NOVEL pH-SENSITIVE MICROPARTICLES
FOR SITE-SPECIFIC DRUG DELIVERY TO THE
GASTROINTESTINAL TRACT

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**Abstract**

Conventional delayed release formulations which utilise pH-sensitive polymers, and take advantage of the aboral increase in gastrointestinal pH, to deliver drugs to either the small intestine or colonic region often suffer from poor site-specificity of drug release in man. This has been attributed to physiological factors including gastrointestinal transit times, pH and free fluid volume within the lumen of the gastrointestinal tract.

pH-sensitive microparticles are expected to offer improved gastrointestinal transit and control of drug release compared to conventional delayed release dosage forms. The aim of this study was to formulate pH-sensitive microparticles to target different regions of the gastrointestinal tract, and to characterise the resulting microparticles both *in vitro* and *in vivo*.

The emulsification/solvent evaporation technique was successfully optimised for the production of Eudragit L55 and L microparticles for small intestinal targeting, and Eudragit S microparticles for colonic targeting. Optimised microparticles were observed by scanning electron microscopy to be spherical and monodisperse, in the size range 30-50 µm. Eudragit L55 microparticles aggregated in acidic media during *in vitro* dissolution testing, which was thought to be due to the low glass transition temperature of the polymer. However, Eudragit L and S microparticles suspended freely without aggregation, and retarded release of a model drug, prednisolone, at acidic pH for a period of two hours, but released it rapidly at small intestinal and colonic pH, respectively, due to the large surface area to volume ratio of the microparticulate dosage forms and, possibly, the amorphous nature of the entrapped prednisolone.

To assess the *in vivo* drug absorption from the microparticulate dosage forms, prednisolone-loaded Eudragit L and S microparticles and a prednisolone control suspension were administered orally to rats which were bled over a period of 8 hours. A faster $T_{\text{max}}$ was achieved with Eudragit L microparticles than with control suspension due to the release of drug in a presolubilised form, close to the absorption window of prednisolone in the small intestine. The relative bioavailability of prednisolone-loaded Eudragit S microparticles was 0.35 compared to the prednisolone suspension. This suggests that drug absorption was incomplete from the Eudragit S microparticles, which was possibly due to the limited fluid volumes in the gastrointestinal tract, demonstrating the limited usefulness of the rat as a model for man.
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Finalement, merci Catherine. Je t’aime.
Plagiarism Statement

This thesis describes research conducted at the University of London School of Pharmacy between 2001 and 2005 under the supervision of Dr. Abdul Basit and Dr. Sudaxshina Murdan. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

Signature

Date

NA Kendall

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<th>Definition</th>
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<tbody>
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<td>ΔH</td>
<td>Enthalpy of heat</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of Variance</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the plasma concentration-time curve</td>
</tr>
<tr>
<td>C&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Maximum plasma concentration</td>
</tr>
<tr>
<td>D&lt;sub&gt;50&lt;/sub&gt;</td>
<td>50% undersize distribution</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>F</td>
<td>Bioavailability</td>
</tr>
<tr>
<td>FDA</td>
<td>Food and Drug Administration</td>
</tr>
<tr>
<td>GE&lt;sub&gt;50&lt;/sub&gt;</td>
<td>Time for 50% of contents to empty from the stomach</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>HLB</td>
<td>Hydrophilic-lipophilic balance</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HPLC-MS/MS</td>
<td>High pressure liquid chromatography-tandem mass spectrometry</td>
</tr>
<tr>
<td>LOD</td>
<td>Limit of detection</td>
</tr>
<tr>
<td>MCC</td>
<td>Microcrystalline cellulose</td>
</tr>
<tr>
<td>MMC</td>
<td>Migrating Myolelectric Complex</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>Na&lt;sub&gt;3&lt;/sub&gt;P&lt;sub&gt;0&lt;/sub&gt;</td>
<td>Tribasic sodium phosphate</td>
</tr>
<tr>
<td>o/o</td>
<td>oil-in-oil emulsion</td>
</tr>
<tr>
<td>RH</td>
<td>Relative humidity</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short chain fatty acid</td>
</tr>
<tr>
<td>SD</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>SPE</td>
<td>Solid phase extraction</td>
</tr>
<tr>
<td>T&lt;sub&gt;g&lt;/sub&gt;</td>
<td>Glass transition temperature</td>
</tr>
<tr>
<td>T&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Time of maximum plasma concentration</td>
</tr>
<tr>
<td>USP</td>
<td>United States Pharmacopoeia</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
<tr>
<td>w/o</td>
<td>water-in-oil emulsion</td>
</tr>
<tr>
<td>XRPD</td>
<td>X-ray powder diffraction</td>
</tr>
</tbody>
</table>
Chapter One: Introduction
1.1 Overview

The oral route is the most popular route of drug delivery, representing the simplest, most convenient and safest method of introducing drugs into the body (York, 1988; Mayersohn, 1996). However, administration of a growing number of drugs as immediate release preparations, designed to disintegrate and release drug in the stomach, is undesirable and can result in suboptimal therapy or the induction of local or systemic side effects. The formulation of such drugs as modified release delivery systems, designed to alter the site and/or time of drug release in an attempt to improve their therapeutic efficacy, renders the dosage form non-disintegrating during passage through some, or all, of the gastrointestinal tract. This in turn can result in delayed and unpredictable gastrointestinal transit (particularly gastric emptying), which in combination with inter- and intra-individual variability in gastrointestinal physiology (particularly pH and fluid volume), can result in variable drug release, and therefore therapeutic performance.

Oral modified release dosage forms are generally presented as either multiple unit or single unit formulations, the latter consisting of numerous subunits such as pellets, granules or mini-tablets either filled into capsules or compressed into tablets. The failings of single unit modified release dosage forms are related to their size. However, contrary to popular belief, multiple unit dosage forms of size 1-1.5mm offer only a limited improvement over single unit modified release formulations in terms of normalising gastrointestinal transit and drug release.

The aim of this work was therefore to formulate modified release microparticles, of size <100μm, which it is proposed may be able to suspend in the liquid contents of the stomach thus emptying rapidly and reproducibly, thereby facilitating a rapid onset of drug action. Furthermore, the large surface area:volume ratio and expected spread of a microparticulate formulation within the gastrointestinal tract would potentially increase the rate of drug release and absorption at site-specific regions of the gastrointestinal tract, particularly the small intestine and colonic region.
1.2 Oral drug delivery
1.2.1 Immediate release
The majority of drugs are still administered as immediate release formulations, which are
designed to disintegrate rapidly into small fragments in the stomach, from which the drug
can readily dissolve in the liquid contents (Rubinstein, 1988), and empty into the small
intestine from where the drug will be absorbed into the systemic circulation. However, for
many drugs, immediate release does not represent the optimal drug delivery strategy, and as
such they are formulated as either delayed or extended release formulations.

1.2.2 Modified release
1.2.2.1 Modified release platform design
The release rate of drugs from modified release formulations is generally controlled by
polymers which are either incorporated into the matrix of, or applied to the external surface
of, the dosage form. Both extended and delayed release formulations may be fabricated as
either single unit (tablets, capsules), or multiple unit (pellets or beads, conventionally of
size 1-1.5mm) systems. Multiple unit formulations are widely accepted to provide more
consistent in vivo performance than an equivalent single unit formulation, being less prone
to dose dumping, which can result in damage of the gastrointestinal mucosa, while
achieving more reproducible drug blood levels (Bechgaard and Nielsen, 1978).

Multiparticulate dosage forms are conventionally prepared by a number of methods which
are summarised briefly below:

- **Solution layering** involves spraying a solution or suspension of drug and binder
  onto an inert core, building the pellet layer after layer. A polymer coat may be
  applied after the drug to impart modified release properties on the final pellet
  (Gamlen, 1985).

- **Wet mass extrusion/spheronisation** requires the drug (and other processing
  excipients) to be granulated with a liquid binder and shaped into cylinders of
  uniform diameter, which are then chopped and spheronised into approximately
  spherical morphology, and dried (Newton, 1990; Vervaet et al., 1995). Wet mass
  extrusion spheronisation is discussed in more detail in Chapter Three.
• *Hot-melt extrusion/spheronisation* (Young et al., 2002; 2005) is similar to wet mass extrusion/spheronisation, except that the application of heat is required prior to shaping of the drug/excipient blend (which includes a thermoplastic polymer) into a cylindrical extrudate.

A limitation of the current approaches of multiple unit dosage form manufacture is that they are unable to fabricate pellets of less than 0.5mm in diameter, the significance of which will be described later.

1.2.2.2 Extended release
Drugs with a short biological half-life or narrow therapeutic index are possible candidates for formulation as extended release products, from which drugs are released at a slow, controlled rate, potentially enhancing therapy by an improvement in patient compliance or a reduction in systemic side effects.

Water-insoluble polymers such as the ammoniomethacrylate copolymers (Eudragit RS and RL), ethylcellulose, cellulose acetate or polyvinyl acetate have been applied as a film coat to core dosage forms, forming a barrier that is semi-permeable to the ingress of gastrointestinal fluid, resulting in a drug "reservoir" from which drug release can occur slowly via diffusion. Drug release rate can be controlled by the polymer grade, coating thickness, or the addition of low molecular weight pore-forming agents into the film coat.

An alternative approach for achieving extended release is to incorporate the drug into a hydrophilic polymer matrix (commonly hydroxypropyl methylcellulose (HPMC) or polyethylene oxide (PEO)) which will gradually swell on contact with gastrointestinal fluid, forming liquid filled pores through which drug can diffuse (Wilding et al., 1995; Conte and Maggi, 1996; Maggi et al., 2000). Drug release rate is determined by the polymer grade, the ratio of drug to polymer, drug solubility, dimensions of the dosage form, and the tortuosity of the fluid-filled pores. However, a disadvantage to this approach is that so far it has only been applied to single unit dosage forms.
1.2.2.3 Delayed release

In contrast to extended release dosage forms which release drug over a prolonged period of time, delayed release dosage forms are designed to release a bolus of drug in a predetermined location, or after a predetermined time. In practice, delayed release dosage forms are designed to deliver drugs specifically to the small intestine, or to the colon.

1.2.2.3.1 Delayed release to the small intestine (enteric coating)

Delayed release to the small intestine, preventing release of a gastric-irritant or acid-labile drug molecule in the stomach, is perhaps the oldest of all modified release technologies, first described by Unna in 1884 (Agyilirah and Banker, 1991). The trigger factor for release from enteric coated dosage forms is the sharp increase in pH from the stomach (pH 1.0-2.5) to the small intestine (pH 6.6±0.5) (Evans et al., 1988).

A polymer with a dissolution threshold pH in the region 4.5 to 6.0 can therefore be considered suitable for achieving delayed release to the small intestine, and a number of such polymers have been developed for this purpose which are either acrylic, cellulosic or polyvinyl derivatives. The pH at which an enteric polymer begins to dissolve is primarily dictated by the number of ionisable acid groups in the molecule, although other factors such as molecular weight and steric hindrance will also play a role in defining this pH. The most commonly used enteric polymers are summarised in Table 1.1. Traditionally these polymers were only available as powders, which were dissolved in organic solvents prior to being applied to a dosage form as a film coat. However, in recent years, environmental concerns regarding the use and disposal of organic solvents have prompted the reformulation of a number of these polymers as aqueous-based dispersions for film coating.
Table 1.1: pH-sensitive polymers commonly used in the production of delayed release oral dosage forms

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Dissolution threshold pH</th>
<th>Manufacturer</th>
<th>Aqueous dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose acetate trimellitate</td>
<td>4.5</td>
<td>Eastman</td>
<td>Not available</td>
</tr>
<tr>
<td>Hydroxypropyl methylcellulose phthalate 50</td>
<td>5.0</td>
<td>Eastman/Shin-Etsu</td>
<td>Not available</td>
</tr>
<tr>
<td>Polyvinyl acetate phthalate</td>
<td>5.0</td>
<td>Not available</td>
<td>Sureteric (Colorcon)</td>
</tr>
<tr>
<td>Hydroxypropyl methylcellulose phthalate 55</td>
<td>5.5</td>
<td>Eastman/Shin-Etsu</td>
<td>Not available</td>
</tr>
<tr>
<td>Hydroxypropyl methylcellulose acetate succinate L</td>
<td>5.5</td>
<td>Shin-Etsu</td>
<td>Aqoat AS-L</td>
</tr>
<tr>
<td>Poly(methacrylic acid, ethyl acrylate) 1:1</td>
<td>5.5</td>
<td>Rohm (Eudragit L55)</td>
<td>Acryl-eze (Colorcon), Eudragit L30-D55 (Rohm)</td>
</tr>
<tr>
<td>Hydroxypropyl methylcellulose acetate succinate M</td>
<td>6.0</td>
<td>Shin-Etsu</td>
<td>Aqoat AS-M</td>
</tr>
<tr>
<td>Poly(methacrylic acid, methyl methacrylate) 1:1</td>
<td>6.0</td>
<td>Rohm (Eudragit L)</td>
<td>Not available</td>
</tr>
<tr>
<td>Cellulose acetate phthalate</td>
<td>6.2</td>
<td>Eastman</td>
<td>Aquacoat (FMC)</td>
</tr>
</tbody>
</table>

A number of factors should be considered before an enteric coating material is chosen for a particular product. These will include the consequences of premature release in the stomach (the pH of which is raised after eating and in certain disease states), which must be considered against the need for a rapid release in the small intestine to maximise bioavailability and therapeutic effect (which may be crucial when the drug is not well absorbed from the small intestine). The nature of the core material (acidity or basicity, or permeability through different enteric polymer films) may also limit the choice of polymer.

Drug release from enteric coated formulations can be influenced by formulation factors such as choice of polymer, coating thickness, surface area:volume ratio of the final dosage form and choice of other processing excipients included in the coating solution/suspension.
1.2.2.3.2 Delayed release to the colon

Drug delivery to the colonic region has attracted a great deal of research interest over the last two decades (Basit, 2005). Primarily, it is recognised that there is a need to better treat localised pathologies in this region which require therapeutic agents to be delivered specifically to the site of disease. Disorders of the colonic region range in severity from irritable bowel syndrome, to the debilitating inflammatory bowel diseases and life-threatening colorectal cancer, which is the third most common cancer-related death in both men and women (American Cancer Society, 2005). More recently, the colon has also been proposed as a site for the systemic delivery of a number of molecules, the most high profile of which are labile protein and peptide molecules which would be degraded by proteolytic enzymes secreted into the proximal gut.

However, the formulation strategy for targeting the colon is not as straightforward as for targeting to the small intestine, and four potential trigger factors have been identified and investigated: time, pressure, bacteria and pH.

Time-responsive delivery

In contrast to other compartments of the gastrointestinal tract, small intestinal transit time is perceived to be relatively constant at $3 \pm 1$ hours, and is largely unaffected by the timing of administration relative to food, or whether the dosage form is single unit or multiple unit in construction (Davis et al., 1986a).

The Pulsincap® system (Wilding et al., 1992a; Stevens et al., 2002) consists of a non-disintegrating half capsule shell fitted with a hydrogel plug that hydrates and swells at a controlled rate on contact with gastrointestinal fluid, ejecting from the capsule body and releasing the contents after a pre-determined lag time of 5 hours, when the dosage form is assumed to have arrived in the colon. The TimeClock® system (Pozzi et al., 1994; Sangalli et al., 2004) works on a similar premise, and comprises a conventional tablet coated with a mixture of high viscosity HPMC and surfactant applied as an aqueous dispersion, which swells and erodes releasing drug after a lag time determined primarily by coating thickness. To overcome the variability in site of drug release of both Pulsincap® and TimeClock®, the application of an enteric coat is required so that swelling/erosion can only commence post gastric emptying. However, even after such a modification, the performance of time
controlled colon delivery systems is poor; although the mean small intestinal transit time of formulations is in the range 3-4 hours, individual values can vary from 1 to 9.5 hours (Davis et al., 1986a) which results in considerable variation in the site of drug release. Furthermore, dispersion of the drug, when it does occur in the distal gut is often limited due to the viscous luminal contents, a problem that is accentuated by the single unit design of the time controlled delivery systems.

**Pressure-responsive delivery**

Despite the fact that little is known about the luminal pressure in different compartments of the gastrointestinal tract, a pressure-controlled colon delivery capsule (PCDC) has been investigated (Takaya et al., 1995; Hu et al., 1998). The PCDC consists of drug suspended in a capsule-shaped portion of suppository base, coated with the hydrophobic polymer ethylcellulose. Once swallowed, the suppository base melts but is retained by the water-insoluble ethylcellulose shell. The device is designed to remain intact in the small bowel, but to rupture under the influence of the more viscous luminal contents and haustral contractions encountered in the colon. However, the *in vivo* targeting potential of the PCDC is yet to be evaluated using a reliable imaging method. Furthermore, the pressure-sensitive colon delivery technology has only been applied to single-unit dosage forms at present.

**Bacteria-responsive delivery**

The bacterial count in the colon is approximately 10 million times higher in the colon than in the proximal gastrointestinal tract. Over 400 different species are present, being mostly anaerobic in nature, and serve to metabolise a wide range of endogenous and exogenous substrates that escape digestion in the upper gastrointestinal tract (Basit, 2005).

The manipulation of bacteria to deliver a therapeutic agent selectively to the colon was first demonstrated in 1972, when the pro-drug sulphasalazine was shown to undergo cleavage at the azo-bond, releasing the anti-inflammatory agent mesalazine, which would otherwise be absorbed from the small intestine, and the carrier molecule sulphapyridine (Peppercorn and Goldman, 1972).
Saffran et al., (1986) were first to investigate the possibility of developing a universal bacterial activated method using synthetic polymers cross-linked with azo-aromatic groups that could be used as a coating material to deliver practically any drug to the colonic region. Although encouraging results were observed in rats, there are concerns as to the safety of synthetic azo-compounds (Van den Mooter et al., 1997), and this has led researchers to investigate alternative substrates for the colonic bacterial enzymes.

Research has focussed on the potential of naturally occurring polysaccharides, particularly those that form part of the human diet or are already utilised as pharmaceutical excipients, and include pectin, chitosan, guar gum, locust bean gum, and amylose (Sinha and Kumria, 2001). Naturally occurring polysaccharides are generally hydrophilic in nature, and to prevent swelling and premature drug release in the upper gut must be either cross-linked or combined with a water-insoluble polymer. Few bacterial-activated colonic delivery systems have progressed further than the bench, only a few have shown promising results in vivo, but none have so far made it onto the market.

**pH-responsive delivery**

Luminal pH is known to increase aborally along the small intestine; being approximately 6.6 in the proximal small bowel and 7.5 in the distal small bowel (Evans et al., 1988). The possibility of using a pH-sensitive polymer to target drugs to the colonic region was first investigated by Dew et al., (1982) using capsules coated with Eudragit S (Rohm Pharma), a polymer which differs from the enteric polymer Eudragit L only in the ratio of carboxylic acid to ester monomer groups, and therefore dissolves at the higher pH of 7.0. Other pH-sensitive polymers evaluated for the potential to deliver drugs to the colonic region include Eudragit FS (Rohm), a polymer with a similar dissolution threshold pH to Eudragit S, but which dissolves at a slower rate, and hydroxypropyl methylcellulose acetate succinate H (Aqoat AS-H, Shin-Etsu), which is soluble at pH>6.8.

Using the pH change along the gastrointestinal tract to target the colonic region is less straightforward than using pH as a trigger factor to target the small intestine, for reasons which will be described later (see section 1.4.2). However, the pH approach remains the only universal method by which drugs are targeted to the colonic region, and a number of products are currently marketed (Asacol MR®, Mesren MR®, Ipocol®) for the treatment of
the inflammatory bowel diseases which affect this region of the gastrointestinal tract (Basit, 2005).

1.3 Gastrointestinal anatomy and physiology with relevance to drug delivery and absorption

The gastrointestinal tract is often simplistically described as a hollow muscular tube, however the anatomy and physiology of the different organs from which it is constituted differ along its length to achieve three specific functions; the digestion of food, absorption of nutrients into the body proper, and removal of waste material.

The basic structure of the wall of the gastrointestinal tract is similar from the mid oesophagus distally. The mucosa of the gastrointestinal tract comprises a single layer of epithelial cells which form the luminal surface, underneath which lies the lamina propria and muscularis mucosa. Beneath the mucosa, lie concentric layers of submucosa, muscularis externa and serosa. Structural differences exist in the histology of the mucosal layer, described later, which are adapted to fulfil the functions of the different regions of the gastrointestinal tract. The structure of the gastrointestinal tract is shown in Figure 1.1.
Figure 1.1: Diagram of the human gastrointestinal tract
1.3.1 Stomach

The primary functions of the stomach are to act as a short term reservoir for food, and to deliver ingested material to the small intestine in a form, and at a rate, at which digestion and nutrient absorption can proceed most efficiently. Anatomically, the stomach is arranged into four parts: the fundus, body, antrum and pylorus.

The characteristically low pH of the stomach is maintained by daily secretion of around 1.5 litres of gastric juice - a mixture of mucus, pepsinogen, and hydrochloric acid - from glands located below the epithelial layer. Maintenance of the pH in the region 1.0 to 2.5 provides conditions for the optimum activity of pepsin, while simultaneously inhibiting bacterial growth. The columnar epithelial cells continually secrete mucus (Washington et al., 2001), which forms a permanent functional layer, about 140μm thick, over the gastric mucosa both preventing it from digestion by its own proteolytic enzymes and serving to lubricate, and facilitate movement of, ingested material.

After consumption of a meal, the stomach expands without significant increase in pressure, through relaxation of the fundus, from a volume of approximately 50mL in the fasted state to a maximum volume of around 1.5 litres (Martini, 1995). The mechanical breakdown of ingested material, initiated in the oral cavity, is continued by the powerful grinding action of the antral muscle, which also serves to mix the solid contents with gastric secretions to form a semi-solid mixture known as chyme (although it is important to note that the contents of the stomach are by no means homogeneous). Movement of solid material to the small intestine is regulated by the pylorus which releases about 1-5mL of chyme once or twice per minute (King et al., 1984) selectively retaining particles above a threshold diameter of 0.5-2mm for further reduction in size (Malagelada, 1977). This occurs via simultaneous contraction of the pylorus and antrum, which effectively homogenises the digestible material, and coincides with retropulsion of other solid particles which fail to pass through the pylorus, thereby subjecting the solid material to shear forces which further assists particle size reduction (Kelly, 1980). In contrast to solid material, the movement of non-calorific liquids is not regulated by the pylorus, and
these empty freely at an exponential rate determined by the pressure difference between the stomach and duodenum (Kelly, 1980; Washington et al., 2001).

After the last of the digestible material has left the stomach, the interdigestive phase of motor activity commences, known as the Migrating Myoelectric Complex, or MMC (Szurszewski, 1969). The MMC describes a series of contractions of varying intensity, usually initiating in the stomach and which can propagate, on occasion, as far as the ileum, which clear the stomach of the remaining indigestible material through a fully relaxed pyloric sphincter. The MMC repeats approximately every 2 hours and is divided into four phases. Phase I is a period of no activity in the stomach and small intestine. During phase II mixing contractions escalate, while in phase III, powerful peristaltic contractions remove residual debris from the stomach. Phase IV is a period of activity intermediate between phases I and III. The MMC has been termed the “interdigestive housekeeper”, and is only interrupted when food is eaten.

The thick mucus barrier which protects the gastric mucosa, considered alongside the relatively modest surface area of the stomach (approximately 1m²), dictate that the stomach is not a major site for drug absorption. Therefore, for the majority of formulations, gastric emptying is a rate limiting step to drug absorption, which considering the way the stomach handles ingested food, would be expected to differ for solutions, non-disintegrating multiple unit formulations, and non-disintegrating single unit formulations. Drug solutions (e.g. effervescent tablets and sachets) would be expected to empty freely from the stomach and achieve a rapid onset of drug action. Non-disintegrating pellets of size 1-1.5mm are small enough to pass through the pylorus (Malagelada, 1977) and would be expected to mix with the digestible material, emptying during the digestive phase of motor activity. However, single unit dosage forms would be expected to be retained during the fed state as they are too large to empty through the pylorus, and only empty with other indigestible debris with phase III contractions of the MMC.
1.3.2 Small intestine

The small intestine is the longest organ of the gastrointestinal tract, and is arbitrarily subdivided into duodenum (0.25m), jejunum (2.8m) and ileum (4.2m), although there are no clear anatomical divisions between the different regions. Small intestinal diameter is approximately 3-5cm (Kararli, 1995; Watts and Illum, 1997).

Although the function of the small intestine is also to continue to mix the chyme with intestinal secretions and transport material in an aboral direction, its overriding role is the absorption of nutrients. The small intestine has a large surface area for nutrient absorption (estimated to be $200\text{m}^2$), being thrown into a number of deep folds known as the plicae circulares, or folds of Kerckring. Absorptive surface area is increased further by a series of finger-like protrusions, termed villi, extending into the lumen of the small intestine, covered by a single layer of columnar cells, which in turn are covered by microvilli. The absorptive capacity of the proximal small intestine is greater than distal regions due to more numerous and prominent plicae circulares and longer villi (Aiache and Aiache, 1985), which is of importance for the delivery of drugs as enteric-coated formulations. Within the lamina propria core of each villus are located an arteriole, venule and lymphatic vessel termed a lacteal, which allows efficient absorption of digested foodstuffs which drain from the capillary bed of the villus into the portal vein.

Although columnar absorptive cells dominate the mucosal surface of the small bowel, the Crypts of Lieberkühn, located between adjacent villi, contain a greater diversity of cells responsible for epithelial renewal, hormone and peptide secretion, as well as secretion of basic mucus. In combination with the secretion of alkaline pancreatic juice containing buffering ions and of bicarbonate ions from the Brunner’s glands into the duodenum, the pH of the acidic chyme passing from the stomach is rapidly neutralised, thus preventing mucosal damage by direct neutralisation of hydrochloric acid and through inactivation of pepsin (Washington et al., 2001). The pH of the luminal contents increases along the length of the small intestine from around 5.5 to 6 in the duodenum, to 7 in the jejunum and approximately 7.5 in the ileum (Evans et al., 1988).
Fed state motility of the small intestine is a combination of segmental and peristaltic contractions. Transfer of chyme from the stomach to small intestine stimulates segmental contractions which involve adjacent segments of the small intestine relaxing and contracting, providing mixing of the chyme and increasing nutrient absorption through contact with the mucosal layer. An increased preponderance of segmental contractions in the upper gut achieves the net effect of moving chyme in an aboral direction, which is facilitated by peristaltic contractions. In the fasted state, the contractile activity of the small intestine is determined by the MMC which serves to remove unabsorbed material from the small intestine. The velocity of the peristaltic waves diminish as they approach the ileum, indicating that the transit of undigestible material which is emptied from the stomach with phase III MMC contractions is more rapid in the proximal small intestine (Kellow et al., 1986). Rapid small intestinal transit associated with phase III MMC contractions may have implications for the *in vivo* performance of enteric coated dosage forms.

Between the small and large intestine lies a region called the ileo-caecal junction (ICJ) which acts as a barrier for the passage of bacteria from the colon into the small intestine. Transit through the ICJ is not controlled by the MMC, as contractions rarely propagate to this region (Quigley et al., 1984). Aiache and Aiache (1985) suggested that the junction opens prior to each peristaltic wave, releasing approximately 2mL of chyme into the large intestine. Prolonged stasis of dosage forms has been reported at the ileo-caecal junction (Khosla and Davis, 1989; Ashford et al., 1993) which has implications for the drug delivery to the large intestine.

While it is acknowledged that active and facilitated mechanisms of drug absorption exist, particularly for molecules with close structural similarity to vitamins, amino acids or monosaccharides, the vast majority of drugs are absorbed passively due to the concentration gradient that exists across the epithelial monolayer (Mayersohn, 1996). Low molecular weight hydrophilic molecules are more likely to diffuse through the tight junctions between adjacent epithelial cells (paracellular absorption), while unionised
lipophilic molecules will more readily dissolve in the lipidic bilayer of the cell membrane, passing through individual enterocytes (transcellular absorption).

The attributes of the small intestine which facilitate the efficient absorption of nutrients also render the small intestine to be the optimal site for the absorption of drugs. Schiller et al., (2005) reported that there is a greater volume of free fluid available in the small than large intestine (105±72mL versus 13±12mL), which should improve the chances of drug dissolution in the luminal contents, a prerequisite for absorption. Furthermore, the large epithelial surface area and rich vasculature of the small intestine facilitate rapid drug absorption and removal from the site of absorption into the systemic circulation, thus maintaining the concentration gradient and driving further absorption.

1.3.3 Large Intestine

The large intestine is anatomically distinct from the small intestine and is subdivided into the caecum, colon, rectum and anal canal. The colon can be further subdivided into ascending (20cm), transverse (45cm), descending (30cm) and sigmoid (40cm) portions.

The primary functions of the colon are the reabsorption of water and electrolytes, and the formation and temporary storage of faecal material. During colonic transit, the luminal contents increase in viscosity due to the reabsorption of water, most of which occurs from the ascending colon. Daily, around 1.5-2 litres of chyme enter the colon compared to approximately 200mL which is excreted as faeces, indicating that water absorption from the colon is indeed an efficient process (Washington et al., 2001).

The average diameter of the colonic lumen is approximately 6cm, being slightly larger than the small intestine (Watts and Illum, 1997). The colonic wall is thinner than the small intestine, and organised as a series of haustra (or pouches), which are more pronounced in the proximal colon, the size and shape of which vary with the contractile activity of the colon. In common with the stomach and small intestine, colonic motility is both peristaltic and segmental. Segmental contractions result in localised agitation of luminal contents, while antiperistaltic contractions result in retrograde movement of the
luminal contents, both of which provide further mixing of the luminal contents and opportunity for water reabsorption. Mass movements of luminal contents also occur in the colon and are induced by prolonged peristaltic contractions. Mass movements occur on average three to four times daily, and serve to move the luminal contents of the distal colon into the rectum, which produces a conscious urge to defaecate. An increase in colonic motility, known as the gastro-colonic reflex, occurs after eating but rather than propelling colonic contents in an aboral direction usually results in mixing of colonic contents (Jian et al., 1984; Bohemen et al., 1989).

As has been mentioned in section 1.2.2.3.2, the colon plays host to a vast and diverse bacterial population not observed in the upper gastrointestinal tract. The bacterial microflora of the colon play an important role in the fermentation of dietary fibre, resistant starches and dietary carbohydrates that escape digestion in the small bowel, and have been estimated to have a metabolic capacity equal to, or greater than, that of the liver (Scheline, 1973). The end products of this fermentation are short chain fatty acids (SCFAs), primarily acetate, propionate and butyrate, which are absorbed and used by the colonocytes, liver or peripheral tissues (Wong et al., 2006). Butyrate is thought to play a key role in colonic health and the prevention of colon cancer. Evans et al., (1988) was the first to demonstrate a fall in luminal pH from the terminal ileum (pH 7.5±0.4) to caecum (pH 6.4±0.4), which is due to SCFA production. Thereafter an increase in pH occurs, the pH of the luminal contents rising to 7.0±0.7 in the sigmoid colon.

For the majority of orally administered drugs, absorption is likely to be complete from the small intestine. However, for drugs which are administered as delayed or extended release formulations, or that are poorly soluble or permeable, the extent of drug absorption from the colon should also be considered. The colon is a poor site of drug absorption compared to the small intestine as it has a comparatively low surface area due to its shorter length and absence of villi. The limited fluid content in this region poses problems for the dissolution of lipophilic drugs, while hydrophilic drugs are less likely to be absorbed by the paracellular route than in the small intestine due to “tighter” tight junctions in the colon (Chadwick et al., 1977; Hayton, 1980). Nonetheless, the extended
residence time when compared to other compartments of the gastrointestinal tract, results in absorption of a number of drugs including theophylline, ibuprofen, nifedipine, oxprenolol, metoprolol, diclofenac and pseudoephedrine (Fara et al., 1989).

1.4 Relationship between modified release dosage form design and gastrointestinal physiology: therapeutic implications

The in vivo performance of modified release dosage forms is dictated by their interaction with the heterogeneous environment of the gastrointestinal tract, and a number of interrelated physiological variables are thought to influence the site, rate and extent of drug release, which in turn impacts drug absorption. Our understanding of the in vivo behaviour of modified release dosage forms has been largely elucidated through the application of gamma scintigraphy; a non-invasive nuclear medicine technique which allows the gastrointestinal transit of radiolabelled formulations to be monitored using a gamma camera. Gamma scintigraphy is particularly useful when combined with pharmacokinetic analysis, enabling the correlation of transit, and dosage form disintegration (where appropriate), with the appearance of drug in the blood (Digenis et al., 1998a,b; Wilding et al., 2001).

This section describes the manner in which the three most important physiological variables – gastrointestinal transit, pH and fluid volume – can impact the performance of conventional modified release dosage forms with an emphasis on drug delivery to the proximal small intestine and ileo-colonic region.

1.4.1 Gastrointestinal transit

1.4.1.1 Gastric emptying

As a comparator for the gastric emptying of modified release formulations, it is useful to define a gastric emptying time of water, which empties at an exponential rate according to the pressure gradient between the stomach and duodenum (Washington et al., 2001). In fasted subjects, the time for 50% gastric emptying (GE50) of 200mL of radiolabelled water was determined to be 10±2 minutes (Wilding et al., 1994), whereas in subjects who consumed a mixed meal consisting of a soft drink, and scrambled egg (radiolabelled with
different markers), half of the liquid content had emptied from the stomach after 30±2 minutes (Feldman et al., 1984).

1.4.1.1 Gastric emptying of modified release dosage forms from the fasted stomach

A summary of the gastric emptying of non-disintegrating single and multiple unit dosage forms of different sizes, from the fasted stomach, is given in Table 1.2. In the fasted state, gastric emptying of single unit dosage forms usually occurs after a mean time of between 0 and 120 minutes (Davis et al., 1986a; Wilding et al., 1992b, 1993; Cole et al., 2002; Tuleu et al., 2002; Ofori-Kwakye et al., 2004; Ibekwe et al., 2006) which supports the assumption that emptying coincides with phase III MMC contractions, which repeat approximately every 2 hours in the fasted state. However, on occasion (Ashford et al., 1993b), non-disintegrating tablets can remain in the stomach for prolonged periods, which could be due to tablets becoming trapped in the less muscular body of the stomach and not being propelled to the antrum from where emptying would occur (Coupe et al., 1991a), or possibly that the MMC had commenced distally to the stomach in some subjects (Kellow et al., 1986).

The random nature of gastric emptying was perhaps best demonstrated by Khosla and Davis (1989). The gastrointestinal transit of five non-disintegrating 5mm tablets, given simultaneously to five fasted volunteers, was evaluated on three consecutive days and was calculated to be 25±9 minutes, 30±13 minutes and 113±70 minutes, exhibiting large inter-day variability. However, multiple tablets were often witnessed to empty as a bolus, which adds strength to the theory that emptying of non-disintegrating dosage forms is under the influence of phase III MMC contractions.

The GE50 times for pellet formulations of size 0.5-1.7mm were found to be in the range 48-186 minutes, broadly similar to the single unit formulations tested. Clarke et al., (1995) found that the emptying of pellets was unaffected by density; the GE50 of 1.5, 2.0 and 2.4g/cm³ pellets was reported to be 185±128, 186±66 and 168±91 minutes respectively, however this was slower than the gastric emptying of similar formulations.
evaluated by other authors (Khosla and Davis, 1987; Yuen et al., 1993; Basit et al., 2004). The smallest pellets evaluated (0.5mm) emptied with a GE\textsubscript{50} of 133±54 mins, which was not significantly different to emptying of 4.75mm pellets (Clarke et al., 1993). Therefore it can be concluded that, contrary to popular belief, pellet formulations do not empty from the stomach with the coadministered water, and if there is a cut-off size for rapid gastric emptying, this is likely to be below 0.5mm.
Table 1.2: Influence of size on the gastric emptying of non-disintegrating dosage forms in fasted subjects

<table>
<thead>
<tr>
<th>Study</th>
<th>Dosage form</th>
<th>GE (mean ± SD in mins)</th>
<th>GE (range in mins)</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tuleu et al., (2002)</td>
<td>Size 0 (21-22mm) coated HPMC capsule</td>
<td>49±30</td>
<td>6-90</td>
<td></td>
</tr>
<tr>
<td>Cole et al., (2002)</td>
<td>Size 0 (21-22mm) coated HPMC capsule</td>
<td>72±54</td>
<td>12-174</td>
<td>Data for Eudragit L55 and FS coated capsules combined</td>
</tr>
<tr>
<td>Davis et al., (1986b)</td>
<td>17x4mm tablet</td>
<td>48±24</td>
<td>25-96</td>
<td>Tablets for young and old combined as no sig. diff. between GE times</td>
</tr>
<tr>
<td>Wilding et al., (1993)</td>
<td>16x7mm tablet</td>
<td>58±57</td>
<td>5-216</td>
<td></td>
</tr>
<tr>
<td>Wilding et al., (1992)</td>
<td>16x7mm tablet</td>
<td>47±51</td>
<td>6-162</td>
<td></td>
</tr>
<tr>
<td>Ofori-Kwakye et al., (2004)</td>
<td>13x7mm tablet</td>
<td>62±17</td>
<td>38-83</td>
<td></td>
</tr>
<tr>
<td>Ashford et al., (1993b)</td>
<td>10mm tablet</td>
<td>144±96</td>
<td>78-366</td>
<td>2/7 tablets resist emptying with MMC</td>
</tr>
<tr>
<td>Ibeke et al., (2006)</td>
<td>8mm tablets</td>
<td>31±18</td>
<td>11-67</td>
<td>Data collated for Eudragit S and Eudragit FS coated tablets</td>
</tr>
<tr>
<td>Khosla and Davis, (1989)</td>
<td>5mm tablets</td>
<td>25±9, 30±13 and 113±70 on three consecutive days</td>
<td>GE&lt;sub&gt;50&lt;/sub&gt; times of five 5mm tablets administered simultaneously on three consecutive days</td>
<td></td>
</tr>
<tr>
<td>Authors (Year)</td>
<td>Description</td>
<td>Mean</td>
<td>SD</td>
<td>Range</td>
</tr>
<tr>
<td>---------------</td>
<td>------------------------------------------------------------------------------</td>
<td>------</td>
<td>-----</td>
<td>-------</td>
</tr>
<tr>
<td>Abrahamsson et al. (1993)</td>
<td>HPMC matrix tablet of undisclosed size</td>
<td>36</td>
<td>6-66</td>
<td></td>
</tr>
<tr>
<td>Wilding et al. (2003)</td>
<td>Enteric coated mesalazine tablet (Claversal)</td>
<td>32±22</td>
<td>1-94</td>
<td></td>
</tr>
<tr>
<td>Davis et al. (1990)</td>
<td>Flexin Continus tablet of undisclosed size</td>
<td>32±16</td>
<td>12-60</td>
<td></td>
</tr>
<tr>
<td>Clarke et al. (1993)</td>
<td>0.5mm pellets 4.75mm pellets</td>
<td>133±54</td>
<td>48-237</td>
<td></td>
</tr>
<tr>
<td>Basit et al. (2004)</td>
<td>1.4-1.7mm pellets</td>
<td>48</td>
<td>30-72</td>
<td></td>
</tr>
<tr>
<td>Clarke et al. (1995)</td>
<td>1.18-1.40mm pellets</td>
<td>188±66</td>
<td>79-261</td>
<td></td>
</tr>
<tr>
<td>Khosla and Davis (1987)</td>
<td>0.5-1.0mm pellets</td>
<td>66±14</td>
<td>47-80</td>
<td></td>
</tr>
<tr>
<td>Yuen et al. (1993)</td>
<td>Pellets of undisclosed size</td>
<td>70±59</td>
<td>8-154</td>
<td></td>
</tr>
<tr>
<td>Wilding et al. (2003)</td>
<td>Mesalazine pellets of undisclosed size</td>
<td>66±40</td>
<td>22-157</td>
<td></td>
</tr>
</tbody>
</table>
1.4.1.1.2 Gastric emptying of modified release dosage forms from the fed stomach

Consumption of a standard breakfast prior to administration of a 17.8mm x 6.4mm enteric coated tablet increased gastric residence time from 41±20 minutes to 276±147 minutes (Ishibashi et al., 1998). Similar results have been obtained in other studies (Abrahamsson et al., 1993; Wilding et al., 1995). However, although it is accepted that the presence of food in the stomach delays gastric emptying of non-disintegrating formulations, the magnitude of the effect for meals and dosage forms of different sizes is not as well understood. Davis et al., (1984) showed that a 3600kJ breakfast delayed the gastric emptying of both a 0.7-1.2mm pellet formulation and an osmotic pump device (Osmet®) to a greater extent than a 1500kJ meal. The effect was more pronounced on the single unit than multiple unit formulation, which failed to empty from the stomach in any of the six volunteers who had consumed the 3600kJ after a period of 10 hours. However, the same authors reported no significant difference in the gastric emptying of two 12mm tablets administered after a light (1500kJ) and full (3000kJ) breakfast (Davis et al., 1988). The results were also contradicted by Coupe et al., (1991b), who observed the gastric emptying of a non-disintegrating 11.5mm ethylcellulose tablet and 0.8-1.1mm pellets to be 90±35 minutes and 105±45 minutes, respectively, after a light breakfast. In all of the subjects, the tablet emptied from the stomach before all of the multiple units had left the stomach. It was also found that intra-subject variability of gastric emptying was less than inter-subject variability for both dosage forms.

