right] \times 100$$

Where C_{rib} = Concentration of solution if all riboflavin were allowed to leach out of the calcium alginate beads.

C_{cs} = Concentration of curing solution.

Losses of riboflavin, noted by the yellow colour of the solution, were also observed during the rinsing process and storing times in glass distilled water. Therefore, in addition to calculating the concentration of the riboflavin in the curing solution, the concentration and hence amount of riboflavin in the rinsing

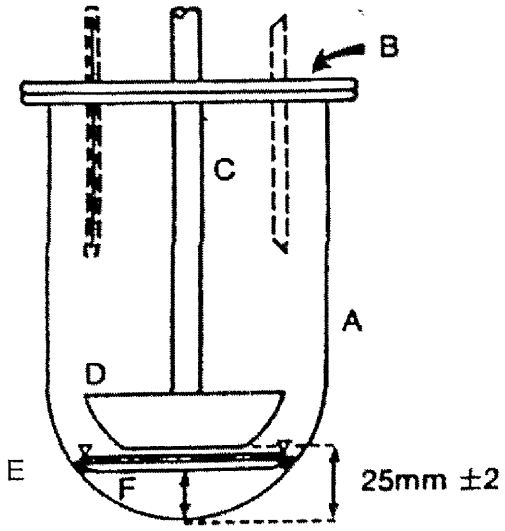
and storing solutions were also calculated. The rinsing and storing of the calcium alginate beads occurred in glass distilled water and values for the concentration of riboflavin were obtained using the equation from the riboflavin in glass distilled water calibration curve, (Appendix III). The amount of drug loss was then calculated according to the Equation 4.2.

4.2.4 *IN VITRO* DRUG RELEASE

The dissolution of a drug from a dosage form is affected by agitation intensity, pH, the type of medium, amount of aeration of the medium and the area of exposure of the drug delivery system to the medium. Traditionally baskets have been used to enclose multi-particulate formulations, but using such a method to assess drug release from the calcium alginate beads results in a reduced exposure of some of the calcium alginate beads to the medium, due to the volume of the basket being taken up by a comparatively large volume of beads.

Performing dissolution studies using the paddle method only is equally unacceptable for floating dosage forms because only a proportion of the dosage form is exposed to the media. In addition, the placement of the beads is not consistent and hence results are not reproducible[114].

Therefore, for the purposes of assessing the *in vitro* release of riboflavin from the calcium alginate beads, the dissolution apparatus used was a standard Erweka Caleva USP XXII device, (Heusenstamm, Germany), that had been modified by placing a ring and mesh assembly in the apparatus as shown in Figure 4.1.



Where A = Dissolution vessel
B = Three hole container cover
C = Stirring shaft
D = Stirring paddle
E = Ring and mesh assembly
F = Distance between ring and mesh assembly and base of dissolution vessel

Figure 4.1. Assembly for in vitro testing of floating dosage forms[114].

The presence of the mesh ensures that the full surface area of the beads was exposed to the dissolution medium[115] and the paddle ensured sufficient agitation of the medium for drug dispersion. Previous studies have shown that the mesh and paddle method provides more reproducible and reliable dissolution profiles compared to other conventional methods[114].

Alternative studies using a similar system modified as Figure 4.1, and a conventional dissolution system were used to examine the dissolution of a model drug from biphasic capsules. The results showed that release profiles were comparable. However, using the standard vessels it was noted that the capsules had a tendency to adhere to the paddles[116]. The use of such a method is therefore inappropriate for gel beads.

The *in vitro* dissolution studies were performed with apparatus consisting of clear glass vessels that fitted into a clear plastic bath. Whilst coating of the vessels would have been possible to protect against the effects of light, such measures would only be considered if preliminary experiments demonstrated it to be absolutely necessary.

Dissolution studies were performed for each sample of beads. The paddles were rotated at 50rpm, the volume of the media used was 900ml and the temperature was maintained at 37°C±1°C. Dissolution studies were performed using the media listed in Table 4.1 in an attempt to simulate the various pH values found throughout the GIT.

Media	pH
0.1M Hydrochloric Acid	1.2
Sørensen's citrate buffer	3.0
0.1M Acetate Buffer	5.0
Singly distilled water	6.7
Sørensen's phosphate buffer	7.4

Table 4.1 Media used for the in vitro dissolution of calcium alginate beads.

Pre-determined quantities of calcium alginate beads were taken from each sample. Once the dissolution study had started, 3ml of medium was removed at appropriate time intervals and assayed using a Cecil 1020 UV spectrophotometer, (Cambridge, England), operating at 267nm and using 10mm quartz cells. The volume removed was replaced by an equal amount of fresh media to prevent the change in volume of dissolution fluid affecting the results due to concentration changes.

Analysis of dissolution profiles

In vitro dissolution tests may be affected by parameters such as day to day variations in equipment set up, laboratory differences, water bath differences and the routine production of the riboflavin loaded calcium alginate beads. Such variations may make for incorrect conclusions to be drawn regarding the similarity or dissimilarity of the dissolution profiles. Therefore, it is necessary to account for the variations using analytical or statistical methods. The f_2 metric is not a statistical method, but is based on experimental design that allows for variation in analytical testing and production variations. The f_2 metric for comparing dissolution profiles where major changes in dissolution profile may be expected is recommended by the FDA[117].

The dissolution profiles obtained in the current work were compared using the f_2 similarity equation, Equation 4.3, below.

Equation 4.3

$$f_2 = 50 * \log\left\{\left[\frac{1}{\sqrt{1+\frac{1}{n}\sum_{t=2}^{n}(R_t - T_t)^2}}\right] * 100\right\}$$

Where R_t = average percent dissolved at time, t, for the reference product

T_t = average percent dissolved at time, t, for the test product

n = number of time-points

Equation 4.3 allows for the percent dissolution, and hence, similarity between two dissolution profiles to be calculated. One curve is considered a 'reference' profile and the curve with which it is compared is known as the 'test' profile. When f_2 vales are between 50 and 100, the dissolution profile of the test product can be considered similar to the reference profile. An f_2 value of 100 indicates that the reference profile is identical to the test profile with regard to the average percentage of drug dissolved.

Using Equation 4.3, only two profiles can be compared at any one time. Therefore, for each dissolution profile from Figure 4.2(a) to Figure 4.6(c), the dissolution profiles have been cross-referenced in the following way:

- Dissolution profiles with suffix (a) were compared with dissolution profiles of suffix (b).
- Dissolution profiles with suffix (b) were compared with dissolution profiles of suffix (c).
- Dissolution profiles with suffix (a) were compared with dissolution profiles of suffix (c).

4.3 RESULTS AND DISCUSSION

4.3.1 RIBOFLAVIN ASSAY

4.3.1.1 SOLUBILITY DETERMINATION OF RIBOFLAVIN IN HYDROCHLORIC ACID

Literature sources confirmed the solubility of riboflavin in alkaline solutions and water[87], but not of riboflavin in HCl. The solubility of riboflavin in 0.1M hydrochloric acid, from the current experimental work, was determined as $0.08mg.ml^{-1} \pm 0.005mg.ml^{-1}$ or 1 in 1250 and can therefore be classified as very slightly soluble. In contrast, the solubility figures of riboflavin in water categorize it as a substance that is very slightly soluble or practically insoluble.

4.3.1.2 DETERMINATION OF WAVELENGTH OF MAXIMUM ABSORPTION FOR RIBOFLAVIN

Literature sources stated that riboflavin has wavelengths of maximum absorption, (λ max), in the UV spectrum of 220 – 225nm[86], 266nm[86], 267nm in aqueous acid[85], 270nm in aqueous alkali[85], 356nm[85], 371nm[86], 444nm[86] and 475nm[86]. The current experimental work showed four maxima when solutions of riboflavin in different media were analysed by UV spectrophotometry. The four maxima occurred at 223nm, 267nm, 354nm and 444nm. The values obtained therefore compare well with literature values. The major peak occurred at 267nm for all solutions of riboflavin. Consequently, all spectrophotometric assays were performed at 267nm.

4.3.1.3 DETERMINATION OF STABILITY OF RIBOFLAVIN IN SOLUTION

UV spectrophotometry

The effect of light on solutions of riboflavin in glass distilled water when left in covered or uncovered clear glass flasks is shown in Table 4.2.

Concentration of riboflavin solution (mcg.ml^{-1})	**Solution left covered or uncovered with aluminium foil**	**Loss of riboflavin from solution (%) at t = 26 hours**	**Loss of riboflavin from solution (%) at t = 98 hours**
20	Covered	0.25 ± 0.11	1.54 ± 0.15
10	Covered	1.83 ± 0.11	3.51 ± 0.15
10	Uncovered	6.18 ± 0.26	17.87± 0.68
4	Covered	1.24 ± 0.04	7.22± 0.19
4	Uncovered	12.47 ± 0.19	36.28 ± 0.46

Table 4.2 Photolytic degradation of solutions of riboflavin in glass distilled water.

The results show that solutions of riboflavin in glass distilled water left in uncovered clear glass containers at room temperature are susceptible to photolytic degradation. Literature sources also confirm that solutions of riboflavin degrade when exposed to visible light[86, 118], with the formation of lumichrome and lumiflavin, (in neutral solutions), and lumiflavin, (in alkali solutions)[86].

Samples of riboflavin that were covered are shown to degrade by less than 10% over a 98-hour period. Such a figure is considered acceptable and therefore all preparations of riboflavin in aqueous solutions were covered with aluminium foil.

4.3.2 DETERMINATION OF DRUG LOSS DURING PRODUCTION OF CALCIUM ALGINATE BEADS

The calculated amount of riboflavin in each curing solution is shown in Table 4.3.

Sample	Citric acid % (w/v)	Original riboflavin % (w/v)	% riboflavin lost during the curing process	Mean
2a	0.00	0.03	13.3	13.5
2b	0.50	0.03	13.0	
2c	0.75	0.03	14.3	
3a	0.00	0.06	6.0	6.8
3b	0.50	0.06	6.8	
3c	0.75	0.06	7.8	
4a	0.00	0.12	3.0	3.3
4b	0.50	0.12	3.2	
4c	0.75	0.12	3. 8	

Table 4.3 Amount of riboflavin lost during the curing process for different formulations of riboflavin loaded calcium alginate beads.

The results show that for samples 2a – 2c containing the least concentration of riboflavin, (0.03% w/v), the mean amount of riboflavin lost was 13.5% w/v. In contrast the results for samples 4a – 4c containing the largest concentration of riboflavin, (0.12% w/v), the mean amount of riboflavin lost was 3.3% w/v.

In retrospect, it is likely that both sets of samples, (i.e. those of the formula 2a – 2c and those with the formula 4a – 4c), have lost the same amount of riboflavin to the curing solution. The following model explains the results. If one set of calcium alginate beads was prepared with 3g of riboflavin and lost 1g of riboflavin to the curing solution, the percentage loss of riboflavin is 34%.

Consider a second set of calcium alginate beads is prepared in the same way, (thereby producing the same size calcium alginate beads), but with 12g or riboflavin. If 1g of riboflavin was lost to the curing solution from the calcium alginate beads, the resulting loss is 8% riboflavin. Such a model reflects the values obtained experimentally.

Subsequent batches of calcium alginate beads were assessed for riboflavin loss to ensure consistency of results, but in particular, losses from the sample range 3a – 3c, containing riboflavin 0.06% w/v that would be used for the *in vivo* studies were monitored. Losses of riboflavin obtained during curing for subsequent batches of calcium alginate beads were consistent with those reported in Table 4.3.

The analysis of the rinsing and storing solutions was also considered. Losses of riboflavin during rinsing into the storing solutions were minimal and in the regions of 0.94% w/v and 0.29% w/v respectively for the riboflavin beads used for the *in vivo* studies. As discussed, the riboflavin may be removed from the outer surfaces of the calcium alginate beads.

The amount of riboflavin lost is unlikely to be attributed to a saturation of the calcium chloride solution by the riboflavin as the concentrations obtained are well below the literature values for the solubility of riboflavin in aqueous solutions. In water, the solubility of riboflavin is 1 in 3000 – 20000[118]. In sodium chloride 0.9% riboflavin is more soluble than in water alone[119]. However, it is likely that the loss of riboflavin may be as a direct result of the curing process of calcium alginate bead production. Riboflavin at the centre of the bead is trapped within the matrix whereas riboflavin on the outer layers of the beads is able to diffuse more rapidly into the curing solution. Further studies are required to determine the exact nature of the loss and drug distribution within the beads. The losses above have been considered when calculating drug loading figures.

4.3.3 DRUG LOADING

The average drug loading figures for the calcium alginate bead samples are shown in Table 4.4.

Sample	Formula	Average Drug Loading (%)	Standard deviation
2a	Riboflavin 0.03% w/v, citric acid 0% w/v	45.7	10.0
2b	Riboflavin 0.03% w/v, citric acid 0.5% w/v	72.2	8.0
2c	Riboflavin 0.03% w/v, citric acid 0.75% w/v	64.4	17.3
3a	Riboflavin 0.06% w/v, citric acid 0% w/v	64.7	4.1
3b	Riboflavin 0.06% w/v, citric acid 0.5% w/v	91.1	1.6
3c	Riboflavin 0.06% w/v, citric acid 0.75% w/v	104.0	4.0
4a	Riboflavin 0.12% w/v, citric acid 0% w/v	62.3	17.6
4b	Riboflavin 0.12% w/v, citric acid 0.5% w/v	92.3	26.4
4c	Riboflavin 0.12% w/v, citric acid 0.75% w/v	118.4	3.1

Table 4.4 Average riboflavin drug loading figures for different formulations of riboflavin loaded calcium alginate beads.

The drug loading figures for samples 3c, 4b and 4c require further investigation and explanation as drug loading values are in excess of 100%. However, it should be noted that regarding the drug loading figures, the calcium alginate bead formulas for samples 3c, 4b and 4c had all been modified to allow for the incorporation of citric acid.

4.3.4 *IN VITRO* RELEASE OF RIBOFLAVIN FROM SELECTED MEDIA

In vitro dissolution studies were carried out using four selected dissolution media. Initially the experiments were carried out over 5.5 hours, but later the

experiments continued for 24 hours to reflect the administration period of a once daily dosage form.

The dissolution profiles are shown in Figures 4.2 – 4.7. The curves start at t = 40 minutes for Figure 4.2(a) and t = 0 for Figures 4.2(b) to 4.7(c). Figure 4.2(a) starts at t = 40 minutes, since no drug had been released before the time-point. The starting point for the curves indicates that all of the drug is contained within the calcium alginate beads. The dissolution profiles show the amount of riboflavin remaining in the calcium alginate beads at specific time-points.

Release of riboflavin from calcium alginate beads in 0.1M HCl, pH1.2

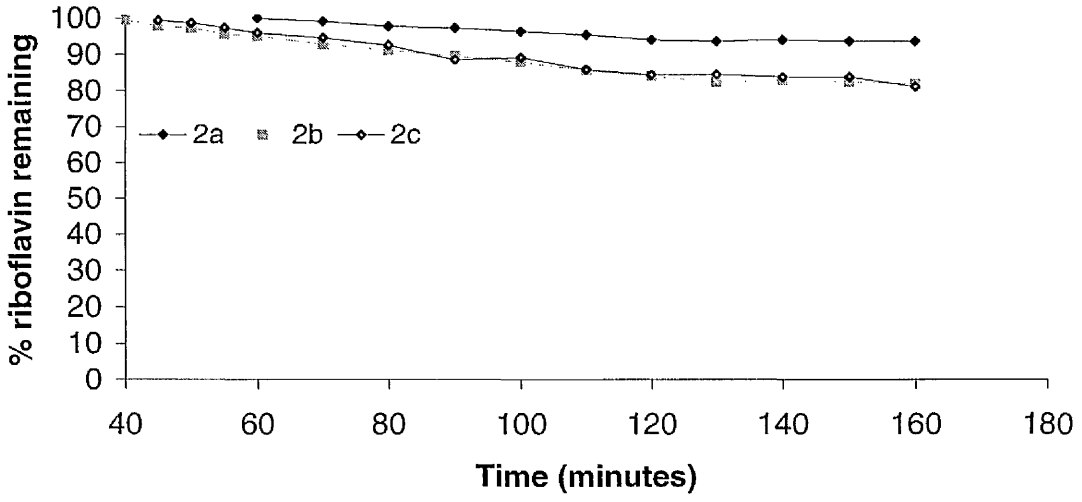


Figure 4.2 (a) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 2a – 2c in 0.1M HCl, pH 1.2.

(2a = 0.03% w/v riboflavin, 0% w/v citric acid, 2b = 0.03% w/v riboflavin, 0.5% citric acid w/v, 2c = 0.03% w/c riboflavin, 0.75% w/v citric acid).

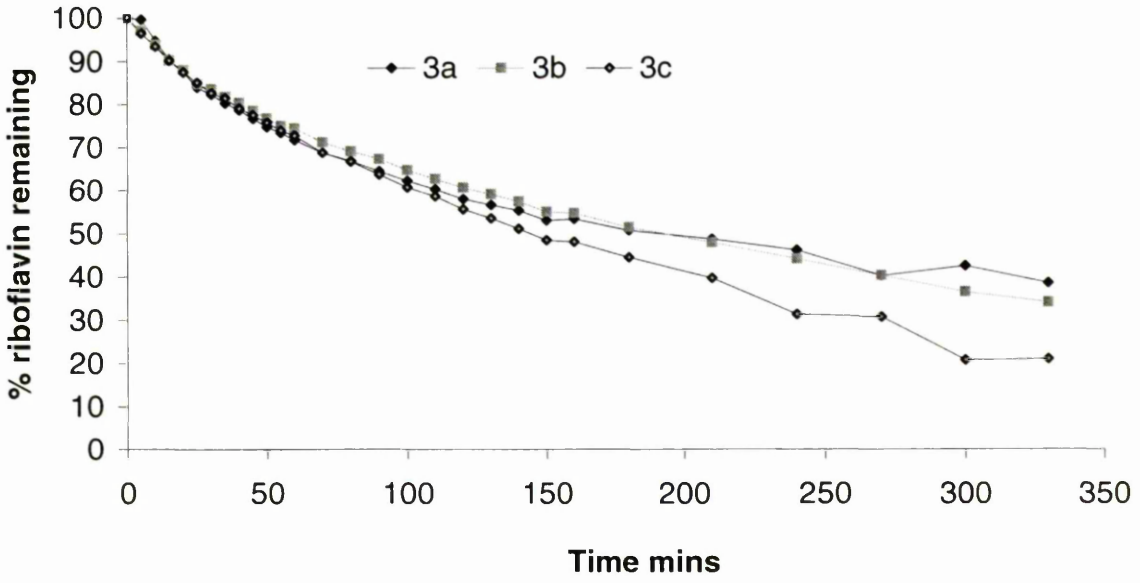


Figure 4.2 (b) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 3a – 3c in 0.1M HCl, pH 1.2.

(3a = 0.06% w/v riboflavin, 0% w/v citric acid, 3b = 0.06% w/v riboflavin, 0.5% citric acid w/v, 3c = 0.06% w/c riboflavin, 0.75% w/v citric acid).

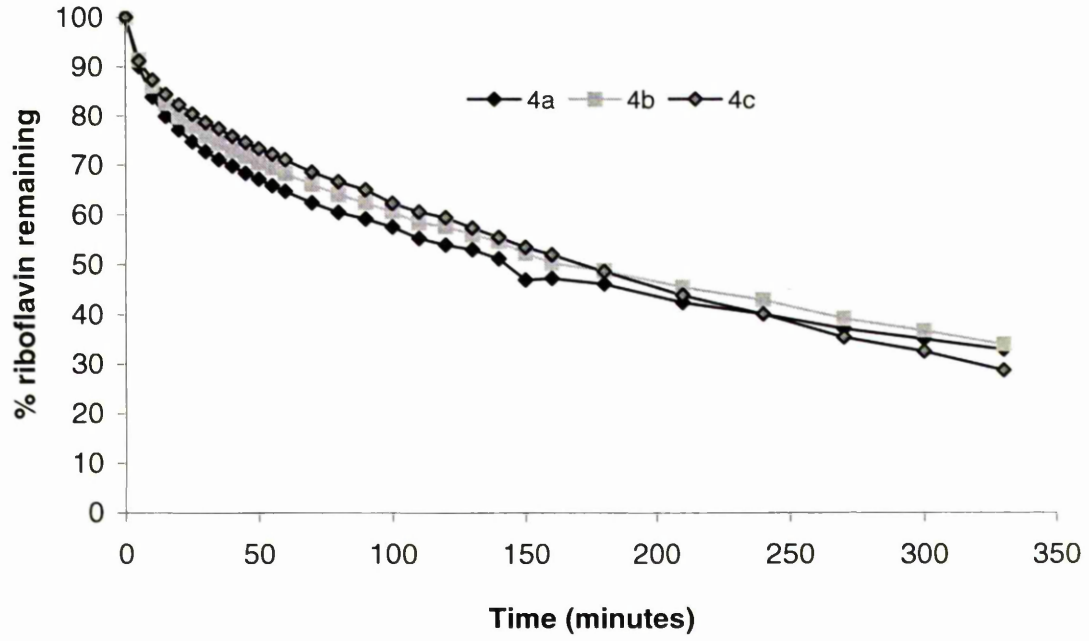


Figure 4.2 (c) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 4a – 4c in 0.1M HCl, pH 1.2.

(4a = 0.12% w/v riboflavin, 0% w/v citric acid, 4b = 0.12% w/v riboflavin, 0.5% citric acid w/v, 4c = 0.12% w/c riboflavin, 0.75% w/v citric acid).

Figures 4.2(a) to 4.2(c) show a steady release of riboflavin from the calcium alginate beads over the time period of the experiment, demonstrating zero order release or riboflavin from the calcium alginate beads. In addition the calcium alginate bead samples prepared with citric acid showed an increased release of riboflavin from the calcium alginate beads.

The reason for the increased release of riboflavin in the presence of citric acid may be attributed to the properties of citric acid. Citric acid is water-soluble and may dissolve from the calcium alginate beads[120], leaving pores. Therefore, a change in the structure of the calcium alginate beads can occur, and riboflavin is able to diffuse through the pores to the solution.

The steady release rate over the study period may be as a result of the formation of alginic acid from the reaction of alginate with acid. Alginic acid is insoluble in media of pH 1.2 and swelling of the calcium alginate beads does not occur[121]. The release of riboflavin can therefore occur by erosion only, compared with a combination of erosion and swelling. Evidence for the insolubility of calcium alginate in acidic media was confirmed when, at the end of the dissolution tests, it was observed that only calcium alginate beads that had been placed in acidic media remained as individual calcium alginate beads in the dissolution vessel. For the *in vitro* tests of calcium alginate beads in acidic media, the end of the tests were of times not exceeding 350 minutes. In contrast, the *in vitro* dissolution tests of calcium alginate beads in media of pH 5.0 showed the calcium alginate beads to form a single mass unit, and, calcium alginate beads studied in media of higher pH were noted to have all dissolved within 350 minutes.

Release of riboflavin from calcium alginate beads in Sørensen's citrate buffer, pH 3.0

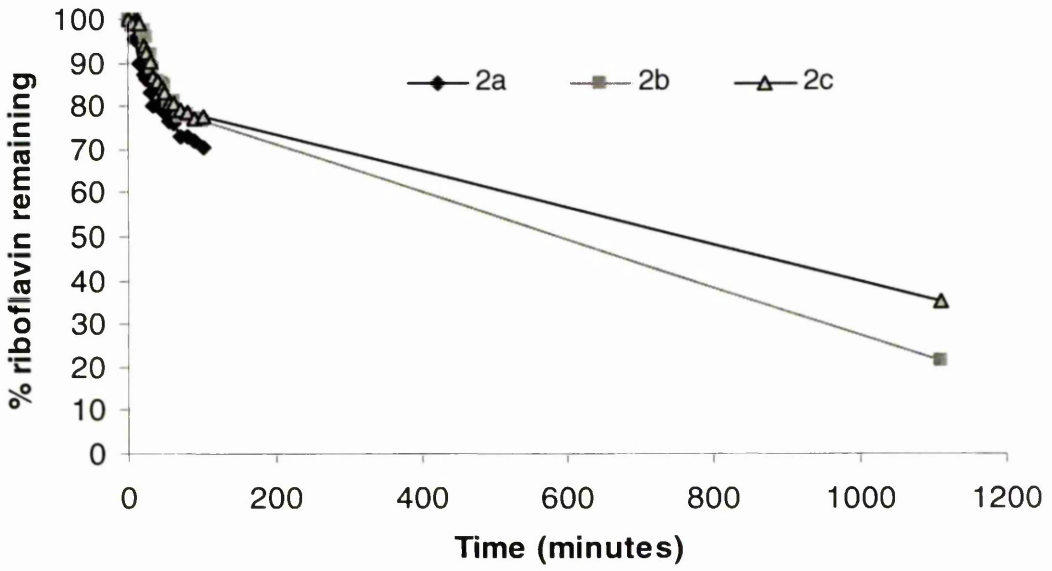


Figure 4.3(a) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 2a – 2c in Sørensen's citrate buffer, pH 3.0. *(2a = 0.03% w/v riboflavin, 0% w/v citric acid, 2b = 0.03% w/v riboflavin, 0.5% citric acid w/v, 2c = 0.03% w/c riboflavin, 0.75% w/v citric acid).*

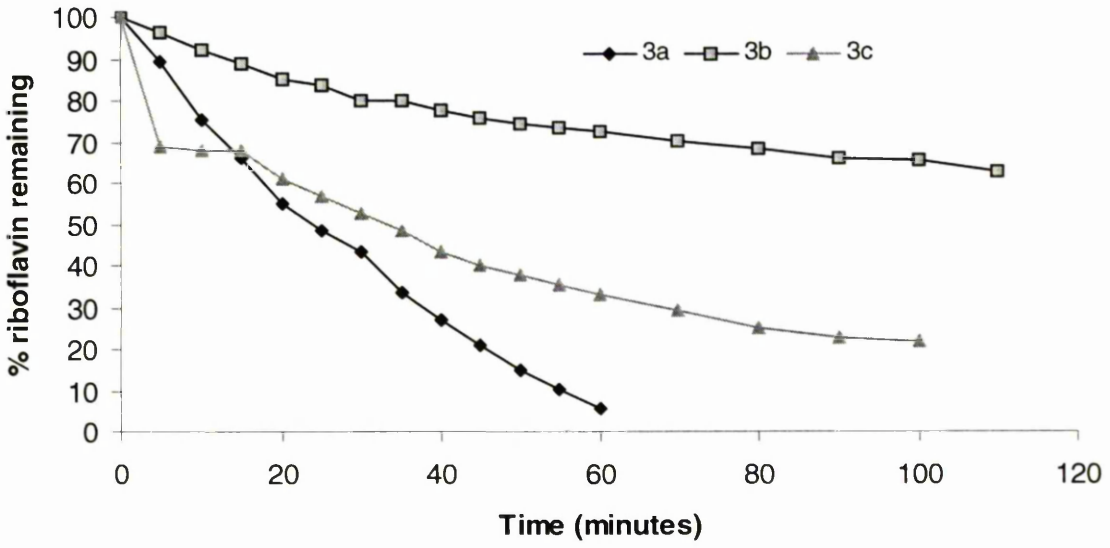


Figure 4.3(b) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 3a – 3c in Sørensen's citrate buffer, pH 3.0. *(3a = 0.06% w/v riboflavin, 0% w/v citric acid, 3b = 0.06% w/v riboflavin, 0.5% citric acid w/v, 3c = 0.06% w/c riboflavin, 0.75% w/v citric acid).*

Release of riboflavin from calcium alginate beads in Acetate Buffer, pH 5.0

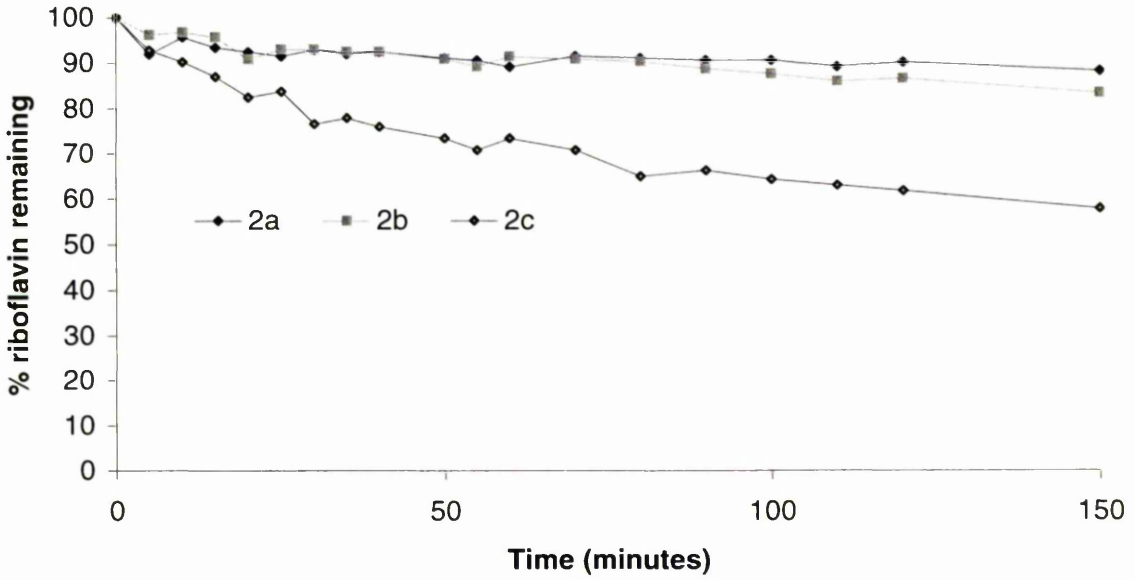


Figure 4.4(a) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 2a – 2c in Acetate buffer, pH 5.0.

