AN INVESTIGATION OF THE IN VITRO AND IN VIVO PERFORMANCE OF pH RESPONSIVE POLYMER SYSTEMS FOR ILEO-COLONIC DRUG DELIVERY

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Thesis submitted for the degree of
Doctor of Philosophy

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2006
Plagiarism Statement

This thesis describes research conducted at The School of Pharmacy, University of London between 2000 and 2004 under the supervision of Dr. Abdul Basit. 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: [Signature] Date: 05 April, 2007
ABSTRACT

This study compared the in vitro and in vivo performance of four pH responsive poly(meth)acrylate polymer systems (Eudragit S organic, Eudragit S aqueous, Eudragit FS aqueous and Eudragit P4135 organic) as film coating systems for ileo-colonic drug delivery; and investigated the effect of food and in situ gastrointestinal pH on performance of Eudragit S organic polymer systems.

Each polymer system was film-coated onto disintegrating prednisolone tablet cores, but difficulties were encountered during film coating with Eudragit P4135 and further work with the polymer was discontinued. Dissolution testing of coated tablets was conducted in different buffer media and pH conditions. At pH 7.4, drug release was rapid and similar in the compendial phosphate buffers, however drug release in bicarbonate buffer was markedly delayed and different for each system as follows: Eudragit S aqueous dispersion > Eudragit FS > Eudragit S organic solution.

Disintegration performance of coated tablets was assessed by gamma scintigraphy in 8 fasted volunteers, with repeat administration of Eudragit FS coated tablets to assess intra-subject performance. Tablets coated with Eudragit S aqueous disintegrated prematurely in the proximal to mid-small intestine of all subjects. Tablets coated with Eudragit S (organic) and Eudragit FS disintegrated in the ileo-caecal junction, but performance of Eudragit S (organic) was variable, disintegrating in 5 out of 8 subjects; whereas Eudragit FS showed reproducible performance with disintegration in 14 out of 16 administrations. Also there was a better correlation between in vivo disintegration data and dissolution in bicarbonate buffer compared to phosphate buffer.

The effect of in-situ gastrointestinal pH and food on disintegration of Eudragit S coated tablets was investigated in a 3-way cross-over study as follows: treatment 1 = coated tablet and pH capsule in fasted state (fasted); treatment 2 = coated tablet and pH capsule 30 minutes before food (pre-feed) and treatment 3 = coated tablet given after food (fed). Tablet disintegration was more rapid in the fed and pre-feed treatments. There was marked inter- and intra-individual variability in measured intestinal pH, but the pH in the mid to distal small intestine was found to be generally above 7 for most volunteers and was not markedly affected by food. Correlation of transit and disintegration data of the coated tablets with in-situ gastrointestinal pH in each subject indicates that pH is the main factor affecting dissolution, although disintegration performance was also markedly affected by transit time through the distal small intestine.
ACKNOWLEDGEMENTS

I will like to express my profound gratitude to my supervisor, Dr Abdul Basit, for his encouragement, guidance and generosity throughout this project - thank you for everything.

I am grateful to all at the School of Pharmacy who have helped in one way or another, especially Sir Keith Barnes for invaluable general support, Brian Bissenden for technical support with dissolution apparatus, Dave McCarthy for the SEMs, Sudershana Dave and the Registry staff for their understanding and support in very difficult moments. I will also like to thank colleagues in the research group – Richard, Matt, Hala, Jon and Fang for their assistance.

I thank Professor David Evans of Wingate Research Institute for the permission to use the gamma camera facilities and helpful discussion of results. Thanks also to Mandeep Khela, of Wingate Institute, for all her assistance during the gamma scintigraphy studies; and to Dr Neil Garvie of Radioisotope unit, Royal London Hospital for his assistance as ARSAC licensee for the studies.

I am grateful to the BBSRC and GSK for funding this PhD programme and to Rohm Pharma and Synectics Ltd for additional support with some aspects of the study.

