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ORAL DELIVERY STRATEGIES FOR THE CONTROLLED RELEASE OF CIMETIDINE

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Doctor of Philosophy

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ASTON UNIVERSITY

ORAL DELIVERY STRATEGIES FOR THE CONTROLLED RELEASE OF CIMETIDINE

by

Khalid Ullah Shah

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Thesis Summary

It is advantageous to develop controlled release dosage forms utilising site-specific delivery or gastric retention for those drugs with frequent or high dosing regimes. Cimetidine is a potent and selective H_2 –receptor antagonist used in the treatment of various gastrointestinal disorders and localisation in the upper gastrointestinal tract could significantly improve the drug absorption. Three strategies were undertaken to prepare controlled release systems for the delivery of cimetidine to the GI tract.

Firstly, increasing the contact time of the dosage form with the mucus layer which coats the gastrointestinal tract, may lead to increased gastric residence times. Mucoadhesive microspheres, by forming a gel-like structure in contact with the mucus, should prolong the contact between the delivery system and the mucus layer, and should have the potential for releasing the drug in a sustained and controlled manner. Gelatin microspheres were prepared, optimised and characterised for their physicochemical properties. Crosslinking concentration, particle size and cimetidine loading influenced drug release profiles. Particle size was influenced by surfactant concentration and stirring speed. Mucoadhesive polymers such as alginates, chitosans, carbopols and polycarbophil were incorporated into the microspheres using different strategies. The mucoadhesion of the microspheres was determined using *in vitro* surface adsorption and *ex vivo* rat intestine models. The surface-modification strategy resulted in highest levels of microsphere adhesion, with chitosan, carbopols and polycarbophil as the most successful candidates for improvement of adhesion, with over 70% of the microspheres retained *ex vivo*. Specific targeting agent UEA I lectin was conjugated to the surface of gelatin microspheres, which enhanced the adhesion of the microspheres.

Alginate raft systems containing antacids have been used extensively in the treatment of gastro-oesophageal disease and protection of the oesophageal mucosa from acid reflux by forming a viscous raft layer on the surface of the stomach content, and could be an effective delivery system for controlled release of cimetidine. Alginate raft formulations were prepared and ratios of excipients optimised for floating *in vitro*. Free drug and gelatin microspheres were incorporated into the rafts and the release evaluated. Drug release from raft formulations was slow with inclusion of microspheres, with 60% of the drug released after 24 hours. The retention of different sizes of microspheres was assessed. Small microspheres of size 4 μm were found to diffuse from the raft after 6 hours.

Swelling-type dosage forms are such that after swallowing, these products swell to an extent that prevents their exit from the stomach through the pyloric sphincter. The dosage form is therefore retained in the stomach for a prolonged period of time and could be used to give controlled release of cimetidine. Freeze-dried porous hydrogels were prepared and swelling studies carried out. Free drug and microspheres were incorporated into the hydrogels and the release evaluated. Drug release was 60% after 8 hours from microspheres inside hydrogels. The level of retention of different size microspheres in the hydrogels was determined. Microspheres of size 6 μm were found to be released from the hydrogel following incubation in SGF.

Key words: Microspheres, mucoadhesion, hydrogels, rafts, gastrointestinal tract

To my beloved parents

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List of abbreviations

BCA.. Bichinchoninic acid assay
BSA.. Bovine serum albumin
CH.. Chitosan
Da.. Daltons
ρ.. Density
DBA.. Dilichos biflorus agglutinin
pKa.. Dissociation constant
FDA.. Federal Drug Administration
FITC.. Fluorescein isothiocyanate
GI.. Gastrointestinal
HMW.. High molecular weight
HPLC.. High performance liquid chromatography
HA.. Hyaluronic acid
HBS.. Hydrodynamically balanced system
HPC.. Hydroxypropylcellulose
HPMC.. Hydroxypropylmethylcellulose
Is.. Internal standard
IPN.. Interpenetrating network
IEP.. Isoelectric point
LCA.. Lens culinaris agglutinin
LMW.. Low molecular weight
MMC.. Migrating motor complex
HNMR.. Nuclear magnetic resonance spectroscopy
LogP.. Octanol-water partition coefficient
pK_a.. Partition coefficient
ppm.. Parts per million
Pas.. Pascal
PNA.. Peanut agglutinin
PEO.. Polyethyleneoxide
PSSA.. Polystyrenesulphonic acid
PVA.. Polyvinylalcohol

t_R	Retention time
rpm	Revolutions per minute
RTP	Room temperature
SEM	Scanning electron microscopy
SGF	Simulated gastric fluid
SIF	Simulated intestinal fluid
SLT	*Solanum tuberosum*
Q_d, Q_w	Swelling ratio
TLC	Thin layer chromatography
TL	Tomato lectin
UEA	*Ulex europaeus* agglutinin
η	Viscosity
WGA	Wheat germ agglutinin

CHAPTER ONE

INTRODUCTION

1.1 ORAL DRUG DELIVERY

The oral route is the most popular route for administration of most therapeutic agents as there are many benefits such as patient compliance, convenience of administration and cost-effective manufacturing. Although oral is the preferred delivery route, many drugs are poorly available when administered by this route.

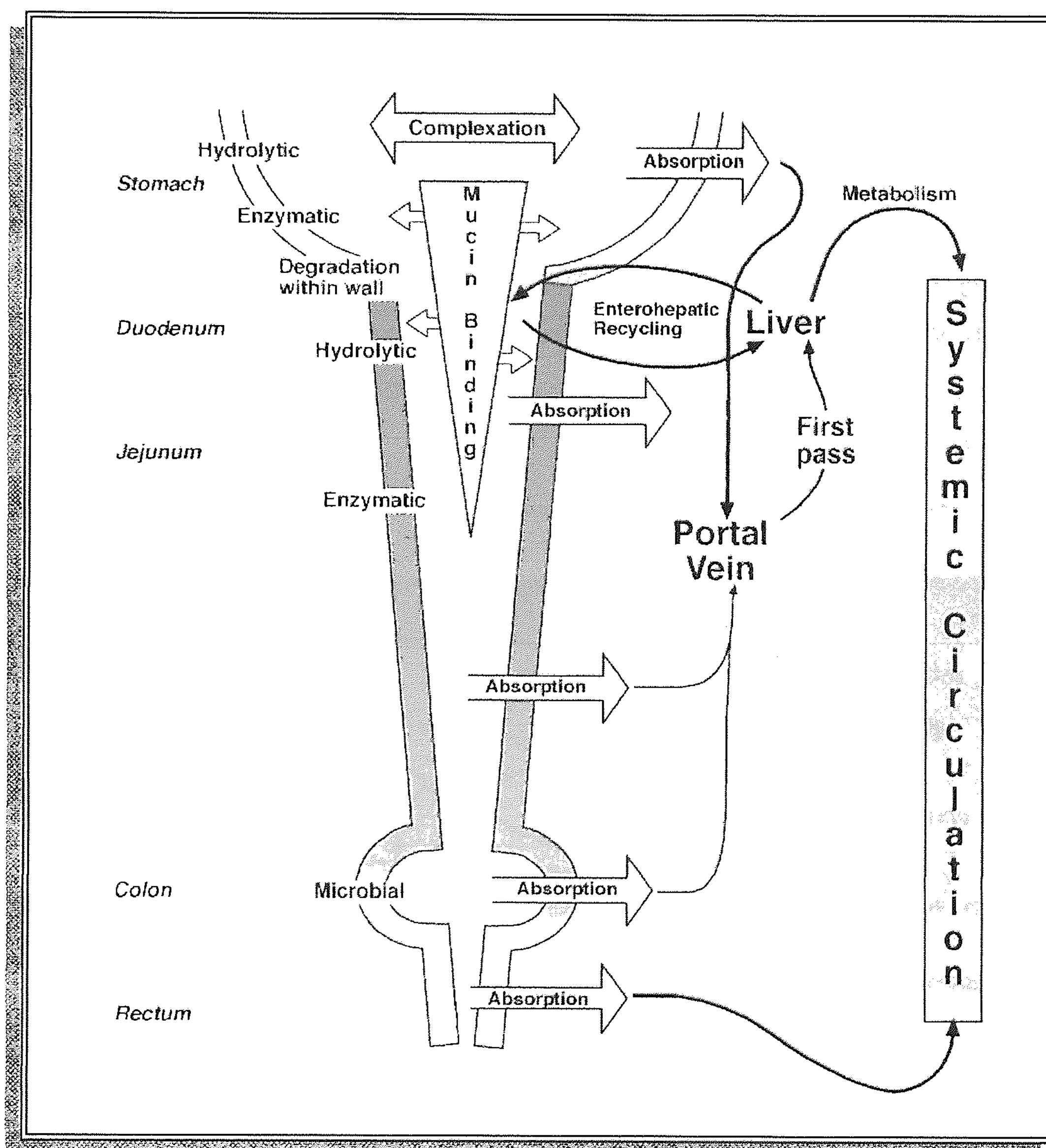


Figure 1.1 Physical models to illustrate various physiological processes encountered by an orally administered drug during the course of gastrointestinal (GI) transit (Adapted from Macadam, 1993)

Following oral delivery, the drug may encounter various physiological processes including complexation, binding to mucin or the mucus lining, and hydrolytic, enzymatic, microbial degradation, illustrated in figure 1.1. Following absorption through the mucosal membrane,

the drug molecules are pooled in the portal circulatory system and liver, where they may be subjected to a first-pass elimination process before being transported to the heart *via* the hepatic vein and inferior vena cava, for systemic circulation through the whole body.

1.2 PHYSIOLOGY OF THE GASTROINTESTINAL TRACT

The gastrointestinal tract (GI tract) is essentially a tube about 9 metres long which runs through the middle of the body from the mouth to the anus (Figure 1.2). The gastrointestinal tract includes the mouth, throat (pharynx), oesophagus, stomach, small intestine (consisting of the duodenum, jejunum and ileum), large intestine (consisting of the caecum, appendix, colon and rectum) and the anus. The wall of the gastrointestinal tract has the same general structure throughout most of its length from the oesophagus to the anus, with some local variations for each region.

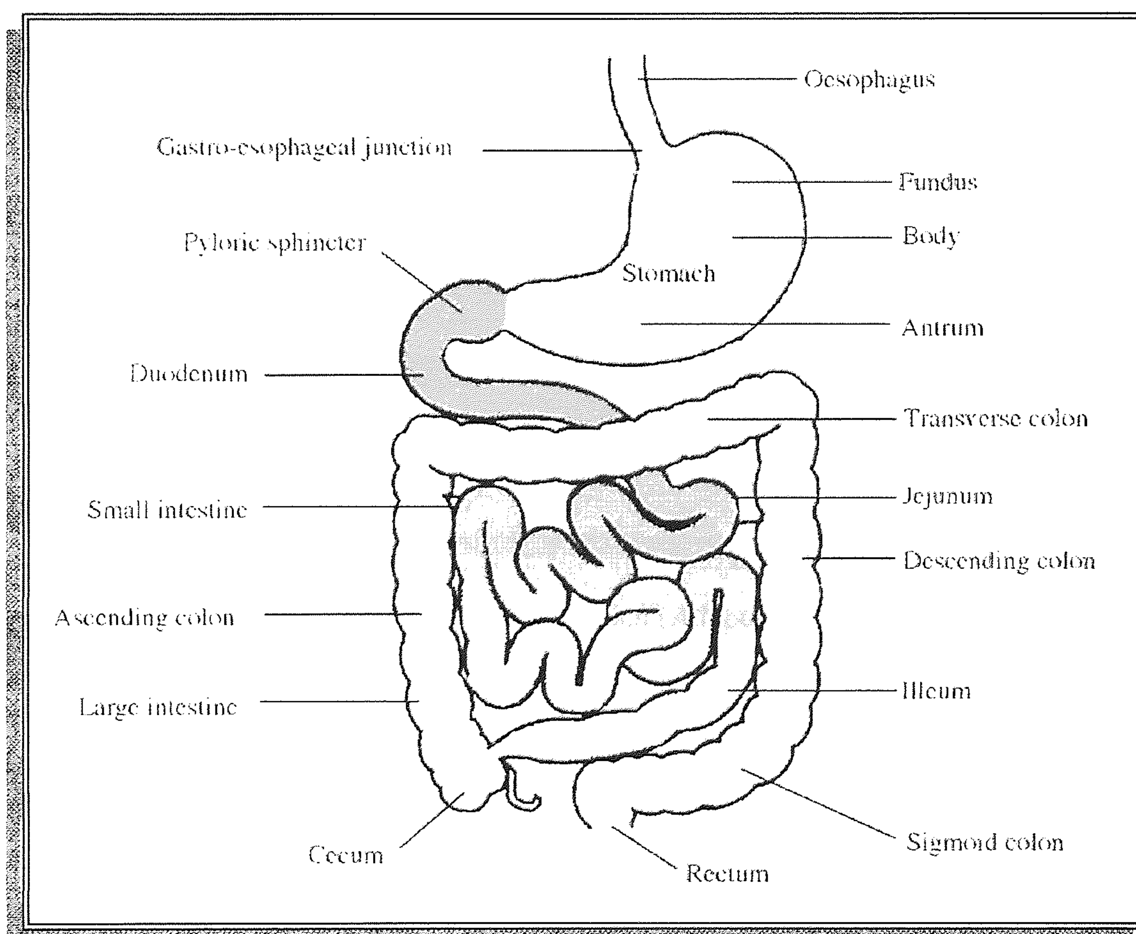


Figure 1.2 Anatomy of the GI tract (Adapted from Macadam, 1993)

The stomach is an organ with a capacity for the storage and mixing (Figure 1.3). Its fundus and body regions are capable of displaying a large expansion for the accomodation of food without much increase in intragastric pressure. The stomach lining is devoid of villi but contains a considerable number of gastric pits contributing to the storage capacity of the stomach. The antrum region is responsible for the mixing and the grinding of gastric contents. Under fasting conditions, the stomach is a collapsed bag with a residual volume of approximately 50 ml and contains a small amount of gastric fluid (pH 1-3) and air (Meyer, 1987). There are two main secretions, mucus and acid, produced by specialised cells (*i.e.* Paneth cells, goblet cells, endocrine cells, etc.) in the stomach lining. The mucus spreads and covers the mucosal surface of the stomach as well as the rest of the GI tract and acts as a protective layer. The purpose of the acid is to breakdown food for digestion.

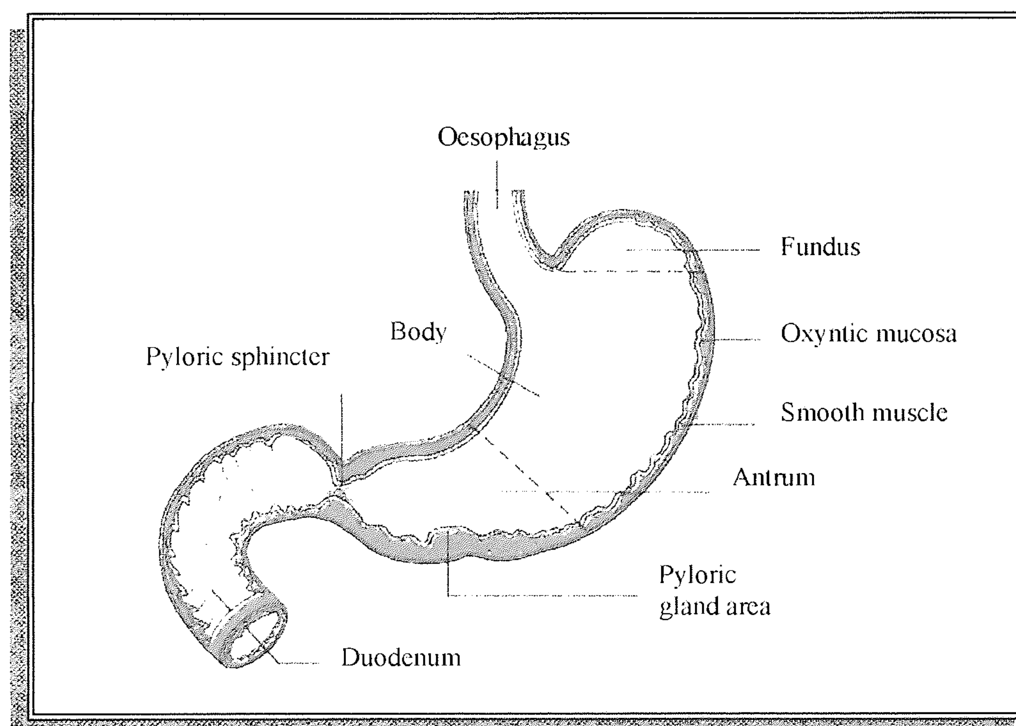


Figure 1.3 Schematic representation of the stomach (Adapted from Sherwood, 1989)

The contents of the stomach are emptied through the pylorus into the proximal duodenal region of the small intestine. In humans, the gastroduodenal junction controls the unidirectional passage from the stomach to the duodenum, even though a duodeogastric reflux occurs in some animal species. In the proximal duodenum, the contents of the gall bladder (*e.g.* bile) and pancreas, as well as some duodenal secretions including bicarbonate, are emptied. The pancreatic juice contributes proteolytic, and carbohydrate-

splitting enzymes. Several enzymes in the intestinal secretion, including leucine aminopeptidase, reside on the striated border of the intestinal absorptive cells as integral parts of the microvilli of the brush border. Unlike the stomach, the small intestine is a tubular viscous organ and has enormous numbers of villi on its mucosal surface that create a huge surface area (4500 m^2 compared to 0.1-0.2 m^2 for the stomach). These villi are minute, finger-like projections of the mucosa and have a length of 0.5-1.5 mm, depending on the degree of distension of the intestinal wall and the state of contraction of the smooth muscle fibres in their own interiors. They cover the entire surface of the mucosa, and are most numerous in the duodenum and proximal jejenum. Following transit through the small intestine, which has a length of approximately 3 metres, the contents are passed through the terminal ileum into the colon *via* a junction called the ileocecal valve. There is a progressive decrease in the surface area from the proximal to the distal region of the small intestine and colon.

The colon lacks villi, and stores indigestible food residues. It also contains a variety of flora, which are normal residents of the GI tract and may degrade the contents of the colon.

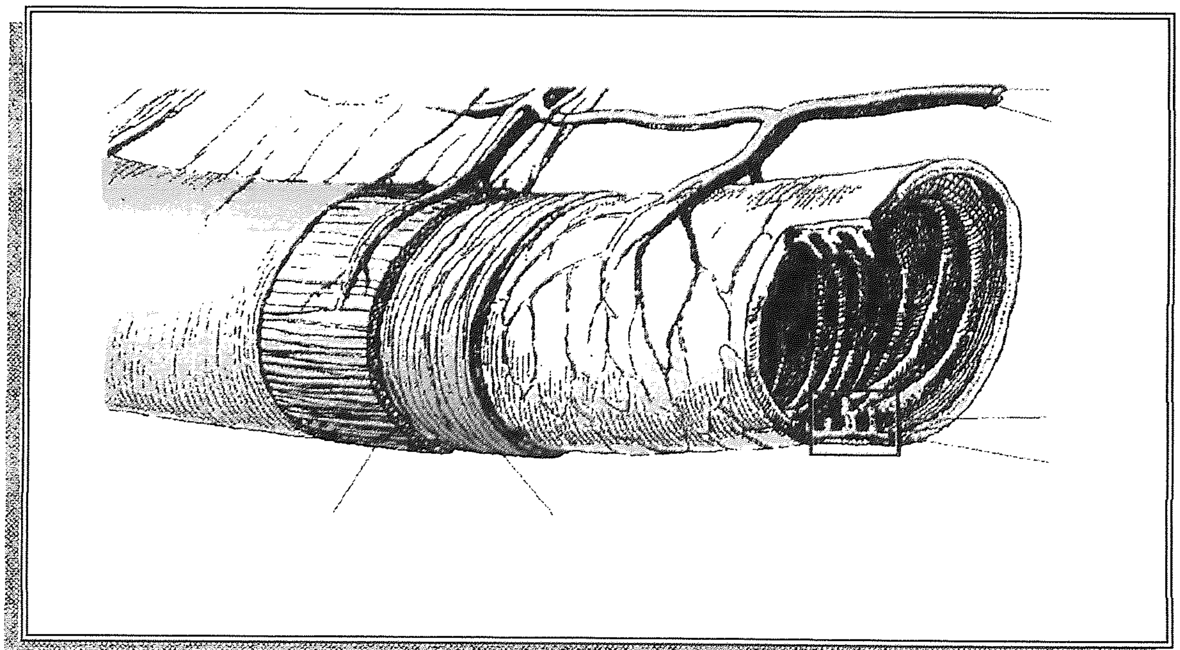


Figure 1.4 Structure of the gastrointestinal wall (adapted from Sherwood, 1989)

The wall of the stomach, the small intestine and the colon consists of four layers (Figure 1.4); the serosa, the muscularis, the submucosa and the mucosa. The muscle layer is divided into two layers; the longitudinal (external) and the circular (internal). The muscle layer controls the peristaltic movements of the GI tract. It is the predominant muscle layer in the pylorus, the caecum and colon. The submucosa consists of loose connective tissue, and the mucosa consists of a muscle layer, glands, subepithelial connective tissue and a single layer of columnar epithelium cells. Between the layers, there is a network of blood vessels which is more dense in the internal than the external layers. As the blood vessels are so close to the mucosa, the epithelium is the major obstacle in the arrival of the drug in the blood capillaries.

A drug molecule may cross from the intestinal lumen into the circulation of the lymphatic system via two major pathways, the transcellular and the paracellular route. The transcellular route may be further subdivided into passive, carrier-mediated and endoctotic pathways. There are active transport mechanisms such as the di-tri-peptide transporter and endocytotic pathways, which include pinocytosis.

Table 1.1 summarises transit times, volume and pH of the GI tract.

Region	Length (m)	Surface area (m^2)	pH fasted state	pH fed state	Residence time
Oesophagus	0.3	0.02	6.8	6.8	> 30 seconds
Stomach	0.2	0.2	1.4 –2.1	3.0-7.0	1-5 hours
Duodenum	0.3	0.02	5.0-6.5	5.0-6.5	> 5 minutes
Jejunum	3	100	4.4-6.6	5.2-6.2	1-2 hours
Ileum	4	100	6.0-8.0	6.0-8.0	2-3 hours
Colon	1.5	3	5.0-8.0	5.0-8.0	15-48 hours

Table 1.1 Comparison of the environment in different parts of the GI tract (Adapted from Dressman *et al.*, 1998; Wilson *et al.*, 2001)

1.2.1 Gastrointestinal motility

The GI tract is in a state of continuous motility. There are two well-known modes of GI motility:

- Interdigestive (fasted) motility pattern,
- Digestive (fed) motility pattern.

The interdigestive motility pattern is dominant in the fasted state with a primary function of cleaning up the residual content of the upper GI tract. It is commonly called the migrating motor complex (MMC) and is organised in cycles of activity and quiescence. Each cycle lasts 90-120 minutes and consists of four phases (Table 1.2).

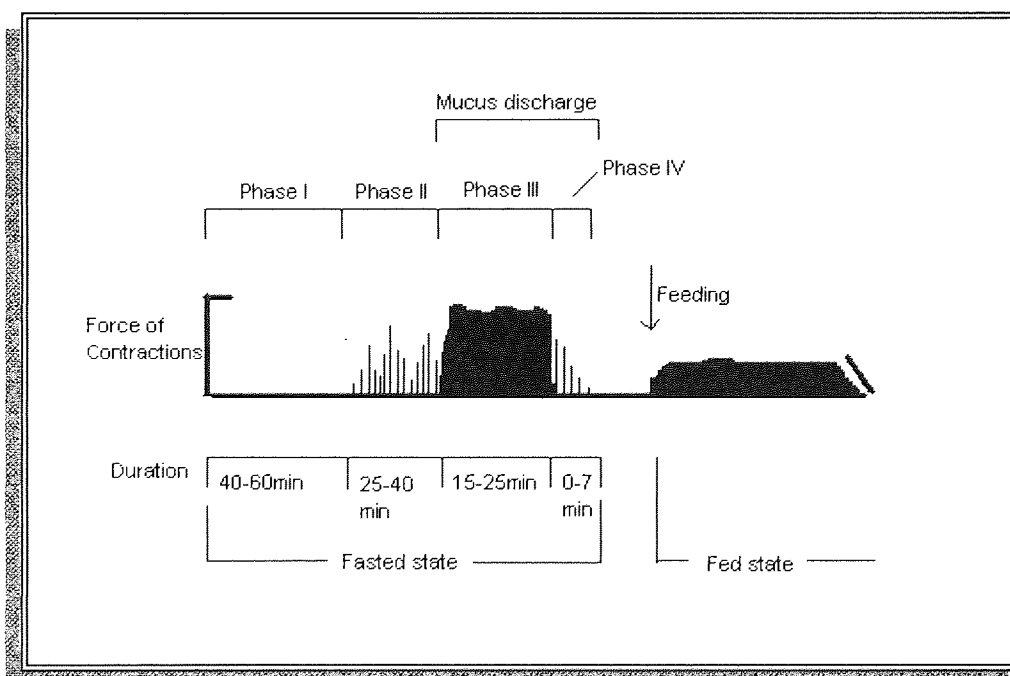


Figure 1.5 Motility patterns of the fasted and fed states of the upper GI tract (Adapted from Macheras *et al.*, 1995)

Phase	Duration (minutes)	Characteristics
I	40-60	Quiescence
II	25-40	Irregular contractions Medium to high amplitude Bile secretion begins Gastric discharge of a small volume of administered fluid Particle/mucus discharge towards phase end
III	15-25	Regular contractions (4-5 *per* min) of high amplitude Continuation of mucus/particle discharge
IV	0-7	Irregular contractions Medium to low amplitude

Table 1.2 Characteristics of the motility patterns in the fasted state (Adapted from Macheras *et al.*, 1995)

In the interdigestive or fasted state, every 90 to 120 minutes, an MMC wave migrates from the stomach down the GI tract. A full cycle consists of four phases, beginning in the lower oesophageal sphincter/gastric pacemaker and propagates over the whole stomach, the duodenum, jejunum and finishing at the ileum (see figure 1.2). Phase III is termed the "housekeeper wave" as the powerful contractions in this phase tend to empty the stomach of its fasting contents and undigestible debris. The administration and subsequent ingestion of food rapidly interrupts the MMC cycle and the digestive phase is allowed to take place. The upper part of the stomach stores the ingested food initially where it is gradually compressed by the phasic contractions.

The digestive or fed state is observed in response to meal ingestion. It resembles the fasting phase II and is not cyclical, but continuous, providing food remains in the stomach. Large objects are retained by the stomach during the fed pattern but are allowed to pass during phase III of the interdigestive MMC. It is thought that the sieving efficiency (*i.e.* the ability of the stomach to grind the food into smaller size) of the stomach is enhanced by the fed pattern and/or by the presence of food (Meyer, 1987).

The gastric emptying of liquids is a function of the volume administered and is controlled by the specific motor activity of the proximal stomach. For a small volume (<100 ml), this is controlled by the existing phasic activity and liquids are emptied at the onset of phase II; most of them are cleared before the arrival of phase III. For volumes larger than 150 ml, liquids are emptied by characteristic discharge kinetics irrespective of phasic activity. The half-life of discharge in dogs is 40-50 min for a small volume of liquid but only 8-12 minutes for a larger volume (Chien, 1992). In humans, volumes of 300 ml emptied twice as fast than 150 ml (Meyer, 1987). A small volume does not affect the existing motility pattern in the stomach, but a large volume converts the fasted state to a fed state, creating the fed-state motility pattern. It is theorised that the emptying of liquids from the stomach is regulated by the tonic contraction of the gastric fundus (Houghton *et al.*, 1988).

The fasted-state emptying pattern is independent of the presence of any indigestible solids in the stomach. Patterns of contractions in the stomach occur such that solid food is reduced to particles of less than 1 mm diameter which are emptied through the pylorus as a suspension. The duration of the contractions is dependant on the physicochemical characteristics of the ingested meal. Generally, a meal of ~450,000 calories will interrupt the fasted state motility for about 3-4 hours. Food residues can be retained in the large intestine for up to 3-5 days and this duration could result in an additional absorption site. It is reported that the antral contractions reduce the size of food particles to ≤ 1 mm, and propel the food through the pylorus (Houghton *et al.*, 1988). However, it has been shown that ingestible solids ≤ 7 mm can empty from the fed stomach in man (Khosla *et al.*, 1989).

The total transit times in humans from the stomach to the ileocecal junction is approximately 3-6 hours in the fasted state and 6-10 hours in the fed state. This means there is a 10 hour limit over which delivered drugs can be absorbed from the small intestine region (Harris *et al.*, 1990a).

1.2.2 Gastrointestinal mucus

1.2.2.1 Physiology of mucus

Gastrointestinal mucus is a viscous, slippery gel and a complex mixture of glycoproteins, water, various serum and cellular macromolecules, electrolytes, micro-organisms, and sloughed cells. Table 1.3 shows the major components of mucus.

Water	> 95%
Glycoproteins	0.5 - 5%
Lipids	< 0.5%
Mineral salts	1%
Free proteins	0.5 - 1%

Table 1.3 Chemical composition of mucus (Johnson *et al.*, 1987).

As described in section 1.2, the role of the stomach is to receive, store, and initiate digestion of all the solid and fluid foodstuffs. In this process, the stomach itself must be protected or defend itself from detrimental effects, including damage from the wide range of ingested substances, as well as from its own secreted HCl, digestive enzymes, and the refluxed contents of the duodenum; this task is relegated to the gastric surface mucus cell (Ito, 1987). These cells synthesise and secrete gastrointestinal mucus which has an unusual consistency and unique capacity in the protection of the delicate epithelial surfaces. This is thought to be due to the major gel-forming glycoprotein components called mucin. The stomach mucosa differs from the small intestine mucosa in that the surface mucosa consists of mainly mucus-secreting cells rather than absorptive cells. The surface of the stomach mucosa is covered by a single layer of mucus-secreting epithelial cells punctuated by gastric 'pits'. There are about 100 pits/mm^2, occupying almost half of the stomach surface. The distance between the centres of the pits is about 0.1 mm. The surface mucus cell forms the free surface of the glandular stomach, lines the gastric pits, and intermingles with parietal cells in the isthmus of the gastric glands. These cells behave similarly to other mucus-secreting cells, such as intestinal goblet cells.

The circular folds of the mucus wall are 8-10 mm long, and are thicker in the region of the jejunum. Villi are projected out of the folds, and their height varies between 0.5 and 1.5 mm, their density being 10-40/mm^2. In each villus, there is a thick network of capillaries, and on the outside a layer of epithelial cells, which are absorptive but also contain some mucus-secreting goblet cells (Ito, 1987).

1.2.2.2 Functions of mucus

The layer of mucus covering the GI tract provides a barrier to acid in the stomach by presenting an unstirred layer into which bicarbonate ions are secreted by the surface epithelium. The thickness of the mucus gel layer is reported to be 25-55 μm (Rubenstein and Tirosh, 1993). The bicarbonate ions, which are actively secreted, neutralise hydrogen ions, which are secreted by parietal or oxyntic cells, as they diffuse towards the epithelium from the lumen (Macadam, 1993). The mucus layer also resists autodigestion of the GI tract by presenting a diffusional barrier to the progress of enzymatic molecules, such as pepsin (Macadam, 1993). From the underlying tissue, mucus is continually secreted from goblet cells, which, immediately upon release, changes in rheological properties from a secretory low-viscosity solution, to a viscoelastic gel. This keeps the mucus layer intact, due to constant loss of mucus from enzymatic degradation and physical erosion. The mucus also allows food to slip over the underlying epithelial layer without causing damage. Furthermore, it helps protect the epithelium from shear forces induced by peristaltic waves and resists HCl and enzyme digestion. The gelling properties which are essential in the function of the mucus are the direct result of the glycoprotein present in the mucosal secretion.

On its luminal surface, the adherent gel layer itself is continuously eroded by mechanical and enzymatic processes. It is reported from experiments in the rat, (as there is no accurate value for the time-scale process in humans), that the turnover time of the mucus gel layer appears to be no longer than 1-4 hours (Lehr *et al.*, 1992 c). This value was obtained using isolated rather than normal gut segments, nevertheless the time-scale would seem fairly short, as with the intestinal epithelial cells, following migration from the crypts to the tips of the villi, renewal is 1-3 days.

Composed mainly of water, the mucus layer has a high molecular weight component which is responsible for the gel formation. The variations in the glycoprotein structure are responsible for the varying properties of the mucus gel.

1.2.2.3 Glycoprotein

Glycoproteins are the main mucus components (after water) and are responsible for the viscosity, adhesive and cohesive properties.

The glycoprotein molecule consists of a protein backbone (made up of approximately 800 amino acids) with oligosaccharide side-chains attached to specific amino acid residues. The molecule is approximately 1,800,000 Daltons (Da) but may vary due to changes in the composition of the oligosaccharide side chains (MacAdam, 1993). Sugar side-chains are attached at about every three residues along the glycosylated regions which results in 200 side chains *per* molecule. The protein content of mucous glycoproteins tends to be low, 12-17% w/w in the human stomach on a dry basis. The subunits of the macromolecule consist of at least two regions. The major region (70-80% of total), is heavily glycosylated and has a high content of serine, threonine, and proline (> 50 mol%), with the remaining amino acids being mainly aspartic, glutamic, valine, glycine and alanine (MacAdam, 1993). The minor region is poorly glycosylated (or "naked") and is susceptible to proteolytic degradation and contains lesser amounts, in contrast to the major region, of serine and threonine. It is however enriched in aspartic, glutamic, glycine, hydrophobic, and basic amino acids.

1.3 ORAL CONTROLLED RELEASE SYSTEMS

The desirability of slow, sustained release oral dosage forms was first discussed by Lipowksi in the early 1950s, where the concept was applied for the first time with several controlled-release products (Yakobi and Walega, 1988). Major advances in the area did not occur for several years due to apparent unreliability of some products as well as limitations of available materials and technologies.

The average development cost of a new chemical entity is approximately £100-300 million (Sastry *et al.*, 2000). It often costs substantially less to develop new methods of administration for an existing drug, which can result in improved efficacy and bioavailability together with reduced dosing frequency and the minimisation of side effects. Therefore, pharmaceutical companies are under constant pressure to maximise the full potential of a drug candidate at the early stage of its life cycle (up to ~15-20 years). This objective can be accomplished by incorporation of the drugs into various delivery systems. This may lead to extended patent life and convenient dosage forms, which can overcome previously presented administration problems. For the last two decades, there has been an enhanced demand for more patient-compliant dosage forms. As a result, there are now approximately 350 drug delivery corporations. The demand for their technology was approximately £10-15 billion in 1995, and is expected to grow by £40 billion annually (Sastry *et al.*, 2000).

The performance of an oral controlled-release dosage form is usually determined by the following three major variables:

- Physico-chemical properties of the drug
- Components and characteristics of the controlled-release dosage form
- Physiological factors

In the design of an oral controlled-release formulation, physiological factors such as gastric emptying, intestinal motility, site of drug absorption and chemical, enzymatic or metabolic degradation can affect the performance of the device. In maximising the potential of an oral controlled-release dosage form, strategies have been designed such as site-specific targeting and gastroretentive strategies.

The number of general controlled-release strategies that are non-specific for the region of GI tract delivery is vast. Drug release duration can be prolonged using a number of approaches, including:

- variation of physical properties of drug such as solubility
- forming a complex of drug molecules with ion-exchange resins
- incorporation of drug into slowly disintegrating or inert porous matrices

• coating drugs with polymers having a barrier function for their diffusion

These delivery devices can consist of tablets or capsules, utilising matrices, resins, microgranules, beads, pellets, micro- or nano-particles, as well as floatable and swellable systems, as the rate controlling mechanism. All the strategies described in this section relate to the release of drugs to target the lower GI tract area, i.e. the small intestine

1.3.1 Drug delivery to the GI tract

An 'oral controlled-release product' by definition, refers to a formulation containing components critical for modulation of the drug-release pattern in a predictable fashion, with control over the location of the drug release in the GI tract, or improvement of the pharmacokinetic profile, usually by improvement of the performance or stability of the oral formulation in the dynamic and 'hostile' environment of the GI tract (Fix, 1999).

Oral controlled-release formulations for the gastrointestinal tract (small intestine and colon in particular) have received considerable attention for many reasons, including pharmaceutical superiority and clinical benefits derived from the drug-release patterns which are not achieved from traditional immediate-release products (Fix, 1999).

In the development of controlled-release, peak-associated side effects have the potential to be reduced or minimised if the formulations could provide lower, but effective, constant plasma levels of the active drug. The ultimate aim has been in the development of true zero-order release formulations, which provide a constant rate of drug release, independent of any remaining drug content in the formulation. Controlled-release systems, which achieve pseudo-first order or first-order drug release can also achieve effective pharmacokinetic and pharmacodynamic results. Development of controlled-release formulations for gastrointestinal delivery is also beneficial where absorption windows exist, which can define the regions of the GI tract from where a potential drug candidate can be absorbed. Where an absorption window exists for a drug candidate, a time- or location- directed formulation could be used in order to achieve adequate drug absorption from the particular region of the GI tract. Drugs susceptible to enzymatic degradation in the stomach, such as peptides and proteins, could be incorporated into designed

formulations, releasing the drug in the lower small intestine or colon to minimise degradation and achieve higher local concentrations of the drug at the absorption surface (Fix, 1999)

The selection of a drug candidate for the design of a controlled-release system is dependant largely on the pharmacological, therapeutic, and pharmaceutical considerations. The main criteria for the selection of drug candidates include, but are not necessarily limited to: short biological half-life, narrow therapeutic index, inefficient GI absorption, low daily dose and lack of first pass metabolism. For some drugs with a relatively short biological half-life, *e.g.* less than 6 hours, controlled-release reduces the dose frequency and maintains constant drug levels. Drugs with relatively narrow therapeutic index may be more efficacious and less toxic if administered in controlled-release form.

Systems for drug delivery to the GI tract can fall into one or more of the following categories:

- pH-independent delivery systems (e.g. Cardizem®) where release of the drug is independent of the delivery device and local pH

- pH-dependant delivery system (e.g. Asacol®) where release of the drug occurs at a specific pH of the GI tract dependant on the polymers used which degrade at the particular pH.

- Prodrug strategy (e.g. Sulindac®), where the synthesis of a chemically-modified version of the parent drug, overcomes the inherent problem of adequate absorption.

- Gastroretentive devices (e.g. Valrelease®), where residence in the stomach can be delayed to give controlled-release (see section 1.5)

- Mucoadhesive delivery systems (no commercial products beyond buccal cavity) where localisation of the drug and formulation components in close proximity to the epithelial barrier of the GI tract can promote drug absorption (section 1.6)

1.3.1.1 pH-independent delivery systems

A delivered solid dosage form in the fasted state, as a result of the physiological mechanisms in the stomach, will remain in the stomach for 15-120 minutes before emptying (this duration depends on which MMC phase is active upon entry of the dosage from into the stomach) (see section 1.2.1). Drugs susceptible to acid hydrolysis may breakdown during the time of their residence in the stomach, thus reducing their potential absorption if the absorptive region were located past the stomach.. This category includes those devices formulated to release an acidic or basic drug in the GI tract at a rate independent of the pH of the GI fluids (which vary from pH 1.2 – 3.5 in the stomach and 6.0 – 7.5 in the lower small intestine, see table 1.1), e.g. time-specific devices. The acidic or basic drug is incorporated along with buffering agents into a tablet core. The influx of the gastric fluids dissolves the buffering agents providing a specific pH climate within the formulation, mediating the drug release. Thus the rate of the drug release would be independent of the pH of the gastric environment.

One strategy of incorporation of a pH-independent (time-specific) element to oral controlled-release systems has been achieved by construction of devices based on the generation of osmotic pressure as the driving force, controlled by water uptake. Osmotic systems incorporate a drug core containing an osmotically active agent and a semi-permeable membrane coating which regulates the influx of water into the device. As the influx of water occurs, the pressure developed as a result, controls the drug release. Drug release occurs through the orfice of the device. A number of these devices have been designed and evaluated. An osmotic system called OROS®, a single unit tablet coated with a semi-permable membrane, provided constant maintenance of the plasma level for metoprolol (Sandberg *et al.*, 1993). Some examples of commercial products based on osmotic effect are Procardia XL® (Pfizer), Glucotrol XL® (Pfizer), Alpress™ (Alza) and Covera-HS® (Alza). Another system, the GITS® was of a push-pull design, which could reportedly be utilised in the delivery of insoluble or poorly soluble drug suspensions (Grundy *et al.*, 1997). This device was reported to release the incorporated drug, nifedipine, at zero-order over 24 hours. It was reported that 2 hours post-administration of the device, detectable drug levels of nifedipine were observed *in vivo*. The drawback of

both these types of devices is that GI transit of the human subjects was reported to be 6-32 hours (Grundy *et al.*, 1997). Therefore, it is likely that total GI transit of the device may occur in a shorter time period than that required for complete release of the drug, resulting in less from the total dose absorbed.

The location of the device in the GI tract and subsequent activation of the release mechanism is the underlying principle behind all the pH-independent delivery systems. There is no influence on the actual residence time, so the device must be 'programmed' to trigger the release while passing through the required absorption region of the GI tract. Since gastric emptying duration can vary inter-subjects and is dependant on the presence of food, it may be difficult for release to occur in the same absorptive region with consistency.

1.3.1.2 pH-dependant delivery systems

In this section are included those devices which mediate the drug release depending on the pH of the environment.

In the formulation of pH-dependant systems, Eudragit® polymers have been used as they are designed to degrade at different pHs allowing the targeting of different regions of the GI tract. For example, they have been used as coating materials for controlled-release from beads (Narisawa *et al.*, 1994). Uncoated and coated beads were prepared from drug/sucrose or drug/succinic acid mixtures and sprayed with Eudragit®/triethylcitrate/water mixture for the coating. It was reported that by using either sucrose or succinic acid as the buffering agent, drug release could be controlled to release higher levels in either acidic or basic medium.

The Eudragit® grades for enteric coatings are based on anionic polymers of methacrylic acid and methacrylates. They contain –COOH as a functional group and dissolve at ranges from pH 5.5 to pH 7. Some examples are listed below

- Eudragit® L 30 D-55 pH-dependent anionic polymer solubilizing above pH 5.5 for targeted drug delivery in the duodenum

- Eudragit® L 100 pH-dependent anionic polymer solubilizing above pH 6.0 for targeted drug delivery in the jejunum
- Eudragit® S 100 pH-dependent anionic polymer solubilizing above pH 7.0 for targeted drug delivery in the ileum

Other pH-dependant polymers have also been used, for example, sodium alginate which is insoluble below pH 3, and forms a swellable gel matrix At neutral or above its pK_a, sodium alginate is soluble and hydrates forming a viscous solution. Therefore, depending on the water solubility of the incorporated drug, varying release rates could be achieved (Fix, 1999). It was reported that for tablets prepared using sodium alginate, lactose and magnesium stearate containing the drug, chloropheniramine maleate (a highly water soluble drug), the release rate was higher in simulated gastric fluid (SGF) than simulated intestinal fluid (SIF). With a poorly soluble drug, hydrochlorothiazide, the effect on release rate was reversed, *i.e.* faster release rate in SIF compared to SGF. This was attributed to the differences in the internal microscopic structure of the microsphere surface layer. It was speculated that at low pH, as the alginate forms a porous gel medium, release rate of highly water soluble drug could be rapid through aqueous pathways.

pH-dependant microspheres have been prepared from polymers such as gelatin or chitosan with polymer coatings such as alginate (Yao *et al.*, 1994; Yao *et al.*, 1996; Narayani and Pandurang Rao, 1994). As chitosan is soluble in dilute acid, and alginate is soluble at higher pH values, the polymers could be used to dictate the drug release in a particular pH environment. For example, with alginate-coated gelatin microspheres, slower release was reported in SGF than in SIF, whereas chitosan-coated microspheres gave the opposite result (Narayani and Pandurang Rao, 1994).

1.3.1.3 Prodrug strategy

Drug absorption limitations due to solubility, stability, aggregation tendency or intrinsic membrane permeability, can be overcome using prodrug strategies (Fix, 1994). Phenytoin is an anticonvulsant with poor bioavailability which is most often attributed to poor water solubility. Prodrugs of phnytoin have been developed including fosphenytion which increased solubility 5000-fold (Varia *et al.*, 1984). Peptide-prodrug strategies have been considered for improving oral delivery. Prodrug strategies for peptides focus on providing

increased protection against proteolytic enzymes in the GI tract because enzymatic degradation is generally a major barrier to effective oral administration efforts. However, in maintaining the therapeutic purpose of the parent drug, the creation of prodrug involves extensive work at the synthesis level.

1.4 MICROENCAPSULATION

1.4.1 Polymers for microencapsulation

Over the past years, a variety of synthetic and natural polymers have been reported to degrade in mammals. The degradation products of some of these polymers can be eliminated from the body by one of two processes; metabolism or kidney filtration. Polymers that undergo degradation in a living environment, through either simple chemical reactions or enzyme catalysed reactions, are defined as biodegradable polymers (Vert *et al.*, 1998) These polymers have become increasingly important in the development of drug delivery systems (Couvreur *et al.*, 1995). They can function either as a matrix in the control of the degradation of the drug, followed by polymer degradation and elimination of degradation products from the body, or they can participate in and control the rate of drug release by polymer hydration and degradation (Dunn *et al.*, 1998).

1.4.2 Gelatin

Gelatin is widely used in pharmaceutical products and as such has received significant attention in controlled-release systems. Many studies have been reported using gelatin in the formation of gels, particles and microspheres. Gelatin is an optimum candidate for the production of controlled-release systems being a good film- and particle- forming material (Cortesi *et al.*, 1998). When the controlled-release duration of the drug is required to be a matter of hours, rather than days or weeks, natural polymers such as gelatin are promising candidates.

Gelatin is a protein obtained by partial hydrolysis of collagen, the chief protein component in skin, bones, hides, and white connective tissues of the animal body. Type A gelatin is produced by acid processing of collagenous raw material; type B is produced by alkaline or

lime processing. Because it is obtained from collagen by a controlled partial hydrolysis and does not exist in nature, gelatin is classified as a derived protein. Such properties as its reversible gel-to-sol transition of aqueous solution, viscosity of warm aqueous solutions, water permeability, and insolubility in cold water, but complete solubility in hot water, are utilised in the food, pharmaceutical, and photographic industries. In addition, gelatin forms strong, uniform, clear, moderately flexible coatings which readily swell and absorb water and are ideal for the manufacture of pharmaceutical capsules.

The main constituents of gelatin are large and complex polypeptides of the same amino acid composition as the parent collagen, covering a broad molecular weight distribution range. In the parent collagen, the 18 different amino acids are arranged in ordered, long chains, each of ~95,000 Da molecular weight. These chains are arranged in a rod-like, triple-helix structure consisting of two identical helical chains, called α_1, and one slightly different chain called α_2 (Chasin and Langer, 1990). These chains are partially separated and broken in the gelatin manufacturing process. Different grades of gelatin have average molecular weights ranging from 20,000 to 250,000. Molecular weight distribution studies have been carried out by fractional precipitation with ethanol or 2-propanol and by complexing with anionic detergent molecules. The coacervate molecules are isolated and recovered as gelatin fractions.

Analysis of gelatin has shown the presence of amino acids from 0.2% tyrosine to 30.5% glycine (Chasin and Langer, 1990). The major amino acids in gelatin are shown in table 1.4.

Glycine	26-30%
Proline	14-18%
Hydroxyproline	13-15%
Glutamic acid	11-12%
Alanine	9-11%

Table 1.4 Major constituent amino acids in gelatin

The remaining amino acids in decreasing order are: aspartic acid, lysine, serine, leucine, valine, phenylalanine, threonine, isoleucine, hydroxylysine, histidine, methionine, and tyrosine (Chasin and Langer, 1990).

One of the most useful properties of gelatin solution is its ability to form heat reversible gel-solutions. When an aqueous solution of gelatin with a concentration greater than 0.5% w/v is cooled to about 35-40°C, the viscosity of the solution increases, and then a gel is formed. The strength of the gel is dependant on the concentration, intrinsic strength of the gelatin sample, pH, temperature and additives. The structure of the gel is a combination of fine and coarse interchain networks. The ratio is dependant on the temperature during the polymer-polymer and polymer-solvent interaction leading to hydrogen bond formation. (Chasin and Langer, 1990)

Gelatin is soluble in hot water and in aqueous solutions of polyhydric alcohols such as glycerol and propylene glycol. The amphoteric character of gelatin is due to the functional groups of the amino acids and the terminal amino and carboxyl groups created during its hydrolysis. In strongly acidic solution, the gelatin is positively charged and migrates as a cation (Chasin and Langer, 1990). In strongly alkaline solution, it is negatively charged and migrates under an electric field as an anion. The intermediate point, where net charge is zero is known as the isoelectric point (IEP), and is designated in pH units. Type A gelatin has an IEP between 7.0 and 10.0 (Sigma-Aldrich data sheets). The viscosity of a gelatin solution is affected by gelatin concentration, temperature, molecular weight of the gelatin sample, pH, additives and impurities. The viscosity of gelatin solution increases with increasing gelatin concentration and with decreasing temperature. Gelatin exhibits its lowest swelling at its isoelectric pH. At pH below the isoelectric point, cations primarily affect the swelling. These are involved in breaking hydrogen bonds, resulting in increased swelling (Chasin and Langer, 1990).

1.4.2.1 Gelatin microspheres

Preparation of gelatin microspheres using single emulsion methods have been reported by a number of workers (Yao *et al.*, 1996; Yin *et al.*, 1996; Leo *et al.*, 1997; Akin and Hasirci, 1995; Leucuta *et al.*, 1997, Vandelli *et al.*, 2001; Narayani and Rao, 1996) using

different techniques. These methods include complex coacervation, phase separation, spray drying and thermal gelation. The simplest and most used methods of preparation of gelatin microspheres is by thermal gelation, where the introduction of a temperature gradient after formation of the emulsion results in the hardening of the gelatin droplets into microspheres. Thermal gelation to prepare gelatin microspheres has been reported by Leucuta *et al.*, (1997) and Vandelli *et al.*, (2001)

Depending on the choice of formulation parameters, microspheres can be produced of any definitive size and with smooth surface morphology. As gelatin is a hydrophilic polymer, encapsulation of hydrophilic drugs would normally be expected to give fast release. As gelatin can be crosslinked by aldehydes (see section 3.3.1.1), with increasing crosslinking of the polymer, the swelling of the polymer is reduced and hence drug release can be controlled. For this reason, gelatin is used widely in microsphere formulations. The occupational exposure standard for glutaraldehyde is 0.2 ppm in the UK (Lund, 1994). It is reported that a 0.5% solution produced skin sensitisation (World Health Organisation, 1987). Oral LD_{50} in rats for 50% glutaraldehyde is 123-77 mg/kg. Since the residual glutaraldehyde was removed from the microspheres in the washing stages (see chapter 3), amount of glutaraldehyde contained in the microspheres is minimal.

It was reported that for gelatin microspheres prepared using phase separation, with increasing aldehyde crosslinker, the size of the microspheres decreased, explained by increasing crosslinking density resulting in the formation of densely packed structure-like helical formation resulting in shrinkage of the particles (Akin and Hasirci, 1995). Increased crosslinking resulted in a slower rate of water evaporation and the formation of microspheres with smooth surfaces. Aggregation increased with increasing amount of glutaraldehyde (Akin and Hasirci, 1995).

The duration of crosslinking of the gelatin matrix can also influence the drug release rate. The release rates of aminophylline and clonidine hydrochloride were influenced by the duration of crosslinking for gelatin microspheres prepared by thermal gelation (Vandelli *et al.*, 1991). Another study showed the effect of crosslinking amount on release rate. Increasing crosslinking concentration was reported to decrease the release rate of oxprenolol (Leucuta *et al.*, 1997). Drug release for 5-flouracil and methotrexate was

reported to follow nearly zero-order kinetics over about 10 days for gelatin microspheres prepared using a polymer dispersion method (Narayani and Panduranga Rao, 1996).

High entrapment levels of drugs can be achieved in gelatin microspheres. Entrapments of 69-72% w/w of 5-flouracil and 76-90% w/w of methotrexate were reported using a polymer dispersion method (Narayani and Panduranga Rao, 1996). Depending on the emulsion used (*i.e.* oil-in-water or water-in-oil for single emulsions), the encapsulation level can be different for soluble and insoluble drugs. Increased levels of water-soluble drugs were encapsulated (95% efficiency) than poorly water soluble drugs (10% efficiency) for microspheres prepared using a thermal gelation technique (Esposito *et al.*, 1996). During the formulation process for water-in-oil emulsions, hydrophobic drugs may partition into the oil phase, thus reducing the encapsulation efficiency.

By varying formulation parameters such as the stirring speed and the level of crosslinker, depending on the drug used and its solubility, particle size and drug release can be controlled (Narayani and Panduranga, 1996; Cortesi *et al.*, 1998; Morimoto *et al.*, 2001). These will be discussed in section 3.3.

1.4.3 Techniques for microencapsulation

Microencapsulation is the technology of entrapping solids, liquids or gases inside one or more polymeric coatings. Encapsulation can be categorised into two major classes:

- Polymerisation during the process of microcapsule preparation
- Controlled precipitation of polymeric solution within which physical changes occur.

There are many different methods for the encapsulation process (Table 1.5) Classification of microencapsulation methods can be difficult as one system may involve two methods or mechanisms simultaneously.

Interfacial polymerisation is the condensation of two polymers at the interface of an organic and aqueous phase. Complex coacervation encapsulation processes use the interaction of two oppositely charged polyelectrolytes in water to form a polymer-rich

coating solution called a coacervate. The coacervate engulfs the liquid or solid being encapsulated, forming an embryo capsule. The coacervate is gelled upon cooling *via* network formation. Gelatin is often used as a primary component for most coacervate systems.

In coacervation, two incompatible polymers are used in the formation of microcapsules. The polymer for the wall of the microcapsule is dissolved in a solvent and a second polymer is introduced, called the phase inducer. Due to the incompatibility of the two polymers, two phases are present. Introduction of drug particles causes one phase to entrap the drug in the coating polymer.

Process	**Coating material**	**Suspended medium**
Interfacial polymerisation	Water-soluble and insoluble polymers	Aqueous/organic solvent
Complex coacervation	Water soluble polyelectrolyte	Water
Coacervation	Hydrophobic polymers	Organic
Salting-out	Water soluble polymer	Water
Hot melt	Hydrophilic/hydrophobic polymers	Aqueous/organic solvent
Phase separation	Hydrophilic/hydrophobic polymers	Aqueous/organic
Thermal denaturation	Proteins	Organic
Solvent removal	Hydrophilic/hydrophobic polymers	Organic solvents
Solvent evaporation	Hydrophilic/hydrophobic polymers	Organic or water
Spray drying	Hydrophilic/hydrophobic polymers	Air/nitrogen

Table 1.5 Classification of microencapsulation methods (Adapted from Mathiowitz *et al.*, 1999)

Hot melt encapsulation does not involve the use of solvents. Salting out involves the addition of salt to an aqueous polymer solution, which causes the polymer to phase separate from the solution. This technique is not used frequently as the potential high concentration of salt may have an adverse effect on the release behaviour from the microparticles.

Phase-separation is a one-step precipitation method of two polymers, which produces double walled microspheres.

Solvent evaporation is one of the most regularly used techniques in the encapsulation of drugs in microparticles. The drug and polymer are dissolved in a water immiscible, volatile organic solvent and the resulting solution is emulsified in an aqueous solution. The solvent is allowed to evaporate, producing solid microparticles. A double emulsion method may also be used, where the aqueous drug phase is emulsified in a polymer-volatile organic solvent solution. The resulting emulsion is emulsified in water, giving a double emulsion. Evaporation of the volatile solvent gives a solid microparticle with an aqueous core. Biodegradable polymers (e.g. polylactic acid) have been regularly used with this technique. Solvent removal is a modified version of the solvent evaporation technique, by using organic solvents as the extracting medium.

Gelation microencapsulation processes cover more than one technique shown in table 1.4. A preformed polymer is precipitated around the core. Examples of this technique include the following: water-soluble polymers such as gelatin can be precipitated with water miscible solvents such as isopropranol; ethylcellulose can be precipitated from cyclohexane upon cooling to form microparticles; sodium alginate can be gellated with aqueous calcium salt solutions.

Spray drying is an important and widely used technology in the pharmaceutical and biochemical industry and has many advantages over conventional microencapsulation techniques. Evaporation of the polymer is achieved using a temperature-controlled cyclone and the microparticles obtained are microspheres or microcapsules, dependant on the drug being either dissolved or dispersed in an organic or aqueous solution of the polymer to be sprayed. A solution, a suspension or an emulsion of polymer and drug substance can be used. The liquid is fed to the nozzle *via* a peristaltic pump, atomisation occurs by the force of compressed air, which disrupts the liquid into droplets (Figure 1.6). The particle size of the droplets and the resulting microparticles can be controlled by adjusting the atomising pressure and the size of the spray nozzle. The droplets, together with hot air, are blown into a chamber where the solvent in the droplets is evaporated and discharged out through an exhaust tube. The solvent in the droplets is removed rapidly due to the heat energy input of the spray drier. The thermal efficiency of the spray drying process is related to the heat

energy input, which is controlled by the inlet temperature and blower, and the heat used during evaporation of the solvent. To reach an optimum spray drying efficiency, it is necessary to achieve a balance between the energy input and the energy needed, related to the sample input. The dry product is collected in a collection bottle. However, in some cases it is necessary to add further drying steps.

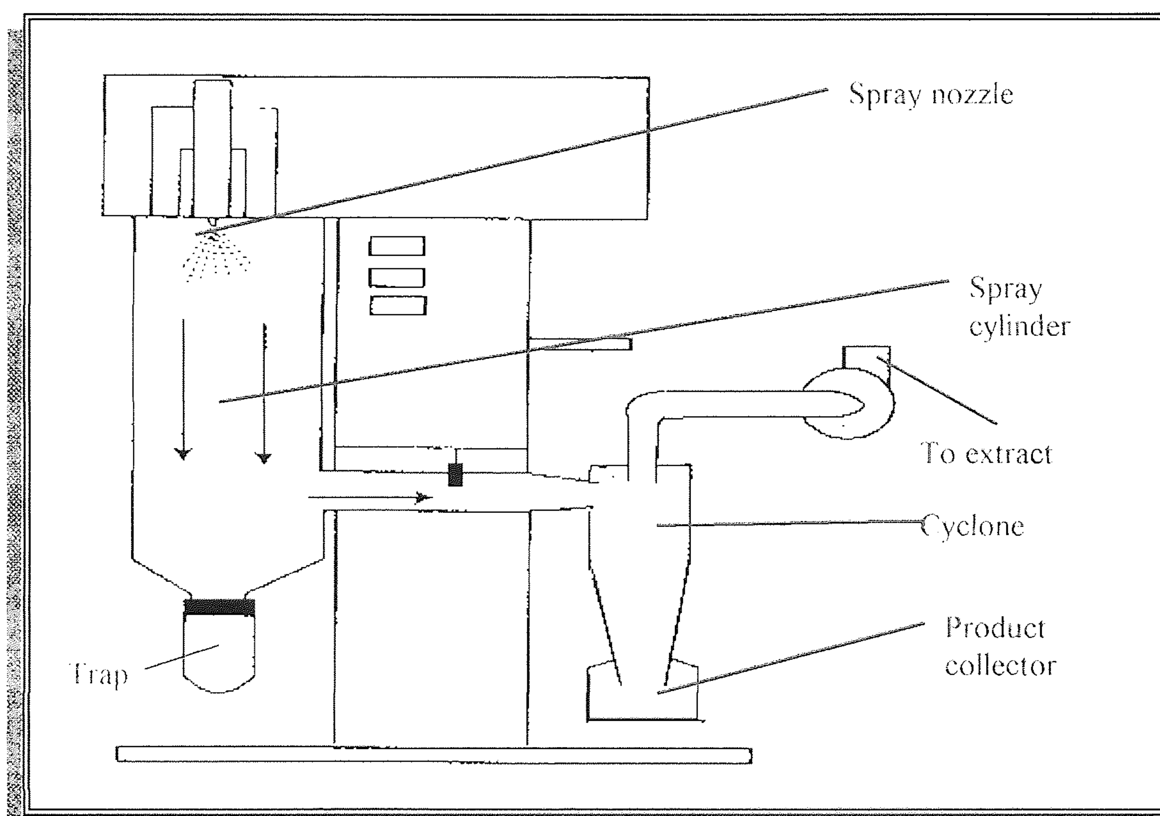


Figure 1.6 Schematic representation of a spray-drier (Mini Buchi 190)

Microspheres have been produced from solutions and emulsions using spray drying with a variety of different polymers. Vidgren *et al.*, (1992) prepared microspheres using simple emulsions of carbopol 934P and reported 3.5 to 5.2 μm sizes. It was thought that the viscosity of the carbopol led to rough surface morphology. Conte *et al.*, (1994) reported that increasing temperature and spray rate had a great effect in reducing microsphere size. He *et al.*, (1999 a) prepared chitosan microspheres using an aqueous solution. The size of the nozzle was reported to affect the sizes of the particles. Microspheres of size 3.63 μm were prepared using 0.5 mm nozzle, and slightly larger sizes of 4.83 μm were reported with 1.0 mm nozzle.