(2a = 0.03% w/v riboflavin, 0% w/v citric acid, 2b = 0.03% w/v riboflavin, 0.5% citric acid w/v, 2c = 0.03% w/c riboflavin, 0.75% w/v citric acid).

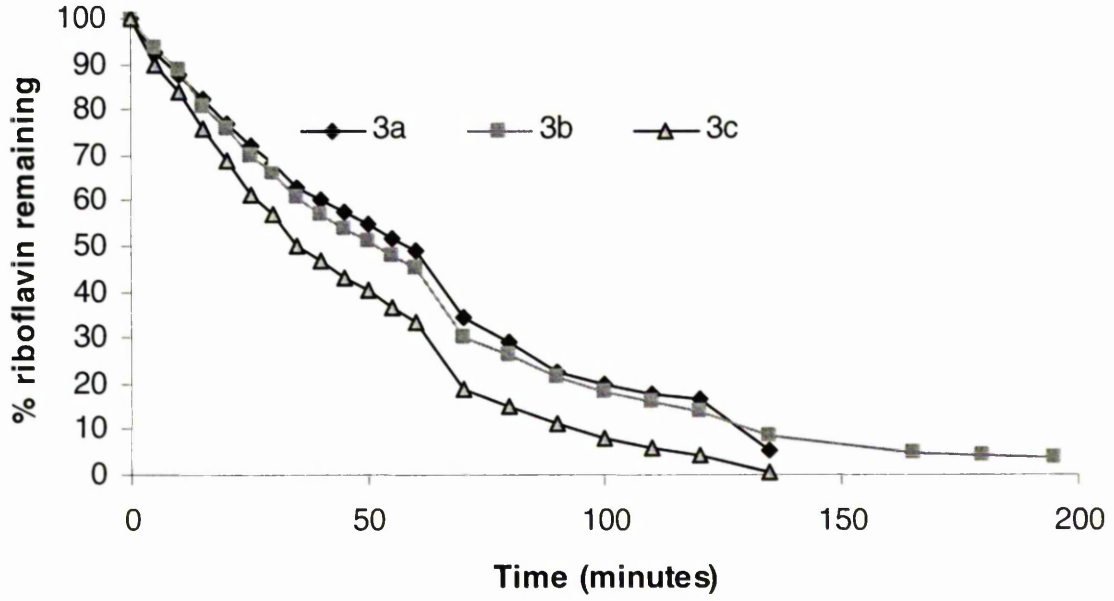


Figure 4.4(b) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 3a – 3c in Acetate buffer, pH 5.0.

(3a = 0.06% w/v riboflavin, 0% w/v citric acid, 3b = 0.06% w/v riboflavin, 0.5% citric acid w/v, 3c = 0.06% w/c riboflavin, 0.75% w/v citric acid).

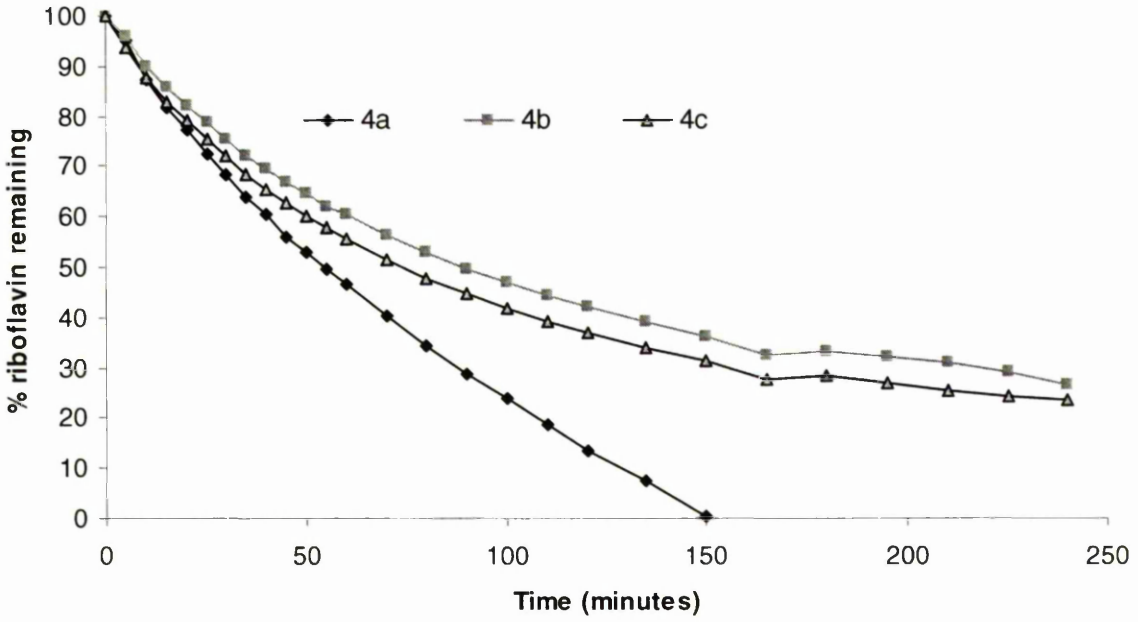


Figure 4.4(c) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 4a – 4c in Acetate buffer, pH 5.0.
(4a = 0.12% w/v riboflavin, 0% w/v citric acid, 4b = 0.12% w/v riboflavin, 0.5% citric acid w/v, 4c = 0.12% w/c riboflavin, 0.75% w/v citric acid).

The release of riboflavin from the calcium alginate beads in a media of pH 5.0 was complete in the initial 200 minutes of the experiment for all formulations. The release of riboflavin from calcium alginate beads in media of pH 5.0 is in contrast to the release of riboflavin from calcium alginate beads in 0.1M HCl. The results also showed that the release of riboflavin from the riboflavin loaded beads containing citric acid was slower when compared to riboflavin loaded calcium alginate beads containing no citric acid.

In media of pH 5.0, it was noted that the riboflavin loaded beads appeared to swell. The swelling is due to the solubility of calcium alginate in media of pH 5.0 and above and its subsequent hydration. The swelling of the alginate is characterised by the formation of a gel layer on the outer surface of the calcium alginate bead that is in contact with the media. The release of riboflavin occurs by diffusion through the swelling/gel layer.

Release of riboflavin from calcium alginate beads in glass distilled water, pH 6.7

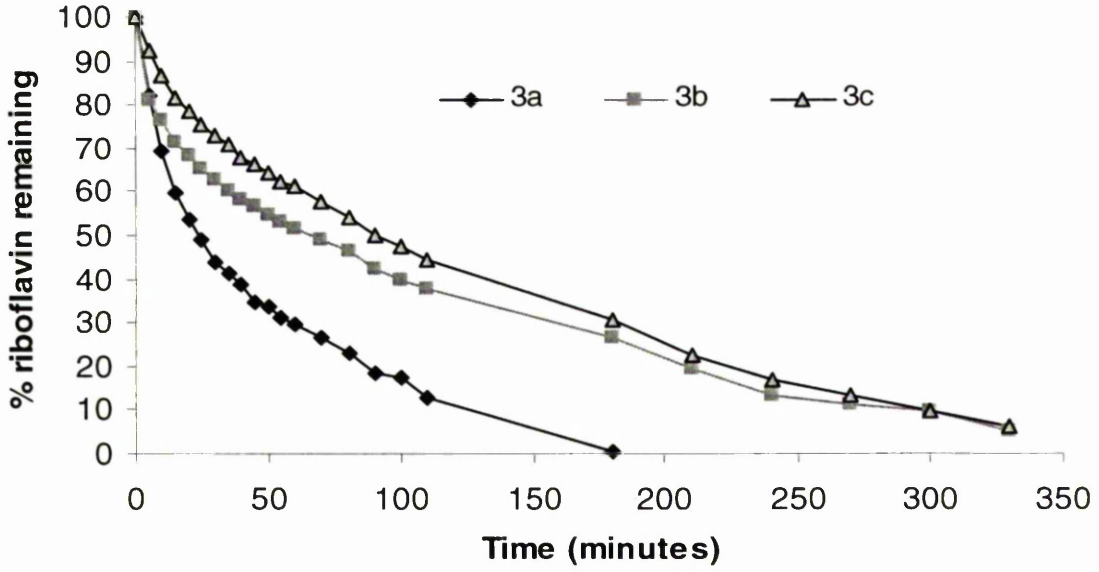


Figure 4.5(a) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 3a – 3c in glass distilled water, pH 6.7.
(3a = 0.06% w/v riboflavin, 0% w/v citric acid, 3b = 0.06% w/v riboflavin, 0.5% citric acid w/v, 3c = 0.06% w/c riboflavin, 0.75% w/v citric acid).

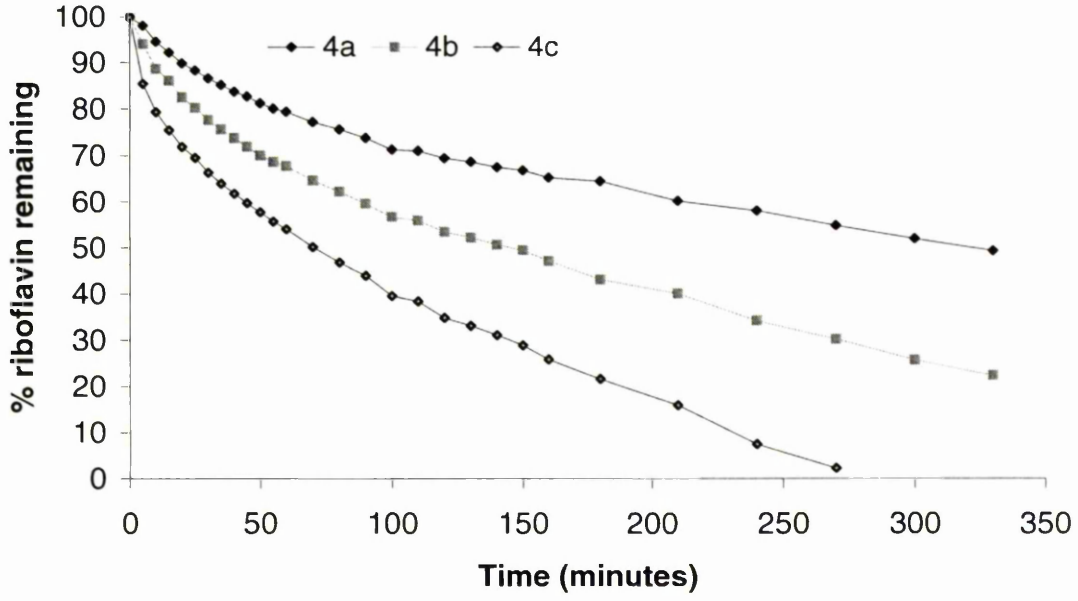


Figure 4.5(b) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 4a – 4c in glass distilled water, pH 6.7.
(4a = 0.12% w/v riboflavin, 0% w/v citric acid, 4b = 0.12% w/v riboflavin, 0.5% citric acid w/v, 4c = 0.12% w/c riboflavin, 0.75% w/v citric acid).

In glass distilled water, all riboflavin was released from the calcium alginate beads within 350 minutes. Over the period of the experiment, riboflavin showed a release rate from the calcium alginate beads typical of first order kinetics. The first order kinetic release profile was confirmed when the data was presented as shown in Figures 4.6(a) and 4.6(b).

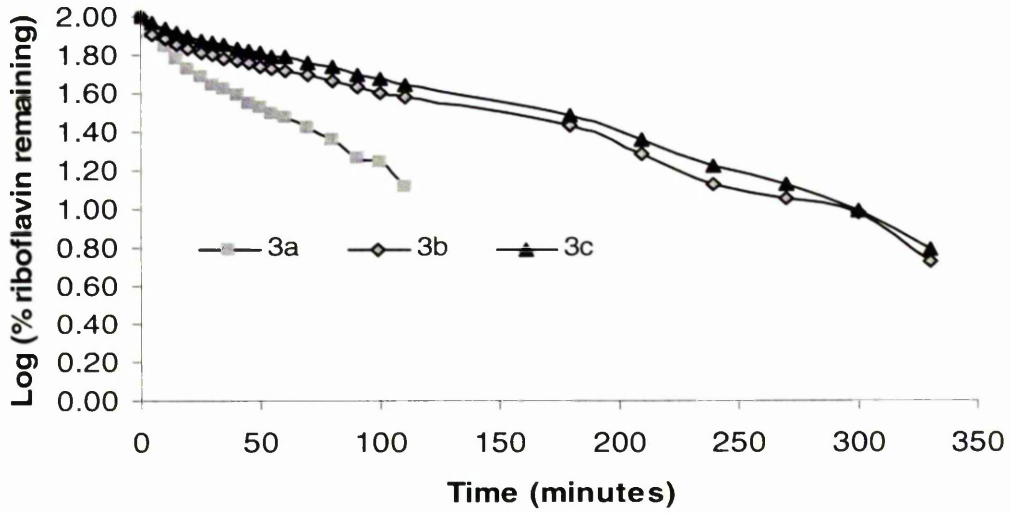


Figure 4.6(a) First order plot of riboflavin release from calcium alginate beads samples 3a – 3c in glass distilled water, pH 6.7.

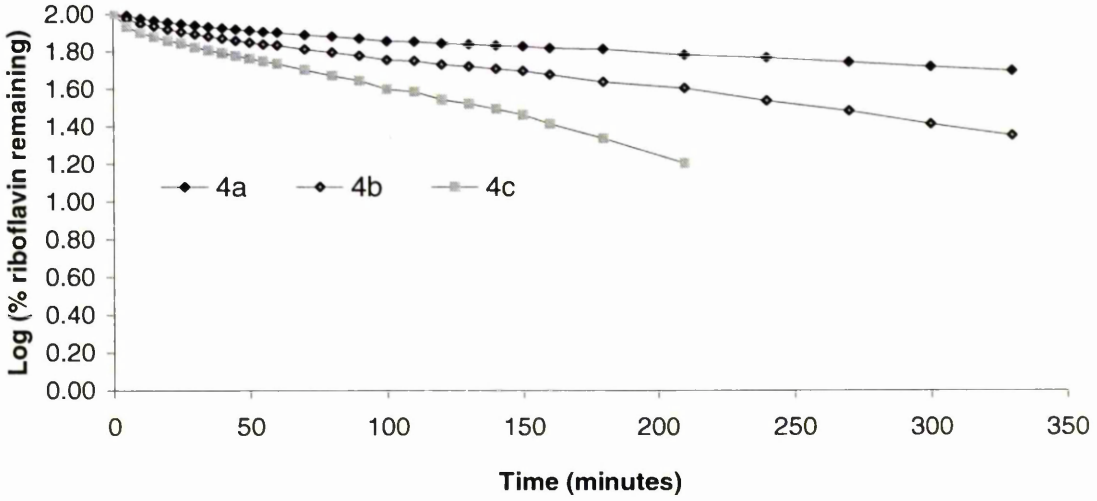


Figure 4.6(b) First order plot of riboflavin release from calcium alginate beads samples 4a – 4c in glass distilled water, pH 6.7.

The formulae of the calcium alginate beads had been modified to allow for the inclusion of citric acid. However, the results of the dissolution tests showed that the effect of citric acid on the amount of riboflavin released was not conclusive. Overall, citric acid neither enhanced nor retarded the release of riboflavin from the calcium alginate beads into media of pH 6.7.

During the dissolution tests in media of pH 6.7, in common with observations made when calcium alginate beads were placed in media of pH 5.0, it was noted that a swelling of the calcium alginate beads occurred. The reason for the swelling of the calcium alginate beads has been discussed previously, (page 160), and the same applies for the tests in media of pH 6.7.

Release of riboflavin from calcium alginate beads in Sørensen's phosphate buffer, pH 7.4

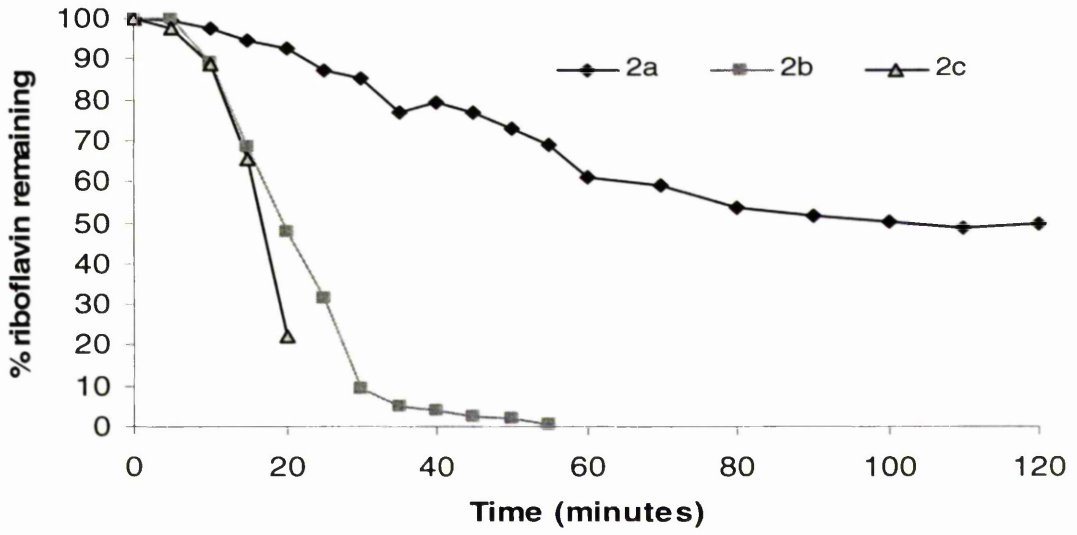


Figure 4.7(a) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 2a – 2c in Sørensen's phosphate buffer, pH 7.4.

(2a = 0.03% w/v riboflavin, 0% w/v citric acid, 2b = 0.03% w/v riboflavin, 0.5% citric acid w/v, 2c = 0.03% w/c riboflavin, 0.75% w/v citric acid).

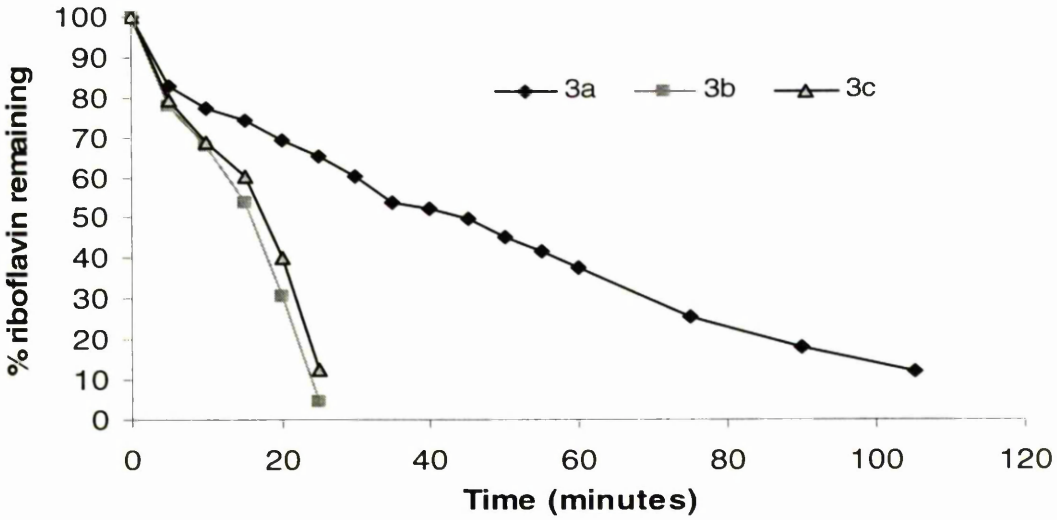


Figure 4.7(b) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 3a – 3c in Sørensen's phosphate buffer, pH 7.4.

(3a = 0.06% w/v riboflavin, 0% w/v citric acid, 3b = 0.06% w/v riboflavin, 0.5% citric acid w/v, 3c = 0.06% w/c riboflavin, 0.75% w/v citric acid).

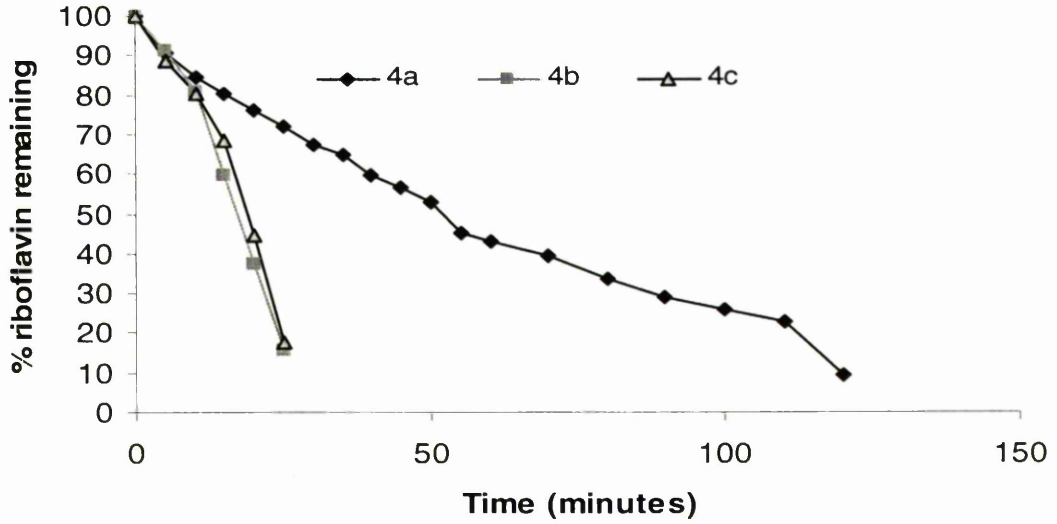


Figure 4.7(c) Percentage of riboflavin remaining as a function of time for calcium alginate bead samples 4a – 4c in Sørensen's phosphate buffer, pH 7.4.

(4a = 0.12% w/v riboflavin, 0% w/v citric acid, 4b = 0.12% w/v riboflavin, 0.5% citric acid w/v, 4c = 0.12% w/c riboflavin, 0.75% w/v citric acid).

In Sørensen's phosphate buffer, pH 7.4 all the riboflavin was released in less than 200 minutes. The presence of citric acid increased the rate of release of riboflavin from the beads. In common with the studies performed at pH 5.0 and pH 6.7, swelling of the calcium alginate beads occurred

Generally, the presence of citric acid within the calcium alginate bead formulation did not enhance or retard the release of riboflavin from the matrix. The results are supported by the calculation of the f_2 metric, (Appendix IV). All calculated f_2 figures were in excess of 50%, thereby demonstrating similarity with regard to the average percentage of drug dissolved. When modifying the calcium alginate bead formula to include citric acid, it would be reasonable to conclude that the presence of citric acid would reduce the pH of the dissolution media. The release of riboflavin from the calcium alginate beads would be retarded due to the insolubility of alginate in acidic media. However, citric acid is a weak acid, and the quantities of calcium alginate beads used in the *in vitro* dissolution studies contained insufficient amounts of citric acid to affect the pH of the media. Prior to completing further *in vitro* studies using calcium alginate beads containing citric acid and riboflavin, consideration should be given to the pKa of citric acid, and the use of Henderson-Hasselbach equation to determine the amount of citric acid required to reduce the pH of physiologically relevant media.

Overall, the release of riboflavin from the calcium alginate beads was complete within 350 minutes, despite extending most studies to 24-hours. Therefore, the *in vitro* studies suggest that the current formula is not suitable for once daily administration and further formula modification is required to alter the rate of drug release from the calcium alginate beads.

Consideration has been given to applying a kinetic model to the release of the riboflavin from the calcium alginate beads. However, although it is not possible to define an exact model or mechanism for the drug release from the calcium alginate beads, it may be possible to consider that drug release occurs as a combination of mechanisms. The reason for this is as follows. Calcium alginate beads are essentially a polymeric drug delivery system. In common

with most polymeric drug delivery systems, drug release occurs by the process of diffusion. However, diffusion of the drug in media of pH 1.2, as shown by the dissolution results, is slow; the release of the drug being determined by the alginate matrix. On contact with acidic media, the outer surfaces of the calcium alginate beads form insoluble alginic acid. However, as demonstrated by the dissolution studies, riboflavin does diffuse from the calcium alginate beads into the media. If the pH of the environment is increased to pH 5.0 and above, reflecting the stomach in the fed condition, then drug release from the calcium alginate beads becomes rapid. First order kinetics are then observed. Therefore the calcium alginate beads do not display zero order kinetics, essential for a gastro-retentive dosage form. In contrast, a similar study using calcium alginate beads loaded with paracetamol showed that the drug release was fastest in acidic media. An initial burst was observed and the drug release profiles demonstrated first order kinetics. However, an explanation for the fast release of the drug from the calcium alginate beads may be that the alginate used to produce the beads was of a low viscosity grade, and different grade alginates will result in different bead properties[69].