I am hugely indebted to my daughter, Ify for her patience and understanding while growing up with a student dad; to my wife, Benete for the support and encouragement to pursue my ambition; and finally to my mum – the text of 1990 is fulfilled.
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<th>Description</th>
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<tbody>
<tr>
<td>5-ASA</td>
<td>5-aminosalicylic acid</td>
</tr>
<tr>
<td>AC</td>
<td>Ascending colon</td>
</tr>
<tr>
<td>ARSAC</td>
<td>Administration of radioactive substances advisory committee</td>
</tr>
<tr>
<td>AUC</td>
<td>Area under the curve</td>
</tr>
<tr>
<td>CAT</td>
<td>Caecal arrival time</td>
</tr>
<tr>
<td>DC</td>
<td>Descending colon</td>
</tr>
<tr>
<td>DSB</td>
<td>Distal small bowel</td>
</tr>
<tr>
<td>DT</td>
<td>Disintegration time</td>
</tr>
<tr>
<td>DTPA</td>
<td>Diethylenetriamine pentaacetic acid</td>
</tr>
<tr>
<td>FASSIF</td>
<td>Fasted state simulated small intestinal fluid</td>
</tr>
<tr>
<td>FESSIF</td>
<td>Fed state simulated small intestinal fluid</td>
</tr>
<tr>
<td>GET</td>
<td>Gastric emptying time</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal transit</td>
</tr>
<tr>
<td>GMS</td>
<td>Glyceryl monostearate</td>
</tr>
<tr>
<td>GRAS</td>
<td>Generally regarded as safe</td>
</tr>
<tr>
<td>HCl</td>
<td>Hydrochloric acid</td>
</tr>
<tr>
<td>ICJ</td>
<td>Ileocaecal junction</td>
</tr>
<tr>
<td>ICJRT</td>
<td>Ileocaecal junction retention time</td>
</tr>
<tr>
<td>MDT</td>
<td>Mean dissolution time</td>
</tr>
<tr>
<td>MFT</td>
<td>Minimum film-forming temperature</td>
</tr>
<tr>
<td>MMC</td>
<td>Migrating motor complex</td>
</tr>
<tr>
<td>MSB</td>
<td>Mid small bowel</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>Ph Eur</td>
<td>European Pharmacopoeia</td>
</tr>
<tr>
<td>PSB</td>
<td>Proximal small bowel</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short chain fatty acids</td>
</tr>
<tr>
<td>SD</td>
<td>Site of disintegration</td>
</tr>
<tr>
<td>SEM</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SITT</td>
<td>Small intestinal transit time</td>
</tr>
<tr>
<td>STDEV</td>
<td>Standard deviation</td>
</tr>
<tr>
<td>$T_{50%}$</td>
<td>Time to dissolution of 50% of the nominal drug content</td>
</tr>
<tr>
<td>TC</td>
<td>Transverse colon</td>
</tr>
<tr>
<td>TEC</td>
<td>Triethyl citrate</td>
</tr>
<tr>
<td>TWG</td>
<td>Total weight gain</td>
</tr>
<tr>
<td>USITT</td>
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CHAPTER 1: INTRODUCTION
1.1 Overview

The oral route is the most common mode of drug administration in man, usually as conventional or immediate release dosage forms from which drug release occurs in the stomach with subsequent systemic drug absorption. While this approach is suitable for many drug substances, in some instances it may be more desirable to extend or delay drug release in the gastrointestinal tract by formulation into modified release systems. Modified release systems may be designed to release the drug in specific sections of the gastrointestinal tract in order to optimise drug absorption or for therapy of localised disease conditions. This can lead to improved therapeutic outcome and/or reduce the incidence of dose-related side effects by enabling the total administered dose to be lowered.