Coupe et al., (1991a), attempted to prove the hypothesis that non-disintegrating single unit dosage forms empty after food, with phase III MMC contractions, by administering five 7mm ethylcellulose tablets along with a radiotelemetry capsule, capable of monitoring the contractile activity of the stomach, after a radiolabelled 1200 kJ breakfast. The emptying of the meal, the 7mm tablets and the radiotelemetry capsule was observed by gamma scintigraphy. In all but one of the volunteers, emptying of some the 7mm tablets occurred due to fed state contractile activity, while all of the ethylcellulose tablets emptied with the food in four of the eight volunteers. Gastric emptying of the 7mm tablets with food is attributed to the phenomenon of fortuitous emptying, whereby it is postulated tablets become trapped in the terminal antrum and are forced through the
partially occluded pylorus by the next contraction (Khosla et al., 1989). Gastric emptying of any remaining tablets occurred with phase II MMC contractions. In all eight volunteers, the radiotelemetry capsule emptied after the food, with activity associated with phase III MMC contractions, although on two occasions the radiotelemetry capsule resisted emptying through at least one series of housekeeper wave contractions, possibly remaining in the less muscular body of the stomach.

Coupe et al., (1993) performed a similar study to investigate the emptying of 0.8-1.1mm pellets, a radiotelemetry capsule and 1200kJ meal. Gastric emptying data was plotted individually for each of the volunteers. Surprisingly, in only two of the eight volunteers did pellet emptying mirror that of the meal, in the remaining subjects the pellets emptied as a bolus, following a lag time in which a large proportion of the meal had already emptied into the small intestine. The signal from the radiotelemetry capsule suggested that the pellets were largely emptied towards the end of the digestive phase, or by phase II and III contractions of the MMC. In all instances, the radiotelemetry capsule emptied from the stomach after all of the food and pellets. The results of this study seem to contradict the widely held belief that pellets mix intimately with, and empty with, the solid content of the stomach.

It is apparent from the data available relating to the gastric emptying of non-disintegrating dosage forms, that it is difficult to predict with a large degree of certainty how long it will take for a dosage form to pass into the small intestine. The two major factors determining gastric emptying appear to be dosage form size and fed state, although on occasion large dosage forms can empty from the fed stomach, and conversely can also resist emptying through one or more series of phase III MMC contractions. Acknowledging that other factors including density, exercise (Ramsbottom and Hunt, 1974), stress (Kaus and Fell, 1984) and body posture will have an influence on gastric emptying, it is clear that the larger the size of a dosage form, the more unpredictable gastric emptying, and therefore the onset of drug action will be.
1.4.1.2 Small intestinal transit of modified release dosage forms

Davis et al., (1986a), compared small intestinal transit data of different formulations that had been evaluated in 201 volunteers (representing 23 studies on solutions, 82 on pellets and 96 on single units). No statistical difference was found between the mean small intestinal transit times of solutions, pellets or single units, the values for which are summarised in Figure 1.2.

This study is, still today, widely cited to describe the consistency of small intestinal transit time in the range 3±1 hours, however the mean data should be interpreted with caution as it masks individual transit times considerably faster and slower than 2-4 hours (see Figure 1.3). Individual small intestinal transit times as rapid as 1 hour were observed, which have also been reported in volunteers in more recent studies (Khosla and Davis, 1989; Khosla et al., 1989; Sugito et al., 1990; Davis et al., 1990; Ashford et al., 1993b; Wilding et al., 1994) for formulations administered to fasted volunteers or after food.
It is likely that this "rapid" transit is occurring as a result of the dosage form being swept along the small intestine by phase III MMC contractions. For enteric coated dosage forms the implication of this would be that, unless dissolution of the enteric coat in the small intestine was extremely rapid, then the dosage form would be located in the ileocolonic region at the time of disintegration, which could potentially impact bioavailability for drugs not well absorbed from the colonic region. It is also likely that the disintegration of a pH-sensitive colon delivery formulation could also be adversely impacted, as there would be less opportunity for ionisation and dissolution of the pH-sensitive film coat prior to arrival in the colon, where the pH may be insufficiently high to affect drug release from the formulation, which would then be at risk of being voided intact.

The mean values reported by Davis et al., (1986a) also mask "slow" transit times of up to 9.5 hours. It would be expected that drug release from a pH-sensitive colon delivery formulation would occur prematurely in this situation, and be completely absorbed from
the distal small intestine resulting in no drug being available to the colonic region, and therefore ineffective therapy. Ashford et al., (1993b) reported the small intestinal transit time of 10mm Eudragit S coated tablets to be longer than the mean values calculated by Davis et al., (1986a), being 6.5±3.8 hours. The authors proposed that differences in reported small intestinal transit times may be due to differences in interpretation of whether a tablet is located at the ICJ or has travelled into the colon proper.

The mean data reported by Davis et al., (1986a) provided the stimulus for the development of the time controlled colon delivery systems described in section 1.2.2.3.2. However, it can be concluded that, given the extent of inter-individual variability in the data reported by Davis et al., (1986a), the concept of using time as a trigger factor to target the colon is fundamentally flawed.

1.4.1.3 Colonic transit of modified release dosage forms

Although the mean residence time of material in the colon is generally longer than in other compartments of the gastrointestinal tract, transit of dosage forms through the colon is variable, and can be affected by eating, diet and disease (Ashford and Fell, 1994).

In terms of drug delivery for either a local or systemic effect (i.e. drug absorption from the colon), the ascending colon represents the optimal site for dosage form disintegration, as the fluid available for drug dissolution is greater than in more distal regions. Residence times in the proximal colon (ascending and transverse colon) are reported to be between 7 and 14 hours (Metcalf et al., 1987; Parker et al., 1988). Hardy et al., (1985) observed that although a 25 x 9mm tablet and 0.5-1.8mm pellet formulation travelled through the stomach and small intestine together, the capsule moved ahead of the pellets in the ascending colon. This phenomenon of colonic sieving has also been described by other researchers (Adkin et al., 1993; Abrahamsson et al., 1996). In contrast, Watts et al., (1990), found similar residence times for 0.2 and 5mm particles in the ascending colon of healthy volunteers. The more rapid movement of larger objects through the colon has been termed "streaming", and is due to the separation of liquid and solid material which move at different rates (Friend, 1998). Ibekwe et al., (2006) reported the rapid colonic
transit of a Eudragit S coated tablet in one volunteer. The so-called "gastrocolonic response" resulted in transit of the tablet from the ileo-caecal junction to the descending colon within 20 minutes of eating. Despite the variability in colonic transit, it is interesting to note that the colonic transit of non-disintegrating tablets was found to be similar in normal subjects and ulcerative colitis patients (Hardy et al., 1988; Davis et al., 1991).

The effects of variable colonic transit should be considered prior to designing a dosage form intended for either extended release or colonic delivery. Figure 1.4 illustrates how variable colonic transit of a single unit extended release formulation can impact systemic drug levels.

Figure 1.4: Plasma concentration-time profiles for oxprenolol delivered using an OROS® device in an individual with a "short" (top diagram) and "long" (bottom diagram) colon transit time (Washington et al., 2001)
Tuleu et al., (2002) also reported dramatic differences in the performance of a bacterially activated (amylose-ethylcellulose coated) capsule in relation to gastrointestinal transit, which was observed using gamma scintigraphy (Figure 1.5).

![Graph showing plasma concentration-time profiles and transit events](image)

**Figure 1.5:** 4-aminosalicylic acid plasma concentration-time profiles and transit events for volunteers with "normal" (top) and "rapid" (bottom) gastrointestinal transit (adapted from Tuleu et al., 2002)

In the top profile, the formulation has performed as expected, delivering 4-aminosalicylic acid (4-ASA) specifically to the colon, i.e. gastric emptying and colon arrival after 1.5 and 7 hours, respectively, followed by digestion by amylase secreted by colonic bacteria and drug release after 8 hours. However, in the bottom profile, the effect of rapid gastrointestinal transit, primarily colonic transit, results in the dosage form being voided intact. It is of interest to note that, in more recent work, the authors have applied the
amylose-ethylcellulose coat to a pellet formulation in an attempt to avoid rapid colonic transit due to the effects of streaming (Basit et al., 2004; Siew et al., 2004).

1.4.2 Gastrointestinal pH

The work conducted by Evans et al., (1988) remains the most comprehensive study into the pH of different regions of the gastrointestinal tract in 66 healthy adults carrying out normal daily activities, i.e. the pH measurements were recorded under physiological conditions in ambulant patients. A representative gastrointestinal pH profile from a normal subject is shown in Figure 1.6, while the mean data for the 66 volunteers is summarised in Table 1.3.

![Gastrointestinal transit and pH profile](image)

Figure 1.6: Gastrointestinal transit and pH profile for a normal, ambulant human volunteer (adapted from Evans et al., 1988)
Table 1.3: Mean gastrointestinal pH profiles (±SD) in normal ambulant human volunteers (Evans et al., 1988)

<table>
<thead>
<tr>
<th>Site</th>
<th>Mean pH (±SD)</th>
<th>Mean pH</th>
<th>Mean pH (±SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal small intestine</td>
<td>6.10</td>
<td>6.63</td>
<td>7.16</td>
</tr>
<tr>
<td>Mid small intestine</td>
<td>7.05</td>
<td>7.41</td>
<td>7.77</td>
</tr>
<tr>
<td>Distal small intestine</td>
<td>7.03</td>
<td>7.49</td>
<td>7.95</td>
</tr>
<tr>
<td>Right colon</td>
<td>5.79</td>
<td>6.37</td>
<td>6.95</td>
</tr>
<tr>
<td>Mid colon</td>
<td>5.78</td>
<td>6.61</td>
<td>7.44</td>
</tr>
<tr>
<td>Left colon</td>
<td>6.37</td>
<td>7.04</td>
<td>7.71</td>
</tr>
</tbody>
</table>

In healthy subjects, the change in pH across the pyloric sphincter (pH 1.0-2.5 to 6.6±0.6) is larger and more predictable than at any other location in the gastrointestinal tract. The pH of the small intestine is still above the dissolution threshold of commonly used enteric polymers (see Table 1.3) when the pH minus one standard deviation of the mean is considered. If pH alone is considered in isolation from the physiological variables of gastrointestinal transit time and fluid volume, drug delivery to the proximal small intestine can be assumed, incorrectly, to be straightforward.

It is apparent that drug delivery to the colonic region using a pH approach is more complicated than to the small intestine. The pH profile through the small intestine will have a significant influence on the final site of dosage form disintegration. Ashford et al., (1993a) simulated the stomach to colon transit of a Eudragit S coated tablet in vitro, under conditions one standard deviation higher and lower than the mean gastrointestinal pH reported by Evans et al., (1988). The in vitro results predicted that the in vivo site of drug release would vary, however it is interesting to note that when the tablets were tested at pH values one standard deviation lower than the mean, drug release did not occur at distal small intestinal pH after a period of 5 hours.

The predicted variability in site of drug release from Eudragit S coated tablets was confirmed using gamma scintigraphy studies in human volunteers (Ashford et al., 1993b), who observed that tablets disintegrated at sites extending from the ileum to the splenic flexure in the ascending colon. The variability in site of disintegration of Eudragit S
coated tablets was confirmed by Ibekwe et al., (2006), who reported the disintegration position of tablets coated with an aqueous dispersion of Eudragit S varied from the proximal small bowel to distal small bowel, whereas tablets coated with an organic solution of Eudragit S reached the colon on all occasions, but did not always disintegrate. The latter findings confirm the observation that commercial Eudragit S coated formulations marketed for the treatment of inflammatory bowel disease, Asacol MR® and Ipocol®, are, on occasion, seen to be voided intact in ulcerative colitis patients (Schroeder et al., 1987; Sinha et al., 2003). That intact Eudragit S coated formulations are observed in the stools is perhaps unsurprising, given that the colonic pH of inflammatory bowel disease patients is lower than healthy subjects (Fallingborg et al., 1993; Sasaki et al., 1997), which further compromises the therapeutic use of these drug products.

Given the fall in pH in the caecal region, and inter-subject variability in both distal small intestinal and colonic pH, it is evident that targeting the ascending colon – the optimal site for drug delivery in the large intestine – is unlikely to be reproducibly achieved. It is apparent that a more realistic goal would be to achieve drug release from the dosage form in the region of the terminal ileum, from where there would be limited opportunity for drug absorption prior to passage of the drug into the colon. Drug delivery to the ileocolonic region would also be of particular benefit to patients with Crohn’s disease, where inflammation of the mucosa of the terminal small intestine is common, as well as the colon (Butcher, 2003).

Given the variable performance of pH-sensitive single unit systems to target the colon, it is surprising that equivalent multiple unit systems have not so far been investigated. It is anticipated that the in vivo performance of a multiple unit pH-sensitive formulation would be better, given the likely spread of these formulations within the colon, reduced vulnerability to the effects of streaming and increased surface area to volume ratio which should facilitate dissolution of the polymer coat.
1.4.3 Gastrointestinal fluid volume

It has long been understood that the luminal contents of the gastrointestinal tract become increasingly viscous as ingested material is transported aborally, due to water reabsorption. Technological advances such as capsule endoscopy (a capsule containing a miniature camera is swallowed by the patients, and colour digital images of the interior of the gastrointestinal tract to a data recorder worn around the patients waist which can subsequently be downloaded to a computer) have facilitated an increased understanding of the heterogenous composition of luminal contents within different regions of the gastrointestinal tract. Recently, however, an innovative use of magnetic resonance imaging (MRI) has allowed an accurate estimation of the volumes and distribution of fluid within different regions of the gastrointestinal tract (Schiller et al., 2005), and the interaction of non-disintegrating dosage forms with this free fluid.

The investigators administered non-disintegrating capsules of size 16.8mm x 4.6mm, containing molten fat, to fasted volunteers at timepoints 0, 3 and 6 hours with 150mL water. Three, two or one 2mm diameter pellets fabricated from an aqueous gel, were placed inside the capsules to allow discrimination between the capsules administered at different timepoints. One hundred millilitres of water was also administered at 1, 2, 4 and 5 hours. The MRI was performed 7 hours after administration of the first capsule. The study was repeated in the same volunteers, except that during the second leg of the crossover study a meal (3381kJ, 900mL homogenised meal volume) was administered immediately prior to the administration of the final capsule (6 hour timepoint). This allowed the effect of food intake on the mean fluid volume within the gastrointestinal tract to be investigated.

The MRI taken at the 7 hour timepoint allowed not only the location of each of the capsules within the gastrointestinal tract to be ascertained, but crucially whether the capsule was “surrounded by liquid”, “partly surrounded by liquid” or “not in contact with liquid”. The number of fluid pockets within the small and large intestine could also be counted, and an estimation of the volume of each fluid pocket was possible by comparison to the internal calibrators, the volume of cerebrospinal fluid and the gall-
bladder. The volumes of free fluid calculated in each compartment of the gastrointestinal tract in the fed and fasted state is summarised in Table 1.4.

<table>
<thead>
<tr>
<th>GI compartment</th>
<th>Fed free fluid volume (mL)*</th>
<th>Fasted free fluid volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>686±93</td>
<td>45±18</td>
</tr>
<tr>
<td>Small intestine</td>
<td>54±41</td>
<td>105±72</td>
</tr>
<tr>
<td>Large intestine</td>
<td>11±26</td>
<td>13±12</td>
</tr>
</tbody>
</table>

* The volume of the stomach reported in the fed state represents the total volume (not only fluid)

Despite a total of 850mL water being consumed in the 7 hours prior to imaging, the findings suggest that there is a surprisingly limited volume of fluid within the gastrointestinal tract. The free fluid was observed to be distributed as small pockets, the mean number of which was significantly greater in both the small and large intestine (6 versus 4, in both compartments) one hour after consumption of the meal, compared to fasting conditions.

As expected more capsules were present in the stomach of volunteers who had been fed one hour prior to imaging (12 capsules) compared to fasted volunteers (5 capsules). All of the capsules resident in both the fed and fasted stomach, were observed to be "surrounded" by fluid. However, the interaction of capsules with fluid was different and less predictable in both the small and large intestine, and is summarised in Table 1.5.
Table 1.5: Location and interaction of capsules with “free fluid” within different compartments of the gastrointestinal tract in fasted and fed volunteers (adapted from Schiller et al., 2005)

<table>
<thead>
<tr>
<th>Contact with liquid</th>
<th>Fasted volunteers</th>
<th>Fed volunteers</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Small intestine (n=28)</td>
<td>Large intestine (n=3)</td>
</tr>
<tr>
<td>Surrounded</td>
<td>50</td>
<td>0</td>
</tr>
<tr>
<td>Partly surrounded</td>
<td>21</td>
<td>0</td>
</tr>
<tr>
<td>Not in contact</td>
<td>29</td>
<td>100</td>
</tr>
</tbody>
</table>

The data demonstrates that single unit dosage forms can only be predicted with confidence to be in contact with “free fluid” in the stomach. The interaction of single unit dosage forms with free water is progressively less likely in the small intestine and large intestine, due principally to the reabsorption of water from the lumen of the gastrointestinal tract. Given the rapid emptying of water, and the delayed and unpredictable emptying of non-disintegrating dosage forms from the stomach (particularly when administered after a meal), the limited interaction of the capsules and fluid in the small intestine and colon is perhaps to be expected.

Given that the pH of the small intestine is sufficiently high for the dissolution of most enteric polymers, the variable interaction of single unit dosage forms with free fluid in the small intestine is probably largely responsible for the lag time of 1.5 to 2 hours, post gastric emptying, that has been observed before an enteric coated tablet or capsule disintegrates (Hardy et al., 1987). In this time it is possible for the dosage form to have travelled to the distal small intestine or ascending colon (Cole et al., 2002), due to the rapid small intestinal transit which is thought to occur with phase III MMC contractions (Kellow et al., 1986), which could impact the bioavailability of molecules that are not well absorbed from this region. The variable association of single unit formulations with water goes some way to explaining the observation that two identical enteric coated tablets emptied from the stomach at the same time, yet the second tablet disintegrated 54 minutes after the first (Wilding et al., 1992). It is likely that there will also be a variable
interaction between pellets, which have been shown to empty from the stomach after a lag time (Coupe et al., 1993), and free fluid in the small intestine, although given the spread of pellets in the gastrointestinal tract this is anticipated to be less problematic than for single unit formulations.

The data further underlines the difficulty of targeting drugs specifically to the colon, where the volume of "free fluid" is extremely low. The limited free fluid content in this region, which is unlikely to be in contact with a single unit dosage form, considered alongside the fall in pH and variable transit from the terminal ileum to ascending colon, suggest that release of drug specifically in the ascending colon cannot be reproducibly achieved.

Finally, the data also has implications for the in vivo performance of extended release dosage forms, which rely on gastrointestinal fluid for swelling and drug release. The degree of interaction of extended release dosage forms with gastrointestinal free fluid would be expected to impact the rate and extent of drug release from these formulations.

1.4.4 Summary of the key issues for targeting to site-specific regions of the gastrointestinal tract using pH-sensitive dosage forms
Drug therapy using delayed release dosage forms is currently suboptimal due largely to the variable physiology of the gastrointestinal tract. The delayed and unpredictable onset of drug action from delayed release dosage forms is due primarily to variable gastric emptying. Furthermore, an interaction between gastrointestinal transit, pH and free fluid can result in drug release from enteric coated dosage forms in the distal small intestine or even the colon, potentially compromising drug bioavailability. The location of drug release from colon targeted delivery systems appears to be more variable still, with dosage form disintegration reported to occur as prematurely as the proximal small intestine, or not at all.
1.5 Modified release microparticles: a paradigm shift?

It is hypothesised that enteric microparticles (e.g. Eudragit L55 or Eudragit L) of size <100μm may be able to suspend in, and empty with, the co-administered water into the small intestine (particularly if they were administered pre-suspended in the water). Remaining in contact with the "free fluid" in the small intestine would facilitate a rapid dissolution of the pH-sensitive microparticles. Furthermore, as the surface area:volume ratio of a 100μm microparticle is 10 times greater than a 1mm pellet, the dissolution of the microparticle formulation should be further improved compared to the pellet formulation. It is therefore expected that drug would be released at a more proximal location in the small intestine, where drug absorption is most efficient due to more numerous and prominent folds of Kerckring and longer villi.

The formulation of Eudragit S microparticles with a large surface area:volume ratio, which would be unlikely to experience rapid transit due to the effects of streaming, would also improve the chances of drug release to the ileo-colonic region, minimising the possibility of the dosage form being voided intact. A rapid gastric emptying would also be beneficial for colonic delivery, increasing the possibility of a rapid, but perhaps more importantly predictable, onset of drug action from the formulation.

It is not possible to produce modified release particles of size <100μm using conventional pharmaceutical technological approaches (e.g. solution layering, extrusion followed by spheronisation) and therefore over the last 20 years numerous attempts have been made to fabricate pH-sensitive microparticles acrylic microparticles by a number of techniques including emulsification/solvent evaporation, spray drying and coacervation/phase separation. However, success has been limited, and a method has yet to be reported which can produce pH-sensitive Eudragit microparticles of the desired size and morphology, which are capable of adequately controlling the release of a model drug to within pharmacopoeial limits.

The gastrointestinal transit of particles smaller than 0.5mm has been investigated in a small number of studies, both directly and indirectly, and so far the results have been
inconclusive. Beten et al., (1995) reported that the GE$_{50}$ of a radiolabelled coevaporate of Eudragit S of particle size 0.1-0.5mm, administered in a capsule with 150mL water, was 67±16 minutes in the fasted state but 308±53 minutes when administered after food. However, the particle size distribution of this formulation was not well defined, and it is not therefore surprising that the gastric emptying of the particles was similar to the 0.5mm pellets reported by Clarke et al., (1993). Furthermore, as capsule disintegration was observed to take up to 15 minutes, this would occur after a portion of the coadministered water had emptied from the stomach in the fasted state, thereby delaying gastric emptying. In the fed stomach, the capsule may have disintegrated in the mass of food, which may and may have resulted in particle emptying at the same rate as the digestible material.

Brown et al., (1998) investigated the gastrointestinal transit of particles of 500mg quantities of particles of diameter 70-80μm, 1-10μm and 500nm in diameter in fasted volunteers using gamma scintigraphy. The reported GE$_{50}$ times of the three formulations were 47±39, 32±16 and 37±25 minutes, respectively, which is among the most rapid of the gastric emptying values summarised in Table 1.2, although the authors reported no details of the composition of the particles or the method of administration.

Sugito et al., (1992) investigated the delivery of pyridoxal phosphate, in volunteers who had been administered granules of size 100, 500 and 1100μm enteric coated with the polymer hydroxypropyl methyl cellulose phthalate 55 (soluble pH > 5.5). Although gastric emptying was not observed directly, the urinary excretion rate of pyridoxic acid was most rapid in the volunteers who had been administered the smallest formulation, suggesting that gastric emptying and/or enteric coat dissolution was most rapid for the 100μm particles.
1.6 Modified release polymers used in this study

The polymers predominantly used in this study are the pH-sensitive Eudragits, although the water-insoluble polymers Eudragit RS and ethylcellulose N7 are also evaluated for their potential to modulate drug release from pH-sensitive microparticles. The chemical structure, molecular weight and solubility of the polymers used in this study are summarised in Figure 1.7.

![Eudragit L55](image)

- Poly (methacrylic acid, ethyl acrylate) 1:1
- Soluble at pH > 5.5
- Molecular weight 250,000 Da

![Eudragit L/S](image)

- Poly (methacrylic acid, methyl methacrylate) 1:1 (Eudragit L) or 1:2 (Eudragit S)
- Eudragit L soluble pH > 6.0, Eudragit S soluble pH > 7.0
- Molecular weight 135,000 Da

![Eudragit RS](image)

- Poly (ethyl acrylate, methyl methacrylate, trimethylammonioethyl methacrylate chloride) 1:2:0.1
- Water insoluble
- Molecular weight 150,000 Da

![Ethylcellulose N7](image)

- Water insoluble
- Molecular weight 55-65,000 Da

Figure 1.7: Summary of modified release polymers used in this study
1.7 Research scope

The aims of this work are:

- to develop novel methods for the formation of pH-sensitive acrylic microparticles for site-specific drug delivery to proximal small intestine and ileo-colonic region

- to develop and utilise *in vitro* test methods to optimise the drug release characteristics of the pH-sensitive microparticles

- to evaluate the *in vivo* performance of the pH-sensitive microparticles using a suitable animal model
Chapter Two: Development of a novel method to fabricate pH-sensitive polymeric microparticles
2.1 Introduction

Due to a combination of safety and processing considerations, the pH-sensitive Eudragits have gained widespread acceptance as film coating materials in delayed release dosage form manufacture. Being fully synthetic polymers, the pH-sensitive Eudragits exhibit a high degree of purity. They are stable, being less prone to hydrolysis than the semi-synthetic cellulose esters, and due to their high molecular weight (135,000-250,000 Da) they are not absorbed from the gastrointestinal tract. Furthermore, they have good film-forming properties from organic solutions or aqueous dispersions, which make them a suitable choice for film coating of pharmaceutical dosage forms (Pharma Polymers, 2001).

The aim of this study was to develop a method for the production of pH-sensitive acrylic microparticles for site-specific drug delivery to the gastrointestinal tract; Eudragit L55 and Eudragit L were investigated for proximal small intestinal delivery, being soluble above pH 5.5 and 6.0, respectively, and Eudragit S was selected for ileo-colonic targeting, as it is soluble above pH 7.0.

2.1.1 Desirable attributes for a method of microencapsulation

The desirable attributes of a microencapsulation process for the fabrication of microparticles for oral delivery are summarised below:

*Microencapsulation process should yield microparticles <500\text{\mum}*

It has been demonstrated that particles of size >500\text{\mum} are selectively retained by the stomach in both the fed and fasted state (Clarke et al., 1993). Therefore, to achieve a rapid gastric emptying, and onset of drug action, microparticle size must be below 500\text{\mum}.

*Microencapsulation process should achieve a monomodal particle size distribution with a spherical and non-aggregated morphology*

Drug release rate from pH-sensitive microparticles should be determined by the rate of polymer dissolution. As this is dependent on the surface area available for dissolution, particle size will dictate the rate of drug release, and a monomodal particle size distribution is therefore desirable. Spherical, non-aggregated microparticles are also desirable to maximise the surface area available for polymer dissolution and drug release.
Microencapsulation process should utilise non-hazardous solvents

Previously reported methods for the microencapsulation of pH-sensitive Eudragit microparticles require the use of organic solvents to dissolve the polymer prior to microparticle formation. Organic solvents employed in the formulation of pharmaceuticals are not usually completely removed by practical manufacturing techniques, and remain in the dosage form in residual quantities (Witschi and Doelker, 2006). Guidelines have been issued by the International Committee for Harmonisation (ICH) as to the acceptable levels of residual organic solvents in dosage forms which have been set following consideration of the toxicity of the solvent. Solvents have been classified into 3 groups according to the potential side effects of the solvent, which are summarised in Table 2.1.

Table 2.1: Summary of the ICH solvent classification (guidance Q3C (R3))

<table>
<thead>
<tr>
<th>ICH solvent class</th>
<th>Toxic potential</th>
<th>Examples</th>
</tr>
</thead>
<tbody>
<tr>
<td>Class I (solvents to be avoided)</td>
<td>Known or strongly suspected carcinogens, environmental hazards</td>
<td>Benzene, carbon tetrachloride, dichloroethane</td>
</tr>
<tr>
<td>Class II (solvents to be limited)</td>
<td>Non-genotoxic animal carcinogens, neurotoxic or teratogenic agents</td>
<td>Acetonitrile, chloroform, methanol, hexane</td>
</tr>
<tr>
<td>Class III (solvents with low toxic potential)</td>
<td>Low toxic potential in man, daily exposure limit &gt; 50mg</td>
<td>Acetone, ethanol, propanol</td>
</tr>
</tbody>
</table>

Unless it can be demonstrated that solvents used in the microencapsulation process are completely removed from the final dosage form, they should be restricted to those in ICH classes II and III.

Microencapsulation process should be simple and rapid

To date, the extent of scientific investigation in the field of microencapsulation is not reflected by the number of products on the market (Burgess and Hickey, 2002), due largely to the considerable capital investment that is required to produce microparticles on a manufacturing scale. Up-scaling is facilitated by the development of methods of
microencapsulation which avoid the need for control of temperature or homogenisation, which are difficult to achieve on a manufacturing scale.

Method of microencapsulation should produce microparticles which control drug release

Furthermore, the resultant pH-sensitive microparticles must be able to control drug release in a manner dictated by the functionality of the pH-sensitive polymers from which they are fabricated.

2.1.2 Literature methods for the fabrication of pH-sensitive acrylic microparticles

Research into the feasibility of producing Eudragit L and S microparticles for oral drug delivery has been undertaken over the last 20 years, and has been driven by an understanding of the theoretical advantages of microparticulate over conventional modified release dosage forms, and the safety and functionality of these polymers. However, to date, a method for the formulation of pH-sensitive Eudragit microparticles has yet to be described which is capable of meeting the criteria listed in section 2.1.1 for a pharmaceutically acceptable microparticulate dosage form.

Spray drying approaches (Palmieri et al., 2002a,b) result in aggregated particles which must be tableted to achieve the possibility of control of drug release in acidic conditions. Emulsification/solvent evaporation approaches often fail to control drug release at acidic pH (Morishita et al., 1991; Obeidat and Price, 2006), utilise ICH class II solvents in the manufacturing process (Esposito et al., 1996; Alavi et al., 2002; Squillante et al., 2003; Lamprecht et al., 2003; Obeidat and Price, 2006), require homogenisation (Alavi et al., 2002; Squillante et al., 2003) or careful control of temperature to achieve emulsion stability (Goto et al., 1986; Alavi et al., 2002; Squillante et al., 2003; Obeidat and Price, 2006) or fail to produce microparticles of size < 500μm (Goto et al., 1986). Furthermore, few of the papers give a representative indication of the morphology of the microparticles produced (scanning electron microscopy images show only a small number of individual microparticles), and the majority of studies in which dissolution data is reported evaluate the in vitro release of acidic model drugs which will assist in the achievement of pH-sensitive release when tested at acidic and neutral pH.
2.1.3 Methods of microencapsulation used in this study

In this study, a number of methods of microencapsulation were investigated for the production of pH-sensitive acrylic microparticles. A simple, non-aqueous coacervation method was investigated, which resulted in the formation of spherical coacervate droplets of Eudragit L, of size < 2μm. However, solidified microparticles could not be harvested as attempts to fully remove the coacervate phase solvent resulted in agglomeration and phase separation. Furthermore, spray drying of organic polymer solutions of Eudragit L55, L and S resulted in collapsed microparticles of size < 50μm, which failed to control the release of a model drug, prednisolone, at acidic pH. The results of the coacervation and spray drying experiments can be found in Appendix One. In this chapter, the development of the emulsification/solvent evaporation method which ultimately led to the formulation of Eudragit L55, L and S microparticles with the desired attributes is described.
2.1.3.1 Emulsification/solvent evaporation

The emulsification/solvent evaporation process is shown diagrammatically in Figure 2.1.

The emulsification/solvent evaporation method is a conceptually simple process, and as the name suggests, is essentially a two step process which involves (i) the formation of an emulsion of microdroplets of drug and polymer solution into a non-solvent phase, (ii) solvent removal to produce solidified microparticles (Watts et al., 1990; O'Donnell and McGinity, 1997; Freitas et al., 2005).

The primary emulsion formed in the emulsification/solvent evaporation technique is generally termed oil-in-water (o/w) or oil-in-oil (o/o), where the internal “oil” phase is actually a solution of polymer dissolved in a suitable organic solvent (which also serves to disperse, or preferably dissolve, the drug). Where the internal phase solvent is water-immiscible (for example chloroform or dichloromethane), an aqueous external phase is
chosen (o/w emulsion) which has the advantage that microparticle harvest and clean-up is easy. When the internal phase solvent is miscible with water, a hydrophobic external phase (o/o emulsion), commonly liquid paraffin, is selected which has the disadvantage that it must be removed from the surface of the harvested microparticles using organic solvents such as hexane (Watts et al., 1990).

The droplet formation step follows the mixing of two immiscible phases which can be achieved by stirring, homogenisation or static mixing (Freitas et al., 2005), and determines the final size and size distribution of the final microparticles (Watts et al., 1990; O'Donnell and McGinity, 1997; Perumal, 2001; Freitas et al., 2005). However, the size of the final microparticles will also be influenced by the concentration of the internal phase polymer solution (Morishita et al., 1991, O'Donnell and McGinity, 1997, Freitas et al., 2005), or the addition of baffles to the walls of the reaction vessel (Perumal, 2001). An emulsifying agent is added to the emulsion to improve the stability of the emulsion, however, this is only a short-term requirement as once adequate solvent evaporation has occurred to produce some hardening of the drug-polymer droplets, coalescence and aggregation should not occur (Watts et al., 1990).

Once droplet formation is achieved, a large surface area is available for diffusion of the internal phase solvent into the external (non-solvent) phase, which evaporates from the surface of the external phase driving the diffusion of further solvent. The volatile nature of the organic solvents employed in the process facilitates the rate at which they evaporate following diffusion into the external phase, and can further be increased by increasing the temperature of the emulsion or reducing the pressure of the atmosphere surrounding the reaction vessel (Freitas et al., 2005). The rate at which solvent evaporation proceeds can determine the final properties of the solidified microparticles, for example a rapid solvent evaporation may improve the entrapment efficiency of a drug which rapidly partitions into the external phase, while a slow evaporation generally produces a denser, less porous matrix, which may affect the drug release (Freitas et al., 2005).

Watts et al., (1990) proposed some important criteria to be considered in the selection of both the polymer solvent and non-solvent phases:
Selection of internal phase solvent:
1. Ability to dissolve chosen polymer,
2. Ideally, the solvent should be able to dissolve the chosen drug, but must at least be able to suspend it,
3. Immiscibility with the continuous phase,
4. Lower boiling point than continuous phase,
5. Low toxicity.

Selection of continuous phase:
1. Immiscibility with dispersed phase,
2. Inability to dissolve polymer,
3. Low solubility toward drug,
4. Higher boiling point than dispersed phase solvent,
5. Low toxicity,
6. Should allow easy clean up and recovery of microparticles.

The final structure and composition of the microparticles produced using the emulsification/solvent evaporation method will be determined by a complex interplay between the polymer, drug, solvent, continuous phase and emulsifying agent (Watts et al., 1990).

2.1.3.2 Choice of initial emulsification/solvent evaporation method for the fabrication of pH-sensitive Eudragit microparticles

In this chapter, we investigate the fabrication of microparticles of pH-sensitive Eudragits using the emulsification/solvent evaporation technique as described in the literature by Morishita et al., (1991) and Lorenzo-Lamosa et al., (1997). Initially, it was attempted to modify the work of Morishita et al., (1991), who reported an emulsification/solvent evaporation method using an aqueous solution of gelatin as the emulsifying agent. Microencapsulation of erythromycin using Eudragit L was investigated as a means of preventing inactivation of drug by gastric acid, while improving the release rate at intestinal pH. The method described by Morishita et al., (1991) was chosen as it fulfils several of the desirable attributes for a method of microencapsulation listed in section 2.1.1; using the relatively non-toxic (ICH class III) solvent ethanol, and liquid paraffin as the internal phase
solvent and non-solvent phase, respectively. Furthermore, it is claimed that the technique requires only the use of magnetic stirring for the emulsification step, and does not require elaborate control of temperature.

The aims of our preliminary experiments using the Morishita method were to maximise the percentage of Eudragit L microparticles in the $\leq 500\mu m$ size fraction, and to assess the potential of the resultant microspheres to control the release of a model drug, prednisolone, at acidic (gastric) pH while releasing it rapidly at (intestinal) pH 6.8.

2.2 Materials

Eudragit L100-55, Eudragit L100 and Eudragit S100 were obtained from Rohm Pharma (Darmstadt, Germany). Micronised prednisolone was purchased from Sanofi-Aventis (Romainville, France). Arlacel 83, Brij 52, Brij 92, oleic acid, Span 20, Span 80 and Span 85 were purchased from Sigma Aldrich, (Poole, UK). All other reagents were of analytical grade and were used as received.

2.2.1 Rationale for choice of prednisolone as a model drug

The chemical structure of prednisolone is shown in Figure 2.2.

![Chemical structure of prednisolone](image)

*Figure 2.2: Chemical structure of prednisolone*

Prednisolone is a white, hygroscopic, crystalline powder with a molecular weight of 360.4 Da. According to the British Pharmacopoeia (2007) it has a solubility of 1 in 1300 in water and 1 in 30 in ethanol.
Prednisolone belongs to the class of compounds known as the glucocorticoids which have a number of therapeutic indications including the treatment of chronic inflammatory conditions, which include the treatment of acute exacerbations of asthma (administered as enteric coated tablets to prevent gastric ulceration) and the treatment of the inflammatory bowel diseases. Therefore, prednisolone is a drug that is required to be targeted to both the small intestine and ileo-colonic region. A therapeutic effect is usually achieved with a dose of 60mg or less (British National Formulary, 2006).