Most models of drug delivery, whether swelling or membrane systems, rely on the fact that the model drug is contained in a matrix within the dosage form. For example, swellable tablets are usually produced with the drug enclosed in a pH independent polymer such as HPMC. Calcium alginate beads do not contain a separate matrix, rather that the drug is dispersed throughout the calcium alginate bead. Nor are they simply a swelling system, since erosion and swelling of the calcium alginate beads gives rise to the release of the drug. Therefore, none of the traditional mechanisms can be applied to the calcium alginate beads when considering drug release rate and mechanism.

Latter studies have attempted to determine the mechanism of drug release from swelling systems where both swelling and eroding fronts occur. Puttipipatkhachorn reported that Hofenberg studied the erosion of a dosage form as the mechanism of drug release, but did not consider the combination of erosion and diffusion as a method of drug release[122]. Other findings suggest that the drug release is affected by the type of polymer[123], additional excipients

and drug substances[124] used in the diffusing front, as well as the position of the swelling and eroding fronts. The release of drug from such a system has demonstrated that initially the eroding front follows non-linear kinetics but subsequent dissolution follows linear kinetics[125]. Therefore, applying the findings to the current work, in order to apply an exact mechanism of drug release to the calcium alginate beads, further knowledge is required regarding the specific polymer, i.e. calcium alginate. A model of drug release from calcium alginate beads can only be suggested when investigations of the swelling and relaxation of the polymer in different media are completed. A study of the gel layer, formed when the calcium alginate beads are placed in media, is critical to such investigations since the thickness of the layer and the drug concentration within it will determine drug release rates and mechanisms.

4.4 CONCLUSION

Further analysis of riboflavin and drug loading calculations of riboflavin in the calcium alginate beads have been performed. Subsequently, initial *in vitro* assessments of the calcium alginate beads in different media designed to reflect changes that occur along the gastrointestinal tract have been completed.

Overall, riboflavin release from the calcium alginate beads was slowest in acidic media, (pH 1.2 – pH 3.0), and quickest in near neutral media, (pH 5.0 – pH 7.4). When considering a gastro-retentive dosage form, it would be advantageous for the calcium alginate beads to be administered under fasting conditions when the environmental conditions of the stomach are acidic. It would also be an advantage for the calcium alginate beads to be resident in the stomach for an extended period of time. Under such conditions, the dosage form would be required to release the drug slowly over a period of time, thereby demonstrating zero order release kinetics. Calcium alginate beads of the current formulation do not show zero order kinetics in all media tested. If the dosage form is to be used for local therapy, such as *H. pylori*, an initial burst of drug from the dosage form is advantageous in order to reach therapeutic concentrations. However,

the continued fast release rate of the drug in media of pH 5.0 and above, typical of the pH of the stomach contents under fed conditions is not desirable as complete release of the drug from the dosage form before the end of the dosing period may result. It would therefore be necessary to administer another dose in order to maintain effective plasma concentrations of the drug. Strategies for retarding the release of the drug from the dosage form are discussed later, (Chapter 6).

Although all formulations have been used for the *in vitro* studies, when considering the *in vivo* studies, formula 3a, (riboflavin 0.06% w/v, citric acid 0% w/v), was selected as the most promising formula with which to proceed. Formulae that contain an amount of citric acid, (i.e. all those formulae with the suffix b or c), would not be considered, as the effect of citric acid within the bead formula requires further investigation. Initial *in vivo* studies should be free from formula modifications so as not to skew or influence the results. Samples of calcium alginate beads of the formula 2a and 4a would also not be considered for *in vivo* studies, the reasons for which are described in Chapter 2, section 2.4.1.

CHAPTER 5 – FLOATING DOSAGE FORMS TO PROLONG GASTRO-RETENTION - *IN VIVO* STUDIES

CHAPTER 5 – FLOATING DOSAGE FORMS TO PROLONG GASTRO-RETENTION – *IN VIVO* STUDIES

5.1 INTRODUCTION

Calcium alginate beads, produced as described in Chapter 2, were assessed in two *in vivo* studies.

Study 1 was a gamma scintigraphy study, the aim of which was to assess the gastro-retention of placebo calcium alginate beads when they were administered under fasting conditions with aqueous vehicles. The first arm of the study investigated the behaviour of the calcium alginate beads when they were administered with 100ml of water. In the second arm of the study the calcium alginate beads were administered with 100ml of citric acid 1% w/v solution in order to determine whether citric acid influenced gastric emptying.

Study 2 consisted of a gamma scintigraphy and a bioavailability study using riboflavin loaded calcium alginate beads that were administered in both fed and fasted states. The aim of the study was to assess the influence of prolonged gastro-retention on the bioavailability of riboflavin from freeze-dried calcium alginate beads under different conditions of food intake.

For both studies, the fed and fasted states are defined as follows:

- Fasted state. Calcium alginate beads were administered in the absence of food with either 100ml of water or 100ml of citric acid 1% w/v solution following a 10-hour overnight fast.
- Fed state. After a 10-hour overnight fast, the calcium alginate beads were administered with 100ml of water following a standard breakfast, (section 5.3.3.2).

Consideration was also given as to a suitable control arm for the *in vivo* studies. Alternative oral pharmaceutical preparations such as tablets and capsules are not comparable dosage forms for the calcium alginate beads since the mechanism and rate of drug release will differ depending on which dosage form is used. An intravenous preparation was not considered for Study 2 as a comparable dosage form since it is not pharmaceutically equivalent to the calcium alginate beads. In addition, the aim of Study 2 was to investigate the bioavailability, rather than the absolute bioavailability, of riboflavin from a gastro-retentive dosage form. Solid high density calcium alginate beads are not suitable since they would require radio-labelling with a separate radio isotope, (e.g. Indium-111), to the technetium-99m that was used to radio-label the floating calcium alginate beads. Indium-111 has a higher energy window than technetium-99m and therefore visualisation of both non-floating and floating calcium alginate beads should be possible. However, in practice the potential exists for radiation in the lower window to contain a contribution from the higher energy window[126]. Hence, separate visualisation of the floating and non-floating calcium alginate beads would not be possible. In the absence of a suitable control arm for the calcium alginate beads, the aim of the first arm of Study 1 was also to provide baseline gastric emptying measurements for the second arm of Study 1 and for the whole of Study 2.

5.2 STUDY 1 – AN *IN VIVO* STUDY USING VOLUNTEERS IN THE FASTED STATE

5.2.1. STUDY BACKGROUND

Calcium alginate beads have been shown to exhibit prolonged gastro-retention when administered in the fed state[126]. However, the behaviour of the calcium alginate beads when administered in the fasted state has yet to be assessed. In addition, literature reports also suggested that certain acids may have the effect of delaying gastric emptying[93]. Consequently, the current study investigated the behaviour of placebo calcium alginate beads when they were administered with water and a non-nutrient liquid after an overnight fast.

The traditional method of delaying gastric emptying is by using fatty acid meals. Fatty acids and lipids are macronutrients that are insoluble in water[128] and digested mainly in the small intestine. They are emulsified by bile and bile salts[128] released into the liver by the gall bladder. Concomitantly, enterogastrones such as secretin and cholecystokinin, are also released, whose function is to inhibit gastric acid secretion and delay gastric emptying[129]. Fats, therefore require longer periods of time, (approximately 4 hours), to be digested[128], than other nutrients such as carbohydrates, (1 hour), and proteins, (2 hours)[128]. In practice, foods containing fatty acids and lipids have demonstrated extended gastric emptying times[5], making them an ideal choice to retard gastric emptying times for the purpose of detecting urease levels and ultimately the presence of *Helicobacter pylori*[94], *(H. Pylori).* However, the disadvantage of fatty acid meals is that they are not palatable and are generally not welcomed by the patient.

H. pylori, the causative organism of chronic gastritis, produces excess quantities of urease that in turn breaks down urea to carbon dioxide and ammonia. When radio-labelled urea is administered to a patient, the level of urease activity can be determined by detecting the amount of carbon dioxide exhaled. Test meals such as the fatty acid meals are given with the radio-labelled urea as they make for increased contact time with the bacterial urease.

A recent study proposed that citric acid drinks might be used as a viable alternative to fatty acid meals that are traditionally used to slow gastric emptying[94]. Citric acid drinks have been used as test meals to diagnose for *H. pylori* and results using such solutions have compared well with those reported after using fatty acid meals[131]. Citric acid is a naturally occurring product found in many fruit species including lemons, where concentrations are in the region of 5-8% w/w[74]. Pharmaceutically, citric acid is used as a flavour enhancing agent in liquid preparations and as an excipient with sodium bicarbonate in the preparation of effervescent granules and tablets.

The mechanism by which citric acid delays gastric emptying has been open to discussion. According to studies by Hunt et al, in dogs, the retardation of

gastric emptying occurs if sufficient oral intake of citric acid occurs to cause the pH of the duodenal contents to fall below pH 6.0 [131]. It was proposed that a local negative feedback mechanism causes the release of bicarbonate and secretin that neutralises the acidic environment and allows gastric emptying to re-commence[131]. In other studies Hunt and Knox also suggested that parameters such as acid volumes, molecular weights of acids[132] and concentrations of acid salts[133] may affect gastric emptying. The findings were substantiated by Leodolter et al who also achieved delayed gastric emptying times using 0.1M citric acid[96].

5.2.2. MATERIALS

Sodium alginate (ISP Alginates, Surrey, England), anhydrous citric acid (Thornton and Ross, Huddersfield, England), calcium chloride (BDH Chemicals, Poole, England), and stannous chloride (BDH Chemicals, Poole, England) were used as received. Technetium-99m ($^{99m}TcO_4$), as pertechnetate in sodium chloride 0.9%, was obtained from The Manchester Royal Infirmary, Department of Nuclear Medicine, (Manchester, England).

Choice of radio-label

Technitium-99m, (^{99m}Tc), is the radioisotope of choice for nuclear medicine imaging studies. It has a short half-life of 6.03 hours and is easy and inexpensive to produce. ^{99m}Tc is eluted as pertechnetate, ($^{99m}TcO_4^-$), with sodium chloride 0.9% from a molybdenum-99 generator.

5.2.3 METHODS

5.2.3.1 PREPARATION OF THE RADIO-LABELLED DOSAGE FORM

Floating radio-labelled calcium alginate beads were prepared as follows. An amount of sodium alginate, (sufficient to make a 2% w/v final solution), was weighed and incorporated into approximately three-quarters of the final volume of glass distilled water. The sodium alginate solution was left overnight to de-aerate. On the day of bead preparation, 1.25ml of stannous chloride, (0.1%

w/v), was removed from a stock solution and placed in a glass vial. The $^{99m}TcO_4$ eluate was added to the stannous chloride, the vial stoppered and the solution shaken to ensure sufficient mixing. The stannous chloride/$^{99m}TcO_4$ mix was added to the sodium alginate solution and stirred. The sodium alginate/stannous chloride/$^{99m}TcO_4$solution was then weighed and made up to volume to give a final concentration of 2% w/v sodium alginate. The resulting solution was passed through a 21G needle from a height of 21cm at a rate of 0.54ml.min^{-1} into a stirred solution of 0.02M calcium chloride. Following curing for 30 minutes, the radio-labelled calcium alginate beads were removed using an Endecotts sieve of mesh size 10 from the calcium chloride solution and 'snap frozen' with liquid nitrogen. The calcium alginate beads were then freeze-dried overnight using an Edwards Modulyo 4 freeze-dryer, (West Sussex, England), that maintained a temperature of –40°C and a pressure of 80 Nm^{-2}.

5.2.3.2 Assessment of the efficiency of the radio-labelling process of the calcium alginate beads

The efficiency of the radio-labelling process of the calcium alginate beads was assessed on the day following completion of the freeze-drying process. A Packard Cobra II Auto Gamma Counter, (Meriden, USA), was used to obtain the counts per minute for sample of calcium alginate beads, (n = 10), and 1ml of calcium chloride supernatant solution that had been used to cure the calcium alginate beads. The counts per minute for the calcium alginate beads and the supernatant were then compared and the amount of radioactivity that was associated with the calcium alginate beads was therefore determined.

5.2.3.3 Investigation of the release of the radio-label into physiologically relevant media

The experimental method by which the calcium alginate beads were produced ensured that the initial sodium alginate solution and resulting calcium alginate beads were readily radio-labelled, (Chapter 5, section 5.2.3.1). In addition, an essential requirement of the radiolabel was that it should also remain attached to the dosage form for the duration of the study. For calcium alginate beads

administered under fed conditions, study periods were expected to be in excess of 5 hours. However, when reviewing the gamma scintigraphic images obtained from the studies, areas of radioactivity were observed in the intestinal region within 30 minutes of swallowing the calcium alginate beads. Since volumes of non-nutrient liquids up to 1000ml have a gastric emptying $t_{½}$ of 10 minutes[23], consideration was also given to the fact that a proportion of the radio-label may have been released from the dosage form into the vehicle used to administer the calcium alginate beads.

The release of the radio-label into selected media was assessed by comparing the radioactive counts of a sample of placebo calcium alginate beads, (n = 10), with 3ml of the selected media. The media selected were the administering vehicles, (water and citric acid solution 1% w/v), and 0.1M HCl, pH 1.2, designed to reflect the environmental conditions of the stomach. For each media, three analyses were performed. From freshly prepared radio-labelled calcium alginate beads, a known quantity were removed and placed in a stoppered vial and the counts per minute obtained using a Packard Cobra II Auto Gamma Counter, (Meriden, USA). An aliquot, (3ml), of selected media was then added to each vial and mixed for 10 seconds at maximum speed using a Hook and Tulcer Rotamixer Deluxe, (Croyden, England). The calcium alginate beads were then separated from the media. The counts per minute were obtained for all calcium alginate beads and media and a percent release of the radio-label from the calcium alginate beads into the media was then calculated.

5.2.3.4 CHARACTERISATION OF DRY CALCIUM ALGINATE BEADS

The characterisation of numerous batches of placebo and riboflavin calcium alginate beads, produced by the method described, (Chapter 2, section 2.3.2.2), showed the production method to be robust and reproducible. Since technetium-99m has a short half-life, a full characterisation of the calcium alginate beads was not possible prior to the start of the study day and following production of the calcium alginate beads. Therefore, the calcium alginate

beads were assessed by visual assessment, weight and for radio-labelling efficiency only on the day they were required.

5.2.3.5 *IN VIVO* STUDY

Five healthy males with ranging ages (28-58), weights (63-79Kg) and heights (163-183cm) were selected and they provided written consent to take part in the study. No volunteers were taking any regular medication or had a history of gastrointestinal disorders. Those volunteers who were smokers abstained during the study. ARSAC, (Administration of Radioactive Substances Advisory Committee), and The University of Manchester Ethics Committee approved the study, reference number, 02144.

The study was designed so that each subject took the requisite number of beads, after a 10-hour overnight fast, to give a dose of approximately 4MBq on a maximum of two occasions in a two way cross over design with a wash out period of at least one week between study days. The calcium alginate beads were placed loosely on the tongue and swallowed with 100 ml water or 100ml of citric acid solution 1.0% w/v. Following administration, the volunteers were instructed to sit or remain standing for the duration of the study to avoid any possibility of posture affecting the gastric emptying of the calcium alginate beads. When taking gamma images, measures were also taken to ensure that volunteers stood in the same position for each image. In addition, providing an adjustable platform on which to stand corrected any major differences in height of the subjects, ensuring that all images were taken with the gamma camera in the same position. Failure to provide such a platform would have necessitated the constant movement of the camera head to allow for height differences of the volunteers and thereby introducing a possible source of error for the results obtained. An initial gamma scintigraphic image, (t = 0), of the stomach was taken immediately after the radio-labelled calcium alginate beads were administered. Successive images were taken at 10-minute intervals until all the calcium alginate beads had left the stomach. No additional food or liquid was consumed until gastric emptying of the dosage form was complete.

5.2.4 ANALYTICAL PROCEDURES

5.2.4.1 THE COLLECTION AND TREATMENT OF GASTRIC EMPTYING DATA

A Ohio Nuclear Sigma 410 single headed gamma camera (Packard Instrument Company, Meriden, USA) that was fitted with a 40cm parallel hole collimator designed to detect 140keV gamma radiation with a 20% energy window was used to image the areas of interest for all the volunteers. The data were recorded using MAPS 2000 software and stored as 128 x 128 pixel images.

The gamma scintigraphic images were assessed by visual examination. Using an acetate sheet, a master outline of the stomach was drawn and placed over subsequent images. The time to the onset of gastric emptying was determined as the time that showed hotspots of radioactivity leaving the stomach and entering the small intestine. When the hotspots depicting the mass of beads no longer appeared in the outline, the calcium alginate beads were deemed to have left the stomach and hence gastric emptying was complete.

5.2.5 RESULTS AND DISCUSSION

5.2.5.1 ASSESSMENT OF THE EFFICIENCY OF THE RADIO-LABELLING PROCESS OF CALCIUM ALGINATE BEADS

Immediately following the freeze-drying process, the efficiency of the radio-labelling process of the calcium alginate beads was assessed. Calcium alginate beads were found to take up in excess of 99% of the radio-label. They were therefore deemed suitable for the purposes of the study. Table 5.1 shows the results obtained for the uptake of the radio-label for a typical sample of calcium alginate beads. The results were representative of all batches of calcium alginate beads produced for the study days.

Sample (Beads and supernatant)	Counts per minute for total number of beads and volume of supernatant	Total counts per minute per sample (number + volume)	% of radio-label associated with calcium alginate beads
Beads (n= 10)	3671444	3688695	99.5
Supernatant (Volume = 3ml)	17250		

Table 5.1 Results of radio-label uptake by calcium alginate beads.

5.2.5.2 THE RELEASE OF RADIO-LABEL INTO PHYSIOLOGICALLY RELEVANT MEDIA

The release of the radiolabel, technetium-99m, into different physiologically relevant media from the calcium alginate beads is shown in Table 5.2.

Media and concentration	% release of radio-label into different media (t = 10 seconds)
0.1M HCl	13.0
Citric acid 1% w/v	13.0
Water	1.0

Table 5.2 Radio-label release into physiologically relevant media when the calcium alginate beads were shaken in different aqueous solutions.

The greater affinity of technetium-99m for acidic media compared with water can be explained as a result of the radio-labelling process. The presence of stannous chloride, (Sn^{2+}), is required to reduce TcO_4^- from an oxidation state of +7 to an oxidation state of +2 and ensure sufficient activity with which to label the sodium alginate solution. Alginate is anionic and carries a negative charge. HCl dissociates to H^+ and Cl^- and in a similar way the citrate ions of the citric acid solution will also carry a negative charge. Therefore the presence of the

HCl may be expected to exchange some of the H^+ with the Tc^{++} resulting in some de-labelling of the negatively charged alginate. Whitehead also noted that leaching of ^{99m}Tc occurred during similar experiments[63] but in addition, it was also reported that approximately 60% of the radio-label was still associated with the calcium alginate beads 5 hours after immersion in 0.1M HCl[1]. Therefore for the purposes of both *in vivo* studies, the calcium alginate beads were considered to retain sufficient radioactivity to enable their detection and subsequent visualisation by the gamma scintigraphy equipment.

5.2.5.3 CHARACTERISATION OF DRY CALCIUM ALGINATE BEADS

All batches of calcium alginate beads produced for the study appeared spherical in shape when viewed with the naked eye. Weights of each batch of calcium alginate beads following the freeze-drying procedure showed that all batches had been dried to in excess of 97% of their wet weight.

5.2.5.4 *IN VIVO* STUDY

Four of the volunteers took part in both arms of the study. The remaining volunteer took part only in the initial stage of the study when the calcium alginate beads were swallowed with water.

Figure 5.1 shows the series of gamma scintigraphic images that were obtained for Volunteer 1 when the calcium alginate beads were swallowed with 100ml of water. Within the series, the position of the beads in the stomach of the volunteer and the passage through to the intestine can clearly be seen. The red areas, indicating greater radioactivity, depict the greater masses of calcium alginate beads. Reduced masses of calcium alginate beads are show as coloured areas of orange and yellow. The images and anatomical visualisations shown in Figure 5.1 were representative of all the volunteers.

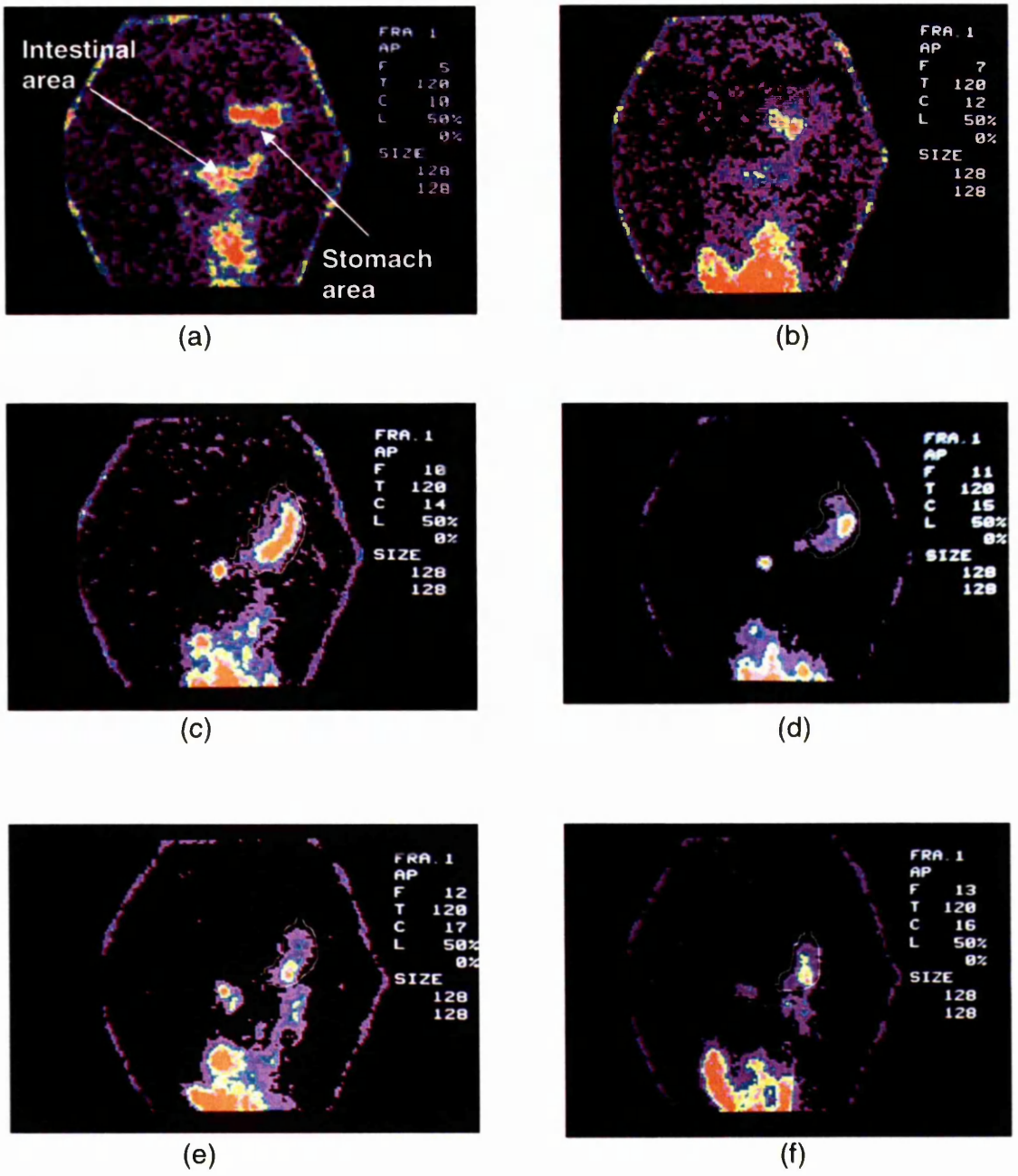


Figure 5.1 Gamma scintigraphic images showing the movement of calcium alginate beads for Volunteer 1 at selected time-points when the beads were administered with 100ml of water.

(a) t = 30 minutes, (b) t = 50 minutes, (c) t = 80 minutes, (d) t = 90 minutes, (e) t = 100 minutes, (f) t = 110 minutes.

Figure 5.1(a), t = 30 minutes, shows distinct areas of radioactivity with both stomach and intestinal areas highlighted. The appropriate identification of the stomach area is also confirmed by outlined images taken at other time-points. The area of radioactivity below the intestinal area in Figure 5.1(a) can be attributed to the vehicle that was administered with the calcium alginate beads. As discussed, (Chapter 5, section 5.2.3.2), radio-labelled calcium alginate beads release some of the radio-label into non-nutrient liquids. Following consumption of non-nutrient liquids under fasting conditions, the MMC is interrupted and the rapid emptying of such liquids occurs.

Gastric emptying was deemed complete when either two successive images of minimal radioactivity were collected or by noting the last time that a percentage of beads were seen in the stomach and the next frame that clearly showed that all calcium alginate beads had left the stomach. The window of time in all cases between the two points was 10 minutes and therefore the measure of gastric emptying gastric emptying was complete within a time error of $\pm$ 5 minutes. The gastric emptying times for volunteer 1 are reflected by Figures 5.1(e) and 5.1(f) for the current study.

The behaviour of the calcium alginate beads depended on whether they were in the stomach or the intestine. In all studies the calcium alginate beads remained as one or two distinct groups whilst in the stomach, but once in the intestine the calcium alginate beads appeared to split up into multiple groups. Such observations were expected, as calcium alginate is insoluble in the acidic stomach media but soluble in the more alkaline intestinal media. The effect of pH on the solubility of the calcium alginate beads was discussed during the *in vitro* studies, (Chapter 4, section 4.2.5).

A summary of the results showing the onset times to gastric emptying that were obtained for the four volunteers taking part in both arms of the study is shown in Figure 5.2.