An application of site-specific drug delivery to a region of the gastrointestinal tract is drug targeting to the ileo-colonic region for topical therapy of localised pathology of the large bowel, notably ulcerative colitis and Crohn’s colitis. Many drug formulation strategies have been investigated for their potential to target drug release to the ileo-colonic region, including dosage forms that rely on changes in pH along the gastrointestinal tract to effect drug release (pH responsive dosage forms). A number of products which rely on pH changes for drug release such as Asacol® MR and the associated ethical generics, Mesren®MR and Ipocol® are approved for therapy of inflammatory bowel disease. These products comprise tablet cores film-coated with the methacrylic acid and methyl methacrylate copolymer, Eudragit S, which dissolves at pH > 7.0.

However, there have been reports of inconsistency in the site and time of drug release from Asacol MR in human subjects, including anecdotal reports of intact tablets in the faeces of some patients (Schroeder et al., 1987; Sinha et al., 2003). The inconsistency in performance has been attributed to inter-subject variability in intestinal pH as well as evidence, albeit unequivocal, of reduced colonic intraluminal pH due to mucosal inflammation. A newer methacrylic acid, methyl methacrylate and ethyl acrylate copolymer, Eudragit FS and its solid derivative Eudragit P4135 has been introduced amidst suggestions of an improved dissolution performance over Eudragit S.
There is a lack of fundamental information on the behaviour and performance features of the pH responsive polymers. In particular, there is a poor correlation between in vitro dissolution and in vivo performance, and the in vivo dissolution pH thresholds of the polymers have not been definitively proven. This study is therefore concerned with a fundamental assessment of the in vitro and in vivo performance of Eudragit S polymer systems in comparison with the newer Eudragit FS or Eudragit P4135. The relationship between dissolution performance and in situ intestinal pH in man is also evaluated using gamma scintigraphy and a novel pH-sensitive radiotelemetry capsule.

1.2 Anatomy and physiology of the human gastrointestinal tract

The gastrointestinal tract has the overall functions of digestion of ingested nutritive materials, nutrient absorption and elimination of unwanted materials. It consists of three regions which are discernible on the basis of anatomy and function as follows: stomach, small intestine and large intestine (figure 1-1); and the essential structural and physiological features of each region differ in accordance with the ascribed function.

1.2.1 Stomach

The stomach is typically a J-shaped sac located in the upper left side of the abdomen immediately below the diaphragm, and consists of three anatomical sections: the fundus, body and antrum. Functionally however, the stomach comprises two regions: proximal and distal region which exist either side of the mid-gastric transverse band (Moore et al., 1986).

The main functions of the stomach as a digestive organ include the temporary storage of ingested materials, biochemical and mechanical digestion of nutritive materials and controlled movement of the resulting chyme into the duodenum (Hellström et al., 2006). The fundus and upper part of the corpus are responsible for the storage function of the stomach and undergo an adaptive relaxation reflex to accommodate large volume of ingesta without a significant rise in intra-gastric pressure (Cannon and Lieb, 1911). The stomach is thus able to expand from a resting volume of about 50 mL to maximum of about 1500 mL after feeding without an increase in intra-gastric pressure (Martini, 1995).
Figure 1-1 Diagram of the human gastrointestinal tract (Capsugel Library)
The ingesta is then held in the stomach to enable partial digestion of nutritive components by the secreted gastric juices and size reduction of solid components and homogenisation into chyme by the muscular contractions of the antrum. Flow of chyme into the duodenum is a physiologically regulated process, known as gastric emptying, which occur at an optimal rate for digestion and absorption of the nutrients (Hunt and Stubbs, 1975).

The stomach mucosa is lined with a layer of simple columnar epithelium, and when empty, forms longitudinal folds known as rugae. The epithelium contains numerous narrow channels known as gastric pits from which arise the gastric glands. The secretory cells of the stomach contribute to a total daily output volume of 1 – 5 L of fluid and the main secretory cells of the stomach include:

**I. Surface mucous cells:** secrete copious quantities of thick mucus that forms a functional barrier layer on the gastric mucosa. The mucus layer, augmented by bicarbonate secretion from the underlying mucosa, protects the mucosa from digestion by secreted pepsin and hydrochloric acid and serves also to lubricate and facilitate movement of ingested material.