Drug encapsulation efficiency is reported as being high using spray drying, but drug release is fast in some cases (Conte *et al.*, 1994; Bain *et al.*, 1999), for example cimetidine encapsulated in chitosan microspheres by spray drying aqueous chitosan solution, reached maximum drug release after 15 minutes. Particle size was formulated in the range of 1-5 μm. Microspheres were reported to gave good sphericity and effect of crosslinking agent on the particle size and zeta potential was reported: lower amount of crosslinking agent, increased the particle size and zeta potential (He *et al.*, 1999a).

1.5 Gastroretentive systems

Gastroretentive systems are designed to delay the removal of the dosage form from the gastrointestinal tract, acting as a platform for controlled release. In the design of these systems, the difficulty in localising controlled-release formulations within desired areas of the GI tract and the variable gastric emptying times of the stomach are the main obstacles to success. Over the last 30 years, various approaches have been pursued to increase the retention of an oral dosage form in the stomach, including floating systems, swelling and expanding systems, bioadhesive systems and high density systems.

1.5.1 Hydrodynamically Balanced Systems

Floating drug delivery systems have a bulk density lower than gastric fluids and therefore remain buoyant in the stomach, without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug is released slowly at a desired rate from the system. After release of the drug, the residual system is emptied from the stomach.

The first proposed type of gastroretentive dosage form using the floating principle was the hydrodynamically balanced system (HBS). The early HBS were non-disintegrating, hydrophilic matrix capsules or tablets (Sheth and Tossounian, 1984) containing the drug mixed in with a polymer (hydroxypropylmethylcellulose (HPMC)) and other excipients (hydrophobic fatty materials) to increase the buoyancy. After contact with gastric fluid, dissolution of the outer shell occurred and water absorption, followed by swelling of the polymer/drug mixture forming a gelled barrier at the outer surface, enabled buoyancy

(Moes, 1993).
In vitro visualisation of the HBS dosage form (Sheth and Tossounian, 1984) after immersion in gastric fluids showed that after 8 hours, there was a visible reduction in size of the device, with relative consistency of the shape and size of the tablet. An *in vivo* study using gamma-scintigraphy on two human subjects (Sheth and Tossounian, 1984) claimed that the HBS showed retention of up to 6 hours whereas a control (conventional tablet) had gastroretention of 1-2 hours. The characteristics and amount of the hydrophilic polymer in the device would be responsible for the floating characteristic of the device (Hwang *et al.*, 1998). This was investigated in another study using agar gel as the hydrophilic ingredient and the drug theophylline (Desai and Bolton, 1993). Compared with a non-floating form *in vitro*, the floating device was shown to have slower drug release attributed to the swelling of the polymer. *In vivo* analysis showed that in the fasted state there was no difference in the gastric emptying times between floating and non-floating forms. Under fed conditions, the floating tablet remained floating for 270 minutes and was reported to settle near the pylorus after 335 minutes, followed by emptying after 392-452 minutes. However, this was also the case for the non-floating form. It was thought that the floating device was propelled towards the pylorus due to the onset of the MMC, and hence its buoyancy did not prevent it from being driven down the stomach with the onset of the contractions (Desai *et al.*, 1993).

Another version of the HBS was developed which consisted of two layers which could increase the floating characteristics of the device, while separately increasing the release rate (Oth *et al.*, 1992). By gammascintigraphy, it was found that since the bilayer device remained in the upper part of the GI tract, it was prevented from emptying during the digestive phase. When subjects had a single meal before taking the bilayer capsule, it was emptied after about 3 hours, the completion of the digestive phase. When subjects were given meals before completion of the previous digestive phase, the device remained in the stomach for over 10 hours (Oth *et al.*, 1992), indicating that the presence of food can increase residence time (Hwang *et al.*, 1998).

These HBS systems have generally proved to be ineffective in prolonging gastroretention in the fasted state, but have led to the development of many other types of floating devices based on the same principles. As one of the main factors responsible for the floatation of these systems is the entrapment of air within the core of the dry material following gelation

of the outer boundary, gas-generating systems have been developed.

1.5.2 Effervescent systems

Effervescent or 'gas-generating' formulations generally include a carbonate- containing compound and sometimes an acidifying agent, which when reacted, rapidly produce carbon dioxide gas. Preparations based on this simple chemical reaction include lozenges, stomach distress preparations, analgesics, dental cleansers, adsorbents, soft drinks and vitamin supplements (Eichman *et al.*, 1997).

The systems can consist of single or multiple layers with the gas-generating phase in any layer. The carbon dioxide-generating component can either be mixed inside the tablet matrix (single-layered tablet) or the gas-generating agent can be contained in one hydrocolloid layer and the drug in another layer (bilayered tablet). More specifically devices where one of the tablet layers contained a carbon dioxide generating blend and a hydrophilic polymer have been developed. As the hydrophilic polymer gellates by action of the gastric fluids, the carbon dioxide is entrapped within the polymer and retains the buoyancy of the device. The second layer and remainder of the device contain the hydrophilic polymer and the drug to be released (Ingani *et al.*, 1987).

Chen and Hao examined porous gelatin capsules for release of verapamil, where gas bubbles were entrapped in the wall of the capsules and effervescence was achieved by incorporation of potassium bicarbonate. The capsules were hardened with formaldehyde, and were reported to float for over ten hours *in vitro* (Chen and Hao, 1998). Examination of such a device (Hilton and Deasy, 1992) in the fed and fasted states, showed no increased bioavalibility compared with a normal tablet. Further *in vivo* studies carried out using gamma-scintigraphy and urinary excretion rates, concluded that increased gastric residence times ≥ 4 hours were found in the lightly fed state but not in the fasted state, where residence times of <2 hours were found. There would seem to be no improvement in these types of devices over single layer ones in the fasted state, with the HBS showing increased gastric residence time (see section 1.5.1).

It has been speculated that gas-generating formulations may directly affect the GI physiological process such as gastric emptying, which could influence the residence time within the stomach. To address this, a study was performed on dogs comparing

effervescent liquid formulations with non-effervescent formulations (Eichman *et al.*, 1997). It was found that highly carbonated solutions created sufficient CO_2 to increase the pressure within the stomach such to change the motility pattern from fasted to pseudo-fed state. Therefore the residence duration of certain effervescent dosage forms is increased due to a direct effect on the motility of the stomach. It is possible that prolonged effect on the motility pattern could permanently affect the digestive system, though there is no evidence for this yet.

Results from *in vivo* studies using effervescent systems have not been consistent. Some studies have shown moderate (up to 25%) increased bioavailability (*e.g.* riboflavin) (Ingani *et al.*, 1987). In another study, the bioavailability of amoxycillin trihydrate was not improved using an effervescent device (Hilton and Deasy, 1992) in either the fed or fasted state compared to a non-effervescent device. Devices have only shown prolonged gastric residence times in the fed state (*i.e.* ≥ 4 hours). Strategies where effervescence have been proved to improve residence duration of devices and have been successfully marketed are raft systems (section 1.5.4).

1.5.3 Low density systems

There have been floating devices other than the HBS and effervescent type which may have more control over the actual floating mechanism by incorporating materials into the core of the device with lower densities. The exterior of the low-density material may be coated with the drug and release-controlling polymers.

Hollow polymeric microspheres with round cavities were examined as floating devices with an outer polymer shell containing the drug, where the internal cavity in the microsphere was formed by generation of a gas phase in the polymer/drug shell. (Kawashima *et al.*, 1991 a). It seemed that due to lower density and increased size (density ~ 0.7 g cm^{-1}, size 500 – 1000 μm) larger particles floated better (Kawashima *et al.*, 1991 a). Davis *et al.*, (1986) reported that light pellets (density 0.94 g cm^{-1}, size 0.7 – 1.0 mm) emptied from the stomach slower than heavy pellets (density 1.96 g cm^{-}1, size 0.7 – 1.0 mm) in the fed state. It was reported that the light pellets floated toward the fundus. However after further duration, the light pellets were reported to empty faster than the heavy pellets.

1.5.4 Raft systems

A floating dosage form that has been successfully marketed is the floating alginate raft, which has been used for the treatment of reflux oesophagitis. Unlike conventional antacids, these formulations form a viscous raft layer on the surface of the stomach contents, protecting the oesophagus from acid reflux. On entering the stomach, a colloidal gel consisting of alginic acid is formed by the sodium alginate reacting with the gastric acid. The incorporation of bicarbonate in the formulation provides gas-production, as on contact with the stomach contents carbon dioxide is formed. The alginate gel entraps the gas bubbles and the resulting foam rises to the surface of the stomach contents and floats. Calcium carbonate aids the gelation of the alginate since the salt dissociates to release divalent calcium ions which interact with the alginate (Johnson *et al.*, 1997). The alginate-containing antacids in raft formulations can reduce gastro-oesophageal disease and protect the oesophageal mucosa.

There are two types of alginate raft formulations. Oral liquid forms of raft-forming antacids contain sodium (or magnesium) alginate in solution (Table 1.5). On entering the stomach the sodium alginate reacts with the gastric acid and the raft is formed. Oral dry preparations are in the form of chewable tablets or powders (Table 1.5). These formulations contain alginic acid and not sodium alginate, since the products are intended to combat gastro esophageal reflux by forming the raft while traveling down the esophageaous forming a coat which protects it from reflux. The exception to this rule is Algicon® where magnesium alginate is used in both the tablets (chewable) and the suspension. This may be due to the presence of aluminium hydroxide-magnesium carbonate co-gel in the formulation, thus not requiring the combination of the chewing action of the mouth and the presence of saliva to form the gel. In all the other formulations, it is apparent from table 5 that sodium alginate is present in the suspensions and alginic acid is present in the tablets. Alginic acid is insoluble in water, but reacts with sodium bicarbonate in the presence of saliva or water to produce carbon dioxide and a viscous solution of sodium alginate (International Specialty Products, 2000). There are no solid oral raft formulations which can be swallowed without chewing.

A problem with alginate raft systems is that the inclusion of particular antacids may cause a marked reduction in cohesive strength. For example, it is reported that magnesium and

aluminium may reduce raft strength (Davies *et al.*, 1994). Not only do these antacids reduce strength and buoyancy, but they may also be unavailable for their neutralisation purpose due to entrapment by the alginate (Washington *et al.*, 1986).

Formulation	Constituents	Amount (mg)
Algicon® Tablets	Aluminium hydroxide-magnesium carbonate co-gel Magnesium alginate Potassium bicarbonate	360 500 100
Algicon® Suspension	Aluminium hydroxide-magnesium carbonate co-gel Magnesium alginate Potassium bicarbonate	140 250 50
Gastrocote® Tablets	Alginic acid Aluminium hydroxide Magnesium trisilicate Sodium bicarbonate	200 80 40 70
Gastrocote® Liquid	Sodium alginate Aluminium hydroxide Magnesium trisilicate Sodium bicarbonate	220 80 40 70
Gaviscon® 500 Tablets	Alginic acid Aluminium hydroxide Magnesium trisilicate Sodium bicarbonate	500 100 25 170
Gaviscon® 250 Tablets	Alginic acid Aluminium hydroxide Magnesium trisilicate Sodium bicarbonate	250 50 12.5 85
Gaviscon® Liquid	Sodium alginate Sodium bicarbonate Calcium carbonate	250 136 80
Gaviscon® Advance Suspension	Sodium alginate Potassium bicarbonate	500 100
Gaviscon® Infant	Sodium alginate Magnesium alginate	225 88
Peptac® Suspension	Sodium alginate Calcium carbonate Sodium bicarbonate	250 80 134
Topal® Tablets	Alginic acid Aluminium hydroxide Magnesium carbonate Sodium bicarbonate	200 30 40 40
Rennie® Duo Suspension	Sodium alginate Calcium carbonate, Magnesium carbonate	150 600 70

Table 1.6 Marketed raft formulations (British National Formulary, 2002)

As an alternative to antacids it has been suggested that alginate-cimetidine therapy could be used for the reduction of the gastric acidity. It has been suggested that the decreased acid secretion from treatment with H_2 -receptor antagonists could inhibit the anti-reflux capacity of the raft (Britton *et al.*, 1991). Using gamma scintigraphy in 12 normal subjects, liquid Gaviscon® was shown to have an increased residence time with a meal during a period of cimetidine treatment and after. Cimetidine pretreatment however only slightly increased the time for half the meal and Gaviscon® to empty from the stomach (Washington *et al.*, 1993).

In general for floating devices to be effective, sufficient amounts of liquids are needed in the stomach (i.e. ~ 150 ml). Devices that do float would be likely to be emptied from the stomach during the housekeeping wave with retention of nearly about three hours at best. Even though there have been countless designs of these types of floating forms, all have been emptied during the housekeeper wave (see sections 1.5.1, 1.5.2 and 1.5.3). With gas generating devices, if the effervescent component is of sufficient amount, the device could have an effect on the MMC state and remain for longer. Some devices which have been reported to reside longer in the stomach in the fed state (Sheth *et al.*, 1984; Ingani *et al.*, 1987; Hilton *et al*, 1992; Oth *et al.*, 1992; Desai *et al.*, 1993).

For successful buoyancy of raft systems, there would have to be sufficient acid activity in the stomach for the raft to form, which would be the case for patients requiring cimetidine treatment. Therefore, since both therapies are used in cases of gastro-oesophageal reflux, in the design of delivery systems for cimetidine, raft systems may be ideal as there is no significant effect on the anti-reflux properties of the raft from cimetidine (see chapter 5).

1.5.5 Swellable hydrogel systems

Swelling type dosage forms are such that after swallowing, these products swell to such an extent that their exit from the stomach through the pyloric sphincter is prevented (i.e. > 1mm)b. The dosage form is therefore retained in the stomach for a prolonged period of time.

Polymers have been found to be promising candidates for development of swellable dosage

forms. These polymers do not behave as intelligent materials responding to the presence of triggering stimuli such as pH, ionic strength and temperature, but respond to the presence of water or biological fluids, which change the polymer structure allowing release of the drug from the dosage form. Swellable materials can be incorporated into devices, enabling expansion on contact with gastric fluids and as they move through the various regions of the gastrointestinal tract, allow drug release. Gastroretention may be achieved since the enlarged device is restricted from passing through the pylorus. The residence time, coupled with available water and local environmental conditions in the different regions of the gastrointestinal tract, can potentially influence the rate and extent of this swelling.

The swellable materials used, maintain three dimensional networks and do not dissolve in water. The aqueous gel networks are called hydrogels, and are usually made of hydrophilic polymer molecules which are crosslinked either by chemical bonds or other cohesion forces such as ionic interaction, hydrogen bonding, or hydrophobic interaction. Hydrogels are elastic solids, which can return to their original configuration following alteration of the form after prolonged periods of time.

Devices may consist of either a solid drug core or crosslinked matrix with the drug in a dispersed or dissolved state. The release of water-soluble drugs from the initially dry hydrogel membrane would be controlled by the membrane swelling following dissolution or core swelling. If the membrane has been originally saturated with drug, then a simultaneous absorption of water and drug release by a swelling-controlled diffusion mechanism is observed (Ravichandran *et al.*, 1997). As water penetrates the membrane, the polymer swells due to ionic repulsion, and the glass transition temperature lowers. Simultaneously, the dissolved drug diffuses through the swollen flexible region into the external release medium.

An interpenetrating network (IPN) is any material containing two polymers, each in network form. A Semi-IPN is an IPN, which consists of one crosslinked and one un-crosslinked polymer. IPN present a means of enhancing the compatibility of polymer components and preventing phase separation (Park *et al.*, 1993). Mechanical strength of the hydrogel can be increased using a relatively hydrophobic polymer as the second polymer. One polymer network or both networks can be made biodegradable (Park *et al.*, 1993).

In drug release from initially dry hydrogels, the drug is immobile in the dry (glassy) material, but becomes mobile when the matrix is swollen by water (*e.g.* in gelatin microspheres or hydrogels). Drug release can be defined as swelling-controlled in two cases. If the release is controlled by penetration of water into the glassy matrix, or if the matrix is penetrated and plasticised rapidly, but then swells slowly. If the matrix is hydrophilic, the increased drug motility can be so great that the swollen layer does not offer much resistance to drug transport, and rate of drug release is dependant on the rate of water penetration. Normally however, as the hydrogel swells, the growing layer represents a significant resistance to drug transport and the overall rate of the drug release is dependant on the rate of water uptake and diffusion of the drug through the swollen gel. If the gel layer continues to swell as the process continues, the diffusion coefficient of the drug in the gel will be increased with increasing water content in the gel. Therefore, the swelling can be a controlling factor in drug release. For hydrogels to be considered as gastroretentive devices, they are required to undergo considerable size expansion to prevent premature gastric emptying and biodegradation. Highly swellable biodegradable hydrogels could suit this purpose (Ravichandran *et al.*, 1997).

Superporous hydrogels differ from conventional hydrogels in that they swell to equilibrium size in minutes due to the water uptake by capillary wetting through numerous interconnected open pores (Chen *et al.*, 2000). Swelling ratios of these hydrogels were reported to be in the hundreds. *In vivo*, it was reported that the hydrogels remained in the stomach for up to 4 hours in the lightly fed state.

Hydrogels have been studied extensively for their biomedical applications due to their high water content, giving good biocompatibility (Kost and Langer, 1986). pH-sensitive hydrogel devices for oral controlled delivery systems, can release the drug in the required location in the gastrointestinal tract, and remain there due to the swelling and increased size of the device. pH-sensitive hydrogels normally contain pendent acidic or basic groups such as carboxylic acids and primary amines, or strong acid and bases, such as sulphonic acids and sodium salts which change ionisation in response to changes in pH, thus altering the properties of the gel (Ravichandran *et al.*, 1997).

pH-sensitive hydrogels targeted for stomach specific delivery were prepared using poly (N-vinyl pyrrolidine-acrylic acid)-polyethylene glycol) (Ravichandran *et al.*, 1997) by free

radical polymerisation, entrapping 5-flouracil. The drug release was found to plateau at 65% after 24 hours. Swelling of the gel was found to be faster in higher pH medium (200% swelling at pH 1.2, 500% swelling at pH 7.4). Initial drug release was attributed to drug entrapped on the surface of the hydrogel, and secondary release was attributed to diffusion.

Hydrogels from crosslinked chitosan and polyether (Yao *et al.*, 1993) showed maximal swelling in pH 3.2 medium. At pH 7.4, the equilibrium swelling of the hydrogel was reduced with increased amounts of polyether, as this affected the intensity of the hydrogen bonding in the network. Cimetidine was entrapped in such a hydrogel and showed very fast drug release plateauing after 45 min (pH 1.0) and after 25 min (pH 6.8) (Yao *et al.*, 1994)

Gelatin-polyethyleneoxide (PEO) semi-IPN hydrogels incorporating riboflavin were prepared by variation of the PEO molecular weight and concentration in the network to optimise the swelling ratio (Amiji *et al.*, 1997). The swelling ratio of gelatin-PEO hydrogels was significantly higher in simulated gastric fluid (SGF) than in simulated intestinal fluid (SIF) regardless of the molecular weight or concentration of the PEO. The full drug load was released in about 8 hours.

Chitosan–polyethyleneoxide semi-IPN hydrogels were prepared by freeze-drying and air-drying, incorporating amoxycillin and metronidazole (Patel and Amiji, 1996). Freeze-dried hydrogels were reported to be highly porous with pore sizes of 8-10µm. The pores had a significant influence on the swelling rate of the hydrogel in SIF and SGF. Freeze-dried hydrogels had a faster release and higher swelling ratios than air-dried hydrogels. For example, after 2 hours, drug release from freeze-dried hydrogel in SGF was 65%, swelling ratio 20; release from air-dried hydrogel was 20% and swelling ratio 4 (Patel and Amiji, 1996).

1.6 MUCOADHESIVE DRUG DELIVERY SYSTEMS

1.6.1 Mucoadhesion

Adhesion is defined as the state where an interface is formed between two bodies such that the molecular forces across the interface resist separation. The materials being joined together are termed adherents or substrates. Therefore an adhesive between two adherents or substrates would be considered an adhesive joint (Zaman *et al.*, 1999). Bioadhesion has been defined as "the attachment of synthetic or natural macromolecules to a biological substrate". If the substrate is a mucosal membrane covered with a coating of mucus, bioadhesive interactions occur mainly within the mucous layer, and this phenomenon is referred to as "mucoadhesion" (Gu *et al.*, 1998).

Mucoadhesion is of interest in development of multi-particulate oral delivery strategies, as immobilisation of the drug-carrying particles at the mucosal surface could result in:

- prolonged residence time at the site of drug action or absorption
- localisation of the delivery system at a given target site
- an increase in the drug concentration gradient due to intense contact with mucosal surface
- direct contact with intestinal cells

There are two ways in which the delivery system could adhere to the mucosal surface, either by (a) binding to the tissue itself or (b) association with the mucus coat (Figure 1.7), which is associated with the tissue surface. Particles which are small enough to be buried inside the mucus may be held securely there, but during mucus secretion, the particles would be pushed further from the mucosal surface until they are removed by the weight of the gel or the luminal contents exertion. Larger particles could be pulled from the mucosal surface due to their weight or dislodged by the contents of the stomach. Scherrer *et al.*, (1993) reported using confocal microscopy that flourescently labelled particles (211 nm diameter) penetrated at least 60 μm deep into the mucus layer of rat intestine. It has also been reported that 200 nm nanocapsules were visualised in close contact with the mucus layer in rats using scanning electron microscopy (Damge *et al.*, 1990). As proposed by Ponchel *et al.*, (1997), it can be generalised that fine particles ≤ 1 μm penetrate the mucus

layer, whereas larger particles (2 μm polystyrene) are associated with the surface of the mucus layer.

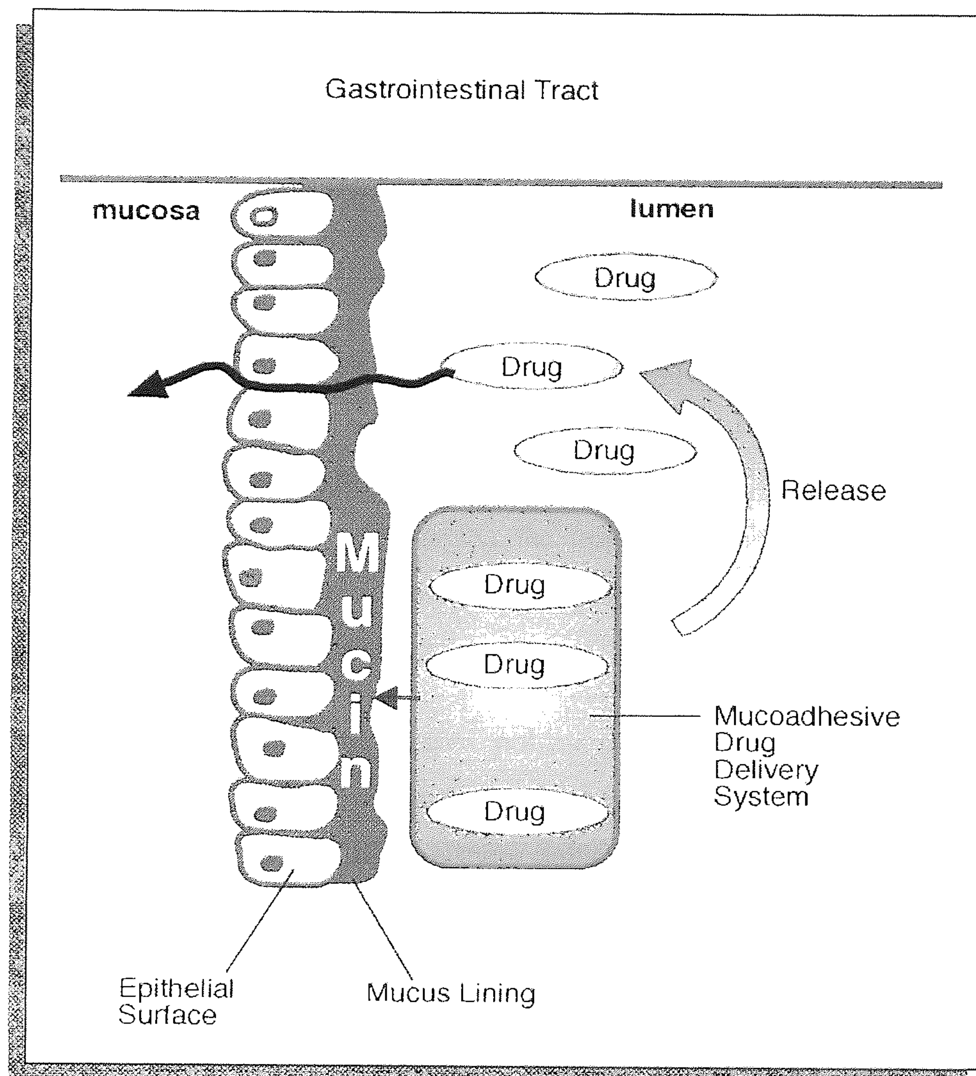


Figure 1.7 Illustration of drug delivery to the GI tract using a mucoadhesive drug delivery system (Adapted from Chien *et al.*, 1992)

1.6.2 Mucoadhesive polymers in drug delivery

Mucoadhesive polymers are synthetic or natural polymers, which interact with the mucus layer, which covers the mucosal epithelial surface and mucin molecules, which constitute a major part of mucus. The concept of mucoadhesives could be used to overcome physiological barriers in drug delivery.

Mucoadhesive polymers may be incorporated into drug delivery devices to retard the GI transit of controlled-release dosage forms for drugs whose absorption is restricted to the

upper regions of the GI tract. Some drugs are absorbed in specific regions of the upper GI tract, e.g. chlorothiazide, riboflavin and cimetidine, which is absorbed from the upper part of the small intestine (Fix, 1999). For most drugs, it is thought that absorption in the colonic region is significantly lower than in the small intestine, likely to be due to differences in the lumenal contents (i.e. decreased fluid volume, see section 1.2) of the colon which can impair the dissolution and diffusion of drugs from the lumen to the epithelial cell wall. The differences could also impose additional degradative pathways, such as bacterial enzyme activity, which could also decrease drug absorption. Due to this problem, controlled-release dosage forms where release times exceed 5-6 hours, are likely to be disrupted from from zero- or first-order kinetics due to the poor absorption. To overcome this problem, and extend effective release time of controlled-release dosage forms, there is great interest in prolonging the upper GI residence time of controlled-release dosage forms, such that a greater proportion of the drug load is released in an environment where reliable absorption can occur.

A disadvantage of known mucoadhesive polymers is their non-specificity, where the mucoadhesives are unable to distinguish between adherent or shed-off mucus, or the surfaces of other gut contents. Therefore these polymers may adhere to surfaces for which they are not intended (Lehr *et al.*, 2000). Furthermore, the mucus gel layer is thought to be renewed at a constant turnover rate of 47-120 minutes, with a range of a few minutes to hours in dogs (Lehr *et al.*, 1992 c; Rubinstein and Tirosh, 1994). It has been suggested that these values differ from human values, with estimated values by mucosal protein turnover rate 1.8 and 2.6 days (O'Keefe *et al.*, 1993).

Duchene *et al.*, (1988) proposed the following two stages in the process of mucoadhesion: Stage 1 is the establishment of an intimate contact between the mucoadhesive polymer and the mucus gel. Stage 2 is where the mucoadhesive polymer could penetrate the mucus gel network, which results in the formation of physical chain entanglements and secondary chemical bonds between the mucus gel and the mucoadhesive material.

The interaction between the polymer and mucin is composed of attraction and repulsion. The magnitude of the phenomenon determines whether they will interact. When the attractive interaction is higher than the non-specific interaction, mucoadhesion occurs. Attractive interactions can arise from Van der Waals forces, electrostatic interactions,

hydrogen bonding, and hydrophobic interactions. Repulsive interactions can occur due to electrostatic and steric repulsions.

The performance of an adhesive material and the measurement of its mucoadhesion can be affected by many factors both *in vitro* and *in vivo*. Different polymers with a wide range of structures have exhibited mucoadhesive properties (Table 1.7). Some of these polymers have been classified with respect to their adhesive properties.

A general consensus is that polymers bearing strong anionic charges and those bearing a number of carboxylic or hydroxylic groups have good binding potential (Zaman *et al.*, 1999). Furthermore, sufficient chain flexibility is necessary for the penetration of mucosal networks and sufficient spreading of the polymer over the mucosal surface. Polyacrylic acids carrying anionic charges have been used in mucoadhesive delivery systems (Longer *et al.*, 1985; Lehr *et al.*, 1992 a; Akiyama *et al.*, 1994; Leucuta *et al.*, 1997) High molecular weight cationic polymers such as chitosan have also been used extensively in mucoadhesive systems (He *et al.*, 1998; Lim *et al.*, 2000). Sodium alginate preparations have also attracted attention due to their ability to interact with the mucus layer (Chickering *et al.*, 1997).

Polymer	**Mucoadhesive ability**
Acacia	Poor
Chitosans	Excellent
Carboxymethylcellulose	Excellent
Carbopol	Excellent
Dextran	Good
Gelatin	Fair
Pectin	Poor
Poly(methyl methacrylate)	Excellent
Poly(acryl amide)	Good
Poly(acrylic acid)	Excellent
Polycarbophil	Excellent
Sodium alginate	Excellent

Table 1.7 Comparison of mucoadhesive properties of polymers (table from Zaman *et al.*, 1999)

1.6.3 Mechanisms of mucoadhesion

The theories of polymer-polymer adhesion can be adapted to polymer-tissue adhesion or bioadhesion, with consideration that bioadhesion differs due to the differing properties of the tissue as opposed to those of the polymer. Different theories exist in explanation of the bioadhesion phenomenon. These theories include electronic theory, adsorption theory and diffusion theory. It is thought that though these theories involve independent mechanisms and are not exclusive (Chickering and Mathiowitz, 1995).

There are three ways in which a mucoadhesive could interact with the mucus layer in terms of actual bonding: physical, secondary chemical or primary (Zaman *et al.*, 1999). Secondary chemical bonding contributes to the adhesion of the mucoadhesive to the mucus layer through Van der Waal's forces and hydrogen bonding. Hydrogen bonds arise from the bonding between hydrophilic functional groups such as hydroxyl groups, carboxyl groups, sulphate groups and amino groups. Primary bonds are formed from the chemical reaction between the functional groups of the adhesive and the mucus layer, resulting in strong covalent bonds.

In the electronic theory, a double layer of electrical charge is present at the interface between the mucoadhesive and the mucus layer, which is resultant from transfer of electrons upon contact. This transfer of electrons occurs due to the difference in structure between the mucoadhesive and the glycoprotein chains in the mucus, and mucoadhesion is the attraction across the electrical double layer (Huang *et al.*, 2000; Zaman *et al.*, 1999). For example, electrostatic attraction has been proposed for adsorption of chitosan mucoadhesive microspheres. It is reported that the adsorption of mucin to chitosan is dominated by the electrostatic interaction between the positively charged chitosan and negatively charged mucin (He *et al.*, 1998).

In the diffusion theory, it is assumed that as soon as the mucoadhesive polymer and the mucus layer have made contact, a process called interpenetration occurs. Polymer chains and the components that make up the thick gel-like structure of mucus, diffuse into one another, which leads to the formation of an intimately mixed layer of polymeric chains and mucus, allowing the creation of a semi-permanent bond (Mikos and Peppas, 1990). It is reported that one of the main structural properties which facilitate the interpenetration of the mucoadhesive into the mucus layer is that the mucoadhesive must have sufficient

polymer chain mobility and flexibility allowing diffusion. Properties of the mucoadhesive such as molecular weight, degree of crosslinking and degree of swelling are contributing factors to the diffusion into the mucus layer (Akiyama *et al.*, 1994; Abd Hameed and Kellaway, 1997; Huang *et al.*, 2000). The mucoadhesive is first brought into intimate contact with the mucus, and over time, the concentration gradient across the interface causes the diffusion of the chains of the mucoadhesive into the mucus layer and the diffusion of the glycoprotein chains of the mucus into the mucoadhesive polymer. The rate of the diffusion is dependant on the chemical potential gradient and the diffusion coefficient of a macromolecule through a crosslinked network. The chains which have diffused across the interface serve as anchors in the attachment of the bioadhesive device in position (Mikos and Peppas, 1990; Huang *et al.*, 2000).

The adsorption theory explains mucoadhesion through secondary forces such as Van der Waals forces and hydrogen bonding. When discussing different phenomena occurring following the administration of mucoadhesive microspheres by the oral route, and resulting adsorption, the following general dynamic description was proposed by Ponchel *et al.*, (1997) (Figure 1.8).

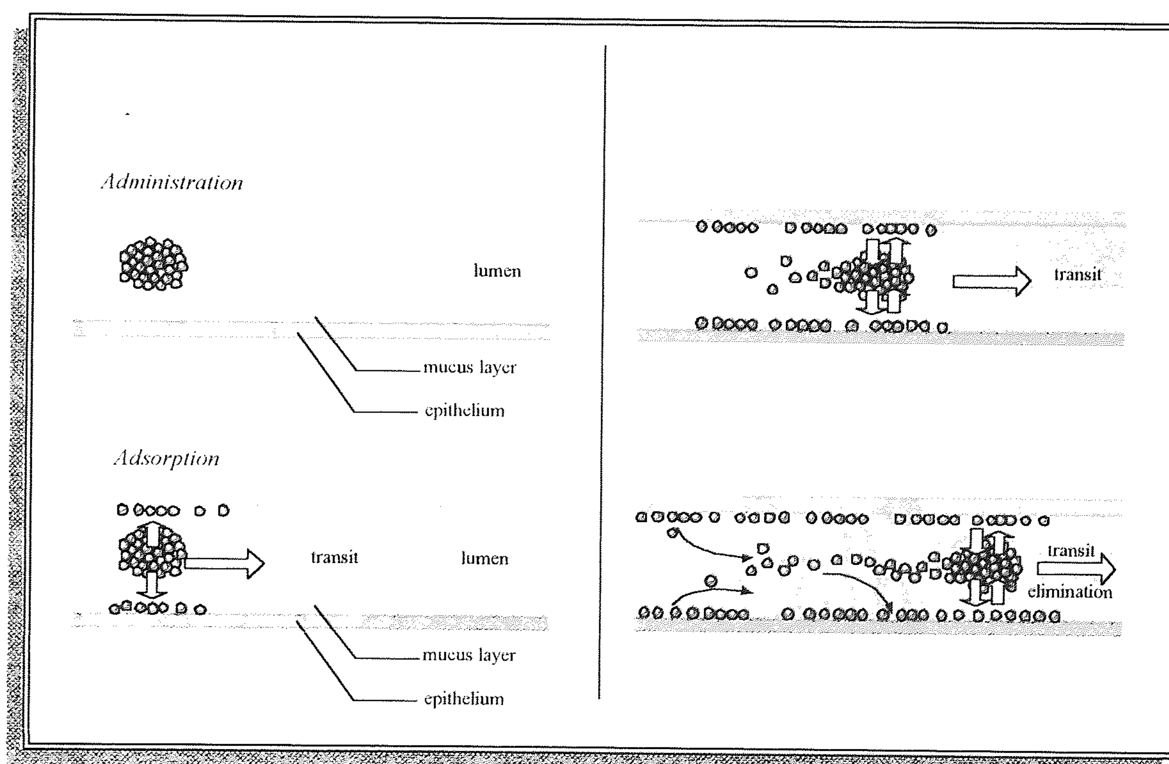


Figure 1.8 Mucoadhesive behaviour of mucoadhesive particles following oral administration (Adapted from Ponchel *et al.*, 1997)

Firstly, the administered particles (in suspension) enter into contact with a portion of the mucosa (step 1). From this point, the concentrated suspension acts as a reservoir of particles and very rapidly, an adsorption process takes place, leading to an adsorption of a fraction of the available particles (step 2). Adsorption occurs with the mucus layer in an irreversible process. However, the luminal particle suspension transits through the whole intestine, sweeping progressively the whole mucosa. The simultaneous adsorption process results in a progressive covering of the intestinal mucosa by adhering particles (step 3). Finally, detachment of the particles from the mucosa begins to occur in the proximal region and is progressively extended to the distal region (step 4). Non-adhering particles from the lumen pool and detached particles from the mucoadherant pool are finally eliminated in the faeces (Ponchel *et al.*, 1997).

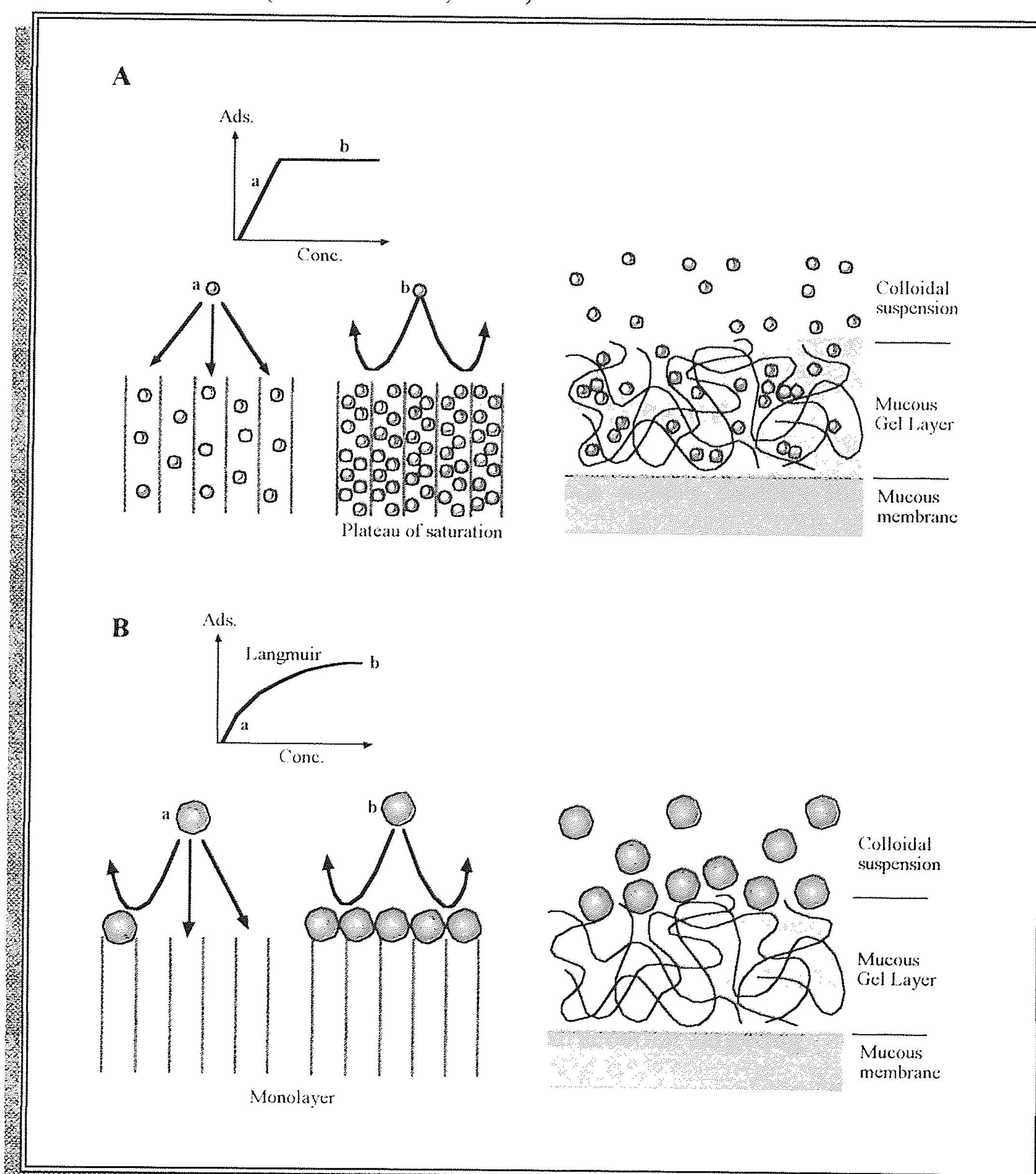


Figure 1.9 Adsorption isotherm shapes and corresponding adsorption models. A: case of particles < 1 μm. B: case of particles > 1μm (Adapted from Takeuchi *et al.*, 2001)

Ponchel *et al.*, (1997) discussed mechanisms for the adsorption of particles to mucus under static conditions. Considering smaller particles below 1 μm, the mucus gel constitutes a porous rather than a smooth adsorbent, and the adsorption kinetics can be controlled by diffusion into the mucus network (Ponchel *et al.*, 1997; Takeuchi *et al.*, 2001). Therefore, particles below 1 μm have the characteristic isotherm shape of adsorbates, which penetrate into the mucus layer (Figure 1.9a). In the case of larger particles, such as 2 μm, adsorption follows a Langmuirian type (Figure 1.9b). The adsorbate then adsorbs in a monolayer on the adsorbent surface, which behaves like a smooth surface.

The wetting theory relates the mucoadhesion of a material to its ability to spread over the surface of the mucus layer allowing intimate contact between the two. The theory uses analysis of the spreading coefficient of a liquid material over a tissue by the displacement of the surrounding gastric fluid. In this theory, the mucoadhesive exhibits a zero or near zero contact angle when applied to the mucus layer, and has a low viscosity so that air is excluded enabling intimate contact (Mikos and Peppas, 1990)

1.6.4 Lectins for prolonging mucoadhesion

The concept of cytoadhesion (where compounds adhere directly to the cell membrane, Lehr *et al.*, 2000) is based on specific receptor-ligand-like interactions, where these bioadhesives bind directly to the cell surface by specific receptor-mediated interactions. The specific binding or adhesion is mainly based on lectin-sugar interactions. The mucous epithelium consists of polarised cells which are connected by tight intercellular junctions. Transport of molecules across the epithelial barrier can occur by passive diffusion *via* transcellular or paracellular pathways, or *via* active processes mediated by membrane-bound carriers or membrane-derived vesicles (endo/trancytosis). Passive cellular diffusion and carrier mediated transport are restricted to small molecules. Desai *et al.*, (1997) reported that particles larger than 1 μm are taken up by CaCO-2 cells by mechanisms other than endocytosis, and particles < 0.1 μm have intracellular targeting.

100 years ago the first plant lectin was recognised which agglutinated red blood cells (Haltner *et al.*,1997). Later on, from the discovery of more substances from various plants,

the term haemagglutinating was recognised, and some of these being specific for blood groups led to the term lectin, which means to select. Lectins share the common ability to bind specifically and reversibly to carbohydrates.

Lectins are found in plants, which constitutes their largest population, and in some vertebrates, referred to as endogenous lectins. They can also be produced from bacteria or invertebrates.

1.6.4.1 Coating of particles with lectins

Tomato lectin (TL) coated polystyrene microspheres (0.98 μm) have shown specific binding to enterocytes *in vitro* (Lehr *et al.*, 1992 b). Fluorescently-labelled polystyrene microspheres were coated with TL and incubated overnight with CaCO-2 cells in an epithelial model. It was observed that the lectin-coated microspheres remained attached to the cells following repeated washings while bovine serum albumin (BSA) coated microspheres were washed off. The binding was not dependant on whether the cells were living, dead, fixed or not fixed.

A drawback with TL prolonging GI delivery is in the potential for cross-reactivity with mucus. It has been suggested that mucus glycoproteins will inhibit lectin binding, due to the existence of a core protein glycosylated with lectin-binding *N*-glycans (Lehr *et al.*, 1992 b). It is also suggested that the TL will act as a mucoadhesive and not bind exclusively to the cell surface (MacAdam, 1993). As TL favours binding at neutral pH, it is more likely to be suited to small intestinal applications.

A study to improve the absorption efficiency of orally administered polymerised liposomes consisted of modification of the liposomal surface with *Ulex europaeus* agglutinin I (UEA I). The lectin has reportedly shown to be M cell specific when exposed to mouse Peyers patches. *In vivo*, it was reported that significantly higher levels of Peyer's patch binding was observed with the lectin-modified particles, and following high dosing levels, the lectin-bearing liposomes were found in the intestinal epithelium (Chen *et al.*, 1996).

In the presence of pig gastric mucin, the binding of the wheat germ agglutinin (WGA) nanospheres to the CaCO-2 cell line increased over time (Gabor *et al.*, 2000). The mucin-WGA interaction was found to be reversible. It was also found that the nanosphere association with CaCO- cells increased with the level of WGA coating.

However some lectins may be toxic and unsuitable for oral delivery. It is reported that wheat germ agglutinin has a negligible peroral toxicity of 300 mg /kg of WGA, the equivalent of that contained in flour (Chen *et al.*, 1996). However it has shown toxicity *in vitro* to certain cells as it has been shown that it induced toxicity in human pancreatic cells in a dose-dependant manner (Schwarz *et al.*, 1990).

It is not known whether simple coating of the microspheres with the lectin could be sufficient to achieve adherence. It is likely that a coating could become removed from the particle during adhesion or before. Other techniques for the attachment of lectins to particles are conjugation or coupling.

1.6.4.2 Attachment of lectins to particles using coupling techniques

Lectin-coupled nanoparticle conjugates were produced using a two-stage carbodiimide process with binding of the lectin to the surface of gliadin (which is a protein) nanoparticles (Arangoa *et al.*, 2000). There are two main methods for coupling lectins to particles; glutaraldehyde coupling and carbodiimide coupling, both involve the activation of carboxylic acid groups to give NH-activated groups, which can react with free amino groups of the ligand polypeptide chains (Irache *et al.*, 1996). In proteins, these carboxylic groups can be found on aspartic and glutamic acid residues. Gliadin has similar amounts of apolar and neutral amino acids, mainly glutamine (~40%) and proline (~14%), is a hydrophobic protein with a low solubility in water due to the presence of both intrapolypeptide disulfide bonds and hydrophobic domains.

Tomato lectin (TL) has been shown to bind selectively to the small intestine epithelium (Carreno-Gomez *et al.*, 1999). In one study, using the everted gut sac model, lectins were coupled to polystyrene microspheres and rates and extent of uptake compared with bovine serum albumin (BSA). It was reported that the uptake of tomato lectin into the serosal fluid

was 8-fold higher than that of BSA (Carreno-Gomez *et al.*, 1999). BSA coated microspheres were shown to have slower uptake rate than TL coated microspheres by factor of 2. Another study was carried out using fluorescent polystyrene nanoparticles (500 nm) and tomato lectin (Lehr *et al.*, 1992b). It was found that significant levels of TL-conjugates remained bound to the intestinal epithelium.

Nanoparticle-lectin conjugates were prepared using the carbodiimide coupling reaction by Ezpeleta *et al.*, (1996). The amino groups of *Ulex europaeus* (UEA I) lectin were coupled to the carbodiimide-activated carboxylic groups of vicillin (a protein) with a coupling efficiency of 20%. UEA I was also conjugated with gliadin nanoparticles (Ezpeleta *et al.*, 1999) using the carbodiimide method with coupling efficiency of ~16%. By this method, the lectin remains firmly attached to the particle because no free lectin was detectable following washing. The time of the carbodiimide activation was found to be an important factor in the conjugation and the ideal contact time was quoted to be 3 hours.

Simple coating of the microspheres with the lectin been used by many workers (section 1.7.1). However it is possible that with a lectin coating, the mechanism of adhesion may not be that strong and the lectin may become unattached from the microsphere during mucoadhesion. The methods for the effective combination of lectin with particles are the coupling methods using glutaraldedhyde or carbodiimide. The reactions are relatively straight forward and lectins have been shown to remain attached to the particles (Ezpeleta *et al.*, 1999). Therefore the coupling technique can be used for the attachment of a lectin to gelatin.

1.7 CIMETIDINE

Cimetidine (Tagamet) is one of the most commonly prescribed drugs in the world. Used by millions of people around the world to treat stomach ulcers and related digestive disorders, this drug was found to have immuno-stimulant, anti-tumour and anti-metastatic effects.

Cimetidine is sold under the brand name Tagamet and is a H_2-receptor antagonist, which inhibits the secretion of basal and gastric hydrochloric acid secretion also reducing the output of pepsin It is commonly used to treat stomach and duodenal ulcers, inflammation

of the oesophagus and other digestive disorders. This drug has been exceptionally effective in management of peptic ulcer disease and other acid-related disorders. Amongst its clinical uses are the treatment of duodenal ulcer, gastric ulcer, reflux oesophagitis, upper gastrointestinal haemorrhage and Zollinger-Elison syndrome However also has an anti androgenic effect; It blocks the binding of dihydrotestosterone to its receptor, and as such has also been used to treat hirsuitism in women (excess facial hair).

The structure of cimetidine is shown in figure 1.10 and the physicochemical properties are summarised in table 1.7.

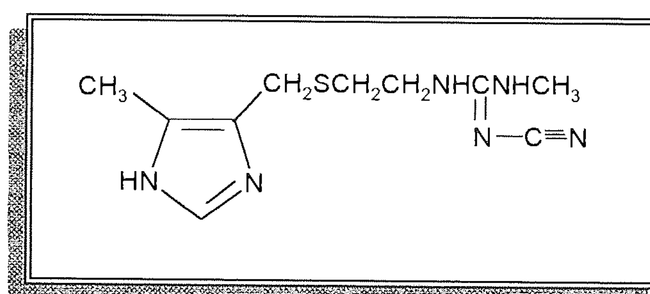


Figure 1.10 Structural formula of cimetidine

Structural formula	$C_{10}H_{16}N_6S$
Solubility, mg/ml	11
pK$_a$	6.8
Molecular weight	252.3
logP	0.40

Table 1.7 Physicochemical properties of cimetidine (GlaxoSmithKline, 1984; Bavin *et al.*, 1984)

Cimetidine has a bitter taste and characteristic odor. It has the following solubility characteristics: soluble in alcohol, slightly soluble in water, very slightly soluble in chloroform and insoluble in ether. Cimetidine hydrochloride is freely soluble in water, soluble in alcohol, very slightly soluble in chloroform and practically insoluble in ether (the liquid and injection dosage forms contain cimetidine as the hydrochloride).

The cimetidine tablets are available in 200, 300, 400 and 800 mg doses. Inactive ingredients consist of cellulose, hydroxypropyl methylcellulose, iron oxides, magnesium stearate, povidone, propylene glycol, sodium lauryl sulfate, sodium starch glycolate, starch, titanium dioxide and trace amounts of other inactive ingredients.

Cimetidine is widely distributed throughout the body, except for the central nervous system, localising in the gastric mucosa (GlaxoSmithKline, 1984). Drug concentrations in the bile are recorded at several times higher than those found in portal and peripheral blood; however, < 2% of the given dose undergoes enterohepatic circulation.

A large proportion of radiolabelled cimetidine is excreted unchanged in the urine following oral or parenteral administration. The principle metabolite of cimetidine is the sulphoxide, which is produced by oxidation of the side chain thioether link (-S). The sulphoxide accounts for about 10% of recovered radioactivity after an oral dose (GlaxoSmithKline, 1984). The 5-hydroxy methyl analogue accounts for about 4%.

Cimetidine is absorbed from the gut after oral administration with a bioavailbility of 70% (GlaxoSmithKline, 1984). Hepatic transformation of the drug is 30-40% of the oral dose. The total protein binding of 13-26% is reported to have no major clinical or pharmacokinetic significance (GlaxoSmithKline, 1984). During the first hour after a standard experimental meal, oral cimetidine 300 mg inhibited gastric acid secretion in duodenal ulcer patients by at least 50%. During the subsequent 2 hours cimetidine inhibited gastric acid secretion by at least 75%. The effect of a 300 mg breakfast dose of cimetidine continued for at least 4 hours and there was partial suppression of the rise in gastric acid secretion following the luncheon meal in duodenal ulcer patients. This suppression of gastric acid output was enhanced and could be maintained by another 300 mg dose of cimetidine given with lunch.

In another study, cimetidine 300 mg given with the meal increased gastric pH as compared with placebo. Mean gastric pH following cimetidine administration after 1, 2, 3 and 4 hours was 3.5, 3.1, 3.8 and 6.1 compared to 2.6, 1.6, 1.9 and 2.2 with placebo.

Peak blood concentrations usually occur within 60-90 minutes in fasting patients and are dose related for doses up to 400 mg. Peak blood levels of cimetidine are lower and occur

later, in the fed state and when taken before or after meals. Cimetidine is usually given three times daily with meals and again at bedtime to inhibit nocturnal and basal acid secretion.

Cimetidine, apparently through an effect on certain microsomal enzyme systems, has been reported to reduce the hepatic metabolism of warfarin-type anticoagulants, phenytoin, propranolol, nifedipine, chlordiazepoxide, diazepam, certain tricyclic antidepressants, lidocaine, theophylline and metronidazole, thereby delaying elimination and increasing blood levels of these drugs (Micromedex, 2002)

Toxicity in adults is reported to include diarrhea, dizziness, myalgia, rash, gynecomastia, confusion or psychosis (reversible), elevated creatinine and possibly neutropenia.

Cimetidine has been reported to increase the pharmacologic effects or toxicity of alprazolam, carmustine, chlordiazepoxide, clorazepate, diazepam, flurazepam, lidocaine, metoprolol, phenytoin, procainamide, propranolol, quinidine, theophylline, triazolam, and warfarin (Micromedex, 2002)

The need for frequent dosing can be reduced using sustained release formulations. The necessity for frequently repeated administration to maintain therapeutically effective plasma drug levels can present problems for patient compliance. Cimetidine, which has a frequent dosing regime can benefit from incorporation into controlled-release dosage systems.

1.8 Aims and Objectives

The aims of the thesis were initially to develop a controlled-release microsphere formulation for the delivery of cimetidine over 12 hours and to determine the maximum loading, particle size range and mucoadhesion. Two assays would be used to measure the mucoadhesion of the microspheres: an *in vitro* model and *ex vivo* model. The microsphere system was then to be further developed into a GI tract targeting system by incorporation of targeting agents such as mucoadhesive polymers by (1) polymer blending of gelatin with modifying polymer and (2) surface modification of the gelatin microspheres. The two

methods would be compared in terms of mucoadhesion, drug release, drug loading and particle size. A suitable lectin would be chosen and incorporated with the gelatin microspheres, its level of incorporation determined and mucoadhesion assessed. Gastroretentive strategies, which could provide retention in the stomach and provide controlled-release of cimetidine were to be developed based on floating and swelling systems. Hydrogels would be formulated, swelling studies carried out and potential of the hydrogels as a platform for the delivery of microspheres assessed. The alginate raft system would be formulated by variation of appropriate excipients. The potential of this system as a platform for the delivery of microspheres would also be assessed.

CHAPTER TWO

MATERIALS AND METHODS

2.1 SUMMARY

This chapter provides an overview of the experimental techniques used. Specific methods relevant to individual chapters are included in the appropriate chapter and section. Unless otherwise stated, results are expressed as the mean ± the standard deviation of at least three values. All materials were obtained from Sigma/Aldrich (Poole, UK) unless otherwise stated and were of analytical grade unless otherwise specified.

2.2 STATISTICAL ANALYSIS

Statistical tests performed include the two-way analysis of variance, and where 3 or more results were compared, Dunnetts test or Tukey test were used. The analysis was carried out using the Primer of Biostats software (version 4.0) by McGraw Hill.

2.2.1 Dissolution profile comparison using similarity factor, f2

Among several methods investigated for dissolution profile comparison, f2 is the simplest. Moore and Flanner (1996) proposed a model independent mathematical approach to compare the dissolution profile using factor f2.

$$f_2 = 50*\log\{[1+(1/n)\Sigma_{t=1}^{n}(R_t - T_t)^2]^{-0.5}*100\}$$ ------------------------------ (Equation 2.1)

where Rt and Tt are the cumulative percentage dissolved at each of the selected n time points of the reference and test product respectively. The factor f2 is inversely proportional to the average squared difference between the two profiles, with emphasis on the larger difference among all the time-points. The factor f2 measures the closeness between the two profiles. F2 is described as the similarity factor. In dissolution profile comparisons, especially to assure similarity in product performance, regulatory interest is in knowing how similar the two curves are, and to have a measure which is more sensitive to large differences at any particular time point.

When the two profiles are identical, f2=100. An average difference of 10% at all measured time points results in a f2 value of 50. FDA has set a public standard of f2 value between 50-100 to indicate similarity between two dissolution profiles

2.3 DRUG RELEASE STUDIES

Unless otherwise stated, drug release for microspheres was carried out in PBS (pH 7.4). 10 mg of the microspheres were incubated in 10 ml PBS at 37 °C under agitation. 1 ml samples were removed at 5, 30, 60, 120, 240, 360, 480, minutes, and 24 hours, placed into eppendorff tubes and following centrifuging at 13500 rpm (MSE, Leicester Ltd., UK), 0.5 ml of the supernatant was removed for analysis by HPLC (see section 2.10.2). 0.5 ml of fresh PBS was replaced with the pellet, resuspended using a whirlimixer (Fisher Scientific, Loughborough, UK) and the entire remaining sample was returned to the release medium. When the release medium was HCl, 0.1 M HCl (pH 1.2) solution was prepared and the assay continued as above.

2.4 DRUG LOADING STUDIES

The microspheres were digested by enzyme degradation using trypsin solution. 10 mg microspheres were incubated in 1 mg ml^{-1} trypsin (type II-S) solution under agitation at 37 °C over 48 hours. The solution was then assayed by HPLC against gelatin standards (section 2.10.2).

Drug loading (%w/w microspheres) was determined according to the following expression:

$$\text{Drug Loading (\%w/w)} = \frac{\text{Cimetidine in sample (mg)}}{\text{Microsphere weight (mg)}} \times 100 \quad \text{------} \quad \text{(Equation 2.2)}$$

2.5 PARTICLE SIZING

Due to swelling of gelatin microspheres in aqueous solution, acetone was used for the particle sizing, carried out using the Malvern Mastersizer/ E particle sizer (Malvern Instruments, Malvern, UK).

Approximately 10 mg microspheres were dispersed in acetone, sonicated for 2 minutes and volume mean determined using the Malvern Mastersizer. For most particle sizing, the instrument was fitted with a 300mm angle lens and a flow cell. When smaller particles were sized (i.e. $< 10\mu m$), a 45 mm angle lens was fitted. The average size of microspheres was determined by a Malvern software package. The mean and SD of three separate values was determined using Malvern software.