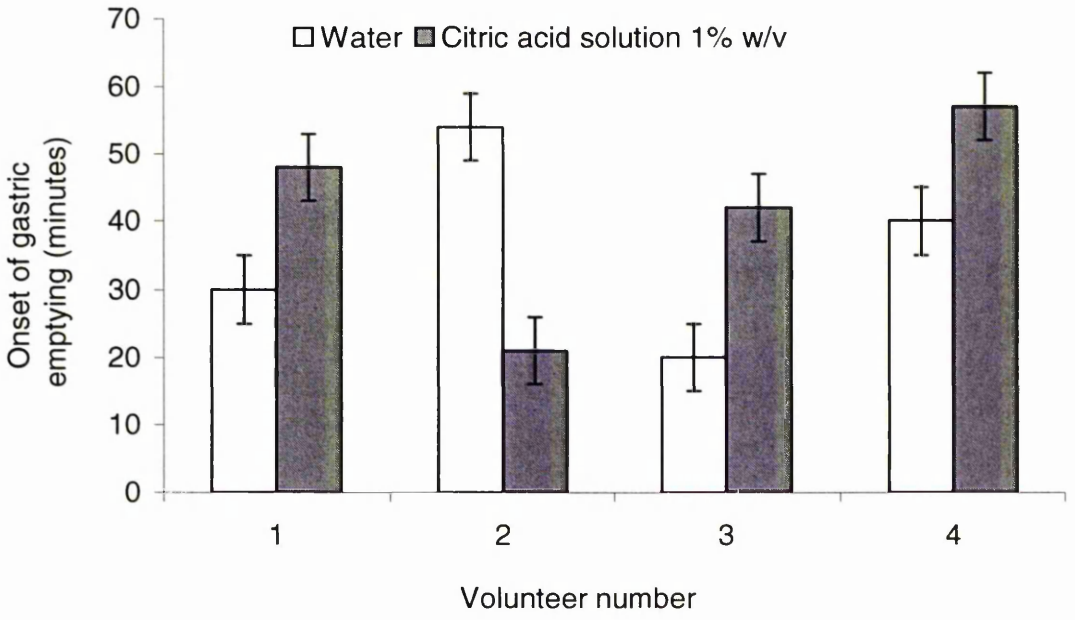


Figure 5.2 Onset of gastric emptying times of calcium alginate beads when administered with and without citric acid.

Overall, Figure 5.2 shows that the calcium alginate beads used in the study can be retained in the stomach for extended periods when administered with a citric acid 1% w/v solution. The retention of the calcium alginate beads was observed in three out of the four volunteers that took part in both arms of the study. When compared with the time to onset of emptying of the calcium alginate beads swallowed with water only, the residence time equates to an increase of approximately 50%.

In addition to the times recorded for the onset of gastric emptying, the total time from swallowing the calcium alginate beads to completion of gastric emptying was also noted, Appendix V, Table AV.1. The analysis of gamma scintigraphic images for the volunteers showed that a proportion of the floating calcium alginate beads were found to remain in the stomach for in excess of 1 hour for 7 out of 9 of the individual tests. In a similar study using calcium alginate micro-balloons administered in the fasted state all the dosage units were found to have emptied from the stomach within 1 hour[108].

The times for the completion of gastric emptying showed no apparent pattern. The erratic completion of gastric emptying times and the decreased residence time of the beads displayed by volunteer two when the calcium alginate beads were swallowed with 100ml citric acid 1% w/v solution can be attributed directly to the Migrating Motor Complex, (MMC). In particular Phase III or the 'housekeeper wave' is the phase that consists of brief powerful contractions that results in an emptying from the stomach of any remaining undigested material or dosage forms. As Phase III will occur at different times for each volunteer, the gastric emptying times will vary considerably, and also affect the total time to emptying.

The assessment of gastric emptying times for calcium alginate beads following administration in the fasted state was in direct contrast to a similar study when calcium alginate beads were administered in the fed state[63]. In the fed state gastro-retention times in excess of 5 hours were achieved for all volunteers[63]. The studies have shown that the calcium alginate beads cannot be retained in the fasted state for the same time period as the fed state.

In practice, the calcium alginate beads may be presented to the patient enclosed in a gelatin capsule. The decision not to enclose the beads in a gelatin capsule was made when considering previous studies that have shown an adherence of the capsule to the gastric mucosa[134, 135].

Consideration should be given to the fact that the delivery of some of the calcium alginate beads to the stomach may be delayed by approximately 30 minutes after swallowing the whole sample. In allowing the calcium alginate beads to be swallowed as a mass of individual calcium alginate beads as opposed to placing in a capsule, some beads may have temporarily adhered to the oesophageal wall. Previous practical work using similar dosage forms has demonstrated such an occurrence[136]. Since the calcium alginate beads adhere to the oesophagus, it should also be considered that the calcium alginate beads may also adhere to the gastric mucosa. Therefore, it is possible to hypothesize that the method of obtaining gastro-retention of the dosage form may occur as a combination of adhesion of the calcium alginate beads to the gastric mucosa,

and, flotation of the calcium alginate beads on the stomach contents. However, such a hypothesis depends on the calcium alginate beads displaying mucoadhesive properties whether in the acidic environment of the stomach or the more alkaline environment of the oesophagus. Ultimately, it is unlikely that gastro-retention is achieved as a result of the mucoadhesion of the calcium alginate beads to the gastric mucosa, as alginate has shown to be a poor mucoadhesive when compared to more traditional mucoadhesives such as chitosan, (Chapter 1, section 1.2.1.3). Studies have shown that when samples of alginate beads were prepared with and without a chitosan coating, 100% of the coated beads adhered to the pig stomach mucosa whilst only 40% of the uncoated alginate beads adhered to the same[49]. Hence, flotation is the method by which gastro-retention is achieved for the current study.

5.2.6 CONCLUSION FOR STUDY 1

The administration of floating calcium alginate beads in the fasted state has been investigated, but a significant delay in gastro-retention has not been achieved when gastro-retention times are compared with those achieved in the fed state. However, in the fasted state, citric acid has been shown to markedly delay gastric emptying times when administered with floating calcium alginate placebo beads.

5.3 STUDY 2 – A COMBINED GAMMA SCINTIGRAPHY AND BIOAVAILABILITY STUDY

5.3.1 INTRODUCTION

The results of Study 1 indicate that prolonged gastro-retention can be achieved in the fasted state when citric acid as opposed to water, is used as a vehicle with which to administer the calcium alginate beads. For Study 2, riboflavin has been selected as the model drug and has successfully been incorporated into the calcium alginate beads.

Riboflavin, also known as Vitamin B_2, is a water-soluble vitamin found in a variety of nutritional sources including yeast, milk, green leafy vegetables, heart, liver and kidney. Commercially, riboflavin is manufactured synthetically. The UK Reference Nutrient Intakes suggest that adult males and females require 1.4mg and 1.2mg per day respectively of riboflavin to prevent deficiency[137].

Therapeutically, riboflavin has been used to prevent and correct riboflavin deficiency, usually as part of a multi B vitamin oral preparation. Physiologically, riboflavin is required to release the energy from food for metabolic reactions[13]. Normal therapeutic doses are in the range of 1-2mg per day although higher doses up to 30mg per day have been recorded[138].

Riboflavin is ideal as a model drug to use in experiments to monitor gastro-retention as it has a specific absorption site within the proximal part of the gastro-intestinal tract. Several authors have also detailed the kinetics and usefulness of riboflavin as a model drug.

Levy and Jusko have shown that when riboflavin is given with food there is a linear relationship between the dose of riboflavin administered and the amount of riboflavin recovered in the urine[139]. However, when given on an empty stomach the amount of riboflavin that was recovered decreased with increasing dose. From their data, they concluded that there was a limited capacity for riboflavin absorption. In later studies that compared the fasted and fed state, the process was shown to be saturable following the administration of large doses[91]. The saturation process was confirmed by figures that initially demonstrated a decrease in the excretion rate of riboflavin when given on empty stomach, but following a meal the excretion rate increased. It was concluded that the absorption mechanism was located solely or mainly in the proximal region of the gastro-intestinal tract. However, the effect was not due to delayed gastric emptying but instead due to re-absorption in the small intestine that in turn was as a result of the presence of food stimulating bile flow[140].

The ingestion of excess quantities of riboflavin results in its renal clearance from the body. Therefore urine collection will make for an ideal non-invasive method

to collect biological samples and hence calculate the amount of riboflavin absorbed.

We have previously reported on the potential of citric acid to prolong the gastric retention of a dosage form[141]. Therefore the use of citric acid to prolong gastro-retention has been extended to the current study.

5.3.2 MATERIALS

Sodium alginate (ISP Alginates, Surrey, England), riboflavin (Merck Darmstadt, Germany), anhydrous citric acid (Thornton and Ross, Huddersfield, England), calcium chloride (BDH Chemicals, Poole, England), ethanol 96% GPR grade (BDH Chemicals, Poole, England), stannous chloride (BDH Chemicals, Poole, England), potassium dihydrogen orthophosphate (BDH Chemicals, Poole, England) and methanol HPLC grade (BDH Chemicals, Poole, England were used as received. Technetium-99m (^{99m}Tc), as pertechnetate in sodium chloride 0.9%, was obtained from The Manchester Royal Infirmary, Department of Nuclear Medicine, (Manchester, England).

5.3.3 METHODS

5.3.3.1 PREPARATION OF THE RADIO-LABELLED DOSAGE FORM

The procedure for the preparation of radio-labelled calcium alginate beads has been described previously, (Chapter 5, section 5.2.3.1). For the current study, floating radio-labelled calcium alginate beads were prepared at an ambient temperature of 25°C by incorporating $^{99m}TcO_4^-$ and stannous chloride, (0.1% w/v), into sodium alginate solution and made up to three quarters of the final volume. Riboflavin was dispersed in 0.25ml w/v of ethanol. The riboflavin/ethanol was then incorporated into the sodium alginate solution and made up to volume with glass distilled water to give final concentrations of 0.06% w/v riboflavin and 2% w/v sodium alginate. The resulting solution was extruded through a 21G needle from a height of 21cm at a rate of 0.54ml.min^{-1} into a stirred solution of 0.02M calcium chloride to precipitate the gel beads.

Following curing for 30 minutes the calcium alginate beads were recovered from the calcium chloride solution and 'snap frozen' with liquid nitrogen. The calcium alginate beads were then freeze-dried overnight using an Edwards Modulyo 4 freeze-dryer, (West Sussex, England), that maintained a temperature of –40°C and a pressure of 80 Nm^{-2}.

5.3.3.2 *IN VIVO* STUDY

Five healthy males with ranging ages (28-51), weights (63-74Kg) and heights (163-175cm) were selected and they provided written consent to take part in the study. No volunteers were taking any regular medication or had a history of gastro-intestinal disorders. Those volunteers who were smokers abstained during the study. ARSAC and The University of Manchester Ethics Committee approved the study, reference number 02144.

The study was designed so that each subject took the requisite number of beads after a 10-hour overnight fast to give a dose of approximately 4MBq of $^{99m}TcO_4^-$. Freshly prepared calcium alginate beads were administered on three occasions in a three way cross over design with a wash out period of at least one week between study days. The calcium alginate beads were administered as Study 1 by placing them loosely on the tongue and swallowing with 100 ml water or 100ml of citric acid 1.0% w/v solution. When the calcium alginate beads were administered under fed conditions, volunteers consumed a standard breakfast, Table 5.3, immediately prior to the start of the study.

Breakfast Item	Amount	Calorific Value[142]
Fried Egg	One	102
Grilled bacon	~75g (3 rashers)	375
Oven cooked sausage	One	230
Toast with butter	One slice	154
Orange Juice or	100ml	35
Tea	150ml	7
Total calorie count		**975 (Orange Juice)**
		947 (Tea)

Table 5.3 Composition of breakfast consumed by volunteers taking part in the fed study.

When the calcium alginate beads were administered under fasting conditions, no additional food or liquid was consumed until gastric emptying of the dosage form was complete. When the calcium alginate beads were administered under fed conditions a soft drink was provided 2.5 hours after the start of the study and lunch was provided 1 hour later, Table 5.4. Volunteers were also instructed to keep a diary for any further food and/or drink consumed for up to 24 hours from the start of the study.

Volunteer Number	Lunch Item	Amount	Calorific Value[142]	Total lunch calories
1	Beef/cheese sandwich	1	1150	**1875**
	Crisps	55g bag	530	
	Soft drink	500ml	195	
2	Tuna roll	1	256	**981**
	Crisps	55g bag	530	
	Soft drink	500ml	195	
3	Chicken roll	1	433	**963**
	Crisps	55g bag	530	
	Water	500ml	0	
4	Ham salad roll	1	227	**952**
	Crisps	55g bag	530	
	Soft drink	500ml	195	
5	Beef/cheese sandwich	1	1150	**1680**
	Crisps	55g bag	530	
	Soft drink	500ml	0	

Table 5.4 Details of lunch consumed by the volunteers when calcium alginate beads were administered under fed conditions.

Volunteers provided a sample of urine prior to the start of each study day that acted as a control for the study samples. Once the study day had begun, the volunteers were then instructed to collect the total volume of voided urine over the 24 hours, noting the time each sample was collected. Following collection of the urine samples, all samples were frozen at -40°C until required for analysis. Riboflavin concentrations in the urine were analysed by HPLC.

5.3.4. ANALYTICAL PROCEDURES

5.3.4.1 GAMMA SCINTIGRAPHY

Gastric emptying times were determined for each volunteer after the collection and analysis of gamma scintigraphic images for each study day, the procedure of which is described for Study 1, sections 5.2.3.5 and 5.2.4.1.

5.3.4.2 HPLC

Reverse phase HPLC is the method of choice for the analysis of urine samples because it allows for the separation and identification of small amounts of analyte in a provided sample.

Method development

An initial method using two mobile phases of different concentrations of buffered water/methanol running over a gradient, was found to be unworkable. Samples of urine were prepared with an amount of riboflavin in glass distilled water added to the urine sample to give a known concentration of riboflavin. Each sample was analysed three times. The method was deemed to be unworkable as, when reviewing the chromatograms for the analysed samples, it was found that regardless of riboflavin concentration, the identification of the peaks was not always conclusive. The identification was not possible because the riboflavin peaks of some samples occurred so close to peaks of other compounds so as to make accurate isolation of one peak impossible. For the samples that did produce adequate peak separation, the actual riboflavin concentration of the urine samples with an added known amount of riboflavin corresponded well with the expected theoretical riboflavin concentrations. Hence, although the method mentioned was not used, it was shown that for those peaks that were identifiable, riboflavin concentrations were not affected when mixed with urine.

An alternative method based on a gradient system using potassium dihydrogen orthophosphate was found to be workable[143]. Once modified, the gradient profile, shown in Figure 5.3 was produced. The system demonstrated that the

riboflavin peak could be separated from the other compounds in the urine samples using a run time of 20 minutes per sample. Subsequently, it was also noted that a similar method has previously been used effectively to determine riboflavin levels in urine by HPLC[144].

A ThermoSpectronic Unicam UV 300 spectrophotometer, (Hampshire, England) with bandwidth 2nm and analysis by Vision 32 software, version 1.25 was used to assess all mobile phases, urine samples taken prior to the start of study days and eluent produced as a result of the HPLC analysis. The examination of all the aforementioned solutions was necessary to determine whether there were any components present within the solutions that would absorb at 267nm, the absorption wavelength of riboflavin.

Apparatus

A Hewlett Packard Series II 1090 LC, (Waldbronn, Germany), with a diode-array detector, (DAD), was fitted with a Luna C18(5μ) column of dimensions 150 x 4.6mm (Phenomenex®, Cheshire, England) for all chromatographic separations.

Mobile Phase Systems

Mobile phase A consisted of potassium dihydrogen orthophosphate dissolved in glass distilled water to give a final concentration of 50mM, (Appendix I). Mobile phase B consisted of 100% methanol, HPLC grade.

Operating Conditions

Both mobile phases were degassed prior to use. The ratios for mobile phase A and mobile phase B were not fixed during the analysis, but run over a gradient, as shown in Figure 5.3.

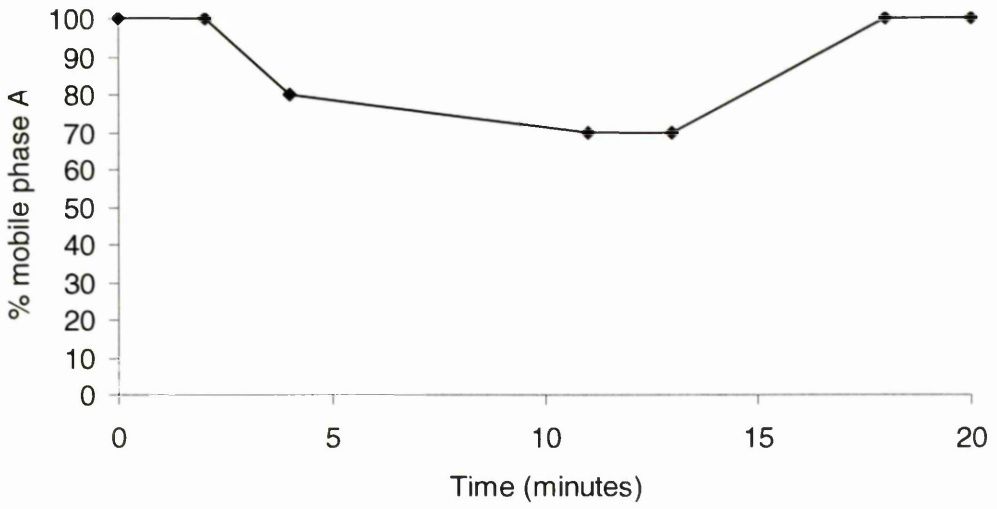


Figure 5.3 HPLC gradient profile for mobile phase A.

The column was conditioned to mobile phase A 15 minutes prior to use and for 2 minutes between samples. The flow rate was 1ml.min^{-1}. The injection volume for each sample was 50µL, (microlitres), and UV detection was performed at 267nm (4nm bandwidth). All analysis were performed at room temperature.

Sample preparation

Samples of urine were removed from the freezer and allowed to defrost overnight before being used for analysis. The urine samples were prepared for HPLC analysis by centrifuging 1.5ml of each sample at 30000rpm for 15 minutes using a Denver Instruments Company 13000g microcentrifuge (Colorado, USA). No further preparation of the urine samples was required before analysis by HPLC[145].

The precision of the analytical procedure was assessed by examining the ability of the method and apparatus to repeat the results of each sample over a short period of time. For the current method, the precision was determined by analysing the results from repeated sampling of known dilutions of riboflavin in glass distilled lab water, (n = 8). The dilutions were run ahead of the urine samples provided by the volunteers.

The detection limit, (i.e. the lowest amount of analyte in a sample that can be reliably detected) was determined. Solutions of riboflavin in glass distilled water with concentrations of 15 mcg.ml^{-1}, 12.5 mcg.ml^{-1}, 10 mcg.ml^{-1}, 7.5 mcg.ml^{-1}, 5 mcg.ml^{-1}, 2.5 mcg.ml^{-1}, 1.2 mcg.ml^{-1}, and 0.6 mcg.ml^{-1} were assessed. Each sample was analysed and the analysis repeated twice.

In order to confirm any change in retention times of the riboflavin peaks and to identify the riboflavin standard peak, between the samples for each volunteer for a particular study, a standard solution of riboflavin in glass distilled water of the minimum detectable concentration was analysed.

Analysis of chromatograms

HPLC chromatograms and all peak areas were obtained for all samples. Identification of the riboflavin peak in the urine samples was made considering the retention times of the riboflavin peak from the standard solutions at the start of the analysis and matching them with corresponding peaks from the urine samples.

5.4 RESULTS AND DISCUSSION

The results for Study 2 are discussed below. The gastric emptying times have been discussed initially followed by the bioavailability results.

5.4.1 GAMMA SCINTIGRAPHY AND GASTRIC EMPTYING RESULTS

Selected gamma scintigraphic images demonstrating the movement of the calcium alginate beads that were obtained for Volunteer 3 when the calcium alginate beads were swallowed with 100ml of water in the fasted state and with 100ml of water in the fed state are shown in Figures 5.4 and 5.5 respectively.

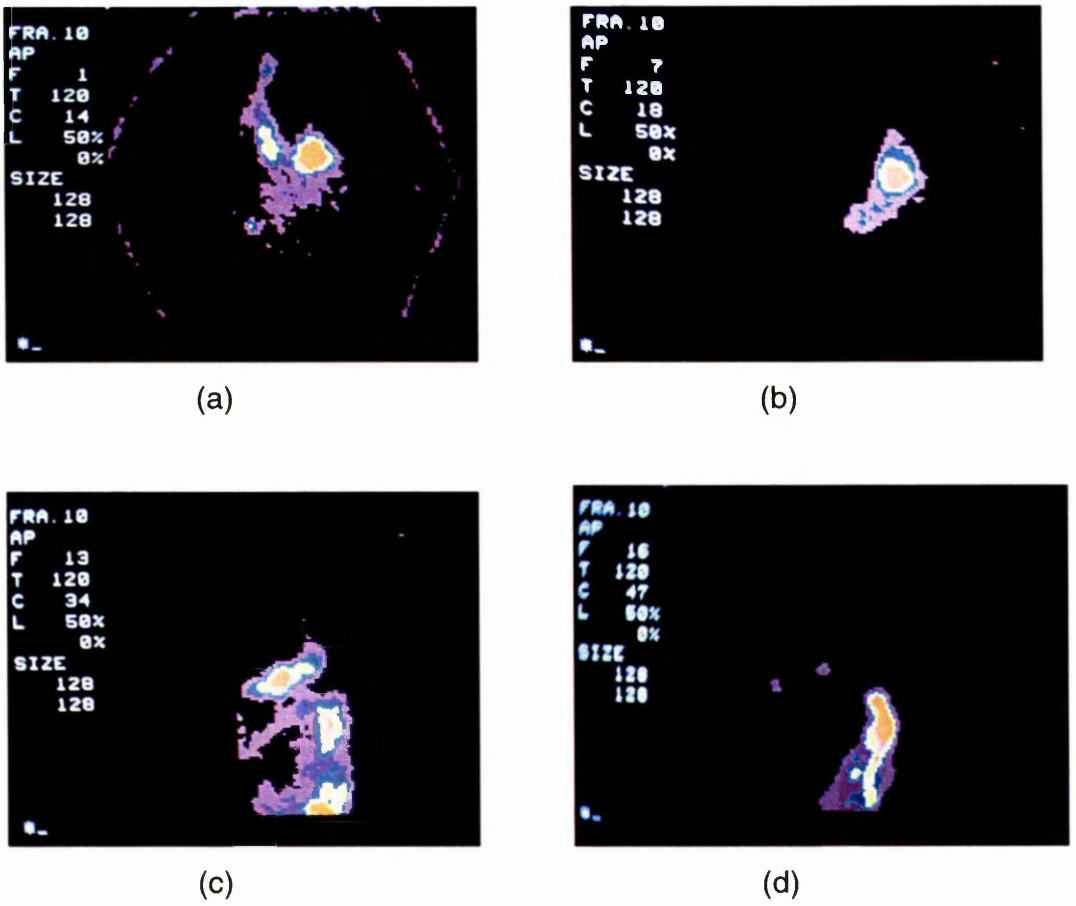


Figure 5.4 Gamma scintigraphic images showing the movement of calcium alginate beads for Volunteer 3 at selected time-points when the beads were swallowed with 100ml of water in the fasted state.

(a) t = 0 minutes, (b) t = 20 minutes, (c) t = 30 minutes, (d) t = 40 minutes.

The series of images in Figure 5.4 shows the behaviour of the beads from the start of the study at t = 0 minutes to the end of the study at t = 40 minutes. At t = 0 minutes the beads had been swallowed immediately before the image was taken and the progression of the calcium alginate beads down the oesophagus to the stomach can be seen. At t = 30 minutes, the calcium alginate beads have started emptying from the stomach and at t = 40 minutes there are no beads remaining in the stomach as the hotspots of radioactivity depicting the calcium alginate beads appear in the small intestine.

The behaviour of the calcium alginate beads for the initial arm of Study 2, Figure 5.4, reflected those from both arms of Study 1. The calcium alginate beads were administered under fasting conditions in all cases, with either 100ml of water or 100ml of citric acid 1% w/v solution. For all the volunteers, the calcium alginate beads remained as one or two distinct groups, the reasons for which have already been discussed, (Chapter 5, section 5.2.5.4).

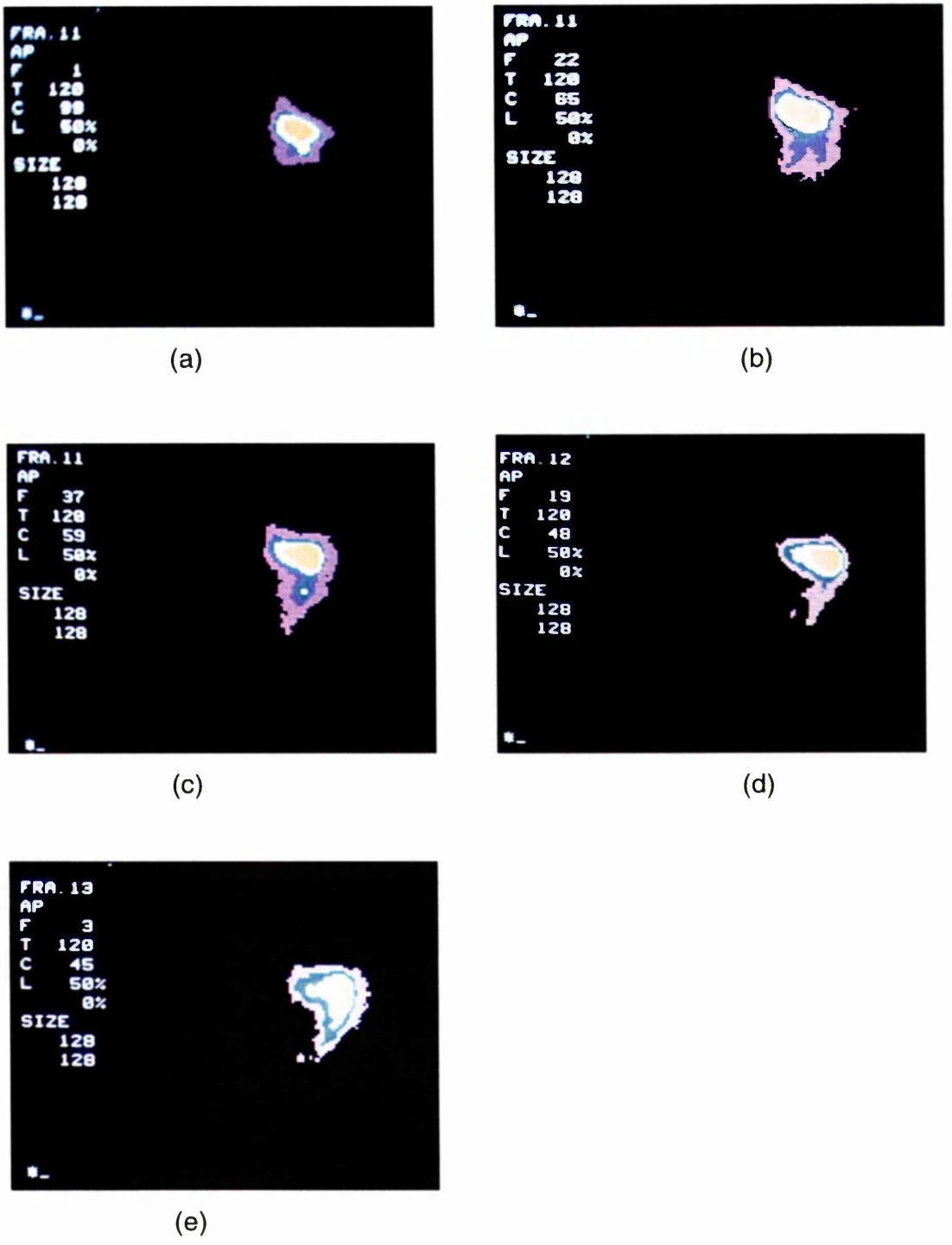


Figure 5.5 Gamma scintigraphic images showing the movement of calcium alginate beads for Volunteer 3 at selected time-points when the beads were swallowed with 100ml of water in the fed state.