**II. Parietal cells:** secrete mainly hydrochloric acid required for conversion of pepsinogen to pepsin and which also has a bactericidal action to kill the microbes ingested with food. Hydrochloric acid secreted by the parietal cells is responsible for the characteristic low pH of the stomach.

### 1.2.2 Small intestine

The small intestine is the longest segment of the gastrointestinal tract and is divided into: duodenum (0.2 – 0.3 m), jejunum (2.5 m) and ileum (3.5 m). The gross anatomy of the small intestine is indistinct, but the different segments have specialised features adapted to their functions in digestion and absorption of nutrient (Washington et al., 2001). The main functions of the small intestine are: secretion of further digestive enzymes, absorption of nutrients and movement of unabsorbed materials towards the large intestine.
The main secretory cells associated with the duodenum include Brunner’s cells located in the sub-mucosa of the intestine and which secrete bicarbonate to neutralise and buffer the incoming acidic chyme. The goblet cells found in the crypts of Lieberkühn also secrete thick protective mucus which has a similar function as in the gastric mucosa. Other secretory cells are located in the crypts of Lieberkühn and are responsible for secretion of digestive enzymes, mucus, entero-endocrine hormones and antibodies.

Further mechanical digestion of solid components of chyme is continued by the contraction of intestinal smooth muscles, while biochemical digestion is achieved by the secretions of intestinal glands which contain a variety of digestive enzymes. The small intestine is the major absorptive region of the gastrointestinal tract and is responsible for over 90% of nutrient absorption (Martini, 1995). The surface area available for nutrient absorption from the small intestine is greatly enhanced by the presence in the mucosa of plicae circulares (folds of kerckring), villi and microvilli, with a total surface area of about 200 m² in a typical adult (Wynsberghe et al., 1996). Plicae circulares are circular permanent folds of the intestinal mucosa and not only increase the surface area but also provide some turbulence to the flow of chyme. The villi are finger-like protrusions of the mucosa, each of which is in turn covered by hundreds of microvilli. The columnar epithelial cells covering the villi and microvilli are responsible for absorption in the small intestine. The plicae circulares and villi are more prominent in the duodenum and jejunum, underlying the greater proportion of nutrient absorption from these two regions in comparison to the ileum.

Digestion and absorption of nutrients is essentially completed by the time the digesta reaches the ileocaecal junction, which acts as a physiological sphincter region, regulating the flow of chyme into the colon and preventing retrograde flow of colonic contents into the small intestine (Basilico and Phillips, 1993).

1.2.3 Large intestine

The large intestine measures approximately 1.2 – 1.5 m in length and comprises the caecum, colon, rectum and anal canal. The most prominent segment is the colon and
this is further sub-divided into the ascending (20 cm), transverse (45 cm), descending (30 cm) and sigmoid colon (40 cm). In comparison to the small intestine, the colon is shorter and has a wider lumen which measures from about 8.5 cm in the caecum to 2.5 cm in the sigmoid colon (Washington et al., 2001). Also the walls of the colon are thinner and the mucosa devoid of well formed villi and microvilli, all indicative of its limited absorptive capacity. The mucosa of the large intestine consists of a single layer of columnar epithelial cells which contain numerous crypts from which arise the absorptive cells, goblets cells, and endocrine secretory cells.

Absorption from the colon is minimal, accounting for only 10% of total absorption from the gastrointestinal tract, and is mainly associated with absorption of water and electrolytes; and it is estimated that for every 2 L of water entering the colon, the residual water in the stools will be less than 200 mL (Washington et al., 2001). Reabsorption of water occurs mainly in the ascending and transverse colons, hence the luminal contents of the large intestine become more viscous beyond the transverse colon. Sodium and chloride ions are also reabsorbed in the colon in exchange for potassium and bicarbonate. Mucus is produced from the goblet cells and acts as a lubricant between the colonic mucosa and the semi-solid contents of the colonic lumen.