2.6 MICROSPHERE SURFACE CHARGE DETERMINATION

Zeta potential measurements were made using a Zetamaster (Malvern Zetamaster, Malvern Instruments Ltd., Malvern, UK). Approximately 10 mg of microspheres were dispersed in 2 ml 10 mM KCl and injected into the instrument. The mean of five separate values was determined from each run.

2.7 SCANNING ELECTRON MICROSCOPY

The surface morphology of the microspheres was examined using scanning electron microscopy. Samples were prepared by mounting on aluminium stubs using carbon tape, and coated with a 20nm gold film for 10 minutes under argon atmosphere in a splutter coater (Emscope, UK). The surface of the particles was studied under magnification with a Stereoscan 590B Scanning Electron Microscope (Cambridge, UK).

2.8 SWELLING STUDIES USING OPTICAL MICROSCOPY

Swelling of the gelatin microspheres was examined using optical microscopy. An optical microscope (Prior, England) was set up with an external light source (× 40 magnification) and connected with an external camera (VideoLabs Inc., USA) to a computer. The optical microscope was calibrated with a stage micrometer. The sample was placed on a glass slide under the microscope and following addition of a few drops of double distilled water, images of the swelling microspheres were taken with the video imager after 1, 5 and 15 and 30 minutes. The swelling ratio (Q) of the microspheres (n = 40) was determined by measurement of the microsphere diameter in the images obtained using the following expression.

$$Q = \frac{D_s}{D_d} \qquad \text{(Equation 2.3)}$$

where D_s is the diameter of the swollen microsphere,
D_d is the diameter of the dry microsphere

2.9 ANALYSIS OF INTERACTION BETWEEN CIMETIDINE AND GLUTARALDEHYDE

To determine whether there was any interaction between cimetidine (GlaxoSmithKline, Harlow, UK) and glutaraldehyde, high performance liquid chrtomatography (HPLC), thin layer chromatography (TLC) and nuclear magnetic resonance spectroscopy (1H-NMR) analysis was used.

2.9.1 HPLC analysis

As glutaraldehyde was normally added in acetone solution to microsphere formulations (see section 3.2.2), 100 mg cimetidine was dissolved in 20 ml acetone to which 1 ml of glutaraldehyde (50 %v/v in aqueous solution) was added under stirring for 6 hours. An aliquot of this solution was assayed using HPLC (section 2.10.2.1).

2.9.2 TLC analysis

The mixture of cimetidine/glutaraldehyde and the two compounds respectively were spotted on silica plates using ethyl acetate/methanol/sodium hydroxide (5 M) in ratios of 10:1:1. One tank was used to develop the plate and the other was filled with iodine to mark the spots (viewed under UV light at 254 nm).

2.9.3 ^{1}H-NMRanalysis

1H-NMRof cimetidine and the mixture of cimetidine/glutaraldehyde was carried out in DMSO. The acetone was evaporated using rotary evaporator at ~ 50 °C and the compounds were then analysed by ^{1}H-NMR(Bruker, UK).

2.10 HPLC TECHNIQUES

2.10.1 HPLC Equipment

The high performance liquid chromatographic system consisted of a Waters 600E Baseline LC pump and systems controller, Satellite WISP 712 autosampler and Waters 484 ultraviolet variable wavelength detector UV absorbance detector. The column purchased from Phenomenex (Cheshire, UK) was a Techsphere ODS-2, 4.6 mm internal diameter by 100 mm length.

The HPLC software generated data and this was then entered into Microsoft Excel 97 and underwent manipulations to generate calibration curves and process sample results.

2.10.2 HPLC methods

All chemicals were reagent grade, solvents were HPLC grade and used as received without further purification. Double distilled water was used in the preparation of mobile phase and reagent solutions. All samples were stored at 4 °C for no longer than 1 week.

Mobile phase was degassed before use. Calibration standards were prepared and run with each assay. Calibration curves were determined by direct linear regression, constructed to relate the peak area ratios of active and internal standard. Adequate chromatographic separation was observed for all compounds and total run time was kept below 10 minutes *per* sample injection.

2.10.2.1 Cimetidine assay

The mobile phase for cimetidine assay consisted of the following: 500 ml acetonitrile, 827.5 µl triethylamine, 2.5 ml acetic acid and 2 L double distilled water (pH 4.1). The mobile phase was developed from Gosh, (1992).

Stock solutions of cimetidine were prepared by dissolving 20 mg in 10 ml methanol and made up to 50 ml with double distilled water. Cimetidine standards were prepared in concentrations between 1 µg ml $^{-1}$ and 60 µg ml $^{-1}$ by diluting the stock solution with mobile phase. Internal standard (is.), ornidazole, was used at concentration 80 µg ml $^{-1}$. Figure 2.1 shows a typical calibration curve constructed from peak ratio against concentration.

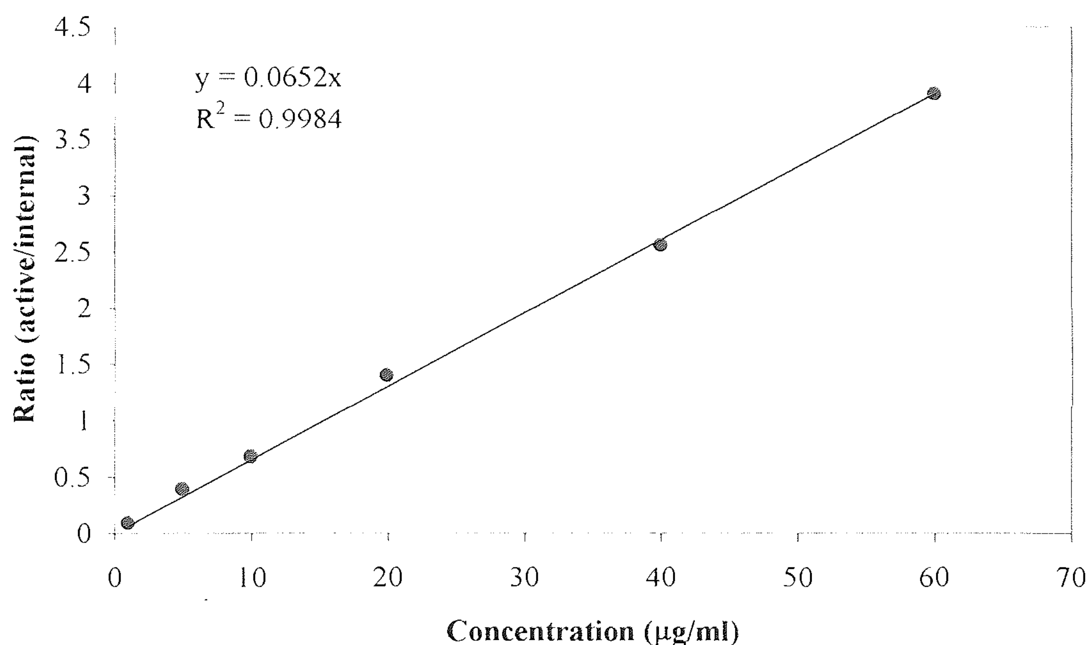


Figure 2.1 Typical calibration graph for cimetidine assay by HPLC determined at 229 nm

Chromatographic conditions for cimetidine assay are shown in table 2.1 and calculated chromatographic parameters in table 2.2. A sample chromatograph is shown in figure A1.1.

Volume	200 μl
Wavelength	229 nm
Aufs	0.01
Flow rate	1 ml min^{-1}
Run time	11 min
Sparge	Helium 15 ml min^{-1}

Table 2.1 Chromatographic conditions for cimetidine HPLC assay

	Active	**Internal**
Theoretical plates	1456	1785
Resolution factor	7.7	7.7
Tailing factor	5.5	5.0
Retention time	5.2 min	8.3 min

Table 2.2 Calculated chromatographic parameters for cimetidine assay

2.10.2.2 Nadolol assay

Mobile phase was prepared consisting of 700 ml methanol, 1 ml 0.1 M HCl, 5.84 mg NaCl and 1300 ml double distilled water (pH 5. 2). The method was developed from Slusarek and Florey, (1986).

Stock solutions of nadolol were prepared by dissolving 20 mg in 50 ml mobile phase. Nadolol standards were prepared in concentrations of 5, 10, 20, 40 and 60 μg ml^{-1} by dilution with mobile phase. The ornidazole concentration (is.) used was 60 μg ml^{-1}.

Figure 2.2 shows the calibration curve constructed from peak ratio against the concentration.

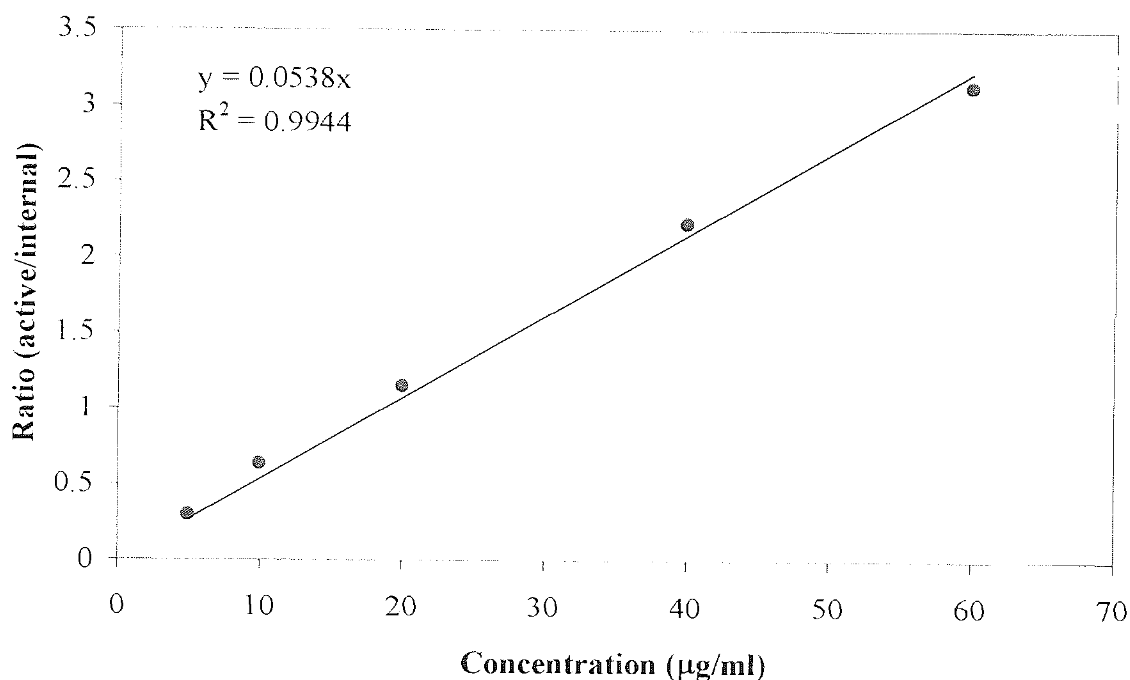


Figure 2.2 Typical calibration graph for nadolol assay by HPLC determined at 254 nm

Chromatographic conditions for nadolol assay are shown in table 2.3 and calculated chromatographic parameters in table 2.4. A sample chromatograph is shown in figure A1.2

Volume	200 μl
Wavelength	254 nm
Aufs	0.10
Flow rate	1 ml min^{-1}
Run time	7 min
Sparge	Helium 15 ml min^{-1}

Table 2.3 Chromatographic conditions for nadolol HPLC assay

	Active	Internal
Theoretical plates	1246	1302
Resolution factor	4.6	4.6
Tailing factor	4.0	2.0
Retention time	2.2 min	5.3 min

Table 2.4 Calculated chromatographic parameters for nadolol assay

2.10.3 Propranolol hydrochloride assay

Mobile phase consisted of 720 ml acetonitrile, 4 g SDS, 144 ml phosphoric acid (0.15 M) and 720 ml methanol (pH 1.6).

Stock solutions were prepared by dissolving 20 mg propranolol hydrochloride in 10 ml methanol, made up to 50ml with mobile phase. Propranolol hydrochloride standards were prepared in concentrations of 1, 5, 10, 20, 40 and 60 μg ml $^{-1}$ by dilution with mobile phase. Ornidazole concentration (is.) used was 60 μg ml $^{-1}$. Figure 2.3 shows the calibration curve constructed from peak ratio against the concentration.

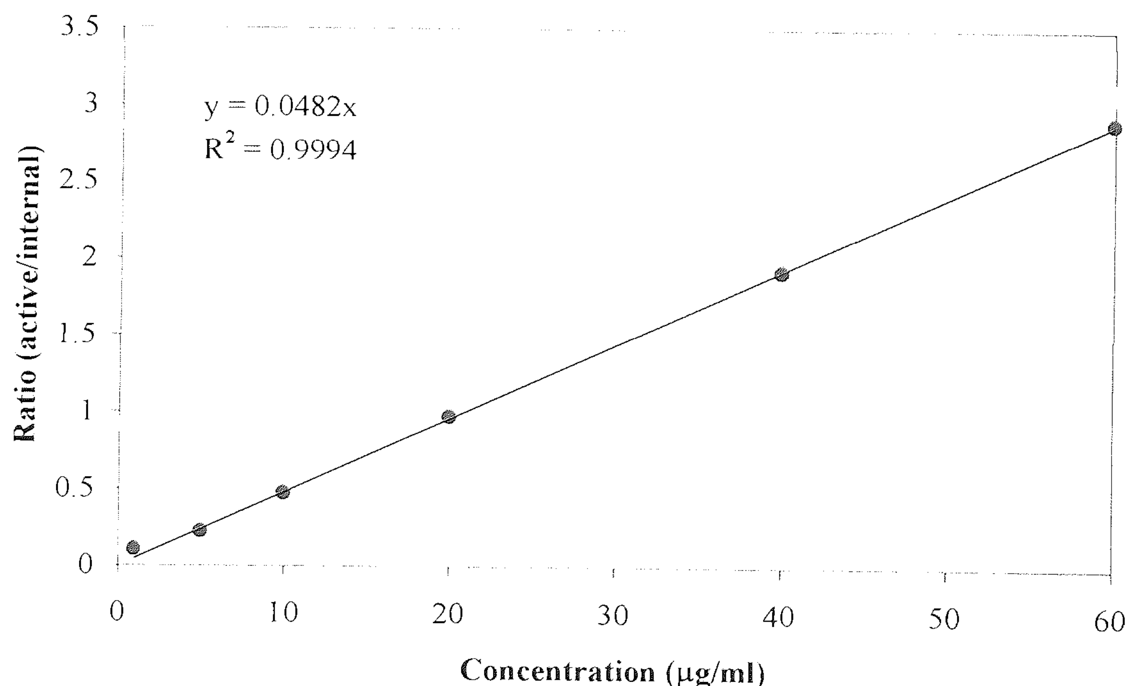


Figure 2.3 Typical calibration graph for propranolol hydrochloride assay by HPLC determined at 290 nm

Chromatographic conditions for cimetidine assay are shown in table 2.5 and calculated chromatographic parameters in table 2.6. A sample chromatograph is shown in figure A1.3

Volume	200 μl
Wavelength	290 nm
Aufs	0.10
Flow rate	1 ml min^{-1}
Run time	9 min
Sparge	Helium 15 ml min^{-1}

Table 2.5 Chromatographic conditions for propranolol hydrochloride HPLC assay

	Internal	**Active**
Theoretical plates	976	1105
Resolution factor	3.2	3.2
Tailing factor	2.5	3.1
Retention time	4.4 min	6.2 min

Table 2.6 Calculated chromatographic parameters for propranolol hydrochloride assay

2.11 SURFACE ADSORPTION ASSAY

The adsorption of mucin to the microsphere surfaces was determined using type I-S mucin (method adapted from He *et al.*, 1998). Type I-S mucin was used due its 12% sialic acid content, as it was reported that with high sialic acid content, adsorption between polymer and mucin is higher than with other types of mucin (He *et al.*, 1998). Two reagents were used; Schiffs reagent containing 500 ml pararosanilline chloride (1 %w/v) and sodium bisulphite (4 %w/v) in HCl (0.25 mol L^{-1}) which stains carbohydrates when used with periodic acid. Periodic acid reagent was freshly prepared by adding 10 μl of 50 %v/v periodic acid solution to 7 ml of 7 %v/v acetic acid solution. 10 mg microspheres were

dispersed in 10 ml of aqueous mucin solution (0.025 mg ml^{-1} to 1 mg ml^{-1}), using a whirlimixer (Fisher Scientific, Loughborough, UK) and incubated at 37 °C for 60 minutes under gentle agitation. 2 ml was removed and centrifuged at 4000 r.p.m. (MSE Ltd, Leicester, UK) and 1 ml of the supernatant used for measurement of the free mucin content. 0.2 ml of periodic acid reagent was added to the supernatant, and samples were incubated at 37 °C for 2 hours. 0.2 ml of Schiffs reagent was then added at room temperature and after 45 minutes the absorbance was determined at 550 nm using a UV spectrophotometer (Wallac Victor 2 Multilabel Counter, Perkin Elmer Life Sciences, Boston USA). Standard mucin concentrations were prepared in the range 0.025 mg ml^{-1} to 1 mg ml^{-1} and assayed similarly (Figure 2.4) and free mucin concentration calculated.

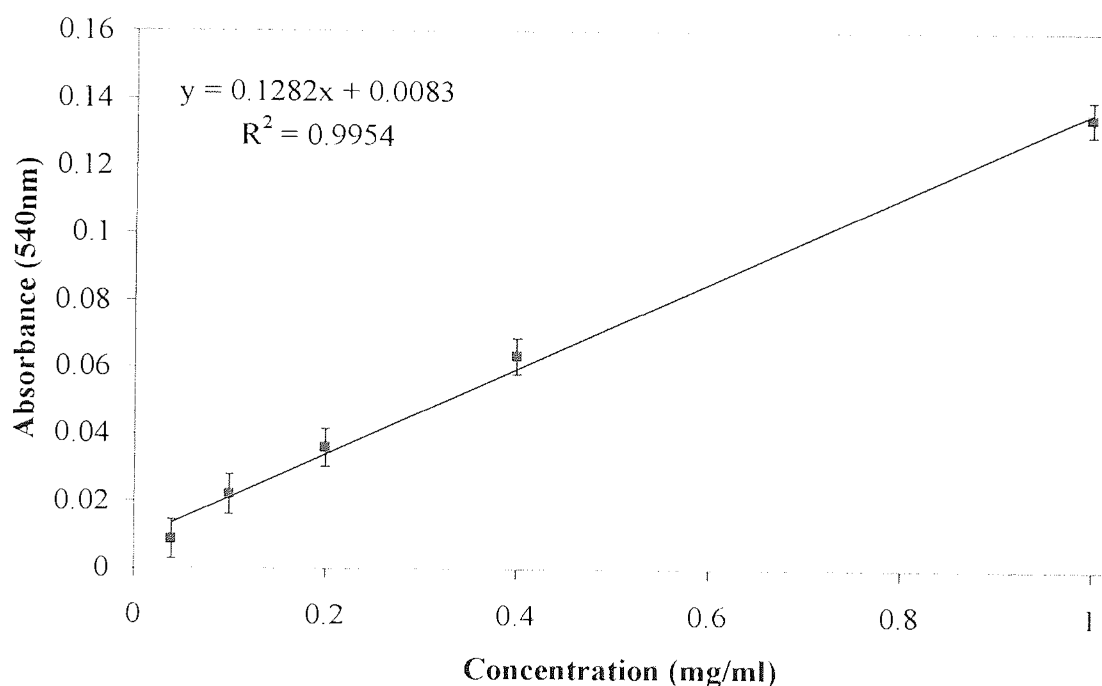


Figure 2.4 Calibration curve for mucin standards (n=3; mean ± sd)

Results of the mucin studies are presented as % mucin adsorbed determined from the difference between the total mucin added and the free mucin remaining in solution at the end of the experiment.

2.12 DETERMINATION OF PARTICLE MUCOADHESION *EX VIVO*

2.12.1 Preparation of intestinal segments

Adult male Wistar rats (500 g) were dissected and the intestine severed below the stomach and above the duodenum. The intestine was removed and cut into 15 cm segments and incubated in Sorensen's phosphate buffer (room temperature) consisting of monopotassium phosphate (0.07 M) and disodium phosphate (0.07 M) until use. Samples could not be frozen as upon thawing, the tissue was found to be considerably softer and leakage of solution was observed. The intestinal segment was washed using a syringe with ~ 100 ml buffer. One end of the intestinal segment was tied. With one end of the intestine loose, it was inverted and subjected to gentle washing with Sorensen's buffer and then reverted.

2.12.2 Addition of microspheres to intestines

6 mg microspheres were suspended in 40 ml PBS and stirred for 30 minutes. From this stock suspension, three 5 ml aliquots were removed and made up to 50 ml with isotonic saline solution, stirred for 30 minutes, then the number of particles in each sample was determined using a Coulter Counter (section 2.12.3). This value was taken as the initial counts. 5 ml of the particle suspension was removed from the stock and injected into the open end of the intestinal segment using a 5 ml syringe which was then tied. The intestines were checked carefully for any leakages and then incubated for 60 minutes in Sorenson's buffer at 37 °C. Following this, the intestines were removed, one end opened and the suspension was allowed to drain from the tissue into a beaker followed by a single wash with 5 ml PBS. The particle suspension was then made up to 50 ml with isotonic saline solution and the number of particles was then counted using the Coulter Counter. The experiment was carried out in triplicate (*i.e.* three intestinal segments were used). With the suspension of microspheres used, particle counts were generally ~ 15000. The number of particles was found to always be in the range shown in figure 2.5.

2.12.3 Particle counting

The numbers of particles were counted using a Coulter Counter (Coulter Electronics Ltd., Luton, UK) which determines the number and size of particles suspended in an electrically conductive liquid. This is achieved by forcing the suspension through a small aperture with

an immersed electrode on either side. As the particle passes through the aperture, it changes the resistance between the electrodes. A voltage pulse of short duration is produced with a magnitude proportional to the particle size. The series of pulses is then electronically scaled and counted.

Isotonic saline solution was filtered through a pore size of 1.6 μm and was the electrolyte used in all Coulter Counter experiments. The Coulter Counter was calibrated with polystyrene latex particles (Polysciences Europe GmbH, Germany) of size 9.17 ± 0.7 μm. The validity of the counting technique was proven by the existence of a linear relationship between the concentration of gelatin microspheres and counts obtained. Known masses of microspheres (1, 2, 4, 6, 8 mg) were added to 40 ml PBS and stirred for 30 minutes. 5 ml of this solution was removed and made up to 50 ml with isotonic saline solution. For all samples, 5 readings were taken with a blank electrolyte each time. From the counts obtained, it was seen that a linear relationship exists between counts obtained and microsphere concentration (Figure 2.5).

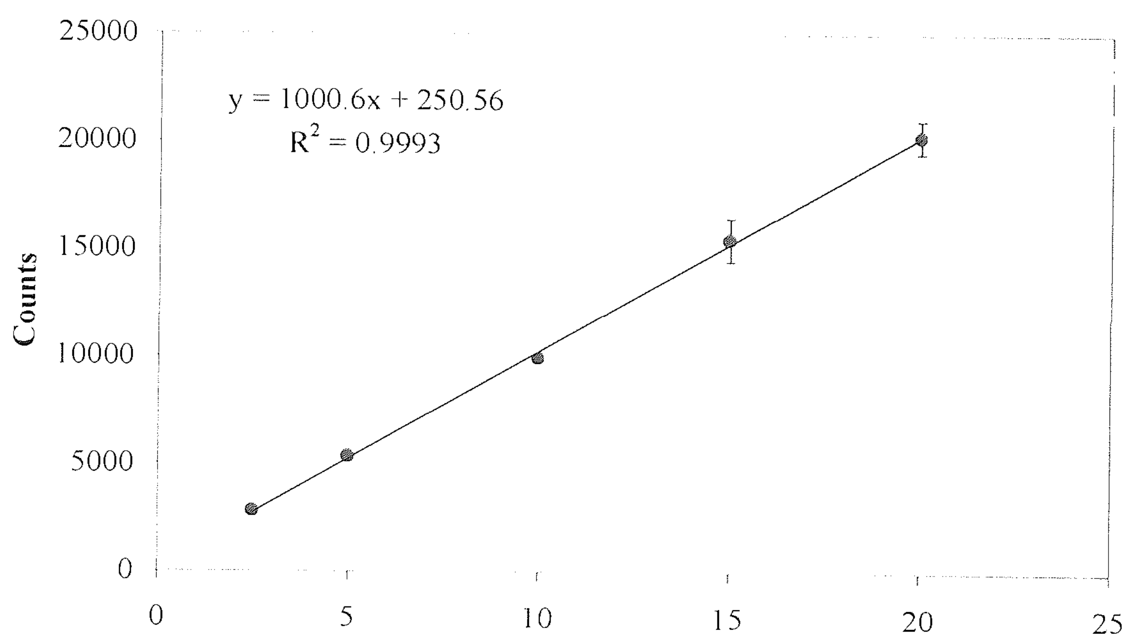


Figure 2.5 Relationship between counts obtained and microsphere concentration obtained using the Coulter Counter (n=3; mean ± sd)

CHAPTER THREE

GELATIN MICROSPHERES: PREPARATION AND CHARACTERISATION

3.1 INTRODUCTION

Many different methods of preparation are available for the production of microspheres. Microencapsulation can be performed without the need for specialised equipment (Akin and Hasirci, 1995) by utilising formulation methods such as solvent evaporation and organic phase separation. The choice of method is dependant on the characteristics of the drug and the polymer.

Preparation of gelatin microspheres using single-emulsion methods has been reported by a number of workers (Akin and Hasirci, 1995; Narayani and Rao, 1996 a and b; Yao *et al.*, 1996; Yin *et al.*, 1996; Leo *et al.*, 1997; Leucuta *et al.*, 1997 a and b; Vandelli *et al.*, 2001) using different techniques. These methods include complex coacervation, phase separation, spray drying and thermal gelation. The simplest and most commonly used method for preparation of gelatin microspheres is thermal gelation, where the introduction of a temperature gradient after formation of an emulsion, results in hardening of the gelatin droplets into microspheres (Leucuta *et al.*, 1997 a and b); Vandelli *et al.*, 1991; Vandelli *et al.*, 2001). As gelatin is easily solubilised by moderate increases in temperature, chemical crosslinking agents can be used to produce stable structures (Akin and Hasirci, 1995). Aldehydes are commonly used in the crosslinking of gelatin, in particular glutaraldehyde (see section 3.3.1.1 for details of mechanism).

In this chapter, gelatin microspheres were prepared using both single and double emulsion techniques. Microspheres were prepared by thermal gelation (unless otherwise stated) using soybean oil as the dispersing medium followed by crosslinking with glutaraldehyde. Soybean oil is also used as an excipient in food and cooking and has previously been successfully used in gelatin microsphere formulations (Cortesi *et al.*, 1998). Soybean oil is most commonly used in pharmaceuticals as a fat source in total parenteral nutrition regimes but has also been used in emulsions for oral and intravenous administration (Wade and Weller[BRC1], 1994)

3.2 MATERIALS AND METHODS

All materials were obtained from Sigma/Aldrich (Poole, UK) unless otherwise stated and were of analytical grade unless otherwise specified.

3.2.1 Preparation of microspheres using crosslinking in oil method

Microspheres were prepared by a single-emulsion (W/O) method. Cimetidine (theoretical load 10-80% w/w gelatin) (GlaxoSmithKline, Harlow, UK) and gelatin type A (bloom strength 300, molecular weight 50000-100000 Daltons) (1.5 g unless otherwise stated) were dissolved in 1% v/v acetic acid (unless otherwise stated) (9 ml), heated to 60 °C and emulsified at 1200 rpm using a Heidolph stirrer (Lab-Plant, Huddersfield, UK) for 10 minutes in 50 ml soybean oil. The emulsion was then cooled under stirring in an ice bath for 30 minutes, acetone was added (37 ml) and stirring continued for a further 5 minutes. Following removal from the ice bath, 30 μl of 50% w/v glutaraldehyde (*i.e.* 1% w/w gelatin) (unless otherwise stated) was added directly to the emulsion under stirring for 5 minutes at room temperature (unless otherwise stated). Microspheres were harvested by filtration, and washed with hexane three times (50 ml volumes) to remove any oil, dried under vacuum overnight at room temperature, and stored in a vacuum desiccator.

3.2.2 Preparation of microspheres using crosslinking in acetone method

Microspheres were prepared by a single-emulsion (W/O) method. Cimetidine (1.5 g) (unless otherwise stated) and gelatin (1.5 g) were dissolved in 1% v/v acetic acid (9 ml) (heated to 60 °C) and emulsified at 1200 rpm (unless otherwise stated) using a Heidolph stirrer for 10 minutes in 50 ml soybean oil, containing 1% v/v Span 80 (unless otherwise stated). The emulsion was then cooled under stirring in an ice bath for 30 minutes, acetone was added (37 ml) and stirring continued for a further 5 minutes. Microspheres were harvested by filtration, and washed with hexane three times (50 ml volumes) for removal of oil, followed by drying in a vacuum oven overnight at room temperature. Crosslinking was then carried out using glutaraldehyde (5% w/w of gelatin) in 20 ml acetone for 5 minutes. Microspheres were then filtered and washed with acetone three times (50 ml), followed by drying in a vacuum oven overnight at room temperature and storage in a vacuum desiccator.

When nadolol or propranolol hydrochloride were formulated, 0.6 g of nadolol or propranolol hydrochloride was dissolved with gelatin in 1% v/v acetic acid (9 ml), and the formulation carried out as described above.

3.2.3 Preparation of microspheres using double emulsion method

Microspheres were prepared by a double emulsion (O/W/O) method. Cimetidine (1.0 g) in 3ml ethanol was sonicated at 120 watts (unless otherwise stated) for 3 minutes using an ultrasound probe (Soniprep 150, MSE, Surrey, UK) into gelatin (1 g) dissolved in water (9 ml) (preheated to 60 °C). The primary emulsion was then added to 50 ml soybean oil, containing 1% v/v Span 80, forming an O/W/O emulsion and emulsified at 1200 rpm using a Heidolph stirrer for 10 minutes. The harvesting and crosslinking was then continued as in section 3.2.2.

3.2.4 Preparation of spray dried microspheres

1.5 g cimetidine was dissolved in 150 ml of 1% v/v acetic acid. 1.5 g gelatin was then added to the solution and stirred for 1 hour. 5% w/w glutaraldehyde (unless otherwise stated) (in 5ml water) was added to the aqueous solution, which was then spray dried using a Buchi 190 spray drier (Buchi Laboratory-techniques Ltd., Flawil, Switzerland). For preparation of smaller microspheres, glutaraldehyde was added (as 5% w/w) in 50ml water. Spray drying conditions were as follows, unless otherwise stated: inlet temperature of 120 ±5 °C; aspirator rate of 10 $m^3 min^{-1}$; pump flow rate of 10 ml min^{-1}; atomising air pressure of 200 mmHg. Microspheres were collected following spray drying and stored in a vacuum desiccator.

3.3 RESULTS AND DISCUSSION

3.3.1 Effect of crosslinking in oil on characteristics of single-emulsion gelatin microspheres (I)

Microspheres were produced in three batches on separate days. Drug release (mean of three readings) and drug loading (mean of three readings) were evaluated using all three batches. Particle size (mean of 3 readings determined using Malvern software), zeta potential (mean of 5 readings determined using Zetamaster software) and surface mucin adsorption studies (mean of 3 readings) were evaluated from individual representative

batches. The preparation of gelatin microspheres using crosslinking in oil was adapted from Yin *et al.*, (1996), where glutaraldehyde was added directly into the emulsion.

3.3.1.1 Effect of glutaraldehyde concentration on physicochemical characteristics of gelatin microspheres

In this section the effect of increasing glutaraldehyde levels on the microsphere characteristics was assessed.

The degree of crosslinking is expected to affect the degree of swelling for microsphere preparations. It has been hypothesised that an increase in the concentration of the crosslinking agent is capable of increasing the crosslinking density (Vandelli *et al.*, 1991). Such an increase would produce a lower degree of swelling and decrease the drug release rate from microspheres.

When gelatin is used, the solidification process normally requires the chilling of an aqueous gelatin solution to 4 °C to form a gel structure. This structure is readily destroyed by moderate temperature increases. Aldehydes can be used to crosslink or fix the protein structures. The rate at which chemicals can travel through the polymer network structure is dependant on its swelling ability, which is in turn influenced by factors such as crosslinking density, crystallinity, porosity and hydrophilicity and chemical structure of the polymer.

The crosslinking of the protein structure by glutaraldehyde can occur by reaction of the amino group of the trifunctional amino acid, lysine with the aldehyde group in formation of a Schiffs base (Digenis *et al.*, 1994) (Figure 3.1).

Minimal levels of glutaraldehyde should be used to reduce residual amounts remaining within or on the surface of the microspheres. It is reported that residual glutaraldehyde remaining on microspheres may result in variable biodegradation, drug release or unexpected *in vitro* cell toxicity (see section 1.4.2.1) (Davis and Illum, 1987). For further work, if microspheres were degraded fully, the level of residual glutaraldehyde could be determined using a colorimetric assay (Boratynski and Zal, 1990).

Increasing concentration of glutaraldehyde reduced drug loading (Table 3.1). This decrease in drug loading could suggest that there may be cross-reactivity of glutaraldehyde with cimetidine as it was a successive decrease. This is discussed in section 3.3.1.2.

This method resulted in a relatively large particle size (Table 3.1), which was found to decrease with increasing glutaraldehyde concentration. The increasing crosslinking density may result in the formation of a densely packed, helical-like structure causing shrinkage of the particles (Akin and Hasirci, 1995). Zeta potential was found to be negative at the highest concentration of glutaraldehyde as expected (He *et al.*, 1999 b), indicating the presence of excess glutaraldehyde on the surface of the microspheres.

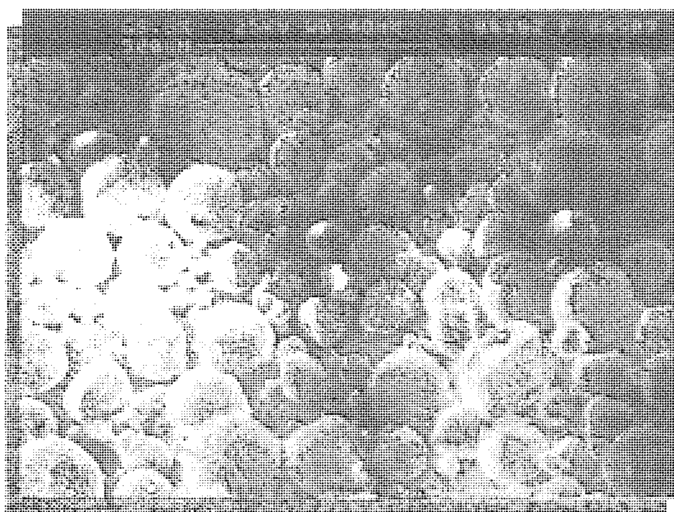


Figure 3.2 SEM image of microspheres prepared using crosslinking in oil method (5% w/w glutaraldehyde, loading 15.3 ± 1.0% w/w)

There was no difference between the release profiles of microspheres crosslinked with 1, 5 or 10% glutaraldehyde (5%, f_2 = 43.9; 10%: f_2 = 48.6) (Figure 3.3), where all preparations had a burst effect (the percentage of drug released from the first reading, *i.e.* after 5 minutes) of 55-75% and released 85-98% in 2 hours. The viscous nature of the oil used as the dispersing medium (density 0.920 gcm^{-3}, Sigma-Aldrich Company Ltd data sheet) may present a barrier against effective cross linking. This method was deemed to be unsuitable for encapsulation of cimetidine as there was insufficient control over the drug release profiles.

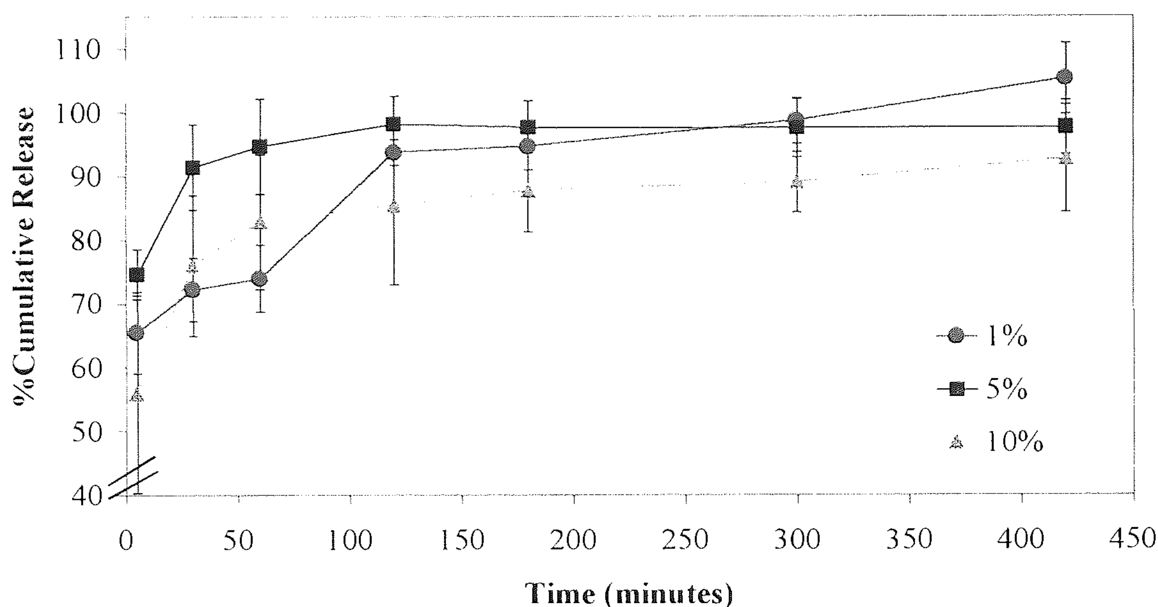


Figure 3.3 The effect of increasing glutaraldehyde (% w/w gelatin) on the release from microspheres formed using the single-emulsion crosslinking in oil method (n=3; mean ± sd)

3.3.1.2 Investigation of potential cimetidine/glutaraldehyde interaction

The possible interaction between cimetidine and glutaraldehyde was investigated by HPLC, TLC and ^{1}H-NMR (section 2.9).

Following mixing of cimetidine with glutaraldehyde, the solution was spiked with a low concentration of cimetidine, prior to assay by HPLC, with a single peak observed (section 2.9.1). There was found to be no splitting of the cimetidine peak or the presence of any other unknown peak (Figure A1.6). This indicated that no reaction of cimetidine with glutaraldehyde had occurred.

By TLC (section 2.9.2), the retention times for the mixture corresponded to the retention times of the individual compounds, indicating that no reaction of cimetidine with glutaraldehyde had occurred (Table 3.2).

Compound	Retention time (t_R)
cimetidine	1.2 cm
glutaraldehyde	7.1 cm
mixture	1.3, 7.1 cm

Table 3.2 Retention times for compounds analysed by TLC

The ^{1}H-NMR spectrum (section 2.9.3) of the mixture was found to be different to the spectrum of cimetidine. The ^{1}H-NMR of cimetidine was identified and the protons labelled (Figure 3.4, table 3.3).

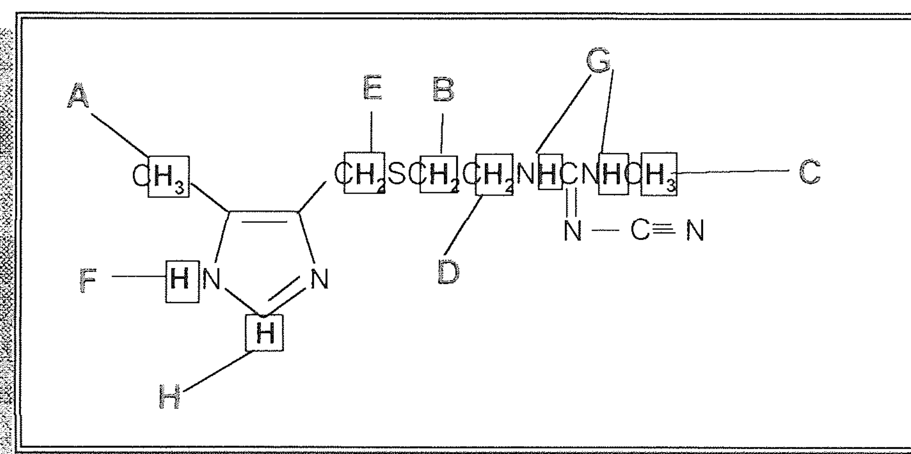


Figure 3.4 Structure of cimetidine with protons labelled

A	singlet (3H)	2.1 ppm
B	triplet (2H)	2.6 ppm
C	singlet (3H)	2.7 ppm
D	doublet (2H)	3.3, 3.5 ppm
E	singlet (2H)	3.6 ppm
F	singlet (2H)	7.2 ppm
G	singlet (2H)	7.5 ppm
H	singlet (1H)	11.9 ppm

Table 3.3 Coupling pattern for protons from spectrum of cimetidine

The potential reaction of glutaraldehyde with cimetidine may result in the formation of C-N bonds from the reaction of the carboxyl group of the aldehyde with the amine groups on

cimetidine by nucleophilic addition. This may result in the possible structure of the new compound shown in figure 3.5, the ^{1}H-NMR for the compound is shown in figure A1.5. The proposed mechanism is shown in figure 3.6. There are two sites where the reaction could occur.

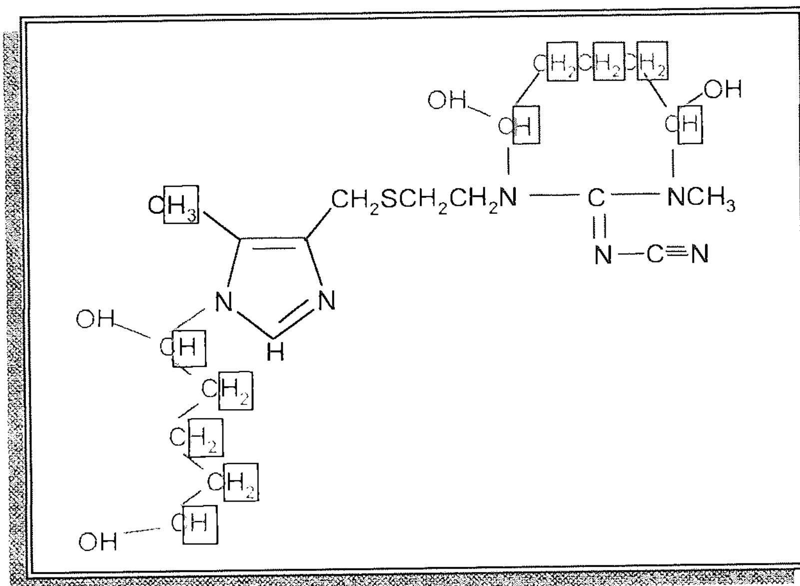


Figure 3.5 Proposed structure for cimetidine-glutaraldehyde complex

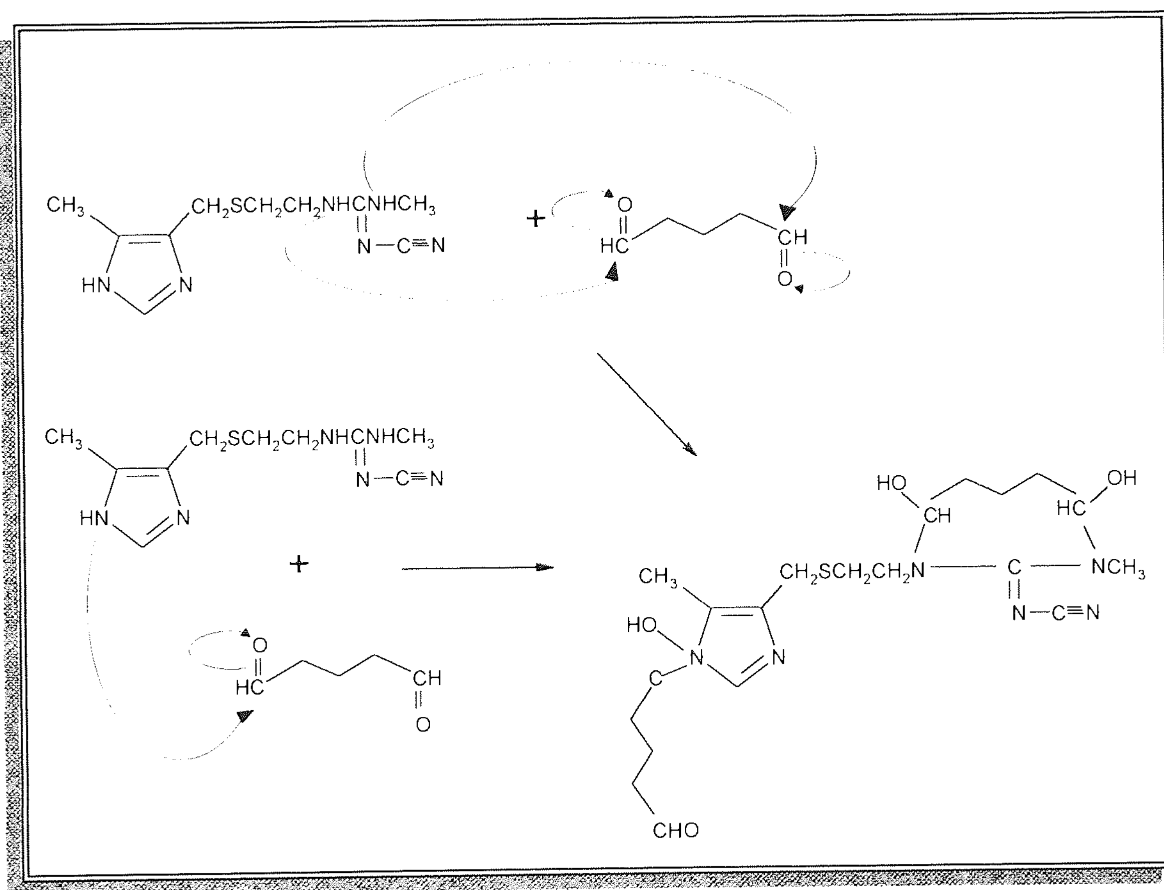


Figure 3.6 Proposed mechanism of reaction of glutaraldehyde with cimetidine

Upon inspection of the ^{1}H-NMR of the cimetidine-glutaraldehyde mixture (Figure A1.5) it is apparent that the peaks representative of cimetidine are still present with about the same chemical shifts, but some new peaks are present at ~ 5 ppm. These peaks are likely to represent the OH groups. Group A (Figure A1.5) is now much wider due to the extra protons highlighted in figure 3.5. The ratio between H and G, and also H and F is reduced but the peaks at 7-8 ppm are still present. These peaks represent protons of N-H of the cimetidine structure. Since these peaks are still present, it is possible that the compounds may not have reacted but the ^{1}H-NMR is showing the presence of both cimetidine and glutaraldehyde. Furthermore, minor changes in the ^{1}H-NMR spectra are not confirmed by HPLC and TLC studies.

3.3.1.3 Analysis of mucoadhesive properties of gelatin microspheres *in vitro*

The adsorption of mucin to the surface of the gelatin microspheres was determined *in vitro* (see section 2.11) (Figure 3.7). Gelatin type A was used for microsphere production with a measured pH of 4.8 (1% w/v) and bloom strength of 300 (Sigma-Aldrich Company Ltd. data sheet), and molecular weight of 50000-100000 Da (Sigma-Aldrich Company Ltd. data sheet). Bloom strength is an indication of the strength of the gel from the solution of gelatin, *i.e.* the higher the bloom strength, the stronger the gel. The gelatin used was type A which is isoionic between pH 7 – 10 (see section 1.4.2 for details on gelatin). The interaction of gelatin with mucin was studied at pH 6.5 (measured pH of mucin solution), where gelatin was positively charged. The surface adsorption assay was adapted from He *et al.*, (1998) where differences in the adsorption of mucin to chitosan microspheres were highlighted. The negative charge of mucin (see section 1.2.2) is due to the ionisation of sialic acid in aqueous environments. The adsorption of mucin to gelatin was expected to be dominated by the electrostatic attraction between the positively charged gelatin and negatively charged mucin. The concentration of glutaraldehyde added was expected to influence the level of adsorption of mucin to gelatin microspheres, due to the effect on the surface charge of the microspheres from the glutaraldehyde. Using the microsphere formulations in table 3.1 it was found that with increasing crosslinker from 5% to 10%, the surface charge, or zeta potential of the microspheres was more negative (at 5%, 7.3 ± 4.6 mV; at 10%, -18.6 ± 0.5). Correspondingly, the adsorption of mucin to microspheres was

significantly decreased from ~11% to ~2.5% (Dunnetts test, P < 0.05). Zeta potentials of microspheres crosslinked with 1% or 5% w/w glutaraldehyde were not significantly different (Dunnetts test, P > 0.05), however the mucin adsorption to the microspheres was significantly lower from ~15% to 11% (Dunnetts test, P < 0.05). The microsphere production method may be a factor in these results, as the microspheres prepared using the crosslinking in acetone method (section 3.3.3.2) exhibited decreased mucin adsorption with increasing negative zeta potential.

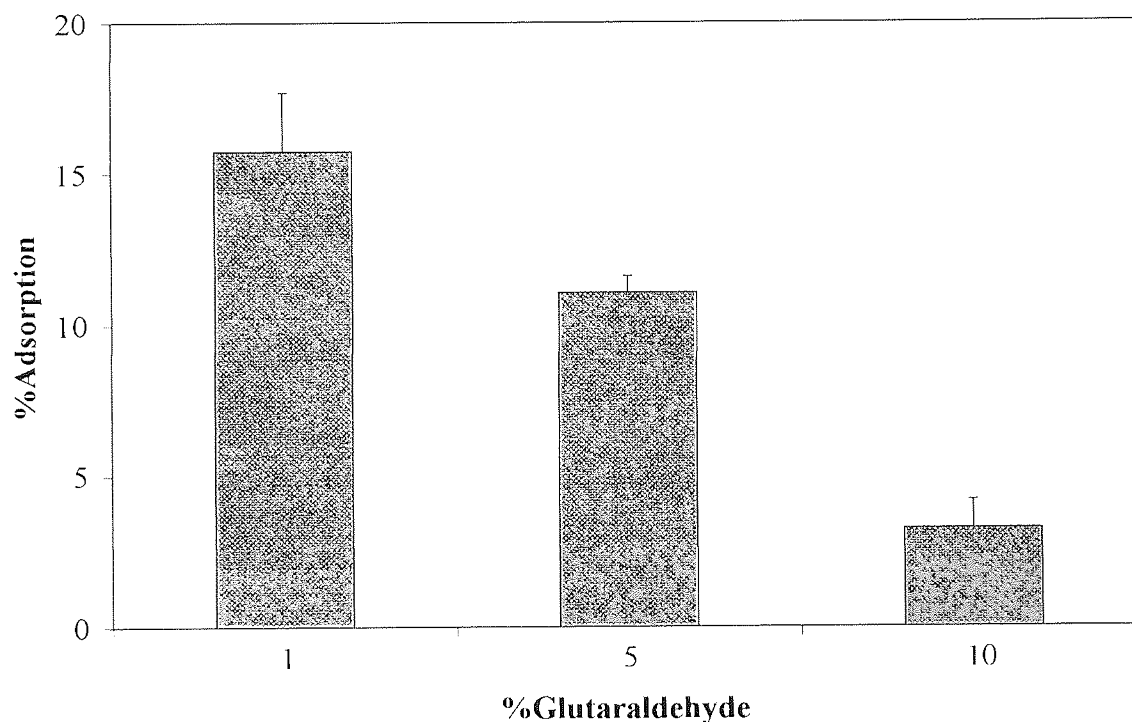


Figure 3.7 The adsorption of mucin to microspheres determined by adsorption assay (n=3; mean ± sd)

3.3.2 Effect of crosslinking in oil on characteristics of single-emulsion gelatin microspheres (II)

In this section one batch each of microspheres was prepared using the single-emulsion crosslinking in oil method. Drug loading, zeta potential, particle size (mean of 3 samples from the same batch), zeta potential (mean of 5 readings determined using Zetamaster software) and particle size (mean of 3 readings determined using Malvern software) were evaluated from these batches. The effect of crosslinking duration, type of polymer, theoretical drug load and particle size on the microsphere characteristics was assessed.

3.3.2.1 Effect of crosslinking time on characteristics of gelatin microspheres

Crosslinking has an important role in the physico-chemical properties of microspheres produced. An increase in the crosslinking time period can increase the crosslinking density of the microspheres, and has been used in modifying the release profiles from gelatin microspheres (Vandelli *et al.*, 1991; Chiao and Price, 1989). The duration for crosslinking of the microspheres using 5 %w/w glutaraldehyde was varied from 5 minutes to 17 hours for microspheres with a theoretical loading of 50% w/w. The only significant difference in loading was between microspheres crosslinked for 5 minutes and microspheres crosslinked for 17 hours duration (Dunnetts test, $P < 0.05$) (Table 3.4). Zeta potential was more negative when the crosslinking duration was increased from 5 minutes to 4 hours (5 minutes: 7.3 ± 4.6 mV and 4 hours: -14.8 ± 1.8 mV). It was not further increased by crosslinking durations beyond 4 hours (Table 3.4).

Crosslinking duration	**Load (%w/w) (n=3; mean ± sd)**	**Zeta potential (mV) (n=5; mean ± sd)**
5 minutes	15.3 ± 1.0	7.3 ± 4.6
4 hours	16.7 ± 0.5	-14.8 ± 1.8
6 hours	16.0 ± 0.4	-15.6 ± 1.2
17 hours	18.2 ± 1.4	-14.1 ± 0.7

Table 3.4 Effect of increase in duration of glutaraldehyde crosslinking on properties of gelatin microspheres prepared by single-emulsion crosslinking in oil

There was no control over the drug release profile with increasing crosslinking duration (Figure 3.8). Using this method, due to the presence of the oil during the crosslinking, it was thought to be difficult to achieve a homogenous distribution of the crosslinker and penetration through the oil to harden the gelatin.

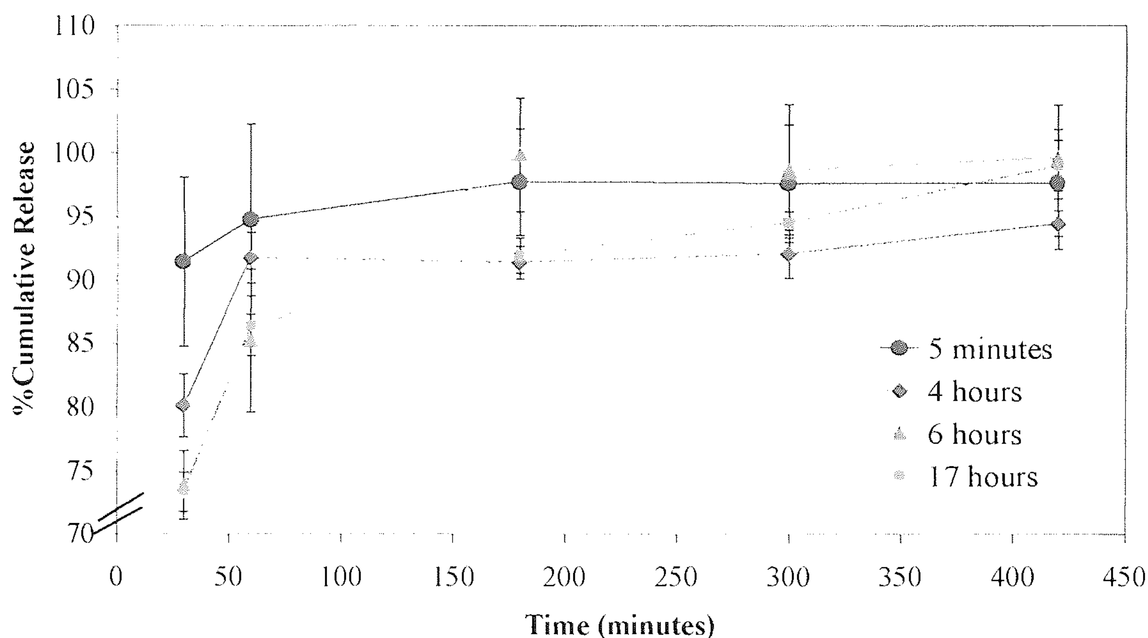


Figure 3.8 Influence of glutaraldehyde crosslinking time on the release profile from gelatin microspheres prepared by single-emulsion crosslinking in oil (n=3; mean ± sd)

3.3.2.2 Influence of drug load on the properties of gelatin microspheres

To investigate the effect of increased theoretical loading on the actual loading and release properties of the microspheres, microspheres (single batches) were prepared with theoretical loading of 10-80% w/w.

Drug added (%w/w gelatin)	Actual load (%w/w microspheres)	Encapsulation efficiency %
80.0	33.9 ± 7.8	42.4
70.0	31.8 ± 7.6	45.4
60.0	28.5 ± 5.5	47.5
50.0	20.5 ± 3.3	41.0
28.6	8.6 ± 1.5	30.1
20.0	5.9 ± 1.0	29.5
10.0	3.1 ± 0.6	21.4

Table 3.5 Theoretical load, actual load and encapsulation efficiency of gelatin microspheres (n=3; mean ± sd)

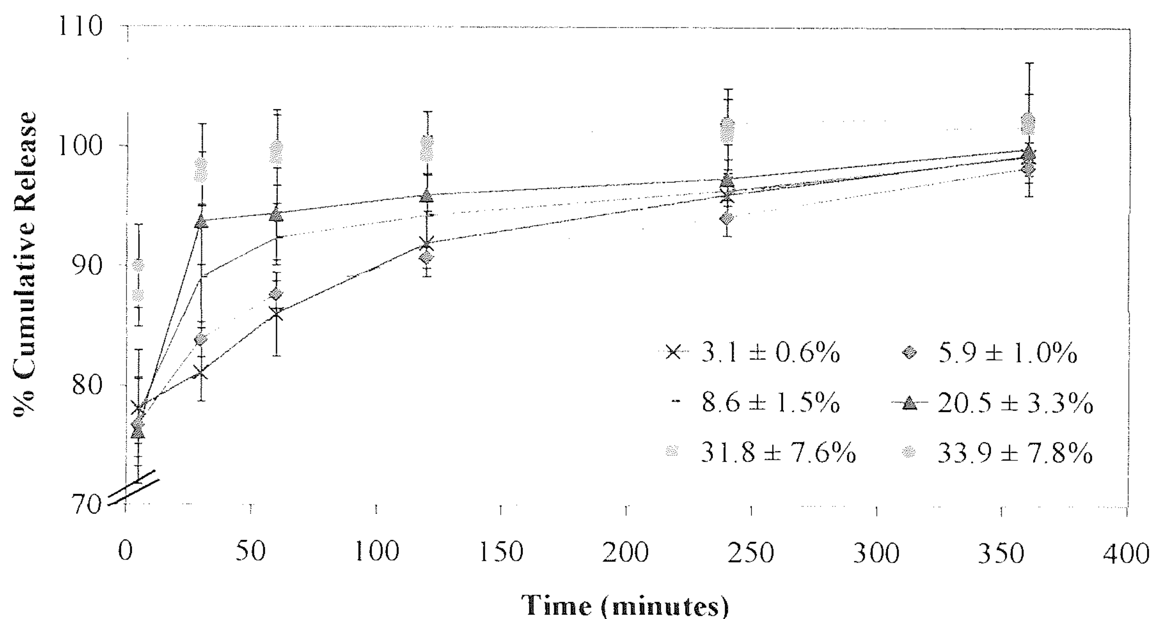


Figure 3.9 Effect of drug loading on the release profile from gelatin microspheres formulated using the single-emulsion crosslinking in oil method (n=3; mean ± sd)

Encapsulation efficiency was increased when theoretical loads were increased from 10% to 60% w/w (Table 3.5). When theoretical drug load was increased over 60% w/w, encapsulation efficiency was not increased further, indicating a maximum level for drug loading of the microspheres. As the theoretical load of the drug is increased, surface loading of the drug is promoted, characterised by increased burst release from the microspheres. There was significant difference between the burst releases from the microspheres, when theoretical loading was increased from 50 to 70% w/w (Figure 3.9). Using the crosslinking in oil method, it was found that the drug loading was not reproducible from batch to batch. For example, in section 3.3.2.1 microspheres with theoretical loading of 50% w/w were loaded with 15.3 ± 1.0% cimetidine. In this section, another batch with theoretical loading of 50% w/w were loaded with 20.5 ± 3.3% drug. Drug loading was variable due to the method of formulation, *i.e.* application of the crosslinker without removal of the oil. It is likely that smaller sized particles would have lower drug loading than larger particles.