(a) t = 0 minutes, (b) t = 70 minutes, (c) = 120 minutes, (d) t = 240 minutes, (e) t = 350 minutes

The image at t = 0 minutes, Figure 5.5, was taken immediately after the beads were swallowed and shows the beads as a single mass in the stomach. At t = 120 minutes the beads can be seen to start emptying into the small intestine. However, the bulk of the beads remain in the stomach. At t = 350 minutes, the main mass of the beads are emptying into the small intestine, indicating that gastric emptying is nearing completion. For all the volunteers, the calcium alginate beads remained in one or two distinct groups throughout the study when the calcium alginate beads were administered in the fed state. However, it was expected that multiple groups of beads might have been seen. As discussed, after administration in the fasted state, the beads remained in one or two groups in the stomach possibly as a result of the insolubility of calcium alginate in acidic pH. The calcium alginate beads administered in the fed state and considered in Figure 5.5 remained in the stomach for the duration of the study period but the presence of food increases the pH of the stomach contents to approximately pH 6.0, thereby making the environment more favourable to permit dissolution of the calcium alginate beads. Hence the presence of multiple groups of beads may have been visualised.

All volunteers for Study 2 were approximately the same height, (155 – 175cm, mean height 163.6cm), and the same procedures as for Study 1 were taken with regard to positioning of the volunteers in front of the gamma camera, (Chapter 5, section 5.2.3.5). However, when calcium alginate beads were administered under fasting conditions compared to the administration of calcium alginate beads under fed conditions, it was noted that in order to obtain images where the masses of calcium alginate beads occurred in the centre of the image, the gamma camera was required to be adjusted upwards. The observation would therefore indicate that the calcium alginate beads are floating on the surface of the stomach contents as opposed to being submerged by the mass of food or adhered to the mass of food.

Using the data obtained for Study 2, (Appendix V), Figure 5.6 displays graphically the gastric emptying times obtained from the gamma camera images when the calcium alginate beads were administered under different conditions of food intake.

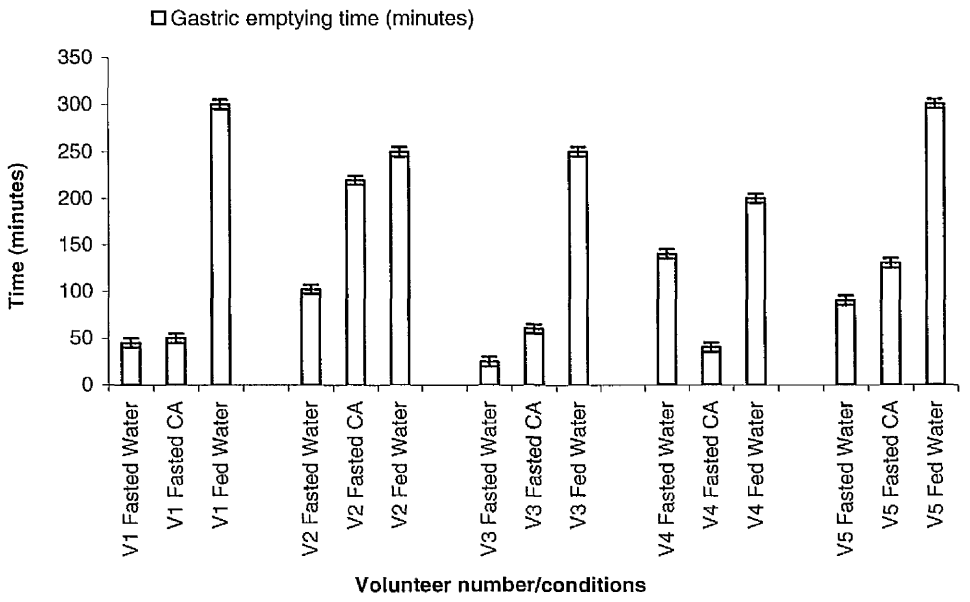


Figure 5.6 Gastric emptying times for volunteers from time of swallowing to time of completion of gastric emptying under different conditions of food intake.

From the data obtained, (Appendix V), considering the total time from swallowing of the calcium alginate beads to completion of gastric emptying, mean gastro-retention times of 80.4 ± 5 minutes and of 100 ± 5 minutes were obtained when the calcium alginate beads were administered with 100ml of water and 100ml of citric acid 1% w/v solution respectively. In all cases the calcium alginate beads emptied from the stomach faster when administered under fasting conditions compared to administration in the fed state regardless of administrative vehicle. The presence of either nutrient or non-nutrient liquid in the stomach interrupts the MMC and initiates the pattern of digestive motor activity so that the stomach is emptied of existing contents and prepared for subsequent food and/or drink intake. Therefore, the results shown in Figure 5.6 were expected.

5.4.1.2 THE INFLUENCE OF CITRIC ACID ON GASTRO-RETENTION

The influence and investigation of citric acid as a pharmaceutical agent to prolong gastro-retention has been discussed previously[139]. The administration of calcium alginate beads with a citric acid 1% w/v solution in the current study showed that a proportion of the calcium alginate beads were retained in the stomach for up to 100 minutes. The figures for the onset of gastric emptying compare well to those previously obtained for Study 1, (Appendix V), and confirm that citric acid has the ability to prolong gastro-retention of the dosage form.

5.4.1.3 THE GASTRIC EMPTYING OF CALCIUM ALGINATE BEADS WHEN ADMINISTERED UNDER FED CONDITIONS AND THE INFLUENCE OF A HIGH CALORIE/HIGH FAT DIET

The foods regimens used in the study were designed to reflect the volunteers' normal eating habits.

When reviewing the breakfast and lunch meals consumed by the volunteers, the following observations can be made. An adult male consumes approximately 2500 calories per day[142]. Therefore, in order to maintain his weight, he should consume at least that amount in a day. The foods consumed for breakfast and lunch provided minimum and maximum calories of 1899 and 2850 respectively, mean 2374 calories, (Chapter 5, section 5.3.3.2). Since only two meals had been consumed, it is likely that when the rest of the food consumed for the day is considered, a total count of 2500 calories will be exceeded, suggesting that the diet is high calorie.

As the breakfast was consumed after an overnight fast, the consumption of a standard breakfast for all the volunteers for the fed state study was necessary to eliminate the possibility of any changes in gastro-retention times being attributed directly to the initial meal of the day. In addition the standard breakfast resembled that used previously[63]. Hence similar gastric emptying times were expected.

The breakfast test meal contained a high proportion of fat. As discussed, fats and lipids are macronutrients, (Chapter 5, section 5.2.1); the composition of which makes for the greatest variability in drug absorption and alteration of gastric emptying. Consequently, high fat meals, such as the one used for the study, are the test meals of choice of the FDA when investigating the food effect for bioavailability studies[146].

When considering the test meal employed in the study, it should be noted that the fat content can be considered as two separate entities; namely the fat introduced into the meal as part of the cooking process and the fat component of the meal itself, e.g. an additional source of fat has been used to fry the eggs but eggs themselves contain a high proportion of fat.

Delays in gastric emptying have been reported following the addition of fat to a meal[18]. Should a meal contain a high proportion of fat both from the food source and the method of preparation, then an increase in gastric pH results and a further delay in gastric emptying is achieved[18].

However, some studies show that high fat meals do not affect gastric emptying or bioavailability. Studies by Penagini et al showed no change in lower oesophageal sphincter activity when comparing standard and high fat meals[147]. The drug methylphenidate, used to treat attention deficit hyperactive disorder in children, has also shown no change in bioavailability when administered with a high fat breakfast[148].

In addition to a high fat breakfast meal, (Chapter 5, section 5.2.1), the lunch meal contained foods such as cheese and crisps that are also high in fat content and therefore increase the amount of total fat within the food regimen. Overall, the food regimens can be considered not only to be high in calories and high in fat content. Considering the combination of a high calorie and high fat meal, the longer period of time required to digest consumed fats and the frequent feeding regimen, the calcium alginate beads displayed extended gastro-retentive times. For the current study, in all cases when the calcium alginate beads were administered under fed conditions with water, gastro-

retention times were in excess of 200 minutes, Figure 5.5, and the values obtained corresponded well to those from a similar study[63]. None of the calcium alginate beads administered under fasting conditions, either with water or citric acid 1% w/v solution, demonstrated gastro-retention times that corresponded with gastro-retention times that were obtained when calcium alginate beads were administered under fed conditions.

The presence of food in the stomach is acknowledged to delay gastric emptying[18] and thereby influence the amount of drug absorbed and the rate at which it is absorbed. In addition, dosage forms designed to float have been shown to maintain their floating abilities when administered after food[14]. Frequent eating patterns, typical of the current study and of a Western diet, have also been shown to maintain floatation of the dosage form.

The results of the current study indicate that maintaining the stomach in the fed state enables the calcium alginate beads to be retained in the stomach, and therefore the beads are emptied more rapidly in the fasted state than in the fed state. However, administration of the beads in the fasted state with citric acid solution rather than water increases the retention time by a maximum of 58%.

5.4.2 HPLC RESULTS AND DETERMINATION OF RIBOFLAVIN BIOAVAILABILITY

HPLC has proved to be a useful analytical tool for the calculation of the amount of riboflavin within a sample, and, ultimately in determining the bioavailability of riboflavin from floating radio-labelled calcium alginate beads.

5.4.2.1 VALIDATION OF THE HPLC METHOD TO ANALYSE URINE SAMPLES

UV scans of methanol, potassium dihydrogen orthophosphate and glass distilled lab water, (Appendix VI), showed no absorption at 267nm, indicating that none of the solutions would interfere with the HPLC analysis. The eluent, (Appendix VI, Figure AVI.V), showed a small amount of absorption at 267nm that was expected. The absorbance is due to the small amounts of riboflavin that were present in the eluent after passing through the HPLC system.

Samples of urine without riboflavin also showed absorbance at 267nm. Many impurities are present within a urine sample, and it is likely that some impurities will show absorbance in the UV spectrum. As a result, the potential existed for the riboflavin peak of interest to be masked by other impurities of the urine that absorbed at the same wavelength. Therefore, it was necessary to adjust the mobile phase gradient profile to that shown in Figure 5.3 in order that the peak of interest was separated from other interfering peaks.

Dilutions of riboflavin in glass distilled lab water were made and analysed by HPLC, the results of which allowed for the construction of a calibration curve, (Appendix VII). The accepted criteria state that the correlation coefficient of an HPLC assay should be ≥0.990. The correlation coefficient value, (R^2), from the calibration curve was 0.999, demonstrating that a high linearity had been achieved. The minimum detectable concentration of riboflavin in glass distilled water was determined as 1.2mcg.ml^{-1}.

The concentration of 1.2mcg.ml^{-1} riboflavin in glass distilled water was selected as the concentration of riboflavin in glass distilled water to be analysed between the samples for each volunteer for a particular study day. Figure 5.7 shows the results obtained when standard samples of riboflavin dissolved in glass distilled water at a concentration of 1.2mcg.ml^{-1} were included at selected sample points throughout the analysis.

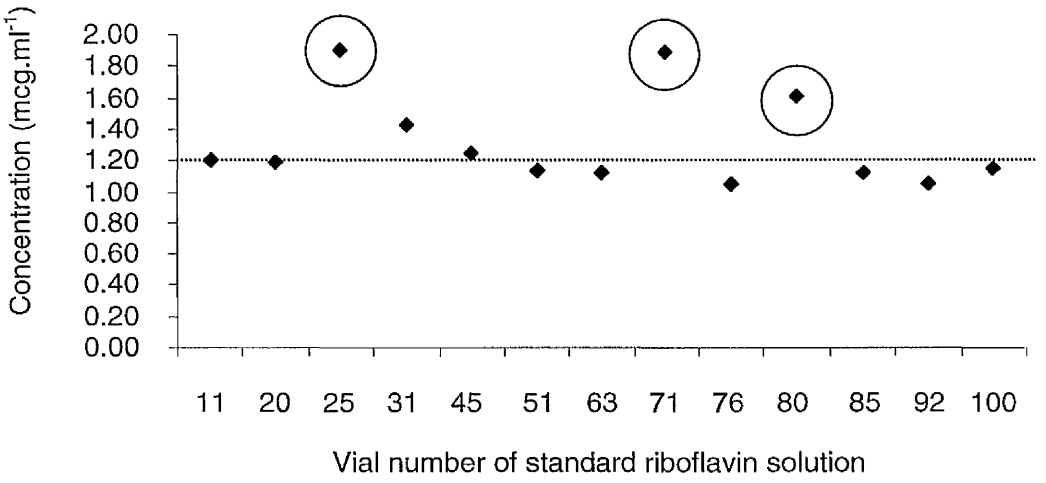


Figure 5.7 Concentration of standard samples of riboflavin in glass distilled water included in the HPLC analysis.

Figure 5.7 shows the samples and respective concentrations of the standard solutions of riboflavin in glass distilled water that were included throughout the HPLC analysis of the volunteers urine samples. The samples of riboflavin in glass distilled water were prepared at a concentration of 1.2mcg.ml^{-1}. Following the construction of a calibration curve, actual concentrations of the analysed riboflavin solutions were calculated. The concentration of all but the three circled samples in Figure 5.7 is 1.2mcg.ml^{-1} ± 0.2mcg.ml^{-1}. The three samples occurring outside the range, circled in Figure 5.7, result in concentrations higher than the intended concentration of 1.2mcg.ml^{-1}. However, this is not significant since the three exceptional values do not form part of a trend of the riboflavin concentrations away from the required concentration of 1.2mcg.ml^{-1} and subsequent values are close to the required concentrations. The differences may be attributed to the build up of impurities on the HPLC column that are consequently cleared by the riboflavin in glass distilled water, a solution that is relatively free of impurities.

The accuracy of the HPLC method is displayed graphically in Figure 5.8. The curve has been produced after analysing samples of known concentrations of riboflavin in glass distilled water and repeating the analysis twice.

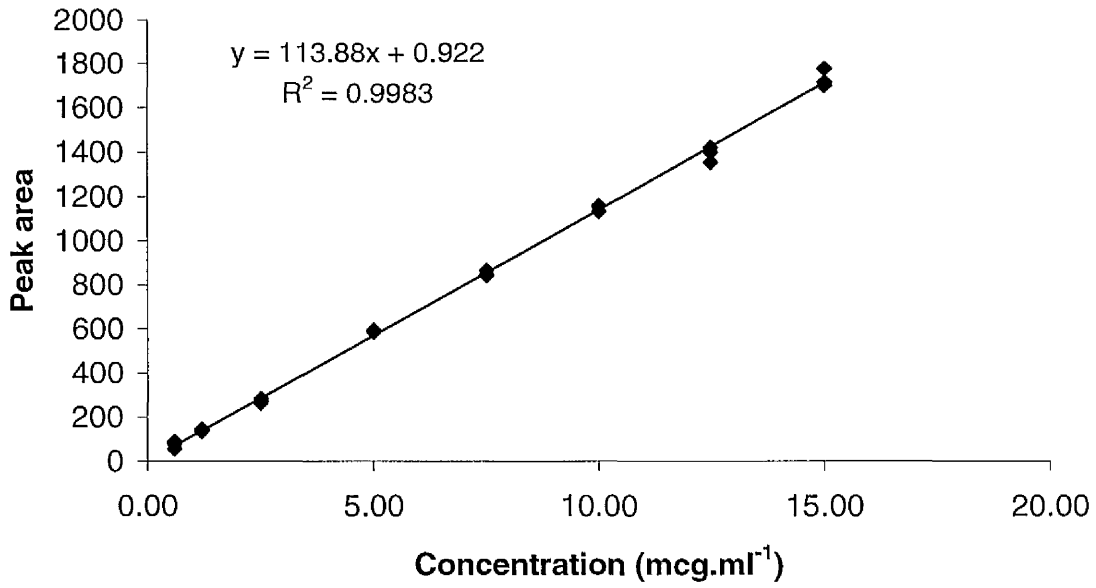


Figure 5.8 Calibration curve of known concentrations of riboflavin in glass distilled water.

With regard to determining the amount of riboflavin in the urine samples, calculations were performed as follows. The weight of the riboflavin loaded calcium alginate beads administered to each volunteer on each occasion was noted. As a result of the drug loading experiments performed, (Chapter 4, section 4.2.4), the actual amount of riboflavin contained within the sample was calculated. The total volumes of urine voided by each volunteer on each study day were recorded. The results of the calibration curve, (Appendix VII), were then used to calculate the amount of riboflavin in the urine samples and hence the total amount of riboflavin absorbed.

Figures 5.9 to 5.11 show the chromatograms of riboflavin in glass distilled water, in urine with an added amount of riboflavin to give a known concentration of riboflavin and a representative sample of urine obtained from a volunteer for Study 2.

Figure 5.9 shows a typical HPLC chromatogram of riboflavin dissolved in glass distilled lab water. The peak is sharp, clearly identifiable and separate to any other peaks.

Figure 5.10 shows a urine sample with a known amount of riboflavin solution added, that resulted in a final theoretical concentration of 33.3 $mcg.ml^{-1}$ w/v riboflavin. The retention time of the riboflavin peak is shown at 17.940 minutes. The peak can clearly be defined as riboflavin as both the retention time and peak area correspond well to that obtained for the standard riboflavin solution shown in Figure 5.9. In practice, riboflavin concentrations from the urine samples of the order of 33.3 $mcg.ml^{-1}$ were not anticipated. Consequently the development of a method that allowed for the isolation of the riboflavin peak was important. Figure 5.10 also demonstrates the importance of the run time of 20 minutes per sample. Prior to obtaining a riboflavin peak at 17 – 18 minutes, peaks of other compounds within the urine sample have been isolated. Such peaks would have caused the possible masking of the required riboflavin peak or made its identification impossible.

With reference to Figure 5.11, the riboflavin peak can be identified as occurring at a retention time of 16.718 minutes. Such identification is possible as when reviewing all the standards that were run initially before all the urine samples, all the riboflavin peaks were observed to have retention times in the range 16.72 – 17.27 minutes.

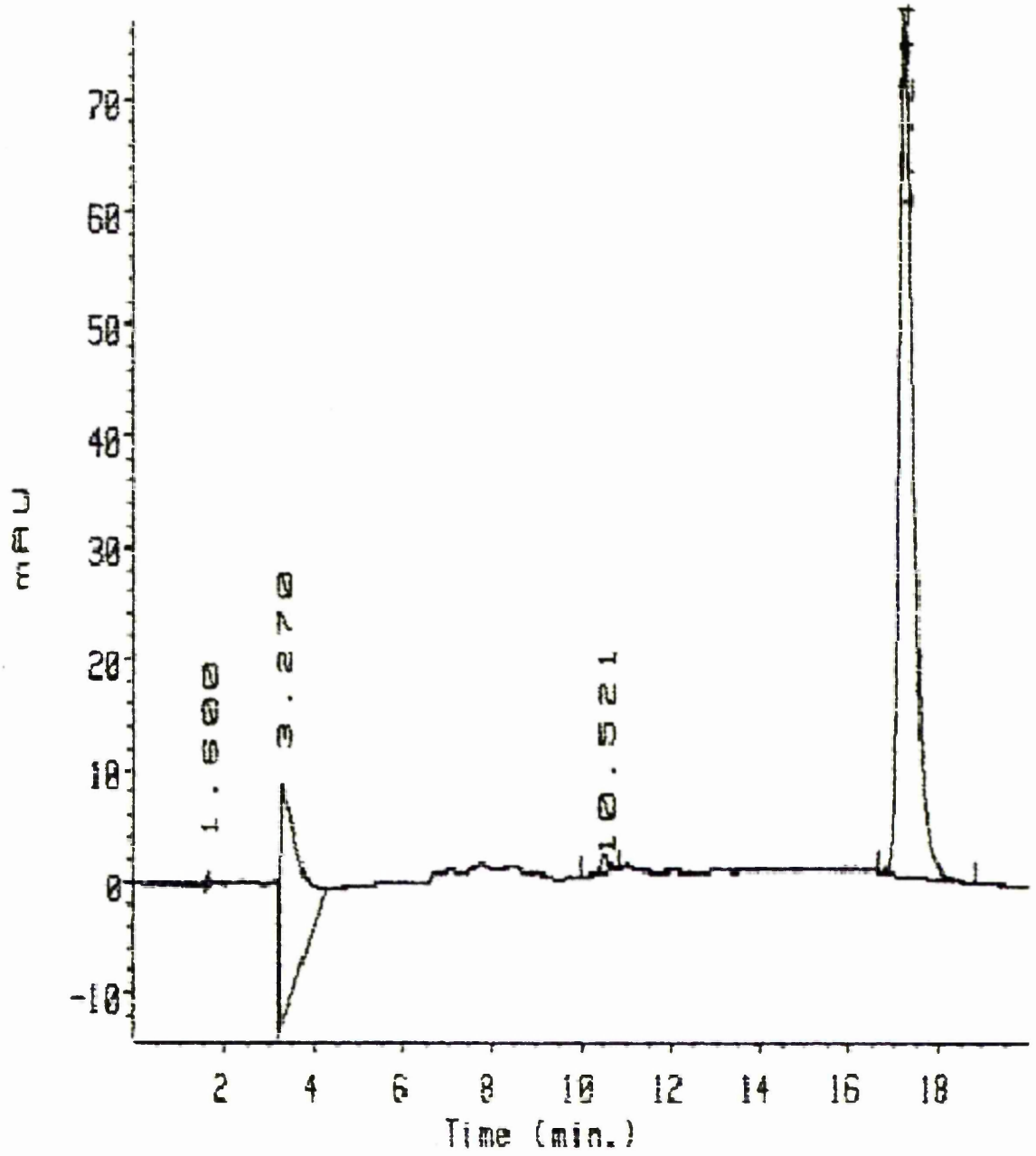


Figure 5.9 Representative HPLC chromatogram of riboflavin in glass distilled water.

(retention time = 17.244 minutes, peak area 1775, concentration 15mcg.ml^{-1}, linearity result from 3 analysis 15.17mcg.ml^{-1})

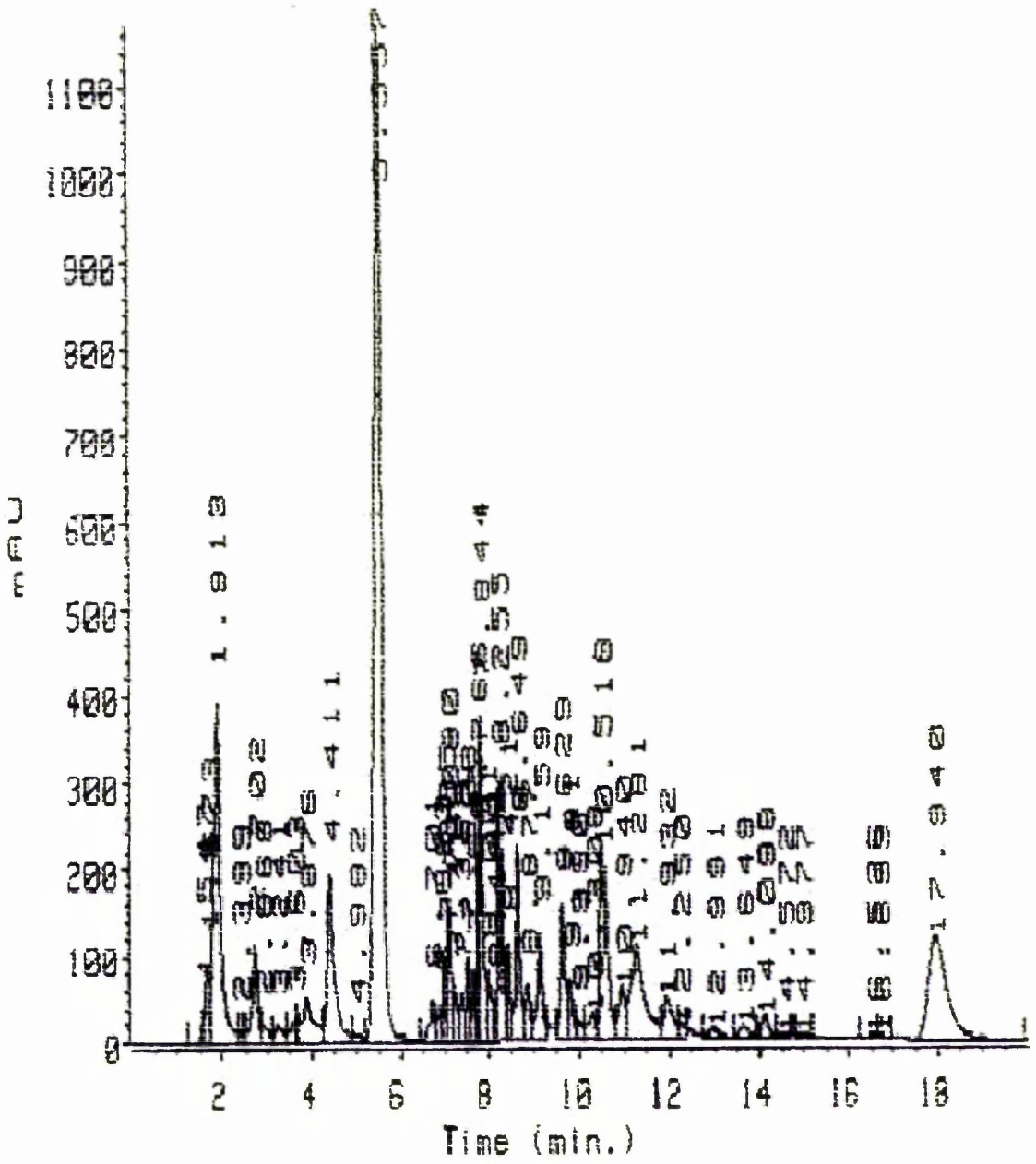


Figure 5.10 HPLC chromatogram of a urine sample with a known amount of riboflavin added.