The colon also has a nominal digestive role, being the site for the digestion of complex carbohydrates such as ‘resistant starch’, fibre, as well as some specific proteins and these arise mainly due to metabolic activities of the diverse microbial population comprising up to 400 species with an estimated total population of $10^{12}$ cfu/mL (Macfarlane and Englyst, 1986). The colonic microflora brings about fermentation of the indigestible components of incoming chyme such as complex carbohydrates, resulting in formation of short chain fatty acids (SCFA) and a variety of other acidic end products.

1.3 Gastrointestinal physiology and performance of oral dosage forms

The gastrointestinal tract is a complex and heterogeneous milieu, in which the secretions, motility and transit of ingested materials are physiologically regulated to
optimise the digestion and absorption processes. Orally ingested solid dosage forms are therefore susceptible to the heterogeneity in motility, transit and intestinal secretions with consequent effect on intraluminal pH, enzyme activity and distribution and composition of gastrointestinal fluid in different segments of the gastrointestinal tract.

Administration of drugs by the oral route is usually in the form of immediate release dosage forms (liquid or solid) formulated for rapid drug release in the stomach and consequent absorption into the systemic circulation. However this mode of delivery is not considered appropriate for many drugs or therapeutic applications, and a more suitable drug delivery strategy would involve formulation as delayed or extended release dosage forms, referred to collectively as modified release dosage forms. Furthermore, modified release dosage forms can be formulated as either single-unit systems such as capsules and tablets or as multiple-unit systems such as pellets, granules and mini-tablets (Bechgaard and Nielsen, 1978). One of the oldest forms of modified release technology is enteric drug delivery systems which delay drug release until the dosage form enters the small intestine (Agyilirah and Banker, 1991), and this concept has now been adapted to delay drug release further until the dosage form reaches the ileo-colonic region.

In contrast to immediate release dosage forms which lead to rapid drug release and systemic absorption, the performance of modified release dosage forms is influenced to a great extent by their interaction with the heterogeneous gastrointestinal features. There are three key physiological attributes of the gastrointestinal tract that can affect the performance of orally administered modified release dosage forms, namely: gastrointestinal pH, fluid volume and transit.

### 1.3.1 Gastrointestinal pH

Basal pH in the gastric lumen is acidic due to secretion of HCl by the parietal cells, although the pH also depends on the neutralising effect of gastric contents. Median gastric pH in healthy young subjects have been reported as 1.7 (range 1.4 – 2.1) in the fasted state, increasing transiently to 5.0 (range 4.3 - 5.4) after meal ingestion.
There is a variation in pH of the different segments of the stomach which is brought about by uneven distribution of parietal cells as well as the extent of mixing of gastric contents (McLaughlan et al., 1989). Gastric pH has also been shown to have a circadian rhythm with acid secretion being highest at night and lowest in the morning (Moore, 1991). Other factors known to influence gastric pH to varying extents include gender, with a higher reported median gastric pH in healthy females (Feldman and Barnett, 1991).

Bicarbonate secretion by the Brunner's cells in the duodenum neutralises and buffers the incoming low pH gastric contents, resulting in a near neutral pH of the intestinal lumen. The pH then rises slowly along the small intestine with a peak in the terminal ileum due to further secretion of bicarbonate in the ileum. The pH of the small intestine has been characterised and reported values for the different segments are: 6.63 ± 0.53, 7.41 ± 0.36 and 7.49 ± 0.46 in the jejunum, mid small bowel and ileum respectively (Evans et al., 1988). On entry into the caecum, intraluminal pH drops by about 1 pH unit compared to the pH of the ileum (table 1-1) and then rises gradually to near neutral pH on moving from the ascending colon to the left colon.