3.3.2.3 Effect of particle size on the properties of gelatin microspheres

Using sieves, particles > 150 μm and < 30 μm were isolated from one batch of microspheres and rate of drug release from each studied (Figure 3.10).

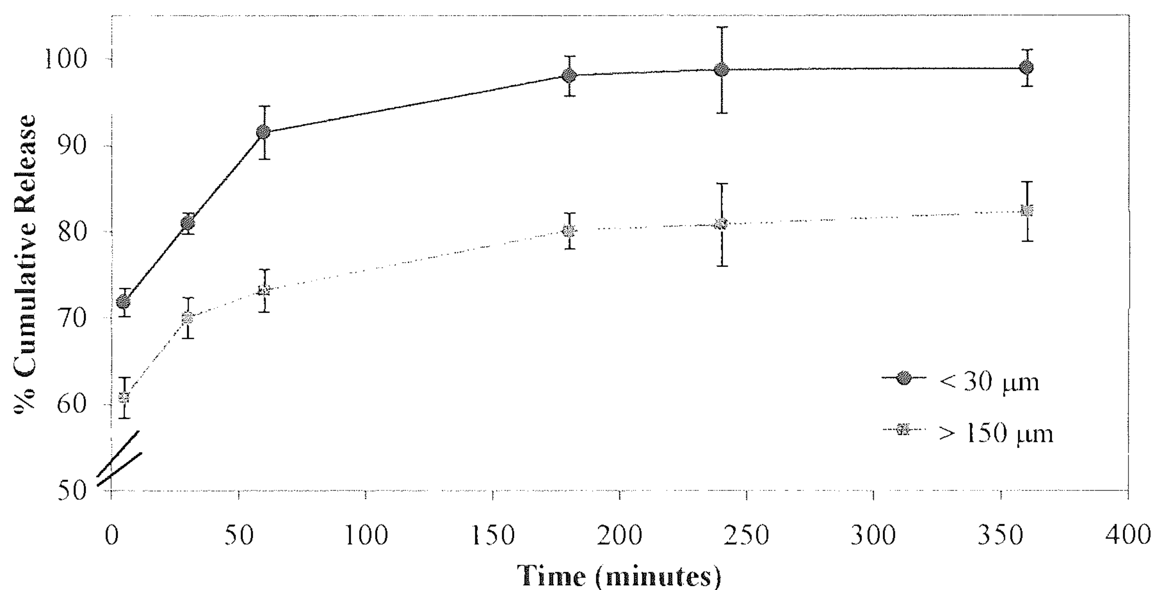


Figure 3.10 Effect of particle size on the release profile from gelatin microspheres formulated using the single-emulsion crosslinking in oil method (n=3; mean ± sd)

Increased particle size led to slow rates of drug release as expected (Figure 3.10). The difference in release profiles was the result of the increased surface area to volume ratio of the small spheres compared to the larger spheres, which gives increased contact with the release medium and a shorter diffusion pathlength. The larger surface area resulted in a greater concentration of cimetidine at or near the surface causing an increased burst release (Lewis *et al.*, 1998).

3.3.2.4 Effect of pH of cimetidine internal phase on the drug release from gelatin microspheres

Cimetidine was dissolved in acetic acid as part of the internal phase, in the preparation of the gelatin microspheres (see section 3.2.1). When cimetidine (in acetic acid) was incorporated in gelatin microspheres, it was found that burst release was high and release was fast (Figure 3.3). It was thought that the drug may be escaping from the inside of the gelatin droplets during the emulsification stage and either lost or result as surface bound. To assess the effect of solubility of the drug on the release characteristics, cimetidine was included in the internal phase in 9 ml water as a suspension.

Cimetidine phase	Load (%w/w microspheres)
Non-acidified	11.4 ± 2.0
Acidified	10.5 ± 2.6

Table 3.6 The effect of pH of cimetidine phase on the drug loading for gelatin microspheres formulated using the crosslinking in oil method (n=3; mean ± sd).

There was no significant difference in the drug loading or release profiles over 1-6 hours (Figure 3.5). This shows that the incorporation of cimetidine as a solution or as a suspension had no effect on the release characteristics using this method and indicates that other factors are responsible for the fast release and high burst (Figure 3.11).

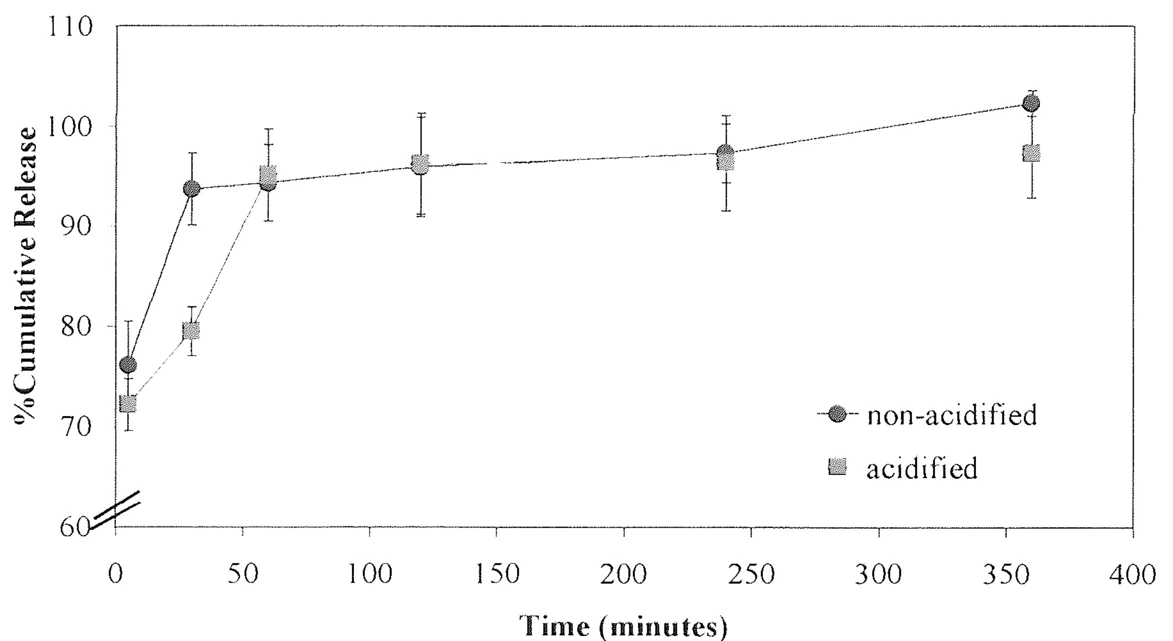


Figure 3.11 Effect of pH of the internal phase on the release profile from gelatin microspheres formulated using the single-emulsion crosslinking in oil method (n=3; mean ± sd)

3.3.2.5 Effect of pH of the medium on the release profile from gelatin microspheres

Normally the rate of drug release of cimetidine from gelatin microspheres would be faster in HCl than in PBS with gelatin reported to exhibit its lowest swelling at its isoelectric pH (7-10) (Chasin and Langer, 1990). Also, cimetidine will be ionised at lower pH and be more soluble (pK_a 6.8, solubility 11mg/ml). At pHs below the isoelectric point, cations

primarily affect the swelling of the polymer. This involves the breaking of hydrogen bonds and protonation of basic amino residues of the gelatin contributing to the increased swelling of microspheres by electrostatic repulsion and counteranion binding. If the microspheres swell more, they will be taking up more water due to uptake of the aqueous medium by capillary action through microsphere matrix. As more aqueous medium is present, more of the drug can be released by diffusion (Vandelli *et al.*, 2001).

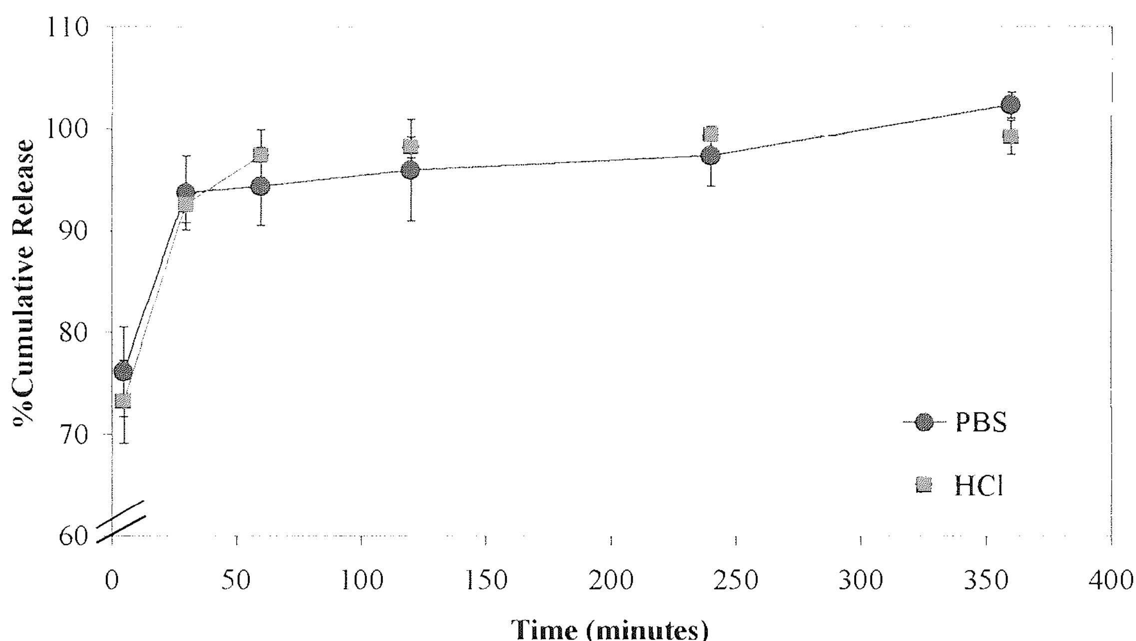


Figure 3.12 Effect of pH of the medium on the drug release from gelatin microspheres formulated using the single-emulsion crosslinking in oil method (n=3; mean ± sd)

There was found no difference between the release profiles in HCl or PBS (Figure 3.12 for microspheres of 269 ± 167 μm size with 15.3 ± 1.0 %w/w loading.. This was thought to be related to the lack of homogenous distribution of the crosslinker and penetration through the oil in the crosslinking process, which could lead to unpredictable swelling and release (see section 3.3.1.1).

3.3.3 Effect of crosslinking in acetone on characteristics of single-emulsion gelatin microspheres

Due to fast release profiles and high burst release with the crosslinking in oil method using different amounts of glutaraldehyde, and low adsorption of mucin to the surface of the microspheres, it was considered that the crosslinking in oil method was not suitable for

preparing crosslinked gelatin microspheres effectively. In an adaptation to the formulation, microspheres were crosslinked in acetone solution after the removal of the oil, similar to Vandelli *et al.*, (2001) and Leucuta *et al.*, (1997 a and b).

Microspheres were produced in three batches on separate days. Drug release (mean of three readings) and drug loading (mean of three readings) was evaluated using all three batches. Particle size (mean of 3 readings determined using Malvern software), zeta potential (mean of 5 readings determined using Zetamaster software) and surface mucin adsorption studies (mean of 3 readings) reported were evaluated from individual representative batches.

3.3.3.1 Effect of glutaraldehyde concentration on characteristics of gelatin microspheres

Microspheres were prepared with different concentrations of glutaraldehyde and the drug loading, drug release, particle size and zeta potential assessed.

Drug loading values were lower than those obtained with the crosslinking in oil method (*e.g.* with 5% glutaraldehyde, crosslinking in oil: 15.3 ± 1.0%, crosslinking in acetone: 9.7 ± 1.0%) (Dunnetts test, $P < 0.05$, compared to 1% formulation) possibly due to the extra washing step (see section 3.2.2). As the glutaraldehyde concentration was increased, there was a significant difference between drug loading obtained from microspheres crosslinked with 5% and 10%w/w (Dunnetts test, $P < 0.05$) (Table 3.7) possibly due to decreased particle size. In the crosslinking in oil method, the glutaraldehyde was added directly into the emulsion and the microspheres were harvested following washing and drying. In the crosslinking in acetone method, the microspheres were washed and dried before the addition of glutaraldehyde. The potential for interaction between glutaraldehyde and cimetidine at high concentrations of crosslinker is reported by He *et al.*, (1999 a) and discussed in section 3.3.1.2).

Glutaraldehyde (%w/w)	Load (%w/w) (n=3; mean ± sd)	Particle size (μm) (n=3; mean ± sd)	Zeta potential (mV) (n=5; mean ± sd)
1%	8.9 ±1.4	121 ± 42	3.5 ± 1.4
5%	9.7 ± 1.0	117 ± 52	-2.9 ± 1.0
10%	6.5 ± 1.7	94 ± 36	-6.9 ± 2.2

Table 3.7 Microsphere loading, particle size and zeta potentials for single-emulsion microspheres crosslinked in acetone

Zeta potential values were found to be more negative with higher glutaraldehyde concentration, indicating increased residual levels (as before) (Table 3.7). Particle sizes were considerably reduced with the crosslinking in acetone method in comparison to the crosslinking in oil method, (*e.g.* 1% w/w: 121 ± 42μm *versus* 371 ± 171μm). Particle size was found to decrease with higher glutaraldehyde concentration (Table 3.7).

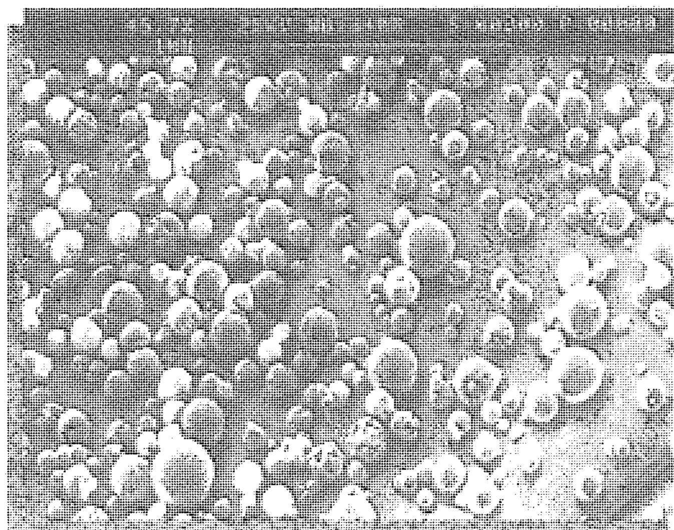


Figure 3.13 SEM image of microspheres prepared using crosslinking in acetone method (5% w/w glutaraldehyde)

Microspheres were generally spherical but some were uneven with rough surfaces (Figure 3.13). This was thought to be from the combination of removal of crystals of the drug from the surface of the spheres during washing, and the effect of filtration under vacuum causing depression on the surfaces of the spheres.

When the glutaraldehyde concentration was increased from 1% w/w to 5% w/w, there was a decrease in the burst release from ~70% to ~50% (Figure 3.14). Increasing the

concentration of glutaraldehyde did not alter the drug release profiles significantly from those obtained at 1% w/w loading (5% w/w, $f_2 = 45.1$, 10% w/w, $f_2 = 42.6$) (Figure 3.14).

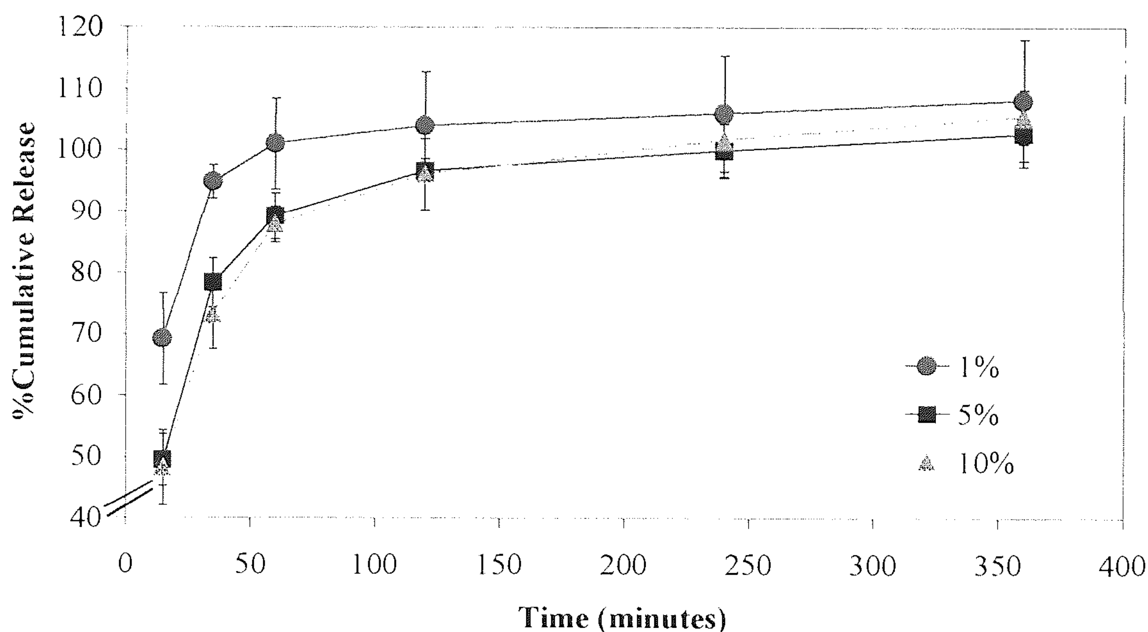


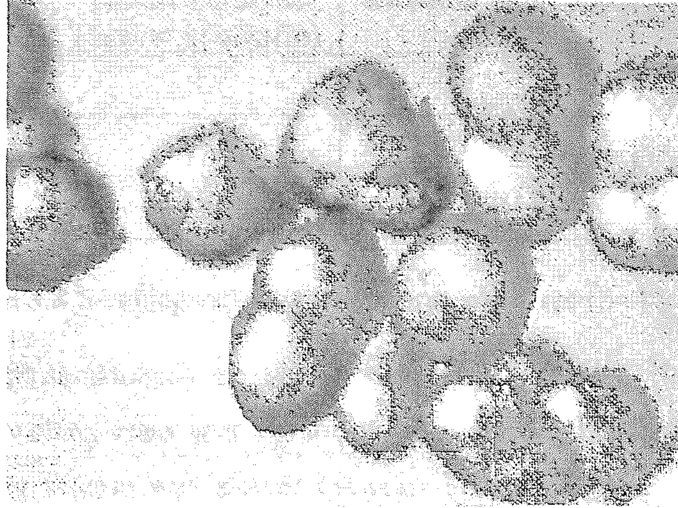
Figure 3.14 The effect of glutaraldehyde (%w/w gelatin) on the release from microspheres formed using the single-emulsion crosslinking in acetone method (n=3; mean ± sd)

3.3.3.1.1 Swelling studies

The rate at which chemicals can travel through the gelatin network structure is dependant on its swelling ability, which is influenced by crosslinking density (Vandelli *et al.*, 2001), due to the change in the number of junctions and the added restrictions of the macromolecular chains (Akin and Hasirci, 1995).

One of the important factors affecting drug release process from gelatin microspheres is reported to be the crosslinking degree (Vandelli *et al.*, 1991). An increase of the concentration of the crosslinking agent is capable of increasing the crosslinking density producing a lower swelling degree and decreases the drug release rate. The swelling of the microspheres was examined by optical microscopy (Section 2.8). The microspheres, crosslinked with 1%, 5% and 10% w/w glutaraldehyde, were found to swell to their maximum size after 15 minutes (similar to Vandelli *et al.*, 2001), and did not increase in size after this. Images of the swelling of 5% crosslinked microspheres are shown in figure 3.15.

(a)



(b)

(c)

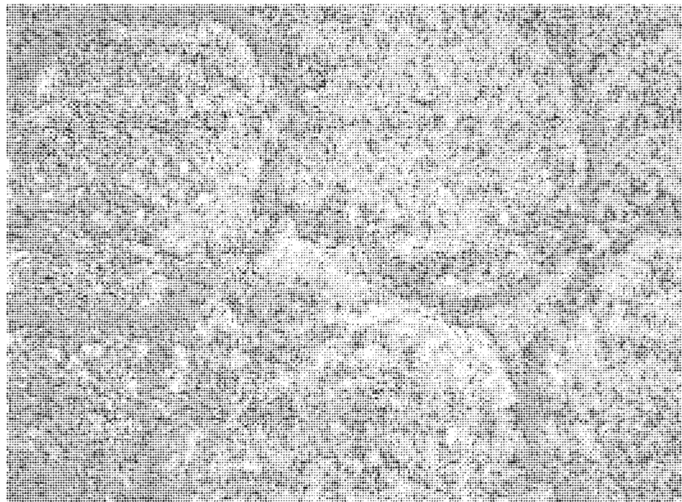


Figure 3.15 Optical microscope images of swelling of gelatin microspheres after (a) 0, (b) 1 and (c) 15 minutes incubation in water (5% w/w glutaraldehyde crosslinked preparation) (bar represents 50μm)

Glutaraldehyde (%w/w of gelatin)	Swelling ratio (Q)
1	3.1 ± 0.4
5	2.4 ± 0.2
10	1.2 ± 0.2

Table 3.8 Swelling ratios of gelatin microspheres (n=40; mean ± sd)

With increasing glutaraldehyde crosslinker concentration (from 1% to 10% w/w) it was found that the swelling ratio was significantly decreased (Dunnetts test, $P < 0.05$), and consequently drug release was slower between 1% and 10% crosslinker concentrations (Table 3.8). Leucuta *et al.*, (1997a) reported a decrease in swelling with increasing crosslinker concentration from 0.1% to 5%. It is reported that swelling is the principal factor for drug release (Cortesi *et al.*, 1999). Microspheres prepared using this method have a relatively rigid structure, and upon contact with water, the interlocked polymer fibres hydrate. Swelling of the polymer is accompanied by an increase in the volume, which aids in the drawing of liquid into the matrix. It is thought that the liquid diffuses into the matrix interior by diffusion through the swollen gel or relaxation *via* the glassy polymer (Leucuta *et al.*, 1997a). With increasing crosslinker concentration and consequently crosslinking density, less volume of liquid can be drawn into the matrix, resulting in the reduction of drug release (Leucuta *et al.*, 1997a). However, the decreased swelling ratio from 5% to 10% did not affect drug release, even though the swelling ratio was lower (Dunnetts test, $P < 0.05$). It can be concluded that variation of the crosslinking concentration can be used as a controlling factor in the rate of drug release up to a certain concentration of crosslinker (*i.e.* 5% w/w).

3.3.3.2 Analysis of mucoadhesive properties of gelatin microspheres *in vitro*

To test the mucoadhesion of different microspheres *in vitro*, the surface adsorption method was used adapted from He *et al.*, (1998). Figure 3.16 shows the effect of glutaraldehyde concentration on the surface mucin adsorption of the microspheres (section 2.11 for method details). There was a clear trend of decreasing adsorption with increasing glutaraldehyde concentration. This trend was similar to that observed with single-emulsion

crosslinked in oil microspheres (section 3.3.1.3). With higher amounts of glutaraldehyde, mucoadhesion of chitosan microspheres has also been reported to decrease considerably (He *et al.*, 1998).

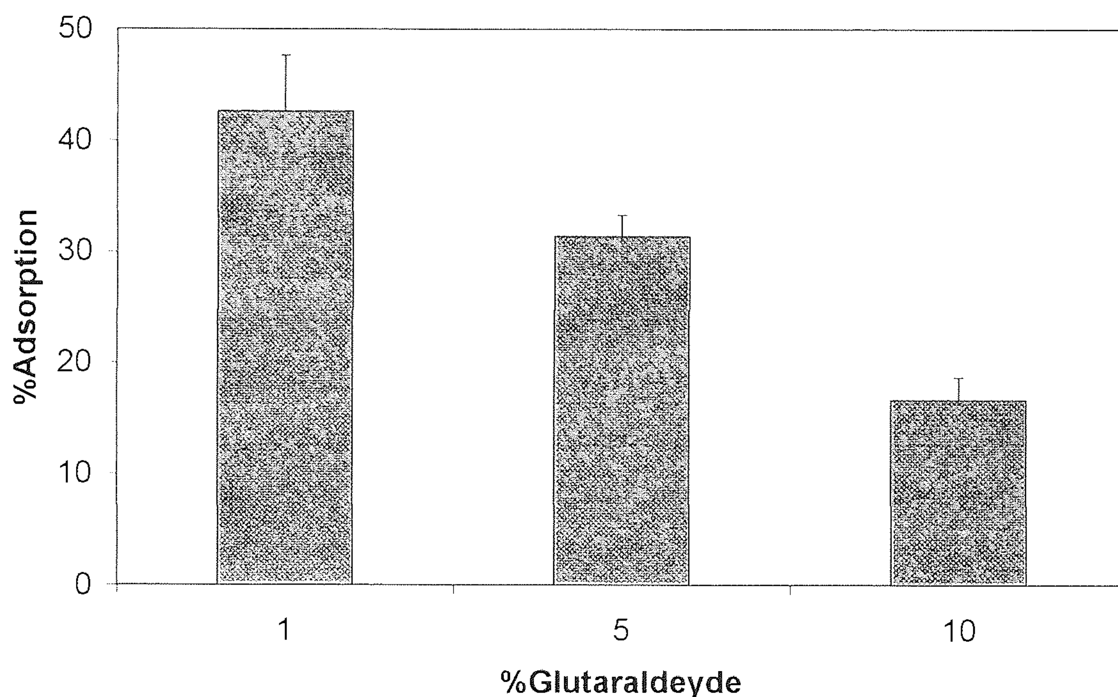


Figure 3.16 The adsorption of mucin to microspheres determined by adsorption assay (n=3; mean ± sd)

With higher concentrations of glutaraldehyde, the surface charge of the microspheres was more negative (Table 3.4). Since the mucus is predominantly negatively charged and binding favours positive charge, hence adsorption of the free mucin to the microspheres decreased with increased crosslinking concentration (see section 1.6.3 for mechanistic details). Furthermore, as one of the factors contributing to mucoadhesion is sufficient chain flexibility, with high crosslinking density of the polymer, the effective chain length which can penetrate the mucus layer is reduced in comparison to low crosslinking density (Akiyama *et al.*, 1994). Therefore with increased crosslinking of microspheres (5% and 10% w/w), the surface mucin adsorption was reduced.

3.3.3.3 Analysis of mucoadhesive properties of gelatin microspheres *ex vivo*

To attempt to mimic the *in vivo* situation more closely, an *ex vivo* rat intestine model was designed (see methods section 2.12) based on He *et al.*, (1998). In designing the method, systems where the intestine was subjected to any tearing or cutting (except when cutting the intestine into portions) were avoided, as this is likely to cause damage to the mucosa of the intestine. For example, Ranga Rao and Buri, (1989) removed the intestine from the rat and then cut it open longitudinally and held it in position with pins, where particles were actually placed on the tissue. In these studies, it was necessary to evert the intestinal segments for gentle washing as it was found that some food particles remaining in the intestine would interfere with the counting of microspheres following their incubation. Following incubation of the microspheres (in PBS) in the intestine at 37 °C for 60 minutes (He *et al.*, 1998), the solution of microspheres inside was allowed to flow out. The intestine was washed only once using PBS, as it was found that further washings resulted in removal of small pieces from the delicate mucus layer (see section 1.6.4.2 for literature comparisons and section 2.12 for details on method).

As a control, polystyrene latex particles were incubated in the rat intestine as latex is reported to have low mucoadhesive properties (Irache *et al.*, 1996). The percentage of particles remaining in the rat intestine for polystyrene latex was 3.2 ± 1.5%. This low value showed the ability of the assay to discriminate between non-mucoadhesive and mucoadhesive particles, as gelatin microspheres were found to have significantly higher levels of adhesion using this method (Figure 3.17). To examine reproducibility, three intestinal segments were used for each set of microspheres.

The retention of the microspheres in the rat intestine with different glutaraldehyde concentrations is shown in figure 3.17. There were no significant differences between the retention of microspheres crosslinked with different levels of glutaraldehyde (Dunnetts test, $P > 0.05$). There was a similar trend however of increasing adhesion with decreased glutaraldehyde concentration as observed with the surface mucin adsorption method, similar to He *et al.*, (1998). *In vivo*, many other factors exist which could affect the mucoadhesion (see section 1.6.4.3).

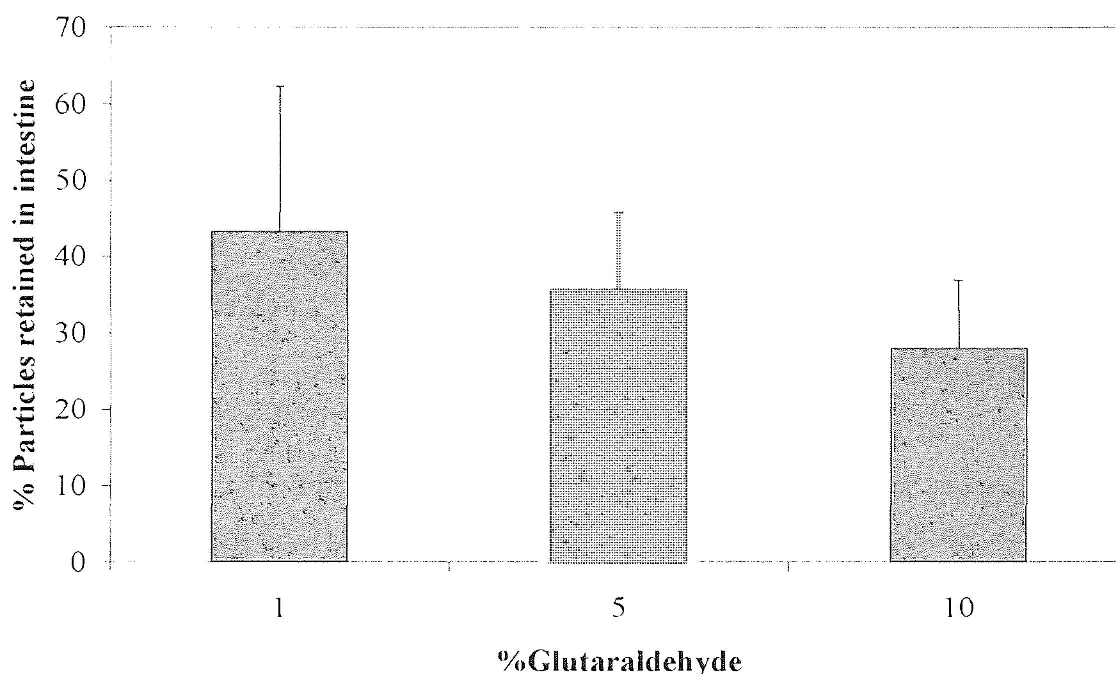


Figure 3.17 The percentage of microspheres remaining in the rat intestine following incubation of the particles in PBS (n=3; mean ± sd)

3.3.3.4 Effect of surfactant on the properties of gelatin microspheres

When there is a large surface to volume ratio for particles dispersed in a liquid medium, this can result in aggregation, and surfactants can be used to reduce this (Florence and Atwood, 1994). Use of surfactants has proven beneficial in stabilising systems where microspheres have uneven surface morphology due to aggregation and clumping (Florence and Atwood, 1994). Surfactant addition can also lead to reduction of gelatin droplet size during the emulsification step, and cause significant reduction of the final microsphere size (Esposito *et al.*, 1996).

To assess the effect of surfactant on the particle size of gelatin microspheres, Span 80 was added to the formulation in varying amounts (Section 3.2.2).

Surfactant %v/v	Particle size (μm) (n=3; mean ± sd)
0	148 ± 62
1	121 ± 42
5	59 ± 35
10	51 ± 32

Table 3.9 Influence of surfactant on the particle size of gelatin microspheres using single-emulsion crosslinking in acetone method

Addition of Span 80 and increasing concentration was found to reduce the particle size of the gelatin microspheres (Table 3.9), similar to Al-Helw *et al.*, (1998) with chitosan microspheres, due to the reduction of the droplet size during emulsification. Loading was not characterised.

3.3.3.5 Effect of stirring speed on particle size of gelatin microspheres

Different stirring speeds were used during homogenisation and the effect on the drug loading was investigated.

Stirring speed (rpm)	Particle size (μm) (n=3; mean ± sd)
600	247 ± 210
1200	121 ± 42
6500	57 ± 33

Table 3.10 Influence of stirring speed on the particle size of gelatin microspheres using single-emulsion crosslinking in acetone method

With increased stirring speeds (from 600 to 6500 rpm), it was found that particle size was successively reduced (Table 3.10), similar to Esposito *et al.*, (1996). At higher speed, turbid flow of the emulsion is reduced, resulting in production of smaller particles (Esposito *et al.*, 1996). This will have an effect on the release profile as seen in section 3.3.2.3, where it was shown that with smaller microspheres, a larger surface area is available, therefore release is faster with smaller particles.

3.3.3.6 Encapsulation of different drugs in gelatin microspheres

Encapsulation efficiency and drug release can be affected considerably by the physical characteristics of the drug. Partition coefficient (logP) is an indicator of hydrophobicity, giving an indication of how likely the drug is to partition into the oil phase from the aqueous phase in the emulsifying step during formulation of the microspheres. To assess the role of some physico-chemical properties on the loading and release of drugs, cimetidine and two β-blockers (nadolol and propranolol) were entrapped in gelatin microspheres

Drug	LogP	Drug loading (%w/w)	Aqueous Solubility (mg/ml)
Cimetidine	0.40 (a)	9.7 ± 1.0	11 (a)
Nadolol	0.71 (b)	6.7 ± 1.6	40 (b)
Propranolol hydrochloride	2.98 (c)	5.2 ± 0.9	50 (c)

Table 3.11 Encapsulation of cimetidine, nadolol, propranolol hydrochloride using single-emulsion crosslinking in acetone method (n=3; mean ± sd)
(a) GlaxoSmithKline, (1982), (b) Slusarek and Florey, (1986), (c) British Pharmacopeia, (1988)

There are two physico-chemical characteristics which would be primarily responsible for the level of entrapment of water-soluble drugs in gelatin microspheres (W/O emulsions). With higher aqueous solubility, it is expected that more drug be entrapped. Increased LogP as an indicator of hydrophobicity, would reduce drug encapsulation, because with higher logP more drug is likely to partition into the oil phase.

As the drug with the lowest loading was that with the highest aqueous solubility (*i.e.* propranolol hydrochloride) (Table 3.11), it is likely that the hydrophobic characteristic of the drug will dominate (Esposito *et al.*, 1996) and the reduced loading was due to the higher logP which causes the drug to partition into the oil phase from the aqueous phase.

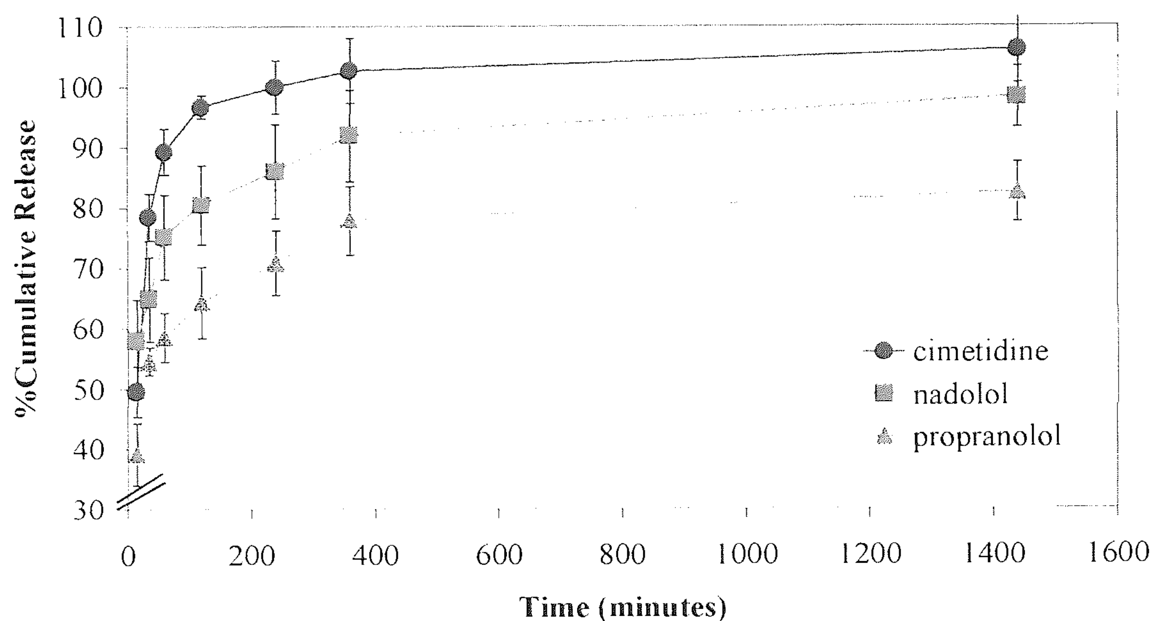


Figure 3.18 Drug release using the single-emulsion crosslinking in acetone method (n=3; mean ± sd)

With lower drug loading, it was found that release was slower (Figure 3.18). This is likely since reduced amounts of drug are available at the surface of the particles. This effect was observed in the analysis of the effect of different loadings on release profile in section 3.3.2.2.

3.3.4 Double emulsion gelatin microspheres

Microspheres were produced in three batches on separate days. Drug release (mean of three readings) and drug loading (mean of three readings) was evaluated using all three batches. Particle size (mean of 3 readings determined using Malvern software) reported was evaluated from individual representative batches.

As the crosslinking in oil method and the crosslinking in acetone single-emulsion methods resulted in fast rates of cimetidine release (where burst release was > 50%, ≥ 90% released after 60 minutes), other methods of cimetidine entrapment were investigated. It was thought that in these methods of gelatin microsphere formulation, the drug was likely to be primarily surface bound where drug release is governed by a substantial initial burst release followed by a fast release profile. It has been reported that slightly soluble

compounds (*e.g.* cimetidine) can benefit from sonication to improve incorporation of the drug particles inside the polymer matrix of microparticle systems (Desai and Bolton, 1993). Sonication has also reported to improve dispersion of microsphere systems and reduce particle sizes considerably (Desai and Bolton, 1993).

Probe sonication, due to the direct input of a sonic probe into the liquid allows a much higher adjustable power than other sonication devices such as ultrasound baths. The ultrasound probe has the ability to induce up to 100 times more ultrasonic energy into a reaction medium than an ultrasound bath (Papadakis, 2000). The frequency of the probe was fixed at 20 KHz, but the amplitude of variation (power) was varied.

Microspheres were prepared using a double emulsion method (O/W/O) involving a combination of the crosslinking in acetone and sonication methods (Section 3.2.3). It was thought that since sonication involves the input of high thermal energy which leads to formation of microspheres, this primary emulsion could promote higher entrapment of the drug inside the polymer matrix, before dispersion in the oily outer phase (soybean oil) where the stable multiple emulsion is formed, prior to hardening of the droplets and crosslinking (Pavanetto *et al.*, 1996). Prior to this, double emulsion methods have not often been used for manufacture of gelatin microspheres, but have been used in preparation of other microspheres including chitosan (He *et al.*, 1999). The amount of gelatin used was reduced from 1.5 g to 1 g when using sonication, as 1.5 g was found to be too viscous to sonicate using the ultrasound probe.

Formulation	**Load (%w/w) (n=3; mean ± sd)**	**Particle size (μm) (n=3; mean ± sd)**
Double emulsion	10.3 ± 0.7	66 ± 31
Single-emulsion	9.7 ± 1.0	121 ± 42

Table 3.12 Drug loading and particle size for microspheres formed using double emulsion and single-emulsion crosslinking in acetone methods

Drug loading was not significantly affected by the suspension of cimetidine in an organic phase (ethanol) and formulation by double emulsion method (Table 3.12). As cimetidine has a similar solubility in ethanol and acetic acid (i.e. ~ 50 $mgml^{-1}$, Bavin *et al.*, 1984), drug loading was not expected to be higher. Particle sizes were smaller with the double

emulsion method, due to the primary emulsion sonication step, which produces smaller particles due to high thermal energy input into the system.

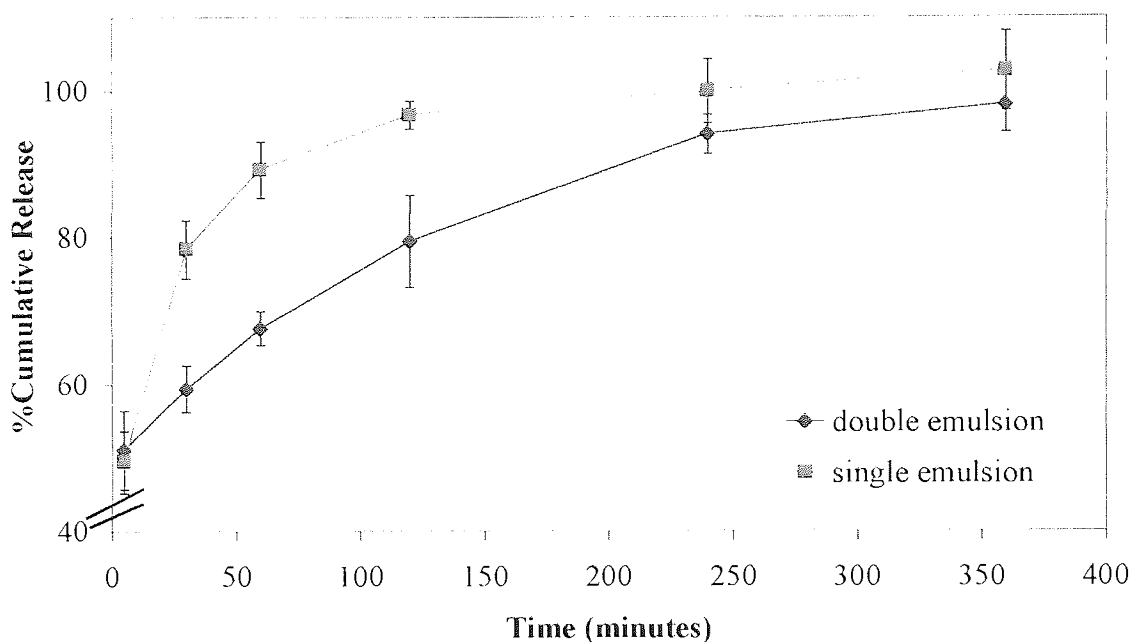


Figure 3.19 Drug release profiles from microspheres formulated by the single-emulsion and double emulsion methods (n=3; mean ± sd)

The drug release from double emulsion microspheres (details of particles size and drug loading in table 3.11) was slower than from those formulated using a single-emulsion method (Figure 3.19). It was previously observed (section 3.3.2.3) that with smaller particles (< 30 μm versus > 150 μm), drug release was slow. However, in this case size does not appear to be a major factor. Release appears biphasic for the drug from both formulations which is characterised by an initial 'burst effect' (phase 1) followed by sustained release (phase 2). Phase 1 is usually attributed to the drug being present at or near the surface of the microspheres whereas the slower second phase of release represents the movement of entrapped drug deeper in the polymer matrix. With the double emulsion method, it is likely that sonication enables more efficient drug distribution within the microsphere matrix, reflected in the drug release profile.

Microspheres were found to be spherical with smooth surface morphology (Figure 3.20).

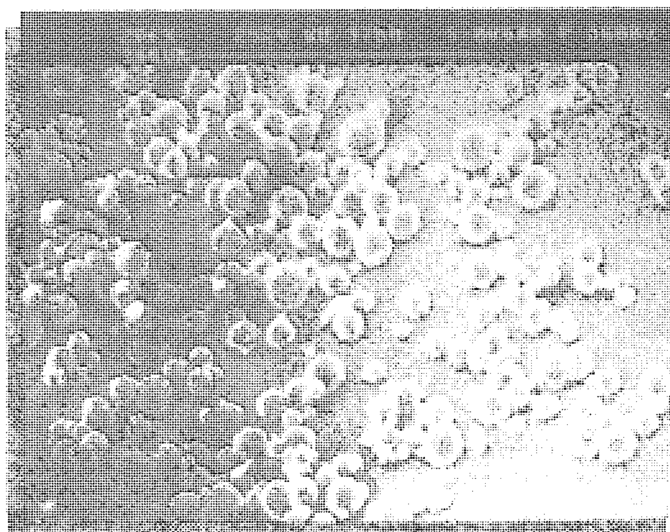


Figure 3.20 SEM image of microspheres prepared using the double emulsion method (5% w/w glutaraldehyde)

3.3.4.1 Effect of sonication variables on microsphere production

To prepare particles of different size using the double emulsion method the sonication power was varied and the loading and release profiles of the microspheres compared.

Power (Watts)	Drug load (%w/w) (n=3; mean ± sd)	Particle size (μm) (n=3; mean ± sd)
120	10.3 ± 0.7	66 ± 31
60	8.6 ± 0.7	66 ± 19
30	12.2 ± 0.9	85 ± 28
15	13.6 ± 1.0	205 ± 161

Table 3.13 Drug loading for microspheres prepared by double emulsion method with variation in sonication power

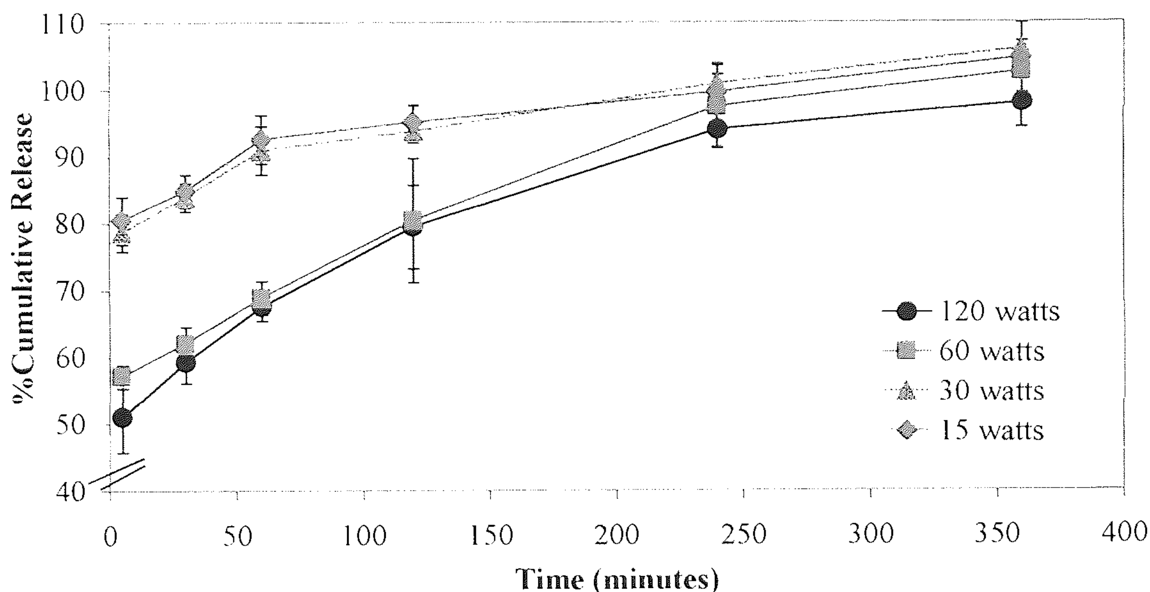


Figure 3.21 The effect of sonication power on the release profile of cimetidine (n=3; mean ± sd)

A slight decrease in the drug loading occurred when power was decreased from 120 watts to 60 watts (Table 3.13). On a further decrease in power however, drug loading was found to increase (Table 3.13) (Tukey test, $P < 0.05$). There was a significant difference in the drug release profiles of microspheres formulated using 60 watts and 30 watts both in the burst release and the rate of drug release apparent from figure 3.21. It is likely that with the lower power, since the thermal energy input during sonication is reduced, drug distribution pattern in the microspheres is altered and more drug is surface bound resulting in faster release (*i.e.* of 60 watts compared to 30 watts). Generally, microsphere size increased with decreasing sonication power. At 120 watts, there was a wider range of particle sizes, as seen by the large standard deviation, and the particle size distribution (Figure 3.22). As the power was decreased to 60 watts, the mean particle size was similar, but the distribution was narrower (Figure 3.22). Further decreases in power to 30 watts showed an increase in the particle size and the standard deviation (Figure 3.23). At 15 watts there was a much broader range of larger sizes, with some aggregates (Figure 3.23). This shows that the particle size can be controlled by variation of the sonication power, and spheres of different sizes can be produced.

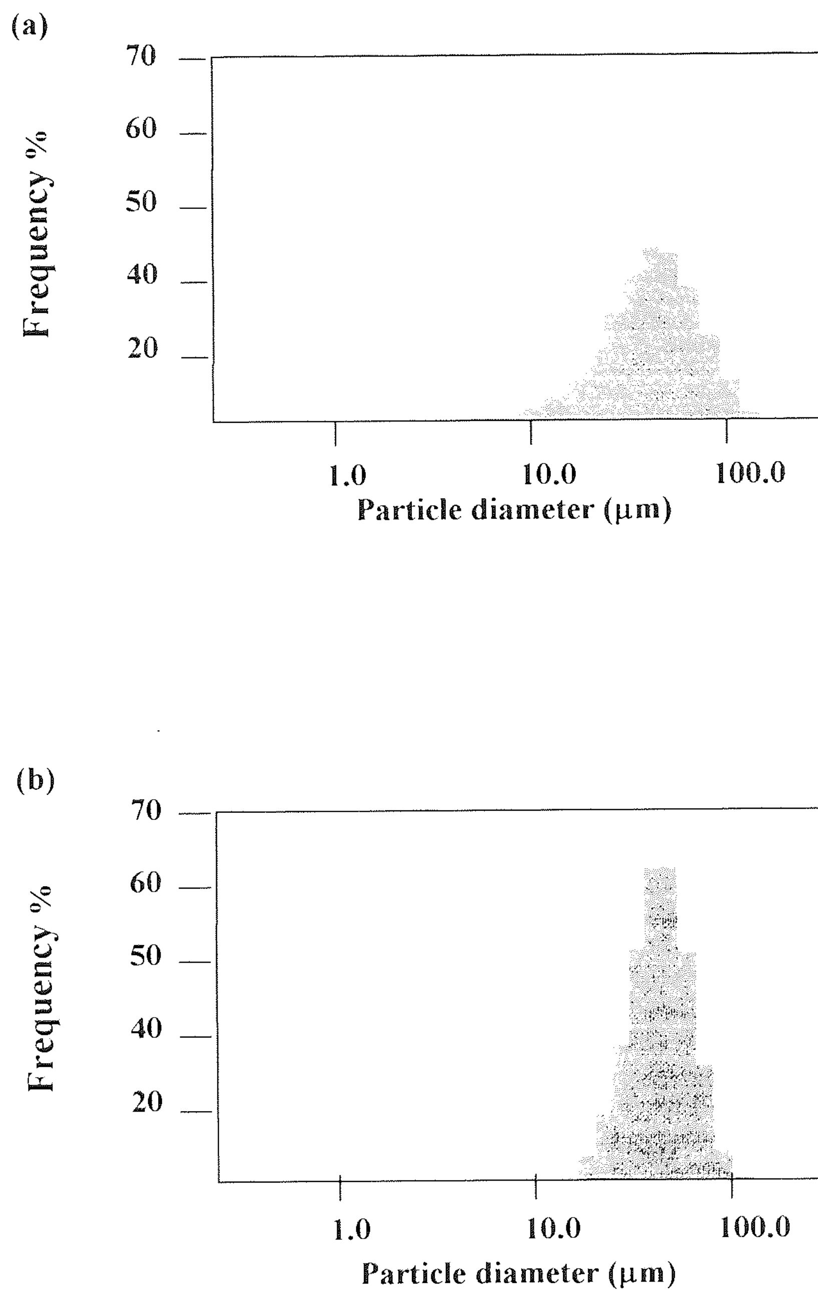


Figure 3.22 Particle size distributions for microspheres produced using the double emulsion method with (a) 120 watts, (b) 60 watts.

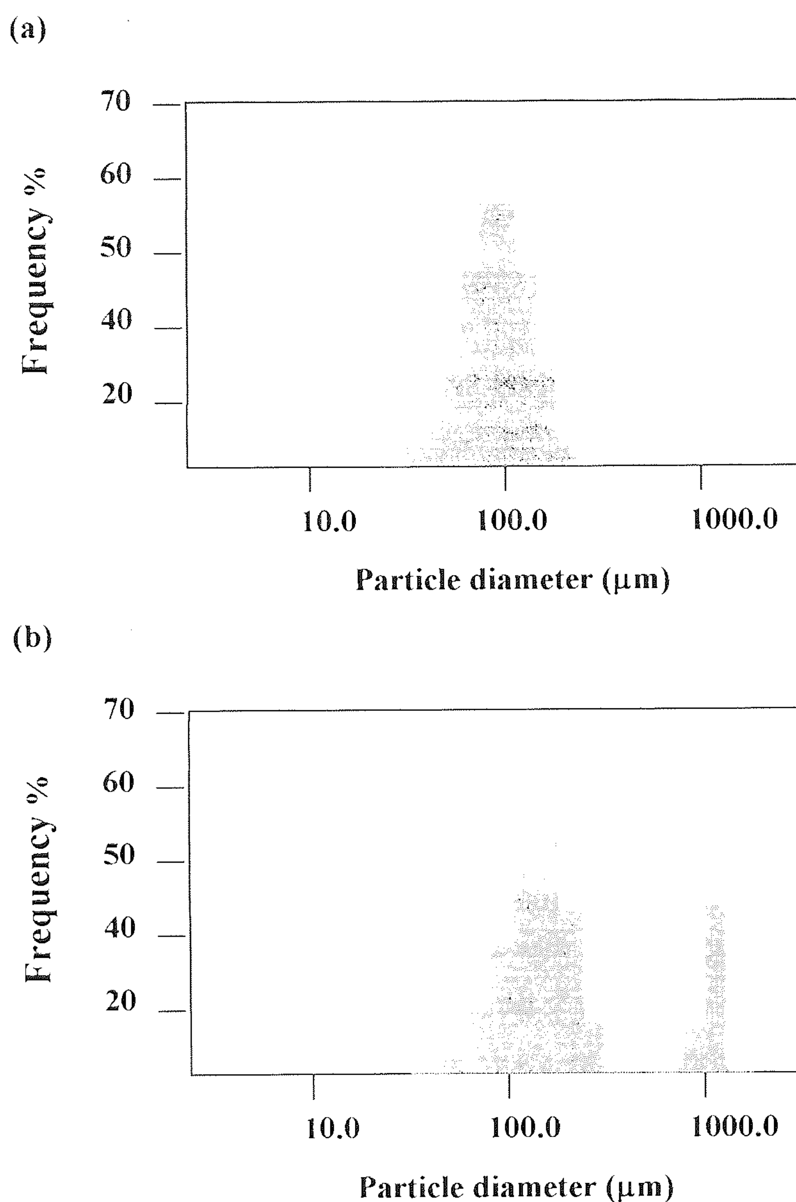


Figure 3.23 Particle size distributions for microspheres produced using the double emulsion method with (a) 30 watts, (b) 15 watts.

3.3.5 Preparation of gelatin microspheres using spray drying and characterisation of microspheres

Spray drying is an important and widely used technology in the pharmaceutical and biochemical industry (see also section 1.4.3). The process is defined by the transformation of a liquid (solution) into a solid (powder). Spray drying for preparation of microspheres from biodegradable polymers has many advantages over conventional microencapsulation techniques, *e.g.* consistent production of microspheres within a narrow size range.

When spray drying is used, the microparticles obtained are microspheres or microcapsules, dependant on whether the drug is dissolved or dispersed in an organic or aqueous solution of the polymer to be sprayed. When the liquid is fed to the nozzle with a peristaltic pump, atomisation occurs by the force of compressed air, which disrupts the liquid into droplets. The droplets, together with hot air, are blown into a chamber where the solvent in the droplets is evaporated and discharged through an exhaust tube. The solvent in the droplets is removed rapidly due to the heat energy input of the spray dryer. The thermal efficiency of the spray drying is related to the heat energy input, which is controlled by the inlet temperature and blower, and the heat used during evaporation of the solvent. To reach an optimum spray drying efficiency, it is necessary to achieve a balance between the amount of energy input and the amount of energy needed, related to the amount of sample input. If the emulsions used contain water, the inlet temperature of the spray-dryer is normally required to be above 100°C for efficient evaporation. The dry product is collected in a collection bottle.

Water-soluble and water-insoluble drugs have been incorporated with high encapsulation efficiency into hydrophilic or hydrophobic polymers by spray drying (Pavenetto *et al.*, 1992). Reproducibility is known to be good with potential for scale-up. A disadvantage is that of low yields in synthesis laboratory small-scale processes, however it is reported that industrial scale spray dryers can achieve yields as high as 90-100% (Bittner and Kissel, 1999). Spray drying is therefore a relatively fast and reliable technique for microencapsulation.

Atomising pressure, nozzle size and flow rate can all be varied to alter the size of the microparticles. The size of the nozzle used will affect the particle size; a 0.5mm nozzle will produce smaller spheres than a 1.0mm nozzle. It is also reported that a low pump flow rate and low air flow results in particles of small size due to the small droplets formed during the process (He *et al.*, 1999 a).

3.3.5.1 Optimisation of conditions using spray drying

Spray-dried microspheres were formulated as in section 3.2.4. In optimising the preparation of gelatin microspheres by spray drying, a number of manufacturing conditions

which could affect the formulation of the microspheres were investigated. The inlet temperature was varied. It was found that below temperatures of 100°C, gelatin microspheres were not formed and some of the gelatin solution passed through the connecting tube between the evaporation chamber and cyclone (see figure 1.9). A minimum temperature setting of 100 °C was required to avoid this. This appears to be the case for formulation of microspheres from other polymers such as chitosan, where it was reported that the temperature must be above the boiling point of water (100 °C) (He *et al.*, 1999 a).

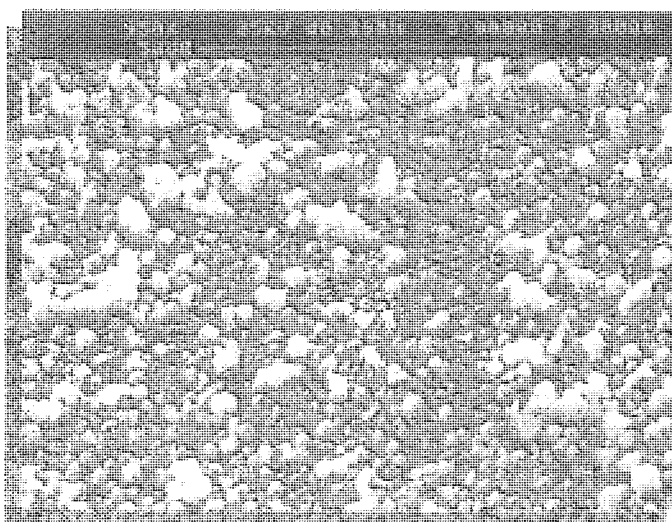


Figure 3.24 SEM image of microspheres produced using spray drying

Microspheres were formed with uniform size and smooth surface morphology using spray drying (Figure 3.24).