(retention time of riboflavin peak 17.940 minutes, peak area 3540, theoretical concentration 33.3 mcg/ml, calculated concentration 31.1mcg/ml)

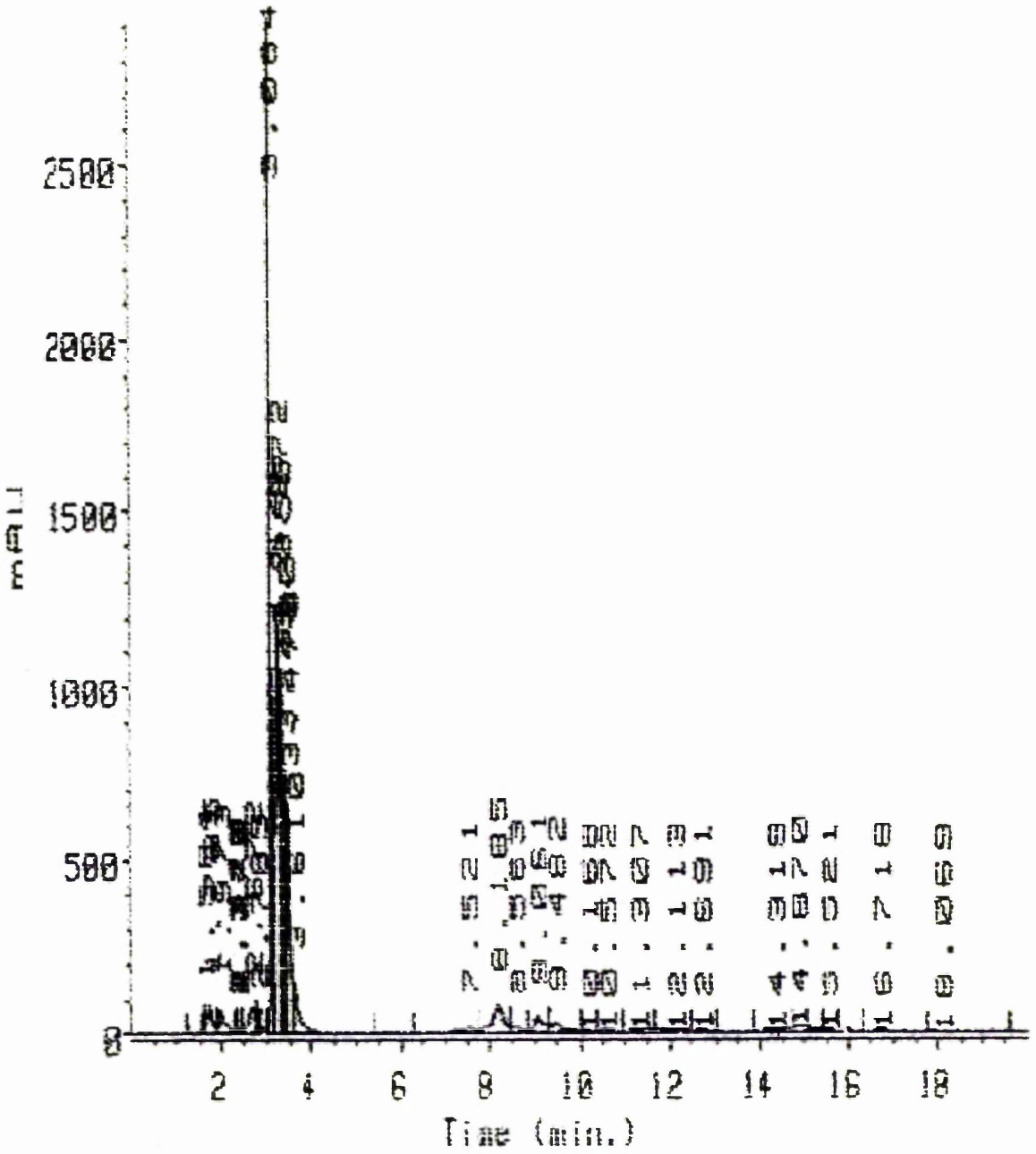


Figure 5.11 Representative HPLC chromatogram of riboflavin in urine.
(retention time 16.718, peak area 472.13,
calculated concentration 2.71mcg.ml^{-1})

5.4.2.2 COMPARISON OF THE AMOUNT OF RIBOFLAVIN ABSORBED IN THE FED STATE WITH THE AMOUNT OF RIBOFLAVIN ABSORBED IN THE FASTED STATE

Figure 5.12 shows the amount of riboflavin absorbed during the study when the calcium alginate beads were administered under the varying conditions of food intake.

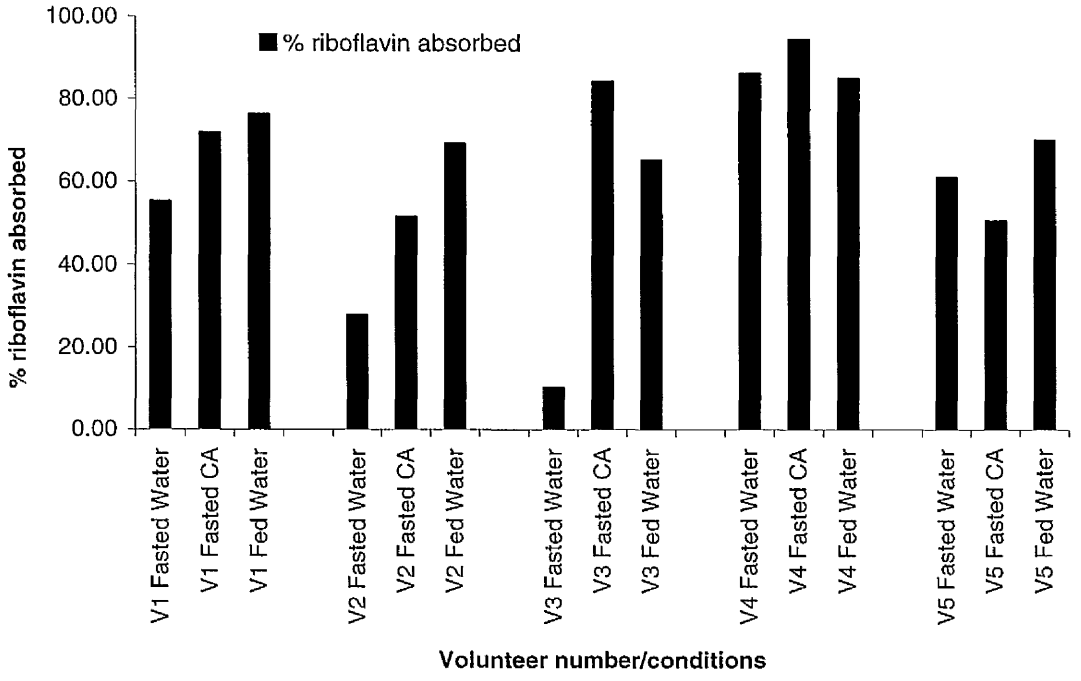


Figure 5.12 Percentage of riboflavin absorbed under different conditions of food intake.

With regards to the administration of oral dosage forms, as discussed, (Chapter 1, section 1.1.6.1), the presence or absence of food in the stomach is a major factor in determining the amount of drug absorbed and also the rate at which it is absorbed. Therefore, the bioavailability of riboflavin was expected to be maximal when the calcium alginate beads were administered under fed conditions. The results shown in Figure 5.12 show this to be true.

As shown, Figure 5.6, administration of the calcium alginate beads under fasted conditions with citric acid 1% w/v solution compared with water increases the retention time of the calcium alginate beads by a maximum of 58%. The increased residence time of the calcium alginate beads in the stomach allows

for an extended amount of time for riboflavin to be released from the calcium alginate beads in the stomach. Therefore increased absorption of riboflavin in the small intestine occurs. Figure 5.12 shows that administering calcium alginate beads under fasting conditions with a citric acid solution 1% w/v compared with water results in an enhancement in the bioavailability of riboflavin by a maximum of 88%, giving an overall bioavailability of riboflavin near to or better than that obtained in the fed state.

When considering the gastric emptying and bioavailability results overall, the figures correspond well. However, in the fasted state the comparatively short gastro-retention times that have been obtained, coupled with maximum riboflavin bioavailability indicate that the riboflavin is released from the beads immediately on contact with aqueous media. The incorporation of a polymer within the formulation may enable the riboflavin release to be retarded but such modification was beyond the scope of the current work.

When the calcium alginate beads were administered in the fasted state to Volunteer 4, a further explanation of the results is warranted. In direct contrast to the gastric emptying times of the rest of the volunteers, the gastric emptying time increases by 71.4% when the calcium alginate beads were administered with citric acid 1% w/v solution compared with water. Furthermore an increase of 10.05% in riboflavin bioavailability has been recorded for Volunteer 4 when the calcium alginate beads were administered with citric acid solution compared with water. A similar pattern was noted when reviewing the behaviour of the calcium alginate beads administered in the fasted state for Volunteer 5. When the gastric emptying times of calcium alginate beads administered with citric acid solution 1% w/v are compared with the administration of the calcium alginate beads with water, an increase of 24% in gastric residence time is noted. However, the corresponding bioavailability decreases by 17.3%. The reason for the differences in riboflavin bioavailability can be attributed to the immediate release of riboflavin from the calcium alginate beads, as stated previously.

With specific regard to Volunteer 4, when administered under fasting conditions, with the citric acid 1% w/v solution compared with water, the calcium alginate beads emptied faster from the stomach. The reverse is true for all other volunteers within the study; calcium alginate beads emptied faster when administered under fasting conditions with water compared to citric acid solution 1% w/v. The difference in the gastric emptying profile of Volunteer 4 compared to the other volunteers can be credited to inter-patient variation. More specifically it is possible that the MMC was displaying Phase III activity for Volunteer 4, whereas for the remaining volunteers, Phase III may not have been commenced.

The amounts of riboflavin absorbed by the volunteers, Figure 5.12, should also be considered with the patterns of drug release from the *in vitro* studies, (Chapter 4, section 4.2.5). When the calcium alginate beads were administered under fasting conditions, the pH of the stomach contents was approximately pH 1.2. The conditions are therefore reflective of the *in vitro* studies completed using 0.1M HCl, pH 1.2. The *in vitro* studies showed that riboflavin release from the calcium alginate beads was slow and occurred over approximately 350 minutes. Overall, the least amount of riboflavin absorbed by the volunteers occurred when the calcium alginate beads were administered in the fasted state with water. Calcium alginate is insoluble at acidic pH and therefore release of the riboflavin from the dosage form is expected to be slow. Therefore, a longer period of time would be required for all of the riboflavin to be released from the calcium alginate bead and pass into the small intestine where the riboflavin will be absorbed. Alternatively, when considering the *in vivo* studies overall, and the gastric emptying times, Figure 5.6, it is possible that the action of the migrating motor complex has emptied the dosage form from the stomach prior to the complete release of the riboflavin.

Calcium alginate is soluble in media of pH above pH 3.0. The *in vitro* studies showed a faster release of the riboflavin from the calcium alginate beads when the selected media had a pH value of pH 3.0 or higher. With regard to the *in vivo* studies, when calcium alginate beds are administered in the fed state, the pH of the stomach contents increases to approximate values of pH 5.0 to pH

7.0. Therefore, the riboflavin release rate from the calcium alginate beads should also increase. Practically, Figure 5.12, an increase in the amount of riboflavin absorbed by the volunteers was noted. The *in vitro* studies have therefore provided an accurate prediction of the release of riboflavin from calcium alginate beads when administered to human volunteers.

It is well documented that the presence of food in the stomach delays the gastric emptying of a dosage form. A longer period of time therefore exists for the drug to be released from the dosage form and to be absorbed by the body. In the current study, whilst the calcium alginate beads were subject to gastric retention, as shown by the gamma scintigraphy results, it is also possible that the riboflavin was released from the calcium alginate beads quickly and absorbed as it passed into the small intestine. Calcium alginate beads remaining in the stomach would therefore contain no riboflavin. The theory is supported by the confocal laser scanning microscopy-FRAP results that showed a rapid leaching of riboflavin from the calcium alginate beads when the calcium alginate beads were placed in media of pH 3.0.

Overall, *in vitro* studies of calcium alginate beads containing riboflavin and citric acid have showed an increase in the amount of riboflavin released when compared to calcium alginate beads containing riboflavin and no citric acid. Similarly the *in vivo* studies have shown that when calcium alginate beads are administered with a citric acid solution compared with water, there is an increase in the amount of riboflavin absorbed by the body.

5.5 CONCLUSION

Floating radio-labelled calcium alginate beads have been produced and used in two *in vivo* studies. Formula modifications allowed for the inclusion of riboflavin as a model drug for the gamma scintigraphy and bioavailability study.

For both of the current studies, when the calcium alginate beads were administered under fasting conditions, gastric retention times in excess of 1

hour were recorded. When calcium alginate beads were administered under fed conditions, gastric retention times were in excess 3.5 hours. The figures compare well with a similar study using microballoons containing riboflavin. The study using the microballoons also showed that the excretion half life for the drug was longer using when floating microballoons were administered, compared with non-floating forms. Therefore a longer gastro-retention time for the floating microballoons was observed, albeit irrespective of whether the microballoons were administered under fed or fasting conditions[149]. However, the structure of the microballoon is such that it contains one spherical cavity of air. In comparison, the calcium alginate beads have multiple cavities. Should the outer surface of the microballoon be compromised then buoyancy will be lost and it will pass into the small intestine, behaving as a normal single unit dosage form. Conversely, if the outer surface of the calcium alginate bead becomes damaged many more cavities are still available to maintain flotation. Hence, the calcium alginate beads are not emptied in an 'all or nothing' nature.

The *in vivo* studies investigated the potential use of citric acid as an excipient to delay gastric emptying. Although citric acid has not been used previously to delay the gastric emptying of a dosage form or to improve the bioavailability of a drug, the use of citric acid to delay gastric emptying for the purposes of the diagnosis of *H. Pylori* is well documented. The *in vivo* studies completed for the current project demonstrate that administering floating calcium alginate beads in the fasted state with citric acid prolongs gastro-retention and improves bioavailability. However, the gastric emptying of the dosage form when administered in the fasted state has not been prolonged when compared to gastric emptying figures for the fed state. Therefore, when using floating calcium alginate beads of the formulation described to deliver drugs, maintaining the fed state is a primary requirement to prolong the gastro-retention of the dosage form.

CHAPTER 6 – GENERAL CONCLUSIONS AND FURTHER WORK

CHAPTER 6 – GENERAL CONCLUSIONS AND FURTHER WORK

A need for a gastro-retentive dosage form has been identified. Advantages of such a dosage form include an improvement in drug bioavailability compared with that of many commercially available immediate release preparations, and, the improved treatment of local conditions, such as *H.pylori*. The basic requirements of a gastro-retentive dosage form are that it should release the drug in such a way that constant plasma concentrations are maintained, and, whilst allowing for once daily oral administration, the dosage form should not be retained by the body at the end of the dosing period. Ideally, the dosage form should be a multiple unit system, since the 'all or nothing' gastric emptying that applies to many single unit systems would be avoided.

The current work has investigated the potential of multi-particulate freeze-dried calcium alginate beads as a suitable floating gastro-retentive dosage form. Alginates are versatile polysaccharides and are therefore ideal to consider using in the manufacture of a gastro-retentive dosage form. Alginates are also biodegradable and will not therefore remain in the body for periods of time in excess of that required. Calcium alginate beads have been developed and the formula was modified to allow for the inclusion of a model drug or other excipients. Riboflavin was selected as the model drug as it has a narrow absorption window in the small intestine. The gastro-retention times of the dosage form was assessed by gamma scintigraphy studies and a bioavailability study.

The physical properties of the calcium alginate beads have been investigated. Using electron microscopy, the internal morphology showed the numerous cavities that enable flotation. The buoyancy properties have been assessed and resultant weight measurements showed that the calcium alginate beads are able maintain their buoyancy in acidic media for periods in excess of 12 hours.

Initial investigations into the rate of drug release from the calcium alginate beads were performed. Results form confocal laser scanning microscopy-FRAP suggested that the diffusion coefficient of riboflavin through a sodium alginate solution was similar to the diffusion coefficient of riboflavin through a calcium alginate matrix when the matrix was placed in media of pH 3.0. Therefore a rapid release of riboflavin from the calcium alginate bead into the media in which it is placed occurs. Further diffusion measurements in other media and hence the rates of drug release have not been determined to date. The rapid release of riboflavin from the calcium alginate matrix is as a result of the properties of alginates. Alginates are pH dependent and in acidic media, alginate on the outer surface of the calcium alginate bead is converted to alginic acid. Since alginic acid is insoluble in aqueous media, the rate of drug release will be slow. When placed in media of pH 3.0 and above, alginates are soluble and swell. The rapid release of drug from the matrix therefore occurs. When considering a dosage form designed for once daily administration, it may be necessary to alter the rate of drug release from the dosage form. Magnesium stearate has been incorporated into the calcium alginate formula for the current work. The hydrophobic property of magnesium stearate may retard the ingress of media into the calcium alginate bead, thereby retarding the rate of drug release. The inclusion of other polymers within the calcium alginate bead formula, such as the pH independent polymer, hydroxypropylmethylcellulose, (HPMC), may also alter the rate of drug release from the dosage form. Further modifications of the calcium alginate beads formula to include such polymers should be undertaken. In addition, the inclusion of a model drug and subsequent *in vitro* experiments will determine if the drug release rate has been successfully retarded.

In vitro studies of calcium alginate beads in different media were completed in order to determine the drug release kinetics. For a once daily orally administered dosage form, zero order kinetics are usually required in order to produce a constant plasma concentration profile and reduce the possibility of the occurrence of side effects. However, when considering a multi-particulate dosage form such as calcium alginate beads, the release rate of the drug may not display strict zero order kinetics. Results from the digital photography

showed that when placed in media of pH 3.0, a gel barrier is formed when the calcium alginate beads is in contact with the media. The surface area is subject to change due to erosion with increasing time. The amount and rate of drug release will occur as a combination of erosion of the surface of the calcium alginate bead and diffusion through the gel barrier. The *in vitro* results showed that the drug was released slowly from calcium alginate beads placed in acidic media, reflective of the stomach environment in the fasted state. When the calcium alginate beads were placed in media of pH 5.0 to pH 7.0, reflective of the stomach environment in the fed state or of the small intestine, the drug release rate increased when compared to the drug release into media of pH1.2. Zero order kinetics were not observed, and the absence of information regarding the movement of drug into the media did not allow for further determination of the kinetic profile. As stated, the inclusion of a pH independent polymer may result in drug release profiles that demonstrate near zero order kinetics. Central to achieving such a profile is a thorough understanding of the gel barrier. The formation, thickness and subsequent erosion will all affect the drug release from the calcium alginate bead. Therefore, further investigations to determine the properties of the gel barrier are required. Such knowledge will also allow for the calcium alginate bead formula to be modified in order that zero order kinetics can be achieved.

The calcium alginate beads produced were used in two *in vivo* studies. For the first study, placebo calcium alginate beads administered under fasting conditions with either water or a citric acid solution. The gastric emptying times of the calcium alginate beads were then recorded. The second study was a gamma scintigraphy and bioavailability study. Calcium alginate beads containing riboflavin were administered under fed and fasting conditions. The effect of citric acid on gastric emptying was also investigated when the calcium alginate beads were administered under fasting conditions. Both studies showed that prolonged gastro-retention times of the calcium alginate beads were achieved when the calcium alginate beads were administered under fasting conditions with citric acid solution compared with water. With regard to the amount riboflavin absorbed, when administered under fasting conditions with citric acid solution, the amount of riboflavin absorbed was near to that

achieved when the calcium alginate beads were administered under fed conditions. Therefore, the studies demonstrated that citric acid has the potential to delay gastric emptying, which in turn results in increased absorption of the drug. However, the gastro-retention times achieved for calcium alginate beads administered under fasting conditions were not equivalent to the gastro-retention times for calcium alginate beads administered under fed conditions.

The incorporation of a polymer may also retard gastric emptying times. HPMC has already been suggested as a polymer with which to alter the drug release profile of the drug. Modifying the calcium alginate bead formula to include HPMC and administering the resulting calcium alginate beads with citric acid solution may delay the gastric emptying time of the calcium alginate beads to a greater extent than that recorded for the current work; the improved gastro-retention times occurring as a result of the swelling/hydration properties of HPMC and the property of citric acid to delay gastric emptying.

APPENDICES

APPENDIX I – BUFFER AND ACID PREPARATION

Acetate Buffer 0.1M, pH 5.0

SOLUTION A - ACETIC ACID 0.2M

Add 11.55ml of glacial acetic acid to 700ml of singly distilled water. Make up to 1000ml with singly distilled water.

SOLUTION B - SODIUM ACETATE 0.2M

Dissolve 27.2g of sodium acetate trihydrate in 700ml of singly distilled water and make up to 1000ml.

ACETATE BUFFER 0.1M

Mix 14.8ml of acetic acid 0.2M with 35.2ml of sodium acetate 0.2M. Adjust to pH 5.0 with glacial acetic acid or sodium acetate and make up to 100ml with singly distilled water.

Calcium chloride 0.02M solution

Dissolve 2.217g of calcium chloride, ($CaCl_2$), in singly distilled water and make up to 1000ml.

Hydrochloric Acid 0.1M, pH 1.2

Add 50ml of concentrated hydrochloric acid (HCl), specific gravity 1.16g.cm^{-3}, purity 31-33% to 4500ml of singly distilled water. Adjust to pH 1.2 if necessary with HCl of sodium hydroxide (NaOH) and make up to 5000ml.

APPENDIX I – CONTINUED

Potassium Dihydrogen Orthophosphate 50mM solution

Dissolve 6.8045g of potassium dihydrogen orthophosphate, (KH_2PO_4), in singly distilled water and make up to 1000ml.

Sørensen's Citrate Buffer, pH 3.0

Solution A – Hydrochloric Acid 0.1M

Add 50ml of concentrated hydrochloric acid (HCl), specific gravity 1.16g.cm^{-3}, purity 31-33% to 4500ml of singly distilled water. Adjust to pH1.2 if necessary with HCl or Sodium Hydroxide (NaOH) and make up to 5000ml.

Solution B – Sodium Hydroxide solution 1M

Dissolve 40g of sodium hydroxide, (NaOH), in singly distilled water and make up to 1000ml.

Solution C – Disodium Citrate solution

Dissolve 42g of citric acid, (CA), in 400ml of NaOH 1M and make up to 2000ml with singly distilled water.

Sørensen's Citrate Buffer, pH 3.0

Add 1875ml of disodium citrate to 2975ml of HCl 0.1M and make up to 5000ml with singly distilled water. Adjust to pH 3.0 if necessary with HCl or Sodium Hydroxide (NaOH) and make up to 5000ml.

APPENDIX I – CONTINUED

Sørensen's Phosphate Buffer, pH 7.4

Dissolve 8.90g of dihydrogen potassium phosphate and 47.76g of disodium phosphate dihydrate in singly distilled water and make up to 5000ml.

APPENDIX II – WEIGHT AND DIAMETER FOR CALCIUM ALGINATE BEADS OF DIFFERENT FORMULATIONS

Diameter

The diameter of the calcium alginate beads was measured using a Moore and Wright 0 – 25mm micrometer. Measurements for each sample were repeated twice.

Calcium alginate bead sample	Average diameter of sample (cm) (n = 10)	Standard deviation
Placebo	0.250	0.06
Riboflavin loaded	0.258	0.01
Placebo with magnesium stearate	0.274	0.21

Table A1.1 Mean diameter of calcium alginate beads of different formulations.

Weight

The weight of the calcium alginate beads was obtained using a Mettler AC 100 balance (Zurich, Switzerland) to weigh the calcium alginate beads. Measurements for each sample were repeated twice.

Calcium alginate bead sample	Average weight of sample (g) (n = 30)	Standard deviation
Placebo	0.0135	0.000351
Riboflavin loaded	0.0157	0.000153
Placebo with magnesium stearate	0.0186	0.000265

Table A1.2 Mean weights of calcium alginate beads of different formulations.

APPENDIX III – BEER-LAMBERT PLOTS OBTAINED FOR RIBOFLAVIN IN DIFFERENT MEDIA USING UV SPECTROSCOPY

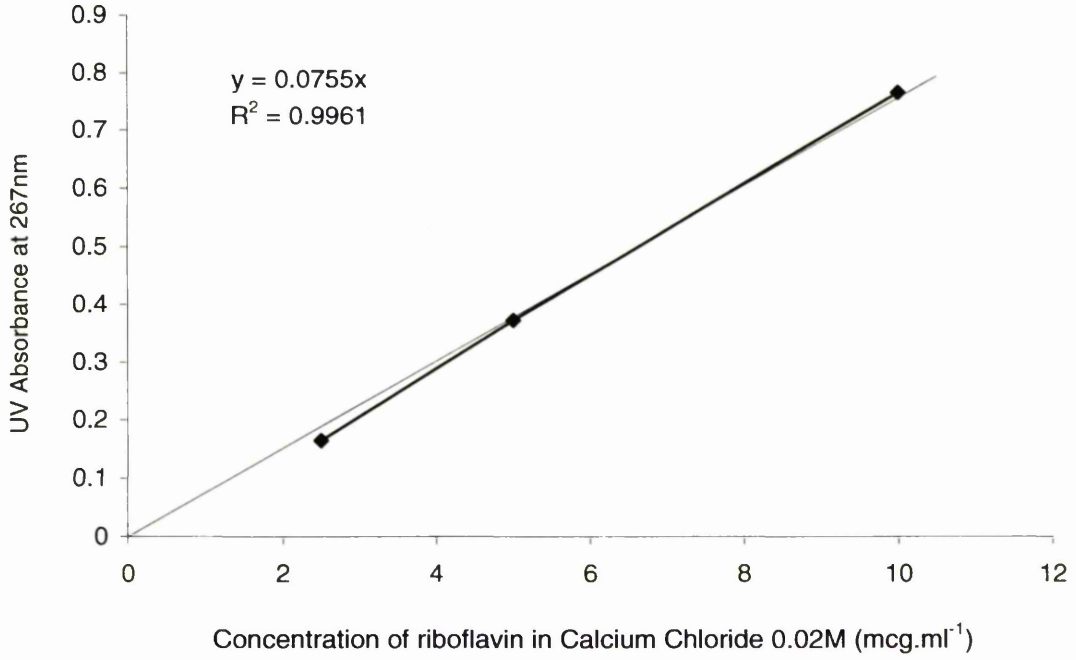


Figure AIII.I Beer-Lambert plot of riboflavin in calcium chloride 0.2M.