Prednisolone was considered a suitable choice of model drug as, being a neutral molecule, its aqueous solubility is not influenced by pH, and therefore any differences observed in the in vitro release rate from pH-sensitive microparticles at different pH values can be attributed to the formulation.
Section One: Fabrication of Eudragit L microparticles using the emulsification/solvent evaporation method and gelatin as emulsifying agent

2.3 Effect of polymer and gelatin concentrations on microparticle formation

It was decided to conduct preliminary experiments using the method of microencapsulation described by Morishita et al., (1991) to investigate the effect of gelatin and Eudragit L solution concentration on the yield, size distribution and morphology of blank Eudragit L microparticles.

2.3.1 Methods

Microparticle formation
To investigate the effect of increasing Eudragit L concentration on microparticle formation, weighed amounts of Eudragit L were dissolved in ethanol to form solutions at concentration 15-30% w/w. Ten millilitres of polymeric solution was slowly added to 100mL liquid paraffin under magnetic stirring at maximum speed (Jenway 1002 Magnetic Stirrer, Dunmow, Essex) followed by the addition of a pre-formed emulsion of 50mL liquid paraffin/8mL 1% gelatin solution. Stirring was continued for 15 minutes, after which further 10mL aliquots of 1% gelatin solution were added seven times, at 10 minute intervals. Stirring was continued for 12 hours to allow complete solvent evaporation, after which the formed microparticles were collected by filtration, and washed 5 times with 50mL portions of hexane.

To investigate the effect of the concentration of gelatin solution on microparticle formation, the above procedure was repeated with 20% w/w ethanolic Eudragit L solutions using gelatin solution at concentration 0, 2, 3 and 5%.

Calculation of yield
The microparticle yield for each batch was calculated using Equation 2.1:

\[
\text{Yield} = \left(\frac{\text{weight of microparticles harvested}}{\text{weight of polymer added}}\right) \times 100 \quad (\text{Eqn 2.1})
\]
Particle size analysis
Microparticles were sized on a bed of sieves of $\sqrt{2}$ progression, from 1000µm to 355µm, with manual shaking for 5 minutes.

Scanning electron microscopy (SEM)
To gain a better understanding of microparticle morphology and surface topography, the microparticles were examined by scanning electron microscopy. Samples of microparticles were fixed on an aluminium stub using double-sided carbon adhesive tape, and coated with gold using a gold sputter module in a high-vacuum evaporator for 3 minutes at 30mA (Emitech K550, Ashford, Kent, England). Samples were examined and micrographs taken using a scanning electron microscope (Philips XL30, Eindhoven, Holland).

2.3.2 Results and discussion
A coarse, white, free-flowing powder was obtained at all concentrations of Eudragit L and gelatin.

Batch yields
The batch yield of microparticles produced from Eudragit L solutions of concentration 15-30% w/w and 1% w/w gelatin solution were variable and are shown in the Table 2.2.

Table 2.2: Batch yields of microparticles fabricated from 15-30% w/w ethanolic Eudragit L solutions and 1% w/w gelatin solution

<table>
<thead>
<tr>
<th>Eudragit L100 concentration (% w/w)</th>
<th>Batch Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>84.2</td>
</tr>
<tr>
<td>20</td>
<td>72.4</td>
</tr>
<tr>
<td>25</td>
<td>99.7</td>
</tr>
<tr>
<td>30</td>
<td>64.3</td>
</tr>
</tbody>
</table>
The batch yield of microparticles produced from 20% Eudragit L solutions using gelatin solution of concentration 0-5% are shown in the Table 2.3.

**Table 2.3: Batch yields of microparticles fabricated from 20% w/w ethanolic Eudragit L solutions and 0-5% w/w gelatin solution**

<table>
<thead>
<tr>
<th>Gelatin concentration (% w/w)</th>
<th>Batch yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>69.7</td>
</tr>
<tr>
<td>0.5</td>
<td>75.9</td>
</tr>
<tr>
<td>1</td>
<td>72.4</td>
</tr>
<tr>
<td>2</td>
<td>76.7</td>
</tr>
<tr>
<td>3</td>
<td>78.1</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
</tr>
</tbody>
</table>

At a gelatin concentration of 5% and above, microparticle formation was not possible.

*Particle size analysis*

The size distribution for the formulations prepared from Eudragit L solutions of different concentration is shown in Figure 2.3.

*Figure 2.3: The effect of Eudragit L concentration in ethanol on particle size using a 1% gelatin solution as emulsifier*
The size distribution for the formulations prepared with gelatin solutions of different concentration is shown in Figure 2.4.

Figure 2.4: The effect of gelatin solution concentration on the size of particles fabricated from a 20% w/w ethanolic Eudragit L solution

Scanning electron microscopy

Figures 2.5 to 2.10 illustrate the gross morphology and surface topography of particles prepared from different concentrations of Eudragit L and gelatin solution. Comparing Figures 2.5, 2.6 and 2.8 it appears that the use of gelatin as an emulsifying agent improves the sphericity of the Eudragit L particles that are formed, and comparing Figures 2.6, 2.9 and 2.10 a 20% w/w Eudragit L solution appears to produce particles of optimal sphericity and porosity.

However, it was decided that the method described by Morishita et al., (1991) was not suitable for the fabrication of Eudragit L microparticles of size <500μm, as for each batch the fraction of microparticles of size <500μm represented less than 30% of the total batch. It was therefore decided to attempt to reduce the microparticle size using homogenisation during the emulsion formation step, and to assess the release of a model drug prednisolone from the microparticles produced.
Figure 2.5: SEM of Eudragit L particles formed from 20% w/w polymer solution and 0% gelatin solution

Figure 2.6: SEM of Eudragit L particles formed from 20% w/w polymer solution and 1% gelatin solution

Figure 2.7: SEM of Eudragit L particles formed from 20% w/w polymer solution and 1% gelatin solution showing surface porosity

Figure 2.8: SEM of Eudragit L particles formed from 20% w/w polymer solution and 2% gelatin solution

Figure 2.9: SEM of Eudragit L particles formed from 15% w/w polymer solution and 1% gelatin solution

Figure 2.10: SEM of Eudragit L particles formed from 25% w/w polymer solution and 1% gelatin solution
2.4 Preparation of prednisolone-loaded Eudragit L microparticles produced using homogenisation

2.4.1 Methods

Ten millilitres of a 20% w/w ethanolic Eudragit L solution, containing prednisolone at a polymer to drug ratio of 30:1, was slowly added to 100mL of liquid paraffin, while homogenising at 8000rpm (Ultra-Turrax T10, IKA, Staufen, Germany). After 5 minutes homogenisation, the beaker was transferred to a magnetic stirrer (Jenway 1002 Magnetic Stirrer, Dunmow, Essex) and an emulsion of 50mL liquid paraffin/8mL gelatin solution (1% w/w) was added, followed by stirring at maximum speed for 15 minutes. Further 10mL aliquots of 1% w/w gelatin solution were added seven times at 10 minute intervals, after which stirring was allowed to continue for 12 hours. Microparticles were collected by vacuum filtration through a Pyrex sintered glass filter (pore size 4, 5-15μm), washed 5 times with 50mL portions of hexane, and dried overnight in a vacuum oven.

Calculation of yield, particle size analysis and SEM were conducted as described in section 2.3.1.

Calculation of prednisolone encapsulation efficiency

Prednisolone encapsulation efficiency was calculated by dissolving 100mg samples of microparticles in 100mL pH 6.8 phosphate buffer. A clear solution was formed obtained, which was filtered through 0.2μm filters, and the UV absorbance was read at 248nm. Prednisolone encapsulation efficiency was calculated in triplicate with reference to a standard curve.

Evaluation of in vitro drug release at gastric and intestinal pH

In vitro release of prednisolone from 300mg microparticles was investigated in 500mL of 0.1M HCl and 500mL pH 6.8 phosphate buffer, prepared according to Appendix 1D of the British Pharmacopoeia 2007 (British Pharmacopoeia online). The dissolution test was carried out using USP II dissolution apparatus at a temperature of 37.0°C (± 0.5) with a paddle speed of 100rpm. Samples were taken every 30 minutes, filtered through 0.2μm filters and assayed for prednisolone spectrophotometrically at 248nm. The amount of prednisolone in the dissolution media was calculated with reference to a standard curve of
prednisolone in pH 6.8 phosphate buffer and 0.1M HCl. The data reported is an average of three runs.

2.4.2 Results and discussion
The batch yield of the Eudragit L microparticles was 71.9%. All of the microparticles were smaller than 355μm in diameter. Prednisolone encapsulation efficiency was calculated to be 48.2±0.2%.

Scanning electron microscopy
Figure 2.11 shows the appearance of microparticles produced with the aid of homogenisation.

![SEM of Eudragit L particles formed from 20% w/w polymer solution and 1% gelatin solution following homogenisation at 8000rpm](image)
**In vitro prednisolone release**

The *in vitro* drug release profiles of the Eudragit L particles in 0.1M HCl and pH 6.8 phosphate buffer are shown in Figure 2.12.

![Graph showing in vitro prednisolone release](image)

*Figure 2.12: In vitro release of prednisolone from Eudragit L particles produced by homogenisation at pH 1.2 and pH 6.8*

The use of homogenisation successfully reduced the size of the Eudragit L particles to less than 500μm, while achieving a satisfactory yield of >70%. However, the homogenised particles did not exhibit the same spherical morphology as particles produced using magnetic stirring alone. Homogenised microparticles released 29.4(±3.0)% of entrapped prednisolone after 2 hours incubation at acidic pH and therefore failed the test for enteric coated dosage forms described in the United States Pharmacopeia and National Formulary 24 (USP), which states that drug release should be <10% after 2 hours in 0.1M HCl. More than 90% of entrapped prednisolone was released after 60 minutes in pH 6.8 phosphate buffer, and it was thought that the slow release was due to the poor wetting of this formulation, with homogenised microparticles observed to be floating on top of the dissolution media.
2.5 Conclusions
The emulsification/solvent evaporation process described by Morishita is complicated, but is thought to proceed as follows:

Addition of an ethanolic Eudragit L solution to liquid paraffin, while stirring, results in the formation of an ethanol-in-oil emulsion. Upon addition of an emulsion of aqueous gelatin solution in liquid paraffin, droplets of gelatin solution will gradually coalesce with the ethanolic Eudragit L solution droplets, both of which are immiscible with oil. As more gelatin solution is added, coalescence continues, resulting in dilution of ethanol and precipitation of polymer. Polymer precipitation/microparticle hardening is probably facilitated by the acidic pH of the gelatin solution (the pH of a 1\% w/w gelatin solution was determined to be 4.95).

The method described by Morishita et al., (1991) could not be applied to produce Eudragit L microparticles which meet the desired attributes listed in section 2.1.1. Using magnetic stirring alone, the average size of the microparticles formed was >500\(\mu\)m, which does not achieve the aim of producing microparticles of smaller size than can be fabricated using conventional pharmaceutical technology approaches. It is thought that the shear forces provided by magnetic stirring are insufficient to form emulsion droplets, and therefore solidified microparticles, in the size range <500\(\mu\)m. The use of homogenisation in the emulsion formation step reduced the particle size to <500\(\mu\)m, although the morphology of the resulting microparticles was non-spherical as shown in Figure 2.11. Furthermore, although the theoretical prednisolone loading was low (only 3.2\% w/w), prednisolone encapsulation efficiency was below 50\% for homogenised microparticles, and release after 2 hours incubation in acid was not within the USP limits for enteric-coated dosage forms.
Section Two: Fabrication of pH-sensitive Eudragit microparticles using emulsification/solvent evaporation with agitation by propeller stirrer and emulsion stabilisation by non-ionic surfactants

2.6 Introduction

It was decided to investigate an alternative emulsification/solvent evaporation method for the fabrication of pH-sensitive Eudragit microparticles, addressing the shortcomings of the Morishita method which were thought to be inefficient stirring and choice of emulsifier. Both of these parameters (discussed in more detail below) are thought to influence the emulsion formation step, which has been reported to influence the final size of the solidified microparticles (Watts et al., 1990; O'Donnell and McGinity, 1997; Perumal, 2001, Freitas et al., 2005).

Optimisation of stirring

The inefficient stirring of the method described by Morishita et al., (1991) was addressed by replacing the magnetic stirrer with an overhead propeller stirrer (Heidolph RZR1, Heidolph Instruments GmbH, Schwabach, Germany), fitted with a 3-blade impeller of diameter 5cm. The overhead stirrer was capable of providing greater shear forces than the magnetic stirrer, which it was hypothesised, should facilitate the formation of polymeric emulsion droplets, and therefore solidified microparticles of smaller size than was possible using magnetic stirring. Furthermore, accurate calibration of stirring speed was possible which should permit greater batch-to-batch reproducibility.

Selection of emulsifying agent

In the emulsification/solvent evaporation process, the stability of the initial emulsion is increased by addition of a surfactant or emulsifying agent which possesses both hydrophobic and hydrophilic regions, and therefore orientates at the interface of the two immiscible liquids, forming a mechanical and/or steric barrier to coalescence of internal phase droplets.
Emulsifying agents are assigned an arbitrary hydrophilic-lipophilic balance (HLB) value from 0-40 related to the balance between the hydrophilic and lipophilic portions of the molecule (Griffin, 1949; 1954). Predominantly hydrophobic surfactants with an HLB value between 3 and 6 are considered suitable for stabilising a w/o emulsion, while surfactants with a HLB value of between 8 and 18 are considered a more appropriate choice for stabilising o/w emulsions.

It is acknowledged that the stabilisation of an o/o emulsion containing a high molecular weight polymer in the internal “oil” phase (which may itself have surface active properties) will be more complicated than a simple w/o emulsion. However, the use of hydrophobic surfactants may be a more suitable choice than an aqueous gelatin solution, with a HLB value of 9.8, given that the organic solvents used to dissolve the pH-sensitive Eudragits are miscible with water. Furthermore, the presence of such a hydrophobic surfactant which is miscible with liquid paraffin would facilitate emulsion stabilisation from the moment of mixing of the internal and external phases.

Lorenzo-Lamosa et al., (1997) described an emulsification/solvent evaporation method for the fabrication of Eudragit L55 microparticles incorporating the antibiotic drug cefuroxime axetil. In the method, a mixture of acetone:ethanol (2:1 v/v) was used to dissolve the polymer, and the non-ionic, hydrophobic surfactant Span 85 (HLB value 1.8) was employed as an emulsifying agent. Propeller stirring was used at 1000rpm to produce the emulsion, and evaporate the solvent. It was attempted to modify the method of Lorenzo-Lamosa et al., (1997) for the production of prednisolone-loaded microspheres of Eudragit L55, L and S.

2.6.1 Methods

The internal phase was prepared by dissolving 3g of Eudragit L55, L or S in 30mL acetone:ethanol (2:1 v/v) using magnetic stirring. An external phase of 200mL liquid paraffin, containing 1% w/w Span 85 as emulsifier was prepared and stirred with a Heidolph RZR1 overhead stirrer, calibrated to 1000rpm and fitted with a 3-blade propeller, for a period of approximately 1 minute. The internal phase was then poured slowly into the external phase over a period of about 15 seconds to form an o/o emulsion. Stirring was
continued for 12 hours to allow solvent evaporation. Microparticles were recovered by vacuum filtration through a Pyrex sintered glass filter (pore size 4 (5-15μm)), washed three times with 50mL portions of hexane, and placed in a vacuum oven overnight. Microparticles were examined by SEM as described in section 2.3.1.

2.6.2 Results and discussion

Microparticles produced using the three acrylic polymers differed significantly in morphology, as shown in figures 2.13 to 2.15. Eudragit L55 microparticles were spherical, free-flowing and unaggregated, whereas L and S microparticles appeared to be present as strings or clusters of smaller, semi-formed microparticles.

Although the morphology of the Eudragit L and S microparticles produced by this method was unacceptable due to the degree of aggregation, the appearance of the Eudragit L55 microparticles was promising being spherical, unaggregated, free flowing and of size <500μm. These microparticles were suitable for further evaluation as a potential drug delivery formulation for targeting the small intestine.

It was therefore decided at this point to focus on improving the morphology of the Eudragit S microparticles in order to achieve a microparticulate delivery system for targeting to the ileo-colonic region which would allow a comparison of two different formulations for drug delivery to the small intestine and ileo-colonic region. It was decided to investigate the use of alternative solvents for Eudragit S, and alternative emulsifying agents to improve the morphology of the Eudragit S microparticles.
Figure 2.13: SEM of Eudragit L55 microparticles formed from 10% w/v polymer solution in acetone:ethanol (2:1) using 1% span 85 as emulsifying agent.

Figure 2.14: SEM of Eudragit L microparticles formed from 10% w/v polymer solution in acetone:ethanol (2:1) using 1% span 85 as emulsifying agent.

Figure 2.15: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone:ethanol (2:1) using 1% span 85 as emulsifying agent.
2.7 Optimisation of internal phase solvent for production of Eudragit S microparticles

Eudragit S has a limited solubility profile in organic solvents, being restricted to acetone and the alcohols. An indication of the solubility of Eudragit L55 and Eudragit S in methanol, ethanol and acetone, is shown in Table 2.4, which lists the time for dissolution of 15g of polymer in 100g of solvent at 20°C (Pharma Polymers, 2001), along with the boiling points of the solvents.

Table 2.4: The dissolution time of Eudragit L55 and Eudragit S in methanol, ethanol and acetone

<table>
<thead>
<tr>
<th>Solvent (boiling point)</th>
<th>Time for dissolution of Eudragit L55 (mins)</th>
<th>Time for dissolution of Eudragit S (mins)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol (65°C)</td>
<td>10</td>
<td>75</td>
</tr>
<tr>
<td>Ethanol (78°C)</td>
<td>20</td>
<td>50</td>
</tr>
<tr>
<td>Acetone (56°C)</td>
<td>30</td>
<td>5</td>
</tr>
</tbody>
</table>

It was hypothesised that the more rapidly the solvent evaporation process proceeds, the less time the emulsion droplets (forming microspheres) would be in a semi-solid and "sticky" state during which time they would be liable to coalesce, and form agglomerates upon complete solvent evaporation. It was considered that the rate of solvent migration from the internal to the external emulsion phase, and therefore degree of microparticle aggregation, would be dictated by the solubility of the polymer in the solvent mixture (i.e. the affinity of the polymer for a particular solvent mixture), and the boiling point of the internal phase solvent(s).

For example, considering the data in Table 2.4, above, it was postulated that Eudragit L55 would be less soluble in a mixture of acetone:ethanol (2:1), used in the method described by Lorenzo-Lamosa et al., (1997), than the same amount of Eudragit S, and consequently migration of solvent mixture from the polymer solution into the liquid paraffin external phase would be slower from Eudragit S emulsion droplets than L55 droplets. This may present more possibility for agglomeration of semi-solidified Eudragit S emulsion droplets, and could possibly explain the agglomeration of Eudragit S microparticles (Figure 2.15) which was not seen for Eudragit L55 microparticles (Figure 2.13).
Therefore it was decided to compare the Eudragit S microparticles formed using different ratios of acetone/ethanol and acetone/methanol as internal phase solvent, to investigate whether this could improve the degree of microparticle agglomeration by increasing the rate of solvent evaporation and therefore reducing the need for emulsion stability.

2.7.1 Methods
The emulsification/solvent evaporation method described in section 2.6.1 was followed, except that 3g Eudragit S was dissolved in a 30mL mixture of acetone/ethanol (2:1 v/v) and 30mL mixtures of acetone/methanol in the ratio 30:0, 5:1, 2:1, 1:1, 1:2, 1:5 and 0:30 v/v. Microparticles were examined by SEM as described in section 2.3.1.

2.7.2 Results and discussion
A comparison of the morphology of the Eudragit S microparticles prepared from mixtures of acetone/ethanol (2:1 v/v) and acetone/methanol (2:1 v/v) is shown in Figures 2.16 and 2.17.

Figure 2.16: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/ethanol (2:1) using 1% Span 85 as emulsifying agent

Figure 2.17: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (2:1) using 1% Span 85 as emulsifying agent

Microparticles prepared from a mixture of acetone/methanol (2:1 v/v) appear to be less aggregated than those produced using a mixture of acetone/ethanol, possibly due to the faster rate of methanol migration from the internal phase to the external phase and evaporation from the external phase. However the microparticles formed using
acetone/methanol (2:1 v/v) are still not very spherical, and slightly aggregated, and it was decided to attempt to further optimise the microparticle morphology by optimising the ratio of acetone to methanol. The SEMs shown in Figures 2.18 to 2.23 demonstrate that changing the ratio of acetone:methanol in the internal phase solvent can affect the morphology of the Eudragit S microspheres, although all of the microparticles are aggregated to some degree.
Figure 2.18: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (0:30) using 1% span 85 as emulsifying agent.

Figure 2.19: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (1:5) using 1% span 85 as emulsifying agent.

Figure 2.20: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (1:2) using 1% span 85 as emulsifying agent.

Figure 2.21: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (1:1) using 1% span 85 as emulsifying agent.

Figure 2.22: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (2:1) using 1% span 85 as emulsifying agent.

Figure 2.23: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (5:1) using 1% span 85 as emulsifying agent.
When methanol alone or acetone/methanol (1:5 v/v) was used as internal phase solvent, the resulting microparticles appear to be hollow, with a thin shell that is easily cracked, which is thought to be due to a too rapid migration of solvent from the internal emulsion phase due to the low affinity of methanol for Eudragit S (see Table 2.4), and its low boiling point. Acetone alone did not produce any microparticles. The optimal ratio of acetone:methanol appears to be in the ratio 1:1 to 5:1 v/v, as shown in Figures 2.21 to 2.23. It was attempted to further optimise the internal phase solvent by investigating mixtures of acetone/methanol in the ratios 23:7, 21:9, 19:11 and 17:13 v/v, however no further improvement in morphology was possible, as determined by SEM (data not shown).

It was therefore decided to select the internal phase solvent acetone/methanol (2:1 v/v) for further investigations, and to attempt to improve the morphology of Eudragit S microparticles by screening alternative hydrophobic emulsifying agents with HLB values in the range 1-9, close to the optimum HLB value for stabilisation of a w/o emulsion, as discussed in section 2.6.

2.8 Optimisation of emulsifying agent for production of Eudragit S microparticles

2.8.1 Methods

The emulsification/solvent evaporation method described in section 2.6.1 was followed, except that 3g Eudragit S was dissolved in 30mL of acetone/methanol (2:1 v/v), and Span 80, Span 20, Brij 92, Brij 52, oleic acid and Arlacel 83 were used as emulsifying agents at a concentration of 1, 2 and 3% w/w in liquid paraffin. Microparticles were examined by SEM as described in section 2.3.1.

2.8.2 Results and discussion

A description of the Eudragit S microparticles produced using the emulsifying agents with HLB values in the range 1-9 is shown in Table 2.5. SEMs of selected microparticles are shown in Figures 2.24 to 2.31.
Table 2.5: Summary of Eudragit S microparticles fabricated from a 10% w/v Eudragit S solution in acetone/methanol (2:1 v/v) using non-ionic surfactants as emulsion stabilisers

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Nonproprietary name</th>
<th>HLB value</th>
<th>Appearance of Eudragit S particles as viewed by SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Span 85</td>
<td>Sorbitan trioleate</td>
<td>1.8</td>
<td>No difference in microparticle morphology or degree of aggregation at a concentration of 1, 2 or 3% (data not shown)</td>
</tr>
<tr>
<td>Span 80</td>
<td>Sorbitan monooleate</td>
<td>4.3</td>
<td>At 1, 2 and 3% concentration no microparticles are formed. Polymer precipitated as non-particulate lumps &gt;1mm diameter (data not shown)</td>
</tr>
<tr>
<td>Span 20</td>
<td>Sorbitan monolaurate</td>
<td>8.6</td>
<td>No particles were formed. Spindle-like fibres formed at 1, 2 and 3% concentration (data not shown)</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>Oleic acid</td>
<td>4.3</td>
<td>At 1% concentration, aggregated microparticles of size &lt;100μm were formed (see Figure 2.24). Aggregation was reduced with increasing concentration, however some aggregation remained and polydispersity appeared to be high (see Figure 2.25)</td>
</tr>
<tr>
<td>Brij 92</td>
<td>Polyoxyl 2 oleyl ether</td>
<td>4.9</td>
<td>At 1% concentration, agglomerated microparticles of size &lt;100μm were formed (see Figure 2.26). A less spherical morphology was obtained at concentrations 2 and 3% (see Figure 2.27)</td>
</tr>
<tr>
<td>Brij 52</td>
<td>Polyoxyl 2 cetyl ether</td>
<td>5.3</td>
<td>Brij 52 is a solid at room temperature and was either dispersed in the Eudragit S organic solution (see Figure 2.28), or dissolved in the liquid paraffin with the aid of heating (see Figure 2.29). Microparticles were more spherical when Brij 52 was incorporated into the external emulsion phase, but aggregation was a problem at all concentrations tested.</td>
</tr>
<tr>
<td>Arlacel 83</td>
<td>Sorbitan sesquioleate</td>
<td>3.7</td>
<td>Spherical, non aggregated microparticles of size ≤50μm were formed at 1, 2 and 3% concentration (see Figures 2.30 and 2.31)</td>
</tr>
</tbody>
</table>
Figure 2.24: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (2:1) using 1% oleic acid as emulsifying agent

Figure 2.26: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (2:1) using 1% Brij 92 as emulsifying agent

Figure 2.28: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (2:1) using 1% Brij 52 as emulsifying agent dispersed in internal phase

Figure 2.25: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (2:1) using 3% oleic acid as emulsifying agent

Figure 2.27: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (2:1) using 3% Brij 92 as emulsifying agent

Figure 2.29: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in acetone/methanol (2:1) using 1% Brij 52 as emulsifying agent dispersed in external phase
The Eudragit S microparticles formed using Arlacel 83 an emulsifying agent were the most promising. Microparticles imaged by SEM were observed to be spherical, non-aggregated, non-porous and also appeared to have a monodisperse particle size distribution. However, the internal phase solvent used was a combination of methanol and acetone, and it was therefore decided to attempt to improve the simplicity of the method, and reduce the use of a toxic ICH class II solvent (methanol) in the process, by attempting to replace the mixture of acetone/methanol (2:1 v/v) with ethanol, a more pharmaceutically acceptable organic solvent which is commonly used as a solubility enhancer in liquid dosage forms.

2.9 Microencapsulation of Eudragit L55, L and S using the emulsification/solvent evaporation method with Arlacel 83 as surfactant and ethanol as polymer solvent

2.9.1 Methods
The emulsification/solvent evaporation method described in section 2.6.1 was followed, except that 3g Eudragit L55, L and S were dissolved in 30mL of ethanol, using 1% w/w Arlacel 83 in liquid paraffin as emulsifying agent. Microparticles were examined by SEM as described in section 2.3.1.

2.9.2 Results and discussion
SEMs of Eudragit S, L and L55 microparticles are shown in figures 2.32 to 2.37.
Figure 2.32: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in ethanol using 1% Arlacel 83 as emulsifying agent (high mag.)

Figure 2.33: SEM of Eudragit S microparticles formed from 10% w/v polymer solution in ethanol using 1% Arlacel 83 as emulsifying agent (low mag.)

Figure 2.34: SEM of Eudragit L microparticles formed from 10% w/v polymer solution in ethanol using 1% Arlacel 83 as emulsifying agent (high mag.)

Figure 2.35: SEM of Eudragit L microparticles formed from 10% w/v polymer solution in ethanol using 1% Arlacel 83 as emulsifying agent (low mag.)

Figure 2.36: SEM of Eudragit L55 microparticles formed from 10% w/v polymer solution in ethanol using 1% Arlacel 83 as emulsifying agent (high mag.)

Figure 2.37: SEM of Eudragit L55 microparticles formed from 10% w/v polymer solution in ethanol using 1% Arlacel 83 as emulsifying agent (low mag.)
Spherical, non-aggregated and monodisperse microparticles were formed from the pH-sensitive polymers Eudragit L55, L and S using Arlacel 83 as emulsifying agent and ethanol as internal phase solvent, i.e. the method was transferable between the pH-sensitive Eudragits. This was not the case for the method described by Lorenzo-Lamosa et al., (1997), described in section 2.6.1, which used Span 85 as emulsifying agent and acetone/ethanol (2:1 v/v) as internal phase solvent. Furthermore, the particle size and particle size distribution appear to be smaller for the Eudragit L55 microparticles produced using Arlacel 83 as surfactant and ethanol as polymer solvent compared to those prepared in section 2.6.1.

It was hypothesised that the beneficial effect of Arlacel 83 on Eudragit microparticle morphology was due to improved emulsion stability in the early stages of the emulsification/solvent evaporation process. This can be confirmed by investigating the stability of the primary o/o emulsion that is formed. Therefore, experiments were conducted using an ethanolic Eudragit S solution as the internal phase, dispersed into liquid paraffin containing 1% w/w of the emulsifying agents Arlacel 83, as well as the non-ionic surfactants Span 80 and Span 85, alone and mixed in the ratio 1:0.32 to produce a combined HLB value of 3.7, the same as Arlacel 83. Emulsion stability was investigated in the absence and presence of continuous propeller stirring.

2.10 An investigation into the rate of creaming of an emulsion stabilised by a variety of non-ionic surfactants and surfactant mixture with HLB of 3.7

2.10.1 Methods

Three grams of Eudragit S were dissolved in 30mL of ethanol containing 20μg/mL methylene blue as contrast agent. A Heidolph overhead propeller stirrer was used to emulsify the blue Eudragit S solution into 200mL liquid paraffin containing 1% w/w of Span 80, or Span 85, or Span80/Span 85 (1:0.32) with combined value HLB 3.7, or Arlacel 83. The emulsion was stirred for two minutes, after which time the contents of the beaker were poured into a 250mL measuring cylinder, which was immediately covered with Parafilm to prevent evaporation of ethanol. The degree of creaming in the measuring cylinder was recorded at 0, 5, 10, 15, 30, 60, 120, 240, 360, 480 and 1440 minutes. Creaming experiments were carried out in triplicate for each surfactant.
2.10.2 Results and discussion

The rate of creaming for each of the surfactants or surfactant mixture is shown in Figure 2.38.

![Figure 2.38: Rate of creaming of an ethanolic solution of Eudragit S in liquid paraffin stabilised by different emulsifying agent(s)](image)

The rate of creaming of the emulsion stabilised by Arlacel 83 was slower than the emulsions stabilised by the other non-ionic surfactants or the surfactant mixture. The emulsion stabilised by the mixture of Span 80/Span 85 (HLB 3.7) is only as stable as the emulsion stabilised by Span 85 alone. This suggests that emulsion stability is not solely determined by the HLB value of the emulsifying agent, and also challenges the well-established theory that a mixture of surfactants better stabilise an emulsion than a single surfactant with the same HLB value, by forming a more rigid, stabilising film at the interface of the two phases. The emulsion stabilised by Arlacel 83 is significantly more stable than the others over the first eight hours. After this time, emulsion stability is not important as solvent evaporation and microparticle solidification will be complete.

Arlacel 83 has been shown to improve o/o emulsion stability following cessation of stirring. However, it is not apparent that such stabilisation will offer advantages during the emulsification/solvent evaporation process, as continual stirring of the o/o emulsion should also help to prevent the coalescence of internal phase emulsion droplets. The experiment below compares the in-process emulsion stability of an o/o emulsion, containing an
ethanolic solution of Eudragit S as the internal phase, and liquid paraffin containing 1% w/w Arlacel 83 or 1% w/w Span80/Span 85 (1:0.32), during propeller stirring at 1000rpm.

2.11 An investigation of in-process emulsion stability

2.11.1 Methods

Three grams Eudragit S were dissolved in 30mL ethanol (containing 20µg/mL methylene blue as contrast aid). Solutions were emulsified into 200mL liquid paraffin containing 1% Arlacel 83 and 1% Span 80/Span 85 (1:0.32). A sample was withdrawn 5, 60, 120, 180, 240, 300 and 360 minutes after commencement of stirring at 1000rpm. The sample was placed on a microscope slide and immediately analysed using an optical microscope (Nikon Microphot FXA) at x4 and x10 objective magnification, and images were captured using a digital camera.

2.11.2 Results and discussion

The appearance of the o/o emulsions stabilised by 1% Arlacel 83 and 1% Span 80/Span 85 (1:0.32) are shown in figures 2.39 to 2.50, below (the bar in each figure is 100µm). The appearance of the o/o emulsions stabilised by Arlacel 83 and the mixture of Span 80/Span 85 are similar for the first hour. However, in the emulsion stabilised by the surfactant mixture, after 2 hours, the size of the ethanolic Eudragit S emulsion droplets appear to be increasing due to coalescence. This process continues, until after 6 hours large agglomerates of semi-solidified microparticles are observed. However, the ethanolic Eudragit S droplets of the emulsion stabilised by Arlacel 83 appear to remain approximately the same size for the duration of the experiment.
Figure 2.39: Appearance of Eudragit S o/o emulsion stabilised by 1% Arlacel 83 after 5 minutes stirring (x10 mag.)

Figure 2.40: Appearance of Eudragit S o/o emulsion stabilised by 1% Span 80/Span 85 (1:0.32) after 5 minutes stirring (x10 mag.)

Figure 2.41: Appearance of Eudragit S o/o emulsion stabilised by 1% Arlacel 83 after 1 hour stirring (x10 mag.)

Figure 2.42: Appearance of Eudragit S o/o emulsion stabilised by 1% Span 80/Span 85 (1:0.32) after 1 hour stirring (x10 mag.)

Figure 2.43: Appearance of Eudragit S o/o emulsion stabilised by 1% Arlacel 83 after 2 hours stirring (x10 mag.)

Figure 2.44: Appearance of Eudragit S o/o emulsion stabilised by 1% Span 80/Span 85 (1:0.32) after 2 hours stirring (x10 mag.)
Figure 2.45: Appearance of Eudragit S o/o emulsion stabilised by 1% Arlacel 83 after 3 hours stirring (x10 mag.)

Figure 2.46: Appearance of Eudragit S o/o emulsion stabilised by 1% Span 80/Span 85 (1:0.32) after 3 hours stirring (x10 mag.)

Figure 2.47: Appearance of Eudragit S o/o emulsion stabilised by 1% Arlacel 83 after 4 hours stirring (x10 mag.)

Figure 2.48: Appearance of Eudragit S o/o emulsion stabilised by 1% Span 80/Span 85 (1:0.32) after 4 hours stirring (x10 mag.)

Figure 2.49: Appearance of Eudragit S o/o emulsion stabilised by 1% Arlacel 83 after 6 hours stirring (x10 mag.)

Figure 2.50: Appearance of Eudragit S o/o emulsion stabilised by 1% Span 80/Span 85 (1:0.32) after 6 hours stirring (x4 mag.)
The key to forming pH-sensitive Eudragit microparticles of acceptable morphology using the emulsification/solvent evaporation method appears to be the use of Arlacel 83 as emulsifying agent. However, the stability conferred on the emulsion is not thought to be due to HLB value alone, as a mixture of Span 80/Span 85 with the same HLB value as Arlacel 83 (i.e. 3.7), failed to stabilise the emulsion, in either the presence or absence of stirring, as effectively as Arlacel 83.

Arlacel 83, a proprietary brand of sorbitan sesquioleate, is an equimolar mixture of sorbitan monooleate and sorbitan dioleate (Lawrence, 2000). It is possible that the two surfactant molecules from which it is constituted combine to form a well-condensed film at the o/w interface of the emulsion, which effectively forms a strong mechanical or steric barrier to reduce coalescence of the ethanolic Eudragit internal phase droplets.

2.12 Chapter Conclusions
A single emulsification/solvent evaporation method was successfully optimised for the production of Eudragit L55, L and S microparticles using the ICH class III solvent, ethanol, as internal phase solvent, and Arlacel 83 as emulsion stabiliser. Microparticles were spherical, non-aggregated, non-porous and of size ≤50μm, fulfilling the attributes that were defined in section 2.1.1.

It was therefore decided to investigate the potential of the optimised pH-sensitive microparticles to deliver a model drug, prednisolone, to the proximal small intestine and ileo-colonic region using suitable in vitro dissolution testing. These experiments are described in Chapter Three.
Chapter Three: *In vitro* characterisation of drug loaded pH-sensitive polymeric microparticles
3.1 Introduction

Although the most reliable test of dosage form performance is a well designed in vivo study in a suitable animal model, it is not ethical or possible to screen a large number of formulations using this approach. Therefore, suitable alternative methods have to be utilised to investigate drug release, a prerequisite for drug absorption, from formulations during dosage form development. In vitro dissolution methods using compendial dissolution apparatus are usually developed to assess drug release from dosage forms prior to in vivo absorption studies.

The main aim of this chapter is to investigate the release of a model drug, prednisolone, from the pH-sensitive microparticle formulations developed in Chapter Two. The potential of Eudragit L and L55 microparticles to deliver drug to the proximal small intestine, and Eudragit S microparticles to deliver drug to the ileo-colonic region, will be evaluated through the development of suitable in vitro dissolution tests, and formulations will be optimised to be taken forward into an in vivo drug absorption study in rats.

Dissolution testing was initially introduced as a quality control check, and although this remains an important application - USP 24 contains around 600 monographs which set out the required specification for a number of immediate and modified release products – dissolution testing is now accepted as an important tool in the development and screening of drugs and dosage forms.

3.1.1 Compendial dissolution apparatus for in vitro testing of oral dosage forms

The USP 24 lists four compendial apparatus for testing of oral dosage forms which are described below (Gray, 2005; United States Pharmacopeia and National Formulary 24, 2006), and summarised in Table 3.1. The apparatus have also been adopted by the European Pharmacopoeia with some minor modifications to the specifications (Kramer et al., 2005).

*USP I (rotating basket) apparatus*

USP I apparatus consists of a closed rotating mesh basket, into which the dosage form is placed, which is immersed in a covered transparent vessel containing the dissolution media and attached to a shaft which is driven by a motor at a controlled speed. In common with
USP dissolution apparatus II, III and IV, the vessels are heated by means of a water bath or heating jacket capable of holding the temperature of the media inside the vessels at 37 ± 0.5°C.

**USP II (paddle) apparatus**

USP II apparatus is identical to USP I, except that the rotating basket is replaced with a paddle of specified dimensions. When floating dosage forms are being tested, an inert sinker may be used to sink the dosage form to the bottom of the dissolution vessel which maintains a constant surface area for dosage form dissolution and prevents mechanical damage of the dosage form upon contact with the rotating paddle. Of all types of dissolution apparatus, types I and II are still the most commonly used due to their simplicity, robustness and wider experience of experimental usage than other types of compendial dissolution apparatus (Kramer et al., 2005).

**USP III (reciprocating cylinder) apparatus**

USP III apparatus consists of a set of cylinders into which the dosage form is placed, closed at the top and bottom with stainless steel mesh, which are reciprocated vertically inside cylindrical, flat-bottomed glass vessels. Commercially available apparatus consists of seven columns of six rows of flat-bottomed outer vessels which facilitate multiple pH changes throughout the dissolution run facilitating the simulation of the aboral increase in gastrointestinal pH that is observed in man. USP III apparatus is considered especially appropriate for the development of extended release formulations due partly to the ease with which pH change methods can be developed, but also because it subjects the dosage form to stronger mechanical forces than other dissolution apparatus, similar to those which may be experienced by the dosage form in vivo, for example during transit through the pyloric sphincter or ileo-caecal region.