Temperature °C	%Yield (n=3; mean ± sd)
100	5.4 ± 0.3
120	13.0 ± 0.3
140	10.6 ± 0.5
160	7.5 ± 0.6

Table 3.14 Yields from study on inlet temperature variation by spray drying (n=3; mean ± sd)

Highest yields were obtained with the inlet temperature at 120 °C (Table 3.14). Other formulations of gelatin microspheres using a Mini Buchi 190 have reported similar conditions (Burgess and Ponsart, 1998).

Aspirator Rate (m^3min^{-1})	**% Yield (n=3; mean ± sd)**
5	7.7 ± 0.7
10	13.2 ± 2.7
20	4.1 ± 0.6

Table 3.15 Percentage yield of microspheres formed by variation of aspirator rate (pump flow rate of 10 $mlmin^{-1}$ and atomising air pressure of 200 mmHg, inlet temperature 120°C) (*n*=3; *mean* ± sd)

The optimum thermal efficiency of the spray drying is related to the heat energy input, which is controlled by the inlet temperature and blower, and the heat used during evaporation of the solvent. Optimum setting of aspirator rate was found to be 10 m^3min^{-1} for production of highest microsphere yield (Table 3.16).

Pump flow rate (ml/min)	**Particle size (μm) (n=3; mean ± sd)**
1	10.3 ± 2.5
5	16.5 ± 4.4
10	24.8 ± 4.6

Table 3.16 Variation in pump flow rate (n=3; mean ± sd)

Particle size was found to increase with increasing flow rate similar to He *et al.*, (1999a) (Table 3.16). Under a faster pump rate, larger droplets are formed leading to increased particle size (He *et al.*, 1999a).

% Glutaraldehyde	Drug load (%w/w) (n=3; mean ± sd)	Particle size (μm) (n=3; mean ± sd)
1	29.5 ± 4.0	24.8 ± 4.6
5	24.4 ± 1.6	26.9 ± 4.8
10	25.3 ± 2.2	28.8 ± 3.5

Table 3.17 Variation in crosslinker (%w/w gelatin) added (5ml volume)

Drug load was found to be considerably higher than with single or double emulsion methods. In spray drying, since the microspheres are not subjected to washing or filtering steps, it is considered a more efficient method in achieving high encapsulation efficiency.

There was no significant difference between the loading at different concentrations of glutaraldehyde (Table 3.17), similar to He *et al.*, (1999a) (Dunnetts test, $P > 0.05$). Particle size was unaffected with changing glutaraldehyde concentration. This contradicts what has been previously observed with microspheres produced using emulsion methods (sections 3.3.1.1 and 3.3.3.1), where higher concentrations of glutaraldehyde resulted in smaller particle sizes. This was thought to be due to the method of formulation used in this section, i.e. spray drying.

In comparison to 5% and 10% crosslinked preparations, gelatin microspheres crosslinked with 1% glutaraldehyde had a faster release profile, similar to microspheres prepared using the crosslinking in acetone method (see section 3.3.3.1). Spray-dried microspheres crosslinked with 1% w/w glutaraldehyde (% w/w of gelatin), also had a higher burst release than 5% and 10% crosslinked formulations (Figure 3.25). High burst release was expected as the drug loading was high. Following the burst release, the release profiles were slow with microspheres prepared using spray drying. The release profiles formulated using gelatin microspheres were slower than those reported with chitosan microspheres with the same drug (He *et al.*, 1999a), indicating efficient spray drying settings for the production of microspheres by this method.

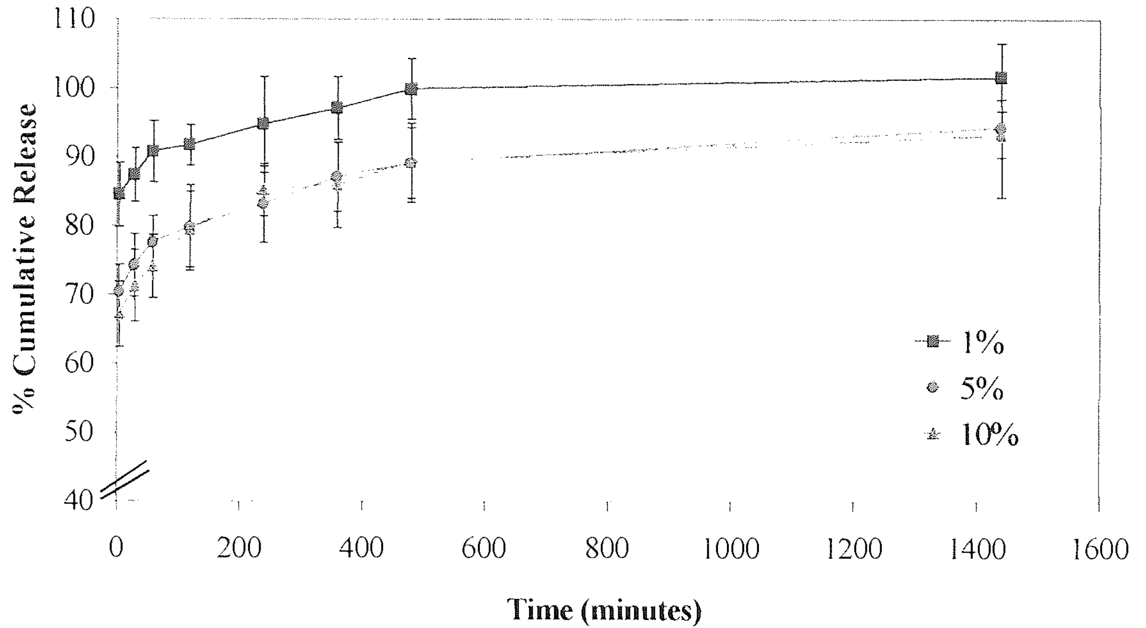


Figure 3.25 Effect of glutaraldehyde concentration on the release profile of gelatin microspheres formulated using spray drying (n=3; mean ± sd)

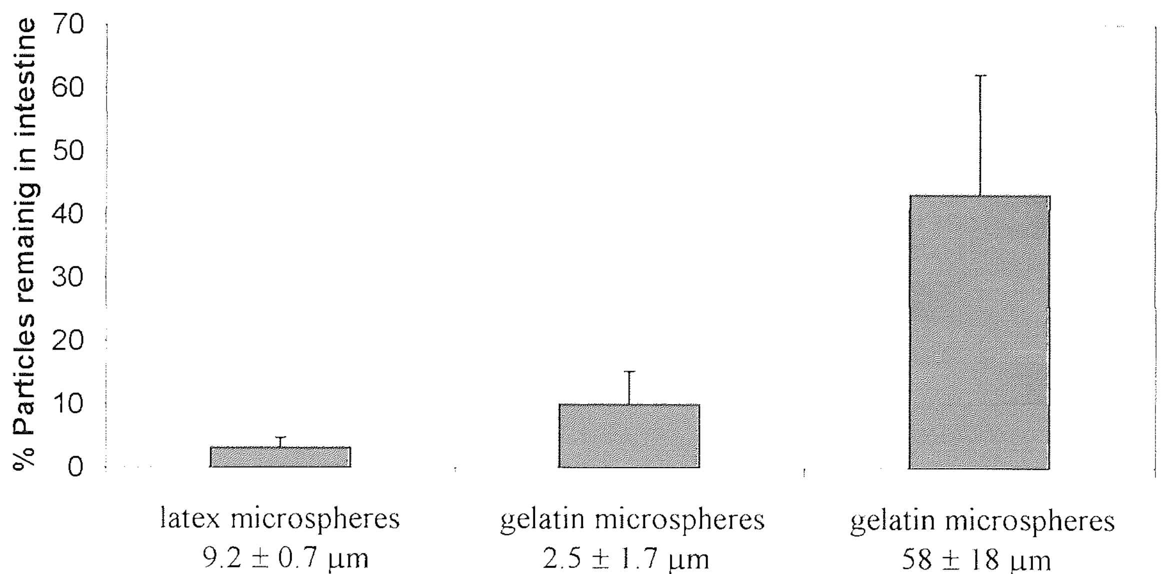


Figure 3.26 The percent of microspheres remaining in the rat intestine following incubation of the particles in PBS (n=3; mean ± sd)

The adherence in the rat intestine model was determined with small and large microspheres (produced by spray drying) (section 3.2.5) and double emulsion (section 3.3.4) using

polystyrene latex microspheres as a control (Figure 3.26). There was no significant difference in the percentage retention of microspheres of small size and latex microspheres in the rat intestine (Dunnetts test, $P > 0.05$). This was an unexpected result, as gelatin is expected to have mucoadhesive properties, as can be seen *in vitro* and *ex vivo* with microspheres of larger sizes, formulated using the double emulsion method. Large particles were retained significantly more than smaller particles (Dunnetts test, $P < 0.05$). Zeta potentials of the microspheres were measured to be –5.9 ± 0.6 mV for the double emulsion method (58 ± 18 μm size) and –16.5 ± 0.2 mV for spray-dried microspheres (2.5 ± 1.7 μm size). Therefore as one of the factors governing the adherence of microspheres to mucus may be electrostatic attraction between positively charged microspheres and negatively charged mucin (He *et al.*, 1999a), the zeta potential alone cannot be responsible for the difference in the retention. Both particles are negatively charged and therefore electrostatic interactions would be reduced compared with a polymer such as chitosan. It is reported that particles less than 1 μm penetrate the mucus layer, whereas particles greater than 2 μm may associate with the surface of the mucus layer instead (Takeuchi *et al.*, 2001). Therefore, in this case, particles of size ~ 2.5 μm would be expected to associate with the mucus layer. With smaller particles, since they have a larger surface area to volume ratio, it would normally be expected that they would adhere more strongly to the mucus layer than larger particles with a smaller surface area to volume ratio. However, in this case larger gelatin microspheres were found to adhere better than smaller particles. He *et al.*, (1998) also reported that larger chitosan microspheres were retained more than smaller particles.

3.4 CONCLUSION

Different methods of producing gelatin microspheres have been examined and the physiochemical properties of the microspheres studied. It was found that the single-emulsion crosslinking in oil method produced microspheres with the highest drug loading and largest particle size of 269 ± 167 μm (Table 3.18).

Method	Emulsion type	Load (%w/w)	Particle size (μm)
Crosslinking in oil	W/O	15.3 ± 1.0	269 ± 167
Crosslinking in acetone	W/O	9.7 ± 1.0	121 ± 42
Double emulsion	O/W/O	10.3 ± 0.7	66 ± 19
Spray-dried	aqueous	24.4 ± 1.6	27 ± 5

Table 3.18 Effect of different formulation methods for production of gelatin microspheres on drug loading (theoretical load of 50% w/w) and particle size (n=3; mean ± sd)

The loading of microspheres could be increased to 33.9 ± 7.8 %w/w by using a theoretical load of 80 %w/w cimetidine. With the crosslinking in oil method, there was found to be no control over the rate of drug release with changing concentration of glutaraldehyde. It was thought that the viscosity of the oil phase may be preventing homogeneous crosslinking of the microspheres. For this reason, crosslinking in acetone was used. Drug loading was reduced using other emulsion methods, and the slowest release profile was found with microspheres produced using a double emulsion method, thought to be due to the sonication step which enabled more efficient drug distribution. Using spray drying, it was found that spherical microspheres of narrow size distribution could be prepared with high drug loading using an aqueous phase. Yields however were low, and drug release was found to be fast with burst release ~ 70% of cimetidine. Highest control over the manufacture of gelatin microspheres with respect to particle size was found with the double emulsion method by the variation in the sonication power (66 ± 31 μm at 120 watts and 205 ± 161 μm at 15 watts). In further studies (chapters 4 and 5), microspheres used were those produced from double emulsion and spray-dried methods.

Formulation method	Percentage retention (%)
Double emulsion	43.3 ± 19.0
Single-emulsion	35.7 ± 10.2

Table 3.19 Percentage of microspheres remaining in the rat intestine for single and double emulsion methods (n=3; mean ± sd)

The difference between microspheres remaining in the rat intestine following incubation for single and double emulsion methods was not significant (Table 3.19). Generally, mucoadhesion *ex vivo* and *in vitro* was found to be low with high crosslinker concentration. It has been theorised that this may be due to a combination of both the more negative zeta potentials of microspheres crosslinked with higher concentrations of glutaraldehyde, and the reduction in the chain mobility of microspheres crosslinked with high concentrations of glutaraldehyde. It was shown that larger microspheres adhered to a higher extent than smaller particles.

CHAPTER FOUR

INCORPORATION OF MODIFYING POLYMERS IN GELATIN MICROSPHERE FORMULATIONS

4.1 INTRODUCTION

Mucoadhesive polymers have been investigated extensively for improvement in performance of oral controlled release drug delivery systems, by prolonging contact time of the delivery device with the mucus layer of the intestine (see section 1.6). Many different polymers have been evaluated for *in vitro* adhesive forces including derivatives of poly (acrylic acid) (*i.e.* carbopol and polycarbophil), chitosans and alginates (Smart *et al.*, 1984; Ch'ng *et al.*, 1985; Hassan and Gallo, 1990; Smart, 1991, Tobyn *et al.*, 1995).

Mucoadhesion can occur between the polymer and the mucin *via* Van der Waals force, hydrogen bonding, electrostatic interactions and physical entanglements (detailed in section 1.6.3). Certain structural requirements of polymers for mucoadhesion are considered as being important. It is reported that for polymers of low molecular weights, interpenetration (or diffusion) of the polymer molecules is favoured, whereas entanglements are favoured for high molecular weight polymers (Mahrag Tur and Ch'ng, 1998). Therefore, for a particular polymer there is likely to be an optimum molecular weight for mucoadhesion. Flexibility of polymer chains is also important for interpenetration of the polymer network, and entanglement with mucin (Mahrag Tur and Ch'ng, 1998). As polymers become crosslinked, the mobility of the individual polymer chains decreases. As the crosslinking density increases, the effective chain length that can penetrate into the mucus layer decreases further and mucoadhesive strength is reduced. This was observed in sections 3.3.1.3, 3.3.3.2 and 3.3.3.3, where the highly crosslinked (with glutaraldehyde) gelatin microspheres, resulted in low mucoadhesion determined *in vitro* and *ex vivo*.

Mucoadhesive microspheres, by forming a gel-like structure in contact with the mucus, should prolong the contact between the delivery system and the mucosa, and should have the potential of releasing the drug in a sustained and controlled manner, thereby possibly increasing the absorption efficiency of the drug. Gelatin has been reported to exhibit moderate mucoadhesion (Zaman *et al.*, 1999) and it was shown in chapter 3 that gelatin microspheres could provide controlled release of cimetidine. These formulations were combined with more mucoadhesive polymers (*i.e.* carbopol, polycarbophil, sodium alginate and chitosan) to potentially enhance the mucoadhesive properties. Microspheres were formulated using two methods: (1) blending of gelatin with modifying polymer prior

to formulation and (2) surface modification of gelatin microspheres with polymers after formation of gelatin droplets in the emulsion. The microspheres were characterised *in vitro* for release, loading, zeta potential, size, swelling ratio, amount of mucin adsorbed, and *ex vivo* for retention of particles in the intestine.

4.1.1 Modelling mucoadhesion

The different methods for assessing mucoadhesion could be considered as falling into three categories: *in vitro*, *ex vivo* and *in vivo*.

4.1.1.1 *In vitro* models for measuring mucoadhesion

A variety of methods have been used to model the mucoadhesive strength of mucoadhesive polymers, *in vitro*. They can be classified as 'bulk' or 'tensile', where bulk techniques include the analysis of polymer/mucin mixtures by oscillatory rheology and penetrometry (Hassan and Gallo, 1990), and tensile methods can include assessment of the maximum detachment force and work of adhesion between polymer gels and mucin gel (Smart, 1991; Tobyn *et al.*, 1995).

4.1.1.1.1 Models for measuring mucoadhesion of polymers

Studies on mucoadhesive polymer strengths using tensiometer instruments have been reported in the literature (Smart *et al.*, 1984; Ch'ng *et al.*, 1985). The method is based on measurement of the separation force between a layer of tissue and the polymer/formulation to be tested. The apparatus normally consists of a balance, chart recorder and water bath (to mimic physiological conditions). The polymer to be tested is placed on a disc and in contact with the mucus segment, load applied, and the force at which it is pulled from the surface is recorded. Factors which can influence this method are: the rate of lowering of the platform, initial contact time of the disc and mucous membrane, and the load applied to the adhesive joint during the initial contact period. Some of the studies on polymers have been modified to test the adhesive strengths of microspheres (section 1.6.4.1.2) (Chickering and Mathiowitz, 1995).

An early experiment in modelling mucoadhesion involved the use of a system based on the Wilhelmy plate method for the determination of surface tension and adaptation for the *in vitro* assessment of mucoadhesive strength of polymer gels. This technology is similar to the tensile stress methods, consisting of a water bath for the incubation of the mucus gel and a platform connected to a plate which remained in contact with the gel and was then raised, the force at which this occurred was determined using a microforce balance. A range of polymers were examined by this technique and the order of mucoadhesive strength for the polymers was found to be carbopol > sodium alginate > hydroxypropylmethylcellulose > gelatin. The contact time of the mucus gel with the coated plate was found to influence the determined mucoadhesive strength (Smart *et al.*, 1984).

The mucoadhesive strengths of polyacrylic acid polymers were measured using a surface tensiometer (Ch'ng *et al.*, 1985), with tissue removed from rabbits. It was reported that with increased crosslinking density of the polymers, there was a decrease in mucoadhesive strength.

An *in vitro* method was used for the assessment of the adhesive force between a disc of test material and a model mucous membrane (Smart, 1991). The intestine slice was obtained from male Wistar rats. Once the test disc material was placed in contact with the mucus intestinal segment, the force at which the disc was pulled from the surface of the mucus layer was recorded. With respect to the accuracy of the assay, when the materials compared were carbopol 934P, HPMC, gelatin and PEG, a minimum ~30% standard deviation error was apparent from the results, from 5 batches. This is highlights the variability of results obtained in this manner with this type of experiment.

A rheological method was used to compare the mucoadhesive strengths of mucin/polymer solutions (Hassan and Gallo, 1990). Viscosity coefficients of polymer solutions with mucin were determined using a viscometer. Proteins such as gelatin and bovine serum albumin were assessed along with polymers such as chitosan and polyacrylic acids. Polyacrylic acid and chitosan recorded the highest adhesion values. Since the values were determined by measuring viscosity, and hence the shear force of stirring the solution after addition of the polymer, the viscosities of the polymers alone led to higher values.

The force of detachment for tablets containing carbopol 934P has been measured in terms of compression weight and compression time (Tobyn *et al.*, 1995) using a tensiometer instrument. Parameters influencing the method of measurement included speed of the separation of the polymer and mucous portions, and the pH of the medium used. Increasing content of sodium chloride in the simulated gastric medium reduced the strength of mucoadhesion of the tablets. Correlations were poor when the work of adhesion was evaluated for 100 tablets, likely due to experimental parameters involved such as speed of separation.

There is a wide variation in published results for *in vitro* mucoadhesion partly due to the lack of universal test methods, particularly due to the lack of standard commercially available apparatus (Tobyn *et al.*, 1995). As was discussed in this section, instrumental parameters and experimental test conditions can affect the observed mucoadhesive results. A number of factors can crucially influence the strength of the observed mucoadhesive bond. For example with the tensile methods, the lowering of the platform, the initial contact time of the disc and mucous membrane, and the load applied to the adhesive joint can be influential on the determined results. As the *in vitro* methods can only realistically be used in the screening of polymers for their potential *ex vivo* or *in vivo* mucoadhesion, the majority of these methods used would be relatively similar in their approximation of the *in vitro* mucoadhesive strength of polymers. For example, Tamburic and Craig, (1997) compared the relative mucoadhesive strengths of carbopol 974P, polycarbophil and carbopol 971P using different tensile and bulk methods and it was found that all the methods gave similar relative results.

4.1.1.1.2 Models for measuring mucoadhesion of microspheres

Techniques used in the measurement of the adhesion of polymers have been modified to measure the adhesion of groups of microspheres or individual microspheres. Some of the methods can be either *in vitro* or *ex vivo*. *In vitro* methods are classified as those using the bulk or tensile methods, e.g. where slices of the intestine are removed and force of adhesion measured, or amount of mucin adsorbed to microsphere surfaces is determined. *Ex vivo* methods are those which closely resemble *in vivo*, where particles are incubated inside or on the intestinal segments, and the resultant level of particles retaining in the intestine determined without tensile or bulk methods.

The mucoadhesive performance of microspheres composed of chitosan and carbopol 934P was studied using a modified tensile method (Abd El-Hameed and Kellaway, 1997). The microspheres were thinly sprinkled onto cellulose nitrate filtration membrane, pre-coated with a thin film of carbopol gel for retention of the microspheres. The carbopol gel film was found to have no effect in the subsequent tensile test measurements. The microspheres were weighed and after drying of the membrane, the detachment force required to separate the membrane from the mucus layer was determined. Microspheres range of ~ 16 μm to ~ 40 μm were used. The relative mucoadhesive strengths were reported to be of the order carbopol 934P > chitosan = Hydroxypropylmethylcellulose = Polyvinylalcohol = lactose. In this assay, many variables could influence the final mucoadhesive strength value calculated, including the rate of lowering of the platform, initial contact time with the membrane and the load applied during the initial contact period. It is likely that the reproducibility with respect to the number of microspheres which were sprinkled on the membrane each time would influence the determined mucoadhesive strength.

Microspheres (315-400 μm) were coated with mucoadhesive polymers, counted and placed onto pig intestinal tissue (Lehr *et al*, 1992 a). The tissue was moved in different directions in solution and the spheres remaining adhered to the tissue were counted. The researchers reported high mucoadhesive properties from polycarbophil samples. Polycarbophil beads as a control were reported to adhere to the pig intestine for ~18 hours.

The detachment force between microspheres and guinea pig small intestinal mucosa was measured by a modified precision balance method (Bogataj *et al.*, 1999). The microspheres were placed on a glass plate and hydrated. The detachment force between the microspheres and mucosa was determined. The mucoadhesive strength of the microspheres was determined to be of the following order: carboxymethlycellulose salt > carbopol > hydroxypropylcellulose >Eudragit > vinylpyrrolidone/vinyl acetate copolymer. It was reported that the volume of solution used to wet the microspheres significantly affected the detachment force of the microspheres from the mucosa, and hence this would affect the determined mucoadhesive strengths.

The *in vitro* mucoadhesive properties of chitosan microspheres were evaluated using turbidimetric measurements between chitosan and mucin in aqueous solutions (He *et al.*, 1998). This method was a simple method involving microsphere incubation in mucin solution, followed by addition of Periodic Acid reagent and Schiffs reagent (see sections 2.1.9 and 3.3.1.3.1.2). Absorbance was used to determine free mucin and by comparison to standards, mucin adsorption to microspheres was calculated. This gave an indication of the levels of mucoadhesion of different chitosan microspheres, and was a simple and effective screen for the relative mucoadhesive strengths of the microspheres. It is also one of the few methods of *in vitro* evaluation of mucoadhesion without using tensile forces to detach the microsphere from the mucus segment.

Durrer *et al.*, (1994) used a turbidimetric method to study the adsorption of latexes to rat intestinal mucosa. It was reported that adsorption increased with the size and the hydrophilicity of the latexes.

4.1.1.2 *Ex vivo* methods for measuring mucoadhesion

In the majority of the techniques for the evaluation of the mucoadhesive potential of microspheres *in vitro*, the microsphere(s) is detached from the mucus sample (in whatever form) to measure the strength of the adhesion. In essence, the adhesive bond formed between the sample and the mucus is destroyed using tensile forces. Therefore, the results obtained may be quantitative but are indirect. Therefore, methods where the level of retention of particles is determined without exertion of external forces (and minimal modification of the intestine layer or segment) are classified as *ex vivo* methods and have been used to closely mimic the *in vivo* situation. Of course, *ex vivo* methods could never be considered to completely reproduce *in vivo* conditions due to the many biological factors *in vivo*, but could be considered a step-up from *in vitro* experiments, particularly when microspheres are being measured.

An *ex vivo* method was designed to test the bioadhesive potential of particles (Ranga Rao and Buri, 1989) using unfasted albino rats. Intestinal segments were cut open longitudinally and held in position with pins. Particles were placed on the tissue and incubated at room temperature for 40 minutes, followed by collection upon drying at 70

°C. A high level of adherance of microparticles to the tissue was reported, with 100% of the polycarbophil particles adhering to the intestine tissue. About 50% adherance using coated particles coated with hydroxypropylmethylcellulose sodium carboxymethylcellulose was found. In the assay, glass beads were used as a control. There would seem to be several limitations with the assay. Cutting of the intestine layer may damage the tissue and subsequently, the mucus layer. Prolonged exposure of the tissue to air may lead to dehydration and damage to the mucus layer. During the assay, an angle of inclination was used on the platform where the tissue was tied, which would affect the rate of flow of the solution and hence washing away of the particles.

Another *ex vivo* model used intestine segments from male Wistar rats (He *et al.*, 1998). Chitosan microsphere (3.3 μm-12.0 μm) suspensions were filled into lengths of intestine and incubated at 37 °C for 60 minutes. The particles present in the suspension were counted using a Coulter Counter. Mucoadhesion of chitosan microspheres was reported to decrease with increased higher levels of glutaraldehyde crosslinker.

Microspheres have also been incubated in everted sacs of the jejunum and colon (Jacob *et al.*, 1995). Following incubation, the microspheres were collected and reweighed, quantifying the percentage of non-adhered microspheres to the mucus layer. Polyanhydride microspheres were employed (sizes 5-10 μm). It was reported that 6% of the microspheres adhered to the descending colon and 36% adhered to the proximal jejenum. A drawback in this assay is the potential damage to the mucus layer which could occur during evertion of the tissue.

The mucoadhesion of microspheres composed of chitosan (CH) and hyaluronic acid (HA) was evaluated by determination of the mucociliary transport rate of the microparticles across an isolated frog palate (Lim *et al.*, 2000). It was reported that HA and HA/CH were the most mucoadhesive microspheres. It was suggested that the mucoadhesive potential of HA combined with the penetration-enhancing effects of chitosan has potential for enhancing mucoadhesive strength.

In a comparison of two methods for measurement of mucoadhesion of particles both the Cahn microbalance method and the everted sac method were used (Santos *et al.*, 1997).

Similar results were reported (microsphere sizes 600-850 μm) In the everted sac method, bound microspheres were determined by weight after collection. The Cahn method used was similar to tensiometer methods (as described previously). Polymers of anhydride copolymers and polycaprolactone exhibiting a high binding percentage in the everted sac assay also demonstrated high fracture strengths when determined by Cahn measurements.

From the different *ex vivo* techniques examined, methods where limited factors can govern the detected mucoadhesion are preferable. For example, methods where the intestine is sliced or cut and exposed to the atmosphere would undoubtedly cause dehydration or some damage the delicate mucus layer. Other methods may better mimic the *in vivo* situation such as the method employed by He *et al.*, (1998).

4.1.1.3 *In vivo* methods for measuring mucoadhesion

In vivo methods which can be used to monitor the fate of a pharmaceutical dosage form in the rat include the administration of radiolabelled particles and the measurement of levels of particles by counting following dissection of the relevant organs. This correlates deposition and transit of the delivery system. This may give some idea of the performance of a pharmaceutical dosage form in pre-human trials.

Radiolabelled poly (hexyl cyanoacrylate) nanoparticles were administered to mice and whole body autodiography was carried out to determine the location of the particles (Ponchel and Irache, 1998). It was found that after 30 minutes, most particles were located in the stomach, and four hours later, the majority of particles were located in the intestine.
The GI transit of adhesive formulations was assessed in rats; polystyrenesulphonic acid (PSSA), hyaluronic acid (HA), hydroxyethyl cellulose were used as aqueous solutions (Harris *et al.*, 1990 b), followed by addition of radiolabel. Carbopol and polycarbophil were used in an acidic solution. The formulations were given by oral gavage in a study using 20 rats, fasted for 18 hours prior to administration. Distribution of the formulations along the GI tract was determined by removal of the organs of the GI tract. It was reported that carbopol 7.5% (w/v) showed some delay in transit to the ileo-caeal junction, 4% and 5% showed significant delay, but 6% carbopol did not. PSSA and HA showed normal transit through the stomach and intestine. It was not conclusively determined whether the

cause of the delays was the mucosal adherance of the polymers or the viscosity of the formulations.

The success of formulation development is often dependent on definition of the variables affecting the *in vivo* behaviour of a drug delivery system. Where *in vitro* testing methods can be used as a screen for the mucoadhesion of polymers or particles and *ex vivo* methods can to a certain extent model the expected performance *in vivo*, there are many other factors which can affect the behaviour of dosage forms *in vivo*. These can include the GI physiology (see section 1.2), chemical nature of the food nutrients present (e.g. caloric value, amount of fat, carbohydrate etc.) and the timing of the dose administration relative to ingestion of a meal.

Gammascintigraphy is the only real technique with which the *in vivo* fate of a pharmaceutical dosage form can be monitored and seen in man. It is a technique by which the transit of a dosage form through its intended site of delivery can be noninvasively imaged *in vivo* via the introduction of a short-life gamma emitting radioisotope (Digenis *et al.*, 1998). Gammascintigraphy has been used in assistance of product development and for the testing of marketed products.

The GI transit of some mucoadhesive formulations in man were studied using gamma-scintigraphy (Harris *et al*, 1990a). The materials used were amberlite IRA-410 (strongly basic ion exchange resin), radioactive marker, carbopol and polycarbophil. with lactose as a control. The delivery vehicle consisted of the resin with the bioadhesive and diluent within a gelatin capsule. 14 human male volunteers were fasted for 12 hours prior to dosing. The lactose was visually seen to readily disperse whereas the carbopol form was seen to appear as clusters of activity, an indication that the adhesive agent remained associated with the labelled resins for some considerable time in the GI tract. The researchers reported that the stomach emptying time for the carbopol formulation was 4 fold longer than lactose in one subject, and was longer than polycarbophil. It was considered that overall, the adhesive polymers did not greatly affect the transit of the formulations, likely due to considerable inter-subject variability in gastric emptying.

In this section some *in vitro*, *ex vivo* and *in vivo* methods were discussed. The *in vitro* methods were concluded to be able to screen particles for their potential mucoadhesive

strengths, however in the majority of the techniques, the adhesive bond formed between the sample and the mucus is destroyed using tensile forces. The technique which was used as a screen for the particles was the surface adsorption technique (see chapter 3 and 4) where the microspheres were incubated with mucin and the level of adherence determined. *Ex vivo*, it is important to mimic the *in vivo* situation as closely as possible. Of course this is difficult as in measurement of the mucoadhesion of microparticles in the rat intestine, the presence of food can affect the determined level of particles when using for example, the Coulter Counter. Therefore it is necessary to wash out the contents of the intestine and sometimes it is necessary to evert it to clean away the food contents. Techniques where the intestine is used as segments and not sliced or cut where the mucus layer may be exposed to the atmosphere dehydrated or damaged. *In vivo* analysis would be preferred as the fate of particles can be monitored using radiolabelled particles, giving reproducible results. However the techniques can be expensive and are likely to be of use in the latter stages of formulation development. In man for example, gammascintigraphy can be used to follow the *in vivo* fate of a formulation.

4.1.2 Carbopol and polycarbophil

Polycarbophil, carbopol 971P and carbopol 974P are polymers of acrylic acid (Figure 4.1), and vary in their crosslinking conditions. These polymers are non-irritant to the skin (man) and non-toxic orally (rats and dogs), with all three polymers considered as GRAS (Generally Recognised As Safe) for oral use by the FDA (BF Goodrich, 1994). Carbopol 971P and 974P, both polymers crosslinked in ethyl acetate, were introduced in the last 10 years as toxicologically preferred benzene-free alternatives to carbopol 934P (BF Goodrich, 1994). Carbopols and polycarbophil are crosslinked with allyl sucrose or divinyl glycol respectively, and are both structurally and chemically similar. They are large polymers with molecular weights above 2×10^6 Daltons, with a high density of carboxylic groups (Slovin *et al.*, 1997). Carbopol 974P is a more crosslinked, higher viscosity analogue of carbopol 971P (Ugwoke *et al.*, 1999). Carbopol 971P is reported to have a 'fish-net' gel structure, whereas the more highly crosslinked polymers, carbopol 974P and polycarbophil, are reported to have 'fuzzball' gel structures (Ugwoke *et al.*, 1999).

Carbopols and polycarbophil in general have reported significantly high mucoadhesive properties determined *in vitro* (Smart *et al.*, 1984; Hassan and Gallo, 1990; Lehr *et al.*, 1992a; Dobrozsi *et al.*, 1999), and *in vivo* (Ch'ng *et al.*, 1984; Longer *et al.*, 1984; Harris *et al.*, 1990a). In particular, polycarbophil, carbopol 971P and carbopol 974P have been reported to have strong mucoadhesive properties (Mortazavi, 1995; Park and Robinson, 1987; Ugwoke *et al.*, 1999). They are known to swell up to 1000 times their original volume (and ten times their original diameter) in water to form a gel when exposed to a pH environment above 4-6, where the ionisation of the carboxylic acid groups of the polymers causes the ionic repulsion of the polymer leading to the swelling (Khan and Zhu, 1999). Carbopol and polycarbophil polymers have a pK_a of 6.0 ± 0.5 (BF Goodrich, 1994). The interaction of the polymers with mucus is related to their structure, as their numerous carboxyl functional groups can form hydrogen bonds with mucus glycoprotein during the process of mucoadhesion (Lim *et al.*, 2000), which is reported to be optimum at acidic pH conditions (Park and Robinson, 1987; BF Goodrich). Furthermore, the high binding ability of carbopol and polycarbophil to mucin in acidic media is mainly due to the presence of a number of carboxylic acid groups in an undissociated form, favouring hydrogen bonding (Mahrag Tur and Ch'ng, 1998).

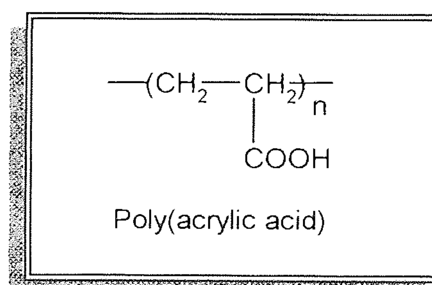


Figure 4.1 Structure of Poly(acrylic acid)

4.1.3 Chitosan

Chitosan is a natural polyaminosaccharide derived from N-deacetylation of chitin, chemically (1→4)-2-acetamido-2-deoxy-β-D-glucan, which is abundant in nature (Figure 4.2). Chitosan is obtained industrially by hydrolysing the aminoacetyl groups of chitin from crabs or shrimps in aqueous alkaline solutions. Chitosan and its derivatives have been explored as membranes for dialysis, blood anticoagulants, wound healing accelerators and

preparations against parasites and carriers for controlled drug delivery (Groves, 1999). Chitosan also shows some pharmacological activity as an antacid and anti-ulcer agent, as well as being able to decrease cholesterol and fatty acid absorption (MacLaughlin and Rolland, 1999). Depending on the degree of deacetylation, chitosan is a polymer of N-acetyl-D-glucosamine and glucosamine units, but the glucosamine units predominate. The deacetylation process destroys the structural regularity of the chitin macromolecule, making the polymer soluble in dilute acid solutions, and yields a rubbery hydrogel in water (Fang *et al.*, 1997).

Chitosan is insoluble in water, alkalis, and organic solvents and requires the aid of select organic acids, such as acetic acid, for solubilisation (MacLaughlin and Rolland, 1999). Due to its high molecular weight and integration of long unbranched chains, chitosan solutions are very viscous. Once in solution, chitosan behaves as a linear polyelectrolyte and a weak base; the amino groups present in the N-deacetylated subunits confer a highly positive charge density. The amino groups on the glucosamine subunits have an intrinsic pK_a of 6.5, and thus chitosan behaves as a polycation at acidic and neutral pH (MacLaughlin and Rolland, 1999).

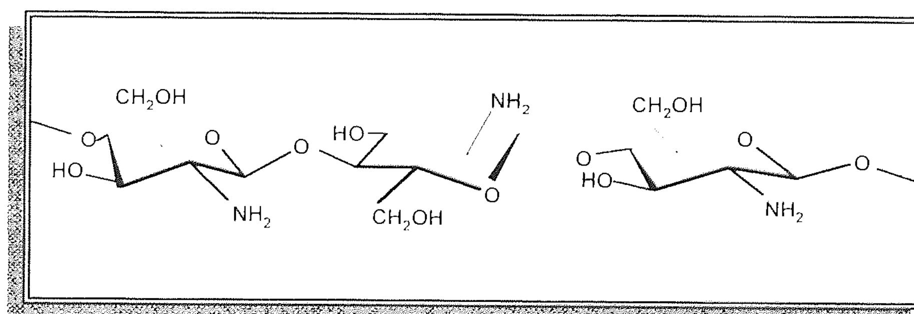


Figure 4.2 Structure of chitosan

Chitosan has been evaluated extensively in microsphere formulations (Jameela and Jayakrishnan, 1995; Liu *et al.*, 1997; Remunan-Lopez *et al.*, 1998; Al-Helw *et al.*, 1998; He *et al.*, 1999 a and b). It has been used to enhance the absorption of poorly bioavailable drugs, such as proteins and peptides across the intestinal epithelia *in vivo*, and *in vitro* (Borchard *et al.*, 1996; LueBen *et al.*, 1997).

From a technological perspective, chitosan has unique properties making it an effective material for microencapsulation. Due to its hydrophilic and cationic nature, it has the

ability to gel upon contact with counter-anions (Remunan-Lopez *et al.*, 1998). The physicochemical properties of the chitosans used in these studies are summarised in table 4.1

	G213	**CL 113**
Molecular weight (gmol^{-1})	460000	110000
%Deacetylation	86	87

Table 4.1 Properties of chitosan polymers

4.1.4 Alginate

Sodium alginate is a polysaccharide salt, and has been used as a food additive, an antacid adjuvant and cell immobiliser (Lee and Min, 1996). In the pharmaceutical industry, alginate has been used as a tablet binder, gastric emptying time delaying substance, and gelling agent due to its protective effect on gastrointestinal mucus and nontoxicity (Lee and Min, 1996). Alginic acid is a linear block copolymer of β-D-mannuronic acid (M) and α-L-guluronic acid (G) residues joined by 1,4 glycosidic linkages (Figure 4.3). Alginate is is a negatively charged polysaccharide with a high charge density. Aqueous solutions of alginates have the ability to form gels on the addition of di- and tri-valent metal ions by a co-operative process involving consecutive residues in the G blocks of the alginate chain (Miyazaki *et al.*, 2000). Alginate has been reported to be mucoadhesive (Gaserod *et al.*, 1998) and has low toxicity and low immunogenicity. Mucoadhesive microspheres have been prepared using alginate (Chickering *et al.*, 1997; Ramdas *et al.*, 1999).

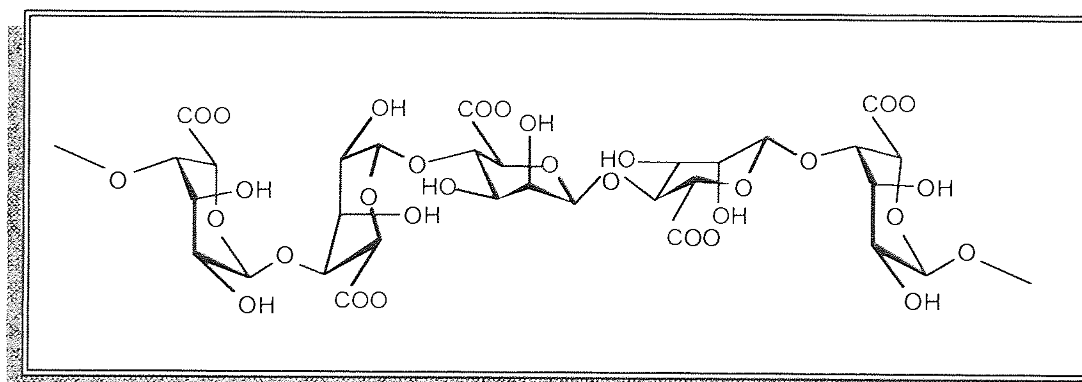


Figure 4.3 Structure of alginate

Two alginate polymers were used in this study: MVM (high molecular weight polymer) and LVG (low molecular weight polymer). A 1% solution of Pronova MVM has a viscosity greater than 200 mPas and the percentage guluronic acid contant is less than 50%. The LVG polymer has a higher guluronic acid content (60%) but a lower viscosity (between 20-200 mPas for a 1% solution).

4.1.5 Lectins

Lectins, a structurally very diverse class of proteins, are found in organisms ranging from viruses and plants (which constitutes their largest population) to humans (see section 1.6.5). They are characterised by their ability to bind to carbohydrates or sugar residues (Haltner *et al.*, 1997). Lectins can be referred to as cytoadhesives, where they bind directly to the cell surface by specific receptor-mediated interactions, where the specific binding or adhesion is reversible without alteration of the covalent structure of any of the recognised glycosal residues (Arangoa *et al.*, 2000). In mammalian mucosa, sugar residues are located either at the surface of epithelial cells or in the mucous layer formed by glycoproteins. Therefore based on lectin-sugar interactions, lectins could bind to these sites of specificity and be used for drug delivery to the gastrointestinal tract, by their incorporation with nanospheres or microspheres.

The association of ligands with nanoparticles or microparticles can be achieved by covalent linkage and adsorption processes. Ideally, the ligand should be conjugated to the particles through a covalent linkage, which is likely to be more stable than adsorption procedures, without affecting the specificity of the lectin. Ligand coupling, which can be used for the conjugation of a lectin to the surface of a protein microsphere (*e.g.* gelatin), can be achieved using glutaraldehyde where coupling occurs *via* the formation of a Schiff base between an amino group and an aldehyde function, by one- or two-steps (Irache *et al.*, 1996).

The lectin used in these studies was *Ulex europaeus* agglutinin I (UEA I). UEA I is obtained from furze seeds and binds to α-l-fucose containing oligosaccharides. Wirth *et al.*, (2002) reported the high binding of UEA I in pig gastric mucin compared to other

lectins, i.e. UEA I > LCA (*Lens culinaris* agglutinin) = STL (*Solanum tuberosum)* > PNA (peanut agglutinin) > DBA (Dilichos biflorus agglutinin). UEA I lectin was chosen for this and other reasons. Firstly, particles composed of proteins such as vicilin and gliadin have been shown to be good substrates for the binding of this lectin to mucin (Ezpeleta *et al.*, 1996; Arangoa *et al.*, 2000). The activity and specificity of this lectin has been preserved following the coupling to particles (Ezpeleta *et al.*, 1999). By the coupling of UEA I to nanoparticles composed of proteins, the interaction with mucin has been enhanced (see section 1.6.5.2) (Ezpeleta *et al.*, 1999).

4.2 MATERIALS AND METHODS

All materials were obtained from Sigma/Aldrich (Poole, UK) unless otherwise stated and were of analytical grade unless otherwise specified.

4.2.1 Preparation of microsphere blends using the double-emulsion method

Microspheres were prepared by a double-emulsion (O/W/O) method. Cimetidine (1.0 g) (GlaxoSmithKline, Harlow, UK), in 3 ml ethanol, was sonicated at 120 watts for 3 minutes using an ultrasound probe (Soniprep 150, MSE, Surrey, UK) into 9 ml of an aqueous solution containing 1 g total of gelatin and mucoadhesive polymer. Microspheres containing carbopol (971P or 974P) (Noveon, BF Goodrich, UK) and polycarbophil (Noveon AA-1, BF Goodrich, UK) were prepared at 10 and 30 % (*i.e.* 0.1 g and 0.3 g, gelatin 0.9 g and 0.7 g respectively). When 30% carbopol, polycarbophil, chitosan CL 113 or chitosan G 213 (Pronova, Norway) were used, 5% acetic acid was used to dissolve the polymer. Alginates MVM and LVG (Pronova, Norway) were dissolved in water. The primary emulsion was added to 50 ml soybean oil, containing 1% v/v Span 80, and emulsified at 1200 rpm using a Heidolph stirrer (Lab-Plant, Huddersfield, UK) for 10 minutes. The emulsion was then cooled under stirring in an ice bath for 30 minutes, acetone was added (37 ml) and stirring continued for a further 5 minutes. Microspheres were harvested by filtration, and washed with hexane three times (50 ml volumes) for removal of oil, followed by drying in a vacuum oven (Gallenkemp, UK) at room temperature overnight. Crosslinking was then carried out using glutaraldehyde (5% w/w of

gelatin) in 20 ml acetone for 5 minutes. Microspheres were then filtered and washed with acetone, dried overnight in a vacuum oven at room temperature and stored in a vacuum desiccator.

4.2.2 Preparation of surface-modified microspheres using the double-emulsion method

The method of surface modification of gelatin microspheres with mucoadhesive polymers was adapted from Leucuta *et al.*, (1997a and b). Microspheres were prepared by a double-emulsion (O/W/O) method. Cimetidine (1.0 g) in 3ml ethanol was sonicated at 120 watts for 3 minutes into gelatin (1 g) in 9 ml water (preheated to 60°C). The primary emulsion was then added to 50 ml soybean oil containing 1% v/v Span 80, and emulsified at 1200 rpm using a Heidolph stirrer for 10 minutes. 0.3 g of the relevant mucoadhesive polymer was suspended in 10 ml soybean oil and added to the emulsion and stirring continued for a further 10 minutes. The formulation was then continued as in 4.2.1.

4.2.3 Preparation of microsphere-lectin conjugates

Lectins were conjugated to gelatin microspheres using the glutaraldehyde activation method (Irache *et al.*, 1996).

35 mg drug loaded (29.5 ± 4.0 % w/w) microspheres (see section 3.2.4 for formulation details) were added to 10 ml PBS (pH 7.4) under stirring for 30 minutes (see section 3.3.5.1 for details on drug release; note: ~ 85% drug release from this formulation after 30 minutes) for complete swelling to occur. 8% w/v glutaraldehyde solution was prepared and 4 ml added to the microsphere suspension. Stirring was continued for 4 hours. Microspheres were then centrifuged at 12500 rpm for 10 minutes and supernatant containing unreacted glutaraldehyde discarded. Microspheres were then resuspended in 7 ml PBS (5 mg/ml microsphere solution). 1 ml was added to 1 ml of fluorescein isothiocyanate (FITC) labelled lectin (0.25 mg/ml) and incubated overnight. Microsphere/lectin conjugates were then centrifuged at 12500 rpm and washed 3 times to remove any unbound lectin. Supernatants were diluted and added to 96-well plates, and absorbances determined by fluorescence at 530 nm using a Wallac Victor 2 Multilabel Counter (Perkin Elmer Life Sciences, Boston USA) (excitation wavelength, 485 nm;

emission wavelength 530 nm). The free lectin concentration in the supernatant was determined against lectin standards. Standard lectin concentrations (0.5, 1.5, 2.5, 3.5 and 5 μg/ml concentrations) were prepared in PBS and assayed at 530 nm using the Wallac Victor (Figure 4.4).

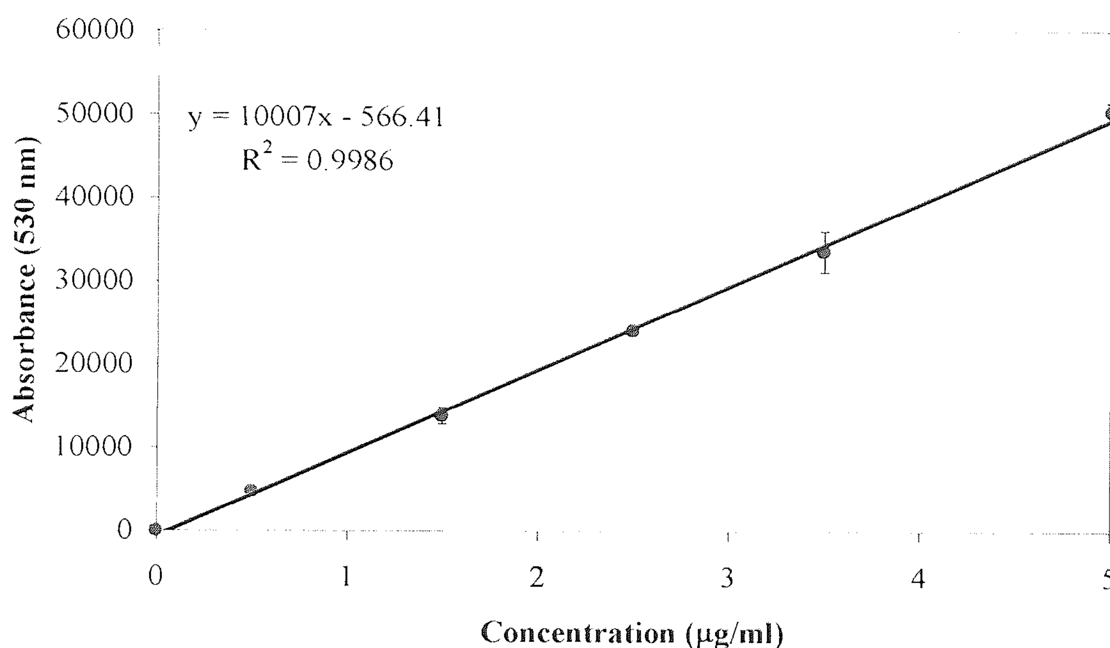


Figure 4.4 Calibration graph for standard lectins determined by fluorescence at 530 nm (n=3; mean ± sd)

4.2.4 Viscosity measurements

The internal phase primary emulsion viscosity (see also section 4.2.1) of the mucoadhesive microsphere formulations was determined using a U-tube (Oswald) viscometer. Following calculation of the densities of the polymer solutions from weight measurements using density bottles, the time taken for the polymer solution to flow through the capillary of the U-tube was determined. As the reference, sucrose (40% w/v) was used. The viscosity was calculated from the following expression:

$$\eta_{\text{unknown}} = \frac{\eta_{\text{reference}} \times t_{\text{unknown}} \times \rho_{\text{unknown}}}{t_{\text{reference}} \times \rho_{\text{reference}}} \quad \text{------} \quad \text{(Equation 4.1)}$$

where η = viscosity, t = time, ρ = density

4.2.5 Determination of protein content in microspheres composed of blends

10 mg microspheres prepared by double-emulsion were incubated in 5 ml (10 M) HCl overnight. This solution was then added to 5 ml (10 M) NaOH and after 1 hour 20 μl was added to 200 μl bichinchoninic acid assay (BCA) reagent containing copper sulphate (4% v/v) (ratio of copper sulphate to BCA 1:50). Following incubation for 1 hour at 37°C, absorbances were determined using a Wallach Victor 2 Multilabel Counter (Perkin Elmer Life Sciences, Boston USA) at 540nm. Figure 4.5 shows the calibration graph.

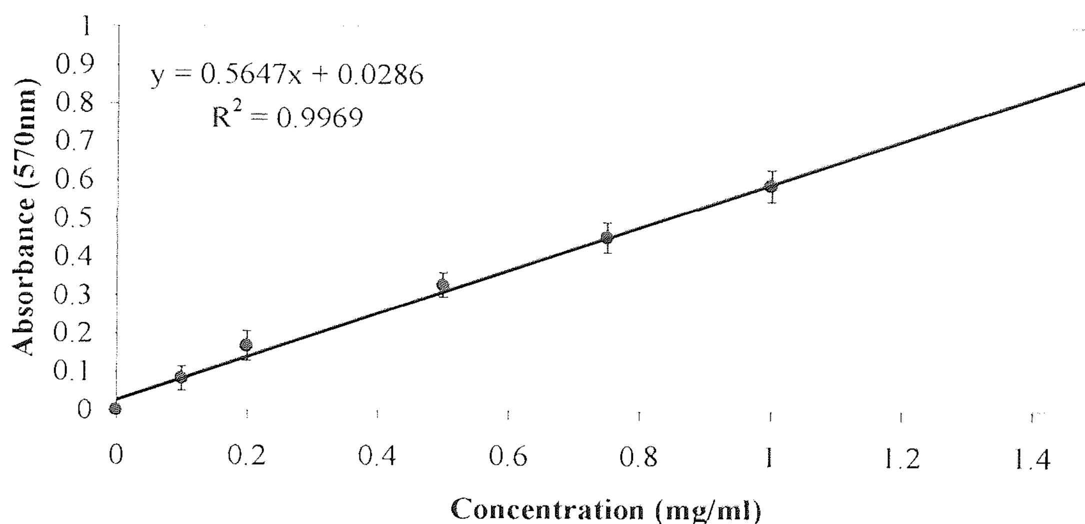


Figure 4.5 Calibration graph for gelatin standards determined at 540 nm (n=3; mean ± sd)

4.2.6 Preparation of ethylcellulose microspheres

The method for preparation of ethylcellulose microspheres was adapted from Guyot and Fawaz, (1998). Cimetidine (1.0 g) in methanol (10 ml) was added to 20 ml dichloromethane (20 ml) containing 1.0 g ethylcellulose. The solution was stirred at room temperature and then added to an aqueous solution of 0.45 % w/v polyvinylalcohol (PVA)

(molecular weight 30000-70000 Da) under stirring at 500 rpm using a Heidoph stirrer. Stirring was continued for 15 ml and temperature was raised to 37 °C using a hotplate stirrer. Stirring was then continued for 3 hours. Microspheres were filtered and washed with hexane (100 ml), dried overnight in a vacuum oven at room temperature and stored in a vacuum desiccator.

4.3 RESULTS AND DISCUSSION

In this chapter, microspheres were produced in three batches on separate days, unless otherwise stated. Drug release (in PBS pH 7.4 unless otherwise stated) (mean of three readings) and drug loading (mean of three readings) was evaluated using all three batches. Particle size (mean of 3 readings determined using Malvern software), microsphere surface charge (mean of 5 readings determined using Zetamaster software), surface mucin adsorption studies (mean of 3 readings; see section 2.11) and rat intestine studies (mean of 3 readings; see section 2.12) reported were evaluated from individual representative batches.

Gelatin consists of large and complex polypeptides of the same amino acid composition as the parent collagen molecule. The protein is an amphoteric molecule due to the functional groups of the amino acids and the terminal amino and carboxyl groups created during its hydrolysis (Chasin and Langer, 1990). Therefore there are many sites on the gelatin molecule where the functional amino or carboxyl groups could interact with functional groups from other polymers such as polymers of poly (acrylic) acid, chitosan or alginate. For example, the different mucoadhesive polymers could interact with gelatin forming coacervates or interact by hydrogen bonding.

4.3.1 Incorporation of carbopol and polycarbophil in gelatin microspheres

Two methods were used in order to formulate microspheres consisting of the mucoadhesive polymer in the core of the microsphere (Section 4.2.1), or the mucoadhesive polymer associated with the surface of the microsphere (Section 4.2.2).

In microspheres composed of blends of two polymers, as the gelatin droplets are formed in the primary emulsion by sonication of the gelatin/mucoadhesive polymer solution, it is anticipated that the matrix will consist of both polymers. Therefore these microspheres are referred to as blended microspheres. In the second method, the mucoadhesive polymer is added to the oil phase containing the gelatin droplets under stirring. It would be expected that the polymer would disperse in the oil phase and would then associate with the surface of the droplets, which are formed into microspheres following hardening and dehydration. The microspheres formed are composed of gelatin and the mucoadhesive polymer is associated with the surface of the microspheres, therefore referred to as surface-modified microspheres.

4.3.1.1 Microspheres composed of blends of carbopol and polycarbophil with gelatin

Drug loading was increased significantly with incorporation of the mucoadhesive polymers compared to gelatin microspheres (Table 4.2) (Dunnetts test, $P < 0.05$). Bogataj *et al.*, (1999) also reported increased drug loading following the incorporation of carbopol into hydroxypropylcellulose (HPC) microspheres encapsulating pipemidic acid, with efficiencies of 16.6 ± 1.8% HPC and 20.0 ± 1.0% (HPC/carbopol).

Modifying polymer	**Load (%w/w)** (n=3; mean ± sd)	**Particle size (µm)** (n=3; mean ± sd)	**Zeta potential (mV)** (n=5; mean ± sd)	**Viscosity (mPas)** (n=3; mean ± sd)
none	10.3 ± 0.7	65.62 ± 31.20	7.8 ± 0.9	24.3 ±2.0
polycarbophil	16.1 ± 0.7	88.72 ± 18.63	-13.5 ± 1.3	360.0 ± 23.4
carbopol 971P	19.0 ± 0.8	97.64 ± 29.42	-6.0 ±1.2	380 ± 21.6
carbopol 974P	16.5 ± 1.2	104.64 ± 28.72	-35.1 ± 3.1	401.0 ± 26.6

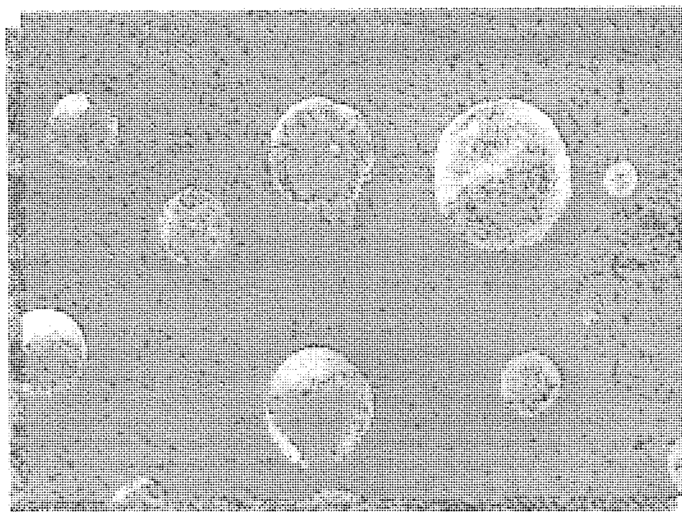
Table 4.2 Microsphere loading, particle size, zeta potential and viscosity of the internal phase for the blended preparations

Viscosities of the internal phase, consisting of gelatin/modifying polymer, were measured. As increase in viscosity would be expected to result in increased drug loading and particle size due to reduced drug loss and decreased mixing efficiency (table 4.2). Although all the

blended preparations had higher loading than gelatin alone and this could be attributed to the high viscosity of the internal phase, there was no discriminating pattern relating viscosity and loading within this group. The presence of acetic acid, used when carbopol and polycarbophil were incorporated, has previously been shown to have no effect on loading (section 3.3.2.4). Gelatin/carbopol 971P microspheres may incorporate more drug than the others due to a lesser degree of crosslinking.