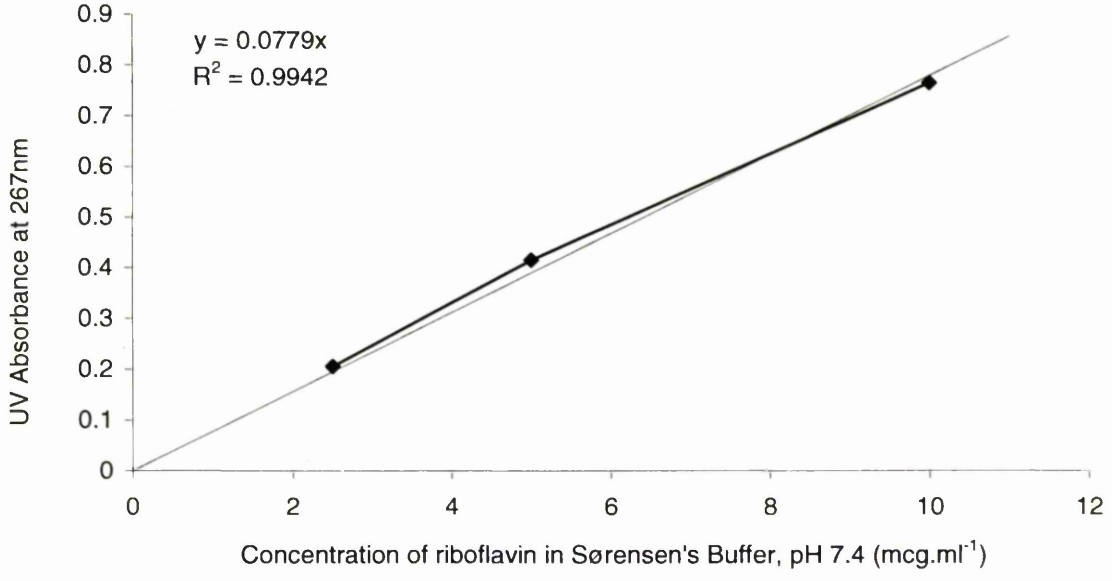


Figure AIII.II Beer-Lambert plot of riboflavin in Sørensen's Phosphate Buffer, pH 7.4.

APPENDIX III – CONTINUED

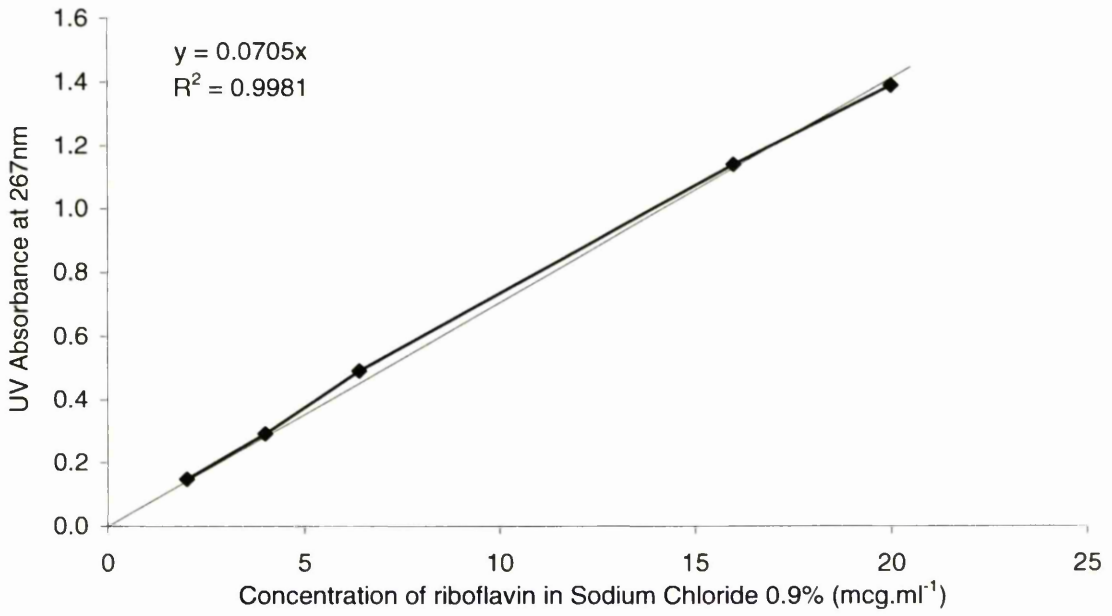


Figure AIII.III Beer-Lambert plot of riboflavin in sodium chloride 0.9%.

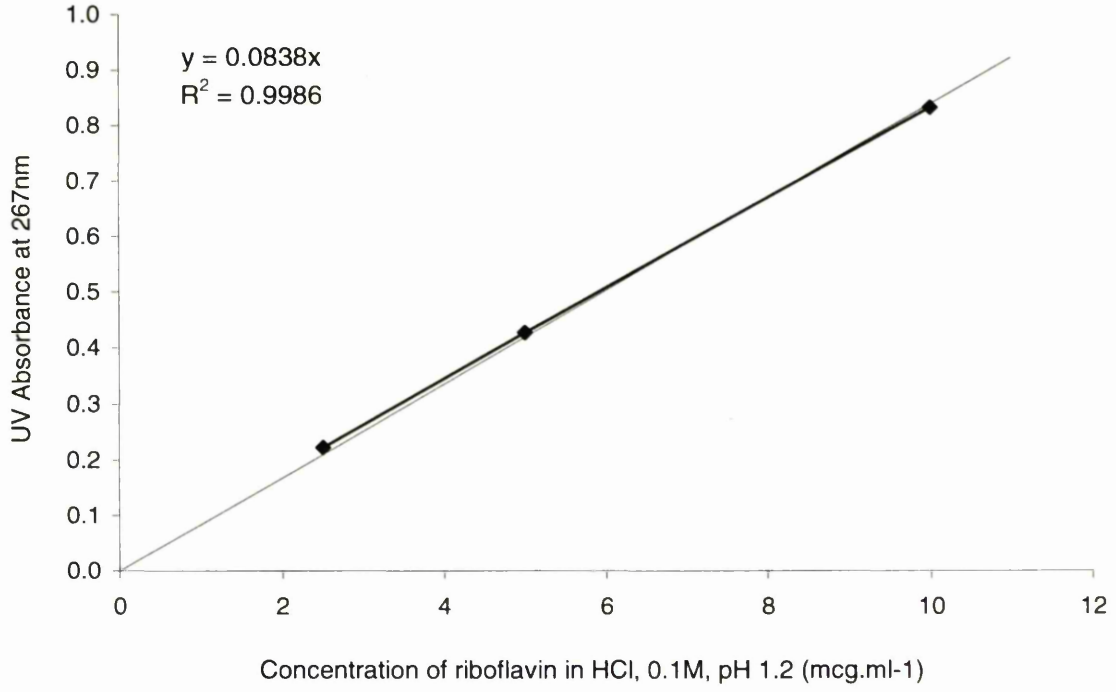


Figure AIII.IV Beer-Lambert plot of riboflavin in 0.1M HCl, pH 1.2.

APPENDIX III – CONTINUED

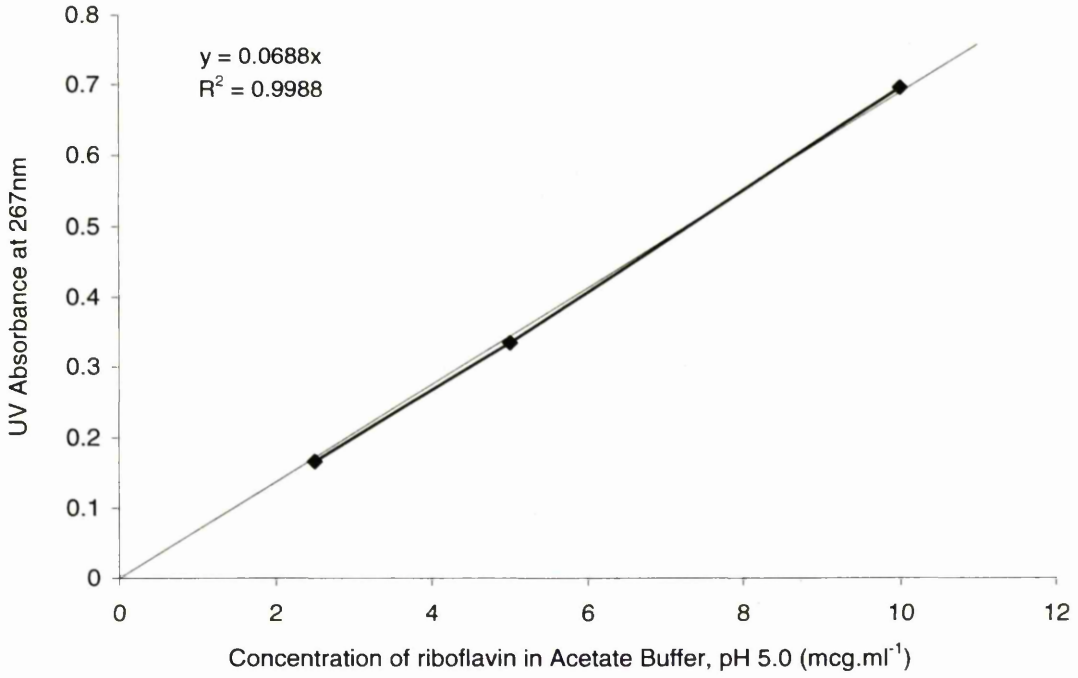


Figure AIII.V Beer Lambert plot of riboflavin in acetate buffer, pH 5.0.

APPENDIX IV – f_2 VALUES FOR *IN VITRO* DISSOLUTION PROFILES

Media	0.1M HCl pH 1.2	Sørensen's Citrate Buffer pH 3.0	Acetate Buffer pH 5.0	Glass distilled water pH 6.7	Sørensen's Phosphate Buffer pH 7.4
***In vitro* dissolution profiles**					
2a – 2b	70.9	70.5	74.5	No	58.5
2b – 2c	80.3	70.5	61.1	Dissolution	70.8
2a – 2c	75.6	73.5	58.6	Profile	55.6
3a – 3b	85.2	54.4	83.1	60.6	58.5
3b – 3c	81.6	56.8	68.4	70.4	74.9
3a – 3c	98.0	71.2	66.4	57.1	60.9
4a – 4b	79.1	No	63.6	86.3	59.7
4b – 4c	82.5	Dissolution	76.3	93.7	80.2
4a – 4c	74.3	Profile	67.0	84.1	61.2

Table AIV.I Table of f_2 values for in vitro dissolution profiles.

APPENDIX V – GASTRIC EMPTYING TIMES OF CALCIUM ALGINATE BEADS FOR *IN VIVO* STUDIES 1 AND 2

The tables show the gastric emptying times obtained from Studies 1 and 2 using the method described, (Chapter 5, section 5.2.3.6).

The following definitions apply:

Time to onset of gastric emptying – Following the administration of the calcium alginate beads with the administering vehicle, the time, (t), in minutes at which the calcium alginate beads were noted to start leaving the stomach and entering the intestine.

Time to completion of gastric emptying – Following the administration of the calcium alginate beads with the administering vehicle, the time (t), in minutes at which all the calcium alginate beads had left the stomach and moved to the intestine.

As discussed, (Chapter 5, section 5.2.5.4), for all times, the time points between each image is 10 minutes, therefore the times noted were subject to an error of ± 5 minutes.

APPENDIX V – CONTINUED

Volunteer	Conditions	Time to onset of gastric emptying (minutes)	Time to completion of gastric emptying (minutes)
1	Fasted, Water	30	55
1	Fasted, Citric acid	48	15
2	Fasted, Water	54	15
2	Fasted, Citric acid	21	15
3	Fasted, Water	20	5
3	Fasted, Citric acid	42	35
4	Fasted, Water	40	50
4	Fasted, Citric acid	57	25
5	Fasted, Water	82	5

Table AV.I Gastric emptying times for volunteers from the first in vivo study.

APPENDIX V – CONTINUED

Volunteer	Conditions	Time to onset of gastric emptying (minutes)	Time to completion of gastric emptying (minutes)
1	Fasted, Water	15	40
1	Fasted, Citric acid	20	45
1	Fed, Water	70	300
2	Fasted, Water	62	97
2	Fasted, Citric acid	104	215
2	Fed, Water	101	250
3	Fasted, Water	12.5	25
3	Fasted, Citric acid	40	55
3	Fed, Water	115	250
4	Fasted, Water	80	135
4	Fasted, Citric acid	20	35
4	Fed, Water	190	200
5	Fasted, Water	40	95
5	Fasted, Citric acid	50	125
5	Fed, Water	70	300

Table AV.II Gastric emptying times for volunteers from the second in vivo. study.

APPENDIX VI – UV SCANS OF MOBILE PHASES AND URINE SAMPLES FOR HPLC ANALYSIS

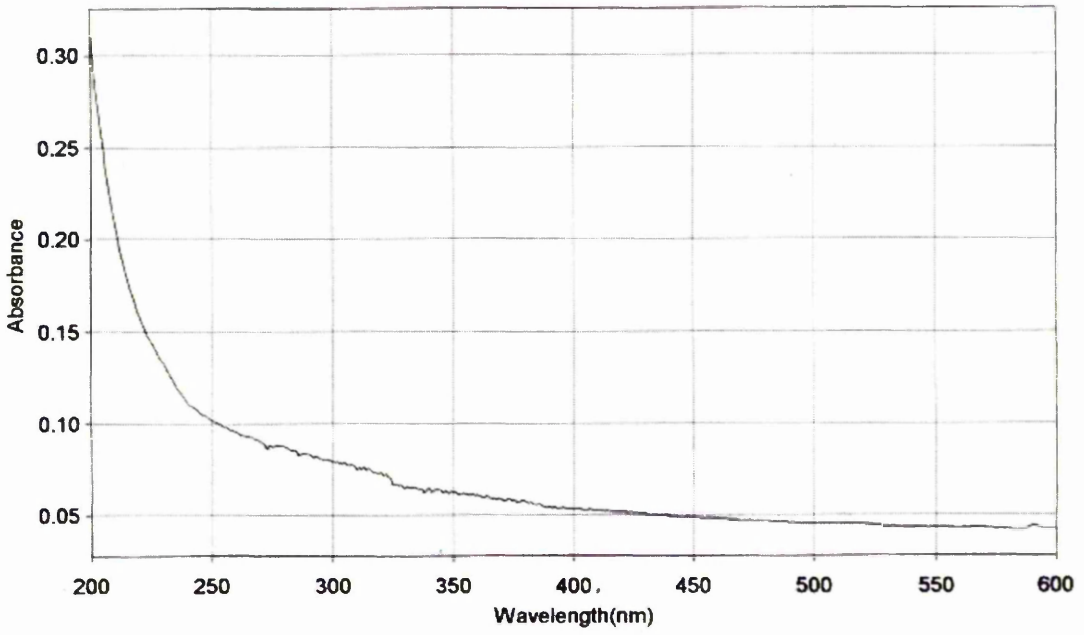


Figure AVI.I UV scan of glass distilled water.

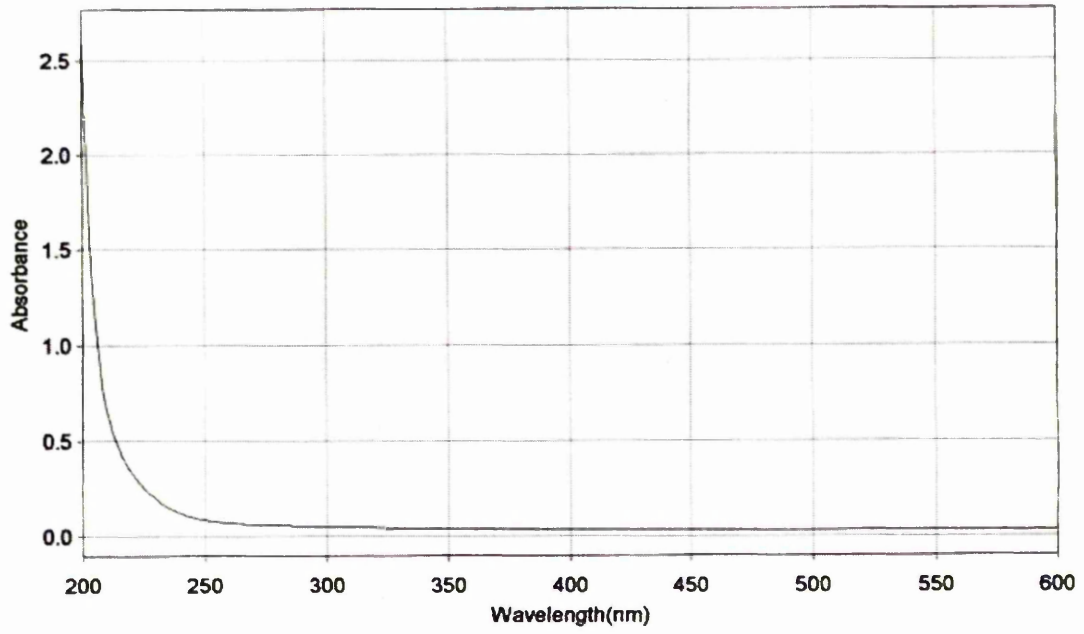


Figure AV.II UV scan of HPLC grade methanol.

APPENDIX VI – CONTINUED

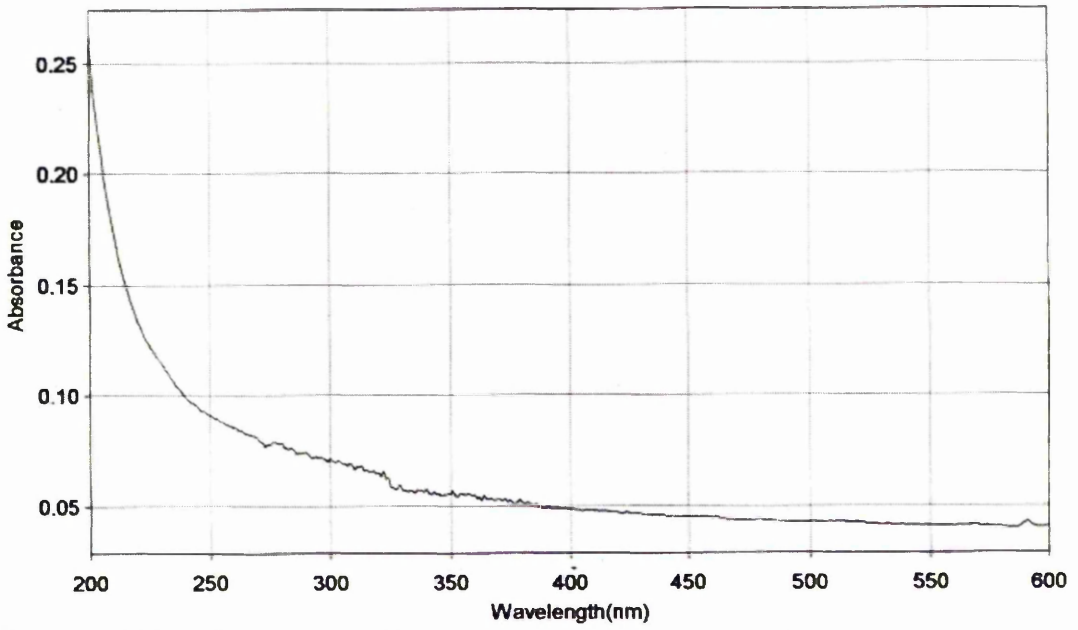


Figure AV.III UV scan of dihydrogen orthophosphate buffer 50Mm.

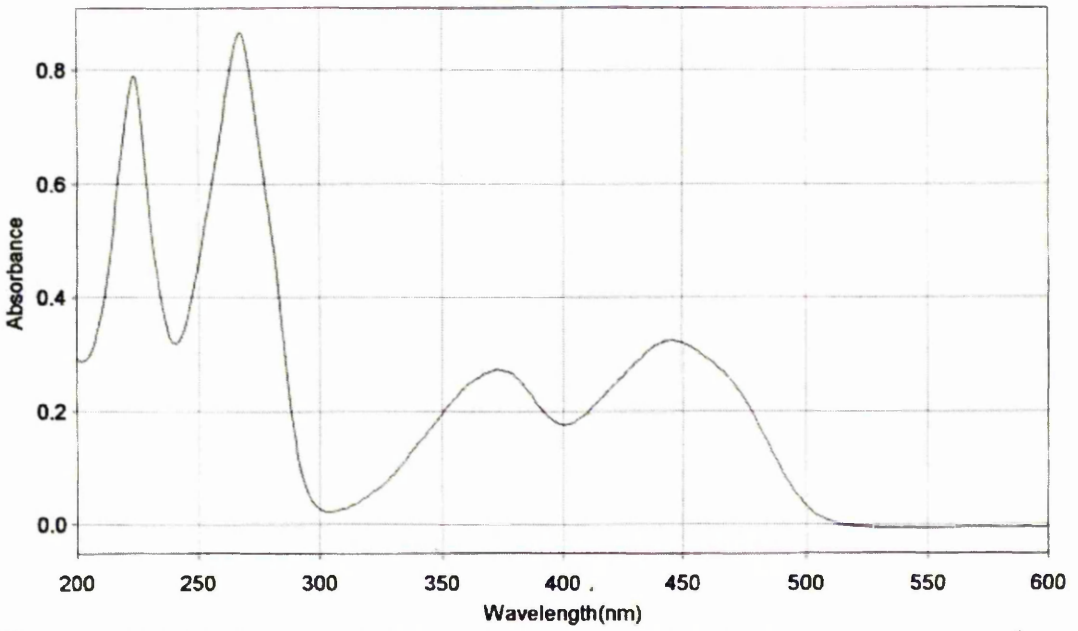


Figure AVI.IV UV scan of riboflavin in glass distilled water 10mcg.ml^{-1}.

APPENDIX VI – CONTINUED

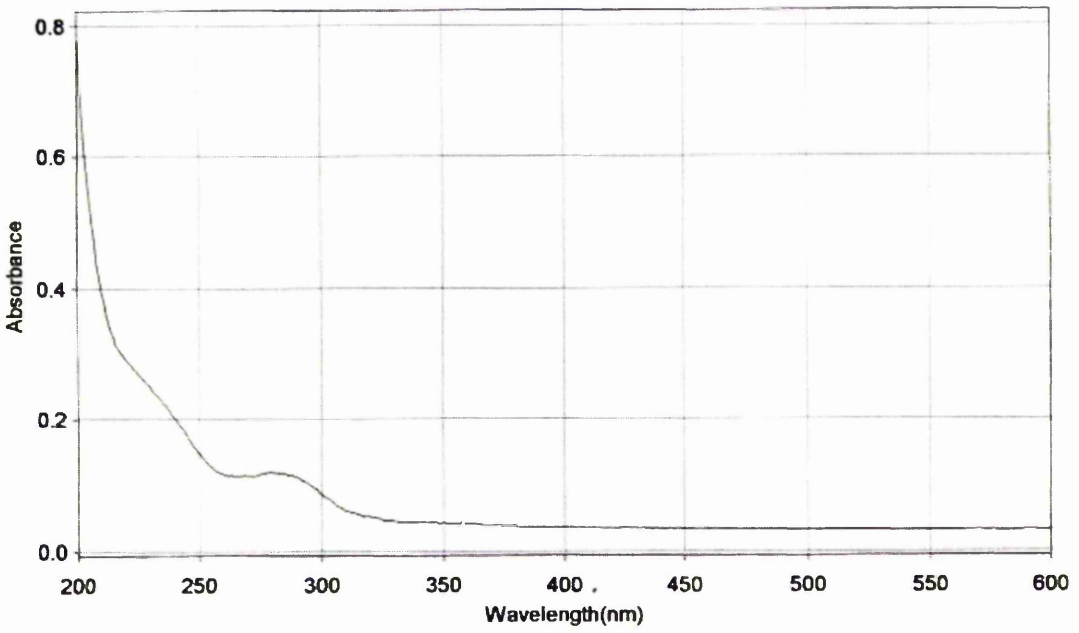


Figure AVI.V UV scan of eluent following HPLC analysis.

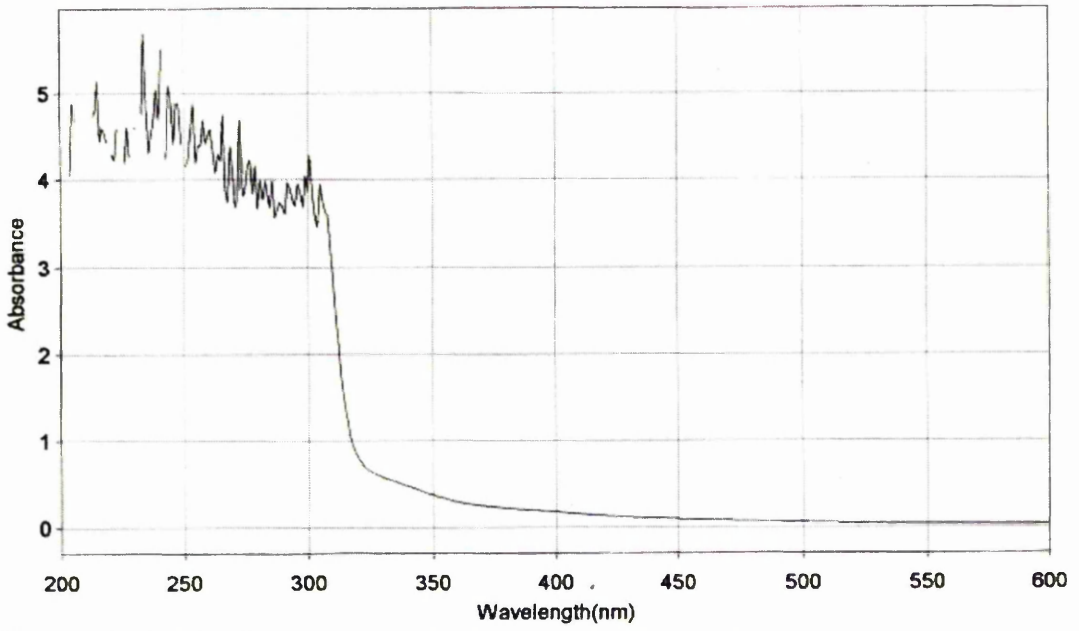


Figure AVI.VI Volunteer 1 urine sample, fasted, water.

APPENDIX VI – CONTINUED

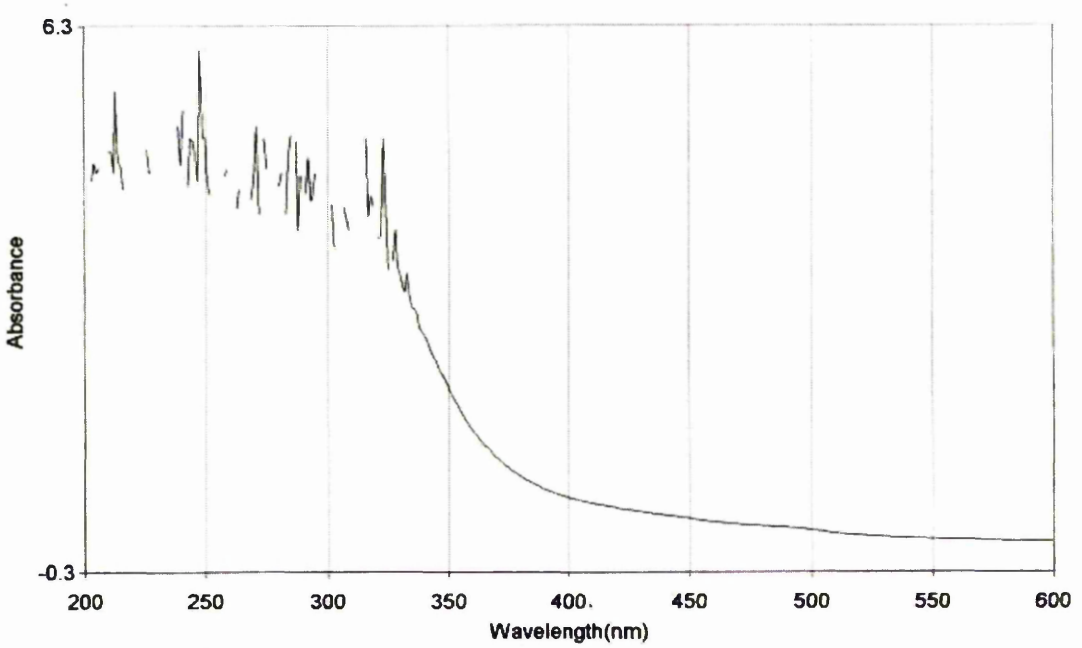


Figure AV.VII Volunteer 1 urine sample, fasted, citric acid.