**USP IV (flow-through cell) apparatus**

USP IV apparatus consists of a vertically mounted transparent cell into which the dosage form is placed, which is fitted with a filter system to prevent release of undissolved particles as dissolution media is pumped through the cell. The system may be operated in either “open loop” mode which offers the advantage of unlimited media supply (of benefit
for the dissolution testing of poorly water soluble drugs), or “closed loop” mode, which permits volumes of dissolution media as low as 15mL to be used.
Table 3.1: Summary of USP I-IV dissolution apparatus

<table>
<thead>
<tr>
<th>Apparatus</th>
<th>Volume of media</th>
<th>Controllable factors affecting dissolution rate</th>
<th>Disadvantages</th>
<th>Advantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>USP I (Basket)</td>
<td>500-2000mL</td>
<td>-Volume of dissolution media -Agitation rate</td>
<td>-Volumes of dissolution media restricted and non-biorelevant -Air bubbles can block basket mesh -Adherence of some dosage forms to basket mesh -Not suitable for very small dosage forms (e.g. microparticles)</td>
<td>-Simple, robust and adequately standardised design -Cost and availability -Equipment can be easily modified to USP II</td>
</tr>
<tr>
<td>USP II (Paddle)</td>
<td>500-2000mL</td>
<td>-Volume of dissolution media -Agitation rate</td>
<td>-Volumes of dissolution media restricted and non-biorelevant -pH-change is slow and difficult to control</td>
<td>-Simple, robust and adequately standardised design -Cost and availability -Lots of monographs -Equipment can be easily modified to USP I -Easy to stagger individual starts</td>
</tr>
<tr>
<td>USP III (Reciprocating cylinder)</td>
<td>200-300mL</td>
<td>-Volume of dissolution media -Speed of reciprocation (dips per minute)</td>
<td>-Not recognised by Japanese Pharmacopoeia -Disintegrating dosage forms can pass through mesh of reciprocating cylinder and will not be carried to next vessel during media change -Not suitable for very small dosage forms (e.g. microparticles)</td>
<td>-Potential for up to six pH changes -Possible to change viscosity of dissolution media -Better simulation of <em>in vivo</em> hydrodynamics -Can accommodate small volumes of dissolution media -Less prone to wobble than USP I and II</td>
</tr>
<tr>
<td>Apparatus</td>
<td>Volume of media</td>
<td>Controllable factors affecting dissolution rate</td>
<td>Disadvantages</td>
<td>Advantages</td>
</tr>
<tr>
<td>-------------------</td>
<td>----------------</td>
<td>-----------------------------------------------</td>
<td>--------------------------------------------------------------------------------</td>
<td>------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>USP IV (Flow-through cell)</td>
<td>≥ 15mL</td>
<td>-Volume of dissolution media</td>
<td>-Increased potential for bubble interference when using UV analysis</td>
<td>-pH change (multiple) is easy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Open or closed system</td>
<td>-Possibility of filter blockage at high material load or small particle size</td>
<td>-Small volumes useful for assay of low dose drugs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Flow rate</td>
<td>-Complex set up</td>
<td>-Large volumes allow sink conditions for poorly soluble drugs</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Presence/absence of glass beads in cell</td>
<td>-No official calibrator tablets</td>
<td>-No evaporation of media from closed system allows testing over prolonged periods</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Cell type</td>
<td>-Dissolution media must be de-aerated</td>
<td>-Glass beads can be mixed with pellets/microparticles if aggregation is an issue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Orientation of dosage form</td>
<td></td>
<td>-Can also be used for suppositories, implants or other novel dosage forms</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>-Retention of undissolved particles within the cell</td>
</tr>
</tbody>
</table>
3.1.2 In vitro/in vivo correlations

In vitro/in vivo correlations (IVIVC) have been defined as "a predictive mathematical model describing the relationship between an in vitro property of the dosage form (e.g. dissolution profile) and an in vivo response" (Kramer et al., 2005), and if they can be achieved early in the formulation development process can reduce the reliance on in vivo testing during the formulation selection process. The achievement of an IVIVC is more likely for drugs for which solubility and not permeability is the rate limiting step to drug absorption.

The development of in vitro dissolution methods which achieve IVIVC may reduce the number of bioequivalence studies that are required during the initial approval process or during scale-up or post-approval formulation changes, and as such this subject has attracted much interest within the pharmaceutical industry. To achieve IVIVC, various modifications have been made to dissolution methods to make them more biorelevant. Modifications include the development of dissolution media to more closely resemble the composition of the gastrointestinal tract contents (Dressman et al., 1998; Galia et al., 1998; Dressman and Reppas, 2000; Horter and Dressman, 2001), multiple pH change methods (Ashford et al., 1993a) or better simulation of the hydrodynamics within the gastrointestinal tract by the use of USP III apparatus (Kramer et al., 2005). The potential of predictive in vitro methods to achieve both monetary and time savings during the formulation approval process has driven the development of complex in vitro models such as the TNO intestinal model (TNO, Utrechtseweg, Netherlands) which closely simulate in vivo fluid volume and flux, hydrodynamics and enzymatic activity of the gastrointestinal tract in either the fed or fasted state, as well as simulating drug absorption via a dialysis mechanism. However, the use of such complicated predictive models is in its infancy and at present does not facilitate rapid testing of formulations during the development process.

3.1.3 In vitro dissolution testing of pH-sensitive dosage forms

The USP 24 lists two methods for the testing of enteric coated dosage forms; method A and method B. Method A is carried out using USP II paddle apparatus, whereby the dosage form is incubated for 2 hours in 750mL 0.1M HCl, after which the pH is increased by the addition of 250mL of 0.2M tribasic sodium phosphate, and the final pH adjusted to 6.8 ± 0.05 by adding 2M HCl or 2M NaOH, with a requirement for pH adjustment to be
completed within 5 minutes. Dissolution is then continued for a further 45 minutes, or until it can be established that drug release is complete. Method B utilises the USP I basket apparatus, initially incubating the dosage form in 1000mL of 0.1M HCl for a period of 2 hours, after which time the baskets are raised, and the dissolution vessel replaced with one containing 1000mL of pH 6.8 phosphate buffer, pre-warmed to 37°C, for a further 45 minutes or until drug release is complete. Unless stated otherwise in the monograph, enteric coated dosage forms tested by either method are required to release <10% of drug after 2 hours in acid, and release at least 75% of drug after 45 minutes at pH 6.8.

3.1.4 Considerations for the in vitro dissolution testing of pH-sensitive microparticles

The use of method B is precluded for the dissolution testing of the pH-sensitive microparticles developed in Chapter Two, as the mesh size of the baskets (0.36-0.44 mm) is too large to retain the microparticles. Although it is possible that baskets could be fabricated with a smaller diameter mesh, as the mesh size decreases bubbles are increasingly likely to become trapped in the mesh, reducing flow of dissolution media to the dosage form and subsequent dissolution rate.

The use of method A also has limitations for the dissolution testing of the microparticles developed in Chapter Two. Firstly, as the method describes only a pH change from 1.2 to 6.8, it is only applicable for the testing of formulations designed to deliver drugs to the small intestine, i.e. enteric-coated products or those which are intended to dissolve at a threshold pH of 4.5 to 6.0, and is not a suitable method for the dissolution testing of dosage forms designed to deliver drugs to the ileo-colonic region using a pH approach. Secondly, method A permits a period of up to five minutes following the addition of 0.2M tribasic sodium phosphate during which the pH of the dissolution media must be adjusted to 6.8 ± 0.05. Exposure of “enteric” microparticles to a pH lower or higher than 6.8 for a period of up to five minutes may result in a drug release rate slower or faster than the true value, which may be particularly significant given the large surface area to volume ratio of the pH-sensitive microparticles. Therefore, the USP pH-change method A would have to be amended for the dissolution testing of Eudragit S microparticles, with a threshold pH of 7.0, if possible facilitating a more rapid and accurate pH change than that described in the USP 24.
3.2 Materials and methods

3.2.1 Materials

Eudragit L100-55, Eudragit L100, Eudragit S100 and Eudragit RS100 were obtained from Rohm Pharma (Darmstadt, Germany). Ethylcellulose N7 was obtained from Aqualon (Dusseldorf, Germany). Micronised prednisolone was purchased from Sanofi-Aventis, (Romainville, France). Budesonide was obtained from AstraZeneca (Macclesfield, UK). Bendroflumethiazide and paracetamol were purchased from Sigma Aldrich, (Poole, UK). Size 0 gelatin capsules were obtained from Capsugel (Colmar, France). All other reagents were of analytical grade and were used as received.

3.2.2 Preparation of media for dissolution testing

Preparation of media to simulate gastric pH

A 0.1M HCl solution was prepared by 50:1 dilution of 5M HCl with water. The pH of this the resultant 0.1M HCl solution was determined to be approximately 1.2 using a pH 211 Microprocessor (Hanna Instruments, Woonsocket, USA), and was considered to be representative of fasted state gastric pH.

Preparation of media to simulate small intestinal pH

Phosphate buffer solutions considered to be in the range of small intestinal pH (6.0 – 7.4) were prepared from volumetrically prepared solutions of 0.2M potassium dihydrogen orthophosphate and 0.1M sodium hydroxide, according to Appendix 1D of the British Pharmacopoeia 2007 (British Pharmacopoeia online). The pH of the buffer solutions were checked and adjusted to ±0.02 of a pH unit, as appropriate, using either 2M HCl or 2M NaOH.

Development of a pH-change method for the dissolution testing of microparticles intended to target the proximal small intestinal and ileo-colonic region

Seven hundred and fifty millilitres of 0.1M HCl were added to 250mL water in a beaker, which was placed on a magnetic stirrer. A pH electrode was secured by a clamp-stand and partially immersed in the acid solution, and stirring commenced. Tribasic sodium phosphate dodecahydrate (Na₃PO₄), with a molecular mass of 380.1, was slowly added to the beaker, which upon dissolution raised the pH of the solution. The quantity of dissolved
Na₃PO₄ which was required to increase the pH of the acidic solution to a pH of 6.0 to 7.4 was recorded, and is shown in Table 3.2 below. The concentration of Na₃PO₄ solution required to achieve the desired pH following addition to 0.1M HCl in a 1:3 ratio is also shown.

**Table 3.2: Concentration of tribasic sodium phosphate solution (250mL) that is required to raise the pH of 750mL 0.1M HCl to a pH of between 6.0 to 7.4**

<table>
<thead>
<tr>
<th>Recorded pH</th>
<th>Weight Na₃PO₄ added (g)</th>
<th>Concentration of Na₃PO₄ solution required (mol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6.00</td>
<td>13.31</td>
<td>0.140</td>
</tr>
<tr>
<td>6.20</td>
<td>13.77</td>
<td>0.145</td>
</tr>
<tr>
<td>6.40</td>
<td>14.33</td>
<td>0.151</td>
</tr>
<tr>
<td>6.60</td>
<td>15.18</td>
<td>0.160</td>
</tr>
<tr>
<td>6.80</td>
<td>16.13</td>
<td>0.170</td>
</tr>
<tr>
<td>7.00</td>
<td>17.30</td>
<td>0.182</td>
</tr>
<tr>
<td>7.20</td>
<td>18.49</td>
<td>0.195</td>
</tr>
<tr>
<td>7.40</td>
<td>19.61</td>
<td>0.206</td>
</tr>
</tbody>
</table>

The concentration of 250mL Na₃PO₄ solution which was required to produce a pH of 6.8 when added to 750mL 0.1M HCl was 0.170 mol/L, lower than the concentration indicated in the USP pH-change method A (0.200 mol/L). This may be accounted for by differences in pH between the water of different laboratories or by the purity of the Na₃PO₄ buffer used. The method developed above facilitates a more accurate pH-change method for the *in vitro* testing of enteric dosage forms using the water and Na₃PO₄ buffer available during this study. Furthermore, it also permits the dissolution testing of pH-sensitive dosage forms designed to deliver drug to the ileo-colonic region, which are likely to release drug slowly at pH 6.8. The addition of 250mL of a 0.206 mol/L Na₃PO₄ to 750mL 0.1M HCl, which effects a pH change from 1.2–7.4, was considered suitable for the *in vitro* evaluation of drug release from Eudragit S microparticles, designed to deliver drug to the ileo-colonic region.
3.2.3 Dissolution apparatus used in this study

The apparatus used in this study was the USP II paddle apparatus. Throughout the course of the study two different USP II apparatus were used, the only difference between the two being the method by which the samples were collected and/or analysed.

Automated sampling and manual UV analysis

A USP II dissolution apparatus (model PTWS, Pharma Test, Hainburg, Germany) was connected to an automated sampler (model PTFC-1, Pharma Test, Hainburg, Germany) which withdrew 3mL samples at predetermined time intervals. Samples were analysed manually using a UV-Vis spectrophotometer (model 554, Perkin Elmer, Ueberlingen, Germany).

Online UV analysis

A second USP II dissolution apparatus (model PTWS, Pharma Test, Hainburg, Germany) was connected to an online UV-Vis spectrophotometer (Cecil CE2021, Cambridge, UK), which was capable of drawing samples into the UV cells, using a peristaltic pump (Icalis PC490, Icalis Data Systems, Finchampstead, UK) at predetermined time intervals and returning the sample to the dissolution vessel following UV analysis. Online UV analysis allows drug release to be assayed without loss of dissolution media (aside from that which is lost to evaporation), which permits more samples to be taken and maintains constant conditions for drug release and dissolution throughout the experiment which may be important when working with poorly water soluble drugs for which sink conditions are difficult to achieve.
Section One: The *in vitro* evaluation of pH-sensitive Eudragit microparticles for site-specific drug delivery to the proximal small intestine

3.3 Methods

3.3.1 Determination of saturation solubility of prednisolone

Excess amounts of prednisolone were added to 10mL quantities of 0.1M HCl and pH 6.8 phosphate buffer in vials, and saturated solutions were prepared by agitation of the vials in a water bath at 37°C for 72 hours. After 72 hours had elapsed, the contents of the vials were filtered through Whatman no. 6 filter paper, and 4mL of the filtrate was diluted volumetrically to 100mL with the appropriate solvent (pre-warmed to 37°C). The UV absorbance of the filtrate was read at 248nm, and the concentration of the filtrate calculated with reference to a standard curve of prednisolone dissolved in 0.1M HCl or pH 6.8 buffer.

The calculated value for the saturation solubility of prednisolone in 0.1M HCl and pH 6.8 phosphate buffer was used to ensure sink conditions in the *in vitro* dissolution studies. That is, the amount of prednisolone contained in the dosage form being tested, when completely dissolved in the dissolution medium, did not exceed 20% of the saturation solubility of prednisolone in the same dissolution medium.

3.3.2 Formulation and *in vitro* evaluation of prednisolone-loaded Eudragit L microparticles

Eudragit L microparticles were prepared in an identical manner to section 2.9.1, except that prednisolone was firstly dissolved in ethanol at levels 30:1, 20:1, 10:1 and 5:1 polymer to drug loading, prior to dissolution of polymer.

To investigate the effect of pH on prednisolone release from Eudragit L microparticles, a 0.1M HCl solution, and pH 6.0, 6.2, 6.4, 6.8 and 7.2 phosphate buffer were prepared as described in section 3.2.2. Nine hundred millilitres of each dissolution media was equilibrated to 37 ± 0.5°C, and 300mg Eudragit L/prednisolone (30:1) microparticles were introduced directly into the dissolution vessels. Three millilitre samples were withdrawn and filtered through 10μm diameter Pharmatest filters, and assayed spectrophotometrically...
at 248nm for prednisolone content, with reference to a standard curve, every 10 minutes for 60 minutes.

To investigate the effect of prednisolone loading on release rate from Eudragit L microparticles, 300mg of Eudragit L/prednisolone microparticles (30:1, 20:1, 10:1 and 5:1 polymer:drug loading) were introduced directly into 900mL of pH 6.0 phosphate buffer, prewarmed to 37 ± 0.5°C. Three millilitre samples were withdrawn, filtered and analysed for prednisolone content every 5 minutes for 60 minutes.

Prednisolone release from 300mg of Eudragit L microparticles (20:1, 10:1 and 5:1 polymer:drug loading) were investigated using the pH-change method described in section 3.2.2. Three hundred milligrams of microparticles were filled into size 0 gelatin capsules and secured inside stainless steel sinkers. Samples were filtered and analysed by online UV spectrophotometry at 248nm, after 60 and 120 minutes incubation in 750mL 0.1M HCl, and every 5 minutes following the pH change using online UV analysis. For comparison, the prednisolone release from a commercial polyvinyl acetate phthalate-coated tablet, Deltacortril Enteric® (Deltacortril Enteric® Patient Information Leaflet), was also studied under identical conditions. Due to the lower dissolution threshold of polyvinyl acetate phthalate (PVAP) compared to Eudragit L (pH 5.0 and 6.0, respectively), it is anticipated that prednisolone release would be more rapid from the Deltacortril Enteric® tablet than from the Eudragit L microparticles.

To gain a more accurate pH-change release profile, 300mg of Eudragit L microparticles (5:1 polymer:drug loading) were investigated using the pH-change method described in section 3.2.2, except that samples were analysed by online UV spectrophotometry at 248nm every 5 minutes during incubation in 0.1M HCl, and every 1 minute following the increase in pH from 1.2-6.8. The pH-change release profile was also evaluated by withdrawing samples manually and filtering through a 0.2μm filter, before reading the UV absorbance manually at 248nm after 2 hours incubation in acid, and every 1 minute (for 10 minutes) following the increase in pH to 6.8.
3.4 Results

3.4.1 Saturation solubility of prednisolone

The saturation solubility of prednisolone was calculated to be 539.2mg/L in 0.1M HCl and 572.5mg/L in pH 6.8 phosphate buffer, which is within the range of solubility listed in the British Pharmacopoeia 2007 (see Table 3.3).

3.4.2 Effect of pH on the rate of prednisolone release from Eudragit L microparticles

Eudragit L microparticles suspended readily throughout the dissolution media due to the agitation of the paddle. The prednisolone release profiles, from 0 to 60 minutes, of the Eudragit L/prednisolone (30:1) microparticles at pH 1.2 to 7.2 are shown in Figure 3.1.

![Figure 3.1: In vitro prednisolone release from Eudragit L microparticles at pH 1.2 to 7.2](image)

At pH 1.2, approximately 2.5% of prednisolone was released after 60 minutes. It is thought that the retardation of prednisolone release at pH 1.2 occurred due to the matrix structure of the microparticles, throughout which prednisolone was dispersed as small pockets surrounded by Eudragit L, which due to its insolubility at pH 1.2 prevented release of entrapped prednisolone. At pH 6.0, drug release was quite slow, as a result of slow polymer dissolution at the threshold pH of Eudragit L. At pH values greater than 6.0, drug release rate increased with pH, and at pH ≥ 6.4, prednisolone release was complete within 10 minutes.
3.4.3 Effect of drug loading on prednisolone release from Eudragit L microparticles

The prednisolone release profiles, from 0 to 60 minutes, of the Eudragit L/prednisolone microparticles (5:1 to 30:1 polymer:drug loading) at pH 6.0 are shown in Figure 3.2.

Figure 3.2: In vitro prednisolone release from Eudragit L microparticles (5:1 to 30:1 polymer:drug loading) at pH 6.0

Prednisolone release from Eudragit L microparticles increases with drug loading. At higher drug loading, more prednisolone is expected to be present on the surface of the microparticles, which once dissolved results in fluid filled channels which facilitate further drug dissolution and also increases the surface area available for polymer dissolution. This process probably proceeds more rapidly at higher drug loading because Eudragit L dissolution is slow at pH 6.0, while dissolution of prednisolone is pH-independent and occurs more rapidly. As the fluid filled pores progress into the matrix of the microparticles, at high drug loading, they may even serve to break up the Eudragit L microparticle matrix thus presenting an even larger surface area of drug to the dissolution media resulting in a rapid completion of drug dissolution from the microparticles.
3.4.4 Evaluation of prednisolone release from Eudragit L/prednisolone microparticles using a pH change dissolution method (5-minute sampling interval)

The pH-change (pH 1.2-6.8) prednisolone release profiles for Eudragit L/prednisolone (5:1, 10:1 and 20:1 polymer:drug loading) microparticles are shown in Figure 3.3, below, along with the pH-change release profile for the commercial product, Deltacortril Enteric®.

![Graph showing prednisolone release profiles for different microparticle formulations](image)

**Figure 3.3:** In vitro prednisolone release from Eudragit L microparticles (5:1 to 20:1 polymer:drug loading) and Deltacortril Enteric® using the pH change method, pH 1.2-6.8. Error bars have been omitted for clarity.

The gelatin capsules containing the Eudragit L microparticle formulations dissolved rapidly, within 5 minutes, in 0.1M HCl and released the microparticles which readily suspended due to the agitation of the propeller.

All formulations pass the specification of the USP 24 for enteric coated products of <10% drug release after 2 hours in acid, and 75% release within 45 minutes of the pH change. The release profiles of the microparticle formulations were similar, with 8.1, 6.2 and 7.3% prednisolone released from the 5:1, 10:1 and 20:1 microparticles, respectively, after 2 hours' incubation in acid. All microparticle formulations released 100% of entrapped prednisolone extremely rapidly, within 5 minutes of the pH being increased from 1.2 to 6.8. This confirms the importance of being able to accurately change the pH of the dissolution vessel without the need for adjustment of pH with 2M NaOH or 2M HCl, as in the time the
USP allows for pH correction, drug release was already complete from the microparticle formulations.

The release profile of Deltacortril Enteric® was different to the Eudragit L microparticle formulations. Drug release in acid was significantly lower for the tablet formulation which released only 1.0% of prednisolone after 2 hours in acid. This was attributed to the inherent differences between the commercial formulation, a film-coated tablet, and the matrix microparticle formulation. The film coat is expected to form a comprehensive barrier to the ingress of acidic media, however, for the microparticle formulations drug is expected to be present throughout the entirety of the matrix, including the outer surface. Combined with a large surface area:volume ratio, release of some drug from close to the surface of the microparticles was not surprising.

Following the pH change, the rate of prednisolone release from the Deltacortril Enteric® tablet was slower than from the Eudragit L microparticles (25 minutes for 100% release, compared to 5 minutes for microparticle formulations), which was somewhat surprising given the difference in the dissolution threshold pH of PVAP (pH 5.0) and Eudragit L (pH 6.0). However, this was probably due to the time required for neutralisation, ionisation, disruption and dissolution of the pH-sensitive coating before which no drug release can occur. The faster release of prednisolone, following the pH change, from the microparticles may be attributable to the much larger surface area:volume ratio. If neutralisation of the microparticle surface is required before dissolution can occur, this would be expected to be completed more rapidly.

3.4.5 Evaluation of prednisolone release from Eudragit L/prednisolone microparticles using a pH change dissolution method (1-minute sampling interval)

The dissolution profile of the Eudragit L/prednisolone (5:1) microparticles determined using the automated sampling method is shown in Figure 3.4.
Following dissolution of the gelatin capsule, a small burst release of approximately 5% of entrapped prednisolone occurred from the Eudragit L microparticles, which slowly increased to 8.1% after 2 hours incubation in acid. It is thought that only prednisolone at, or close to, the outer surface of the microparticles which is not completely surrounded by Eudragit L is released during incubation in acid. Following the increase in the pH of the dissolution media from 1.2 to 6.8, a peak was seen in the apparent drug release profile of approximately 150% release at 121 minutes, followed by a reduction in apparent drug dissolution over the next 2 minutes. The "false peak" at 121 minutes was attributed to adherence of a number of microparticles to the surface of the Pharmatest filter attached to the sampling probe, which occurred as dissolution media containing the suspended Eudragit L microparticles was drawn through the filter. These microparticles did not dissolve during the acid phase of the dissolution experiment, but did so following the pH change. However, dissolution of microparticles from the filter surface was slower than those suspended in the pH 6.8 dissolution media due to a lower exposed surface area to the dissolution media. The false high readings from 121 to 125 minutes are therefore a due to a combination of absorbance due to the prednisolone released as Eudragit L microparticles dissolve in the dissolution vessel, and due to microparticles dissolving and releasing prednisolone as pH 6.8 media is drawn through the dissolution filter. As the volume of media contained in the tubing between the filter and UV cell is low (estimated to be in the region 10-15mL), this resulted in a disproportionately high value for prednisolone release,
which fell to a plateau of approximately 100% once dissolution of microparticles adhering to the filter was complete.

To confirm this hypothesis for the “false peak” of percentage prednisolone released following the pH change, the dissolution method was repeated with manual sampling after 2 hours’ incubation in acid, followed by sampling every 1 minute following the pH change. The dissolution profile of the Eudragit L/prednisolone (5:1) microparticles determined using the manual sampling method is shown in Figure 3.5.

![Graph showing prednisolone release](image)

**Figure 3.5:** *In vitro* prednisolone release from Eudragit L/prednisolone (5:1) microparticles using the pH change method, pH 1.2-6.8 (manual sampling and analysis)

After 2 hours exposure to 0.1M HCl, 6.9% prednisolone was released from the microparticles. Following pH change, prednisolone release was rapid, being 92.4 and 99.5% complete at 121 and 122 minutes, respectively. The absence of a “false peak” in the dissolution profile confirms that adherence of the Eudragit L microparticles to the Pharmatest dissolution filters during the acid phase was resulting in the “false peak” following the pH change. However, although the manual dissolution method provides a more accurate *in vitro* dissolution profile for the Eudragit L microparticles than the automated method, due to the difficulty of running multiple samples simultaneously using the manual method, it did not represent a practical solution and therefore could not be used in future dissolution studies.
3.5 The *in vitro* evaluation of pH-sensitive Eudragit L55 microparticles for site-specific drug delivery to the small intestine using the pH change method

Introduced in 1985, Eudragit L55 has largely superseded Eudragit L as the acrylic enteric-coating polymer of choice for proximal small intestinal drug delivery. This is in part due to its slightly lower dissolution threshold pH of 5.5 (compared to 6.0 for Eudragit L), but also because it is known to form flexible and non-brittle films. The *in vitro* dissolution behaviour of Eudragit L55/prednisolone microparticles will now be investigated and compared to Eudragit L/prednisolone microparticles.

3.5.1 Methods

Eudragit L55 microparticles were prepared in an identical manner to section 2.9.1, except that prednisolone (5:1 polymer:drug loading) was firstly dissolved in ethanol prior to dissolution of polymer. Three hundred milligram samples of the resultant microparticles were filled into size 0 gelatin capsules, and secured inside stainless steel sinkers. The pH-change dissolution methodology (pH 1.2-6.8) described in section 3.2.2 was used, with automated sampling and analysis by online UV spectrophotometry every 5 minutes for 120 minutes in acid, and every five minutes for 360 minutes following the pH change.
3.5.2 Results and discussion

The prednisolone release profile for Eudragit L55/prednisolone (5:1) microparticles, at pH 1.2-6.8, is shown in Figure 3.6.

![Figure 3.6: In vitro prednisolone release from Eudragit L55/prednisolone (5:1) microparticles using the pH change method, pH 1.2-6.8](image)

The behaviour of the Eudragit L55 microparticles in the dissolution vessels was observed to be very different to the Eudragit L microparticles. Rather than suspending freely following capsule disintegration, the Eudragit L55 microparticles were retained within the sinker as a single aggregate, which dissolved slowly following the pH change from 1.2 to 6.8. Differences in degree of aggregation can be explained by the different physicochemical properties of the two polymers, which are influenced by their chemical structures (shown in Figure 3.7).

![Figure 3.7: Chemical structure of Eudragit L55 and Eudragit L](image)
Although in both Eudragit L and Eudragit L55, the ratio of carboxylic acid to ester groups is 1:1, the different monomers from which they are comprised impart different physicochemical properties on the two polymers. The excellent film-forming behaviour of Eudragit L55 can be related to the relatively low glass transition temperature (Tg) of 107°C, and therefore increased molecular mobility, compared to Eudragit L which has a Tg of ~200°C (Pharma Polymers, 2001). The lower Tg of Eudragit L55 compared to Eudragit L is due to the ethyl acrylate content which due to the absence of a methyl group attached to the polymer backbone allows more opportunity for rotation of the polymer backbone and interaction between different polymer chains. Although the Tg of Eudragit L55 is still above the temperature of the dissolution media in which it is immersed, it is thought that water is acting as a plasticiser to lower the Tg further, resulting in increased molecular mobility at 37°C which results in microparticle aggregation.

Differences are seen between the prednisolone release from Eudragit L55 and L microparticles at both pH 1.2 and 6.8. Despite the fact that microparticle aggregation in acid reduces the surface area available for dissolution, the L55/prednisolone microparticles released approximately double the amount of prednisolone in acid (16.8% compared to 8.1%), and therefore did not meet the USP 24 specification for enteric-coated dosage forms. It is thought that this was due to increased molecular mobility increasing prednisolone release close to the surface of the microparticles. Following the increase in pH from 1.2 to 6.8, prednisolone release is slower than from Eudragit L55 than Eudragit L microparticles (prednisolone release is complete 240 and 2 minutes after pH change, respectively), due to a greatly reduced surface area for polymer dissolution. The variability in the release profiles is due to differences in the structure of the aggregate that forms in the six replicate vessels, which result in differences in the extent to which dissolution media can penetrate the matrix of the microparticle aggregate and effect drug release.

There have been previous reports of aggregation of multiparticulate Eudragit L55 formulations. De Jaeghere et al., (2001) observed that Eudragit L55 spray dried microparticles exhibited poor wettability and aggregation; vigorous vortex mixing and sonication was required to break up the microparticle aggregates prior to particle sizing. Similarly, ethyl acrylate-derived polymers are often observed to be “tacky” during film-coating or subsequent curing. Erdmann et al., (2003) investigated a variety of anti-tack
agents to reduce the plastic behaviour of poly (ethyl acrylate, methyl methacrylate). Rudolph et al., (2001) reported that aggregation of pellets coated with Eudragit FS, a polymer with a dissolution threshold pH of 7.0 which also contains ethyl acrylate monomer groups, during curing at the relatively low temperature of 40°C. Problems due to tackiness may be worsened by the addition of plasticisers to polymer dispersions, which are utilised to lower the minimum film forming temperature of a polymer. In general, it seems that a difficult balance has to be struck between formation of a flexible, non-brittle film, and preventing aggregation of the dosage form during coating and/or curing. Aggregation of Eudragit L55 microparticles in the dissolution bath is accentuated by the large surface area available for polymer:polymer interaction. Interestingly, however, Lorenzo-Lamosa et al., (1997) did not describe any aggregation problems for cefuroxime axetil-loaded Eudragit L55 microparticles, also produced using the emulsification solvent evaporation method, although dissolution was only carried out in pH 5.2 and 6.0 phosphate buffer, and not at more acidic pH.

3.6 Attempts to reduce the aggregation behaviour of Eudragit L55 microparticles by the addition of talc and Eudragit L

During film coating of tablets, capsules and pellets with Eudragit L55, the addition of talc as an anti-tack agent has been recommended to prevent sticking together of individual dosage forms during the coating or curing process (Pharma Polymers, 2001). It was therefore decided to incorporate talc into the Eudragit L55 microparticles to attempt to reduce particle aggregation following capsule dissolution. It was also decided to investigate Eudragit L55/L blends, as both polymers can be used for site specific delivery of drugs to the small intestine, and no aggregation of Eudragit L microparticles had been observed in the dissolution bath.

3.6.1 Methods

Talc was added to Eudragit L55 microparticles at 10% (0.300g), 25% (0.750g) and 50% (1.500g) of dry polymer weight. Eudragit L55/Eudragit L blends were prepared in the ratio (75:25, 50:50, 25:75, 10:90, 5:95). All microparticles were manufactured by the method described in section 2.9.1, and the ratio of total polymer:prednisolone was 5:1 for each formulation. Samples were analysed by SEM as described in section 2.3.1. Samples of microparticles containing a theoretical quantity of 50mg drug were filled into size 0 gelatin
capsules, and drug release was evaluated using the pH-change dissolution method, pH 1.2-6.8, sampling every 5 minutes during the acid phase, and every 2 minutes following the pH change.

3.6.2 Results
The SEMs of Eudragit L55/talc (10% and 50%) microparticles are shown in Figure 3.8 and 3.9.

![Figure 3.8: SEM of Eudragit L55 microparticles (+ 10% talc)](image1)

![Figure 3.9: SEM of Eudragit L55 microparticles (+ 50% talc)](image2)

The SEMs of the L55/prednisolone/talc microparticles show an increased presence of talc protruding from the surface of the particles with increasing talc content within the particles. It is anticipated that talc will be present throughout the matrix structure of the particles as well as on the surface. Even at 50% talc content, aggregation of Eudragit L55 microparticles in 0.1M HCl was not prevented.

The in vitro dissolution profile of Eudragit L55 microparticles, with and without the incorporation of 10% talc, is shown in Figure 3.10. When talc was incorporated at levels >10%, the filters of the dissolution bath became blocked, and it was not possible to determine the release rate of prednisolone. However, all formulations were observed to aggregate following capsule dissolution.
Although there was no apparent difference in degree of aggregation of the L55 microparticles, with and without talc, release in acid is slightly greater when talc is present (20.8% versus 16.8%). Similarly, release rate following pH change seems to be slightly more rapid, suggesting that the presence of talc may be reducing aggregation and increasing the surface area available for polymer dissolution and drug release.

Figure 3.11 compares the in vitro dissolution profile of Eudragit L55/L blended microparticles. The dissolution profiles of Eudragit L55/L (10:90 and 5:95) are omitted for clarity, and Eudragit L55/prednisolone microparticles have been included for ease of comparison from Figure 3.6.
Figure 3.11: In vitro prednisolone release from Eudragit L55/L microparticles (5:1 polymer:drug loading) using the pH change method, pH 1.2-6.8

Microparticle aggregation, following the dissolution of the gelatin capsule, was observed when the ratio of Eudragit L55:Eudragit L ratio was 1:1 or greater whereas at ratios of Eudragit L55:Eudragit L of 1:3 or below, prednisolone release in acid was < 10%, and release was rapid following the pH change, meeting the USP 24 specification for enteric coated products. However, no improvement was possible on the release profile of Eudragit L/prednisolone (5:1) microparticles.

3.7 Section One Conclusions

The rate of prednisolone dissolution from the Eudragit L microparticles increased with both pH and drug loading. Despite having a higher dissolution threshold pH, Eudragit L microparticles show a greater potential for drug targeting to the proximal small intestine as they suspend freely without aggregation in acid, while retarding release of prednisolone to within pharmacopoeial limits (<10%), even at a relatively high polymer:drug loading of 5:1.
Section Two: *In vitro* evaluation of pH-sensitive Eudragit microparticles for site-specific drug delivery to the ileo-colonic region

3.8 Methods

3.8.1 Microparticle fabrication

Prednisolone-loaded Eudragit S microparticles were prepared in an identical manner to that described in section 2.9.1, except that prednisolone was firstly dissolved/dispersed in ethanol at 30:1 to 2.25:1 polymer:drug loading.

Prednisolone-loaded tailored release (delayed-extended release) microparticles (5:1 polymer:drug loading) were prepared by blending Eudragit S with the water insoluble polymers ethylcellulose N7 and Eudragit RS, using the method described in section 2.9.1. Ethylcellulose N7 was incorporated into the microparticles by dissolving 0.6g prednisolone in 30mL ethanol followed by 1.5g ethylcellulose N7 and 1.5g Eudragit S. Eudragit RS was incorporated into the microparticles by dissolving 0.6g prednisolone in 20mL acetone/10mL ethanol followed by 1.5g Eudragit RS and 1.5g Eudragit S.

Bendroflumethiazide, budesonide and paracetamol-loaded Eudragit S microparticles (5:1 polymer:drug loading) were prepared by the method described in section 2.9.1 after dissolving 0.6g of the respective drug and 3g of Eudragit S in 30mL ethanol.

3.8.2 *In vitro* dissolution testing and SEM

Prednisolone release from Eudragit S/prednisolone microparticles (30:1) was tested at pH 1.2, 6.8, 7.0, 7.2 and 7.4, using 0.1M HCl and phosphate buffer solutions as described in section 3.2.2, by introducing 300mg samples of microparticles directly into the dissolution media. Three millilitre samples were withdrawn, filtered and analysed for prednisolone content at 0, 5, 10, 15, 20, 30, 60, 120, 240, 480 and 720 minutes.

The effect of drug loading on prednisolone release rate was determined using the method described to achieve a pH change from 1.2-7.4, as described in section 3.2.2. All microparticles were filled into size 0 gelatin capsules, and secured inside sinkers.
Prednisolone release was determined by online UV analysis after 60 and 120 minutes in acid, and every 1 to 5 minutes following pH change.

Eudragit S/prednisolone (30:1) microparticles were also evaluated using a progressive pH change method. Microparticles were initially tested in 750mL 0.1M HCl for 2 hours after which time the pH was increased to 6.8 as described in section 3.2.2. After 3, 4 and 5 hours, respectively, the pH was increased to 7.0, 7.2 and 7.4 by the dropwise addition of 5M NaOH. Prednisolone release was determined by online UV analysis.

Eudragit RS/Eudragit S (1:1) and ethylcellulose N7/Eudragit S (1:1) microparticles were evaluated using the pH change method (pH 1.2-7.4) described in section 3.2.2, and prednisolone release determined by online UV analysis every 5 minutes.

Bendroflumethiazide, budesonide and paracetamol-loaded Eudragit S microparticles of 5:1 polymer:drug loading, containing 9.5mg, 33.3mg and 50.0mg theoretical content of model drugs, respectively, were evaluated using the pH change method (pH 1.2-7.4) described in section 3.2.2. All drugs were detected by online UV spectrophotometry; bendroflumethiazide at 324nm, budesonide at 247nm and paracetamol at 243nm. Drug release profiles were calculated with reference to a standard curve at the respective UV wavelength used for the detection of each drug.

SEM was carried out as described in section 2.3.1.
3.9 Results

3.9.1 Effect of pH on rate of prednisolone release from Eudragit S microparticles

Figure 3.12, below, compares the in vitro dissolution profile of Eudragit S/prednisolone (30:1) microparticles at pH 1.2 to 7.4.

Figure 3.12: In vitro prednisolone release from Eudragit S microparticles at pH 1.2 to 7.4

In common with Eudragit L microparticles, prednisolone release rate from Eudragit S microparticles increases with the pH of the dissolution media. Only 1% prednisolone release had occurred after 12 hours in 0.1M HCl. At pH 6.8, 56% of prednisolone was released from the microparticles after 12 hours. The time for 100% release was approximately 4, 2 and 1 hour(s) at pH 7.0, 7.2 and 7.4 respectively.

3.9.2 Effect of drug loading on rate of prednisolone release from Eudragit S microparticles, using a pH change method (pH 1.2-7.4)

The pH change dissolution profile of Eudragit S/prednisolone microparticles (30:1 to 2.25:1 drug loading) at pH 1.2-7.4 is shown in Figure 3.13.
A pH-responsive release profile is observed at all drug loadings, with prednisolone release in acid being restricted to less than 5% after 2 hours incubation in acid for all formulations of drug loading 2.5:1 and below (and being less than 1% at drug polymer:drug loadings of less than 10:1). Eudragit S microparticles appear to be more resistant to prednisolone release after 2 hours at pH 1.2 than Eudragit L microparticles, a result that can possibly be attributed to the larger size of the former microparticles, and therefore a lower surface area to volume ratio, as viewed by SEM (see Figures 2.32 and 2.34).