Zeta potentials were found to be more negative with gelatin/carbopol 974P microspheres due to the higher extent of crosslinking of the carbopol 974P polymer. The particle size of the microspheres was found to increase with increasing viscosity of the internal phase, with the gelatin/carbopol 974P blend having the largest particle size (105 ± 29 μm). SEM analysis of gelatin/carbopol and gelatin/polycarbophil microspheres showed spherical structures with uneven surfaces (Figure 4.6)

(a)



(b)

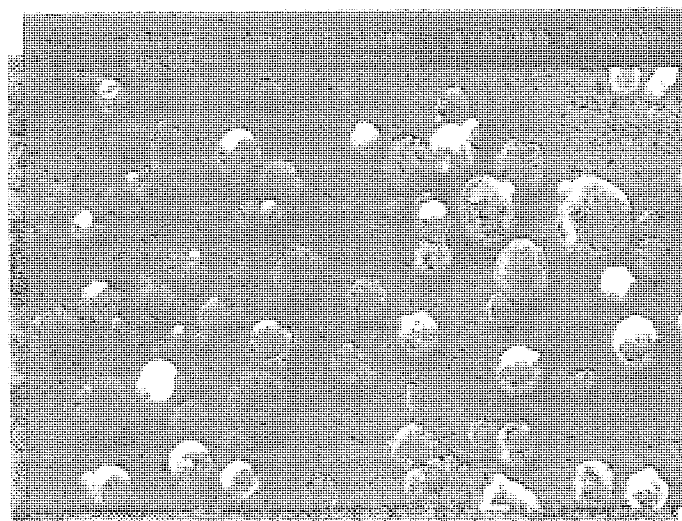


Figure 4.6 SEM images of (a) gelatin/carbopol 974P and (b) gelatin/polycarbophil microspheres

The drug release profiles for the microsphere blends show no differences (Figure 4.7). The swelling (see section 4.3.4) of gelatin microspheres was not significantly changed by the incorporation of carbopol 974P (gelatin: Q = 2.45 ± 0.23; gelatin/carbopol 974P: Q = 3.10 ± 0.62; t-test, P = 0.164) (Figure 4.22). Nevertheless, it has been seen that with high crosslinking concentrations, the cimetidine followed a slow rate of release (sections 3.3.1.1 and 3.3.3.1). In the case of the modifying polymers, when carbopol 971P (a lightly crosslinked polymer) was blended with gelatin, drug loading was high and cimetidine release was faster than from plain gelatin. When carbopol 974P, a highly crosslinked polymer, was blended with gelatin, drug loading was lower (than with 971P) and drug release was reduced. The differences in loading were not large enough to affect release rates (section 3.3.2.2), therefore the crosslinking level of the modifying polymers was thought to influence the loading and release rates of the microspheres, though differences in swelling were not visible using microscopy. Furthermore, as the carbopol/polycarbophil polymers are anionic, their potential for interaction with gelatin cannot be ruled out. This may result in formation of a stable matrix and contribute to higher encapsulation of cimetidine in microsphere blends.

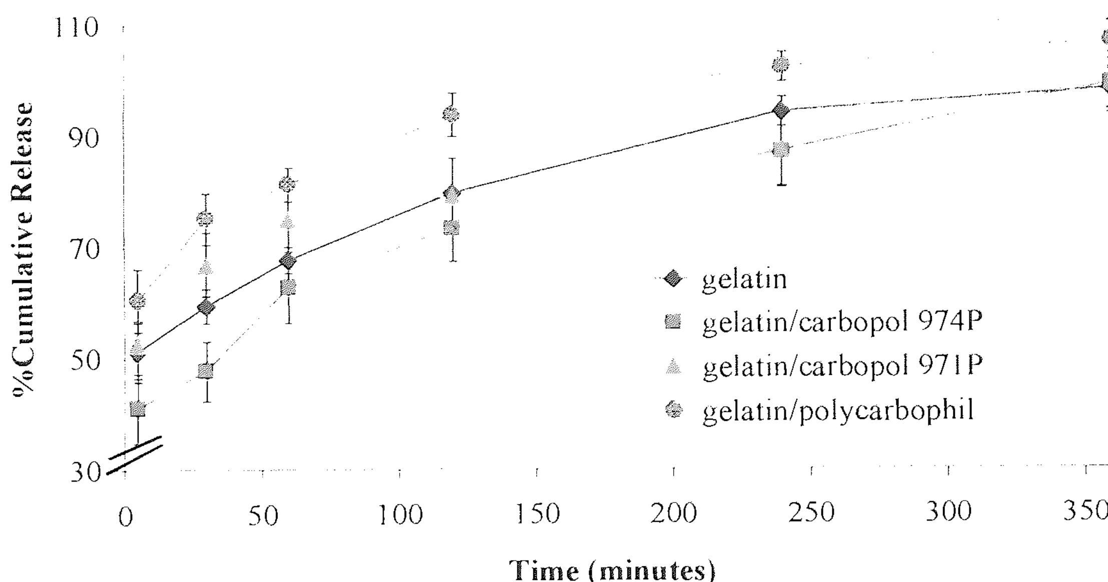


Figure 4.7 Drug release from microspheres composed of gelatin and modifying polymers (n=3; mean ± sd)

4.3.1.1.1 Effect of different concentrations of carbopol and polycarbophil on the drug loading of gelatin microspheres

Both 10% w/w and 30% w/w carbopol and polycarbophil were incorporated into the microspheres to assess the effect of the percentage of modifying polymer on the drug loading (Section 4.2.1).

Modifying polymer (% w/w)	**Drug load (% w/w)**
None	10.3 ± 0.7
10% polycarbophil	16.9 ± 2.3
30% polycarbophil	16.8 ± 0.7
10% carbopol 974P	13.7 ± 0.8
30% carbopol 974P	17.7 ± 1.4
10% carbopol 971P	20.7 ± 1.1
30% carbopol 971P	19.7 ± 1.1

Table 4.3 The effect of levels of incorporated polymers on cimetidine loading of microspheres (n=3; mean ± sd)

Increasing the amount of incorporated polymer was seen to increase the drug loading for gelatin/carbopol 974P microspheres only (table 4.3). This was as expected due to increased levels of the viscous polymer. However, no increases in drug loading were detected for the other preparations. Loading may already have reached a maximum achievable level with these formulations, these being the highest loads achieved at this theoretical load (*i.e.* 50% w/w).

4.3.1.1.2 Effect of medium on drug release from gelatin/carbopol and gelatin/polycarbophil microspheres

In this section, drug release results are represented from single batches of microspheres.

As it is reported that carbopol and polycarbophil polymers swell less at low pH (BF Goodrich, 1994), cimetidine release from gelatin/carbopol and gelatin/polycarbophil microspheres was assessed, to determine whether the incorporation of the mucoadhesive polymer in the microsphere matrix had any effect on the drug release profile in low pH medium. It was previously observed with gelatin microspheres that the release profile was similar in low and high pH (section 3.3.2.5).

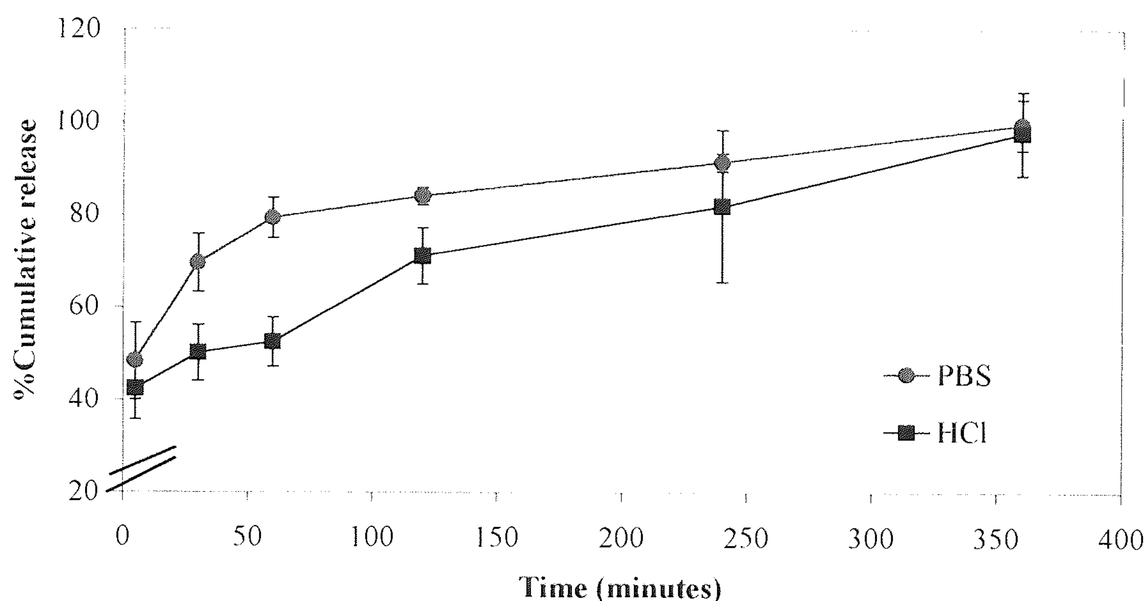


Figure 4.8 The effect of pH of the medium on cimetidine release from gelatin/carbopol 974P microspheres (n=3; mean ± sd)

With gelatin/carbopol 974P microspheres it was found that drug release was slower in HCl (pH 1.2) than in PBS (pH 7.4) (Figure 4.8). Carbopol and polycarbophil polymers swell to a lesser extent in low pH (section 4.1.1), since the acidic nature of the solution protonates the free carboxylate groups from the polymers, resulting in overall minimizing of repulsive force and reduced swelling or collapse of the polymer network (Mortazavi *et al.*, 1995). pH of the release medium was found to have no effect on the drug release rate of cimetidine from gelatin microspheres (see section 3.3.2.5), so it can be concluded that this is a function of the incorporated carbopol. This effect was similar for polycarbophil also (Figure 4.9). Therefore, even though the swelling of gelatin/carbopol microspheres versus gelatin microspheres was indifferent (see section 4.3.4), the potential for the interaction between gelatin and carbopol, and subsequent effect on the release rate of cimetidine from the matrix cannot be discounted.

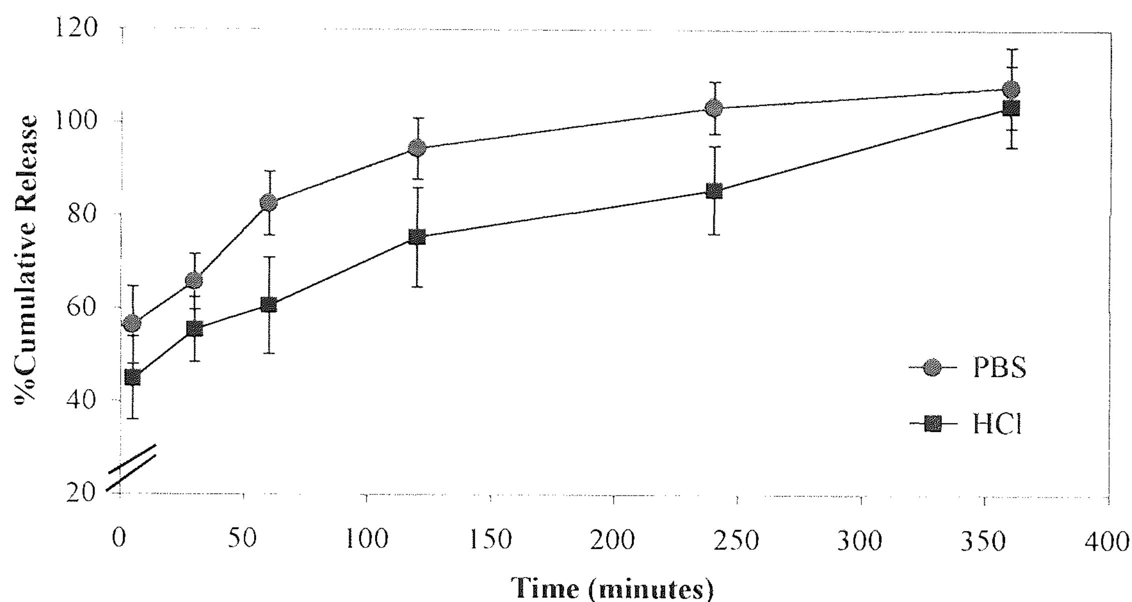


Figure 4.9 The effect of pH of the medium on cimetidine release from gelatin/polycarbophil microspheres (n=3; mean ± sd)

4.3.1.1.3 Analysis of mucoadhesive properties of gelatin/carbopol microspheres and gelatin/polycarbophil microspheres in vitro and ex vivo

Gelatin/carbopol 971P microspheres were found to adsorb the most mucin (~78%) by the surface adsorption assay, which was significantly different to ethyl cellulose microspheres (unloaded non- mucoadhesive microsphere control) (particle size 290.25 ± 80.93 μm), which adsorbs only 20% (Figure 4.10). Gelatin microspheres did not adsorb significantly more mucin than ethylcellulose microspheres (Dunnetts test, $P > 0.05$).

Carbopol and polycarbophil, bearing strong anionic charges and a number of carboxylic or hydroxyl groups are known to have good binding potential to mucin, and by the incorporation of these polymers with gelatin, the mucoadhesive properties of these microspheres were expected to be significantly enhanced in comparison to the mucoadhesive properties of gelatin microspheres.

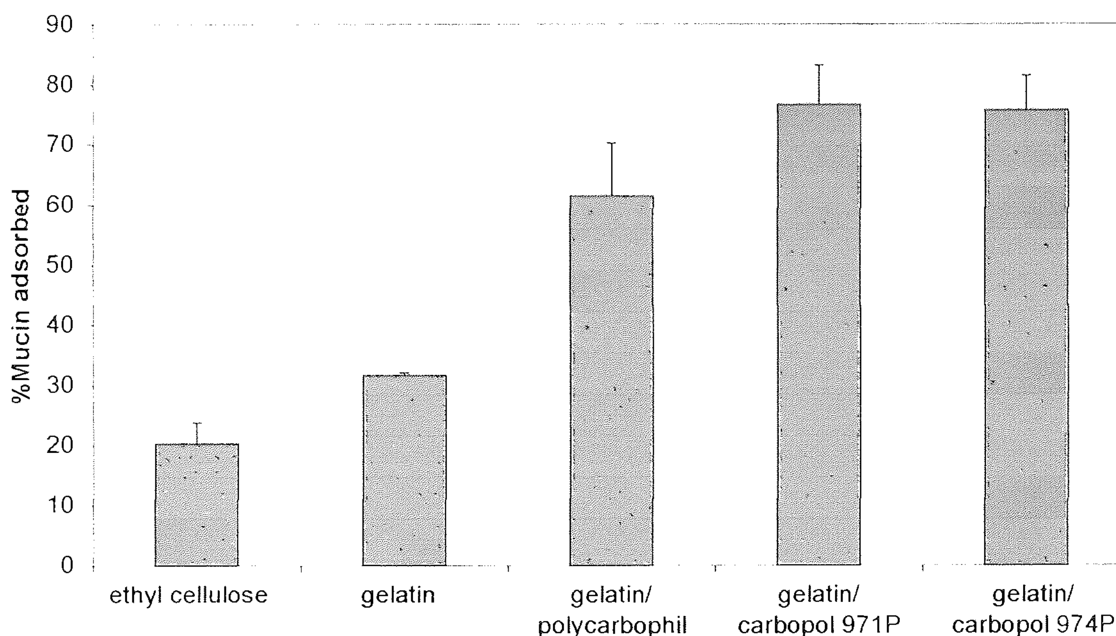


Figure 4.10 The adsorption of mucin to microspheres determined by adsorption assay (n=3; mean ± sd)

Gelatin/carbopols and gelatin/polycarbophil microspheres adsorbed more mucin than gelatin (Tukey test, P > 0.05). There were no significant differences in the percentage of mucin adsorption (at pH 6.5) between gelatin/carbopol 974P, gelatin/carbopol 971P or gelatin/polycarbophil microspheres (Tukey test, P > 0.05) (Figure 4.10).

Ex vivo, significantly more gelatin microspheres modified with carbopol (971P and 974P) and polycarbophil polymers were found to be retained in the rat intestine (Figure 4.11), than un-modified gelatin microspheres (Dunnetts test, P < 0.05). The rat intestine model was designed to mimic the *in vivo* situation as closely as possible. Due to other variables existing in the *ex vivo* model (such as shed-off mucus, etc., see section 1.6.4.2), differences observed *in vitro* may not be reflected *ex vivo*. pH would be expected to influence mucoadhesion, as the carbopols and polycarbophils are reported to be highly mucoadhesive at low pH. There were no significant differences between the values *in vitro* and *ex vivo*. Therefore, the incorporation of these modifying polymers with gelatin at the 30% level, was sufficient to improve the mucoadhesive properties of gelatin microspheres. There were no differences in the mucoadhesive properties of gelatin/carbopol 974P, gelatin/carbopol 971P and gelatin/polycarbophil microspheres *in vitro* or *ex vivo*. In

conclusion, the blending of carbopols and polycarbophils with gelatin in microspheres significantly enhanced the mucoadhesion determined *in vitro* and *ex vivo*, compared to gelatin microspheres. This demonstrated that microspheres prepared by this method of combining gelatin with other more mucoadhesive polymers has the potential for both slow release of the drug cimetidine and mucoadhesion possibly leading to retention within the GI tract.

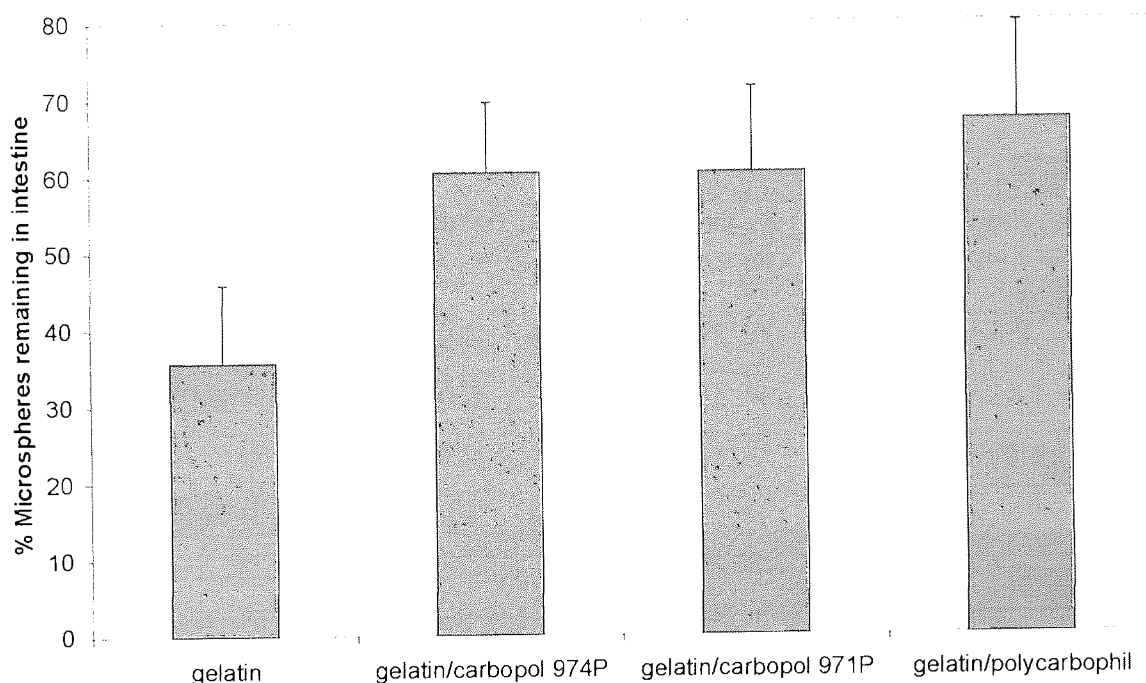


Figure 4.11 The percentage of microspheres retained in the rat intestine following incubation of the particles in PBS (n=3; mean ± sd)

4.3.1.2 Surface-modified gelatin microspheres with carbopol and polycarbophil

To compare the effect of incorporation of mucoadhesive polymer in the matrix of the microsphere or on the surface, gelatin microspheres were prepared by surface modification with carbopol and polycarbophil, similar to Leucuta *et al.*, (1997 a and b) (Section 4.2.2).

Modifying polymer	Microsphere blends	Surface-modified microspheres
polycarbophil	16.1 ± 0.7	12.5 ± 0.7
carbopol 971P	19.0 ± 0.8	14.4 ± 0.8
carbopol 974P	16.5 ± 1.2	13.4 ± 0.6

Table 4.4 Drug loading for microspheres composed of blends and surface-modified microspheres using carbopols and polycarbophil (n=3; mean ± sd)

Surface modifying polymer	Load (%w/w) (n=3; mean ± sd)	Particle size (μm) (n=3; mean ± sd)	Zeta potential (mV) (n=5; mean ± sd)
None	10.3 ± 0.7	65.62 ± 31.20	7.8 ± 0.9
polycarbophil	12.5 ± 0.7	79.43 ± 21.24	-62.2 ± 2.6
carbopol 971P	14.4 ± 0.8	77.43 ± 18.79	-46.0 ± 3.5
carbopol 974P	13.4 ± 0.6	82.16 ± 25.63	-38.0± 6.7

Table 4.5 Microsphere drug loading, particle size and zeta potential for gelatin microspheres surface-modified with mucoadhesive polymers

Drug loading was significantly lower with surface-modified microspheres than blends (Tukey test, $P < 0.05$) (Table 4.4). However, drug loading was higher with surface-modified microspheres than with plain gelatin microspheres (Dunnetts test, $P < 0.05$) (Table 4.5). The combination of the polymers as blends in the microspheres was thought to increase the drug loading due to interaction between the two polymers (Section 4.3.1.1). In this case, when the modifying polymer was added after formation of the gelatin droplets, before hardening and dehydration steps (Section 4.2.2), it may reduce the diffusion of the drug from the gelatin droplets into the oil phase during emulsification, where some drug loss is likely to occur. Zeta potentials for all the modified microspheres were more negative than gelatin alone (7.8 ± 0.9 mV) (Table 4.4). With negatively-charged polycarbophil and carbopol present as a coating on the particles, the negative charges were generally higher than the blended preparations, indicating their presence on the surface. Particle sizes with surface-modified microspheres were found to be lower than with polymer blends in microspheres, possibly due to viscosity differences.

The release of cimetidine from carbopol and polycarbophil surface-modified microspheres is shown in figure 4.12. It was envisioned that these microspheres might have a slower release profile than unmodified microspheres. As the mucoadhesive polymer was thought to be on the surface of the microspheres, this could slow the diffusion of the drug from the microsphere due to the presence of an additional barrier. However, the release profiles from gelatin and surface-modified microspheres were similar, despite any differences in loading. The incorporation of the modifying polymer carbopol 974P was found to have more a pronounced effect on the release profile of the microspheres when incorporated into the matrix, rather than on the surface of the particles. This may be as a result of interaction with the gelatin.

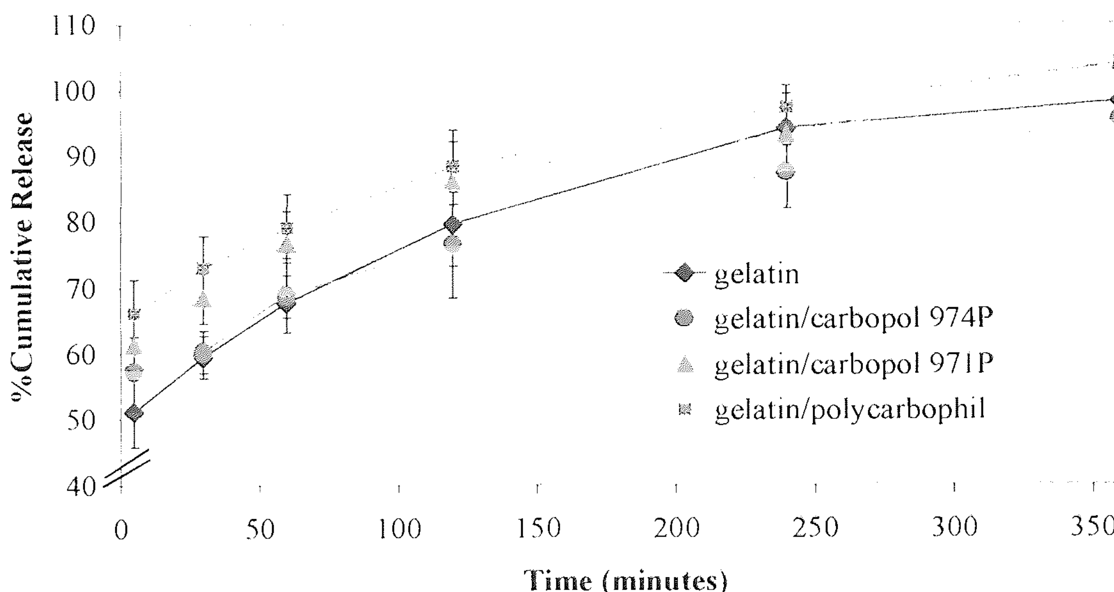


Figure 4.12 Cimetidine release from gelatin microspheres surface-modified with carbopol and polycarbophil (n=3; mean ± sd)

4.3.1.2.1 Analysis of mucoadhesive properties of carbopol and polycarbophil surface-modified gelatin microspheres ex vivo

The results for the *ex vivo* mucoadhesive assessment of carbopol and polycarbophil surface-modified microspheres are shown in figure 4.13. There were significant differences

between retention of unmodified and carbopol (and polycarbophil) surface-modified microspheres (Dunnetts test, $P < 0.05$).

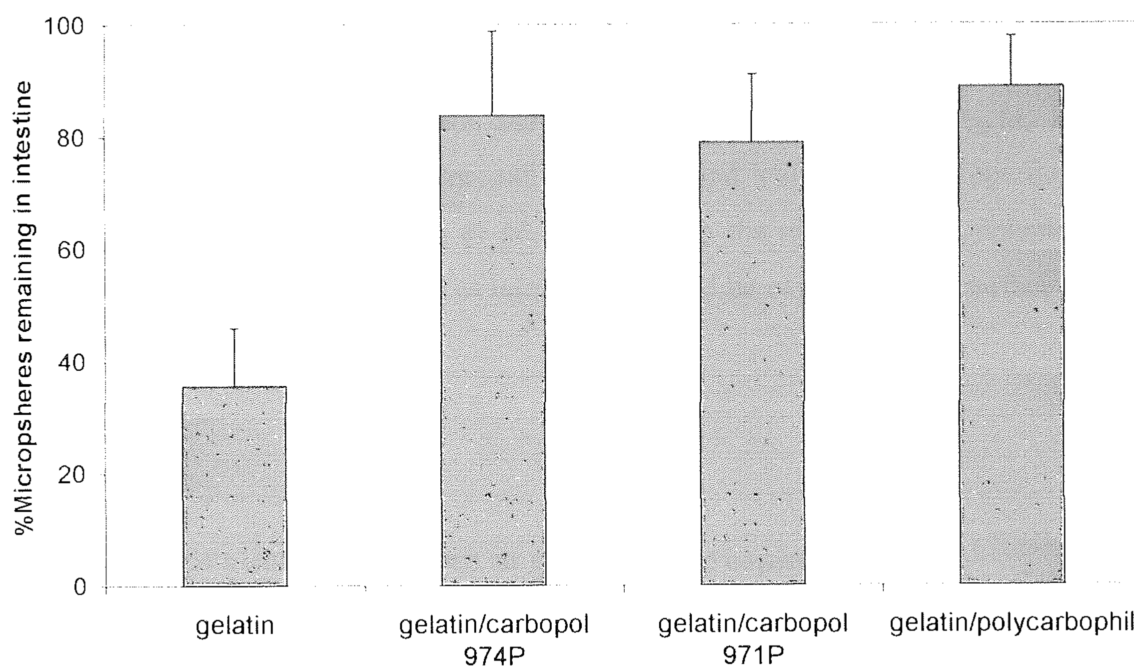


Figure 4.13 The percentage of microspheres remaining in the rat intestine following incubation of the particles in PBS (n=3; mean ± sd)

Modifying polymer	Blends	Surface-modified
carbopol 971P	60.4 ± 11.1%	79.1 ± 12.0%
carbopol 974P	60.4 ± 9.2%	83.9 ± 15.0%
polycarbophil	67.2 ± 12.7%	89.0 ± 8.8%

Table 4.6 The percentage of particles remaining in the rat intestine for blends and surface-modified microspheres (n=3; mean ± sd)

In the ex-vivo retention model, the surface-modified particles were retained to a greater extent than the corresponding blends (table 4.6), though these increases were not significant (Tukey test, $P > 0.05$).

Mucoadhesion is defined as an adhesion phenomenon occurring between a mucosal membrane covered with mucus and non-biological materials consisting of two steps. The first step is considered to be an interfacial phenomenon influenced by the surface energy effects and spreading of both the mucus and the mucoadhesive particle. The second step involves interdiffusion or interpenetration of polymer chains of both phases. This step requires hydration of the polymers and is influenced by molecular weight, molecular mobility, viscosity of the adhesive and swelling (and gel-forming) properties of the adhesive and the mucus (Akiyama *et al.*, 1994). With the mucoadhesive polymer on the microsphere surface, a higher level of interaction would be expected from surface modified microspheres than blends.

4.3.2 Incorporation of chitosan in gelatin microspheres

Chitosan has been used in microsphere formulations as the single polymer (Jameela and Jayakrishnan, 1995; Al-Helw *et al.*, 1998; He *et al.*, 1999 a and b), in combination with other polymers in microspheres (Yin *et al.*, 1996; Liu *et al.*, 1997; Remunan-Lopez *et al.*, 1998), or as a coating on microspheres (Gaserod *et al.*, 1998). It has been reported to have significant mucoadhesive properties (Hassan and Gallo, 1990; Zaman *et al.*, 1999). In this study microspheres were prepared from a blend of gelatin and chitosan, or gelatin microspheres, surface-modified with chitosan in order to formulate particles composed of the two polymers (Section 4.2.1 and 4.2.2)

4.3.2.1 Microspheres composed of blends of chitosan with gelatin

Loading was not significantly affected by addition of the modifying chitosan polymers to gelatin (table 4.7) (Dunnetts test, $P > 0.05$). Loading was significantly lower with chitosan -modified microspheres than carbopol- (or polycarbophil-) modified microspheres, similar to Abd El-Hameed and Kellaway, (1997). This is attributed to the higher viscosities of the carbopol/polycarbophil polymers and their possible interaction with gelatin resulting in higher load.

Modifying polymer	Load (%w/w) (n=3; mean ± sd)	Particle size (μm) (n=3; mean ± sd)	Zeta potential (mV) (n=5; mean ± sd)	Viscosity (mPas) (n=3; mean ± sd)
none	10.3 ± 0.7	65.62 ± 31.20	7.8 ± 0.9	24.3 ± 2.0
chitosan CL113	11.8 ± 1.9	49.52 ± 8.79	39.2 ± 4.1	113.6±12.6
chitosan G213	11.9 ± 1.4	58.64 ± 12.46	36.4 ± 0.7	113.8± 9.2

Table 4.7 Microsphere loading, particle size, zeta potential and viscosity of the *internal* phase for the blended preparations

When higher molecular weight (HMW) chitosan was used (G213), microspheres composed of blends were larger than with lower molecular weight chitosan (Table 4.7). similar to He *et al.*, (1999 b). Under similar preparation conditions, HMW chitosan resulted in larger droplet formation and therefore larger particles than lower molecular weight chitosans (He *et al.*, 1999 b). Al-Helw *et al.*, (1998) also reported smaller mean particle sizes with low molecular weight (LMW) chitosan. Zeta potentials of gelatin microspheres modified with chitosan were more positive than gelatin microspheres, as chitosan is cationic and positively-charged. SEM showed gelatin/chitosan microspheres of spherical structures and smooth surface morphology (Figure 4.14)

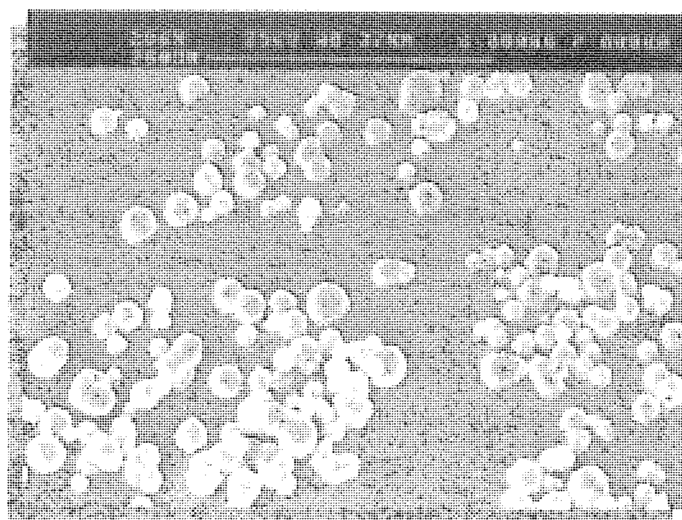


Figure 4.14 SEM image of microspheres of gelatin/chitosan CL113 loaded with cimetidine

The presence of chitosan in the formulation had no significant effect on the release of cimetidine in PBS (Figure 4.15). Chitosan is a positively charged polymer and it is reported that the presence of chitosan can effectively prolong the release profile of drugs in

negatively charged alginate microspheres, due to interaction between opposite charges of the polymers (Liu *et al.*, 1997). However, gelatin is amphoteric and in strongly acidic solution, gelatin is positively charged, and in strongly alkaline solution, it is negatively charged (Section 1.4.2). It may be expected that gelatin may interact with chitosan, in a similar way to alginate, but there was no evidence for this from the release profiles, perhaps due to the conditions not being strongly basic or acidic in these experiments (i.e. for acidic conditions pH 1.2, and basic conditions pH 7.2). An interaction of chitosan with gelatin could be indicated by a low release rate as the drug could be more efficiently distributed within the matrix.

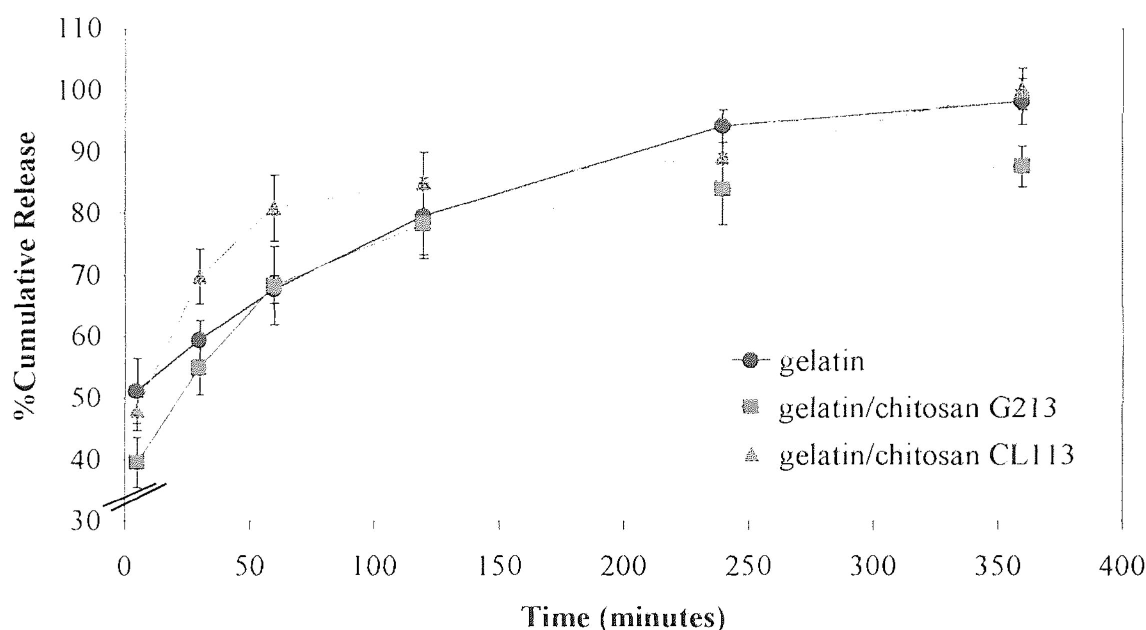


Figure 4.15 Drug release from gelatin microspheres modified by blending gelatin with chitosan polymers G213 and CL113 (n=3; mean ± sd)

4.3.2.1.1 Analysis of mucoadhesive properties of chitosan/gelatin microspheres in vitro and ex vivo

Gelatin/chitosan microspheres were found to adsorb significantly more mucin than gelatin microspheres and ethylcellulose microspheres (Table 4.8) (Dunnetts test, $P < 0.05$). This adsorption was however significantly less than the adsorption of mucin to gelatin/carbopol microspheres (Table 4.8) (Tukey test, $P < 0.05$).

Polymer(s)	Percentage adsorption %
ethyl cellulose	20.3 ± 3.5
gelatin	31.7 ± 0.4
gelatin/chitosan CL113	54.3 ± 2.6
gelatin/chitosan G213	58.5 ± 4.5
gelatin/carbopol 974P	75.8 ± 5.7
gelatin/carbopol 971P	76.8 ± 6.5
gelatin/polycarbophil	61.6 ± 8.7

Table 4.8 Percentage adsorption of free mucin to microspheres composed of blends (n=3; mean ± sd)

Similar rank orders of mucoadhesion have been reported with microspheres of carbopol and chitosan polymers respectively (Abd-Hameed and Kellaway, 1997; Ferrari *et al.*, 1997) *in vitro*, but levels of mucoadhesion were reportedly higher than those observed in these experiments. In this case, that the ability of glutaraldehyde to crosslink chitosan, as well as gelatin, affected the chain flexibility of the polymers and their ability to physically interact by binding with the mucus layer compared to the literarure.

It was reported that with chitosan microspheres, particles with larger sizes (~12 μm *versus* 3.3 μm) and less glutaraldehyde incorporation, showed the highest mucin adsorption *in vitro* and retained the most particles in rat intestine *ex vivo* (He *et al.*, 1998). In this study, there were no significant differences in the amount of gelatin/chitosan and gelatin microspheres in the rat intestine (Dunnetts test, $P > 0.05$) (Figure 4.16). Less gelatin/chitosan microspheres were retained in the rat intestine *ex vivo*, than expected from the *in vitro* results (Table 4.8) Studies *in vitro* were carried out at pH 6.5, and *ex vivo* at pH 7.2, which may be a contributing factor to the lower mucoadhesion observed, as chitosan is more mucoadhesive at low pH (MacLaughlin and Rolland, 1999).

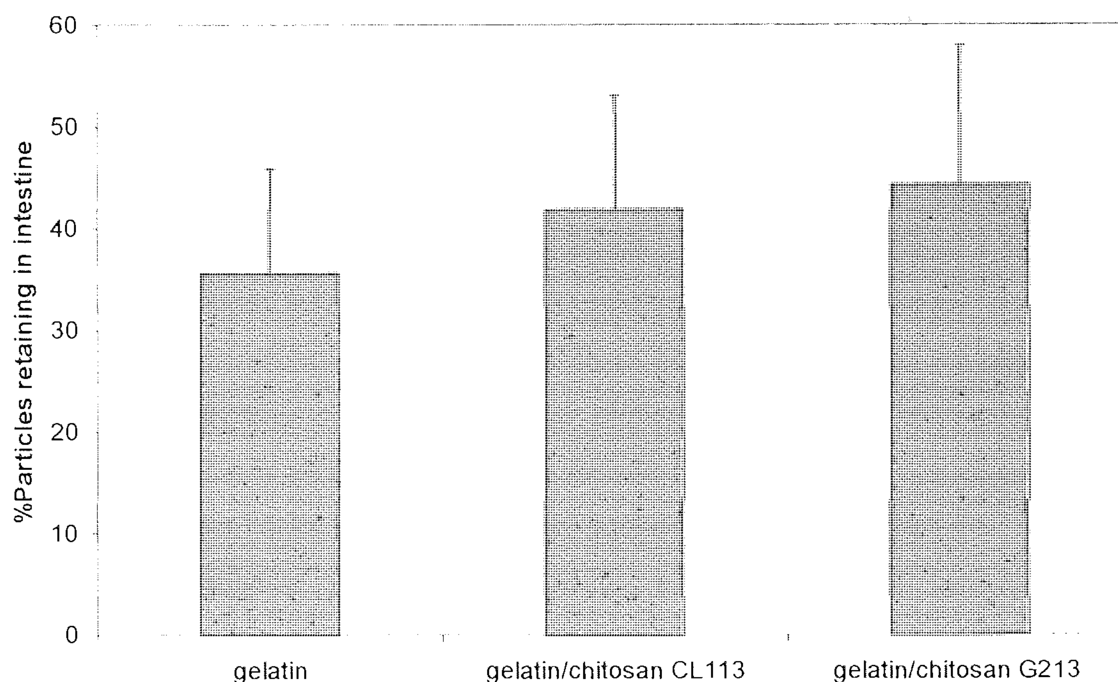


Figure 4.16 The percentage of microspheres remaining in the rat intestine following incubation of the particles in PBS (n=3; mean ± sd)

4.3.2.2 Surface-modified gelatin microspheres with chitosan

To compare the effect of incorporation of mucoadhesive polymer in the matrix of the microsphere or on the surface, gelatin microspheres were surface-modified with chitosan, in a similar method to Leucuta *et al.*, (1997 a and b) (Section 4.2.2).

Modifying polymer	Load (%w/w) (n=3; mean ± sd)	Particle size (μm) (n=3; mean ± sd)	Zeta potential (mV) (n=5; mean ± sd)
none	10.3 ± 0.7	65.6 ± 31.2	7.8 ± 0.9
CL113	12.4 ± 0.3	58.3 ± 21.4	25.1 ± 2.0
G213	12.1 ± 0.3	64.4 ± 19.8	28.7 ± 1.7

Table 4.9 Loading, particle size and zeta potential of chitosan-modified gelatin microspheres (n=3; mean ± sd)

Drug loading for chitosan-modified gelatin microspheres was significantly higher than gelatin microspheres (Table 4.9), whereas there was no significant difference between drug loading of gelatin/chitosan blends compared to gelatin or surface modified microspheres (Dunnetts test, $P < 0.05$) (Table 4.7). This was also seen for carbopol-modified

preparations (see section 4.3.1.2). This increase could be due to reduced diffusion of the drug from the gelatin droplets to the oil phase during emulsification, by the presence of an additional barrier on the gelatin droplets (*i.e.* the mucoadhesive polymer). Zeta potentials were less positive with surface-modified microspheres (25.1 ± 2.0 mV and 28.7 ± 1.7 mV) than microspheres composed of polymer blends (39.2 ± 4.1 and 36.4 ± 0.7mV) using chitosan. This could indicate reduced incorporation of positively-charged chitosan on the surface of the microspheres than in blended form, as it is expected that the zeta potential of surface-modified microspheres should be higher than blended microspheres, as the modifying polymer is on the surface of the particle. Protein recovery was carried out on the microspheres but does not indicate for surface-modified microspheres the level of mucoadhesive polymer present, though gelatin recovery was 100% (see section 4.3.6). This was true when negatively-charged carbopols and polycarbophils were used, where zeta potentials were more negative with surface modification than with incorporation as blends (*i.e.* polycarbophil blend: −13.5 ± 1.3 mV, surface-modified: -62.2 ± 2.6 mV, carbopol 971P blend: -6.0 ± 1.2 mV, surface modified −46.0 ± 3.5 mV). This would suggest that surface modification of microspheres with chitosan is not optimised under these conditions. Particle sizes were similar with modified and un-modified microspheres, unlike with carbopol/polycarbophil polymers where the blends had larger particle sizes than surface- modified. This may be due to viscosity differences or due to chitosan not being adsorbed efficiently to the microsphere surface.

Surface modification using chitosan had no effect on the release profile of cimetidine from the gelatin microspheres. It was thought that the incorporation of chitosan on the surface of the gelatin microspheres might present an extra barrier to drug release from the gelatin microspheres, therefore release from these microspheres would be slower than release from gelatin microspheres. However this was not the case. It was suggested earlier, that the incorporation of chitosan on the surface of the microspheres might be lower than expected, since the zeta potential of these microspheres should be higher than the gelatin/chitosan microspheres. This may be why there was a reduced effect of chitosan on the release profile of gelatin microspheres when surface-modified.

4.3.2.2.1 Analysis of mucoadhesive properties of chitosan surface-modified *microspheres ex vivo*

Ex vivo studies were used to assess the mucoadhesive properties of the chitosan surface-modified gelatin microspheres (Section 2.12).

There was a two-fold difference in the retention of CL113 surface-modified microspheres in the rat intestine compared to unmodified microspheres (Table 4.10), and a significant difference between the CL113 surface-modified preparation and the blended preparation (Tukey test, $P < 0.05$). There were no significant differences between the retention of the other chitosan surface-modified microspheres or gelatin/chitosan microspheres, and the retention of unmodified microspheres (Table 4.10).

Modifying polymer	Blended preparation	Surface-modified preparation
none	35.7 ± 10.2%	35.7 ± 10.2%
chitosan CL113	42.0 ± 11.1%	71.8 ± 10.2%
chitosan G213	44.4 ± 13.6%	68.0 ± 16.1%

Table 4.10 The percentage of microspheres retained in the rat intestine for chitosan blended and surface-modified preparations (n=3; mean ± sd)

It was expected that surface modification of gelatin microspheres with chitosan would increase the adherence of the microspheres *ex vivo* over gelatin microspheres, as this has been reported with chitosan-coating on alginate beads (Gaserod *et al.*, 1998). The adherence of surface-modified chitosan microspheres was expected to be higher than blends, as the presence of the polymer on the microsphere surface may offer better interaction with mucus, as seen with carbopols and polycarbophil surface modified preparations (Section 4.3.1.2.1). When comparing the adherence of microspheres surface-modified with chitosan and gelatin/chitosan blends, the adherence was highest with CL113 surface-modified microspheres. This may be due to molecular weight, as chitosan CL113 is of lower molecular weight (110 kDa) than chitosan G213 (460 kDa). Molecular weight of polymers is an important factor in mucoadhesion. For example, polymers of low molecular weight favour the interpenetration (or diffusion) of the polymer molecules; whereas entanglements are favoured for high molecular weight polymers (Mahrag Tur and Ch'ng, 1998). Therefore, for a particular polymer there is likely to be an optimum molecular weight where mucoadhesion can be highest.

From the mucoadhesive results it can be concluded that chitosan is present as an association of the polymer with the surface of the microspheres. The other *in vitro* studies do not corroborate this however, e.g. there was no difference in drug load or drug release. This may be due to other reasons.

4.3.3 Incorporation of alginate into gelatin microspheres

Alginate microspheres have been prepared by emulsification using gelation methods (Funduanu *et al.*, 1998; Lemoine *et al.*, 1998; Chickering *et al.*, 1997; Ribeiro *et al.*, 1999). Alginate, being an anionic polymer, is reported to exhibit swelling properties, which are sensitive to pH changes, ionic strength and specific ionic composition of the medium. For example, drug release from alginate matrix tablets was reported to be faster in acidic medium than in phosphate buffer, and release from alginate matrixes was faster in 0.1M HCl than in water (Ostberg *et al.*, 1994). It is reported to be a mucoadhesive polymer due to its numerous carboxylic groups (Zaman *et al.*, 1999). For this reason, microspheres were prepared from a blend of gelatin and alginate, or gelatin microspheres were surface-modified with alginate in order to formulate particles composed of the two polymers (Section 4.2.1 and 4.2.2).

4.3.3.1 Microspheres composed of blends of alginate with gelatin

Modifying polymer	Load (%w/w) (n=3; mean ± sd)	Particle size (μm) (n=3; mean ± sd)	Zeta potential (mV) (n=5; mean ± sd)	Viscosity (mPas) (n=3; mean ± sd)
none	10.3 ± 0.7	65.62 ± 31.20	7.8 ± 0.9	24.3 ± 2.0
LVG	12.6 ± 2.4	78.41 ± 30.24	-66.7 ± 1.8	250 ± 18.1
MVM	9.7 ± 2.6	88.62 ± 29.87	-62.0 ± 0.7	334 ± 24.7

Table 4.11 Microsphere loading, particle size, zeta potential and viscosity of the internal phase for gelatin/alginate blends

Drug loading was not significantly different between gelatin and gelatin/alginate microspheres (Dunnetts test, $P > 0.05$) (Table 4.11), as was the case with gelatin and chitosan blends. Particle size was found to marginally increase with internal phase viscosity, as expected. Lemoine *et al.*, (1998) reported production of larger microspheres using high viscosity alginate compared to low viscosity alginate, as was the case here, with gelatin/MVM (viscosity ~334 mPas) producing a mean size of ~88 μm, and gelatin/LVG (viscosity ~ 250 mPas) producing a mean size of ~ 78 μm (Table 4.11). Zeta potentials of gelatin/alginate microspheres were more negative with incorporation of negatively-charged alginate polymers as expected confirming their successful incorporation. SEM images of the gelatin/alginate microspheres showed spherical particles with uneven surfaces (Figure 4.18).

Figure 4.18 SEM image of microspheres of gelatin/alginate MVM loaded with cimetidine

The release of cimetidine from gelatin/alginate (MVM) microspheres was slower than from gelatin/alginate (LVG) and gelatin microspheres respectively (MVM blend: $f_2 = 23.2$; LVG blend: $f_2 = 6.2$) (Figure 4.19). This may be due to a number of reasons. The gelatin/alginate microspheres would have had more crosslinker available to crosslink the gelatin in these formulations (i.e. since there was 70% gelatin in these formulations) than gelatin microspheres alone. With increased crosslinking, there is slower drug release (see section 3.3.3.1). Alginate MVM is a higher viscosity polymer (viscosity of a 1% solution is 234 mPas; FMC Biopolymer, 2002) than alginate LVG (viscosity 156 mPas) and combination of the former polymer with gelatin produced an internal phase with higher

viscosity (334 ± 24.7mPas) than combination of gelatin and alginate LVG (250 ± 18.1mPas) and gelatin alone (24.3 ± 2.0 mPas). The viscosity difference may account for the slower release from gelatin/alginate (MVM) microspheres, as the higher viscosity polymers may reduce the diffusion of the drug through the microsphere matrix. Also, the incorporation of alginate in the matrix of the microspheres with gelatin may form a stable structure with gelatin. Since gelatin has numerous NH_2 groups, which can be formed into peptide bonds on reaction with aldehdydes in crosslinking, they could also react with the numerous carboxylic groups in sodium alginate leading to formation of a stable structure between gelatin and alginate (Liu *et al.*, 1997), contributing to the slower release profiles of the gelatin/alginates.

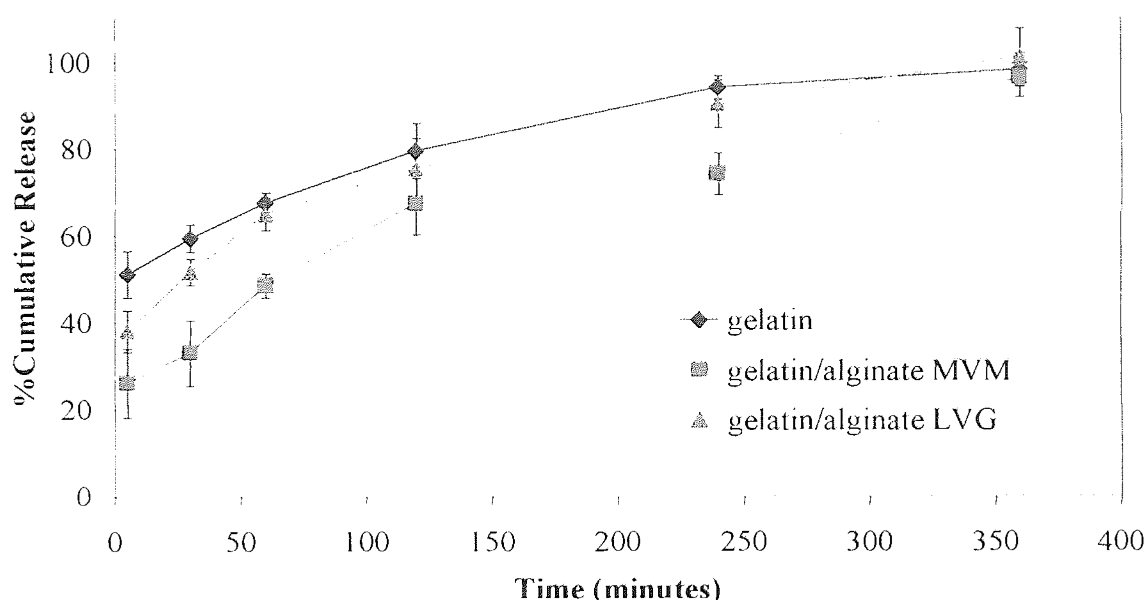


Figure 4.19 Drug release from gelatin microspheres modified by blending gelatin with alginate polymers, LVG and MVM (n=3; mean ± sd)

4.3.1.1.1 Analysis of mucoadhesive properties of alginate/gelatin microspheres in vitro and ex vivo

Gelatin/alginate microspheres adsorbed significantly more mucin than gelatin microspheres *in vitro*, but not as much as chitosan and carbopol combinations (Tukey test, $P < 0.05$) (Table 4.12).

Carbopols have been reported to show more mucoadhesion *in vitro* than alginates (Smart *et al.*, 1984). It is thought that since carbopol particles are viewed as a network structure of

polymer chains interconnected by crosslinks, with molecular weights in the billions, the polymers can interact more with the mucus glycoproteins to a higher extent than alginates (BF Goodrich, 1994).

Modifying polymer	% Mucin adsorbed
alginate MVM	48.3 ± 3.4
alginate LVG	46.2 ± 2.6
chitosan CL113	54.3 ± 2.6
chitosan G213	58.5 ± 4.5
carbopol 974P	75.8 ± 5.7
carbopol 971P	76.8 ± 6.5
polycarbophil	61.6 ± 8.7

Table 4.12 Adsorption of mucin to microspheres using adsorption assay for microsphere blends

Ex vivo, there was no significant difference between percentage of particles retained in the rat intestine for gelatin and gelatin/alginate microspheres (Dunnetts test, $P < 0.05$) (Figure 4.20), corroborating the *in vitro* data.

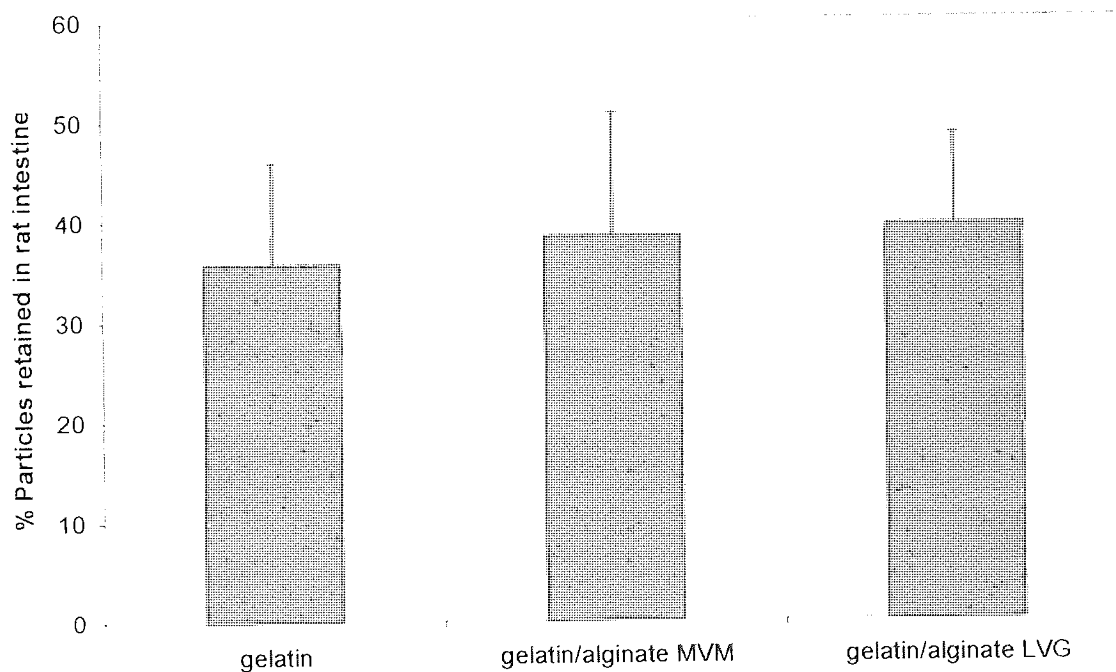


Figure 4.20 The percentage of microspheres remaining in the rat intestine following incubation of the particles in PBS (n=3; mean ± sd)

4.3.3.2 Surface-modified gelatin microspheres with alginate

To compare the effect of incorporation of mucoadhesive polymer in the matrix of the microsphere or on the surface, gelatin microspheres were prepared surface-modified with alginate, similar to Leucuta *et al.*, (1997 a and b) (Section 4.2.2).

Modifying polymer	**Load (%w/w)** (n=3; mean ± sd)	**Particle size (μm)** (n=3; mean ± sd)	**Zeta potential (mV)** (n=5; mean ± sd)
none	10.3 ± 0.7	65.62 ± 31.20	7.8 ± 0.9
alginate LVG	9.7 ± 0.6	80.42 ± 22.69	-32.1 ± 4.4
alginate MVM	10.2 ± 1.7	84.26 ± 27.13	-38.0 ± 1.7

Table 4.13 Loading, particle size and zeta potential of gelatin microspheres surface-modified with alginate polymers

Drug loading was not significantly different between modified and unmodified gelatin microspheres (Dunnetts test, $P > 0.05$) (Table 4.13), as was the case with gelatin/alginate blends (Table 4.11). Therefore the addition of alginate to gelatin in the form of surface modification or blending did not affect the drug loading. With addition of the negatively charged alginate, zeta potentials were more negative, as expected. Particle sizes were found to be larger with alginate modified microspheres than unmodified microspheres, as was the case using other anionic polymers, *i.e.* carbopol and polycarbophil (Table 4.5).

The release profile of cimetidine from both alginate MVM and LVG surface-modified microspheres was slower than from unmodified gelatin microspheres (MVM: $f_2 = 35.2$; LVG: $f_2 = 57.2$) (Figure 4.21), similar to the differences in the release profiles from gelatin/alginate blends and gelatin microspheres. The addition of a polymer on the surface of the microsphere would be expected to present an extra barrier to drug release. As alginate MVM is of higher molecular weight than alginate LVG, this may explain the differences between the two surface-modified microspheres (see section 4.1.3).

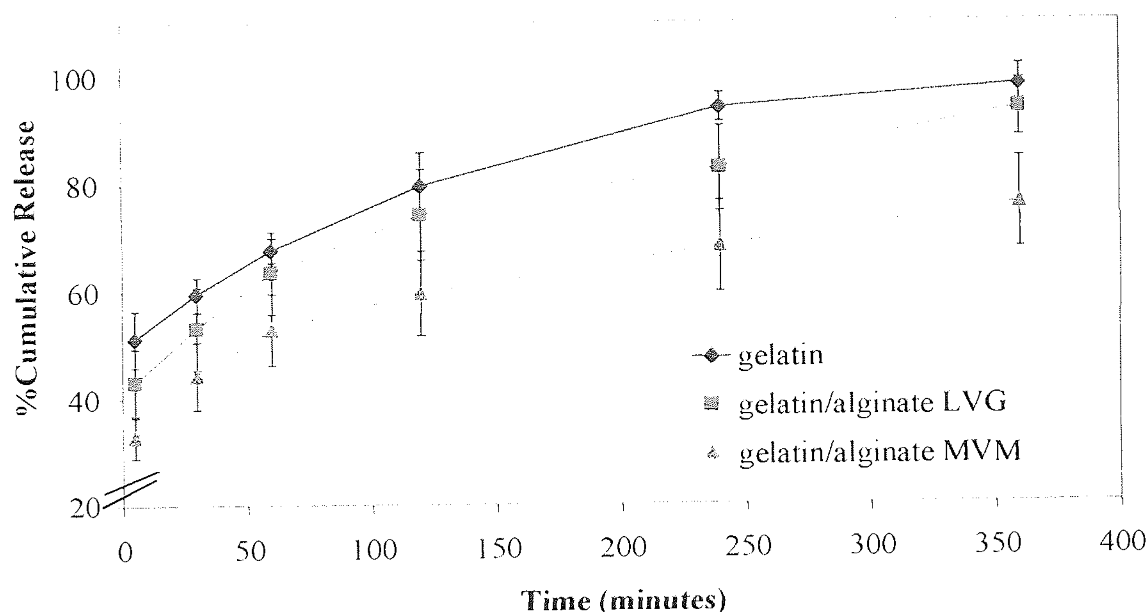


Figure 4.21 Drug release from gelatin microspheres surface-modified with alginate polymers LVG and MVM (n=3; mean ± sd)

4.3.3.2.1 Analysis of mucoadhesive properties of alginate coated microspheres ex- vivo

As was the case with gelatin/alginate LVG blends, the percentage of alginate LVG surface-modified gelatin microspheres remaining in the rat intestine was not significantly different from gelatin (Dunnetts test, $P > 0.05$) (Table 4.14). Factors reducing the potential mucoadhesion of the surface-modified microspheres are effect of chemical crosslinking on the gelatin content of the gelatin/alginate microspheres, *i.e.* since the same amount of crosslinker was used for all formulations, it can be considered that 100% gelatin microspheres would be less crosslinked than 70% gelatin/30%alginate, since alginate is not crosslinked by glutaraldehyde.