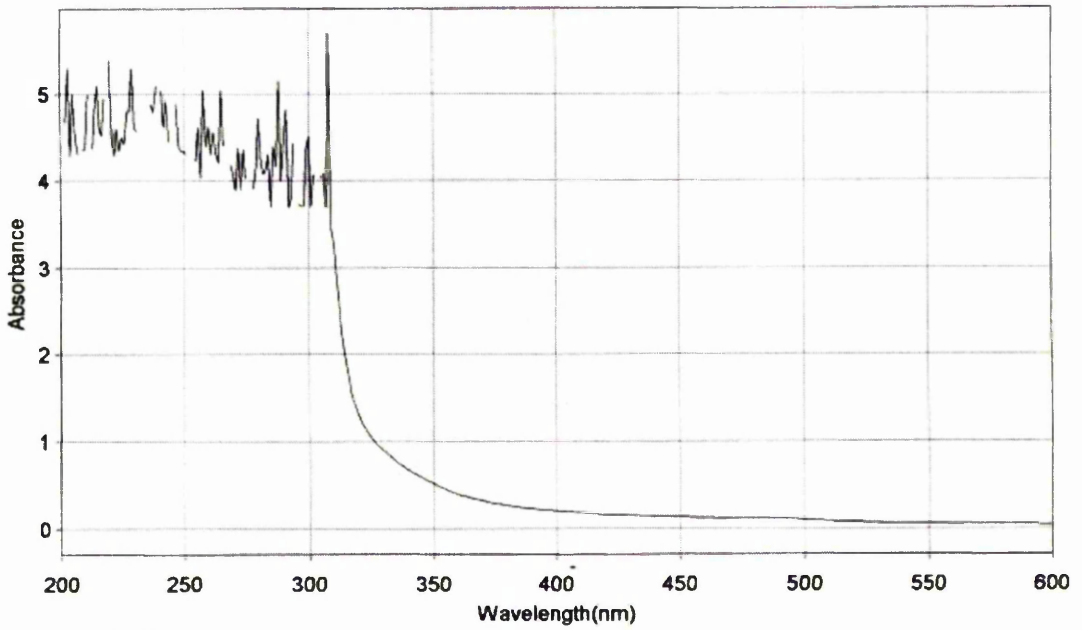


Figure AVI.VII Volunteer 1 urine sample, fed, water.

APPENDIX VI – CONTINUED

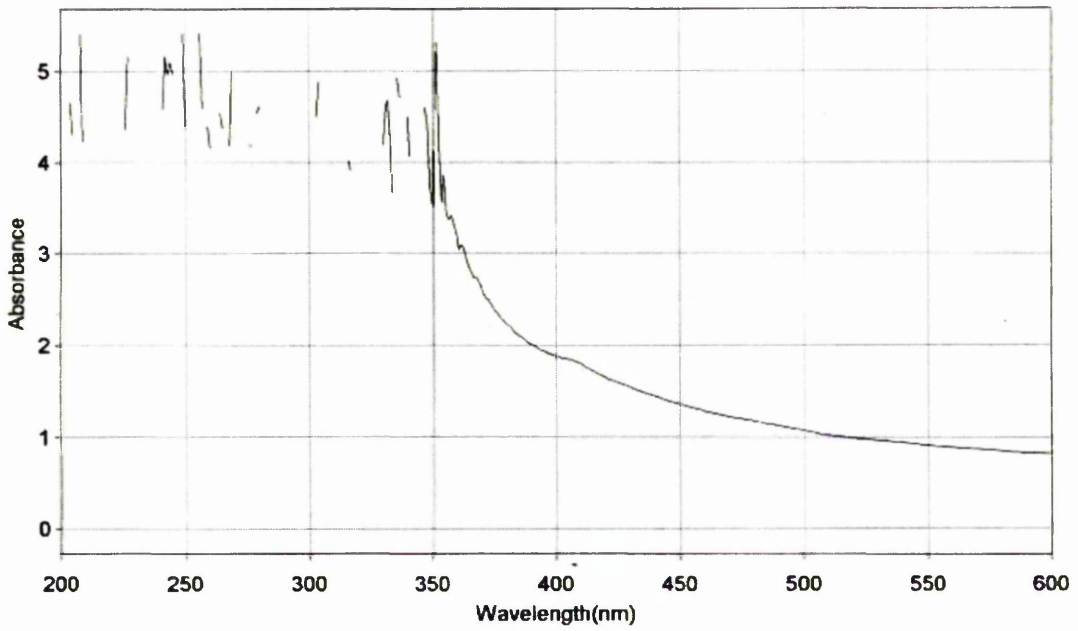


Figure AVI.VIII Volunteer 2 urine sample, fasted, water.

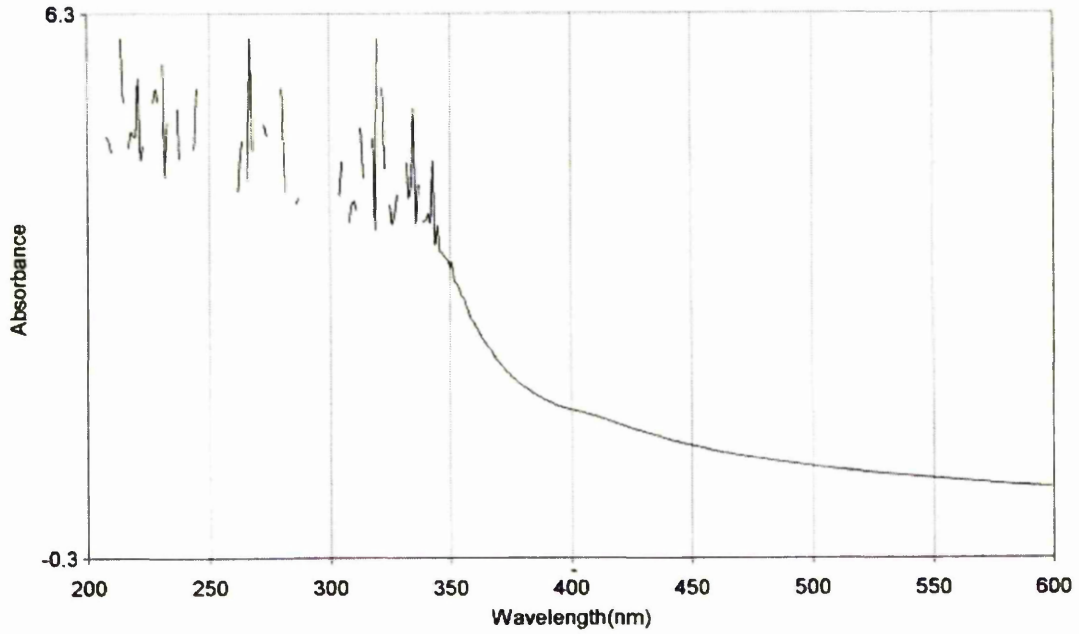


Figure AVI.IX Volunteer 2 urine sample, fasted, citric acid.

APPENDIX VI – CONTINUED

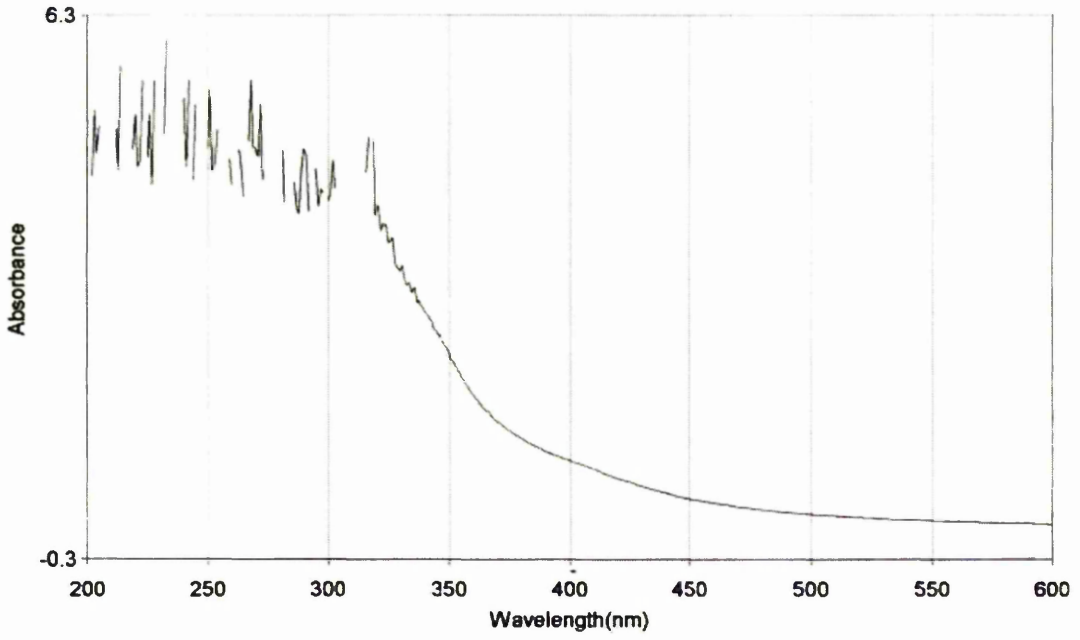


Figure AVI.X Volunteer 2 urine sample, fed, water.

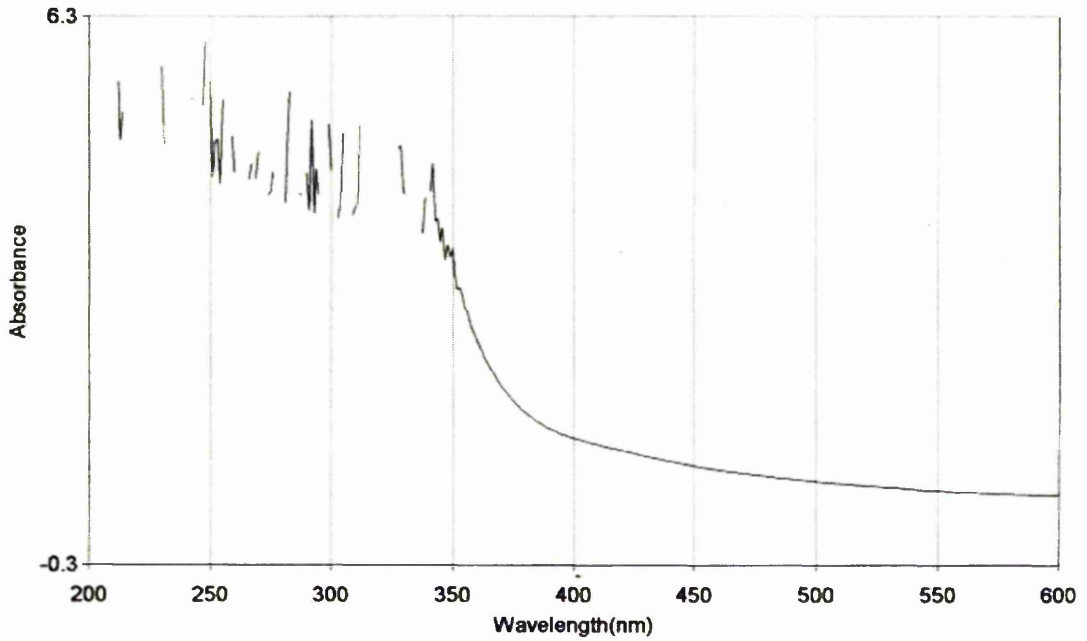


Figure AVI.XI Volunteer 3 urine sample, fed, water.

APPENDIX VI – CONTINUED

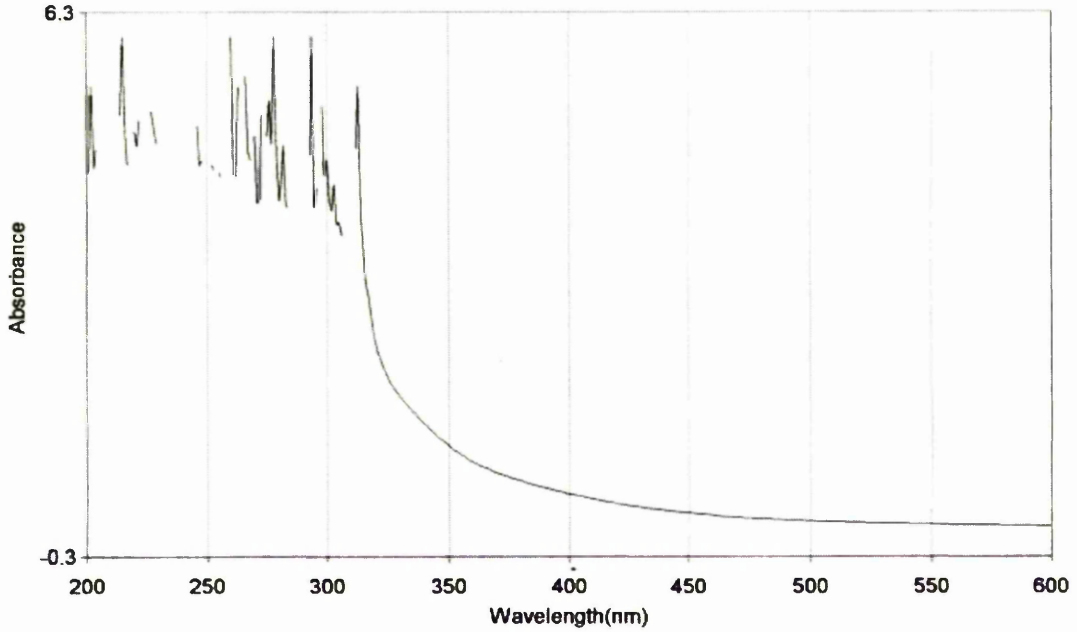


Figure AVI.XII Volunteer 4 urine sample, fasted, water.

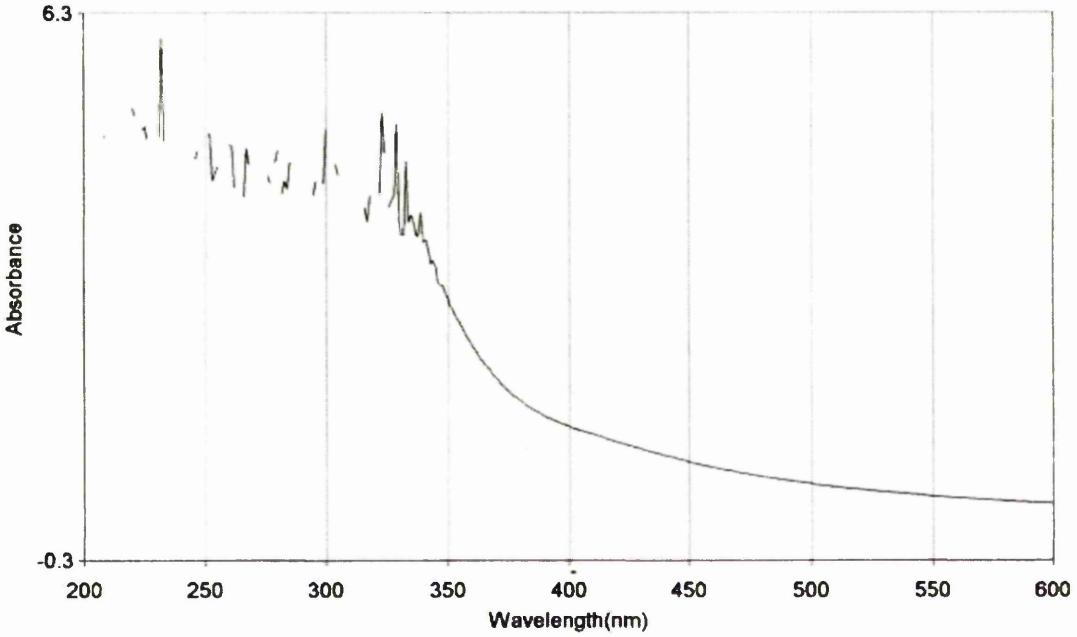


Figure AVI.XII Volunteer 4 urine sample, fasted, citric acid.

APPENDIX VI – CONTINUED

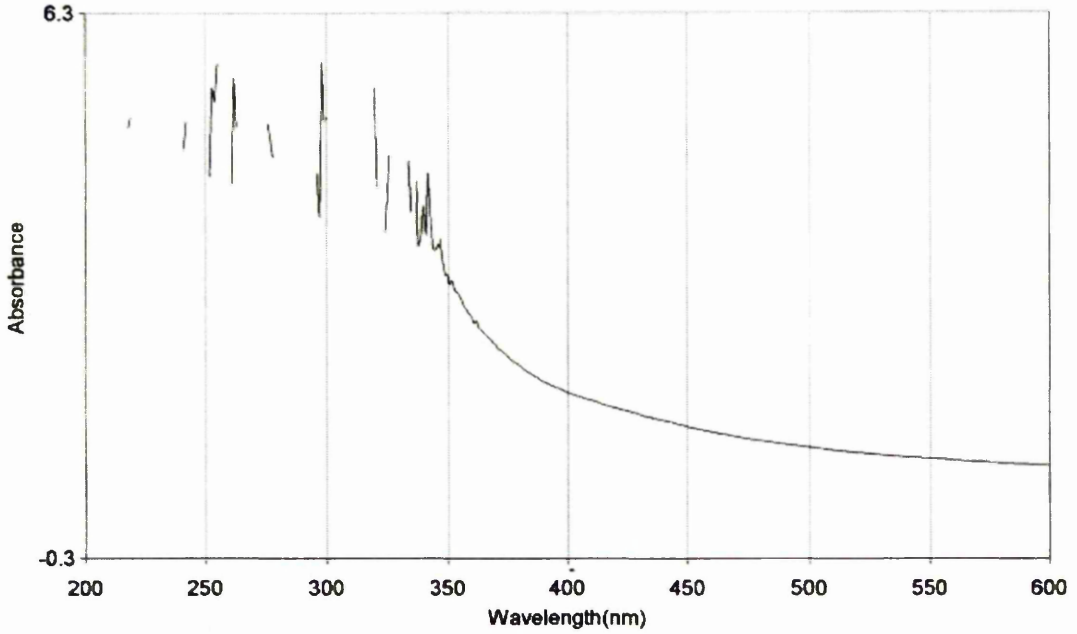


Figure AVI.XIII Volunteer 4 urine sample, fed, water.

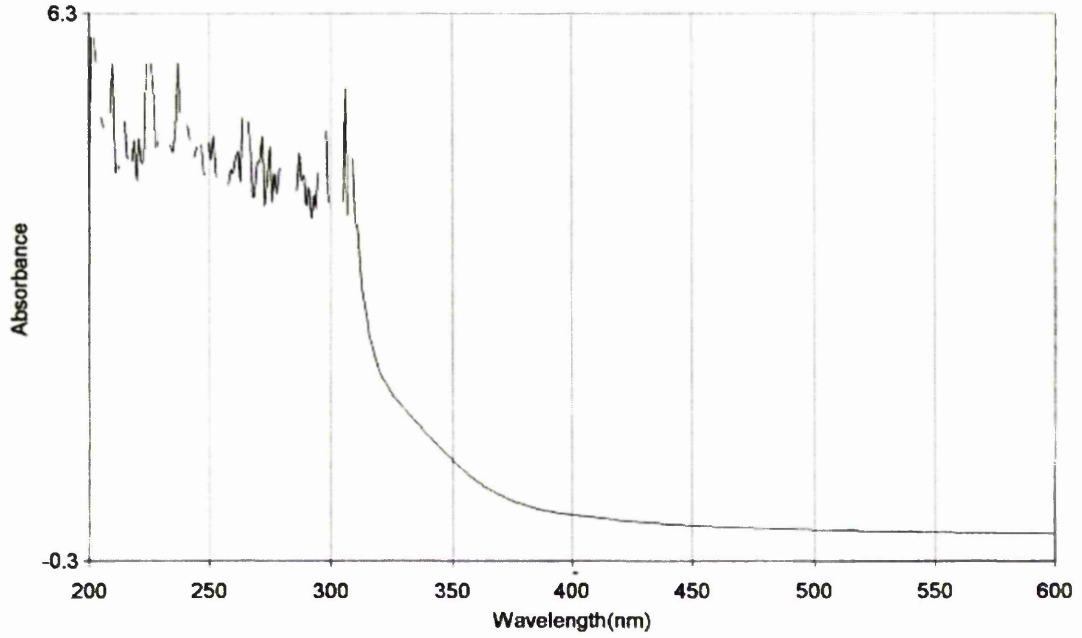


Figure AVI.XIV Volunteer 5 urine sample, fasted, water.

APPENDIX VI – CONTINUED

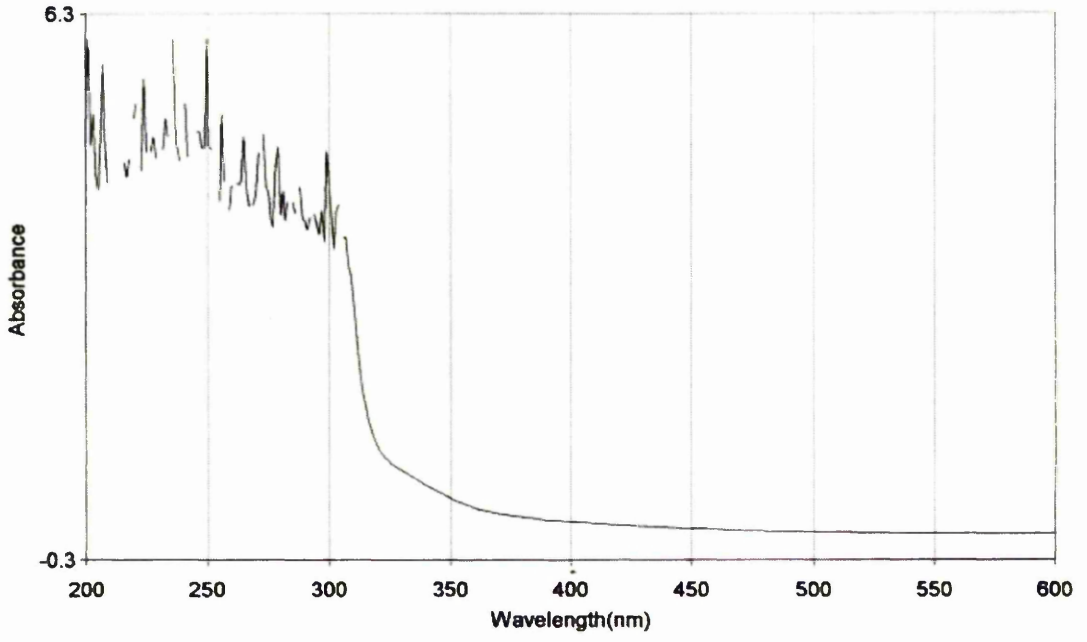


Figure AVI.XIV Volunteer 5 urine sample, fed, water.

APPENDIX VII – BEER-LAMBERT PLOT FOR RIBOFLAVIN IN GLASS DISTILLED WATER USING HPLC

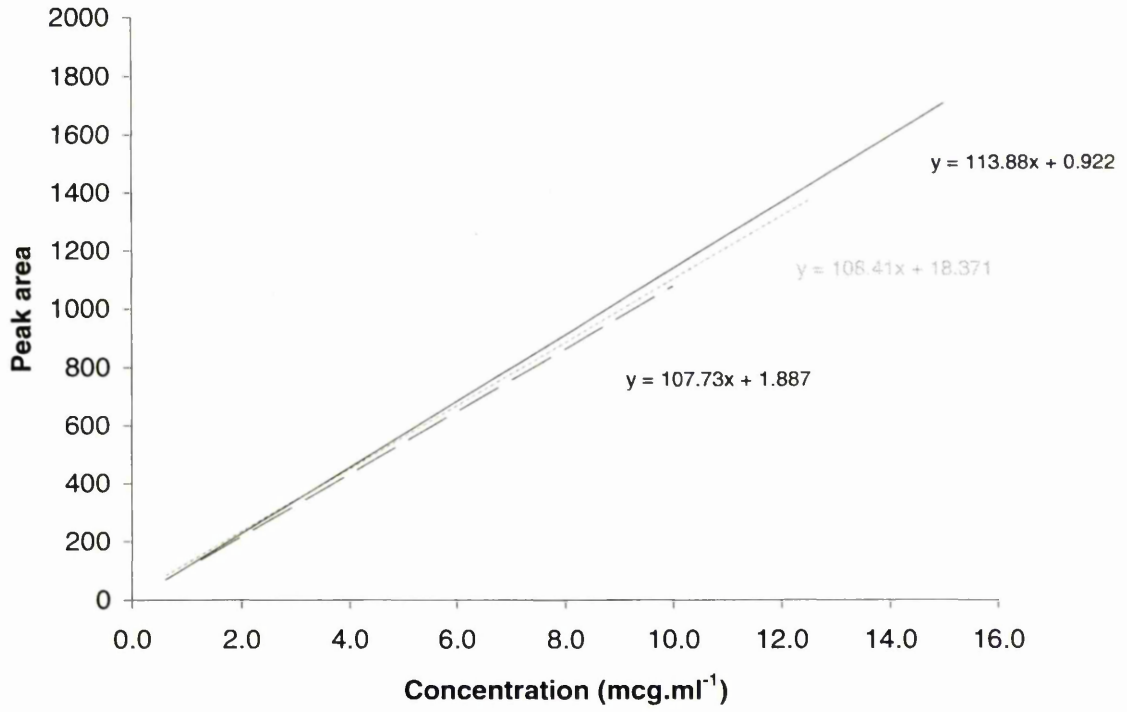


Figure AVII.I Calibration curve proving precision of HPLC as an analytical method for the analysis of urine samples.

(The three different analysis were carried out on three separate days. For each day, samples of different concentration of riboflavin in glass distilled water were freshly prepared.

The equation y = 113.88x + 0.922 was produced from the following concentrations: 15mcg.ml^{-1}, 12.5mcg.ml^{-1}, 10mcg.ml^{-1}, 7.5mcg.ml^{-1}, 5mcg.ml^{-1}, 2.5mcg.ml^{-1}, 1.2mcg.ml^{-1}, 0.6mcg.ml^{-1}. The aforementioned samples formed the initial part of the analysis of the urine samples for the gamma scintigraphy and bioavailability study, Chapter 5, section 5.2).

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