Eudragit S/prednisolone 2.25:1 microparticles fail the USP test for enteric coated products, given that 50.0% prednisolone was released after 2 hours in acid. SEM reveals that the 2.25:1 (Figure 3.14) microparticles exhibit a different morphology to microparticles of lower drug loading (Figure 3.15 and 3.16). Microparticles of drug loading 2.25:1 are of similar size and are approximately spherical, however they do not possess the smooth surface morphology that is evident for microparticles of lower prednisolone loading. The flaky surface morphology is possibly due to crystalline prednisolone which, if present extensively on the surface, may account for the burst release of these microparticles in acid. At a polymer:drug loading of 2.25:1, prednisolone is present at a level above its saturation solubility in ethanol (calculated to be 1052.9g/30mL at room temperature), which may
result in prednisolone crystallising out before the Eudragit S, resulting in the presence of increased amount of crystalline drug on the surface of the microparticles.

Figure 3.14: SEM of surface topography of Eudragit S/prednisolone (2.25:1) microparticles

Figure 3.15: SEM of surface topography of Eudragit S/prednisolone (3:1) microparticles

Figure 3.16: SEM of surface topography of and cross section of Eudragit S/prednisolone (5:1) microparticles

Following the increase in pH from 1.2 to 7.4, the rate of prednisolone dissolution from the microparticles increased with drug loading, taking approximately 45 minutes for complete release of prednisolone from Eudragit S microparticles of 30:1 prednisolone, and 5 minutes for particles of 5:1 drug loading and higher (the dissolution profile contains the same false peaks described for Eudragit L microparticles in section 3.4.5). The rapid dissolution of prednisolone at pH 7.4 could possibly be due to the encapsulation of prednisolone within the microparticle matrix as the amorphous form, as no crystallinity is evident in Figure 3.16, which shows a cross section through Eudragit S/prednisolone 5:1 microparticles. The
physical form of prednisolone within the microparticles will be investigated in Chapter Four.

3.9.3 Prednisolone release from Eudragit S microparticles (pH 1.2-6.8-7.0-7.2-7.4)

Although the pH change method from 1.2-7.4 was considered adequate for the optimisation of drug loading experiments, a multiple pH change profile potentially mimics the in vivo transit of microparticles from the stomach to the ileo-colonic region more closely. The in vitro prednisolone release from Eudragit S/prednisolone (30:1) microparticles is shown in Figure 3.17 using the progressive pH change method.

![Figure 3.17: In vitro prednisolone release from Eudragit S microparticles (5:1 polymer:drug loading) using a progressive pH change method](image)

It is interesting to note that, assuming a small intestinal transit time of 4 hours, and a relatively constant increase in pH from 6.8 to 7.4, release of a significant portion of prednisolone from the microparticles will have occurred prior to arrival in the colon, in the region of the mid to distal small intestine. For some indications, such as the topical treatment of Crohn’s disease which affects both the mucosa of the distal small intestine as well as the colon, such a release profile may be close to ideal. Other indications such as the treatment of ulcerative colitis or colon carcinoma, or the delivery of protein and peptide drugs may require drug delivery specifically to the colonic region. To investigate the possibility of avoiding a premature drug release and possibility of complete drug absorption
into the systemic circulation prior to arrival of the microparticles in the ileo-colonic region, microparticles were fabricated from a mixture of Eudragit S, and the water-insoluble polymers ethylcellulose N7 and Eudragit RS.

3.9.4 Tailored-release (delayed-extended) release microparticles for topical drug delivery to the colon
Eudragit RS/Eudragit S and ethylcellulose N7/Eudragit S microparticles appeared to be slightly aggregated compared to Eudragit S microparticles. The \textit{in vitro} dissolution profiles (pH 1.2-7.4) of Eudragit RS/S (1:1) and ethylcellulose N7/Eudragit S (1:1) microparticles of polymer:drug loading (5:1) are shown in Figure 3.18.

![Figure 3.18: In vitro prednisolone release from Eudragit RS/Eudragit S and ethylcellulose N7/Eudragit S microparticles (5:1 polymer:drug loading) using the pH change method, pH 1.2-7.4](image)

The \textit{in vitro} release profile of Eudragit RS/S (1:1) microparticles is similar to that of Eudragit S microparticles, with prednisolone release being restricted to less than 10% after 2 hours in acid, and being approximately 85% complete 45 minutes after the increase in pH to 7.4. However, the release profile of the ethylcellulose N7/Eudragit S microparticles is significantly different from the Eudragit S microparticles, with a burst release of approximately 40% of entrapped prednisolone after 2 hours in acid, and an extended release of the remainder of the drug following the pH change. The burst release in acid was attributed to prednisolone on the surface of microspheres incorporating ethylcellulose N7.
(see Figure 3.19), which was not observed on those containing Eudragit RS (see Figure 3.20), and is probably related to a slower precipitation of ethylcellulose than Eudragit S or RS. The more rapid prednisolone release from the microspheres containing Eudragit RS, following the pH change, may be due to the rapid swelling of this polymer, which would occur following dissolution of Eudragit S at pH 7.4.

Figure 3.19: SEM of surface of ethylcellulose N7/Eudragit S/prednisolone (1:1:0.4) microparticles

Figure 3.20: SEM of surface of Eudragit RS/Eudragit S/prednisolone (1:1:0.4) microparticles

3.9.5 In vitro release of drugs with different physicochemical properties from Eudragit S microparticles

The in vitro dissolution profile (pH 1.2-7.4) of the bendroflumethiazide, budesonide and paracetamol-loaded Eudragit S microparticles are shown in Figure 3.21. The chemical structures and physicochemical properties of bendroflumethiazide, budesonide, paracetamol and prednisolone are shown in Figure 3.22 and Table 3.3, respectively.
Figure 3.21: In vitro release of bendroflumethiazide, budesonide and paracetamol from Eudragit S microparticles (5:1 polymer:drug loading) using the pH change method, pH 1.2-7.4

Figure 3.22: Chemical structures of the model drugs bendroflumethiazide, budesonide, paracetamol and prednisolone (adapted from the British Pharmacopoeia, 2007)
Like prednisolone, release of bendroflumethiazide and budesonide was restricted to less than 10% after 2 hours’ incubation in 0.1M HCl, being 1.1 and 6.8%, respectively. This was particularly surprising for bendroflumethiazide which is a weakly basic drug, and as such is expected to be more soluble at acidic pH. However, 76.2% of the weakly acidic drug, paracetamol, was released under the same conditions, which was probably due to the lower molecular weight and higher aqueous solubility of this molecule in comparison to the other model drugs evaluated.

Following the increase in pH from 1.2 to 7.4, a false high peak was observed for budesonide which was similar to the false peak observed in the dissolution profile of the chemically similar model drug prednisolone, which was due to adherence of the microparticles to the dissolution filter during incubation in acid. However, due to the lower aqueous solubility of budesonide compared to prednisolone, it took longer for the budesonide which had adhered to the filter to completely dissolve, and false high values are detected for 20 minutes following the pH change. Following the pH change, bendroflumethiazide dissolution was also complete within 25 minutes, despite the weakly basic nature and low aqueous solubility of this drug. The release profile of
bendroflumethiazide shows promise for the delivery of poorly soluble basic molecules to the small intestine, which when administered as immediate release formulations are understood to readily dissolve in the stomach contents, but precipitate out in the neutral environment of the small intestinal lumen as a poorly soluble form, which can impact negatively on drug bioavailability (Kostewicz et al., 2002).

3.10 Section Two Conclusions
In common with Eudragit L microparticles, prednisolone release rate from Eudragit S microparticles was observed to increase with pH and drug loading. Prednisolone release in acid was restricted to <10% at drug loadings of 2.5:1 and below. The incorporation of a water-insoluble polymer into the Eudragit S matrix retarded drug release at pH 7.4. It was also possible to achieve a pH-responsive release of a range of model drugs with different physicochemical properties, although it was not possible to retard the release of the low molecular weight, hydrophilic drug, paracetamol, in acid.
Section Three: Preparation and in vitro evaluation of a conventional delayed release pellet formulation to target the proximal small intestine and ileo-colonic region

3.11 Introduction

In sections one and two of this chapter, the potential of pH-sensitive Eudragit microparticles to deliver a model drug, prednisolone, to the proximal small intestine and ileo-colonic region, respectively, has been investigated using in vitro dissolution testing. The aim of this section is to fabricate, by conventional methods, a pellet dosage form designed to deliver drugs to these site-specific regions of the gastrointestinal tract, and to compare the in vitro dissolution of these conventional dosage forms to the pH-sensitive microparticles.

Pellets of size 1-1.5mm are conventionally prepared by extrusion followed by spheronisation or pelletisation. Extrusion of a blend of drug and suitable processing excipients to form a cylindrical extrudate follows heating above the melting temperature of the blend, i.e. hot-melt extrusion (Young et al., 2002; 2005) or following wet-massing with a liquid binder, i.e. conventional extrusion (Newton, 1990; Yuen et al., 1993; Peh and Yuen, 1995; Krogars et al., 2000; Sousa et al., 2002). The method of extrusion chosen is influenced by both the physicochemical properties of the drug, including solubility, sensitivity to heat or water, and the availability of processing equipment. The cylindrical extrudate formed during the extrusion step is then chopped and/or spheronised into pellets of approximately spherical form.

Conventional extrusion followed by spheronisation was used for the preparation of core pellets in this study as the available equipment could be readily modified for the production of pellets of size <1mm in diameter, which would have a larger surface area:volume ratio than pellets of size 1–1.5mm which are usually prepared by this technique. Pellets of size <1mm in diameter would therefore represent a better formulation for comparison of in vitro dissolution with the pH-sensitive microparticles evaluated in sections one and two. Furthermore, a single batch of core pellets could be coated with different quantities of Eudragit L and S, which would allow the minimum quantity of polymer coat required to
deliver drug selectively to the small intestinal and colonic region, respectively, to be determined. It was anticipated that this would facilitate the formulation optimisation of modified release pellets to achieve a rapid drug release at either small intestinal or colonic pH.

The selection of suitable solid excipients and binder for the wet-massing stage is of critical importance during the extrusion step. The wet mass must be sufficiently plastic to be forced through a die, without sticking to the extruder or itself, while retaining the shape of the die following extrusion. Most drugs when used alone with a binder do not exhibit this property, and so have to be pre-blended with other excipients that permit the extrusion process to occur. One of the most frequently utilised materials in the extrusion spheroidisation process is microcrystalline cellulose which has the ability to absorb large quantities of water, thus becoming malleable while retaining a degree of rigidity. It is commonly combined with the water soluble sugar, lactose, which serves to increase the rate of drug release from the pellets (Newton, 1990).

The resultant extrudate is then processed using a spheroidiser, which usually comprises a rotating cross-hatch plate at the bottom of a closed cylinder which firstly chops the extrudate into pieces of uniform size, which are then rounded into a spherical shape by a combination of the frictional forces of adjacent material and the cross-hatches of the spheroidiser plate. The properties of the initial wet mass also influence the product of the spheroidisation step. Over-wetting of the wet mass prior to extrusion can result in uncontrollable aggregation of the extrudate during the spheroidisation stage. On the other hand, an extrudate that is not sufficiently wet may fragment during spheroidisation (Gamlen, 1985).

3.12 Methods
3.12.1 Preparation of core pellets
Two hundred grams of microcrystalline cellulose, 160 grams of lactose and 110 grams of prednisolone were dry mixed for 5 minutes in a planetary mixer (Kenwood, Havant, Hampshire, UK). Mixing was continued and 250 grams of water was gradually added in 10 to 20 gram portions over a period of 5 minutes. Mixing was continued for a further 5
minutes after the addition of the last of the water. A spatula was used, when required, to remove any material adhering to the sides of the mixing bowl.

The wet powder mass was transferred to a barrel (length 20.30 cm, diameter 2.54 cm) fitted with a die of 0.5 mm diameter and 5.0 mm length using a funnel, and gently consolidated using a Perspex® rod. A piston was inserted into the barrel, and the barrel, die and piston assembly was mounted onto a rigid support positioned beneath the crosshead of the ram extruder (MX 50, J.J. Lloyd, Southampton, Hampshire, UK). The crosshead was driven down at a constant rate of 200 mm/min and the force exerted by the piston recorded as a function of time via a computer. Extrudate was collected in a plastic bag positioned between the die and the support.

The product of the extrusion process was stored in sealed bags and spheronised as soon as possible following the extrusion process. A rotating plate spheroniser (GB Caleva Ltd., Sturminster Newton, UK) with a 20.3 cm radial plate, rotating at 1000 rpm, was used to process the extrudate. The resultant pellets were then dried in a temperature controlled oven at 40°C for 24 hours, and sieved using a nest of British standard sieves with a $\sqrt{2}$ progression ranging from 0.355mm to 1.00mm using a mechanical sieve shaker (Endecott Ltd., London, UK) for 10 minutes. Prednisolone content of core pellets was calculated by dissolving accurately weighed samples (15-20mg) of core pellets in 500mL 0.1M HCl with the aid of sonication, filtering, and analysing the filtrate using UV spectrophotometry at 248nm with reference to a standard curve.

3.12.2 Preparation of coating solutions/suspensions

3.12.2.1 Ethanolic coating solutions

Ethanolic coating solutions of Eudragit L and S were prepared by gradually adding 30.0 grams of the appropriate polymer to 480mL absolute ethanol under magnetic stirring. An emulsion of 0.6 grams Tween 80 and 1.5 grams glycerol monostearate in 20mL water was added, under constant stirring, along with 3.0 grams triethyl citrate. The beaker was covered and stirring continued for 24 hours.
3.12.2 Aqueous coating dispersions
Aqueous dispersions of Eudragit L and S were made prepared according to the manufacturer’s instructions (Pharma Polymers, 2001) as described in Table 3.4.

Table 3.4: Composition of Eudragit L and S aqueous dispersions used for film-coating

<table>
<thead>
<tr>
<th>Eudragit L aqueous dispersion</th>
<th>Eudragit S aqueous dispersion</th>
</tr>
</thead>
<tbody>
<tr>
<td>50.0g Eudragit L100</td>
<td>57.6g Eudragit S100</td>
</tr>
<tr>
<td>17.0g NH₄OH (1.7%)</td>
<td>29.2g NH₄OH (1.7%)</td>
</tr>
<tr>
<td>25.0g Triethyl citrate</td>
<td>28.8g Triethyl citrate</td>
</tr>
<tr>
<td>250.6g water</td>
<td>172.2g water</td>
</tr>
</tbody>
</table>

3.12.3 Film coating
An Aeromatic AG Strata-1 fluidised bed coating machine (ACM Machinery, Tadley, UK) was used for all coating runs, using pellets of size 500-710 μm. The coating process was optimised using placebo lactose/microcrystalline cellulose pellets prior to coating of prednisolone-loaded pellets. Coating was stopped every 25 minutes during coating with the Eudragit L dispersion, and every 15 minutes when coating with the Eudragit S dispersion. The theoretical weight gain (TWG) was calculated at each time point from the weight of the pellets at the beginning and end of each coating step, following curing of the pellet coat at 60°C for a period of one hour.

3.12.4 Scanning electron microscopy
SEM of intact and cross-sectioned pellets was carried out as described in section 2.3.1.

3.12.5 In vitro dissolution testing
In vitro dissolution testing was carried out using the pH-change dissolution method described in section 3.2.2 at pH 1.2-6.8 for Eudragit L coated pellets and pH 1.2-7.4 for Eudragit S coated pellets.
3.13 Results and discussion

3.13.1 Preparation of core pellets

The wet-massed material extruded well, forming short cylindrical strands of extrudate with a smooth surface. A typical force-time profile for the extrusion process is shown in Figure 3.23. The force-time profile indicates that the initial downward force of the piston served to fully consolidate the wet mass, and after a period of approximately 20 seconds, material began to be forced through the die. The force recorded by the computer rose sharply at this point to a maximum of approximately 20kN. This force remained relatively constant for the duration of the extrusion process, indicating that water was retained by the formulation, and not squeezed out of the wet mass.

![Figure 3.23: Typical force-time extrusion profile](image)

A spheronisation time of 3 minutes was required to achieve wet pellets of approximately spherical morphology, within the range of 2–15 minutes required of a good formulation (Vervaet et al., 1995).
The weight of the pellets before and after drying was 487.9 grams and 325.3 grams, respectively. The theoretical prednisolone loading of the core pellets following drying to a constant weight was therefore 22.9%. This was verified by sonicating weighed amounts of pellets in 500mL of 0.1M HCl for 1 hour, filtering the resulting suspension through 0.2µm filters and analysing the filtrate for prednisolone using UV spectrophotometry at 248nm. Calculated prednisolone loadings were 24.50±0.36%, corresponding well to theoretical values. The calculated value of 24.50% was used in all future calculations for drug loading of coated pellets.

The size fractions of the dry pellets determined by sieving analysis are shown in Table 3.5.

Table 3.5: Size distribution of core pellets

<table>
<thead>
<tr>
<th>Sieve fraction</th>
<th>% oversize</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1000µm</td>
<td>5.3</td>
</tr>
<tr>
<td>1000-710µm</td>
<td>41.1</td>
</tr>
<tr>
<td>710-500µm</td>
<td>47.7</td>
</tr>
<tr>
<td>500-355µm</td>
<td>5.6</td>
</tr>
<tr>
<td>&lt;355µm</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Over 90% of the core pellets were below 1000µm in diameter, i.e. in the microparticle size range. The pellets in the 500-710µm size fraction were selected for film coating, as this was the smallest size fraction obtained in sufficient quantity to permit film coating.

3.13.2 Film coating

It was not possible to coat the 500-710µm pellets with either the ethanolic Eudragit L or S coating solutions. This was due to sticking of the pellets to the side of the coating cylinder as a result of build up of static on the surface the fluidised pellets, which was accentuated by the large surface area to mass ratio of the pellets. Earthing the coating chamber or the use of an anti-static gun did not resolve the problem.

It was however possible to coat a 50 gram charge of 500-710µm pellets with the aqueous coating dispersions prepared in section 3.12.2.2, which was thought to be due to dissipation
of charge by the water content of the coating dispersion. However, the bed temperature of the coater had to be kept low - an inlet temperature of 30°C was used - and coating was only possible at a flow rate of 1mL/min.

### 3.13.3 Scanning electron microscopy

Figures 3.24 and 3.26 show the surface topography of pellets coated to a 5.9% and 29.8% TWG with an aqueous dispersion of Eudragit L, respectively. Figures 3.25 and 3.27 show a cross section through pellets taken from the same batches, demonstrating that the coating thickness is approximately 10μm and 40-50μm on pellets coated to a 5.9% and 29.8% TWG, respectively.

![Figure 3.24: SEM of surface of prednisolone-loaded pellet coated to a TWG of 5.9% with Eudragit L aqueous dispersion](image1)

![Figure 3.25: Cross section of prednisolone-loaded pellet coated to a TWG of 5.9% with Eudragit L aqueous dispersion](image2)

![Figure 3.26: SEM of surface of prednisolone-loaded pellet coated to a TWG of 29.8% with Eudragit L aqueous dispersion](image3)

![Figure 3.27: Cross section of prednisolone-loaded pellet coated to a TWG of 29.8% with Eudragit L aqueous dispersion](image4)
3.13.4 *In vitro* dissolution testing

The *in vitro* release profile of core prednisolone-loaded pellets and pellets coated to a 5.9 to 29.8% TWG with Eudragit L, and 6.1 to 26.7% TWG with Eudragit S, containing 50mg prednisolone, are shown in Figures 3.28 and 3.29.

![Figure 3.28: In vitro prednisolone release from core pellets and pellets coated to a TWG of 5.9% to 29.8% with an aqueous dispersion of Eudragit L (pH 1.2-6.8). Error bars are omitted for clarity.](image)

![Figure 3.29: In vitro prednisolone release from core pellets coated to a TWG of 6.1% to 26.7% with an aqueous dispersion of Eudragit S (pH 1.2-7.4). Error bars are omitted for clarity.](image)
Prednisolone release rate from the core pellets decreases with time, taking 180 and 760 minutes for 50% and 90% release, respectively. The release profile follows that which is commonly described for drug release from polymeric matrices, being initially rapid as drug is released from close to the matrix surface, but slowing as dissolution media has to travel further into the matrix, dissolve the drug, and the drug solution diffuse out of the matrix. The slow rate with which prednisolone is released from the core pellets is probably related to the lipophilic nature of the drug, and tortuosity of the diffusion pathway within the pellet matrix.

However, a TWG of 29.8% Eudragit L and 18.8% Eudragit S is required to limit the burst release of prednisolone from coated pellets to less than 10% after 2 hours in 0.1M HCl. This is far in excess of the 4.45% TWG recommended by the manufacturer to impart enteric properties on 7mm diameter tablets when coating with aqueous Eudragit L30D-55 dispersions. This is primarily due to the larger surface area of the 500-710μm pellets in comparison to a tablet formulation. Furthermore, the film coating process had to be carried out close at a temperature of 30°C, and was therefore close to the minimum film forming temperature of the Eudragit L and S aqueous dispersion, which is between 25°C and 35°C (Pharma Polymers, 2001). This may have resulted in a brittle film with a tendency to crack, as can be observed on the surface of the Eudragit L coated pellets (see Figures 3.24 and 3.26) which in turn may have resulted in leakage of prednisolone from the matrix of the coated pellets during the acid phase of the pH-change dissolution.

The similarity of the in vitro release profile for pellets coated with Eudragit S to a TWG of 18.8% and 26.7% TWG suggests that dissolution of polymeric coat is rapid following pH change. If this were not the case, the pellets coated to an 18.8% TWG would be expected to release prednisolone more rapidly than pellets coated to a 26.7% TWG following the pH change.

It is also of interest to note that the Eudragit L coating thickness of 40-50μm required to restrict prednisolone release in acid to <10% is greater than the diameter of the Eudragit L microparticles (approximately 30μm) which can achieve the same result. This is in agreement with previous findings which suggest that organic pH-sensitive film coats form a
more impervious barrier to drug release at pH below the dissolution threshold than do aqueous films (Ibekwe et al., 2006).

3.14 Section Three Conclusions
pH-sensitive pellets of size <1000μm could be produced by extrusion/spheronisation followed by film coating. However, it was not possible to replicate the release profile of the pH-sensitive Eudragit L and S microparticles using this conventional approach. Large quantities of Eudragit L and S were required to restrict prednisolone release to <10% in acid. Thereafter, prednisolone release was slow due to the non-disintegrating matrix structure of the core pellets and low aqueous solubility of prednisolone. The use of hot melt extrusion/micropelletisation of a blend of pH-sensitive Eudragit and prednisolone may result in a formulation more closely resembling the pH-sensitive microparticles tested in section one and two of this chapter, where prednisolone release would be controlled by polymer dissolution, and merits further investigation.
Section Four: Further characterisation of optimised pH-sensitive microparticles for drug delivery to the proximal small intestine and ileo-colonic region

3.15 Introduction

3.15.1 Selection of optimised microparticles for in vivo evaluation

Eudragit L/prednisolone (5:1) microparticles were selected for in vivo evaluation of drug delivery to the proximal small intestine, as they suspended freely, without aggregation, during in vitro dissolution testing and passed the USP test for drug release in acid, while releasing prednisolone completely within 2 minutes following pH change to 6.8. Although Eudragit S microparticles of higher drug loading were capable of controlling prednisolone release in vitro, Eudragit S/prednisolone (5:1) microparticles were selected for in vivo evaluation of drug delivery to the ileo-colonic region as prednisolone release from these microparticles had been shown to be rapid at pH 7.4 (within 5 minutes), and also this would permit a better comparison with the Eudragit L microparticles which were tested at the same drug loading.

3.15.2 Further characterisation of optimised prednisolone-loaded microparticle formulations

The in vitro dissolution of the pH-sensitive microparticles has already been described in this chapter. However, it was also decided to investigate other characteristics that define the efficiency of the microencapsulation process (microparticle yield and prednisolone encapsulation efficiency), determine the possible methods of administration (flow properties), or, perhaps most importantly, are known to affect gastrointestinal transit (particle size and density). The further characterisation of the optimised pH-sensitive microparticles is described below.

3.16 Methods

3.16.1 Microparticle preparation

Three batches of Eudragit S/prednisolone (5:1) microparticles, and 3 batches of Eudragit L/prednisolone (5:1) microparticles were prepared as described in section 2.9.1.
3.16.2 Microparticle yield

The yield was calculated for each of the three batches of each formulation, using equation 3.1.

\[
Yield = \left( \frac{\text{wt. microparticles}}{\text{initial wt. polymer} + \text{initial wt. drug}} \right) \times 100 \quad \text{(Eqn. 3.1)}
\]

The three batches of microparticles were then blended using a Turbula mixer for 15 minutes, and the mixed samples used for all further characterisation.

3.16.3 Calculation of prednisolone encapsulation efficiency

Prednisolone encapsulation efficiency was calculated by dissolving 30mg of microparticles in 10mL methanol with the aid of sonication. Methanolic solutions were made up to 100mL with 0.1M HCl which induced precipitation of the pH-sensitive polymer, after which the samples were sonicated for 10 minutes. Samples were filtered through 0.2\( \mu \)m filters and analysed for prednisolone content spectrophotometrically at 248nm, with reference to a standard curve of prednisolone in 10% methanol in 0.1M HCl. Prednisolone encapsulation efficiency was calculated in triplicate for each formulation.

3.16.4 Characterisation of powder flow using tap density

Sufficient sample material was gently poured into a 10mL measuring cylinder (graduated to 0.1mL), to achieve a fill volume of between 8.0 and 9.0mL. The mass (M) of material and poured volume (\( V_0 \)) were recorded. The cylinder was placed into the adaptor of a Vankel Tap Density Meter (Varian Ltd., Oxford, UK), which was tapped at a speed of 300 taps per minute, from a drop height of 14mm. The volume of material was recorded after 10, 50, 100, 200, 500, 750 and 1250 taps. The volume of material after 1250 taps (\( V_1 \)) was used to calculated the tap density. The initial bulk density, tapped bulk density and Hausner ratio for Eudragit L/prednisolone (5:1) microparticles, Eudragit S/prednisolone (5:1) microparticles and prednisolone were calculated using the equations 3.2 to 3.4.
Initial bulk density (IBD) = $\frac{M}{V_0} (g/cm^3)$  
(Eqn. 3.2)

Tapped bulk density (TBD) = $\frac{M}{V_1} (g/cm^3)$  
(Eqn. 3.3)

Hausner ratio = TBD/IBD  
(Eqn. 3.4)

3.16.5 Density
The density of Eudragit L100 and S100 powder, prednisolone, and Eudragit L/prednisolone 
(5:1) and Eudragit S/prednisolone (5:1) microparticles was measured using a gas 
pycnometer (Accupyc 1330, Micromeritics, Norcross, USA). Each sample was repeated in 
duplicate.

3.16.6 Particle size analysis
Particle size analysis was carried out by laser diffraction using a Malvern Mastersizer X 
with a 45mm lens (Malvern Instruments Ltd., Malvern, UK). A sample of microparticles 
was first suspended in 0.1M HCl by vigorous vortex mixing for 30 seconds, which was 
then added dropwise into the magnetically stirred small volume diffraction chamber, also 
containing 0.1M HCl, of the Mastersizer, until an obscuration of 10 – 15% was achieved. 
Particle size analysis was conducted in triplicate for both Eudragit L and S microparticles, 
and the values for mean volume diameter at the 10%, 50% and 90% undersize are recorded.
3.17 Results and discussion
The appearance of the Eudragit L and S microparticles harvested was similar to those that had been previously produced, i.e a white, free flowing powder was obtained for all six batches.

3.17.1 Microparticle yield
The mean calculated yield for the Eudragit L microparticles was 96.4% (range 95.7-97.0%) and for the Eudragit S microparticles was 97.1% (range 96.9-97.3%).

3.17.2 Prednisolone encapsulation efficiency
The mean prednisolone encapsulation efficiency for Eudragit L microparticles was calculated to be 86.4% (range 86.0-87.1%) and for the Eudragit S microparticles was 90.0% (range 89.8% to 90.4%).

3.17.3 Evaluation of powder flow using tapped density
For each sample, the tapped volume after 1250 taps was assumed to be the final tapped volume, as the difference between the tapped volume following 750 taps and 1250 taps was less than 2%. The values for IBD, TBD and Hausner ratio for the 3 powders are summarised in Table 3.6.

<table>
<thead>
<tr>
<th></th>
<th>Prednisolone</th>
<th>Eudragit L microparticles</th>
<th>Eudragit S microparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>IBD</td>
<td>0.38</td>
<td>0.68</td>
<td>0.66</td>
</tr>
<tr>
<td>TBD</td>
<td>0.66</td>
<td>0.79</td>
<td>0.76</td>
</tr>
<tr>
<td>Hausner ratio</td>
<td>1.58</td>
<td>1.16</td>
<td>1.15</td>
</tr>
</tbody>
</table>

The Hausner ratio is commonly used to estimate the degree of flowability of a powder using the scale shown in Table 3.7.
The scale of flowability as determined using Hausner ratio (Carr, 1965) is shown in Table 3.7.

<table>
<thead>
<tr>
<th>Hausner ratio</th>
<th>Flow character</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.00-1.11</td>
<td>Excellent</td>
</tr>
<tr>
<td>1.12-1.18</td>
<td>Good</td>
</tr>
<tr>
<td>1.19-1.25</td>
<td>Fair</td>
</tr>
<tr>
<td>1.26-1.34</td>
<td>Passable</td>
</tr>
<tr>
<td>1.35-1.45</td>
<td>Poor</td>
</tr>
<tr>
<td>1.46-1.59</td>
<td>Very poor</td>
</tr>
<tr>
<td>&gt; 1.60</td>
<td>Very, very poor</td>
</tr>
</tbody>
</table>

The flow of prednisolone is classified as very poor, which was unsurprising given the cohesive nature of prednisolone powder. However, the flow of both Eudragit L and S prednisolone-loaded microspheres is good. This suggests that as well as the possibility of formulating the microspheres as a suspension for oral administration, the microspheres could be readily filled into capsules or compressed into tablets, where good flow is required to ensure uniformity of dose and physicomechanical properties (Staniforth, 2000).

3.17.4 Density

The density of the microsphere formulations, polymer and prednisolone are summarised in Table 3.8. The calculated values were similar for all of the samples, lying in the range 1.2-1.3 g/cm³.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Density (g/cm³)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit L100 powder</td>
<td>1.27</td>
</tr>
<tr>
<td>Eudragit S100 powder</td>
<td>1.25</td>
</tr>
<tr>
<td>Prednisolone</td>
<td>1.30</td>
</tr>
<tr>
<td>Eudragit L microparticles</td>
<td>1.26</td>
</tr>
<tr>
<td>Eudragit S microparticles</td>
<td>1.22</td>
</tr>
</tbody>
</table>
3.17.5 Particle size analysis

A summary of the laser diffraction data for the Eudragit L and S microparticles is given in Table 3.9.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Mean $d_v(0.1)$ (range)</th>
<th>Mean $d_v(0.5)$ (range)</th>
<th>Mean $d_v(0.9)$ (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit L microparticles</td>
<td>21.97 $\mu$m (21.34-23.09 $\mu$m)</td>
<td>31.28 $\mu$m (31.04-31.54 $\mu$m)</td>
<td>43.38 $\mu$m (41.57-46.25 $\mu$m)</td>
</tr>
<tr>
<td>Eudragit S microparticles</td>
<td>36.57 $\mu$m (35.21-38.23 $\mu$m)</td>
<td>50.04 $\mu$m (49.54-50.74 $\mu$m)</td>
<td>66.91 $\mu$m (63.20-71.13 $\mu$m)</td>
</tr>
</tbody>
</table>

The particle size distribution of both the Eudragit L and S microparticles was narrow, with a mean span value of 0.68 and 0.61, respectively. The particle size of the microparticles calculated by laser diffraction is in good agreement with the size of the microparticles which was approximated using SEM. The small size of the microparticles, in combination with a density of 1.26 and 1.22 for Eudragit L and S microparticles, respectively, provides an explanation for the suspension of the microparticles throughout the dissolution media in the USP II dissolution apparatus. The density and size of the Eudragit L and S microparticles should also be appropriate for suspension in the liquid contents of the stomach, therefore facilitating a rapid gastric emptying.

3.18 Section Four Conclusions

The batch yields and prednisolone encapsulation efficiency is high for both Eudragit L and S microparticles suggesting that the emulsification/solvent evaporation method developed in Chapter Two represents an efficient process. The physical properties of the pH-sensitive microparticles are ideal for the intended purpose of oral administration. The good flow properties should facilitate ease of filling into capsules as well as compression into tablets as well as the possibility of administering the microparticles as a suspension. The microparticles may be particularly suitable for administration as the latter formulation given their small size and low density. It is likely that the size and density of the pH-sensitive microparticles may facilitate their suspension in the liquid contents of the stomach which may permit a rapid gastric emptying, thus overcoming the delayed and unpredictable
gastric emptying associated with conventional modified release dosage forms. Furthermore, the narrow particle size distribution should result in reproducible microparticle dissolution and drug release.

3.19 Chapter Conclusions

In vitro dissolution testing of prednisolone-loaded Eudragit L and S microparticles, of theoretical drug loading 5:1, suggests that they are able to deliver prednisolone to the small intestine and ileo-colonic region. Furthermore, these microparticles exhibit excellent physical properties which suggest that gastrointestinal transit may be improved in comparison to conventional delayed release dosage forms. The physical form and stability of prednisolone within the pH-sensitive microparticles will be tested in Chapter Four, while the in vivo absorption of prednisolone from these microparticles will be determined in Chapter Five.
Chapter Four: An investigation into the physical form, and stability, of prednisolone within Eudragit L and S microparticles
4.1 Introduction

The formulation of drugs within microparticles has previously been shown to change the physical form of the entrapped drug (De Jaeghere et al., 2001). Perhaps the most significant physical change is that of the crystalline to amorphous form, which can impact physicochemical properties of the drug including melting temperature, vapour pressure and, perhaps most importantly, dissolution rate (often confused with solubility). The latter attribute of the amorphous form has resulted in substantial research interest for the improvement of oral bioavailability of poorly soluble drugs via an increase in the \textit{in vivo} dissolution rate within the lumen of the gastrointestinal tract (Jachowicz, 1987; Byrn et al., 1995; Jung et al., 1999; Yu, 2001; Ambike et al., 2004; Horoz et al., 2004). However, the amorphous form of a drug is usually unstable, and tends to revert to a more stable crystalline form on storage (Lane and Buckton, 2000).

The rapid dissolution of prednisolone from the Eudragit L and S microparticles described in chapter three, and lack of crystallinity on the surface or within the matrix of the pH-sensitive microparticles, at a theoretical drug loading of 5:1 (see Figure 3.16), indicates that prednisolone may be entrapped as the amorphous form within the microparticles.

The aim of this chapter is to investigate methods for the determination of the physical form of prednisolone within Eudragit L and S microparticles, and to relate any changes in the physical form of the drug upon storage to \textit{in vitro} prednisolone release using the pH-change dissolution method developed in chapter three.

4.2 The crystalline and amorphous state of solid materials

A pharmaceutical ingredient, in common with all other matter, can be classified depending on the state in which it exists as solid, liquid or gas. The majority of pharmaceutical ingredients (both actives and excipients) are in the solid state when taken by patients. The solid state is characterised by closely packed molecules held tightly together by intermolecular linkages, primarily hydrogen bonding and Van der Waals forces, and is much more ordered than the liquid or gaseous states. However, molecules of the same drug in the solid state can adopt a variety of different packing arrangements which result in different physical forms of a solid material. These different physical forms are categorised as crystalline, solvated or amorphous and are described in more detail below. The different
physical forms in which a solid material may exist is dictated by molecular structure and method of preparation (Rustichelli et al., 2000; Brittain, 2002).

4.2.1 Crystalline state
The crystalline state is characterised by both short and long range order, with the molecules organised as characteristic and repeating unit cells which give a crystal its recognisable three-dimensional structure or habit. A material is said to exhibit polymorphism when it may exist as two or more crystalline forms having different molecular arrangements or conformations within the crystal lattice. The different crystal forms are said to be polymorphs of the same compound, and may exhibit different physicochemical properties, for example dissolution rate, stability and melting point.

4.2.2 Solvated state
Solvates, also known as pseudo polymorphs, are crystal forms containing either stoichiometric or non-stoichiometric amounts of a solvent. When the solvate is water, the solvate is termed a hydrate (Byrn, 1982).

4.2.3 Amorphous state
“Amorphous” literally means lack of form or shape, and in contrast to the crystalline form, the amorphous form consists of a disordered arrangement of molecules, possessing only short-range order over a few molecular dimensions (Hancock and Zografi, 1997). The amorphous form has been compared to the liquid state; the molecules having a higher molecular mobility and more random arrangement than in the crystalline form, however molecules cannot easily move past one another. The physicochemical properties (e.g. density, viscosity, hygroscopicity, dissolution rate) of the amorphous state are therefore very different to those of the crystalline state. The amorphous state has a higher free energy, and is therefore thermodynamically unstable, in comparison to the crystalline form. Over time, the amorphous form will transform spontaneously to the most thermodynamically stable crystalline form, possibly after transition through metastable forms. Amorphous materials lack a well-defined melting point of crystalline material, however they do possess a glass transition temperature (Tg), below which the material is described as being in the glassy state, and above which, the rubbery state due to the
increased molecular mobility. The increased molecular mobility above Tg can ultimately result in recrystallisation of an amorphous material (Hancock and Zografi, 1994).

In order to achieve reproducibility of physical, chemical and biological properties, great efforts are traditionally made during the drug development process to isolate a new chemical entity with a high level of crystallinity, and as its most thermodynamically stable polymorph. However, in recent years, the use of combinatorial chemistry and high throughput screening in the drug development process has led to a significant increase in the number of poorly soluble molecules in development (Leuner and Dressman, 2000). This has driven research interest into methods to generate and maintain the more rapidly dissolving amorphous form within pharmaceutical formulations, which may improve the bioavailability of drugs for which solubility is the rate limiting step.

4.3 Methods for generation of the amorphous state
A number of methods by which the amorphous form of a drug may be generated have been described, but the most common are spray drying, super-cooling of the melt (quench cooling) and milling (Hancock and Zografi, 1997).

4.3.1 Spray drying
The process of spray drying is described in Appendix Two. In essence, the crystalline form of a material is dissolved in a suitable solvent, which is atomised, followed by rapid solvent evaporation in a stream of drying gas. The rapidity of solvent removal does not allow sufficient time for the material to organise as the crystalline form, leaving the material in the disordered amorphous form.

4.3.2 Super-cooling of the melt (quench cooling)
The principles of quench cooling are similar to spray drying. The liquid form is induced by heating a material slowly beyond its melting point, and the rapid cooling (usually achieved by decanting into liquid nitrogen) does not allow sufficient time for crystallisation to occur.