Modifying polymer	Percentage of microspheres remaining in the rat intestine (%)
none	35.7 ± 10.2
alginate LVG	56.0 ± 13.6
alginate MVM	61.0 ± 13.1
chitosan CL113	71.8 ± 10.2
chitosan G213	68.0 ± 16.1
carbopol 971P	79.1 ± 12.0
carbopol 974P	83.9 ± 15.0
polycarbophil	89.0 ± 8.8

Table 4.14 Percentage particles retained in the rat intestine for surface-modified gelatin microspheres (n=3; mean ± sd)

From table 4.14, it can be concluded that alginate LVG modification did not significantly improve the mucoadhesive properties of the gelatin microspheres as this was not reflected with *ex vivo* results (Dunnetts test, $P > 0.05$), however alginate MVM, chitosan, carbopol, polycarbophil coatings did improve the mucoadhesive properties of gelatin microspheres significantly (Dunnetts test, $P < 0.05$). It was reported by Akiyama *et al.*, (1994) that coating of microspheres with polymers could not enhance the mucoadhesion of microspheres as the coating would stick to the mucus layer and the microsphere would be removed. However, this does not seem to be the case here. The order of mucoadhesion was unmodified < alginate < chitosan < carbopol/polycarbophil. Therefore surface modification of gelatin microspheres has the potential for retention in the GI tract using mucoadhesion.

4.3.4 Swelling studies with blended and surface-modified microspheres

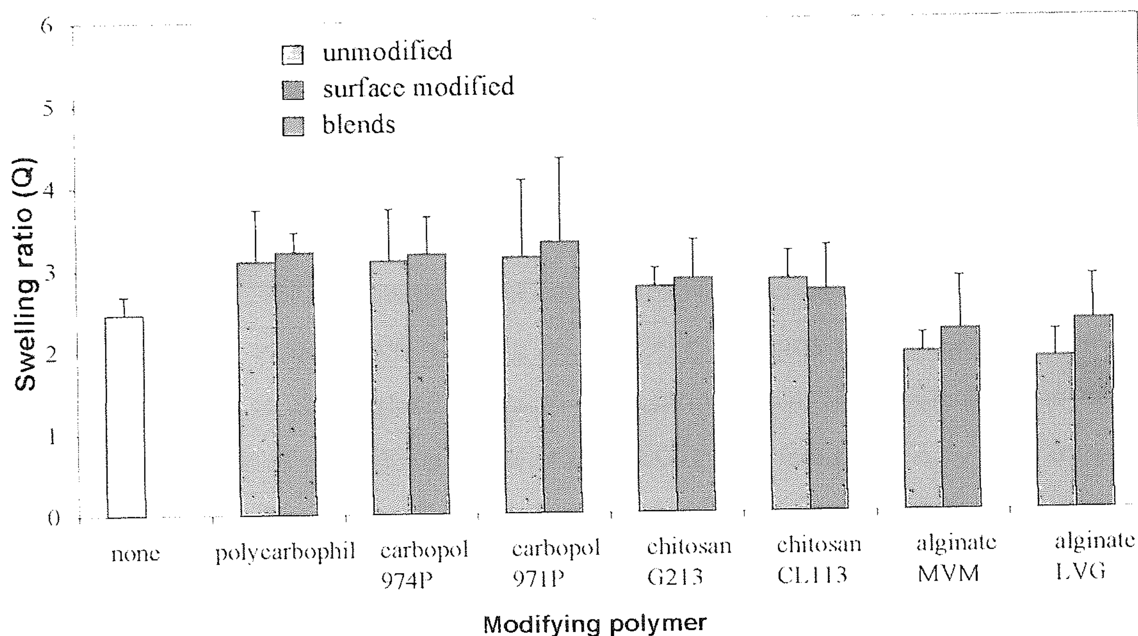


Figure 4.22 Swelling ratios of gelatin/modifying polymer microspheres and surface-modified gelatin microspheres (Q) (n=3; mean ± sd)

Figure 4.22 shows the swelling ratio (Q) of the blended and surface-modified microspheres after 15 minutes in distilled water (see section 2.8). As the swelling ratios were similar for microsphere blends and surface-modified microspheres, statistical analysis (multiple comparisons using Tukey test) was carried out and showed that there were no significant differences between non-modified gelatin microspheres and modified microspheres (blends or surface modified).

Polycarbophil and carbopol polymers are known to swell highly in a pH environment above 4 (Khan and Zhu, 1999). It is reported that the swelling capacity of polyacrylic acids is greater than that of crosslinked gelatin (Khan and Zhu, 1999). However the possibility of gelatin and carbopols/polycarbophil forming a stable structure may have reduced the swelling ability of these polymers in the microspheres. At this pH (7.4) it was thought that gelatin/alginate microspheres would swell more than gelatin microspheres as alginate would swell at higher pH. However, this effect was not seen, which may be due to the higher level of crosslinking of the gelatin content of the gelatin/alginate microspheres.

4.3.5 Lectin-gelatin microsphere conjugates

Due to the presence of numerous functional groups (*i.e.* amino and carboxylic residues), proteins are considered excellent candidates for the preparation of conjugates formed by the attachment of molecules to the surface of microparticles, such as lectins (see section 1.6.5).

Proteins have been evaluated as potential conjugates with lectins in nanoparticles (Irache *et al.*, 1994; Ezpeleta *et al.*, 1996; Epeleta *et al.*, 1999; Arangoa *et al.*, 2000). By covalent coupling of lectins to the surface of micro- or nanoparticles, the residence time may be increased at the site of absorption (Lehr *et al.*, 2000). The lectin, UEA I, was conjugated with gelatin microspheres (size 2.5 ± 1.7 μm) prepared by spray drying (Section 3.3.5). The method of glutaraldehyde activation was used for the coupling of the lectin to the gelatin microsphere (Section 4.2.3).

The level of coupling of lectins to the microspheres was found to be 39.9 ± 1.9% (*i.e.* the amount of added lectin coupled) which was determined from the washings, as 60.1 ± 1.9% of the lectin was unbound. Following the second washings, no more unbound lectin was detected. Reported literature for coupling of UEA I to other protein nanoparticles was reported at ~16% (Ezpeleta *et al.*, 1999), therefore the method of coupling used here was considered efficient for binding of the lectin with gelatin microspheres. The actual amount of bound lectin was calculated to be 20.3 ± 1.1 μg lectin per mg of microspheres. The particles were assessed *ex vivo* to determine whether this level of coupling to the microspheres of size 2.5 ± 1.7 μm would be sufficient to increase the mucoadhesion over gelatin microspheres.

Significant increases in the retention of conjugated microspheres in the rat intestine were observed *ex vivo* (t-test, P = 0.048) compared to non -conjugated microspheres (Table 4.15). This result shows that lectins can be coupled to gelatin microspheres and their coupling efficiency is sufficient to cause a significant improvement in the mucoadhesive potential of unmodified gelatin microspheres *ex vivo*.

Modifying lectin	Percentage of microspheres remaining in the rat intestine (%)
None	10 ± 5.3
UEA I	21.4 ± 4.6

Table 4.15 Percentage particles remaining in the rat intestine for lectin-conjugated microspheres and gelatin microsphere control

4.3.6 Protein recovery from modified microspheres

Microspheres formulated with gelatin and modifying polymers were degraded fully (see section 4.2.6), to determine the protein content and ratio of gelatin to modifying polymer in the microspheres, ensuring that all microspheres had the same level of gelatin and hence, modifying polymer.

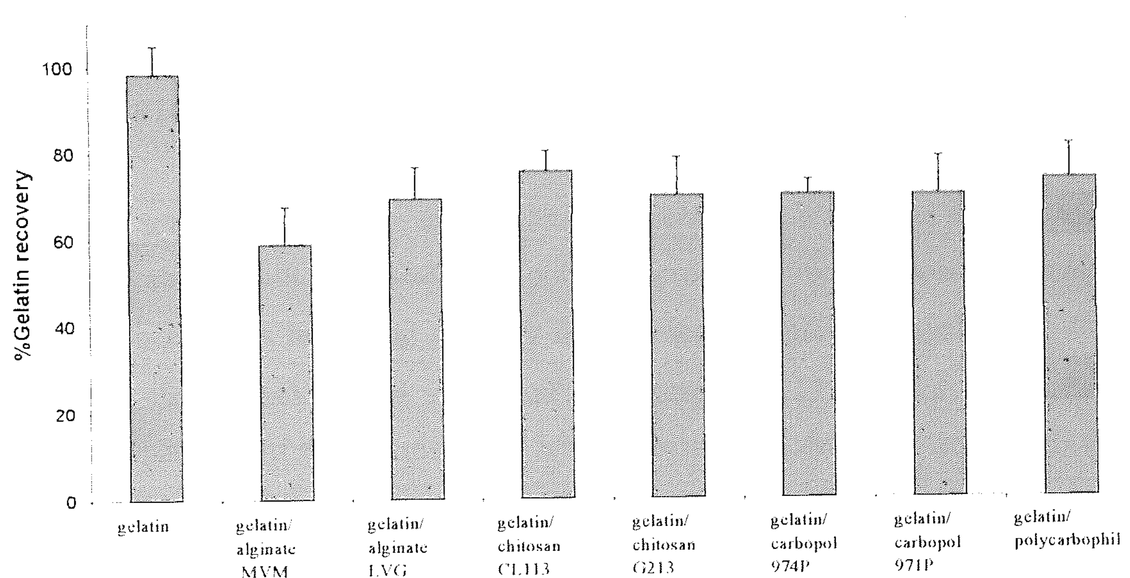


Figure 4.23 Protein recovery following degradation of microsphere blends (n=3; mean ± sd)

Protein recovery was ~70% (Figure 4.23) with no significant differences between the microsphere blends (Tukey test, $P > 0.05$) indicating that all modifying polymers were incorporated in the microspheres to the same level, as expected.

4.4 CONCLUSION

In this chapter, gelatin microspheres were successfully modified with mucoadhesive polymers, both as blends with gelatin in the matrix and as surface-modified preparations. As different polymers were used, carbopols, polycarbophil, chitosans and alginates, the incorporation of these polymers with gelatin produced microspheres with different physicochemical properties.

The highly-crosslinked polymers, carbopol 974P and polycarbophil increased the loading significantly (16.5 ± 1.2% and 16.1 ± 0.7% respectively) compared to gelatin microspheres (10.3 ± 0.7%) most likely due to the higher viscosities of these internal phase solutions compared to gelatin alone. The incorporation of the lightly-crosslinked carbopol 971P polymer with gelatin in microspheres, was found to produce microspheres with the highest loading (19.0 ± 0.8%) than any other blend or surface-modified formulation. Therefore the extent of carbopol crosslinking was thought to affect the formulations in terms of drug loading and drug release, as drug loading was higher and release was fast with the lightly crosslinked 971P polymer, and drug loading was lower and release slow with the highly crosslinked 974P polymer.

In terms of surface morphology, gelatin/chitosan microsphere blends had smooth surfaces, but incorporation of other mucoadhesive polymers produced microspheres with rough and uneven surfaces. With increasing viscosity of the internal phase (carbopol 974P: 401.0 ± 26.6 mPas, alginate MVM: 334 ± 24.7 mPas, chitosan CL113: 113.6 ± 12.6 mPas) the surface morphology of the microspheres was increasingly uneven and depressed, due to stirring of the emulsion, where the high internal phase viscosity may cause increased turbidity in the emulsion. This may lead to small quantities of polymer aggregating at the microsphere surfaces, resulting in the uneven surface morphology.

The slowest drug release profile was found when the high viscosity alginate, MVM, was used as the modifying polymer, both in blending with gelatin in microspheres, and surface-modified gelatin thought to be due to the potential interaction between gelatin and alginate, and the higher crosslinking level of gelatin in the gelatin/alginate formulations.

It can be concluded that the *ex vivo* rat intestine model is capable of distinguishing between modified and unmodified microspheres, however it does not indicate whether these differences would also be evident *in vivo*.

The presence of other particles in the rat intestine inevitably interferes with counting of the number of particles using the Coulter Counter following incubation. However, the *ex vivo* data corroborated the *in vitro* data (for gelatin microspheres: *in vitro* 31.7 ± 0.4 % and *ex vivo* 35.7 ± 10.2 % adherence) (see section 3.3.3.3 for more details).

Examination of all *ex vivo* data (Table 4.14) shows significant differences between chitosan CL113, carbopol 971P, carbopol 974P and polycarbophil surface-modified microspheres and unmodified microspheres. It can be concluded that these polymers were successfully combined with gelatin to enhance the mucoadhesive properties of gelatin microspheres *ex vivo*.

Generally, the mucoadhesion of surface-modified microspheres were found to be higher than the blends, as the polymers are not as intimately in contact and may be more available for interaction with the mucus layer. Where chemical crosslinking of the polymers was possible (i.e. chitosan) or higher crosslinking of gelatin in gelatin/alginate formulations was possible (i.e. alginate) it was found that resultant mucoadhesion was low. Using combination of carbopols and polycarbophil with gelatin, mucoadhesion was found to be optimum.

UEA I lectin was successfully incorporated onto the surface of gelatin microspheres using glutaraldehyde coupling, and was found to significantly increase the mucoadhesion probably due to specific receptor-ligand interactions.

It has been shown that by incorporation of polymers such as alginates with gelatin, slow drug release can be achieved over 6 hours. In terms of mucoadhesion, carbopols/polycarbophil surface-modified gelatin microspheres gave adherence values of > 80%. Desirable microsphere properties (e.g. slow release and increased mucoadhesion) may therefore be achieved by blending more than one polymer.

CHAPTER FIVE

GASTRORETENTIVE DEVICES

5.1 INTRODUCTION

Oral gastroretentive dosage forms are designed to delay the removal of the dosage from the gastrointestinal tract, acting as a platform for controlled release.

Development of oral gastroretentive devices is limited by physiological uncertainties such as the localisation of the delivery system in the desired area within the GI tract, and the highly variable nature of the gastric emptying process (Khosla *et al.*, 1989, Rouge *et al.*, 1996) (see section 1.2.1). Depending on the physiological state of the subject (*i.e.* fasted or fed state) and the design of the pharmaceutical formulation, the gastrointestinal residence time can last from a few minutes to several hours (Chien, 1992).

Various approaches have been followed to increase the retention of an oral dosage form including floating systems (section 1.5.1), swelling and expanding systems (section 1.5.5), and bioadhesive systems (section 1.6). Swellable type dosage forms such as highly swellable biodegradable porous hydrogels, can undergo size expansion by swelling rapidly upon contact with water, in order to control their residence times and removal from the GI tract (Ravichandran *et al.*, 1997). Gastric emptying is prevented, as the hydrogels are unable to exit the stomach through the pyloric sphincter due to their increased size (Ravichandran *et al.*, 1997). The rate and extent of the swelling of the device will be influenced by the residence time of the device and local environmental conditions in the different regions of the gastrointestinal tract (Slovin and Robinson, 1997).

Floating systems are those with a bulk density below gastric fluids and may thus remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system is floating on the gastric contents, the drug can be released in a controlled manner. Floating systems can based on the following:

- Hydrodynamically balanced Systems (section 1.5.1)
- Gas-generating systems (section 1.5.2)
- Low-density systems (section 1.5.3)
- Raft systems (section 1.5.4)

It has been shown that microspheres are an effective multiparticulate carrier system, but

may be rapidly removed from the stomach, due to their small size (section 1.4). The incorporation of particular mucoadhesive polymers and targeting agents into microspheres has been examined as a means for targeting within the GI tract (section 1.6.2, 1.6.5 and chapter 4). However, if microspheres were incorporated within floating or swellable systems, the delivery device may be retained in the stomach with controlled release occurring from the microspheres within the device. The aim of this chapter is to formulate hydrogels and alginate rafts containing gelatin microspheres and to assess their properties including their ability to retain microspheres once in gastric fluid, and their effect on the release profile of a model drug, cimetidine.

5.1.1 pH Sensitive Hydrogels for Gastroretention

Hydrogels have been studied and used extensively in biomedical applications as their high water content enables good biocompatibility, for example they are used in contact lenses (Kost and Langer, 1986). Hydrogels as swellable systems are discussed in section 1.5.5. pH-sensitive hydrogels normally contain acidic or basic groups such as carboxylic acids and primary amines, or strong acid and bases, such as sulphonic acids and sodium salts which change ionisation in response to changes in pH, thus changing the properties of the gel, which makes them ideal for targeting specific areas of the GI tract (Ravichandran *et al.*, 1997).

Superporous hydrogels are a type of hydrogel that have numerous super-size pores inside in contrast to conventional hydrogels, which can have few or none (see section 1.5.5). The pores of the hydrogel remain connected to each other to form capillary channels, enabling the hydrogel to swell on contact with water. Superporous pH sensitive hydrogels have been considered as delivery systems by number of researchers (Patel and Amiji, 1996; Chen *et al.*, 2000).

It has been reported that chitosan/polyethylene oxide (PEO) hydrogels prepared using freeze-drying methods, are formed with pore sizes in the range of 8-10μm (Amiji *et al.*, 1997). Since these hydrogels are easy to synthesise and have reported slow release of entrapped drugs (Patel and Amiji, 1996), they may be used as a gastroretentive delivery system. The potential of such systems to provide controlled release of cimetidine has been

assessed in this chapter. Microspheres have been incorporated into these hydrogels, to serve as a carrier for the particles in the environment of the stomach, enabling gastroretention. Freeze-dried hydrogels were prepared similar to Patel and Amiji, (1996), their swelling properties investigated and their potential as a carrier for microspheres studied.

Hydrogels were prepared using chitosan and chitosan/polyethylene oxide, in order to form a semi-interpenetrating network matrix, which would provide controlled release of the drug upon water uptake and swelling. It is likely that the semi-interpenetrating network is formed (in acid conditions) *via* the crosslinking reaction of amino groups on chitosan and aldehyde groups of glyoxal, in the presence of PEO, and intermolecular association through hydrogen bonding between the amino hydrogen and oxygen from PEO (Yao *et al.*, 1993) (Figure 5.1).

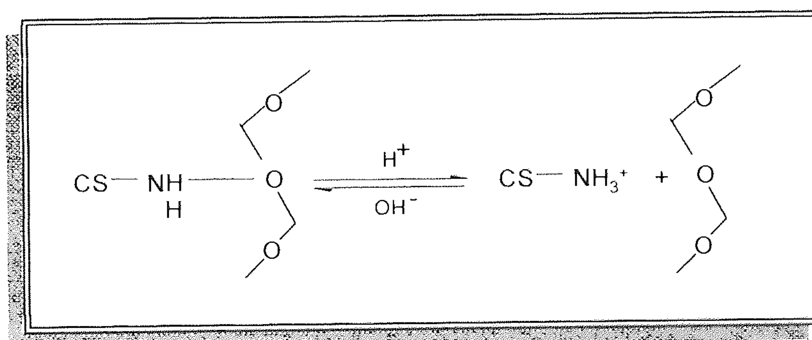


Figure 5.1 Semi-interpenetrating network of chitosan and PEO through intermolecular association by hydrogen bonding between amino hydrogen and oxygen from polyether (CS = chitosan)

Hydrogels prepared by air-drying have been reported to be less porous than those prepared by freeze-drying (Patel and Amiji, 1996). The highly porous nature of the hydrogel has a significant influence on the rate and extent of its swelling and drug release in SGF and SIF. Ionisation of the glucosamine residues in chitosan, and the osmotic effect from the high molecular weight PEO, forces the uptake of the aqueous medium, by capillary action, through the open channels in the hydrogel matrix, increasing the swelling of the semi-interpenetrating hydrogel compared to hydrogels composed of chitosan, alone (Patel and Amiji, 1996). Chitosan hydrogels will therefore be used as a control system, for this investigation.

5.1.2 Alginate Raft Systems

Another strategy used in achieving gastric retention is the alginate raft system, which has been used both for prevention and treatment of disease and heartburn (see section 1.5.4). There are two classes of alginate-containing preparations; those containing alginate as the principal active ingredient (Gaviscon®) and those containing alginate in combination with a significant amount of antacid (Algicon®) (Lambert *et al.*, 1990). Formulations containing sodium alginate, sodium bicarbonate and calcium carbonate have been reported to form rafts in the fasting and fed human stomach, and have been effective in reducing symptoms of gastro oesophageal disease (Lambert *et al.*, 1990). Raft formulations act by forming a viscous raft layer on the surface of the stomach contents formed by the precipitation of sodium alginate in the acidic environment of the stomach. The incorporation of bicarbonate provides gas-production, as on contact with the stomach contents, carbon dioxide is formed. The alginate gel entraps the gas bubbles and the resulting foam rises to the surface of the stomach contents and floats. Calcium carbonate aids the gelation of the alginate since the salt dissociates to release divalent calcium ions, which interact with the alginate forming the raft (Johnson *et al.*, 1997).

In marketed formulations such as Gaviscon®, the strength of the alginate raft formed is dependant on several factors, including the amount of carbon dioxide generated and entrapped in the raft, and the presence of aluminium or calcium in the antacid components of the formulation. It is reported that antacid-alginate suspension combinations perform poorly *in vivo* (Washington *et al.*, 1987). As the intention in this chapter was for the design of the raft formulation principally as a gastroretentive strategy, and not for antacid therapy, aluminium or other antacids were excluded from the formulations. Calcium carbonate was included for gelation of the alginate.

Stronger rafts are reportedly formed with liquid Gaviscon® formulations than solid formulations, where dissolution of the alginates can be either slow or incomplete (Washington *et al.*, 1986). In this chapter however, the requirement of the gastroretentive strategy is for slow release from the dosage form in the GI tract, with raft formation in the stomach. For this reason, only solid formulations were considered as the incorporation of microspheres in a liquid raft would lead to microsphere swelling and immediate drug release. As alginate rafts are reported to float on the stomach contents as a viscous foam,

they have potential for not only the delivery of cimetidine but also the delivery of microspheres to the GI tract. Rafts were optimised in terms of constituents, and the drug release and microsphere retention in the rafts was evaluated.

5.2 MATERIALS AND METHODS

All materials were obtained from Sigma/Aldrich (Poole, UK) unless otherwise specified and all chemicals used were of the analytical grade unless otherwise specified.

5.2.1 Preparation of freeze-dried hydrogels

The method was adapted from Patel and Amiji, (1996). Chitosan/PEO hydrogels were were prepared by addition of 0.8 g chitosan (High molecular weight, 800-2000cps) to 50ml acetic acid (1% v/v) under stirring for 3 hours using a Heidolph stirrer (Lab-plant, Huddersfield, UK). 0.2 g PEO (Molecular weight 1 kDa) was added to the chitosan suspension to give chitosan to PEO of 80:20 using a Heidolph stirrer at 1200 rpm. Stirring was continued for 1.5 hours, followed by addition of glyoxal (2 ml of a 40% in water solution) and the mixture was filtered through glass wool. Chitosan hydrogels were prepared as controls by addition of 0.8 g chitosan to 50 ml acetic acid under stirring for 3 hours using a Heidolph stirrer. Glyoxal was added and the formulation continued as above. The hydrogels were then poured into petri dishes and incubated overnight at 37 °C for hardening. Following this, the gels were cut into discs (2.5 cm diameter, weight ~0.10-1.2 g), washed with 0.1 M NaOH and then double distilled water. The hydrogels were then frozen using a mixture of dry ice and acetone, and freeze-dried using an Edwards Modulo freeze dryer (Boc Ltd., Sussex, UK) for 24 hours.

5.2.2 Preparation of air-dried hydrogels

The hydrogels were formulated as in 5.2.1. Following hardening of the gels, they were cut into discs (2.5 cm diameter) and washed with 0.1 M NaOH and then double distilled water. Hydrogels were dried under vacuum (Gallenkamp, UK) at room temperature, for 48 hours.

5.2.3 Preparation of drug-loaded hydrogels

Drug loaded hydrogels were prepared using chitosan/PEO hydrogels. 50 mg of cimetidine (GlaxoSmithKline) was dissolved in 50 ml acetic acid (1% v/v). 0.8 g chitosan followed by 0.2 g PEO were added to the solution. The hydrogels were then prepared as in 5.2.1. Drug-loaded chitosan hydrogels were also prepared according to the loadings above and in 5.2.1.

5.2.4 Hydrogel swelling studies

Hydrogels were incubated in 50 ml enzyme-free simulated gastric fluid (SGF, pH 1.2) (USP, 1996) or simulated intestinal fluid (SIF, pH 7.2) (USP, 1996) at 37°C using a hot water bath with shaker. After 5, 30, 60, 120, 240, 360, 480 minutes, and 24 hours, samples were removed from the medium, and blotted on tissue to remove excess surface moisture, weighed (or the diameter measured manually using a ruler) and then re-immersed in the fluid.

The swelling ratio (Q) of chitosan and chitosan/PEO hydrogels was determined according to the following expression:

$$Q = \frac{W_s}{W_d} \quad \text{------------------------------} \quad \text{(Equation 5.1)}$$

where W_s is the weight of the swollen hydrogel,

W_d is the weight of the dried hydrogel.

When swelling ratio was determined using size measurements the following expression was used:

$$Q = \frac{D_s}{D_d} \quad \text{------------------------------} \quad \text{(Equation 5.2)}$$

where D_s is the diameter of the swollen hydrogel,

D_d is the diameter of the dried hydrogel

5.2.5 Incorporation of microspheres into hydrogels

Hydrogels were formulated as in section 5.2.1. Following addition of 0.8g chitosan to 50 ml acetic acid (1% v/v) and mixing with 0.2 g PEO, 335 mg gelatin microspheres (loading 15.4 ± 0.4% unless otherwise stated, equivalent to 50 mg drug) were added, followed by addition of glyoxal (final concentration 16 mg/ml). The hydrogels were then formulated as 5.2.1.

Different sizes of microspheres (335 mg), i.e. 6 ± 3 μm (loading 24.8 ± 3.2 %), 19 ± 10 μm (loading 30.0 ± 4.3 %) and 85 ± 28 μm (loading 15.4 ± 0.4 %), were incorporated into the hydrogels to determine the effect of size on the microsphere retention in the hydrogel.

5.2.6 Assessment of microsphere retention

To assess the microsphere retention in the raft, the hydrogel discs containing the microspheres were weighed prior to immersion in gastric fluid. After two hours incubation with agitation at 37 °C in SGF, the hydrogels were removed and subjected to freeze-drying. The hydrogels discs were weighed again and the difference between the initial and final weight was determined to assess the weight difference and retention of the microspheres in the hydrogel discs. As controls, hydrogels without microspheres were weighed, freeze-dried, and reweighed.

5.2.7 Drug release from hydrogels

The release from drug-loaded hydrogels was calculated as a function of the theoretical drug amount incorporated. Drug release from microsphere-loaded hydrogels was calculated as a function of the actual loading of the microspheres.

Hydrogels were incubated in 50 ml SGF (unless otherwise specified) at 37°C under agitation. 1 ml samples were removed at 5, 30, 60, 120, 240, 360, 480, minutes, and 24 hours, placed into eppendorff tubes and following centrifuging at 13500 rpm (MSE, Leicester ltd., UK), 0.5 ml of the supernatant was removed for analysis by HPLC (see

section 2.10.2.1). 0.5 ml of fresh buffer was added and the sample was returned to the medium.

5.2.8 Simulation of *in vivo* transit on the drug release from hydrogels

To simulate stomach conditions, hydrogels formulated with free drug and microspheres were incubated in 50 ml SGF (pepsin and pancreatin were omitted as they have been reported to have no significant effect on the swelling behaviour of hydrogels, Patel and Amiji, 1996) at 37 °C for 2 hours, under agitation. The gel was then crushed and stirred vigorously with a glass rod. The solution was filtered to collect the gel from the SGF. The SGF solution was discarded and the gel was then incubated in 50ml SIF for a further 22 hours. 1 ml samples were removed at 5, 30 minutes, 1, 2, (in SGF) 4, 6, 8 and 24 hours (in SIF) from the medium, and the procedure was continued as 5.2.7

5.2.9 Alginate raft preparation

Raft powder blends were prepared by mixing sodium alginate (200 mg), calcium carbonate (10 mg) and potassium bicarbonate (220 mg), unless otherwise stated, using a pestle and mortar.

5.2.10 Assessment of raft formation

Rafts were formed in 250 ml beakers, with 50 ml SGF. After 1 hour, the raft was removed, blotted on a strip of tissue and the thickness from the centre of the raft, and raft diameter was measured manually using a ruler.

5.2.11 Incorporation of free drug and microspheres into rafts

Free drug (10, 50, 200 or 400 mg) was mixed with 200 mg sodium alginate, 10 mg calcium carbonate and 220 mg potassium bicarbonate. 335 mg microspheres (loading 15.5 ± 1.9%

equivalent to 50 mg drug) or 575 mg (loading 8.7 ± 0.2%, equivalent to 50 mg drug) were mixed with 50 mg sodium alginate and 100 mg calcium carbonate using pestle and mortar.

5.2.12 Assessment of microsphere retention in rafts

As the hydrogels were solids, they were easily weighed and the retention of microspheres inside them was determined by weighing before and after freeze-drying. Since alginate raft formulations containing microspheres formed a viscous foam, they could not be dealt with in this manner. Therefore the level of retention of microspheres in the rafts was determined by measuring protein levels using a bichinchoninic acid assay (BCA assay).

Microspheres and rafts containing microspheres (335 mg microspheres) were incubated in SGF and 1 ml samples were removed after 2, 4, 6, 8 and 24 hours, added to 4 ml (10 M) HCl for microsphere degradation followed by addition of 10 ml (5 M) NaOH. Protein levels were determined by BCA, where 20 μl sample was added to 200 μl BCA reagent containing copper sulphate (4% v/v) (ration of copper sulphate to BCA 1:50). Following incubation for 1 hour at 37°C, absorbances were determined using a Wallach Victor 2 Multilabel Counter (Perkin Elmer Life Sciences, Boston USA) at 540nm. Figure 5.2 shows the calibration graph.

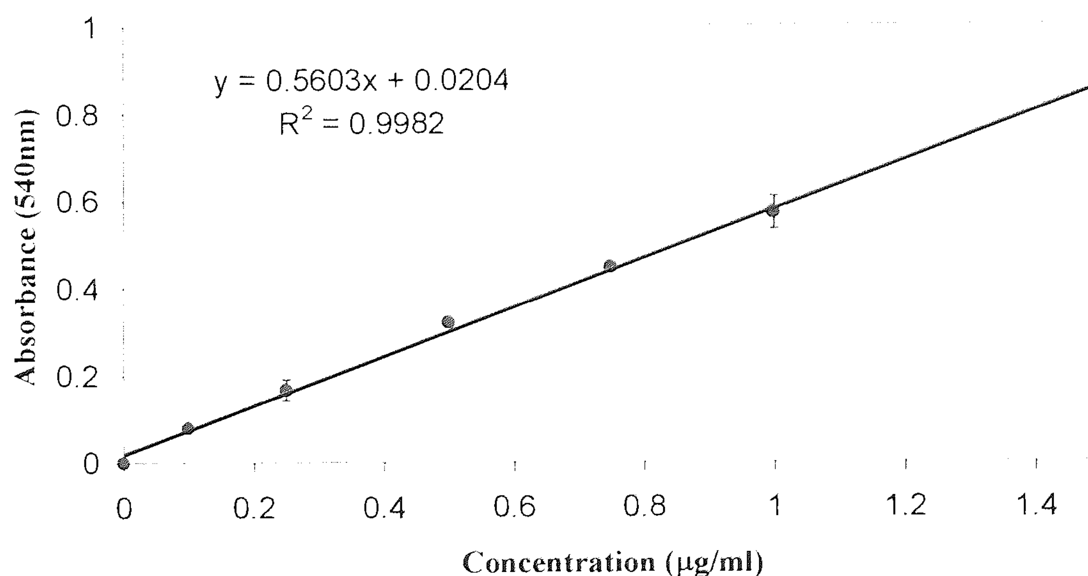


Figure 5.2 Calibration graph for gelatin standards determined by BCA assay (n=3; mean ± sd)

5.2.13 Drug release from raft formulations

Drug release from raft formulations and those containing microspheres were investigated. In order to comply with the sensitivity of the HPLC method, a 25 ml volume was used rather than 50 ml for the release studies. This was not expected to have any major effects on raft strength and formation.

Rafts were incubated in 25 ml SGF at 37°C, under stirring, and 1 ml samples were removed at 5, 30, 60, 120, 240, 360, 420 minutes, and 24 hours, placed into eppendorffs and following centrifuging at 13500 rpm, 0.5 ml of the supernatant was then removed for analysis by HPLC (see section 2.10.2). 0.5 ml of fresh buffer was replaced back into the eppendorff, which was vortexed and returned to the medium.

5.3 RESULTS AND DISCUSSION

5.3.1 Freeze-dried and air-dried hydrogels

5.3.1.1 Swelling of chitosan and chitosan/PEO hydrogels

The swelling ratios of air-dried and freeze-dried hydrogels were evaluated to confirm the higher swelling of hydrogels in SGF than in SIF, and higher swelling ratios of freeze-dried hydrogels than air-dried hydrogels, as reported by Patel and Amiji, (1996).

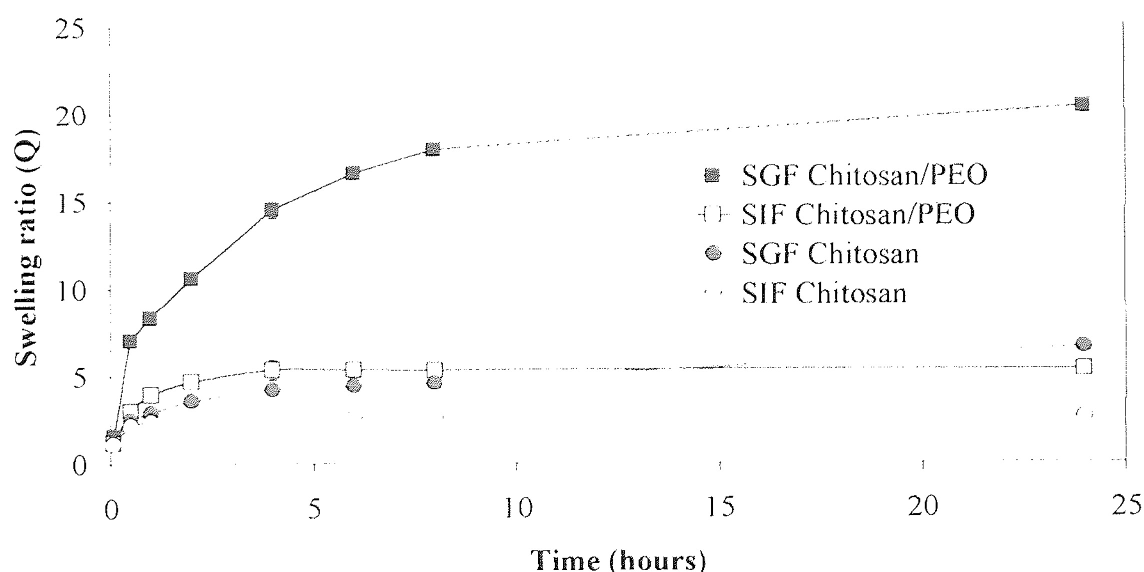


Figure 5.3 Swelling ratio (by weight) of chitosan and chitosan/PEO hydrogels prepared by air-drying in simulated gastric acid (SGF) and simulated intestinal fluid (SIF) (n=3;mean ± sd)

Air-dried chitosan/PEO hydrogels swelled more in SGF than in SIF (Figure 5.3). The chitosan/PEO hydrogels swelled more than chitosan hydrogels in both media. The faster rate, and higher extent of swelling for chitosan/PEO hydrogels than chitosan controls in SGF, was due to the formation of the semi-interpenetrating network between the chitosan and the PEO. In the low pH environment of SGF, basic amino residues of the chitosan will be ionised and contribute to the increased swelling of hydrogels by electrostatic repulsion and counteranion binding (Amiji *et al.*, 1997). The considerable difference in swelling of chitosan and chitosan/PEO hydrogels in SGF indicates the effect of the PEO in modification of the properties of the hydrogels. As part of the network, PEO functions either as an osmotic agent, facilitating the initial uptake of the medium into the hydrogel, or to decrease the crystallinity of the chitosan matrix by interpenetration to facilitate the transition from a glassy to a rubbery state in the aqueous medium (Amiji *et al.*, 1997).

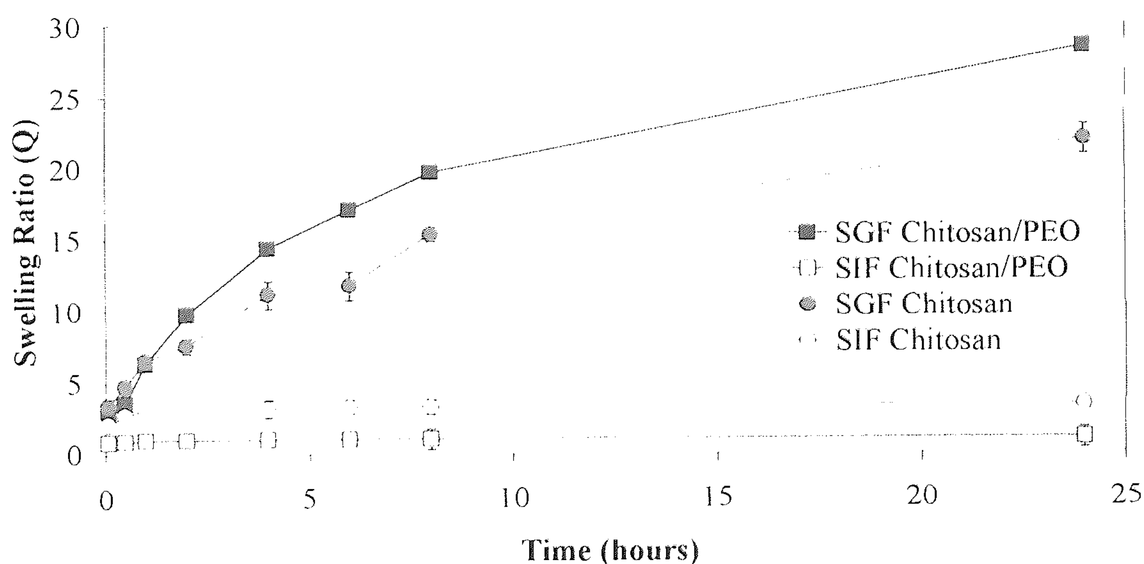


Figure 5.4 Swelling (by weight) of chitosan and chitosan/PEO hydrogels prepared by freeze-drying in simulated gastric acid (SGF) and simulated intestinal fluid (SIF) (n=3; mean ± sd)

Chitosan and chitosan/PEO hydrogels prepared by freeze-drying had a greater swelling potential in SGF than hydrogels prepared by air-drying (Figures 5.3 and 5.4), for example after 8 hours, freeze-dried: chitosan Q = 15, chitosan/PEO Q = 20, air-dried: chitosan Q = 5, chitosan/PEO Q = 18. The addition of the PEO in air-dried hydrogels increased the swelling such that it was similar to the freeze-dried formulation. From the literature (Amiji *et al.*, 1997), it was expected that the swelling difference between air-dried and freeze-dried hydrogels be more pronounced. It may be that the matrix of the freeze-dried hydrogel

formulated in this chapter was less porous compared to Amiji et al., (1997) thus enabling less water uptake and forcing reduced swelling. There was a considerable difference in swelling between air-dried and freeze-dried chitosan hydrogels in SGF, for example at 2 hours: air-dried = 5, freeze-dried = 10, as the highly porous structure of the freeze-dried hydrogels enables faster uptake of aqueous solution through the channels in the matrix (Patel and Amiji, 1996).

Swelling ratios of the hydrogels were also determined using size measurement (section 5.2.4) (using equation 5.2). The mass change was found to be much more significant than the diameter changes, mainly due to the additional weight from the uptake of solution, for example at 4 hours for chitosan/PEO hydrogels: by diameter change, Q = 1.3; by mass change, Q = 14.5 (Figure 5.5).

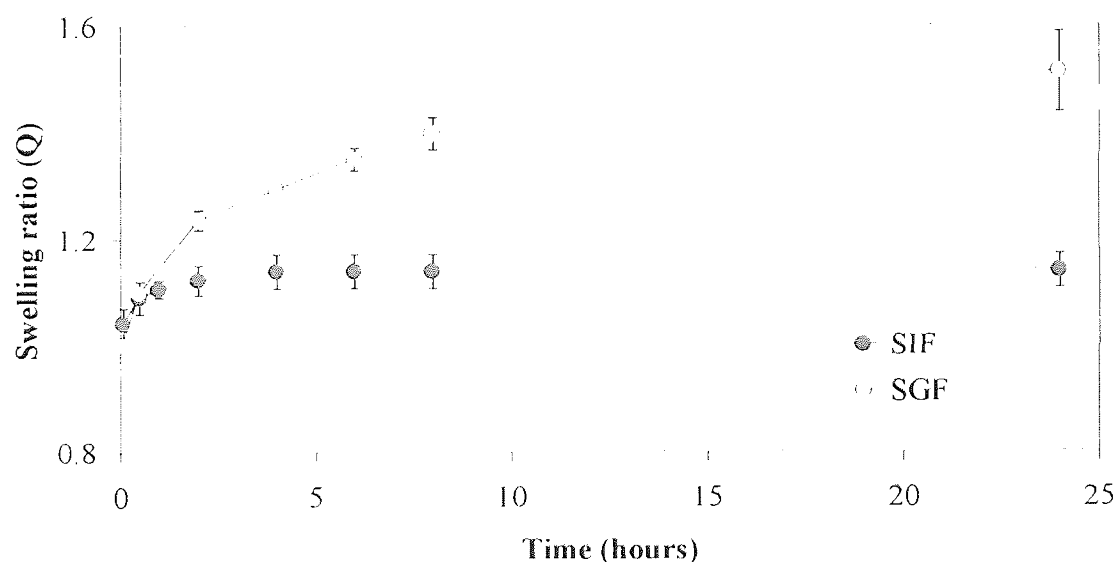


Figure 5.5 Swelling of freeze-dried chitosan/PEO hydrogels in simulated gastric acid (SGF) and simulated intestinal fluid (SIF) determined by diameter changes (n=3; ± mean sd)

In man, it is reported that solids up to 7 mm diameter can be emptied from the stomach (Khosla *et al.*, 1989). The sizes of the hydrogels used in these studies were much larger (~20mm diameter) than this suggested value, and increased in size upon swelling (up to ~ 30 mm after 8 hours) (Figure 5.5). Therefore this size of hydrogel would be expected to retain in the stomach and not pass through the pyloric sphincter until it was reduced in size to below 7 mm by the grinding and sieving actions of the antrum (see also section 5.3.1.3.3 and 1.2.1). It is expected that to be viable for an oral formulation, the hydrogels could be formulated inside capsules.

5.3.1.2 Release of cimetidine from freeze-dried hydrogels

In chitosan/PEO semi-IPN hydrogels, the large amount of water taken up by the hydrophilic matrix provides the main diffusional pathway for the drug through the swollen hydrogel. Solute transport through the hydrogel is generally described in terms of two mechanisms: the pore mechanism and the solute-diffusion or partition mechanism. In the pore mechanism, the solute is presumed to diffuse through microchannels within the membrane matrix. The diffusion rate of the solute is related to the average pore size of the hydrogel matrix (note: the water filled pores or channels in the matrix are changing in size due to swelling and are not in fixed or definite locations). In the partition mechanism, the solute dissolves in the hydrogel, and diffusion rate is dependant on water solubility of the solute and physiochemical properties of the hydrogel. Therefore drug release from the swollen hydrogel will take place by the action of both mechanisms together, where one may dominate over the other (*e.g.* in the case of freeze-dried hydrogels, the pore mechanism may dominate) (Fang *et al.*, 1997).

Freeze-dried chitosan/PEO hydrogels were prepared loaded with cimetidine (section 5.2.3). Drug release from chitosan/PEO hydrogels was similar in SGF and SIF (Figure 5.6). The release of the drug from the hydrogels appeared to be unrelated to their swelling ratios, as hydrogels were swollen more in SGF than SIF. This result was not expected from the theory, and has not been reported in any of the literature. It can be concluded that swelling is not the major factor affecting release from this system. Furthermore, in the environment of the stomach, the drug release from the hydrogel would be unpredicatable and would not be dependant on the amount of fluid uptake into the hydrogel. However, since the hydrogels are primarily under consideration as a platform for controlled drug delivery (i.e. with the incorporation of microspheres inside the hydrogel) drug release would be mediated from the microspheres. In both media, the release rate of cimetidine was slower than free cimetidine dissolution (in SGF: f_1 = 33.0; in SIF: f_1 = 36.8) (Figure 5.6). The initial burst effect (20%) may be attributed to the release of drug entrapped toward the surface of the gel matrix. These results are similar to those reported by Ravichandran *et al.*, (1997).

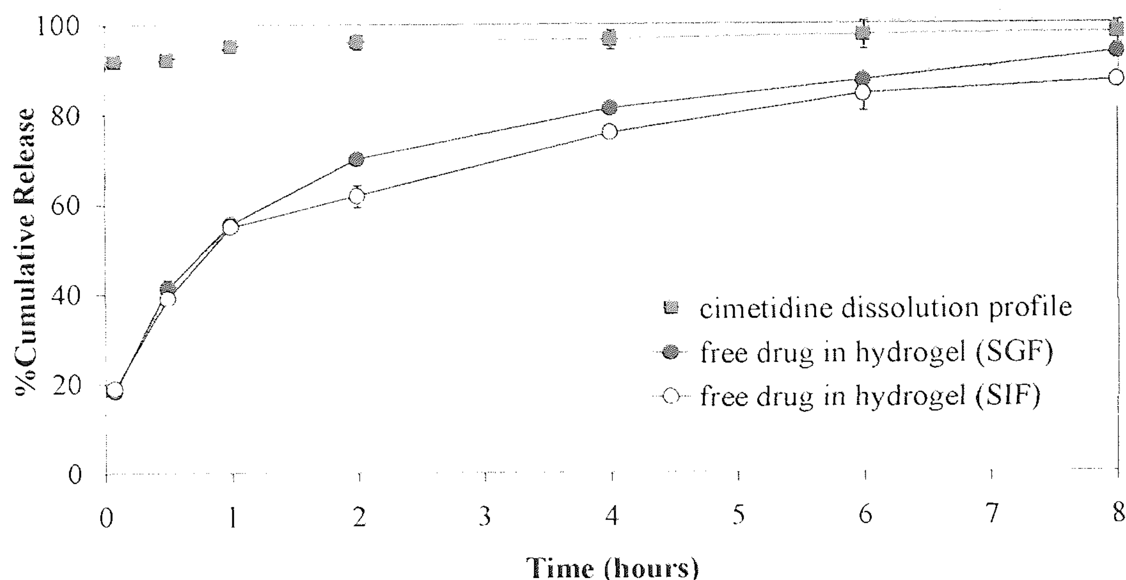


Figure 5.6 Cimetidine release from chitosan/PEO hydrogels prepared by freeze-drying in simulated gastric acid (SGF) and simulated intestinal fluid (SIF) and cimetidine control (SGF) (n=3; mean ±sd)

After 2 hours in SGF, drug release for cimetidine-loaded hydrogels was 70%. Literature sources have reported release from similar hydrogels using metronidazole (drug release of 60%) and amoxycillin (drug release of 65%) after 2 hours (Patel and Amiji, 1996) (solubilities: cimetidine 11 mg/ml, metronidazole 10.5 mg/ml, amoxycillin 4mg/ml). A chitosan/polyether network hydrogel containing cimetidine showed maximum drug release in ~30 minutes in an acidic medium (Yao *et al.*, 1994). In this case, chitosan/PEO hydrogels prepared incorporating cimetidine showed a slower release over 6 hours, with a burst release of 20%. Therefore they have potential as a platform for delivery of cimetidine in the GI tract and were further evaluated by microsphere incorporation.

5.3.1.3 Incorporation of microspheres into hydrogels

Microspheres prepared by the double-emulsion method previously showed a controlled release over 6 hours (section 3.3.4). The chitosan/PEO hydrogels also showed a degree of controlled release of cimetidine (Figure 5.6). There are two ways in which the incorporation of microspheres into hydrogels could provide an increased barrier to the drug release. Firstly by the drug diffusion from the microsphere matrix, followed by diffusion through the hydrogel matrix, or secondly with the drug diffusing initially from the

microspheres inside the hydrogel, and then diffusion from the microspheres, once they themselves are released from the hydrogel following swelling of the matrix. It may be possible that microspheres larger than the pore size of the hydrogel could be retained inside the hydrogel, and particles smaller than the pore size may diffuse from the hydrogel. Freeze-dried hydrogels in the dry state were reported to have a highly porous structure, of 8-10μm in diameter (Patel and Amiji, 1996).

Gelatin microspheres with 15.4 ± 0.4% w/w load and particle size of 85 ± 28 μm were used in all the following studies, unless otherwise stated.

5.3.1.3.1 Drug release from microspheres incorporated into hydrogels

Microspheres were incorporated into freeze-dried hydrogels and release assessed in SGF and SIF (see method sections 5.2.5 and 5.2.7). A control, free drug was incorporated into hydrogels (see method section 5.2.3).

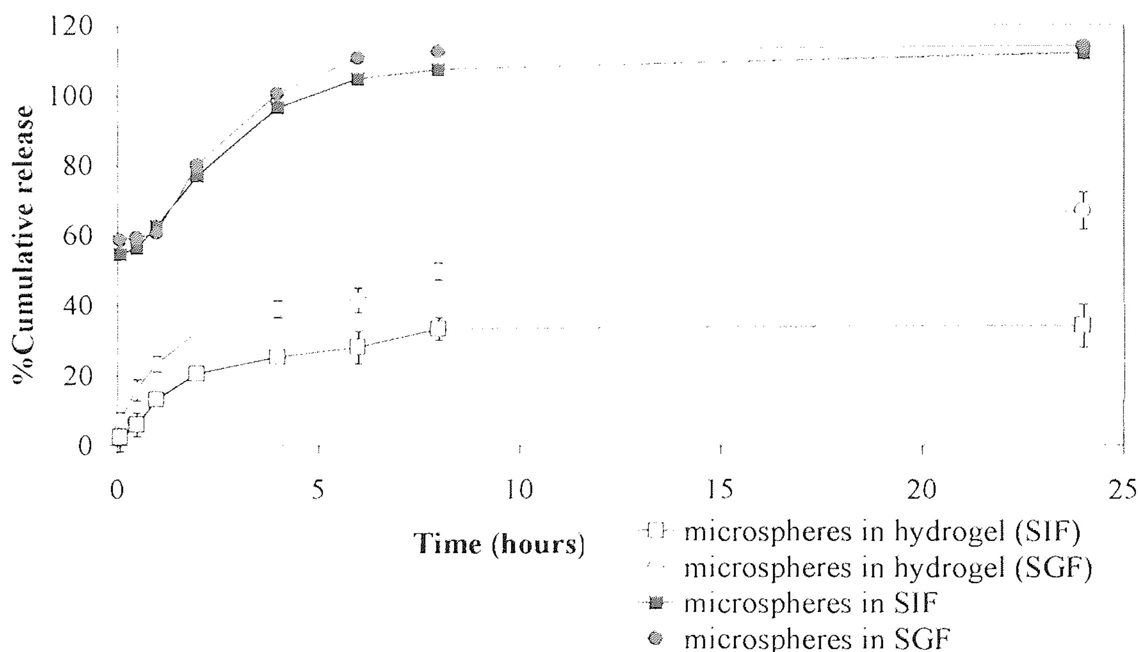


Figure 5.7 Drug release from gelatin microspheres and gelatin microspheres inside chitosan/PEO hydrogels in simulated gastric acid (SGF) and simulated intestinal fluid (SIF) (n=3; mean± sd)

Hydrogels were observed to remain intact over 48 hours. With hydrogels (containing microspheres) in SIF, there was no further release after 8 hours. It was thought that this could be due to non-uniform distribution of microspheres in the hydrogels, prior to cutting into discs. Drug release from microspheres incorporated into hydrogels demonstrated a slower rate of drug release than from microspheres alone (Figure 5.7). This suggests that the microspheres were retained inside the hydrogel and drug release was from the microspheres and then through the hydrogel matrix as it swelled. This was confirmed in section 5.3.1.3.4. Figure 5.8 shows the modification of the drug release profile following incorporation into the various controlled release systems, with microspheres inside hydrogels providing the slowest rate of drug release in SGF. Microspheres were found to release over 100%. This was thought to be due inconsistency in drug loading between batches of microspheres resulting in overall higher drug released.

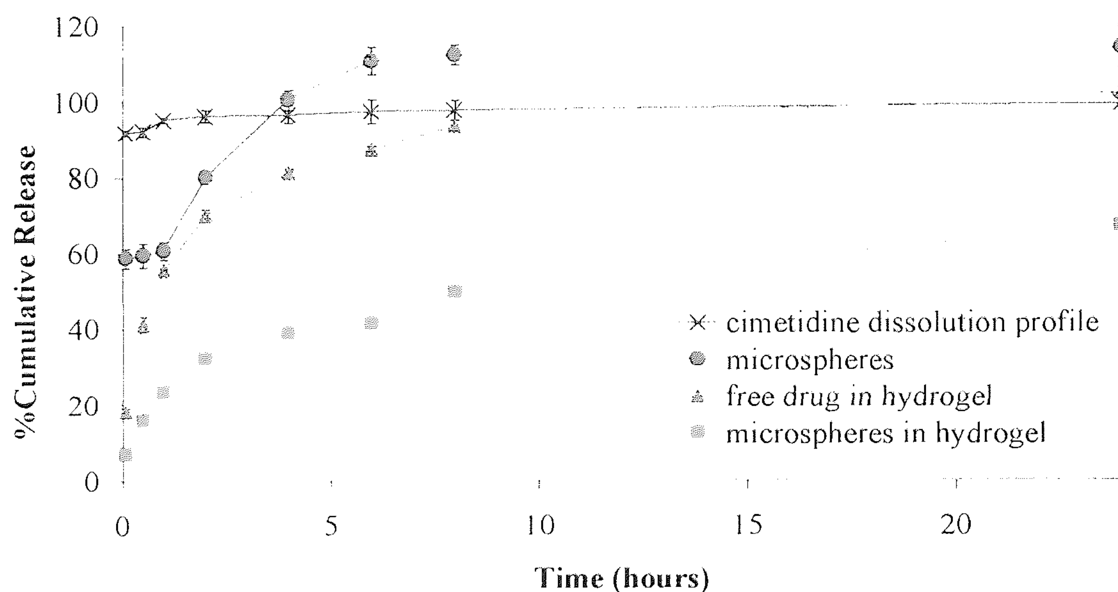


Figure 5.8 Cimetidine release from microspheres in chitosan/PEO hydrogel (50 mg drug), free drug in chitosan/PEO hydrogel (50 mg), free microspheres (microsphere control, 10 mg) and cimetidine dissolution profile (n=3 mean ± sd from separate batches) all in SGF (n=3; mean ± sd)

5.3.1.3.2 Effect of microspheres on swelling of hydrogels

The effect of microspheres on the swelling of hydrogels was determined by weight (Figure 5.9).

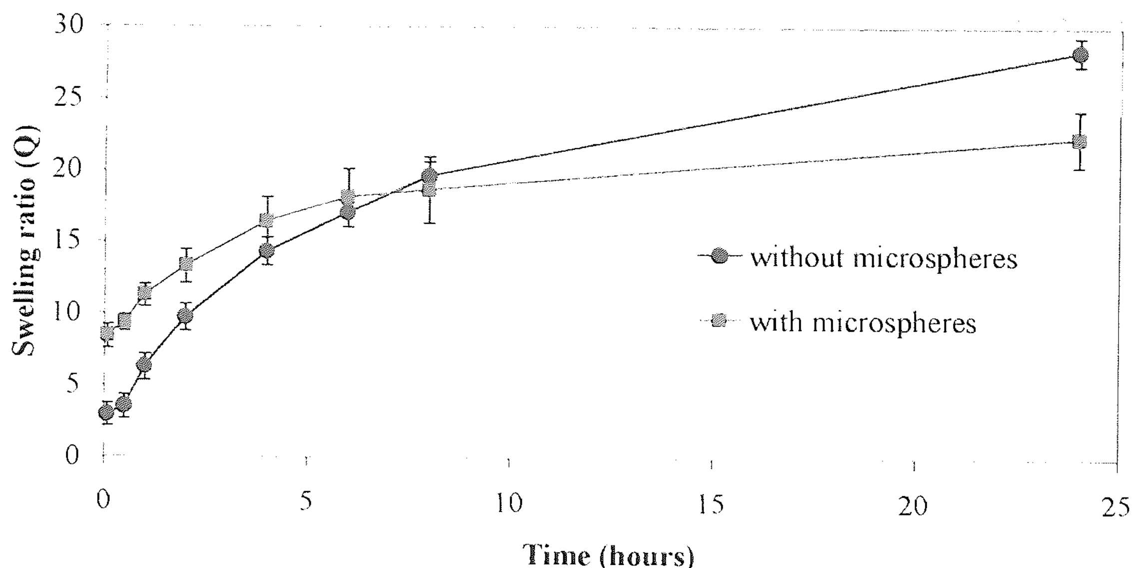


Figure 5.9 Effect on swelling of chitosan/PEO hydrogels in SGF upon incorporation of microspheres, determined by mass change (n=3; mean ± sd)

Overall, the swelling for hydrogels containing microspheres was less than the rate of swelling of hydrogels without microspheres since the microspheres in the hydrogel matrix may disrupt the swelling of the matrix (Figure 5.9). However, hydrogels containing microspheres showed an initial faster rate of swelling, indicating a more rapid uptake of water within the timeframe suitable for gastroretention. It is possible that the microspheres may physically prevent the water uptake through some of the pores of the hydrogel at later points, as in the microsphere retention studies (section 5.3.1.3.4) it was found that larger sizes of microspheres (85 ± 28 μm) were found to be retained in the hydrogels after 2 hours.

5.3.1.3.3 Simulation of in vivo transit of hydrogels incorporating microspheres or free drug

The lower part of the stomach (antrum) is the prime propeller, grinder, and siever of solid food (Meyer, 1987). Furthermore, vigorous antral contractions repeatedly propel chunks of food back and forth in the stomach, and food is fragmented by its repeated contact with the walls of the contracting muscular antrum (Meyer, 1987). Also, the pyloric segment in the stomach is considered a functional sieve, responsible for solid-liquid discrimination. Therefore, it is expected that a delivery vehicle such as a hydrogel would be subjected to

this grinding and sieving effect, until it was small enough to be emptied from the stomach (below 7 mm size) (Khosla *et al.*, 1989).

In vivo transit of the freeze-dried hydrogels was simulated using hydrogels loaded with both drug and microspheres respectively (see section 5.2.11). To simulate *in vivo* conditions, hydrogels were incubated in SGF for 2 hours (as the gastric emptying time was reported to be 90-160 minutes for pellets in the fed state, Khosla *et al.*, 1989), crushed and transferred to SIF (Figure 5.10) (see section 5.2.8).

Release of cimetidine from both formulations was slow following breaking up of the gel and incubation in SIF. This is due to the remaining drug still releasing from the gel and the lower swelling ratio in SIF compared to SGF, where in the low pH environment of SGF basic amino residues of the chitosan will be ionised and contribute to the increased swelling of hydrogels by electrostatic repulsion and counteranion binding The pH of the medium has previously been shown not to have a significant effect on the drug release from gelatin microspheres or hydrogels (section 3.3.2.5 and figure 5.6). In SIF, freeze-dried hydrogels were shown to swell less than in SGF (Figure 5.4). Therefore it can be concluded, that the slower release following incubation may be due to the actual duration of the crushing and filtering stage, during which drug release was still occurring.

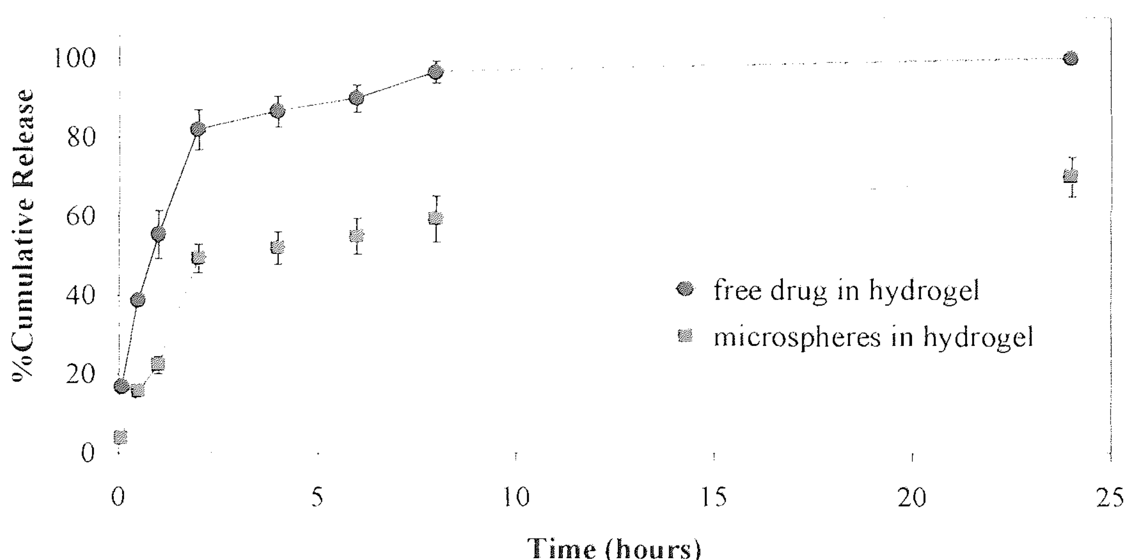


Figure 5.10 Effect of simulated *in vivo* transit on release of cimetidine from chitosan/PEO hydrogels and chitosan/PEO hydrogels containing microspheres (SIF and SGF) (n=3; mean ± sd)

5.3.1.3.4 Effect of microsphere size on retention in hydrogels

To determine whether different sizes of microspheres were retained in hydrogels to the same extent, hydrogels were prepared containing a range of microsphere sizes (Table 5.1). Release studies were not carried out as the purpose of this study was to assess the level of retention of the microspheres within the hydrogel. For each hydrogel formulation, three hydrogel discs from each batch were used in the experiment (9 discs in total).

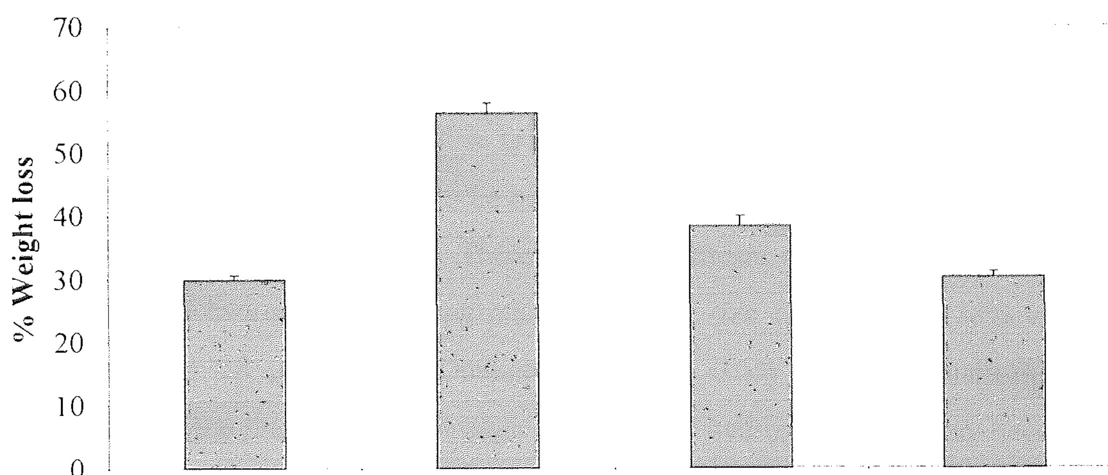


Figure 5.11 Chitosan/PEO hydrogel weight loss following incubation in SGF for 2 hours (n=9; mean ± sd)

Microsphere size (μm)	Preparation method	Loading (% w/w)
6 ± 3	Spray-dried	24.8 ± 3.2
19± 10	Spray-dried	30.0 ± 4.3
85 ± 28	Double-emulsion	15.4 ± 0.4

Table 5.1 Particle sizes, drug loadings and preparation methods for microspheres incorporated into hydrogel formulations

A total of 335 mg of microspheres were added to each hydrogel in order to maintain consistency. As controls, hydrogels without microspheres were prepared. The weight of the hydrogel discs was measured prior to incubation in SGF (e.g. weight of hydrogel disc without microspheres: 70 mg; weight of hydrogel disc with microspheres: 113 mg).

Following 2 hours incubation, hydrogels were removed and freeze-dried, and the weight of the discs determined. Figure 5.11 shows the percentage weight loss from the hydrogels. Control hydrogels were found to undergo some weight loss (30%) following freeze-drying, which could be attributed to surface erosion during swelling over the two hours, or mass loss during removal and handling of hydrogels. Hydrogels containing microspheres of size 6 ± 3 μm were found to undergo a greater weight loss (56%) (which was greater than the weight of the microspheres, however controls showed weight loss also) compared to hydrogels containing microspheres of size 19 ±10 μm (38%). There were no significant differences in weight loss between control hydrogels and hydrogels containing microspheres of size 85 ± 28 μm (Dunnetts test, $P > 0.05$). The results indicate that small microspheres (6 ± 3 μm) may diffuse from the raft during incubation, through the pores of the hydrogel, which are reported to be of size 8-10μm in the dry state, and likely to grow in size during the uptake of water. There was a significant difference between percentage weight loss of hydrogels containing microspheres of size 19 ± 10 μm (38% weight loss) and size 85 ± 28 μm (30%) (Dunnetts test, $P < 0.05$). This may indicate loss of a percentage of the microspheres through pores of the hydrogel, which may have expanded to a size large enough to allow microspheres to pass through. Large microspheres were retained in the hydrogel. In conclusion, if the hydrogel pores are large enough to allow particles of size 6 ± 3 μm to escape, these microspheres could be used to release the drug in the GI tract through mucoadhesion, and the hydrogel would continue to provide controlled release until it passed through the pylorus following reduction in size by the grinding and sieving action of the stomach. If the application required microspheres to be retained in the hydrogel, the larger particles should be used.

5.3.2 Raft formulations

For formulations in this study, sodium alginate was used as the raft-forming agent. From studies of the literature and currently marketed products, it was found that generally liquid raft formulations have been prepared using sodium alginate, and solid formulations (available as chewable tablets) have been prepared using alginic acid (see section 1.8.4). The reason for the inclusion of the alginic acid in solid formulations is because it forms a viscous gel on contact with water and saliva, through the chewing actions of the mouth,

whereas in liquid preparations, the sodium alginate salt precipitates in the acidic environment of the stomach forming a viscous gel. Without the chewing action alginic acid would not form adequate rafts, therefore sodium alginate was used in these preparations, as the raft was required to be formed in vitro in simulated gastric environment. Furthermore, as gelatin microspheres have been shown to swell following incubation in solution (section 3.3.3.1.1), preparation of liquid formulations containing gelatin microspheres would mean that the microspheres would begin swelling and initiate drug release. Dry formulations were preferred, as this would prevent premature drug release from incorporated microspheres.

Potassium bicarbonate was included for the gas generation, as opposed to sodium bicarbonate as it was reported that inclusion of potassium bicarbonate resulted in formation of stronger rafts (Johnson *et al.*, 1997).

5.3.2.1 Optimisation of rafts by variation of ingredients

To optimise the raft formulations, ratios from Gaviscon® 250 tablets (alginate 250 mg, sodium bicarbonate 85 mg, calcium carbonate 20 mg, ratio of sodium bicarbonate to calcium carbonate = 4.5) were formulated and the raft size determined in terms of the thickness (at the centre of the raft) and diameter, after 1 hour (raft thickness 4.8 ± 1.5 mm and diameter 31.2 ± 2.0 mm). The method for measurement of raft thickness and diameter was adapted from Johnson *et al.*, (1997), where raft formulations were formed in measuring cylinders and the thickness of the raft measured (see methods section. 5.2.10). In observation of rafts developing in the beaker, it was apparent that not only the height and the width of the raft was important, but also the amount of foam entrapping the alginate gel as a result of the production of the carbon dioxide. This quality was thought to give the raft its buoyancy and was only a visual observation. It was thought that contact of the raft with the sides of the vessel may influence its physical dimensions. Therefore, rafts were formulated in 100 ml beakers and both the thickness and the diameter of the rafts were measured 1 hour after formation. To optimise the carbonate content in the raft, keeping the weight of the raft constant, the amounts of potassium bicarbonate and calcium carbonate were varied (Table 5.2). The sodium alginate level was reduced from 250 mg to 200 mg to decrease the overall weight of the raft. Even though the raft formulated with 200

mg (with the same ratio of bicarbonate to carbonate, *i.e.* potassium bicarbonate 185 mg, calcium carbonate 45 mg, ratio ~ 4 and in Gaviscon®: sodium bicarbonate 85 mg, calcium carbonate 20 mg, ratio ~ 4) was similar in dimensions (*i.e.* thickness t-test, P = 0.857 and diameter t-test, P = 0.158), it was visually apparent that buoyancy was better-achieved following formation. However, following optimisation of raft height and thickness with potassium carbonate and calcium carbonate, the amount of sodium alginate was also varied from the 200 mg value to investigate this further.

The optimum amounts of potassium bicarbonate and calcium carbonate were found to be 220 mg and 10 mg respectively in terms of raft thickness and height. Further decrease beyond 10 mg in calcium carbonate level reduced the raft height and diameter, as this level was not sufficient for the sufficient activation of the raft mechanism in terms of generation of bubbles by the carbonate and entrapment within the alginate matrix. Generally, there was found to be a relationship between the increasing ratio of potassium bicarbonate to calcium carbonate with increasing strength of raft (in terms of physical dimension). Using this optimum ratio of potassium bicarbonate to calcium carbonate, the sodium alginate amount was varied (Table 5.3).

Potassium bicarbonate (mg)	Calcium carbonate (mg)	Height of raft (mm)	Diameter of raft (mm)
130	100	2.3 ± 0.6	15.7 ± 1.5
140	90	3.0 ± 1.0	25.0 ± 3.0
180	50	3.7 ± 0.6	24.7 ± 2.5
185	45	5.0 ± 1.0	33.7 ± 1.5
200	30	5.3 ± 1.6	29.7 ± 3.2
210	20	7.3 ± 1.5	31.0 ± 1.0
220	10	9.7 ± 1.5	37.7 ± 1.5
225	5	6.3 ± 1.5	32.7 ± 1.5

Table 5.2 Optimisation of raft formulations (sodium alginate = 200 mg) based on thickness and diameter of rafts formed (n=3; mean ± sd)

Sodium alginate (mg)	Height of raft (mm)	Diameter of raft (mm)
10	1.7 ± 0.6	18.7 ± 2.1
50	2.7 ± 1.2	20.0 ± 1.7
100	5.3 ± 2.5	31.0 ± 3.6
200	9.7 ± 1.5	37.7 ± 1.5
300	8.0 ± 1.0	33.0 ± 1.0

Table 5.3 Variation of sodium alginate in optimisation of raft formulation (potassium bicarbonate 220mg, calcium carbonate 10mg) (n=3; mean ± sd)

The optimum amount of sodium alginate was found to be 200 mg with respect to the dimensions of the rafts formed (Table 5.3). When sodium alginate content was increased to 300 mg, the raft height and diameter were reduced. This was interesting as the quantity of material was greater, yet the raft was smaller. It was thought that in this formulation, with higher alginate content there was not enough calcium carbonate to aid the gelation, and also not enough potassium bicarbonate to form the bubbles in the gel, hence there was less foam produced and the dimensions were smaller (note: dimensions were measured from one side of the raft to the other and included the foam). With reducing alginate content, the strength of the raft was reduced in terms of the thickness and diameter. Therefore, alginate rafts were prepared using 200 mg alginate, 220 mg potassium bicarbonate and 10 mg calcium carbonate. This would seem to be the correct amounts to formulate rafts with the optimum dimensions.

5.3.2.2 Cimetidine release from alginate rafts

The free drug was incorporated in the rafts (section 5.2.11) and drug release in SGF was assessed (section 5.2.13). With increasing levels, the amount of drug released in an initial burst effect was higher (60% released with 400 mg free drug; 4% released with 10 mg free drug) (Figure 5.12). As raft mixture used was 430 mg, incorporation of 400 mg cimetidine was expected to have a higher burst release, due to relatively high drug levels in the device. Incorporation of 200 mg and below showed more controlled release, indicating that drug was entrapped in the raft structure.

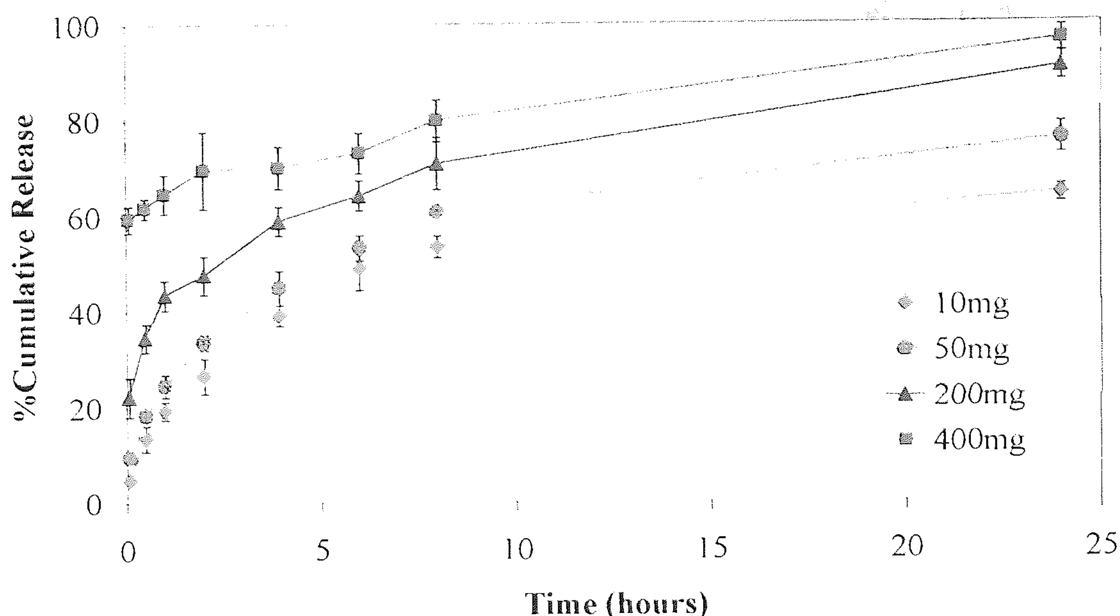


Figure 5.12 Cimetidine release from powder raft formulations in SGF, containing different quantities of free drug (n=3; mean ± sd)

5.3.2.3 Incorporation of microspheres into raft systems

The raft formulation consists of a discontinuous alginate gel, with bubbles of carbon dioxide entrapped within the gel network. To successfully incorporate microspheres into the raft formulation, it is necessary for the microspheres to become entrapped within the gel matrix.

Since high quantities of microspheres were required to incorporate 50 mg drug (*e.g.* 335 mg with 15.5% w/w loaded microspheres), when microspheres were incorporated into raft systems, it was found that the strengths of the rafts were reduced in terms of raft thickness and raft diameter (Table 5.4). This is because the microspheres absorb water, swell (see section 3.3.3.1.1) and become physically very bulky as constituents in the raft. Therefore, the inclusion of the microspheres has a diluting effect on the raft, which reduces the strength of the raft, affecting the thickness and the diameter. The effect of inclusion of microspheres can be considered similar to the effect of inclusion of aluminium hydroxide, which is similarly reported to reduce the strength of the raft (Washington *et al.*, 1986). For this reason it was thought necessary to optimise the rafts containing microspheres to obtain the raft with the optimum dimensions.

Raft formulation	Height of raft (mm)	Diameter of raft (mm)
with microspheres	3.0 ± 1.0	25.0 ± 1.7
without microspheres	9.7 ± 1.5	37.7 ± 1.5

Table 5.4 Dimensions of raft formed with microspheres (containing 335 mg microspheres), and without microspheres (containing 200 mg sodium alginate, 220 mg potassium bicarbonate and 10 mg calcium carbonate) (n=3; mean ± sd)

5.3.2.3.1 Optimisation of excipients for raft formulations containing microspheres

The sodium alginate level was reduced as the microspheres were bulky and heavy, disrupting the delicate raft forming ability of the raft. It was thought that by reducing the amount of sodium alginate to a specific quantity, the gelling of the raft might be sufficiently achieved to entrap the microspheres in the alginate gel with sufficient foam and bubbles formed from the sodium bicarbonate and calcium carbonate. In table 5.3, with decreasing alginate amount it was found that the rafts became smaller. This was not the case when microspheres were incorporated (Table 5.5). When sodium alginate was changed to 50 mg, the raft was found to foam and gel better, which is why the raft had a larger height compared to other alginate quantities. (note: even though the actual mass of the raft may have been less, when measuring the raft the actual amount of foam produced around the raft was taken into account)

Sodium alginate (mg)	Height of raft (mm)	Diameter of raft (mm)
10	3.7 ± 0.6	23.3 ± 2.1
50	7.3 ± 1.5	27.3 ± 2.1
100	2.7 ± 1.2	23.3 ± 1.5
200	3.0 ± 1.0	25.0 ± 1.7
300	2.3 ± 0.6	22.0 ± 1.0

Table 5.5 Effect of alginate content on dimensions of rafts formed (335 mg microspheres, potassium bicarbonate 220 mg, calcium carbonate 10 mg) (n=3; mean ± sd)

Other excipients were varied to optimise them also. With variation of calcium carbonate and potassium bicarbonate levels, there was not as much of a pronounced change in raft dimensions as seen earlier. With calcium carbonate at 100 mg and potassium bicarbonate

at 130 mg, raft dimensions were optimum with the largest raft height and diameter (Table 5.6). It would seem that the inclusion of the microspheres in the raft affected the mechanism of raft formation as the same effects of carbonate/bicarbonate variation were not observed

Potassium bicarbonate (mg)	Calcium carbonate (mg)	Height of raft (mm)	Diameter of raft (mm)
130	100	9.3 ± 1.2	32.3 ± 1.5
140	90	8.3 ± 1.5	26.0 ± 3.6
180	50	7.3 ± 0.6	24.0 ± 1.7
190	40	7.0 ± 1.7	24.7 ± 1.5
200	30	8.0 ± 3.6	24.7 ± 2.9
220	10	7.3 ± 1.5	27.3 ± 2.1

Table 5.6 Dimensions of raft formed with microspheres (containing 335 mg microspheres, 50 mg sodium alginate (n=3; mean ± sd)

In summary, with the free raft formulations, it was found that increasing bicarbonate and sodium alginate, and decreasing carbonate produced rafts with the largest physical dimensions. With rafts containing microspheres, the effects were different. Increasing alginate resulted in decrease of the raft diameter. This may be because the microspheres swelled in the SGF and as a result became physically bulky and reduced the raft strength. There was also no real effect on the dimensions of the raft with variation of the carbonate/bicarbonate ratio. This was likely due to the lower level of alginate than previously used (i.e. 50 mg here *versus* 200 mg in table 5.2) where there was only a small amount of gel which could be formed.

5.3.2.3.2 Drug release from microspheres incorporated into raft systems

Particle size (μm)	Drug loading (%w/w)	Preparation method
65 ± 31	15.5 ± 1.9	double-emulsion (section 3.2.3)
4 ± 2	8.7 ± 0.2	spray-drying (section 3.2.4)

Table 5.7 Particle size and drug loading of microspheres used in raft formulations

Initially, microspheres of sizes 65 ± 31 μm were incorporated into the raft mixture (as outlined in section 5.2.11 and detailed in table 5.7). Figure 5.13 shows the effect of different formulation methods on the release of cimetidine. Incorporation of microspheres into the raft system was seen to effectively slow the release of drug from the microspheres. The raft system could be used in the delivery of the microspheres to the GI tract, and provide slow release from the microspheres inside. As free cimetidine was released in a controlled manner also, the dose could be tailored to provide sufficient therapeutic levels of the drug. As after 4 hours some microspheres escaped from the raft structure (Figure 5.16), the system could be used to release mucoadhesive microspheres from the raft which could continue to provide controlled release even after the removal of the raft device through the pylorus. However as the gastric residence duration of the raft system in the fed state is reported to be ~ 3.5 hours (Washington *et al.*, 1998) it is unlikely that microspheres would be released from the raft system before the raft is removed from the stomach.

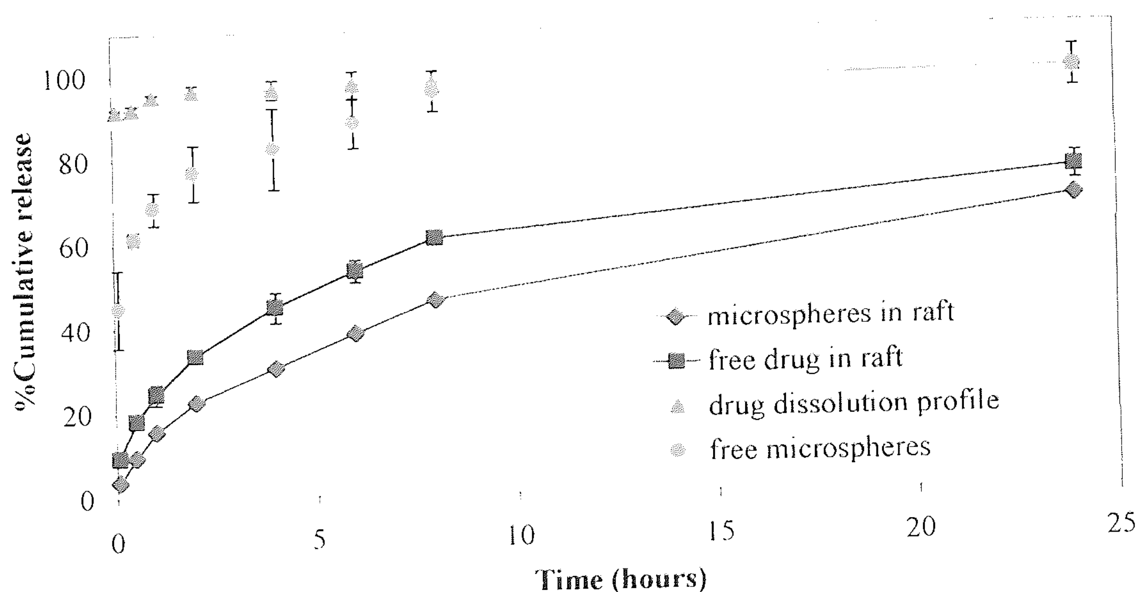


Figure 5.13 Drug release from microspheres in raft (50 mg drug), free drug in raft (50 mg), free microspheres and cimetidine dissolution profile in SGF (n=3; mean ± sd)

5.3.2.3.3 Drug release from different sizes of microspheres incorporated into raft systems

To compare the effect of microsphere size on the release profile from the raft formulations, microspheres of size 4.0 ± 1.7 μm were incorporated into the alginate rafts. Since these microspheres had a lower drug loading than the larger microspheres (Table 5.7), to

incorporate the same equivalent amount of drug into the rafts, a larger amount of microspheres were mixed in with the formulations (*i.e.* 535 mg).

Incorporation of microspheres of size 4.0 ± 1.7 μm into raft systems also resulted in slow release of cimetidine (Figure 5.14), even though the weight of the raft was higher than rafts prepared using larger microspheres.

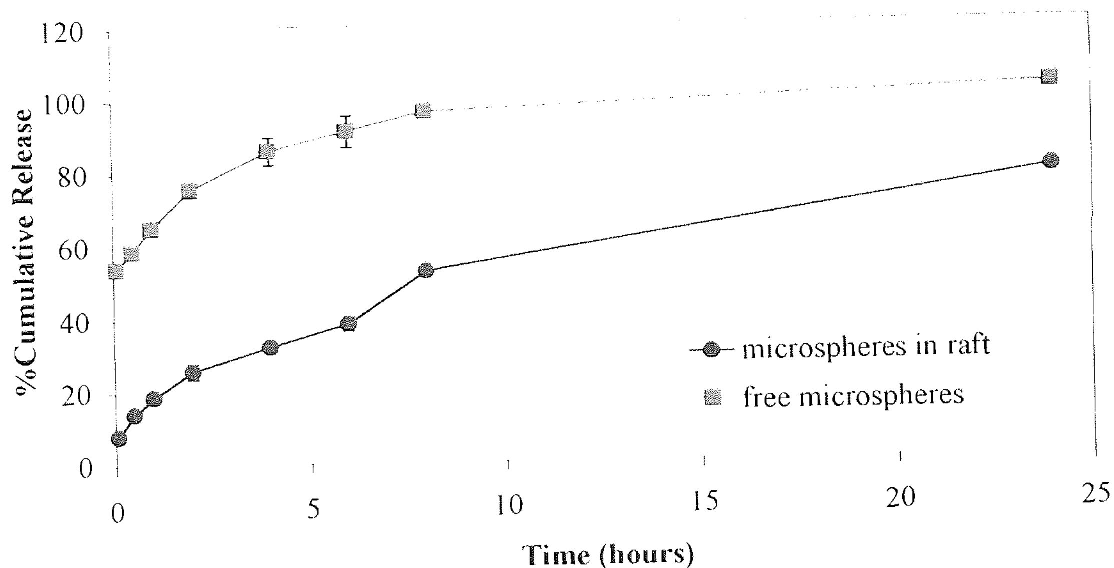


Figure 5.14 Drug release from microspheres and microspheres in rafts in SGF (microsphere of size 4.0 ± 1.7μm) (n=3; mean ± sd)

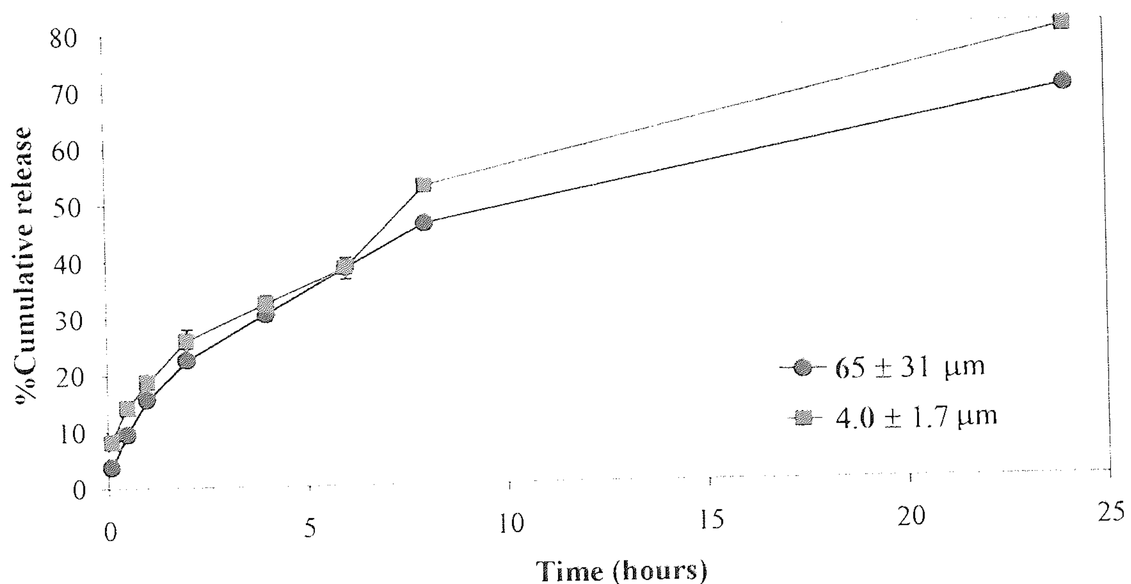


Figure 5.15 Drug release from raft formulations with microspheres of sizes 65 ± 31μm and 4.0 ± 1.7μm (n=3; mean ± sd)

There was no difference in the drug release profiles from small and large microspheres when incorporated into alginate rafts up to 4 hours (Figure 5.15), indicating that the microspheres were retained in the raft to a similar extent. Release of microspheres from the raft system may be indicated by a change in the gradient of the release profile, as drug is released from both microspheres and microspheres in rafts. After 4 hours, there was a small change in the gradient of the drug release profile indicating that this may be the case. To further investigate this, the level of retention of microspheres in the rafts was determined.

5.3.2.3.4 Determination of retention of microspheres in raft systems

To determine the level of retention of microspheres, rafts were formulated using 335mg of microspheres of large (65 ± 31 μm) and small microspheres (4.0 ± 1.7 μm) respectively were incubated in SGF and the extent of microsphere release from the raft determined by BCA assay (section 5.2.12). This experiment was carried out only to determine whether different sizes would behave differently in the raft matrix, therefore the same quantity of the microspheres were used in the rafts. As a control, free microspheres were used, which gave 100% recovery of gelatin (Figure 5.16). After 6 hours, more protein was detected using the smaller particles than the larger particles (14.9 ± 8.7% *versus* 1.4 ± 0.5%). This indicates that the crosslinked alginate matrix has a certain pore size, which allows small particles to escape. It could also indicate the loss in structure of the raft, though visually the rafts remained intact for 8 hours. However, it is unlikely that the alginate raft system would be resident in the stomach for this length of time, so it is not likely that the microspheres would be released from the raft before gastric emptying or biodegradation. The actual dose of drug released is a small amount due to the low loading of the small microspheres (*i.e.* ~ 5 mg of drug). However, this amount could be increased with incorporation of higher levels of microspheres or free drug. To achieve this, the amounts of excipients would have to be varied as it was seen that incorporation of microspheres in the raft changed the effect of carbonate/bicarbonate on the physical dimensions.

If the smaller particles diffuse from the raft after 4 hours to some extent, this could be important in formulation of a delivery system based on rafts containing microspheres. The

raft could be used as a platform for the delivery of cimetidine, which could be achieved by a synergistic effect. Mucoadhesive microspheres could be contained in the raft which upon their release from the raft, could adhere to the mucosa and prolong the release of cimetidine following the removal of the raft from the stomach.

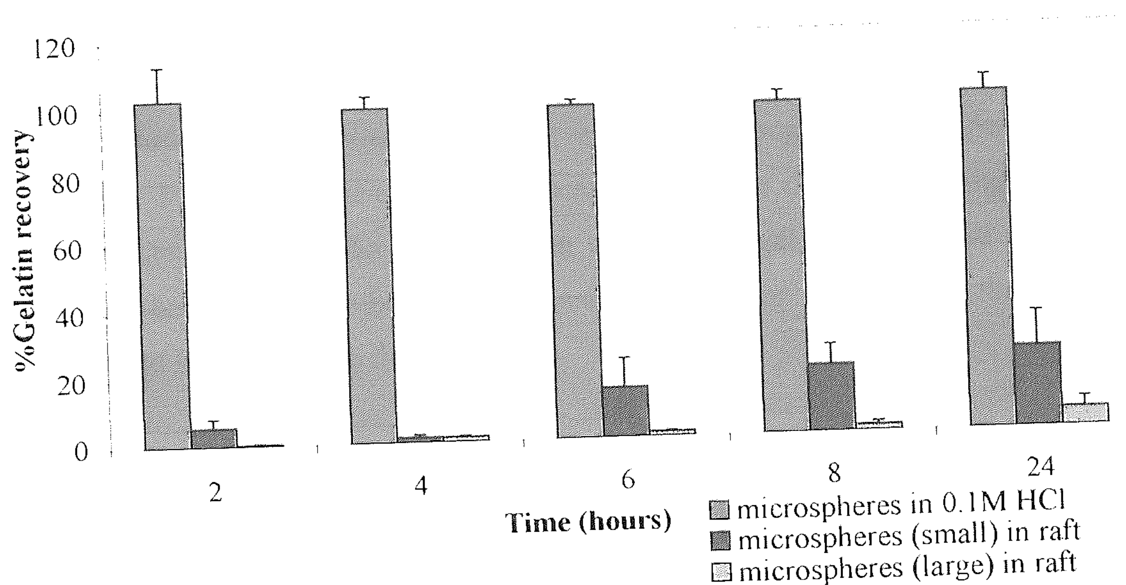


Figure 5.16 The percentage of gelatin recovered following incubation in SGF of small and large microspheres of sizes 4.0 ± 1.7 μm and 65 ± 31μm in raft systems and free microspheres (n=3; mean ± sd)

5.4 Conclusions

It was found that the faster rate, and higher extent of swelling for chitosan/PEO hydrogels than chitosan controls in SGF, was attributed to the formation of the semi-interpenetrating network between the chitosan and the PEO. The highly porous structure of freeze–dried hydrogels was thought to contribute to faster swelling in comparison to air-dried hydrogels. It was due to the porous nature of the freeze-dried hydrogels, that they were used in further work with microspheres.

The diameter of the hydrogels was 20 mm, and increased in size upon swelling. This size was thought to be sufficient for retention in the stomach as it was reported that the cut-off size for gastric emptying from the stomach is 7 mm (Khosla *et al.*, 1989). Freeze-dried hydrogels incorporating free drug showed controlled release with 100% released over 8 hours, and burst release of 20%. Furthermore, incorporation of microspheres inside hydrogels was found to provide slower drug release (compared to hydrogels with free drug), where 60% was released after 8 hours with burst release of 8%. Drug release was slower from hydrogels containing microspheres, as the drug had to diffuse through two barriers: through the microsphere matrix and then through the hydrogel matrix. The swelling of hydrogels containing microspheres was slower than the swelling of hydrogels containing microspheres, due to disruption of the hydrogel matrix by the microspheres. This was thought to contribute to the lower rate of drug release from these hydrogels compared to hydrogels with free drug.

From hydrogel weight loss studies after 2 hours it was determined that loss of 6 μm sized particles from the hydrogel had occurred. The results indicated that small microspheres (6 ± 3 μm) could diffuse from the hydrogel during incubation, through the pores of the hydrogel, which may have expanded to a size large enough to allow microspheres to pass during the uptake of water. Since it is reported that gastric emptying times for tablets of size 7 mm was 90-160 minutes, the hydrogel could allow controlled release of the drug as it expands in the stomach, and as the pore sizes increase, the mucoadhesive microspheres could be released and adhere to the mucosa, enabling controlled release from the microspheres also. The delivery system could be custom designed to consist of a certain amount of free drug in the hydrogel to provide the initial controlled release of a required dose (Figure 5.8). Therefore using this strategy, it may be possible to control where and when and how much drug may be released in the GI tract.

Alginate rafts were formulated following optimisation of the excipient levels, i.e. sodium alginate, potassium bicarbonate and calcium carbonate. With increasing free drug (*i.e.* 10 mg to 400 mg) the burst release was found to increase (5% to 60%), and the release profile was found to follow a faster rate (60% vs 100% released over 24 hours). Incorporation of microspheres into the raft system was seen to effectively slow the release of drug from the

microspheres (~3% burst and ~60% released after 24 hours) in comparison to free drug (~7% burst and ~67% released after 24 hours) by a synergistic effect.

The combination of microspheres entrapped in either rafts or hydrogels, gave similar slow release profiles; showing that both mechanisms could provide effective controlled release of cimetidine in the GI tract. The results indicate the importance of formulation optimisation depending on drug loading.

CHAPTER SIX

FINAL CONCLUSIONS

One of the initial goals of the thesis was the design and production of gelatin microspheres with maximum drug loading to provide sustained release of cimetidine over a period of 12 hours. In the formulation of the gelatin microspheres (chapter 3), a double emulsion method was used as it produced microspheres closest to these objectives. By variation of the primary emulsion parameters, microspheres of sizes 66 ± 31 μm were produced. It was also possible to create larger microspheres in the range 205 ± 161 μm. Maximum loading of the microspheres was found to be 33.9 ± 7.8 %w/w by using a theoretical load of 80 %w/w cimetidine. Controlled release of cimetidine over 6 hours was achieved using the double emulsion method with burst release of 50%.

Another goal of the thesis was the selection and use of suitable *in vitro* and *ex vivo* models to predict mucoadhesion of the microspheres. By use of an *in vitro* surface adsorption assay, the microspheres were easily and quickly screened and design of the rat intestine *ex vivo* model served to compare mucoadhesive levels between microspheres in a more realistic environment.

A goal of the thesis was the formulation of mucoadhesive microspheres using other polymers in combination with gelatin to improve on properties of the gelatin microspheres in terms of mucoadhesion, drug loading and drug release profiles. In chapter 4, gelatin microspheres were incorporated with modifying mucoadhesive polymers carbopols, polycarbophil, chitosans and alginates, both as blends with gelatin in the matrix and on the microsphere surface to determine which method produced microspheres giving higher mucoadhesion. Surface modification of gelatin microspheres (35.7 ± 10.2% mucoadhesion) by polymers such as chitosan CL 113 (71.8 ± 10.2%), carbopols (79.1 ± 12.0% and 83.9 ± 15.0%) and polycarbophil 89.0 ± 8.8%) produced microspheres with high levels of mucoadhesion determined by the *ex vivo* rat intestine model. The incorporation of polymers such as carbopol and polycarbophil with gelatin increased the drug loading from 10.3 ± 0.7% (gelatin microspheres) to 16.5 ± 1.2% (gelatin/carbopol) and 16.1 ± 0.7% (gelatin/polycarbophil). Controlled release from the gelatin microspheres was slow with incorporation of high viscosity alginate MVM, with burst release of ~ 32% and 70% released after 6 hours. This was a slower profile than that obtained with gelatin microspheres.

Another objective of the thesis was to attempt to incorporate lectins with gelatin microspheres and determine whether it had any affect on mucoadhesion. *Ulex europaeus* agglutinin I lectin was incorporated onto the surface of gelatin microspheres by covalent coupling with efficiency of 39.9 ± 1.9%. The lectin-coupled gelatin microspheres were found to give higher mucoadhesive levels (21.4 ± 4.6 %) than unbound gelatin microspheres (10 ± 5.3%).

A goal of the thesis as the formulation of porous hydrogels to achieve gastroretention in the stomach. These hydrogels were to be used as a gastroretentive platform for the delivery of mucoadhesive microspheres in the GI tract. The drug release was to be determined and retention of different sizes of microspheres inside the hydrogel was to be assessed. In chapter 5 freeze-dried porous hydrogels were produced from chitosan and polyethylene oxide incorporating free drug and different sizes of microspheres. Hydrogels with free drug showed sustained release with 100% released over 8 hours, and burst release of 20% from the hydrogels. Drug release from microspheres inside hydrogels was slower where 60% was released after 8 hours with burst release of only 8%. Small microspheres (6 ± 3 μm) diffused from the raft during incubation through the pores of the hydrogel, while larger microspheres (19 ±10 μm and 85 ± 28 μm) were retained inside the hydrogel. Therefore, it may be possible for the hydrogel to be used as a platform for the delivery of mucoadhesive microspheres to the GI tract since smaller microspheres were released from the hydrogel.

Another goal was to design and incorporate microspheres into alginate raft systems to see if they could also be used as a platform for the delivery of microspheres to the GI tract. Incorporation of microspheres into the raft system was seen to effectively slow the release of cimetidine from the microspheres with 3% burst release and 60% released after 24 hours, where free drug burst release was 7% and 67% was released after 24 hours. After 6 hours, microspheres of size 4.0 ± 1.7 μm were found to be released from the raft matrix. By this time, the raft would be expected to have emptied from the stomach. The raft system could be used for the controlled release of cimetidine as drug release was slow and the rafts have demonstrated their gastric retention ability in the literature (see section 1.5.4). However, there may be no advantage of incorporation of microspheres over free drug into the rafts as drug release was similar in both cases.

FURTHER WORK

Microspheres were formulated using combinations of gelatin with other mucoadhesive polymers with the intention of site-specific delivery to the GI tract. *In vivo* studies could be carried out which would indicate whether this targeting strategy has any effect on improving bioavailability or therapeutic effect. As part of the further work the mucoadhesive microspheres could be incorporated into a tablet or capsule and the transit of the microspheres assessed using radiolabelling.

Gastric retention by utilising swelling or floating systems was another strategy for the controlled release of cimetidine. Evaluation of hydrogels showed controlled release over 8 hours with small burst release. Peristaltic forces in the stomach can degrade dosage forms. The maximum pressure in the fasted and fed state is known to range from 50 to 130 cm H_2O in humans (Chen *et al.*, 2000). Therefore, measuring the breaking point of the hydrogel discs in further studies could better indicate the potential performance *in vivo*. For further work, the hydrogels could be compressed and placed into capsules. Since compression could affect the properties of the hydrogel in terms of swelling and drug release, these effects would need to be evaluated. For further work with the raft formulations, a method of measuring the strength of the raft based on a stress measurement could aid in the prediction of the performance under the peristaltic forces in the stomach. The next step in the raft formulation is compression into tablets and assessment of the raft *in vitro*.

Using gammascintigraphy, the transit of the dosage through its intended site of delivery can be noninvasively imaged *in vivo* via the introduction of a short-life gamma-emitting radioisotope. As it has been used in assistance of product development and for the testing of marketed products, it may be useful to assess the effectiveness of the hydrogel and raft formulations following the compression work.

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APPENDIX

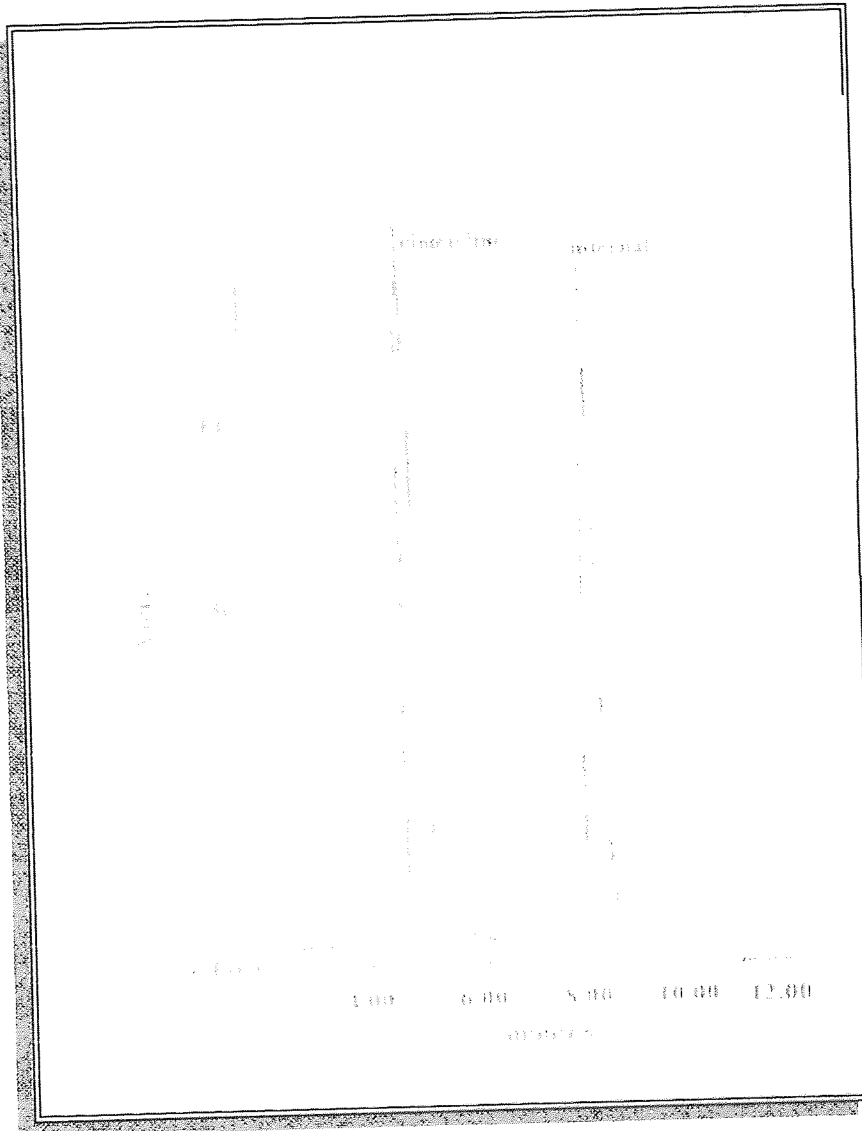

Figure A1.1 Typical HPLC chromatograph of the cimetidine peak with the ornidazole internal standard peak

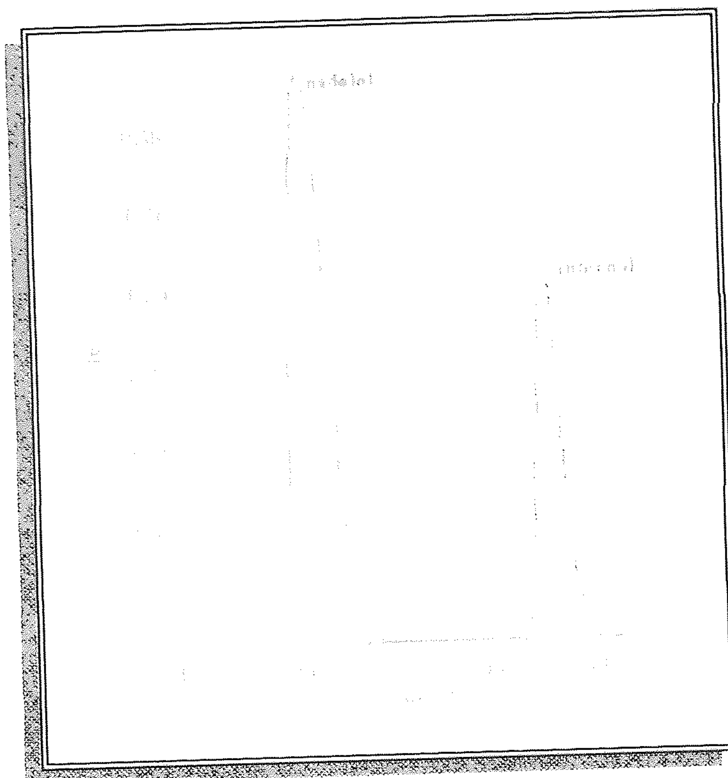

Figure A1.2 Typical HPLC chromatogram of the of nadolol peak with the ornidazole internal standard

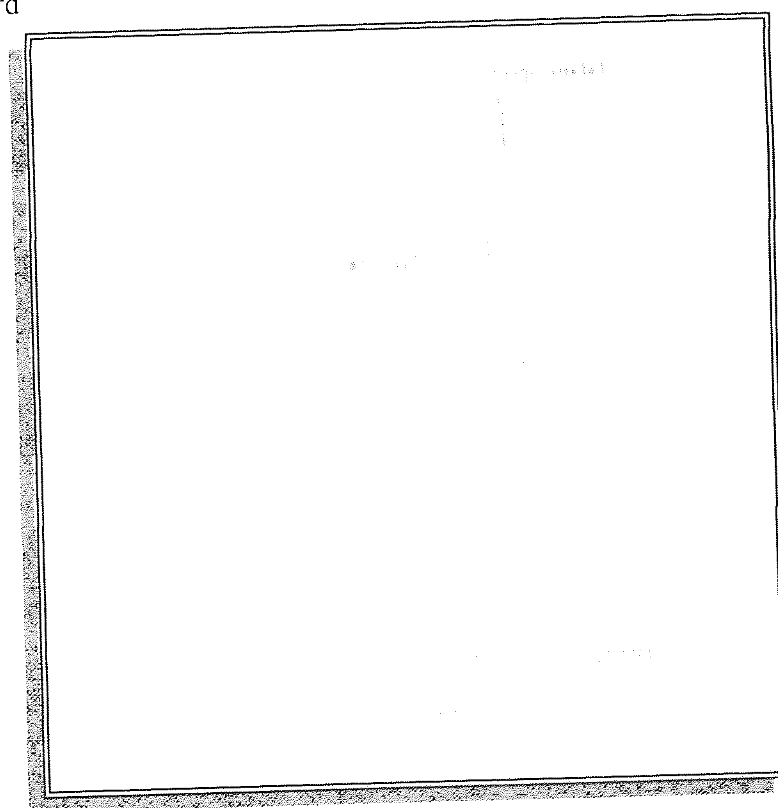

Figure A1.3 Typical HPLC chromatograph of the propranolol hydrochloride peak with the ornidazole internal standard

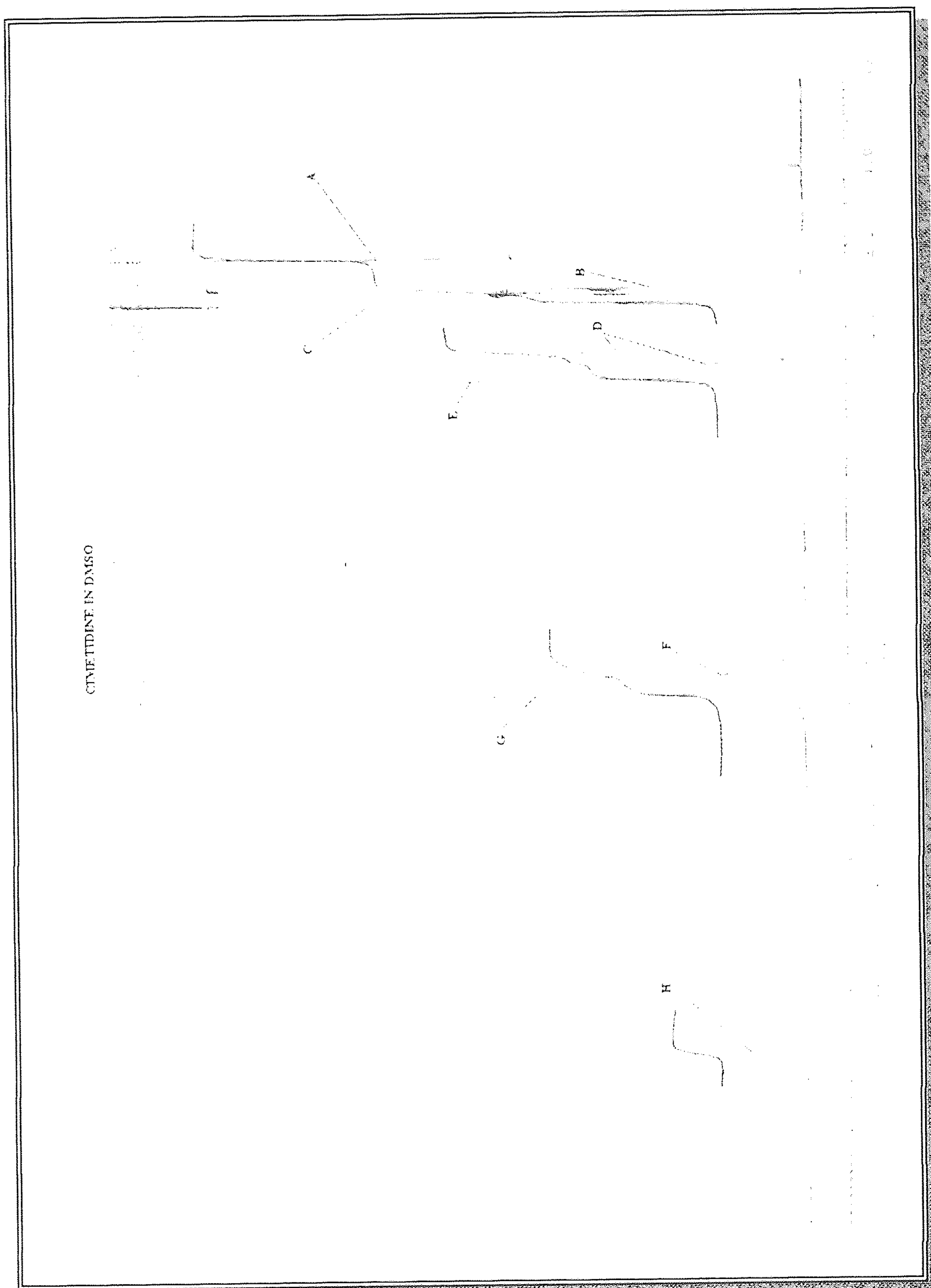

Figure A1.4 H-NMR spectrum of cimetidine

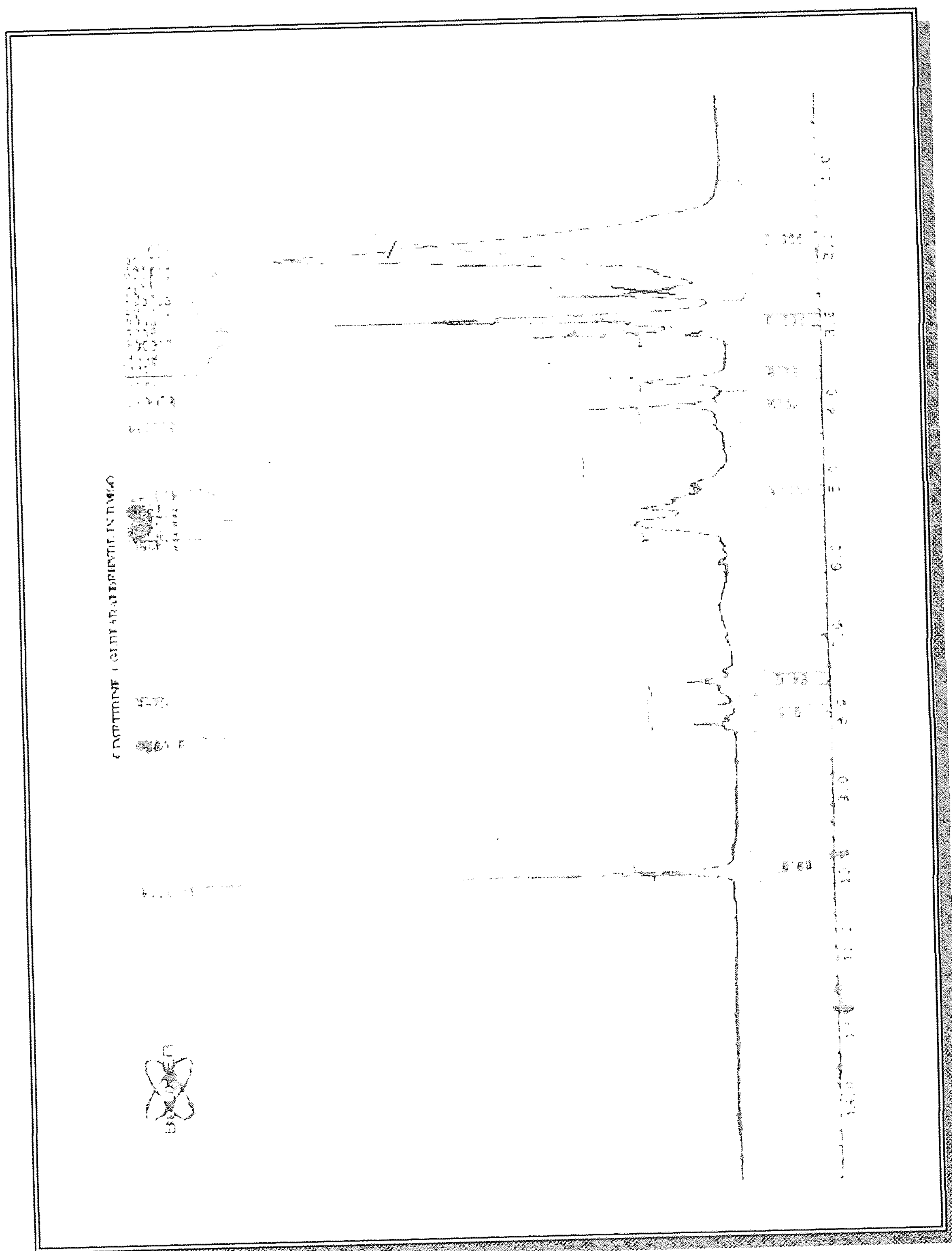

Figure A1.5 H-NMR spectrum for cimetidine-glutaraldehyde mixture

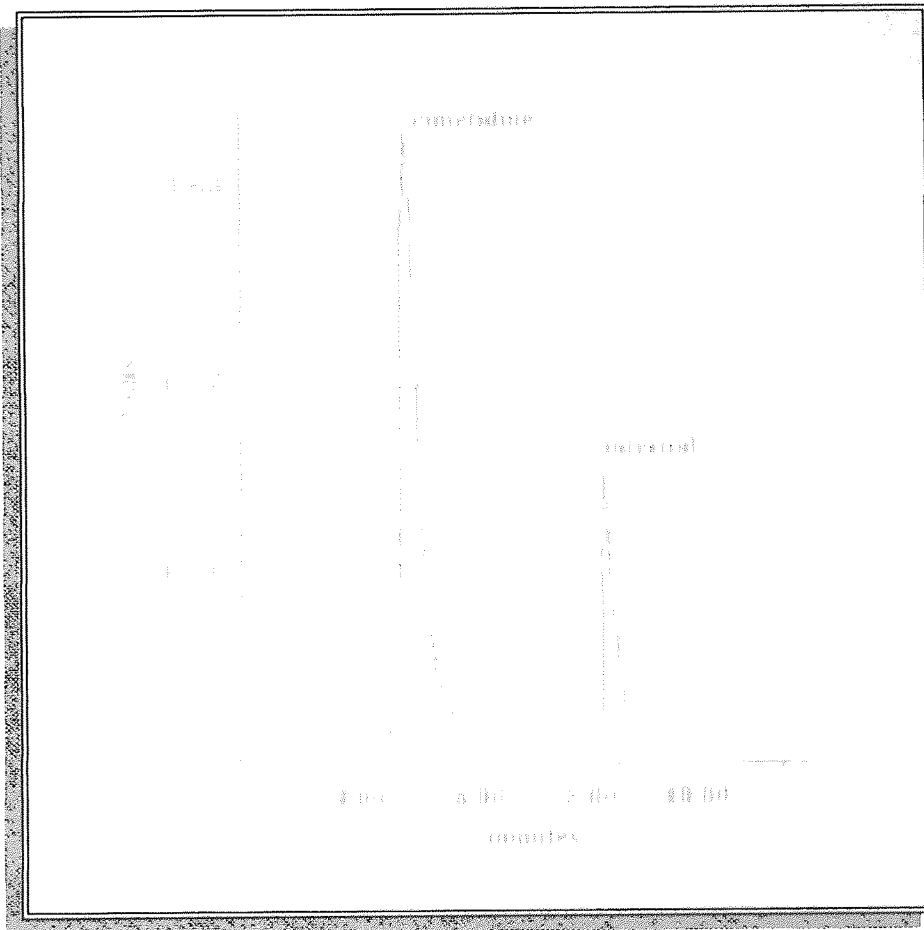

Figure A1.6 HPLC chromatograph for cimetidine-glutaraldehyde mixture