4.3.3 Milling (micronisation)
Air-jet milling and mechanical grinding are used to reduce particle size, which may be important to improve the dissolution rate of a drug, or to deposit the drug at a specific site
of action within the body as is the case, for example, with dry powder inhalers. The energy imparted on the material by the milling process may increase the disorder of the system to the extent that the material converts partially or completely to the amorphous form.

4.4 Methods for stabilisation of the amorphous state

Recently, it was estimated that around half of drugs in development in the pharmaceutical industry are poorly soluble (Lipinski, 2002). Due to the inherent solubility advantage of the amorphous over the crystalline form, it is perhaps unsurprising that a great deal of research has been undertaken to find methods of stabilising the amorphous form.

The incorporation of a drug as a solid dispersion or solid solution within the matrix of an amorphous polymeric carrier material is the most widely used strategy attempted to improve the stability of amorphous drugs. This is generally achieved by rapid cooling of a co-melt of drug and polymer (Sekiguchi and Obi, 1961), or by solvent evaporation from a co-solution of drug and polymer. When a polymeric material with a high Tg is employed as the matrix forming polymer, the stability of the entrapped amorphous drug can be improved due to an antiplasticising effect of the polymer which raises the Tg of the system somewhere between that of the individual components (Ambike et al., 2004). A further advantage of amorphous solid dispersions is that drug release rate is controlled by the rate of polymer dissolution, which serves to increase the surface area of the drug that is presented to the dissolution medium, improving the dissolution rate of hydrophobic drugs for which wettability is usually poor (Leuner and Dressman, 2000).

4.5 Characterisation of the amorphous state

The differences in a number of material physical properties (density, viscosity, heat capacity, X-ray diffraction) allow the amorphous form of a drug to be distinguished from the crystalline form (Hancock and Zografi, 1997), and the rubbery amorphous state to be distinguished from the glassy. Some methods are sensitive enough to allow a quantification of the amorphous content to be made. Techniques which have been utilised for the quantification of amorphous material include X-ray powder diffraction (XRPD) (Clas et al., 1995; Yuksel et al., 1996; Jung et al., 1999), differential scanning calorimetry (DSC) (Yuksel et al., 1996; Jung et al., 1999; Jiang et al., 2002; Saunders et al., 2004; McGregor et al., 2004; Hurtta and Pitkanen, 2004), isothermal microcalorimetry (Dilworth
et al., 2004), solution calorimetry (Gao and Rytting, 1997) and spectroscopic methods (Campbell Roberts et al., 2002).

The techniques of XRPD and DSC were used in this study to investigate the physical form of prednisolone within Eudragit L and S microparticles, and are described below.

4.5.1 Differential scanning calorimetry (DSC)

In DSC, two identical furnaces control the heat flow to a sample and reference pan which subject the two pans to identical temperature-controlled programs. The difference in heat flow required to maintain the pans at the same temperature is measured as the sample temperature is increased, or decreased, generally at a linear rate. The difference in power output between the sample and reference furnace is equal to that of the energy of the thermal event that is taking place in the sample pan over a specific temperature range. DSC is widely utilised to quantify changes in state (including crystalline transitions, melting, sublimation and evaporation) as a function of either temperature or time.

DSC is a useful technique as it requires only small sample size (3-5mg), and at high scan rates is an expedient process. DSC is a sensitive technique for the detection of small amounts of crystalline material, however crystallinities of <2% are difficult to detect (Leuner and Dressman, 2000).

Whereas, conventional DSC utilised scanning rates of the order of 10°C/min, hyper (high speed) DSC employs scan rates of up to 500°C/min (Saunders et al., 2004; McGregor et al., 2004; Hurtta and Pitkanen, 2004). It is claimed that hyper-DSC provides greater sensitivity than conventional DSC for the determination of small amounts (1-2%) of amorphous content, as recrystallisation during heating is hindered (Pijpers et al., 2002). Furthermore, low sample masses of around 1mg are used, and when considered alongside a faster run times than conventional DSC, it is evident that hyper-DSC is a more efficient technique than conventional DSC.
4.5.2 X-ray powder diffraction (XRPD)

X-rays are generated when a beam of electrons is accelerated onto a metal target, usually copper. The generated X-rays are collimated and directed onto a sample, at a range of angles. When X-rays are scattered (diffracted) by the ordered environment of a crystal, information regarding the arrangement and spacing of atoms in a crystalline material can be determined using Bragg's Law (Equation 4.1):

\[ n\lambda = 2dsin\theta \]  

Eqn 4.1

Bragg's Law, where \( n \) is the order of the diffracted beam, \( \lambda \) is the wavelength of the incident X-ray beam, \( d \) is the distance between the adjacent planes of atoms and \( \theta \) is the angle of incidence of the X-ray beam.

The X-ray diffraction pattern is unique for each individual crystalline substance, including different polymorphic forms of the same material, and the XRPD pattern can therefore be regarded as a “fingerprint” for the identification of crystalline solids. Amorphous solids, however, lack the long-range three-dimensional order of the crystalline state, and diffract X-rays in an irregular manner, with the resulting trace (often described as a “halo”) containing no distinct peaks.

Prior to evaluation by XRPD, the material to be characterised is preferably ground into a fine homogeneous powder, gently, so as not to induce the amorphous state. This ensures that the crystals are orientated in a random order which permits that a sufficient number of these will fulfill the Bragg condition for reflection from every possible interplanar spacing. The finely ground crystalline powder is then mixed with an appropriate non-crystalline binder, if necessary, and packed into a mould with the powder mass having a smooth flat surface. The surface of the crystalline powder is then scanned over a range of angles.

Although XRPD is often used to provide qualitative determination on the physical form of a solid material (i.e., amorphous, crystalline, partially crystalline), XRPD has also been used for the quantitative determination of crystalline content, although sensitivity is limited and crystallinities below 5-10% cannot generally be detected (Leuner and Dressman, 2000).
4.6 Stability of pharmaceutical dosage forms

In the time between manufacture and dosing by a patient, changes may occur to the active ingredient or other excipients which constitute a pharmaceutical dosage form. Such changes may be chemical, physical, biological or microbiological and result from exposure of the dosage form to a number of environmental influences such as temperature, light, humidity or bacteria and can result in changes in therapeutic efficacy and/or toxicity.

The shelf life of a pharmaceutical dosage form is defined by the ICH guidelines (Q1A-F) as "the time period during which a drug product is expected to remain within the approved shelf life specification, provided that it is stored under the conditions defined on the container label." The shelf life assures the efficacy and safety of a dosage form at the time of dosing, and is assigned after appropriate stability testing.

Stability testing usually involves both long term testing for a minimum of 12 months at either 25±2°C and 60±5% relative humidity (RH) or 30±2°C and 65±5% RH, to mimic ambient conditions, and stressed testing at 40±2°C and 70±5% RH. Stability testing under stressed conditions allows the generation of accelerated stability data, and may also provide confidence in the stability of the drug product during short term excursions outside the label storage condition, e.g., during shipment and handling.

This chapter focuses on the physical stability of prednisolone-loaded Eudragit L and S microparticles at temperatures and humidities representing both ambient (25°C/60% RH) and stressed (40°C/70% RH) conditions, for a period of 13 weeks. The techniques of XRPD and DSC will be used to investigate the physical stability of the encapsulated prednisolone within Eudragit L and S microparticles, and the influence of any physical instability of entrapped prednisolone on drug release and dissolution will be investigated using the in vitro pH-change dissolution method described in Chapter Three.
4.7 Materials

Eudragit L100 and Eudragit S100 were obtained from Rohm Pharma (Darmstadt, Germany). Micronised prednisolone was purchased from Sanofi-Aventis (Romainville, France). Size 0 gelatin capsules were obtained from Capsugel (Colmar, France). Indium and zinc (99.999% purity) were obtained from PerkinElmer Instruments (Beaconsfield, UK). All other reagents were of analytical grade and were used as received.
Section One: Investigation of methods for the determination of the physical form of prednisolone within pH-sensitive microparticles

4.8 Methods
The techniques of DSC and XRPD were investigated to determine the sensitivity of each for the quantification of crystalline prednisolone in physical mixtures of prednisolone and Eudragit polymer.

4.8.1 Differential scanning calorimetry (DSC)
Physical mixtures of Eudragit S and prednisolone (0:1, 1:1, 5:1, 10:1, 15:1 and 20:1) were prepared by Turbula mixing for 10 minutes, followed by gentle blending for 15 seconds with a mortar and pestle. Before conducting a DSC scan at a new scan rate, the instrument was calibrated using high purity standards of indium and zinc which have well defined temperature and energy transitions. The instrument was corrected for any difference from the literature values for the melting points of indium and zinc.

4.8.1.1 Instrumentation for standard scan rates
A DSC 7 Differential Scanning Calorimeter (PerkinElmer Instruments, Beaconsfield, UK) was used for experiments conducted at conventional scan rates. Samples of mass 4-5mg were accurately weighed (to 2 decimal places) directly into non-hermetically sealed pans which were subsequently crimped. A blank, crimped pan was used as a reference in all experiments. Following an isothermal hold for 1 minute, a temperature scan at 10°C/min was conducted to a final temperature of 300°C. Data was recorded and analysed using Pyris Thermal Analysis Software (version 3.81).

4.8.1.2 Instrumentation for hyper-DSC
A Pyris 1 Differential Scanning Calorimeter (PerkinElmer Instruments, Beaconsfield, UK) was used for hyper-DSC. Samples were weighed into pans as described for conventional DSC, except that smaller sample sizes of 1-2mg were accurately weighed (to 2 decimal places), in order to reduce the effect of thermal lag in the sample. Following an isothermal hold for 1 minute, a temperature scan at 100°C/min or 200°C/min was conducted to a final
temperature of 300°C. Data was recorded and analysed using Pyris Thermal Analysis Software (version 3.81).

4.8.2 X-ray powder diffraction (XRPD)
Physical mixtures of Eudragit L and prednisolone containing 100, 50, 25, 10, 5, 4, 3, 2, 1 and 0% prednisolone were accurately weighed out into glass vials and blended for 10 minutes using a Turbula mixer, followed by gentle blending for 15 seconds with a mortar and pestle. Samples were gently compressed into a round disc sample holder and smoothed with a Perspex block. Samples were analysed using a Philips PW3710 Scanning X-ray Diffractometer (Philips, Cambridge, UK) with a Cu Kα filter generated at 30mA and 45kV, and a scan rate of 0.02°/sec from 5° to 85° 2θ for prednisolone, and 5° to 25° 2θ for all other samples. Samples were scanned in triplicate to minimize the effects of preferred orientation, with the sample being repacked between repeats. The peak height and peak area was calculated for all samples using X’Pert HighScore data analysis software (version 2.0a).
4.9 Results and discussion

4.9.1 Differential scanning calorimetry

(10°C/min)

The DSC traces obtained at 10°C/min are shown in Figure 4.1. At a scan rate of 10°C/min, prednisolone showed an endothermic peak (melting transition) at 236°C (onset), 243°C (peak). This was in good agreement to the melting peak of 238°C determined at a scan rate of 10°C/min reported by Taylor and Langkilde (2000). No endothermic peak was obtained for a physical mixture of Eudragit S100/prednisolone (1:1), which is probably due to prednisolone dissolving in the polymer chains of Eudragit S above its Tg of 160°C. It was decided to employ higher scan rates to reduce the time for dissolution to occur.

![DSC traces of Eudragit S, prednisolone and physical mixtures (10°C/min)](image)

Figure 4.1: DSC traces of Eudragit S, prednisolone and physical mixtures (10°C/min)
The DSC traces obtained at 100°C/min are shown in Figure 4.2. At a scan rate of 100°C/min, prednisolone showed a sharp endothermic peak (melting transition) at 251°C (onset), 258°C (peak). An endothermic peak was also observed for a physical mixture of Eudragit S/prednisolone (1:1), however no endothermic peak was observed for a 5:1 physical mixture, which was probably due to dissolution of prednisolone within the polymer chains when it is present at lower concentrations. It was concluded that, at a scan rate of 100°C/min, DSC was not sensitive enough for the detection of crystalline prednisolone at levels representative of drug loading within the microparticles.

Figure 4.2: DSC traces of Eudragit S, prednisolone and physical mixtures (100°C/min)
200°C/min

The DSC traces obtained at 200°C/min are shown in Figure 4.3. At a scan rate of 200°C/min, prednisolone showed a sharp endothermic peak (melting transition) at 253°C (onset), 261°C (peak). Physical mixtures of Eudragit S and prednisolone at 5:1 and 10:1 ratios showed endothermic peaks at 222°C (onset), 235°C (peak) and 223°C (onset), 232°C (peak), respectively. A standard plot (see Figure 4.4) of average ΔH for Eudragit S and prednisolone physical mixtures 0:1 to 10:1 showed a good correlation ($r^2 = 0.983$), however ΔH values were extremely variable for 5:1 and 10:1 physical mixtures (6.78±4.41 J/g and 1.77±0.70 J/g, respectively). The variability in ΔH could be due to variable distribution of prednisolone, and hence variable contact area of prednisolone and Eudragit S, within physical mixtures of the same ratio. This would in turn impact the quantity of prednisolone dissolving into the polymer chains above the Tg of Eudragit S and also the quantity of prednisolone remaining in the crystalline form. It was decided that at a scan rate of 200°C/min DSC was still not sensitive enough to allow the quantification of crystalline prednisolone in the quantities present within the Eudragit S microparticles.

![Figure 4.3: DSC traces of Eudragit S, prednisolone and physical mixtures (200°C/min)](image-url)
Figure 4.4: Standard plot of $\Delta H$ versus prednisolone concentration in physical mixtures of Eudragit S and prednisolone

4.9.2 X-ray powder diffraction (XRPD)

The X-ray powder diffractograms for physical mixtures of Eudragit L and prednisolone containing 100, 50, 25, 10, 5, 4, 1 and 0% prednisolone are shown in Figure 4.5.

Figure 4.5: X-ray powder diffractograms for Eudragit L and prednisolone physical mixtures

The major XRPD peak for prednisolone was determined to be located at $15.4^\circ 2\theta$ which was in agreement with previous work carried out by Jachowicz (1986). A plot of peak height versus prednisolone concentration (Figure 4.6) gave a better correlation than peak area versus prednisolone concentration (Figure 4.7). At prednisolone concentrations of 5%
and 4% w/w, coefficient of variation was 9.9% and 10.3%, respectively. Although approximately 25% of the prednisolone within the microparticles would have to be crystalline before it could be detected with the XRPD method, the sensitivity of the method was in the upper range reported for XRPD, which is generally understood to have a limit of detection for crystalline material of 5-10% (Leuner and Dressman, 2000). It was therefore decided to use XRPD for the quantification of crystalline prednisolone in the Eudragit microparticles in the stability study.

Figure 4.6: Standard plot of peak height (15.4° 2θ) versus prednisolone concentration in physical mixtures of Eudragit L and prednisolone

Figure 4.7: Standard plot of peak area (15.4° 2θ) versus prednisolone concentration in physical mixtures of Eudragit L and prednisolone
Section Two: Stability study

4.10 Methods

4.10.1 Preparation of desiccators for control of temperature and relative humidity
Saturated salt solutions can be used to maintain specific relative humidities in closed chambers for stability studies. A saturated solution of sodium bromide will maintain a relative humidity of 57.5±0.177 at 25°C and a saturated solution of sodium chloride will maintain a relative humidity of 74.7±0.149 at 40°C (Nyqvist, 1983), within the ranges for ambient and stressed conditions described in the ICH guidelines of 25±2°C and 60±5% RH, and 40±2°C and 70±5% RH, respectively. Saturated solutions of sodium bromide and sodium chloride were prepared by dissolving an excess of salt in 400mL water at 60°C, and cooling to room temperature before transferring to air-tight glass desiccators. The desiccators containing saturated sodium bromide and sodium chloride solutions were maintained in temperature controlled ovens at 25°C and 40°C, respectively.

4.10.2 Preparation of a drug product containing prednisolone-loaded Eudragit L and S microparticles and choice of primary pack
Six batches of Eudragit L and S/prednisolone (5:1) microparticles were prepared as described in section 2.9.1. The six batches of each formulation were combined in separate 60mL amber glass jars and blended for 15 minutes in a Turbula mixer. 300±1mg samples of each formulation were accurately weighed out into 90 size 0 gelatin capsules.

The stability of a drug product is a composite of the final dosage form, as it is to be given to the patient, and the primary pack. Therefore, in order to maximise the possibility of dosage form stability, an amber glass jar was chosen as the primary pack, being as hermetic as possible to the conventional degradation routes of exposure to humidity, oxygen and light.

Four amber glass jars (one for each stability timepoint) containing 10 capsules of each formulation were stored in a desiccator containing a saturated sodium bromide solution maintained in a temperature controlled oven at 25°C (ambient condition (25°C/60% RH)). Four amber glass jars containing 10 capsules of each formulation were stored in a desiccator containing a saturated sodium chloride solution maintained in a temperature controlled oven at 40°C (stressed condition (40°C/75% RH)). A further 10 capsules of each
formulation were set aside for immediate analysis to provide baseline (time zero) data. Samples were analysed as described below at 2, 4, 8 and 13 weeks after microparticle preparation.

4.10.3 Determination of drug loading of Eudragit L and S microparticles

The prednisolone encapsulation efficiency of the blended Eudragit L and S microparticles was calculated as described in section 3.16.3.

4.10.4 X-ray powder diffraction (XRPD)

At time points 0, 2, 4, 8 and 13 weeks post-microparticle manufacture, the contents of two capsules were emptied into a round disc sample holder and smoothed with a Perspex block. Samples were analysed in triplicate following repacking as described in section 4.8.2 from 5 to 25° 2θ, and peak heights calculated using X’Pert HighScore data analysis software. Samples of Eudragit L and S (5:1) prednisolone-loaded microparticles which had been stored in an amber glass jar, at ambient conditions, for 18 months were also subjected to XRPD analysis as described in section 4.8.2.

4.10.5 In vitro dissolution studies

The pH-change dissolution method described in section 3.2.2 was used to assess the in vitro dissolution of the Eudragit L and S/prednisolone (5:1) microparticles. At each time point 10 litres of 0.1M HCl was prepared, and 2 litres of Na₃PO₄ buffer solution containing 129.0g/2l (for pH 6.8) and 156.9g/2l (for pH 7.4). Prior to the dissolution experiment, 750mL 0.1M HCl were added to 250mL buffer solution and the pH checked. Capsules containing 300mg Eudragit L/prednisolone (5:1) microparticles were tested for 2 hours in 750mL 0.1M HCl and 1 hour at pH 6.8, and Eudragit S/prednisolone (5:1) microparticles tested in 750mL 0.1M HCl for 2 hours and pH 7.4 for 1 hour.
4.11 Results

4.11.1 Drug loading of Eudragit L and S microparticles
Free flowing Eudragit L and S microparticles were harvested as previously described. Prednisolone encapsulation efficiency was calculated to be 86.7±0.7% and 88.2±2.0% for the Eudragit L and S microparticles, respectively. This equates to an actual drug loading within the microparticles of 14.5% for the Eudragit L and 14.7% for Eudragit S. Furthermore, this indicated that for crystalline prednisolone to be detected, 27.7% of the prednisolone within the Eudragit L microparticles would be required to crystallise; for the Eudragit S microparticles the value was 27.2%.

4.11.2 X-ray powder diffraction (XRPD)
No peak was seen at 15.4° 2θ for either Eudragit L or S microparticles at either storage condition after 2, 4, 8, or 13 weeks, indicating that the amount of crystalline prednisolone within the microparticles was less than 27.7% and 27.2% of the total prednisolone contained within the Eudragit L and S microparticles, respectively, for the duration of the study. Furthermore, no crystallinity was detected in the samples stored at ambient conditions for 18 months. Figures 4.8 and 4.9 show the X-ray diffractograms of Eudragit L and S polymer, respectively, plotted against the X-ray diffractograms of the respective microparticles (freshly prepared) and following 13 weeks storage at 25°C/60% RH and 40°C/75% RH.
Figure 4.8: X-ray diffractograms of Eudragit L powder, Eudragit L/prednisolone (5:1) microparticles (freshly prepared) and after 13 weeks storage at 25°C/60% RH and 40°C/75% RH

Figure 4.9: X-ray diffractograms of Eudragit S powder, Eudragit S/prednisolone (5:1) microparticles (freshly prepared) and after 13 weeks storage at 25°C/60% RH and 40°C/75% RH

4.11.3 In vitro dissolution studies

The in vitro dissolution profiles of the Eudragit L and S microparticles at each stability time point for both storage conditions are shown in Figures 4.10 to 4.13. The in vitro release profile of freshly prepared Eudragit L and S microparticles and those stored in gelatin capsules at both storage conditions, as well as those stored for 18 months in glass vials prior to capsule filling, are shown in Figure 4.14 and 4.15, respectively. A summary of the dissolution data is given in Table 4.1.
Figure 4.10: Change in dissolution profile of Eudragit L microparticles stored at 25°C/60% RH

Figure 4.11: Change in dissolution profile of Eudragit L microparticles stored at 40°C/75% RH
Figure 4.12: Change in dissolution profile of Eudragit S microparticles stored at 
25°C/60% RH

Figure 4.13: Change in dissolution profile of Eudragit S microparticles stored at 
40°C/75% RH
Figure 4.14: Dissolution profile of Eudragit L microparticles after 13 weeks’ storage at 25°C/60% RH and 40°C/75% RH and 18 months ambient storage in comparison to freshly prepared microparticles.

Figure 4.15: Dissolution profile of Eudragit S microparticles after 13 weeks’ storage at 25°C/60% RH and 40°C/75% RH and 18 months ambient storage in comparison to freshly prepared microparticles.
Table 4.1: Summary of in vitro prednisolone release in acid and buffer for Eudragit L and S microparticles after 13 weeks' storage at 25°C/60% RH and 40°C/75% RH and 18 months ambient storage in comparison to freshly prepared microparticles

<table>
<thead>
<tr>
<th>Sample</th>
<th>Prednisolone release after 2 hours at pH 1.2 (%)</th>
<th>Time for maximum prednisolone release following pH change (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit L microparticles day 1</td>
<td>9.1</td>
<td>1</td>
</tr>
<tr>
<td>Eudragit L microparticles 13 weeks storage (25°C/60% RH)</td>
<td>10.1</td>
<td>1</td>
</tr>
<tr>
<td>Eudragit L microparticles 13 weeks storage (40°C/75% RH)</td>
<td>8.5</td>
<td>1</td>
</tr>
<tr>
<td>Eudragit L microparticles 18 months storage (ambient)</td>
<td>9.4</td>
<td>1</td>
</tr>
<tr>
<td>Eudragit S microparticles day 1</td>
<td>5.0</td>
<td>1</td>
</tr>
<tr>
<td>Eudragit S microparticles 13 weeks storage (25°C/60% RH)</td>
<td>4.2</td>
<td>4</td>
</tr>
<tr>
<td>Eudragit S microparticles 13 weeks storage (40°C/75% RH)</td>
<td>3.7</td>
<td>20</td>
</tr>
<tr>
<td>Eudragit S microparticles 18 months storage (ambient)</td>
<td>3.9</td>
<td>4</td>
</tr>
</tbody>
</table>
4.12 Discussion: The stability of entrapped prednisolone within Eudragit L and S microparticles

The XRPD traces did not detect any crystalline prednisolone within the Eudragit L or S microparticles stored at either 25°C/60% RH or 40°C/75% RH for a period of 13 weeks, or at ambient conditions for a period of 18 months. This suggests that if recrystallisation of prednisolone is occurring under these storage conditions then it is limited to less than 28% of the prednisolone content of the microparticles.

However, the change in the pH-change dissolution profile of the Eudragit L and S microparticles over the same period of time is very different between the two formulations. In terms of prednisolone release after 2 hours in acid, and prednisolone release rate following the increase in pH from 1.2 to 6.8, the dissolution profile of the Eudragit L microparticles remains practically identical after storage at either 25°C/60% RH or 40°C/75% RH for a period of 13 weeks, or at ambient conditions for a period of 18 months (Figure 4.14). In contrast, although the prednisolone release from the Eudragit S microparticles in acid after 2 hours is similar at all storage conditions, prednisolone release rate following the pH change from 1.2 to 7.4 decreases at all storage conditions. Possible explanations for this are discussed below.

Although the XRPD method described in section 4.8.2 for the detection of crystalline prednisolone in Eudragit L exceeds that of 5-10% commonly quoted for quantitative XRPD, when the drug loading of the particles is considered, approximately 28% of total prednisolone would be required to recrystallise for this to be detected. Therefore the extent of prednisolone recrystallisation could differ greatly between the Eudragit L and S microparticles, and it is not inconceivable that prednisolone is remaining essentially amorphous in the Eudragit L microparticles, while 28% of prednisolone has recrystallised in the Eudragit S microparticles. However, when the pH-change dissolution profile of the Deltacortril Enteric® tablets in section 3.4.4 is considered, the extent of prednisolone recrystallisation that could occur without detection in the Eudragit S microparticles, is unlikely alone to explain the slow down in prednisolone dissolution rate following storage for 13 weeks at 40°C/75% RH. Prednisolone is likely to be contained almost entirely as the crystalline form within the Deltacortril Enteric® tablets, and yet release following the pH change from 1.2 to 6.8 is complete within 25 minutes, a similar period of time to the
Eudragit S microparticles stored for 13 weeks at 40°C/75% RH. It may however be possible that prednisolone may be recrystallising as a poorly soluble crystalline polymorphic form, with a lower dissolution rate in aqueous media than the polymorphic form contained within the commercial formulation.

Another possible explanation for the slow down in prednisolone dissolution rate from the Eudragit S microparticles could be conformational changes in the polymer over time. Such changes may affect either the dissolution rate of the polymer itself, which is primarily controlling the dissolution of entrapped prednisolone, or alter the way in which prednisolone is entrapped which may alter the release of prednisolone from within the Eudragit S microparticle matrix.

Whether the differences in change of prednisolone release rate/dissolution from the Eudragit L and S microparticles following storage is due to changes in the physical form of the drug or changes in polymer conformation, the probable explanation for the differences in stability of the two formulations is a difference in the molecular mobility of the two polymers, particularly at 40°C/75% RH. For amorphous materials, molecular mobility is greater above the Tg than below, due to a lower viscosity of the rubbery amorphous form. As the Tg of Eudragit L and S is 200°C and 160°C, respectively (Pharma Polymers, 2001), it is apparent that this is well above the temperature of the stability study storage conditions and molecular mobility of both polymers would be expected to be low when considering the polymers alone. However, as has been observed during the dissolution testing of Eudragit L55 microparticles in section 3.5.2, the Tg of a polymer can be lowered due to plasticisation by water. Amorphous materials are generally hygroscopic in nature, readily absorbing water from their surroundings (Hancock and Zografi, 1994) which dissolve into the amorphous solid disrupting hydrogen bonding between the solid molecules and increasing the free volume of the solid. Additionally, as the Tg of water is low, being 135 K (or -138°C) (Sugisaki et al., 1968), this will tend to reduce the Tg of composite system comprised of water and a material which is solid at room temperature.

It follows therefore that the plasticising effect of water may be reducing the Tg of the Eudragit S microparticle system, which would be a composite of Eudragit S/prednisolone/water, to such an extent that molecular mobility would be increased at the
storage temperatures used in this study. Molecular mobility would be expected to be
greater for the Eudragit S than Eudragit L microparticles, and greater at 40°C than at 25°C.
This increased molecular mobility may be inducing conformational changes on the acrylic
polymer, or may be permitting reorganisation of prednisolone molecules in the crystalline
form. The recrystallisation step occurs following dissolution of drug molecules in water,
and it is anticipated that this process will also proceed more readily at elevated temperature
and elevated humidity.

The water that is absorbed by the microparticles may be drawn from either the air within
the dessicator or from the gelatin capsule in which the microparticles are contained. It is
interesting to note that the reduction in prednisolone dissolution rate is similar in the
Eudragit S microparticles stored in gelatin capsules inside a glass jar at a controlled
25°C/60% RH for 13 weeks in a dessicator, and for 18 months at ambient conditions (close
to 25°C/60% RH), in a glass jar only. This similarity of the change in dissolution at these
storage conditions suggests that water from the gelatin shell may be contributing more
significantly to the physical instability of the prednisolone within the Eudragit S
microparticles than water from the air. With the benefit of hindsight, it would have been of
interest to monitor any change in water content of the gelatin shell at each timepoint to
establish if water is indeed being lost from the gelatin shell to the amorphous
microparticles. The extent of water uptake by the Eudragit L and S microparticles at each
time point would also be of interest, and would allow an estimation of the change in Tg of
the system due to water absorption (using the Gordon-Taylor equation), which would verify
if the Tg of the plasticized Eudragit S/prednisolone microparticles is indeed lowered to
approximately 40°C, accounting for the physical instability of this formulation under the
stressed storage condition.

The different monomer composition of Eudragit L and S provides a second possible
mechanism for the differences in physical stability of prednisolone entrapped within the
acrylic microparticles. The ratio of carboxylic acid-containing methacrylic acid
groups:methyl methacrylate groups is 1:1 and 1:2 for Eudragit L and S, respectively. It is
possible, therefore, that there is an increase in hydrogen bonding between Eudragit L and
prednisolone compared to Eudragit S, which may help to stabilise the entrapped drug
within the amorphous form. However, considering the ratio of monomer groups, it is
probable that Eudragit L is the more hydrophilic of the two polymers and perhaps a little surprising that water uptake by the Eudragit L microparticles does not also result in a reduction in prednisolone dissolution rate at pH 6.8 on storage, particularly when the larger surface area of Eudragit L is also taken into account.

The XRPD and dissolution data allow a shelf life to be assigned for the Eudragit L microparticles. Eudragit L microparticles demonstrated no change in the physical form of the drug, as determined by XRPD (although the usefulness of this data is highly questionable), and no change in dissolution rate for 13 weeks at 40°C/75% RH, and as such would be granted a shelf life of 6 months according to ICH guidelines (ICH guidance Q1E), which could possibly be extended following an extension of the stability study period. However, although no change in the physical form of prednisolone was detected following 13 weeks storage at either 25°C/60% RH or 40°C/75% RH for a period of 13 weeks, the rate of prednisolone dissolution from the Eudragit S microparticles at pH 6.8 is slower following 13 weeks storage at either stability condition and as such no shelf life could be assigned.

The stability of a drug within a dosage form is the composite of the drug, the physical form of the drug, the formulation, the packaging and the storage conditions. It could therefore be attempted to improve the stability of the drug within the Eudragit S microparticles by the use of suitable water-impermeable packing materials, possibly incorporating a desiccating agent in conjunction with HPMC capsules which are less prone to cracking in the presence of desiccating agents, reducing the drug loading of the microparticles, or reducing the temperature at which the Eudragit S microparticles are to be stored.
Chapter Five: *In vivo* absorption of prednisolone from pH-sensitive microparticles in rats following oral administration
5.1 Introduction

The need for in vivo proof of efficacy of a drug delivery system has arisen from an increased understanding that no amount of in vitro testing is able to predict how a new chemical entity or drug delivery system will perform in man. Although the most reliable model for investigating the performance of a drug delivery system in man is the target organism itself, i.e. man, a new chemical entity or delivery system is usually investigated in an animal model prior to clinical studies in man.

5.1.1 Animal models to estimate oral drug absorption

The anatomical, metabolic and physiological similarities and differences that exist between the gastrointestinal tract of man and the animal model being used must be fully understood when interpreting the data from animal experiments as physiological factors such as gastrointestinal pH, fluid volume, motility, bile and pancreatic juice can have an effect on the gastrointestinal transit, drug release and dissolution rate, and hence absorption from dosage forms (Kararli, 1995).

A wide variety of animal models have been used, with larger animals being favoured to evaluate drug absorption from a formulations, particularly single unit systems, intended for human use (Dressman and Yamada, 1991). Beagle dogs and pigs are perhaps the mammalian animal models that are most commonly used, primarily due the similarities that exist between the gastrointestinal tract of these species and of man, but also because of their relatively docile nature and ease of handling. However, the cost of upkeep of such animals, alongside the stringent regulations for the use of animals in scientific experiments, means that other animal models must be considered.

Smaller animals such as the rat, mouse and guinea pig are generally used to investigate the mechanism(s) of drug absorption and to determine bioavailability from powders and solutions. However, a number of researchers have administered pellets (Tuleu et al., 1999; 2001) and microparticles (Jeong et al., 2001; Wong et al., 2006) to this species. The small size, low cost of upkeep and ease of handling of the rat make it an attractive animal model (Dressman and Yamada, 1991). The rat was the animal model used in our in vivo experiments and the gastrointestinal tract of the rat and man is therefore compared below.
5.1.2 A comparison of the anatomy of the human and rat gastrointestinal tract

There are strong morphological similarities between the human and rat GI tract on the microscopic level (DeSesso and Jacobson, 2001). In both species, the GI tract is lined by a mucosal layer which serves as a barrier to the entry of materials into the body proper. The mucosa is composed of a layer of epithelial cells overlying the lamina propria, a layer of connective tissue accommodating blood and lymphatic capillaries. Furthermore, the basic structural organisation of the gastrointestinal tract is similar in most mammalian species, including the rat and human, in that it consists of a stomach, small intestine, caecum, colon and rectum. However, gross anatomical differences in the relative surface areas available for absorption mean that the human GI tract is capable of absorbing materials faster and to a greater extent than that of the rat (DeSesso and Jacobson, 2001). An overview of the anatomy of the human and rat GI tract is shown in Figure 5.1.

![Figure 5.1: Overview of the anatomy of the human (left) and rat (right) GI tract, not to scale (adapted from DeSesso and Jacobson, 2001)](image)

5.1.2.1 Stomach

Whereas the human stomach is completely glandular, the rat stomach can be divided into two grossly discernible regions: glandular and non-glandular. The non-glandular forestomach of the rat is thin walled and transparent, being lined by a layer of stratified squamous epithelium. The forestomach acts as a site of entry for the oesophagus, and as a store for food and a site for bacterial digestion (DeSesso and Jacobson, 2001). In contrast,
the glandular portion of the stomach is thick walled and covered by columnar epithelia. The lamina propria of the glandular portion is occupied by simple tubular gastric glands containing mucus secreting neck cells, pepsinogen secreting chief cells, and parietal cells which secrete hydrochloric acid. In contrast to the human stomach which can accommodate volumes up to 1.5 litres, the capacity of the rat stomach is in the region of several milliliters. The exact volume that the rat stomach can accommodate will vary according to the age and size of the animal, but volumes of up to 1-2mL liquid previously been dosed by oral gavage with no reports of any adverse effects (Palin et al., 1982; Mori et al., 1989; Ciftci and Groves, 1996; Eyles et al., 1997).

5.1.2.2 Small intestine
In common with humans, the rat small intestine represents the major organ for the absorption of nutrients, water, electrolytes and drugs into the systemic circulation. Most absorption occurs from the duodenum and proximal half of the jejunum by processes including passive and facilitated diffusion, active transport, pinocytosis and solvent convection (DeSesso and Jacobson, 2001). The post mortem small intestinal length of humans and rats is approximately 7 metres and 1.0-1.5 metres, respectively, while the average diameter of the small intestine is 5cm in humans and 0.25-0.5cm in rats (Kararli, 1995). The relative lengths of the duodenum, jejunum and ileum is different between the two species, which has to be taken into account when the absorption of drugs with regional absorption windows is being investigated, as bioavailability is expected to differ between the two species (Kararli, 1995).

One major difference between man and rat is the much larger surface area in man, which is estimated to be 200 times that in rat despite the fact that the length of the human small intestine is only five times the length of the rat small intestine. The smaller surface area in rats is due principally to the absence of folds of Kerckring, which, in man, increase the surface area of the small intestine by a factor of 3 (DeSesso and Jacobson, 2001). The absorptive surface area of the rat small intestine is increased by the presence of 20-40 villi per square millimetre of mucosa, the villi being more abundant in the ileum than the jejunum (Taylor and Anderson, 1972). The shape of the villi differ slightly between the species, being finger shaped in humans and tongue shaped in rats (Kararli, 1995). Mirovilli are present in both species.
The relative surface area of the gastrointestinal tract of man and rat, obtained by dividing the absolute surface area of each compartment of the gastrointestinal tract by the body surface area, allows a more meaningful comparison of absorptive surface area in each organism. The relative surface area of the small intestine in man is more than 4 times that of the rat, and it would therefore be anticipated that drugs which are poorly absorbed in both species will exhibit better bioavailability in humans than in rats (DeSesso and Jacobson, 2001).

5.1.2.3 Caecum
The rat caecum is proportionally much larger than that of humans, representing 26% and 5% of the total length of the large intestine (DeSesso and Jacobson, 2001) respectively. This is perhaps unsurprising given that the primary function of the caecum is digestion of cellulose by the resident bacterial species, and that rats are herbivorous, in contrast to omnivorous man. The rat caecum is sacculated, capacious and never empty (Tuleu et al., 1999), in comparison to the human caecum which is poorly defined and continuous with the colon. Residence of pellets in the rat caecum has been shown to be prolonged (up to six hours), which implies that the caecum acts as a trap in which pellets sink to the bottom and resist passage into the colon (Tuleu et al., 1999).

5.1.2.4 Colon
The colon of both species has a role to play in the absorption of water, sodium and other minerals, and the production and absorption of volatile fatty acids (Kararli, 1995). The colon is proportionally shorter in rats than in humans, measuring approximately 10cm and 150cm respectively, or representing approximately 6% and 18% of the length from duodenum to colon. In contrast to humans, the rat colon is unsacculated. The majority of nutrient processing that occurs in the colon is via the resident bacterial microflora and little nutrient processing occurs as a result of the secretory activity of the intestinal mucosa (DeSesso and Jacobson, 2001).
5.1.3 A comparison of the physiology of the human and rat gastrointestinal tract

5.1.3.1 Gastrointestinal pH

The pH differential along the gastrointestinal tract is similar in humans and rats. In the stomach, secretion of hydrogen ions by the parietal cells of both species results in an acidic pH. However, the pH is lower in the glandular portion of the rat stomach (2.3±0.2) than the non-glandular portion (4.5±0.2) due to the localisation of parietal cells in this region. In humans, the pH of the stomach contents increases in the fed state, in rats feeding results in an increase in pH of the non-glandular forestomach, but a slight reduction in the pH of the glandular portion (Kararli, 1995). In general, the pH of the rat stomach is slightly higher than that of humans, but still below the dissolution threshold of the Eudragit L microparticles (pH 6.0).

The pH of the duodenum is significantly higher than that of the stomach in both species, due to secretion of bicarbonate from the pancreas. pH increases to a maximum in the ileum of both species, before falling again in the caecum due to the fermentation of dietary fibre to short chain fatty acids. Interestingly, despite the herbivorous nature of rats, and therefore an expected greater production of short chain fatty acids, the fall of pH in the rat caecum is not greater than that which has been observed in humans. Colonic pH is similar in both species (Evans et al., 1988; Kararli, 1995).

For drugs with a pKa in the physiological range, the pH of the luminal contents can affect the degree of ionisation, solubility and dissolution rate of the drug in the gastrointestinal contents, although drugs are more readily absorbed across the gastrointestinal mucosa when in the unionised form. The differences in pH of the various components of the gastrointestinal tract, and the differing lengths and transit times through these regions in rats and humans, may therefore complicate the extrapolation of drug absorption from a rat model to man.

Table 5.1 summarises the anatomy and physiology of the different compartments of the gastrointestinal tract of the rat and man.
Table 5.1: Comparison of the gastrointestinal anatomy and physiology in the rat and man

<table>
<thead>
<tr>
<th>Region</th>
<th>Post-mortem length (mm)</th>
<th>Post-mortem diameter (mm)</th>
<th>Relative surface area (regional surface area/body surface area)</th>
<th>Mean residence time of non-disintegrating pellets (hours)</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Forestomach</td>
<td>200</td>
<td>150</td>
<td>0.016&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;d&lt;/sup&gt;</td>
<td>4.5&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>• Glandular</td>
<td></td>
<td></td>
<td>0.029&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.1&lt;sup&gt;e&lt;/sup&gt;</td>
<td>2.3&lt;sup&gt;f&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>1.0-2.5&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Small intestine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Duodenum</td>
<td>95-100&lt;sup&gt;a&lt;/sup&gt;</td>
<td>250&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2.5-3&lt;sup&gt;a&lt;/sup&gt;</td>
<td>25&lt;sup&gt;c&lt;/sup&gt;</td>
<td>6.9&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>• Jejunum</td>
<td>900-1350&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3000&lt;sup&gt;b&lt;/sup&gt;</td>
<td>4-5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>111&lt;sup&gt;c&lt;/sup&gt;</td>
<td>5.5-6.0&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>• Ileum</td>
<td>25-35&lt;sup&gt;a&lt;/sup&gt;</td>
<td>3000&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3.5&lt;sup&gt;a&lt;/sup&gt;</td>
<td>50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>7.8&lt;sup&gt;f&lt;/sup&gt;</td>
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<td></td>
<td></td>
<td></td>
<td>7.0-7.5&lt;sup&gt;g&lt;/sup&gt;</td>
</tr>
<tr>
<td>Caecum</td>
<td>50-70&lt;sup&gt;a&lt;/sup&gt;</td>
<td>100-300&lt;sup&gt;b&lt;/sup&gt;</td>
<td>10&lt;sup&gt;a&lt;/sup&gt;</td>
<td></td>
<td>6.7&lt;sup&gt;f&lt;/sup&gt;</td>
</tr>
<tr>
<td>Colon</td>
<td>90-110&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1500&lt;sup&gt;b&lt;/sup&gt;</td>
<td>3-10&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.85&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.1&lt;sup&gt;f&lt;/sup&gt;</td>
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<td></td>
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<td></td>
<td>50&lt;sup&gt;b&lt;/sup&gt;</td>
<td>0.19&lt;sup&gt;c&lt;/sup&gt;</td>
<td>7.0-7.5&lt;sup&gt;g&lt;/sup&gt;</td>
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<sup>a</sup> Hebel and Stromberg (1976);<sup>b</sup> Ritschel (1991);<sup>c</sup> DeSesso and Jacobson (2001);<sup>d</sup> Tuleu et al., (1991);<sup>e</sup> Clarke et al., (1993);<sup>f</sup> Ward and Coates (1987);<sup>g</sup> Evans et al., (1988)
5.1.3.2 Bile fluid

Bile is a variable mixture of water, bile acids, phospholipids, cholesterol, bilirubin and inorganic solutes necessary for the emulsification and absorption of fats. Bile also aids the dissolution of lipophilic drugs in the luminal contents by improving the wetting properties of the drug or increasing the solubility of the drug in micellar solutions. Bile salts are formed in the liver by the breakdown of cholesterol, and in humans, are secreted into the duodenum via the gall bladder. Rats do not possess a gall bladder, and dilute bile fluid is continually secreted into the duodenum in large volumes. Bile flow is higher in the rat (48-92 versus 2.2-22.2 mL/kg/day in man). However the composition of bile is more variable in humans, containing 3-45mmol/L of bile salts, compared to 17-18 mmol/L in rats (Kararli, 1995).

5.1.3.3 Gastrointestinal motility and transit

Food, as well as the vast majority of drugs, is not absorbed from the stomach in significant quantities. The fasted state motility of the stomach in humans characterised by the migrating myoelectric complex (MMC), has already been discussed in section 1.3.1. Rats, in common with other laboratory animals display this cyclic motility pattern in the fasted state (Kararli, 1995). Following food intake, the rat stomach returns to a level of activity less than phase III MMC contractions, with mixing and grinding contractions facilitating the breakdown of food to a particle size small enough to aid digestion.

The gastrointestinal transit of dosage forms in humans has been discussed in Chapter One. A number of researchers have investigated the gastrointestinal transit of non-disintegrating dosage forms in the rat. While in humans non-invasive imaging techniques are now the preferred mechanism for elucidating GI transit, the studies in the rat usually involve sacrificing an animal at a specific time point post-dose, and recovering the non-disintegrating dosage form from the GI tract of the dead animal. Repeating this for several animals at different time points allows a picture of the distribution profile of the dosage form with time to be built up.

Mori et al., (1989) investigated the mouth to caecum transit of non-disintegrating granules of diameter 0.8mm and specific gravity 0.9-1.85 in fasted rats, fed rats, and rats given soft
food. The presence of food in the stomach, and administration of low or high density pellets delayed gastric emptying. However, small intestinal transit time was always around 3 hours, irrespective of dosage form or fed status, which correlates well with the small intestinal transit time in humans (Davis et al., 1986a). It should be noted, however, that although small intestinal transit times in humans and rats are similar, the speed of transit is much slower in the rat small intestine which is 1.2-1.7m in length compared to 6-7m in humans (Kararli, 1995).

Tuleu et al., (1999) performed a similar investigation in fed rats, but follow whole gut transit of low (0.9g/cm$^3$) and high (1.5g/cm$^3$) density pellets of size 0.7-1mm and 1.25-1.6mm. Calculated small intestinal transit was in agreement with (Mori et al., 1989), however caecal residence time was prolonged (~2-11 hours), and increased for large and dense formulations.

Ciftci and Groves (1996) investigated the gastrointestinal transit of barium sulphate-loaded granules of size 425-500µm in fasted female Sprague-Dawley rats. Granules were located in the stomach for the first 2 hours, in the small intestine and caecum from 2-4 hours, in the ascending colon at 4-6 hours, in the ascending and transverse colon from 6-8 hours and in the descending colon and rectum from 12-24 hours.

In summary, gastrointestinal pH, gastric emptying and small intestinal transit time are similar in the rat and man. The small size of the microparticles, and the non-ionisable nature of prednisolone, along with the ease of handling and low cost of the rat make the rat a potential model for preliminary investigations into the \textit{in vivo} performance of Eudragit L and S microparticles.
5.1.4 Pharmacokinetics of prednisolone

Absorption
Like many drugs, prednisolone is most efficiently absorbed from the proximal small intestine. Nakayama et al., (1999) measured the absorption of prednisolone from the different regions of the rat small intestine, by injecting 1mL of 100μM prednisolone solution directly into a 10cm loop of duodenum, jejunum and ileum and measuring the unabsorbed fraction. After 30 minutes, 97.5±2.2, 72.6±4.1 and 44.9±4.4% of the administered dose had disappeared from the duodenal, jejunal and ileal loops, respectively. The limited absorption of prednisolone from the distal small intestine has been attributed to the activity of P-glycoprotein in this region, for which a number of steroid hormones, including prednisolone, are substrates.

Following oral administration of prednisolone tablets in humans, prednisolone is efficiently absorbed from the gastrointestinal tract, with 75-98% of the dose being absorbed into the systemic circulation (Vogt et al., 2006). Peak plasma levels (C_{max}) are reported to occur 1 to 2 hours post dose (Pickup, 1979; Al Habet and Rogers, 1980; Rose et al., 1981a; Frey and Frey, 1990) and the half life of prednisolone in the plasma has been calculated to be between 2 and 4 hours (Ali, 1992).

When repeated administration of prednisolone is required, it is usual for the drug to be delivered as an enteric coated dosage form to avoid the risk of gastric ulceration. The use of enteric coated prednisolone tablets or administration of immediate release prednisolone tablets with or after food may delay and reduce plasma C_{max}, although bioavailability is not generally affected (Bloor et al., 1989).

Distribution
In blood, prednisolone is extensively bound to plasma proteins, mainly to the corticosteroid binding globulin, transcortin, (high affinity, low capacity) and albumin (low affinity, high capacity). It appears that prednisolone exhibits dose dependent pharmacokinetics, whereby volume of distribution and plasma clearance increase with increasing dose, which can be related to the non-linear binding of prednisolone to plasma proteins at increasing doses (Ali, 1992; Czock et al., 2005; Vogt et al., 2006). An increase in the free prednisolone in the blood is observed once transcortin binding sites become saturated, thought to occur at
prednisolone concentrations above 400ng/mL which follow administration of doses in excess of 20mg in humans (Czock et al., 2005).

**Metabolism**

Although the metabolic pathways of prednisolone are far from clearly defined, prednisolone, in common with other corticosteroids, is mainly metabolised in the liver. Major metabolic pathways for prednisolone involve oxidation-reduction at the C-11 position, reduction of the 20-keto group, cleavage of the dihydroxyacetone side-chain, reduction of A ring double bonds and hydroxylation at the C-6 carbon (Ali, 1992). Plasma clearance of prednisolone is rapid in the rat; Boudinot and Jusko (1986) calculated the half life to be just 0.5 hours after intravenous doses of 5mg/kg and 50mg/kg prednisolone sodium succinate.

**Excretion**

Prednisolone is excreted in the urine as free and conjugated glucuronic acid and sulphate metabolites together with an appreciable proportion of unchanged prednisolone representing between 7 and 30% of the administered dose (Ali, 1992). Thus, administration of prednisolone to patients with impaired hepatic or renal function, or those concomitantly taking one or more drugs which inhibit prednisolone metabolism, such as cyclosporine, results in a longer elimination half life and therefore higher plasma drug concentrations with the risk of toxicity. The opposite effect is seen with enzyme-inducing drugs such as rifampicin, carbamazepine and the barbiturates (Czock et al., 2005, British National Formulary 52). Interestingly, smoking has not been shown to affect prednisolone pharmacokinetics (Rose et al., 1981b).
5.2 Materials
Micronised prednisolone was purchased from Sanofi-Aventis (Romainville, France). Eudragit L100 and S100 were obtained from Rohm Pharma (Darmstadt, Germany). Sorbitan sesquioleate was purchased from Sigma Aldrich Company (Poole, UK). Methylcellulose E4M was obtained from Colorcon (Dartford, England). Male Wistar rats of weight 280-350 g were purchased from Harlan (UK).

5.3 In vivo absorption studies using the rat oral gavage model
The aim of the in vivo studies described in this chapter was to investigate the absorption of prednisolone from a suspension of prednisolone-loaded Eudragit L and Eudragit S microparticles, in comparison to a control suspension of prednisolone.

5.3.1 Preparation of prednisolone and microparticle suspensions
Three batches of Eudragit L/prednisolone (5:1) and Eudragit S/prednisolone (5:1) microparticles were prepared as described in section 2.9.1. The three batches of Eudragit L microparticles were mixed in a Turbula mixer for 15 minutes, and the three batches of Eudragit S microparticles were mixed in a Turbula mixer for 15 minutes. The pooled microparticles were stored under vacuum inside sealed amber glass jars until required. The prednisolone encapsulation efficiency of the blended Eudragit L and S microparticles was calculated as described in section 3.16.3, and was 86.4% and 90.0%, respectively.

Micronised prednisolone (Sanofi-Aventis, Romainville, France) was used as received, the particle size of which was <5μm (see Figure 5.2).
A 0.5% w/v methylcellulose solution was prepared as the suspending agent for both the Eudragit L and S microparticles, and prednisolone drug substance. A mass of each formulation equivalent to 300mg/kg prednisolone was suspended in 3mL of methylcellulose solution with the aid of a Rotamixer (and brief sonication for prednisolone suspension), of which 2mL of suspension (containing a dose of 200mg/kg prednisolone) was administered to rats by oral gavage. The study was conducted in 3 legs, which were as follows:

- **Leg 1 (prednisolone suspension):** 2mL of 0.5% w/v methylcellulose solution containing 200mg/kg prednisolone drug substance was administered orally to a group of five rats
- **Leg 2 (Eudragit L microparticle suspension):** 2mL of 0.5% w/v methylcellulose solution containing 1389mg/kg Eudragit L microparticles (equivalent to 200mg/kg prednisolone) was administered orally to a group of five rats
- **Leg 3 (Eudragit S microparticle suspension):** 2mL of 0.5% w/v methylcellulose solution containing 1333mg/kg Eudragit S microparticles (equivalent to 200mg/kg prednisolone) was administered orally to a group of five rats

### 5.3.2 Dosing and blood sampling from rats

Male Wistar rats weighing 280-350g were housed in cages in groups of three or four and allowed free access to food and water. The night before dosing, the rats’ diet was restricted to four pellets of standard rat chow, but they continued to have free access to water. As gastric emptying is known to be delayed in proportion to the quantity of food that is present...
in the stomach, a restricted diet was enforced in an attempt to avoid a prolonged gastric residence of microparticles. As rats are corprophagic, i.e. they will eat their own faeces in the absence of alternative food, and it was not possible to place the rats in a metabolic cage under the terms of the project licence, a restricted diet was considered to be the best way of standardising and hastening the gastric emptying of the microparticle and drug suspensions. Rats were used for experiments after a one-week acclimatisation period.

On the morning of the study, rats were taken from the group cages and placed in isolation, where they were allowed free access to water, but not fed again until 8 hours post dose. Rats were weighed to allow a calculation of the dose of prednisolone or microparticle suspension to be administered.

Rats were dosed by oral gavage with 2mL of a suspension containing a 200mg/kg dose of prednisolone in either Eudragit L or S prednisolone-loaded microparticles or prednisolone crystals in 0.5% w/v methylcellulose solution. At 0, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8 hours post dose, approximately 200μL of blood was collected from the rat tail vein into heparinised tubes (Microvette CB300, Sarstedt, UK), and stored on ice. Immediately following collection of the 8 hour sample, blood samples were centrifuged at 13,000 rpm for 10 minutes (Eppendorf Centrifuge 5415D, Eppendorf AG, Hamburg, Germany), and the supernatant plasma was collected in Eppendorf tubes, and stored at -20°C until analysis.

5.3.3 Quantification of prednisolone in rat plasma
It was firstly attempted to quantify prednisolone in rat plasma using a HPLC-UV method (described in Appendix Two). However, the sensitivity of the method was low due to a combination of the small volume of plasma which can be sampled from the rat, interference due to co-eluting peak in the HPLC trace (thought to be cortisol), prednisolone metabolism reducing the quantity of parent drug in the plasma, and the poor UV chromophore of prednisolone. It was therefore decided to quantify prednisolone in the plasma samples using HPLC/tandem mass spectrometry (MS-MS).

In mass spectrometry (MS), a sample is bombarded by an electron beam to produce gaseous charged molecules. The generated ions can be manipulated by the application of either electric or magnetic fields and separated by their mass to charge ratio. A mass
spectrum details the amounts of different ions formed under specific conditions plotted against the mass:charge ratio. MS-MS splits the molecule to be analysed into a number of daughter ions, which can be used as a fingerprint for a particular molecule. HPLC-MS-MS is therefore a much more specific technique than HPLC-UV, to an extent that reduces the need for total separation of the various compounds in a sample by chromatography alone. The application of HPLC-MS-MS facilitates the detection and quantification of drug substances from biological matrices in the sub ng/mL range (Watson, 1999).

5.3.3.1 Solid Phase Extraction (SPE) and HPLC-MS/MS method for the quantification of prednisolone in rat plasma

The plasma samples collected as described in section 5.3.2 were analysed for prednisolone by Tepnel Scientific services, Glasgow, UK, using the SPE and HPLC-MS/MS method described below.

5.3.3.1.1 Solid phase extraction

A solid phase extraction was carried out using Varian Focus (10mg/mL) SPE cartridges as follows:

**Conditioning step:** 500µL acetone/acetonitrile (1:1), followed by 500µL methanol, and finally 500µL 0.1% formic acid

**Sample loading step:** 20µL plasma was diluted with 20µL dexamethasone solution (2µg/mL) as internal standard and 600µL of 0.1% formic acid

**Interferent washing step:** 500µL 0.1% formic acid followed by 500µL acetonitrile/deionised water (1:9)

**Elution step:** 500µL acetone/acetonitrile (1:1)

The acetone/acetonitrile (1:1) was evaporated to dryness under a stream of nitrogen gas at 40°C and reconstituted in 80µL of 10mM ammonium acetate:acetonitrile (70:30 v/v), and transferred to sample vials for HPLC-MS/MS analysis.
5.3.3.1.2 HPLC-MS/MS analysis

Ten microlitres of the reconstituted sample from section 5.3.3.1.1 was injected onto a ACT ACE 5AQ 50 x 2.0mm (5μm i.d.) column. The mobile phase consisted of a mixture of 10mM ammonium acetate:acetonitrile (60:40 v/v) delivered isocratically at a flow rate of 200μL/min. Tandem mass spectrometry was carried out on a PerkinElmer Series API 3000 mass spectrometer (PerkinElmer Instruments, Beaconsfield, UK) using the TurboIonSpray source in negative ionisation mode. The probe temperature was set at 350°C and ion spray voltage set at -3000V, with a collision energy of -22V. Mass transitions of prednisolone and dexamethasone were 419.23 to 328.83 and 451.21 to 361.12, respectively.

5.3.4 Data analysis

The area under the plasma concentration-time curve from 0 to 8 hours (AUG (0-8 hours)) was calculated in each of the rats using the trapezoidal rule. Mean values for the pharmacokinetic parameters AUG (0-8 hours), C\textsubscript{max} and T\textsubscript{max} were calculated for each of the groups. One way analysis of variance (ANOVA) was carried out for each of the pharmacokinetic parameters with planned comparisons back to control.

5.4 Results

The limit of quantification of the HPLC-MS/MS method was 10ng/mL, which compared extremely favourably to the limit of detection of the HPLC-UV method for prednisolone in rat plasma described in Appendix Two.

The plasma prednisolone concentration-time profiles for the groups of rats dosed with each of the three test formulations are shown in Figures 5.3 to 5.5. Figures 5.6 and 5.7 summarise the average concentration-time profiles for all three formulations. A summary of pharmacokinetic data for each of the test formulations is shown in Table 5.2.
Figure 5.3: Plasma concentration-time profile for prednisolone suspension

Figure 5.4: Plasma concentration-time profile for Eudragit L/prednisolone (5:1) microparticles
The AUC (0-8 hours) for rat LF is at least 4 times the value of any other rat in the Eudragit L microparticle group (see Figure 5.4). It was therefore decided to consider rat LF as an outlier and recalculate the mean plasma prednisolone concentration-time profile of the other four rats of the group only. The corrected mean plasma prednisolone concentration-time profiles, excluding rat LF are shown below in Figure 5.7.
A summary of the pharmacokinetic parameters AUC (0-8 hours), $C_{\text{max}}$ and $T_{\text{max}}$ are shown in the Table 5.2 (the data is shown for the Eudragit L microparticle group with rat LF both included and excluded).

**Table 5.2: Summary of mean pharmacokinetic data (±SD) for the three test formulations (n = 4 or 5).** * denotes statistical significance ($p < 0.05$) between prednisolone suspension(control) and microparticle formulations.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>AUC (0-8 hours) (ng.h/mL)</th>
<th>Relative bioavailability</th>
<th>$C_{\text{max}}$ (ng/mL)</th>
<th>$T_{\text{max}}$ (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisolone suspension</td>
<td>15372 (±2377)</td>
<td>1</td>
<td>4152 (±829)</td>
<td>102 (±45)</td>
</tr>
<tr>
<td>Eudragit L microparticles</td>
<td>11288 (±1963)*</td>
<td>0.73*</td>
<td>4330 (±609)</td>
<td>45 (±15)*</td>
</tr>
<tr>
<td>(excluding LF)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eudragit L microparticles</td>
<td>20435 (±18377)</td>
<td>1.33</td>
<td>8704 (±8765)</td>
<td>48 (±15)*</td>
</tr>
<tr>
<td>(including LF)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eudragit S microparticles</td>
<td>5414 (±1570)*</td>
<td>0.35*</td>
<td>1376 (±444)*</td>
<td>72 (±24)</td>
</tr>
</tbody>
</table>
There were also statistically significant differences between Eudragit L and Eudragit S microparticles for the pharmacokinetic parameters AUC (0-8 hours), and therefore relative bioavailability, and C_{max} (p < 0.05). The difference in the T_{max} of the Eudragit L and Eudragit S microparticles was not statistically significant.

5.5 Discussion

5.5.1 Prednisolone absorption from control suspension

The absorption of prednisolone into the systemic circulation of the rat from the control suspension (T_{max} 102±45 minutes) was similar to the absorption of prednisolone into the systemic circulation of man following administration of an immediate release tablet, for which C_{max} has been reported to occur 1-2 hours post dose (Pickup, 1979; Al Habet and Rogers, 1980; Rose et al., 1981a; Frey and Frey, 1990).

Before prednisolone can be absorbed into the systemic circulation from the control suspension, crystalline prednisolone must dissolve in the gastrointestinal contents. The dissolution step may occur in either the stomach or small intestine, with prednisolone absorption predominantly occurring from the small intestine. It is not anticipated that either mechanism will be favoured as prednisolone is a neutral molecule and does not exhibit pH-dependant solubility, although as is the case for most drugs, absorption will be maximised if prednisolone is already in solution before it reaches the optimal absorption site, which has been shown to be the proximal small intestine (Nakayama et al., 1999).

The gastric emptying of radiolabelled polyvinyl pyrrolidone (PVP) solution was investigated in rats, and found to be 75% complete after 15 minutes (Nilsson and Johansson, 1973), while the small intestinal transit times of multiparticulate dosage forms in rats has been shown to be about 3 hours (Mori et al., 1989; Tuleu et al., 1999; Jeong et al., 2001). The timing of the maximum plasma prednisolone concentration at 102±45 minutes therefore probably coincides with the majority of prednisolone, in solution or suspension, being located in the mid small intestine.

At 3 to 4 hours post dose, the mean prednisolone plasma concentration begins to fall. This could be due to reducing prednisolone levels in the lumen of the GI tract (suggesting absorption is nearly complete), or because the prednisolone in solution/suspension has
travelled to the ileo-caecal region where absorption of prednisolone is less efficient, partly attributed to the expression of P-glycoprotein in this region (Nakayama et al., 1999).

It has been reported that prednisolone is absorbed from the rat colon, though to a lesser extent than from the small intestine; intracolonic administration of 10 mg/kg doses in saline producing an AUC of 785±180 ng.h/mL compared to 4800±600 ng.h/mL for the same dose given orally (Yano et al., 2001; 2002). Absorption of prednisolone from the rat colon following oral administration of prednisolone suspension is also likely to be complicated by the absorption of the majority of water from the co-administered methylcellulose solution, so absorption of prednisolone from the lumen of the rat colon in this study is unlikely to be as effective as was observed by Yano et al. (2002).

A double peak was seen in the plasma concentration-time profile of rats PA, PC and PE after 3 or 4 hours. The presence of two peaks has been observed for other drugs including ranitidine, cimetidine, famotidine, furosemide and penicillamine (Mummaneni et al., 1995; Suttle and Brouwer, 1995a,b; Basit et al., 2002). There are three possible explanations for the double peak phenomenon; firstly that prednisolone may have more than one absorption window within the gastrointestinal tract, or, secondly, that enterohepatic recycling of prednisolone is occurring, or finally that variable gastric emptying of the prednisolone suspension is occurring. These theories are discussed in more detail, below.

Tuleu et al., (1999) demonstrated that non-disintegrating pellets experienced prolonged residence in the caecum which acted as a large trap restricting the transit of pellets into the colon. If that was also the case with the prednisolone control suspension, it is possible that any undissolved prednisolone particles may remain in the caecum for sufficient time to dissolve in the relatively abundant luminal contents of this region. Therefore, although prednisolone absorption from this region is not as rapid as from the proximal small intestine, this could be compensated by the available fluid for drug dissolution and extended residence time providing a second "absorption window".

Enterohepatic recycling occurs when drug accumulates in the bile prior to secretion into the small intestine where it would be available for reabsorption into the systemic circulation. The double peak phenomenon has not been described following administration of
prednisolone to humans, however bile flow is higher in the rat than in humans which would make the second peak in the prednisolone concentration-time profile more prominent in rats if enterohepatic recycling is indeed occurring.

Variable gastric emptying of the suspension may also provide an explanation for the double peak. It is possible that some prednisolone particles may become physically entangled in the folds of the stomach during gastric emptying of the suspending medium. This retained portion may resist gastric emptying until cleared by phase III MMC contractions when they would pass into the small intestine and be available for absorption. However, the timing of the second peak at six hours probably occurs too late to be a result of phase III contractions which would be likely to occur 3 to 4 hours after administration of the control suspension.

5.5.2 Prednisolone absorption from Eudragit L microparticles

The data for the Eudragit L microparticle group is discussed with rat LF being excluded. Prednisolone absorption from Eudragit L microparticles was significantly more rapid than from the control suspension, with peak plasma concentrations occurring after 45±15 minutes compared to 102±45 minutes (p < 0.05). Given that the gastric pH of the rat is well below the threshold pH of Eudragit L, the particles are not expected to dissolve in the stomach and drug release can therefore only occur after emptying of the Eudragit L microparticle suspension into the small intestine. Assuming that the gastric emptying of the Eudragit L microparticles suspended in methylcellulose solution proceeds at a similar rate to the PVP solution evaluated by Nilsson and Johansson (1973), this suggests that particle dissolution and prednisolone release is occurring rapidly after stomach emptying.

In the rat small intestine, rapid dissolution of the Eudragit L microparticles is expected to occur as the pH of the luminal contents exceeds the threshold pH for polymer dissolution, being 6.9±0.1 and 7.1±0.1 in the fed and fasted state, respectively (Kararli, 1995). Following microparticle dissolution, prednisolone may be presented to the duodenal contents in the amorphous (essentially pre-solubilised) form, which facilitates rapid dissolution. Once in solution, prednisolone is available for absorption at or close to its absorption window in the upper small intestine.
There is a second peak in the plasma prednisolone profiles for rats LA, LB and LD at six hours, however this peak is less prominent than for the rats which were dosed the control suspension. If enterohepatic recycling is responsible for the double peak, it would be reasonable to expect the peak to be the same size or larger in the Eudragit L microparticle group as prednisolone is absorbed more rapidly from the microparticle formulation. The timing of the double peak at six hours is unlikely to be the result of phase III MMC emptying of a portion of the formulation. More plausible is the absorption of prednisolone from the distal gut; most likely the caecum. The size of the double peak may be smaller for the Eudragit L microparticles than the control suspension due to a smaller proportion of unabsorbed prednisolone in the caecum.

The AUC for the Eudragit L microparticles was significantly smaller than from the control suspension (p < 0.05). The reasons for this are unclear given that prednisolone is released from the microparticles close to the absorption window in the gastrointestinal tract in the freely soluble amorphous form. It is conceivable that, as the gastrointestinal contents are not homogeneous with regard to pH or liquid content, a portion of microparticles empty from the stomach entrapped in a solid mass of partially digested rat chow in which polymer dissolution and drug release is not favoured resulting in incomplete prednisolone release and absorption from the Eudragit L microparticles.

It should not go unmentioned that the \textit{in vivo} performance of the Eudragit L microparticles is very different to conventional enteric coated dosage forms in man. A lag time of one to two hours is generally observed, post gastric emptying before drug is available for absorption from enteric coated tablets or pellets. This delay relates to the time for neutralisation, ionisation and disruption of the enteric polymer coating, which is a prerequisite for dosage form disintegration. In the rat, this lag time may reasonably be expected to be longer, due to the much lower volumes of luminal contents. However, rapid drug release and dissolution in the rat small intestine is probably facilitated by the large surface area of the microparticles, and the amorphous rather than crystalline nature of the drug compared to that which is contained in tablet and pellet dosage forms.

The reasons for the unexpectedly large AUC in rat LF are not clear. Possible sources of operator error would include the administration of prednisolone instead of Eudragit L
microparticles which would account for an approximate six-fold overdose of the intended quantity of prednisolone, although this is not thought to be the case, or contamination of the blood samples during extraction or HPLC analysis. Physiological factors such as the fluid volume in the gastrointestinal tract of different rats prior to dosing may also result in variability in AUC. The quantity of prednisolone administered to the rats was large, and it is possible that incomplete absorption was occurring from all three formulations. It may be the case that rat LF had consumed more water than the other rats immediately prior to dosing which would provide a more favourable environment for prednisolone dissolution and absorption. Similarly, bile flow may have been greater in rat LF which would also have facilitated prednisolone dissolution and absorption.

5.5.3 Prednisolone absorption from Eudragit S microparticles

Prednisolone absorption from the Eudragit S microparticle suspension is significantly lower than from the control suspension \((p < 0.005)\) and from the Eudragit L microparticles \((p < 0.005)\). Relative bioavailability is only 35% of the control suspension, and 48% of the Eudragit L microparticles. The reduced prednisolone absorption from Eudragit S compared to Eudragit L microparticles is probably related to the amount, or the rate, at which prednisolone is released from the formulation.

The Eudragit S microparticles do not appear to deliver prednisolone specifically to the ileocolonic region of the rat, given that the \(T_{\text{max}}\) is only 72±24 minutes, which suggests that the microparticles would be in the mid-small intestine. Premature release of a model drug, ellagic acid, in the rat small intestine was also reported from microspheres fabricated from Eudragit P4135, a pH-sensitive polymer with a dissolution threshold pH of 7.0, designed to deliver drug to the colonic region (Jeong et al., 2001).

Although drug release from the Eudragit S microparticles is likely to be slow in the rat duodenum where the pH is around 7.0 (considering the \textit{in vitro} release profiles of Eudragit S microparticles at pH 7.0 (see Figure 3.12)), any drug released from near the surface of the microparticles in this region of the small intestine is likely to be absorbed rapidly. Conversely, in the distal small intestine, luminal pH increases to a value of around 7.8 in the jejunal/ileal region, where conditions are more favourable for microparticle dissolution and drug release, but absorption of prednisolone is less rapid. The slow release, and
therefore drug absorption, from the Eudragit S microparticles could explain the plateau in the plasma concentration-time profile as drug is absorbed and eliminated at approximately equal rates.

A double peak phenomenon is seen to some extent in all rats dosed with Eudragit S microparticles, with the exception of rat SB. The appearance of a second peak at 6 hours for rats dosed with Eudragit L and Eudragit S microparticles may indicate that the gastrointestinal transit of the microparticle dosage forms is similar.

5.6 Chapter Conclusions

The pH-sensitive microparticle formulations show significantly different prednisolone plasma concentration-time profiles to the control suspension.

Eudragit L microparticles reduced the time to maximum plasma concentration compared to a control suspension of prednisolone, thought to result from release of encapsulated drug in a highly soluble form, close to the absorption window of prednisolone. This is not usually the case when drugs are administered as enteric coated formulations. However, the relative bioavailability of the Eudragit L microparticles was still significantly lower than the control suspension, possibly due to incomplete drug release from the microparticles as a result of heterogeneous mixing and composition of the gastrointestinal contents.

Eudragit S microparticles produce an extended release profile in the rat. This is probably due to slow polymer dissolution in the upper small intestine, where prednisolone is well absorbed, but slow absorption of prednisolone from the lower gastrointestinal tract where the drug is likely to be released more rapidly due to the slightly higher pH. The maximum prednisolone plasma concentration is significantly reduced in comparison to Eudragit L microparticles or control suspension, and relative bioavailability is only 35% of the control suspension.

Differences in size and pH of the gastrointestinal tract of the rat and man limit the usefulness of rat as a model for drug absorption in man. The Eudragit L microparticles, in particular, have shown promise to improve the drug delivery of poorly soluble compounds to the small intestine, and warrant further investigation in man.
Chapter Six: General discussion and future work
6.1 General discussion and conclusions

Conventional delayed release dosage forms designed to deliver drugs to site-specific regions of the gastrointestinal tract suffer from an unpredictable onset of drug action and poor targeting specificity, which is attributed to the physiological variability of the gastrointestinal tract, particularly with regard to transit, pH and fluid volume. The primary aim of this work was to engineer and evaluate pH-sensitive microparticle formulations for drug delivery to the proximal small intestine and ileo-colonic region, which it is proposed may be able to overcome the limitations of conventional delayed release dosage forms.

Using a simple, non aqueous coacervation method it was possible to form a spherical coacervate of Eudragit L, but attempts to solidify the coacervate by complete removal of the coacervate phase solvent resulted in agglomeration and phase separation. Spray drying organic solutions of pH-sensitive Eudragits did produce microparticles, but morphology was collapsed and yields were invariably low. Furthermore, when prednisolone was incorporated into spray dried Eudragit L particles at a theoretical drug loading of 3.2%, prednisolone release after 2 hours' incubation at pH 1.2 exceeded the USP limit of 10% for enteric coated products.

With some difficulty, a novel yet simple émulsification/solvent evaporation method was successfully developed for the fabrication of Eudragit L55, L and S microparticles using the pharmaceutically acceptable ICH class III solvent, ethanol. The critical parameter was the inclusion of the surfactant Arlacel 83 (sorbitan sesquioleate) into the o/o emulsion, which permitted the formation of spherical, unaggregated microparticles of mean size \( \leq 50\mu m \). The beneficial effect of Arlacel 83 on microparticle formation was shown to be due to improved emulsion stability, however, it was shown that this was not due to the HLB value of the surfactant alone.

Eudragit L and L55 microparticles were tested in vitro for their ability to target prednisolone to the proximal small intestine. The rate of prednisolone release from Eudragit L microparticles increased with both drug loading and pH. Using a pH change dissolution methodology to simulate transit from the stomach to the small intestine (pH 1.2-6.8), Eudragit L/prednisolone microparticles of 5:1, 10:1 and 20:1 drug loading each released less than 10% of entrapped drug after 2 hours' incubation in acid, but completely
released drug within 5 minutes following the pH change, which was faster than a commercial formulation (Deltacortril Enteric®). Eudragit L55 microparticles were considered to be unsuitable for further evaluation, given that they aggregated, and failed to control the release of prednisolone, in acidic media. Both of these shortcomings were attributed to the low glass transition temperature (107°C) of this polymer in comparison to Eudragit L (200°C), which it was hypothesised resulted in increased molecular mobility of Eudragit L55 at 37°C due to the plasticising effect of water.

Eudragit S microparticles were evaluated in vitro for their ability to deliver model drugs to the ileo-colonic region. Similarly to Eudragit L microparticles, prednisolone release rate increased with drug loading and pH. Eudragit S microparticles were evaluated using a pH change dissolution methodology more suitable to the higher dissolution threshold pH of this polymer (pH 1.2-7.4). The release of prednisolone could be restricted to <10% at pH 1.2 at drug loadings of 2.5:1 and below. At a drug loading of 2.25:1 prednisolone is present within the internal oil phase of the emulsion above its saturation solubility, and the morphology of the microparticles is seen to be different to microparticles of lower drug loading, possibly with prednisolone being present on the surface of the microspheres in a crystalline form. The incorporation of the water insoluble polymers, ethylcellulose or Eudragit RS, into the matrix of the Eudragit S microparticles retarded drug release at pH 7.4, which may be useful for the topical delivery of drugs to a larger area of the colonic mucosa than is possible using Eudragit S alone. Control of release of the model drugs bendroflumethiazide and budesonide was also possible, which was particularly surprising for the former molecule given the basic nature of this drug. However, when paracetamol was investigated as a model drug over 75% drug release occurred after 2 hours’ incubation in acid, which is thought to be due to the low molecular weight of this drug.

The fabrication of prednisolone loaded pellets of size <1mm using the technique of extrusion followed by spheronisation was also possible. However, due to the large surface area of the pellets, theoretical weight gains of 29.8% Eudragit L and 18.8% Eudragit S were required to limit prednisolone release in acid to within USP limits. Furthermore, following the pH change and dissolution of the polymer coat, prednisolone release was slow from the pellet matrix.
The rapid release of prednisolone from the Eudragit L and S microparticles, above the threshold pH of the respective polymers, was hypothesised to be related to the large surface area of the microparticles and, possibly, the amorphous nature of the entrapped drug. However the technique of XRPD could not confirm that the entrapped prednisolone was present entirely as the amorphous form because, although the sensitivity of the developed method for the detection of crystalline prednisolone in Eudragit L was in the upper range of that reported for the technique (4%), this meant that prednisolone could be present in the crystalline form at a level of up to 28% of total drug content and not be detected. Furthermore DSC, generally regarded as a more sensitive tool than XRPD for the quantification of crystalline material, was not able to characterise the physical form of the drug within the microparticles as crystalline prednisolone was thought to dissolve in the Eudragit polymer chains above their Tg, thus reducing the observed ΔH value for the melt.

Eudragit L and S microparticles (5:1 polymer:prednisolone loading) were filled into gelatin capsules and subjected to stability testing at 25°C/60% RH and 40°C/75% RH for 13 weeks. During that time, the in vitro dissolution profile of prednisolone from Eudragit L microparticles (pH 1.2-6.8) remained unchanged, while the dissolution profile of Eudragit S (pH 1.2-7.4) microparticles slowed down considerably following the pH change. Such changes in the in vitro dissolution rate are commonly a result of a change in the physical form of the drug (from amorphous to crystalline), however the XRPD method failed to detect crystallinity in any of the stability samples, further highlighting the unsuitability of this technique for the quantification of crystalline prednisolone within the microparticles. Nevertheless, it was hypothesised that the instability of the Eudragit S /prednisolone (5:1) microparticles was due to the lower Tg of this polymer (160°C) than Eudragit L (200°C). It is thought that plasticisation of the Eudragit S polymer resulting from water uptake from the controlled atmosphere inside the desiccator, or perhaps more likely the capsule shell, increased the molecular mobility of the polymer chains in the microparticle matrix, particularly at 40°C. This, in turn, could have resulted in recrystallisation of amorphous prednisolone, or affected the conformation of the polymer chains, either of which could potentially have led to a decrease in prednisolone dissolution rate at pH 7.4.

The in vivo performance of the Eudragit L and S microparticles in comparison to a prednisolone control suspension was evaluated using the rat oral gavage model. The in
in vivo performance of the Eudragit L microparticles was extremely encouraging, as prednisolone absorption into the systemic circulation of the rat was significantly more rapid than from the control suspension, occurring after 45±15 minutes compared to 102±45 minutes. This was thought to be due to prednisolone release from the particles in the amorphous (pre-solubilised form), close to its absorption window in the upper small intestine. However total prednisolone absorbed from Eudragit L microparticles was significantly lower than from the control suspension (relative bioavailability is 0.73) for reasons which are not clear.

It is unlikely that the Eudragit S microparticles delivered prednisolone specifically to the ileo-colonic region of the rat gastrointestinal tract, given that the T<sub>max</sub> of this formulation was only 72±24 minutes when the formulation would be expected to be in the mid-small intestine. Compared to the prednisolone suspension and Eudragit L microparticle formulations, plasma prednisolone levels are fairly constant from 1 to 7 hours post dose, which was thought to be due to slow release and efficient absorption of prednisolone in the proximal small intestine, and more rapid release (due to increased luminal pH) and reduced absorption efficiency (compared to the proximal small intestine) in the ileo-colonic region.

Overall, the aims of the study were achieved. A universal method for the production of pH-sensitive Eudragit microparticles was developed that compares favourably to any reported in the literature in terms of microparticle morphology, in vitro control of drug release and potential for scale-up. In addition, the Eudragit L microparticles have been shown to be physically stable for a period of 13 weeks at accelerated storage conditions, and have shown, in vivo, the potential to achieve a rapid drug release in the small intestine.

### 6.2 Future work

- It would be of interest to confirm the reason for the agglomeration of Eudragit L55 microparticles that occurred in 0.1M HCl following capsule disintegration. A possible method to achieve this would be the use of immersion dynamic mechanical thermal analysis (immersion DMTA), which could measure the Tg of the microparticles while immersed in, and plasticised by, the dissolution medium.
• The physical form of prednisolone within the microparticles could not be elucidated in this study using either DSC or XRPD, due to a lack of sensitivity of these methods. It would be useful to develop a method to characterise the physical form of the drug, which could be correlated with *in vitro* dissolution in the stability testing protocol, and would be able to determine at what point prednisolone recrystallisation (if it is responsible for changes in dissolution rate) will begin to affect dosage form performance.

• The effects of alternative packaging on the stability of the acrylic microparticles could be readily investigated. It would be of particular interest to confirm whether the use of HPMC capsules (which contain less residual moisture than gelatin capsules) and moisture occlusive packaging such as PVC/Aclar® or cold form aluminium blister packaging could achieve stability of Eudragit S microparticles upon storage.

• Last but not least, the promising *in vitro* dissolution data (and perhaps to a lesser extent, *in vivo* data) suggests that the Eudragit L and S microparticles warrant investigation in human volunteers. A joint pharmacokinetic and gamma scintigraphic study would unambiguously prove whether or not the formulations developed in this study are capable of overcoming the limitations of conventional delayed release dosage forms. An investigation of how different methods of microparticle administration (capsule, small and large volume suspension) affect gastrointestinal transit and drug release would aid the rational design of future modified release formulations.
Appendix 1
Section One: Microencapsulation by coacervation

A1.1 Introduction

Coacervation can be described as the macromolecular aggregation (or controlled phase separation) process brought about by the partial desolvation of fully solvated macromolecules (Arshady, 1990). The gradual reduction in solubility of a dissolved macromolecule (or polymer), by addition of a chemical, or change of temperature or pH, gradually increases the interactions between the polymer molecules, resulting in the formation of aggregates or coacervates around an insoluble nucleus (Arshady, 1990; Magdassi and Vinetsky, 1996). This partial desolvation is distinct from the total or exhaustive desolvation that is observed during precipitation. In all coacervation systems, the polymer-rich phase is termed the “coacervate phase” and the polymer poor phase the “coacervation medium” (Arshady, 1990). Coacervation differs from the related emulsification/solvent evaporation method of microencapsulation (see section 2.1.3.1), as the solvent components of the polymer-poor and polymer-rich phases are essentially the same, as opposed to two immiscible liquids.

Coacervation is generally employed as a method of fabricating microcapsules, where polymer coacervation proceeds around solid nuclei that are insoluble, and suspended, in the coacervation medium. However, under certain circumstances it has been demonstrated that this procedure can be utilised to form microspheres (Ruiz et al., 1989; Benoit et al., 1996).

Coacervation processes can be aqueous or non-aqueous, depending on whether the agent used to initially solvate the polymer is aqueous, or not. Aqueous coacervation systems can be further subdivided into simple and complex systems. Simple coacervation involves only one polymer, whereas complex coacervation requires two polymers, commonly of opposite charge, which interact to form an insoluble complex which induces phase separation. The most significant application of coacervation has been in the manufacture of carbonless copy paper, which is achieved by a complex coacervation technique (Thies, 1996).

Pharmaceutical uses of complex coacervation generally employ gelatin as the cationic polymer, which can interact with a number of anionic polymers, commonly gum arabic.
Microcapsules with a core loading as high as 80-90% can be formed by this method, however microcapsules usually have to be cross-linked before they can be isolated from the coacervation medium (Thies, 1996).

Polymers most commonly used for the fabrication of microcapsules by the coacervation approach include gelatin, acacia, alginates, starch and ethylcellulose (Arshady, 1990), although the Eudragits have also been investigated (El Sayed et al., 1982; Benita et al., 1985a,b; Dong and Bodmeier, 2006).

It was attempted to design a simple, non aqueous coacervation method for Eudragit L, a polymer soluble in a number of water-miscible organic solvents (acetone, ethanol, methanol, propan-2-ol). It was hypothesised that the solubilisation of Eudragit L in these solvents followed by the gradual addition of water would result in the formation of a coacervate due to diffusion of organic solvent which is more miscible with water than with the acrylic polymer.

A1.2 Materials
Eudragit L100-55, Eudragit L100 and Eudragit S100 were obtained from Rohm Pharma (Darmstadt, Germany). Micronised prednisolone was purchased from Sanofi-Aventis, Romainville, France). All other reagents were of analytical grade and were used as received.

A1.3 Methods
Preliminary experiments had shown that the gradual addition of water to a solution of Eudragit L in ethanol or propan-2-ol/acetone (6:4 v/v) could result in the formation of a coacervate (unpublished data). Weighed amounts of Eudragit L were dissolved in 10mL of propan-2-ol/acetone (6:4 v/v) or ethanol to form solutions at 1-5% w/v. Ten millilitres of water was added dropwise, under continued magnetic stirring, and the mixtures were examined for the appearance of a coacervate using optical and scanning electron microscopy (SEM). Complete evaporation of the coacervation medium to solidify the coacervate phase droplets into solid microparticles was attempted using prolonged stirring under ambient conditions for 12 hours, and at 40°C under reduced pressure using a Rotary Evaporator (Büchi Rotavapor R-114, Büchi Labortechnik AG, Flawil, Switzerland).
A1.4 Results

SEM confirmed that coacervate droplets were formed when both the propan-2-ol/acetone (6:4 v/v) (see figures A1 and A2) and ethanol (see figures A3 and A4) were used as coacervate solvents for Eudragit L in concentrations of up to 4.5% w/v and 5% w/v respectively. Above these concentrations, the addition of water to the organic polymer solutions resulted in rapid desolvation of the polymer and precipitation, rather than coacervate formation.
Figure A1: SEM of coacervate formed upon addition of water to a 1% w/v solution of Eudragit L in isopropanol/acetone (6:4) v/v

Figure A2: SEM of coacervate formed upon addition of water to a 4.5% w/v solution of Eudragit L in isopropanol/acetone (6:4) v/v

Figure A3: SEM of coacervate formed upon addition of water to a 2% w/v solution of Eudragit L in ethanol

Figure A4: SEM of coacervate formed upon addition of water to a 4% w/v solution of Eudragit L in ethanol

Figure A5: SEM of coacervate formed upon addition of water to a 2% w/v solution of Eudragit L in isopropanol/acetone (6:4) v/v before rotary evaporation

Figure A6: SEM of coacervate formed upon addition of water to a 2% w/v solution of Eudragit L in isopropanol/acetone (6:4) v/v after 15 minutes rotary evaporation
As can be seen from figures A1 to A4, the coacervate particles all appear to be <2\(\mu\)m in diameter, i.e., they are in the lower range of particle size usually produced by this method (5-5000\(\mu\)m) (Kas and Oner, 2000). The use of ethanol as organic solvent resulted in smaller particles than propan-2-ol/acetone (6:4 v/v). For both solvents, an increase in Eudragit L concentration resulted in an increase in particle size.

It was not possible to form solid microparticles by complete evaporation of coacervate solvent. Continued stirring at room temperature, or short periods of rotary evaporation result in an increase in turbidity of the coacervate medium (Figures A5 and A6 show the appearance of coacervate droplets before and after 15 minutes rotary evaporation, respectively), which is due to an increase in particle size. Prolonged rotary evaporation or stirring under ambient conditions resulted in precipitation of Eudragit L as a large agglomerate.

A1.5 Discussion and conclusions

A simple organic coacervation method using water as a non-solvent led to the formation of a Eudragit L coacervate using the solvents propan-2-ol/acetone (6:4 v/v) and ethanol. This method therefore avoids the use of ICH class I (Benzene) (El Sayed et al., 1982) and class (chloroform, cyclohexane) solvents that have been used in previously reported methods (Benita et al., 1985a,b).

It has been demonstrated that the more concentrated the organic polymer solution, the larger the coacervate formed upon addition of water. However, the solidification of the coacervate droplets to microparticles could not be achieved by gradual solvent evaporation at room temperature, or accelerated solvent evaporation using a rotary evaporator. Following initial addition of water to the polymer solution under magnetic stirring, the organic solvent begins to diffuse out of the polymer solution initiating the formation of a polymer-rich phase and a polymer-poor phase. The volatile organic solvent evaporates from the coacervation medium, resulting in a diffusion gradient for the migration of further organic solvent. As the processes of solvent diffusion and evaporation proceed, the coacervate phase increases in viscosity. As Eudragit L is a hydrophobic polymer, agglomeration of coacervate droplets will occur as most of the organic solvent evaporates, eventually resulting in complete phase separation.
The phenomenon of total phase separation following the formation of a coacervate has previously been described. Arshady (1990) noted that in the absence of any stabiliser and stirring, droplet coalescence continues unabatedly and leads to the formation of large coacervate droplets and finally to macroscopic phase separation. Ruiz et al., (1989) investigated the coacervation of poly (lactic acid-co-glycolic acid) (PLGA) dissolved in dichloromethane upon addition of silicone oil. A small “stability window” was described in which the addition of a certain quantity of silicone oil would induce the formation of a stable dispersion of PLGA coacervate droplets. Addition of further silicone oil induced extensive aggregation of coacervate droplets, and polymer precipitation. Solidified PLGA microspheres could only be obtained by transferring the PLGA coacervate to a non-solvent medium which caused them to harden.

Recently, an improvement of the simple organic coacervation method described in this section has been reported (Dong and Bodmeier, 2006), using aqueous solutions of hydrophilic polymers as the non-solvent phase. Solutions of hydroxypropyl methylcellulose were thought to act as a stabiliser and thickening agent to prevent coalescence of the coacervate droplets formed from ethanolic solutions of Eudragit L55, L or S. Spherical, drug loaded microparticles of size \( \leq 30 \mu m \) have been shown to be formed, although so far no drug release profiles have been reported.

It was decided that as the coacervation method described above was not capable of producing solidified microparticles, other methods for the microencapsulation of pH-sensitive Eudragits would be explored.
Section Two: Microencapsulation by spray drying

A1.6 Introduction

Spray drying involves the transformation of a liquid feed material (solution, suspension, emulsion) into a dried particulate form by spraying into a stream of hot gas (Masters, 1990). Although it is often described as a one-step process, in reality spray drying can be sub-divided into four separate stages

(i) atomisation of liquid feed material
(ii) spray-hot gas contact
(iii) solvent evaporation (particle drying)
(iv) product recovery

Atomisation of the liquid feed material is achieved by pumping it through a circular nozzle, generally of diameter <1mm, located in the centre of the spherical outlet for the atomising gas to form droplets of size up to a few hundred microns (Farid, 2003). The droplets formed during the atomisation process are then dried quickly on contact with the hot gas in the drying cylinder as shown in Figure A7.
Farid proposed that after atomisation, an individual droplet is heated by the drying air with no significant evaporation until it reaches the air wet-bulb temperature (A-B). A period of shrinkage then occurs as solvent evaporates (B-C), with the droplet remaining at the wet-bulb temperature. Following the period of shrinkage, solvent evaporation continues but with the formation of a crust on the outer droplet surface surrounding a wet core (C-D), a process which continues until the end of the drying phase (E). After the completion of drying, a slight heating of the solidified particle occurs (E-F).
The final diffusion and evaporation of the solvent through the crust may result in the particle remaining intact, or fracturing, inflating or collapsing (Farid, 2003). Finally the dried particles are separated in the cyclone which uses centrifugal force to separate particles by size (hence the fine particle fraction passes the cyclone and ends up in the filter, while the larger particles will empty into the collecting jar).

Rapid conversion of the spray dried drug from the liquid to solid state has been shown to induce the production of the more soluble amorphous form, as insufficient time is available for the drug molecules to organise into crystal lattices. However, as has been discussed in Chapter Four, this form is thermodynamically unstable, and will tend to revert to the crystalline state upon storage (Yonemochi et al., 1999; Colombano et al., 2002; Ambike et al., 2004). A common approach to improving the physical stability of amorphous drugs, is to spray-dry composite solutions of drug with a carrier material, commonly a polymer of higher Tg (Ambike et al., 2004; 2005), which often results in a solid dispersion of drug entrapped within a polymer matrix. Although the solubility of the drug may be increased, its dissolution rate will now be dictated by that of the matrix forming polymer. Improved stability of composite systems can occur through mechanisms thought to include increasing the Tg of the system well above the storage temperature, hydrogen bonding between the drug and polymer, the antiplasticising effect of the polymer, and physical entrapment of the drug molecules within a polymer matrix.

Interestingly, Corrigan et al., (2003) demonstrated that the physical stability of bendroflumethiazide was reduced when co-spray-dried with the hydrophilic polymer PEG 4000, which is in contrast to the authors previous findings with lactose/PEG 4000 co-spray-dried material (Corrigan et al., 2002). The instability of PEG 4000/bendroflumethiazide spray-dried composites was attributed to the plasticising effect of the polymer, which served to lower the Tg of the system.

Factors affecting spray dried product morphology
A number of process variables are known to affect the morphology of the spray dried product, a summary of how these may affect the properties of the spray dried product is shown in Table A1 below. However, it should be noted that spray drying parameters are interdependent, and changing one variable may result in changes in other variables, for
example increasing feed rate usually results in a lower outlet temperature if all other variables are kept constant (Masters, 1990).

Table A1: Summary of the processing parameters which influence spray dried particle characteristics

<table>
<thead>
<tr>
<th>Process variable</th>
<th>Effect on spray dried particle characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atomising pressure</td>
<td>An increase in atomising pressure will generally decrease particle size via efficient break-up of the liquid feed</td>
</tr>
<tr>
<td>Feed rate</td>
<td>Increasing the flow rate of the feed solution will increase particle size, and residual moisture content (wetness) of the product</td>
</tr>
<tr>
<td>Air flow</td>
<td>Increasing the air flow rate will reduce drying, with the risk of increased product wetness</td>
</tr>
<tr>
<td>Inlet temperature</td>
<td>If the inlet temperature is too high (≥ solvent boiling point) a porous product with a low bulk density can be expected, lower inlet temperatures may result in crystallisation due to slow solvent evaporation</td>
</tr>
<tr>
<td>Outlet temperature</td>
<td>Low outlet temperatures may result in product wetness (and crystallisation)</td>
</tr>
<tr>
<td>Concentration of feed solution</td>
<td>Increasing concentration of feed solution can increase particle size and bulk density</td>
</tr>
<tr>
<td>Solvent</td>
<td>Choice of solvent can affect particle morphology, size or degree of agglomeration (Esposito et al., 2000)</td>
</tr>
</tbody>
</table>
Choice of spray dryer

Two different spray dryers were used in this study: the Büchi 191 mini spray dryer and the Niro SD Micro (see Figure A8). The design of the Büchi and Niro spray dryers are essentially the same, the major difference is that the drying gas used in the Niro is nitrogen, which is fed from a nitrogen generator and permits spray drying of solutions of organic solvents. This is not possible using the Büchi which only allows the processing of aqueous feed solutions/suspensions for which air is employed as the drying gas. A further advantage of the Niro is that the fluid dynamics more closely mimic those of industrial spray dryers, facilitating ease of scale up.

![Diagram of spray dryers](image)

Figure A8: A comparison of the Büchi 191 Mini Spray Dryer and Niro SD Micro spray dryer

A1.7 Microparticle production using Büchi 191 Mini Spray Dryer

A1.7.1 Method

It was attempted to prepare microspheres by spray drying both a solution and suspension of polymer and drug. A 30mg/mL suspension of Eudragit L in water, containing a ratio of 60:1 polymer: prednisolone, was prepared using a magnetic stirrer. A 30mg/mL solution of Eudragit L, containing 60:1 polymer:drug loading of prednisolone, was prepared in 1M NaOH. The solution and suspension were spray dried using the following process parameters:

- Inlet temperature: 105°C
- Outlet temperature: 66°C
- Feed rate: 10%
- Air flow rate: 600 L/h
A1.7.2 Results

Figure A9 and A10, respectively, show the morphology of Eudragit L as received and following spray drying from an aqueous suspension. It seemed that to change the structure of the Eudragit L polymer and form microparticles, the polymer would have to be dissolved rather than merely suspended in the liquid to be spray dried.

![Figure A9: SEM of Eudragit L100 (as received)](image)

![Figure A10: SEM of Eudragit L spray dried from a 30mg/mL suspension using the Buchi 191 Mini Spray Dryer](image)

Spray drying of the solution of Eudragit L/prednisolone in 1M NaOH resulted in recovery of a fine powder in the collection vessel. However, when the spray dried powder was suspended in 0.1M HCl, the powder dissolved, raising the pH of the media, which suggested that NaOH had been co-spray dried with the Eudragit L and prednisolone. It was therefore concluded that producing spray dried Eudragit L drug-loaded microparticles from a solution (in NaOH) or suspension was not feasible, and that it would be attempted to produce microparticles by spray drying organic solutions of pH-sensitive Eudragits using the Niro SD spray dryer.

A1.8 Microparticle production using Niro SD Micro spray dryer

The Niro SD Micro spray dryer uses nitrogen as opposed to air as the drying gas, and therefore permits the spray drying of organic solvents. This has utility for the generation of the amorphous form of poorly water soluble drugs from flammable organic solutions, but more importantly for this project allows solutions of the acrylic polymers to be spray dried, which have a solubility profile limited largely to flammable organic solvents.
The aim of this work was to investigate the feasibility of the use of spray drying for the fabrication of pH-sensitive Eudragit microparticles, and to investigate the effect of concentration of polymer solution, polymer solvent, processing temperatures, and the incorporation of a model drug, prednisolone, on microparticle morphology.

A1.8.1 Methods
Two hundred millilitres of solutions of Eudragit L55, L and S (2.5-15% w/v) were prepared in acetone, methanol or ethanol, with and without the addition of prednisolone (summarised in Table A2). Inlet temperatures were maintained below the boiling point of the solvent. The atomiser and nitrogen flow settings were retained at 25kg/h and 2.5kg/h, respectively. Yield was calculated for all batches. The morphology of the spray-dried product was examined by SEM.

A model drug was incorporated into the spray dried particles by dissolving prednisolone at 30:1 polymer:drug loading into selected formulations. The pH-responsive release of prednisolone loaded Eudragit L microparticles were evaluated in 500mL 0.1M HCl and 500mL pH 6.8 phosphate buffer (prepared as described in section 3.2.2) using USP II dissolution apparatus. Samples were filtered and withdrawn at 30 minute intervals and analysed spectrophotometrically at 248nm for the presence of prednisolone with reference to a standard curve.
### Table A2: Spray drying runs conducted using the Niro SD Micro spray dryer

<table>
<thead>
<tr>
<th>Run</th>
<th>Polymer</th>
<th>Polymer concentration (% w/v)</th>
<th>Solvent</th>
<th>Drug concentration</th>
<th>Inlet temp (°C)</th>
<th>Outlet temp (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Eudragit L</td>
<td>15</td>
<td>Ethanol</td>
<td>0</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Eudragit L</td>
<td>10</td>
<td>Ethanol</td>
<td>0</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>3</td>
<td>Eudragit L</td>
<td>10</td>
<td>Ethanol</td>
<td>3.2</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>4</td>
<td>Eudragit L</td>
<td>7.5</td>
<td>Ethanol</td>
<td>0</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>5</td>
<td>Eudragit L</td>
<td>5</td>
<td>Ethanol</td>
<td>0</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>6</td>
<td>Eudragit L</td>
<td>5</td>
<td>Ethanol</td>
<td>3.2</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>7</td>
<td>Eudragit L</td>
<td>2.5</td>
<td>Ethanol</td>
<td>0</td>
<td>70</td>
<td>50</td>
</tr>
<tr>
<td>8</td>
<td>Eudragit L</td>
<td>2.5</td>
<td>Ethanol</td>
<td>0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>9</td>
<td>Eudragit L</td>
<td>2.5</td>
<td>Ethanol</td>
<td>0</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>10</td>
<td>Eudragit L</td>
<td>2.5</td>
<td>80% ethanol in water</td>
<td>0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>11</td>
<td>Eudragit L</td>
<td>2.5</td>
<td>70% ethanol in water</td>
<td>0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>12</td>
<td>Eudragit L</td>
<td>2.5</td>
<td>60% ethanol in water</td>
<td>0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>13</td>
<td>Eudragit S</td>
<td>5</td>
<td>Ethanol</td>
<td>0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>14</td>
<td>Eudragit S</td>
<td>5</td>
<td>Acetone</td>
<td>0</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>15</td>
<td>Eudragit S</td>
<td>5</td>
<td>Ethanol/acetone (3:1)</td>
<td>0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>16</td>
<td>Eudragit S</td>
<td>5</td>
<td>Ethanol/acetone (1:1)</td>
<td>0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>17</td>
<td>Eudragit S</td>
<td>5</td>
<td>Ethanol/acetone (1:3)</td>
<td>0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>18</td>
<td>Eudragit L55</td>
<td>5</td>
<td>Ethanol</td>
<td>0</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>19</td>
<td>Eudragit L55</td>
<td>5</td>
<td>Acetone</td>
<td>0</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>20</td>
<td>Eudragit L55</td>
<td>5</td>
<td>Methanol</td>
<td>0</td>
<td>60</td>
<td>40</td>
</tr>
</tbody>
</table>
A1.8.2 Results

A white, cohesive powder was recovered from the collection vessel for all of the spray drying runs. Batch yields were less than 10% for each formulation. Low yields are not uncommon for spray drying, however it was thought that the thermoplastic nature of the acrylic Eudragits results in sticking of spray dried product to the sides of the spray cylinder, cyclone and tubing in between, resulting in a particularly low yield of product available for collection from the collecting vessel. Sticking of spray dried material to the walls of the spray dryer may also be occurring due to build up of static, which is anticipated to be worse for organic than aqueous solutions for which the charge is dissipated by the presence of water. Furthermore, the Niro SD Micro is designed to spray dry a minimum of 100-200g of solid material, rather than the 5-30 g of material which were contained in the solution listed in Table A2, which may have further contributed to the low yields.

Effect of polymer concentration

The size of spray dried Eudragit L microparticles formed from ethanolic solutions increases with increasing polymer concentration (see Figures A11 and A12).Collapsed microparticles were formed at all concentrations of Eudragit L, however at concentrations of 10% w/v and above, stringy aggregates were also formed which could be prevented by dilution of the feed solution to below 5% w/v.

Figure A11: SEM of 10% w/v Eudragit L in ethanol spray dried using SD Niro spray dryer (inlet 60°C, outlet 40°C)

Figure A12: SEM of 2.5% w/v Eudragit L in ethanol spray dried using SD Niro spray dryer (inlet 60°C, outlet 40°C)
It was therefore decided that, to avoid the formation of stringy aggregates, the effect of further spray drying parameters would be investigated using feed solutions containing ≤5% w/v Eudragit in organic solvent.

**Effect of organic solvent type**

Eudragit L55 microparticles fabricated by spray drying a 5% w/v Eudragit L55 in ethanol, methanol and acetone are shown in figures A13 to A15.

Eudragit L55 microparticles formed from 5% w/v solutions in ethanol, methanol or acetone are of size ≤10μm, exhibiting a collapsed morphology.
Eudragit S microparticles fabricated from 5% w/v solutions of Eudragit S in ethanol, acetone or mixtures of ethanol and acetone are shown in figures A16 to A20 below. The size of the Eudragit S microparticles appears to be similar to Eudragit L55 microparticles formed from a 5% w/v solution shown in figures A13 to A15, and the Eudragit S microparticles exhibit a similar collapsed morphology.
Figure A16: SEM of 5% w/v Eudragit S in ethanol spray dried using SD Niro spray dryer (inlet 60°C, outlet 40°C)

Figure A17: SEM of 5% w/v Eudragit S in ethanol/acetone (3:1) spray dried using SD Niro spray dryer (inlet 60°C, outlet 40°C)

Figure A18: SEM of 5% w/v Eudragit S in ethanol/acetone (1:1) spray dried using SD Niro spray dryer (inlet 60°C, outlet 40°C)

Figure A19: SEM of 5% w/v Eudragit S in ethanol/acetone (1:3) spray dried using SD Niro spray dryer (inlet 60°C, outlet 40°C)

Figure A20: SEM of 5% w/v Eudragit S in acetone spray dried using SD Niro spray dryer (inlet 50°C, outlet 40°C)
Effect of incorporation of prednisolone as a model drug

Eudragit L microparticles fabricated from 5% w/v solutions of Eudragit L in ethanol, with and without the addition of prednisolone (30:1 polymer:drug loading), respectively, are shown in figures A21 and A22.

Figure A21: SEM of 5% w/v Eudragit L in ethanol spray dried using SD Niro spray dryer (inlet 70°C, outlet 50°C)

Figure A22: SEM of 5% w/v Eudragit L in ethanol (containing 3.2% prednisolone loading) spray dried using SD Niro spray dryer (inlet 70°C, outlet 50°C)

Microparticle size appears to be similar with and without the addition of prednisolone. Furthermore, there appears to be no sign of crystallinity on the surface of the microparticles, suggesting that prednisolone is incorporated within the matrix of the collapsed Eudragit L microparticles.
The effect of processing temperature on microparticle morphology and yield

Eudragit L microparticles formed from 2.5% w/v solutions in ethanol, at spray drying inlet temperature 70°C/outlet temperature 50°C and inlet temperature 50°C/outlet temperature 40°C are shown below in Figures A23 and A24, respectively, and can be compared to Eudragit L microparticles fabricated from a 2.5% solution in ethanol at inlet temperature 60°C/outlet temperature 40°C shown in Figure A12.

The size and morphology of Eudragit L microparticles formed from spray drying a 2.5% w/v ethanolic solution at inlet and outlet temperatures of 70°C/50°C, 60°C/40°C and 50°C/40°C are similar. The SEMs demonstrate that reducing the drying temperatures does not improve the collapsed morphology of the microparticles. Furthermore, microparticle yield was below 10% at all three drying temperatures, suggesting that sticking of spray dried material to the walls of the spray dryer due to the thermoplasticity of the polymer is occurring even at the relatively low inlet/outlet temperatures of 50/40°C.
The effect of the addition of water to organic feed solutions

The appearance of Eudragit L microparticles formed from spray drying a 2.5% w/v solution of 80% ethanol in water at inlet and outlet temperatures of 70°C/50°C is shown in Figure A25.

![SEM of 2.5% w/v Eudragit L in 80% ethanol in water spray dried using SD Niro spray dryer (inlet 70°C, outlet 50°C)](image)

A comparison of Figures A12 and A25 demonstrates that the addition of water to the ethanolic Eudragit L solution appears to be increasing the aggregation of the microparticles formed by spray drying. This can be attributed to slow evaporation of water from the spray dried droplets, resulting in aggregation of individual microparticles prior to crust formation in the drying chamber.
In vitro dissolution

The pH-responsive release of Formulation 3 (10% w/v Eudragit L in ethanol containing 3.2% prednisolone) in 500mL 0.1M HCl (pH 1.2) and 500mL pH 6.8 phosphate buffer is shown in Figure A26.

![Graph showing prednisolone release from spray dried Eudragit L microparticles](image)

**Figure A26: Prednisolone release from spray dried Eudragit L microparticles**

17.3% prednisolone was released from the particles after 2 hours in acid, in excess of the USP 24 limit for 10% drug release in acid for enteric dosage forms. This may be attributable to the small particle size which was shown by SEM to be ≤50μm, presenting a large surface area for drug dissolution, as well as the porous nature of particles often produced by spray drying, due to the rapid drying, although the SEMs reveal no evidence of surface porosity. Approximately 90% of prednisolone is released after 30 minutes in pH 6.8 phosphate buffer, indicating that polymer dissolution and drug release is rapid above the threshold pH of the Eudragit L (pH 6.0), however the release rate may be slowed slightly by the cohesive nature of the particles which results in aggregation in the dissolution media.
A1.9 Discussion and conclusions

The insolubility of the pH-sensitive Eudragit polymers in water presents problems when spray drying aqueous solutions or suspensions of these polymers using the Büchi 191 Mini spray dryer. It has been demonstrated that microparticle formation is not possible when Eudragit L is suspended in water. It seems that a change in morphology from Eudragit powder to microparticles can only occur when the polymer is spray dried from solution. This is in agreement with work carried out by Esposito et al., (2000), who spray dried aqueous suspensions of Eudragit L, RS and RL incorporating ascorbic acid as a model drug. Release rate of ascorbic acid from Eudragit L, RL or RS microparticles was not significantly different from free ascorbic acid, suggesting that ascorbic acid was present only on the surface of particles of acrylic polymer which remained unchanged by the spray drying process.

Spray drying solutions of pH-sensitive Eudragits (L55, L and S) from solutions in organic solvents using the Niro SD Micro spray dryer resulted in the formation of microparticles within the desired size range. However microparticles were of collapsed morphology which resulted in poor flow, and yields were invariably low (less than 10%), which could not be improved by optimisation of polymer concentration, polymer solvent, or drying temperatures.

Low yields are commonly achieved using spray drying (De Jaeghere et al., 2001). The extremely low yields in this study are attributed to the thermoplasticity of the acrylic polymers, which results in product adhering to the walls of the spray dryer. Furthermore, the use of non-aqueous solvents may further reduce yields due to static build up. Some spray dryers are fitted with vibrators, moving rakes or sweeper arms to prevent product sticking to the chamber walls and assist in collecting the product. However, neither the Büchi 191 Mini Spray Dryer nor the Niro SD Micro are equipped with such a facility.

Even at a low theoretical prednisolone loading of 3.2%, Eudragit L microparticles produced by spray drying failed to control drug release in acid. The failure of spray dried Eudragit L microparticles to adequately control drug release in acidic media has previously been reported. Palmieri et al., (2001) demonstrated that, following tableting, Eudragit L microparticles containing 11.1% theoretical paracetamol loading released >20% of drug
after 2 hours in acid. The same authors showed that ketoprofen release could be restricted within pharmacopoeial limits after 2 hours in acid while releasing all of the drug within 20-30 minutes at pH 6.8. However it should be noted that ketoprofen is an acidic molecule which itself exhibits a pH dependent solubility profile (Palmieri et al., 2002a,b).

A spray drying method which permits the formation of spherical pH-sensitive microparticles which control the release of a suitable model drug within pharmacopoeial limits has yet to be described. Esposito et al., (2000) conducted a thorough investigation of the factors which affect the morphology of microparticles produced by spray drying a solution of the water-insoluble acrylic polymer, Eudragit RS. A factorial design was used to optimise the spray drying parameters polymer solvent, feed rate, air flow rate, drying temperature and aspirator flow rate for the production of Eudragit RS microparticles. However, although Eudragit RS microparticles of spherical morphology and narrow size distribution of size approximately 5μm could be obtained, when the optimised parameters were applied to Eudragit RL, L, S and E, collapsed microparticles were produced, which were characterised by a non-spherical and irregular shape. This indicates that optimal spray drying parameters are not transferable between, and must be optimised individually for, chemically similar polymers.

Spray drying is a widely available, continuous, one-step process which is readily scaled-up, and has therefore provoked interest as a method of microencapsulation. However, although it is possible to produce Eudragit L55, L and S microparticles in the desired size range, the low yield, poor morphology and failure of the resultant particles to control drug release at acidic pH limit the utility of spray drying for the fabrication of pH-sensitive Eudragit microparticles. The emulsification/solvent evaporation technique was therefore investigated as an alternative method for the fabrication of pH-sensitive Eudragit microparticles.
Appendix 2
A2.1 Introduction
This appendix describes the validation of a SPE and HPLC-UV method for the quantification of prednisolone in rat plasma, and the unsuccessful application of this method to investigate the prednisolone absorption in rats from Eudragit L and S microparticles, and a prednisolone suspension.

Section One: SPE and HPLC-UV method validation
A2.2 Methods
A2.2.1 Preparation of prednisolone-spiked plasma samples
One millilitre of plasma was spiked with 25μL ethanolic prednisolone solutions to yield plasma samples containing 0, 1.25, 2.5, 5, 10, 25 and 50μg/mL prednisolone. Fifty microlitres of plasma samples were diluted with 600μL water prior to solid phase extraction as described below.

A2.2.2 Solid phase extraction (SPE)
Solid phase extraction was carried out using Strata C18E (30mg/mL) cartridges (Phenomenex, Macclesfield, UK) and a Phenomenex extraction manifold was used to draw liquids through the cartridge as follows:

**Conditioning step:** 1mL acetonitrile/acetone (1:1), followed by 1mL methanol, followed by 1mL water was allowed to flow slowly through the sorbent, and drain to waste

**Sample loading step:** the diluted plasma sample prepared in section A2.2.1 was allowed to flow slowly through the sorbent, and drain to waste

**Interferent washing step:** 1mL dichloromethane was allowed to flow slowly through the sorbent, to waste, after which the cartridge was dried for 15 minutes under a pressure of 20 inches Hg

**Elution step:** Prednisolone was eluted from the column with 1.5mL acetonitrile/acetone (1:1), which was collected in 2mL a glass vial.

The acetonitrile/acetone mixture was evaporated to dryness under a stream of nitrogen at 40°C using a DriBlock DB3 heater fitted with a SC-3 sample concentrator (Techne,
Cambridge, England). The residue was reconstituted with 250μL mobile phase (water/acetonitrile (18:7 v/v)).

A2.2.3 HPLC-UV analysis
A Hewlett-Packard 1050 series HPLC system (Agilent Technologies, UK) was used to analyse the reconstituted samples, using the method described by Yano et al., (2002). Sixty microlitres of reconstituted sample was injected onto a 250mm x 4.6mm i.d. ODS (5μm particle size) column (Grace Vydac, Hesperia, CA, USA). The mobile phase (water/acetonitrile (18:7 v/v)) was pumped at a flow rate of 1mL/min and prednisolone was detected at 242nm. Detector response was confirmed by regular injection of a prednisolone standard solution in mobile phase.

A2.3 Results
The retention time of prednisolone was 10.5 minutes. The extraction efficiencies for the recovery of prednisolone in the spiked plasma samples are shown in Table A2.1.

<table>
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<tr>
<th>Sample</th>
<th>1.25μg/mL</th>
<th>2.5μg/mL</th>
<th>5μg/mL</th>
<th>10μg/mL</th>
<th>25μg/mL</th>
<th>50μg/mL</th>
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<tr>
<td>i</td>
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<td>79.5</td>
<td>93.0</td>
<td>79.7</td>
<td>88.7</td>
<td>82.4</td>
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<td>ii</td>
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<td>101.8</td>
<td>89.2</td>
<td>88.2</td>
<td>86.7</td>
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<tr>
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<td>106.2</td>
<td>85.1</td>
<td>95.8</td>
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<td>80.0</td>
</tr>
<tr>
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<td>98.7</td>
<td>88.8</td>
<td>92.7</td>
<td>85.3</td>
<td>87.5</td>
<td>82.9</td>
</tr>
<tr>
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<td>2.7</td>
<td>3.9</td>
<td>0.9</td>
<td>2.6</td>
</tr>
<tr>
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<td>10.7</td>
<td>2.9</td>
<td>4.6</td>
<td>1.0</td>
<td>3.1</td>
</tr>
</tbody>
</table>

The extraction efficiency of prednisolone was between 82 and 99% in the concentration range 1.25 to 50μg/mL. Calculated extraction efficiencies were higher at low prednisolone concentrations, which was due to the presence of a co-eluting peak (thought to be cortisol) observed in blank plasma samples which were subjected to the same solid phase extraction and HPLC-UV analysis as prednisolone-spiked samples. The AUC of the co-eluting peak was small but extremely variable, and could represent up to 50% of the AUC of the 1.25μg/mL prednisolone-spiked plasma sample. The limit of detection (LOD) of prednisolone (4.5 times baseline noise) would therefore be approximately 5μg/mL.
It was therefore decided to administer large doses of prednisolone to overcome the poor sensitivity of the method. Yano et al., (2002) administered 10mg/kg oral doses of prednisolone to rats and reported a $C_{\text{max}}$ of between 2 and 2.5μg/mL, although no mention was made of the co-eluting peak at the retention time of prednisolone. It was decided to administer 200mg/kg doses of prednisolone in these in vivo studies to attempt to increase the anticipated plasma prednisolone concentrations well above the LOD for the HPLC-UV method. Two hundred milligram per kilogram doses were considered to be acceptable, given the LD$_{50}$ for acute oral doses of prednisolone in mice of 1680 mg/kg (Sanofi-Aventis MSDS).

Section Two: Prednisolone absorption studies in rats

A2.4 Prednisolone absorption studies in rats and quantification by HPLC-UV

A2.4.1 Methods

A single batch of prednisolone-loaded Eudragit L and S (5:1 polymer:drug loading) microparticles were prepared as described in section 2.9.1, and prednisolone encapsulation efficiency determined as described in section 3.16.3. Two millilitres of 0.5% methylcellulose solution containing 200mg/kg quantities of prednisolone as Eudragit L or S microparticles or prednisolone powder was administered to rats as described in section 5.3.2. Blood samples were collected from the tail vein of rats, and the plasma separated and stored as described in section 5.3.2.

The frozen samples were allowed to thaw at room temperature, and vortex mixed for 5 seconds. Samples were analysed for prednisolone content using the SPE and HPLC-UV method described in section A2.2.2 and A2.2.3.
A2.4.2 Results

The prednisolone encapsulation efficiency of the Eudragit L and S microparticles was 87.1% and 91.0%, respectively. Therefore, the rats which were dosed with Eudragit L and S microparticles were given 1378mg/kg and 1318mg/kg microparticles, respectively. The plasma concentration-time profiles for the Eudragit L, Eudragit S and prednisolone suspension formulations are shown in Figure A2.1.

![Figure A2.1: Plasma concentration-time profiles for the three test formulations as determined by HPLC-UV (n = 1)](image)

The prednisolone concentrations in the rat plasma following administration of 200mg/kg doses of prednisolone were much lower than anticipated from the literature (Yano et al., 2002), who reported plasma concentrations of approximately 2500ng/mL following a 10mg/kg oral dose. This was thought to be explained by an additional peak in the HPLC trace, which eluted before the parent compound (6.9 and 10.5 minutes, respectively), suggesting that prednisolone was being metabolised to a conjugated polar metabolite, thus reducing the amount of prednisolone in the plasma. An example HPLC trace is shown in Figure A2.2.
It was concluded that the HPLC-UV method was not sensitive enough for the quantification of prednisolone in rat plasma as the $C_{\text{max}}$ for each of the formulations was close to the LOD of the method (5µg/mL). The low sensitivity of the method was attributed to a combination of the small volume of plasma that was available for assay (50-100µL), the metabolism of prednisolone and the limited UV absorbance of the prednisolone chromophore. It was therefore decided to use a HPLC-MS/MS method for the quantification of prednisolone in rat plasma, which is described in Chapter Five.
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