<table>
<thead>
<tr>
<th>Intestinal site</th>
<th>Mean pH ±SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Jejunum</td>
<td>6.63±0.53 (55)</td>
</tr>
<tr>
<td>Mid small intestine</td>
<td>7.41±0.36 (52)</td>
</tr>
<tr>
<td>Ileum</td>
<td>7.49±0.46 (58)</td>
</tr>
<tr>
<td>Right colon</td>
<td>6.37±0.58 (66)</td>
</tr>
<tr>
<td>Mid colon</td>
<td>6.61±.83 (51)</td>
</tr>
<tr>
<td>Left colon</td>
<td>7.04±0.67 (50)</td>
</tr>
<tr>
<td>Mean small intestine</td>
<td>7.30±0.34 (51)</td>
</tr>
<tr>
<td>Mean colon</td>
<td>6.63±0.67 (48)</td>
</tr>
</tbody>
</table>

Table 1-1 Luminal pH measured along the intestine by radiotelemetry capsule

[(Adapted from Evans et al., 1988); Values in parenthesis represent number of subjects].
This drop in pH of the caecum is due to short chain fatty acids (SCFA) resulting from metabolic activities of colonic microflora. The higher concentration of the SCFA in the caecum and ascending colon is due to the abundance of substrates in the incoming materials from the small intestine. SCFA are metabolised by the epithelium leading to falling intraluminal levels distally from the caecum (Roediger, 1980) and the lower levels of SCFA and bicarbonate secretion contribute to the rising pH profile along the distal colon (Nugent et al., 2001).

The major significance of gastrointestinal pH is related to the performance of enteric coated dosage forms which are formulated to remain intact in the stomach, but dissolve in the higher pH environment of the small intestine. Traditional enteric coating formulations usually dissolve at pH > 5 and other polymer coatings which dissolve at pH ≥ 7 are now used to target drug release to the distal small intestine and colon. Commonly reported basal gastric pH in healthy subjects is sufficiently low to ensure all enteric coatings remain intact in the stomach. However, gastric pH is occasionally reported to be up to pH 6.0 which could be due to achlorhydria in the study population (Lindahl et al., 1997).

Ingestion of food causes a transient rise in gastric pH, but return to basal value is usually very rapid, although the extent and duration of change is dependent on the constitution of the meal, with high protein content meals causing a higher pH change than other equicalorific meals. Hardy et al. (1987) reported following administration of naproxen tablets to fed subjects, that the post-cibal transient rise in gastric pH did not affect the integrity of the enteric coating. Nevertheless, knowledge of changes in gastric pH following meals and in different pathological conditions should be considered in the formulation development of enteric dosage forms and administration with respect to meal time for instance.

Reported pH in the different regions of the gastrointestinal tract is sufficiently high to ensure drug release from enteric coated dosage forms. However, it is important to consider the prevailing pH in pathological conditions. Gilbert et al. (1988) found that jejunal pH in children with cystic fibrosis was lower than in healthy subjects, and which has a potential therefore to cause a delay in drug release or even failure of
enteric coated dosage forms. A similar situation applies to the performance of pH coatings for drug delivery to the distal intestine and colon, with results showing that the luminal pH of the colon in the target pathological conditions is lower than in healthy controls (Raimundo et al., 1992, Fallingborg, 1993).

1.3.2 Gastrointestinal fluid

The amount of fluid available for drug dissolution in the gastrointestinal tract has been the least studied physiological factor affecting dosage form performance, as is evidenced by a paucity of published investigational data. It is pertinent to note that drug dissolution and diffusion to the mucosa is considered essential whether the drug is intended for local or systemic action, and both processes require a minimum amount of free fluid depending on the physicochemical nature and quantity of the drug.

Fluid volume is variable as the net water flux across the gastrointestinal tract mucosa is dependent on the tonicity of the luminal contents, with hypertonic meal leading to a net influx of water and higher volume and vice versa (Fordtran and Locklear, 1966). The amount of fluid consumed per day varies considerably between climatic regions and individual habit although an estimate of 2 L per day is often quoted. Pancreatic secretions into the intestine account for about 1 – 2 L with a further 600 mL arising from bile secretions per 24 hour period (Diem and Lentner, 1970). In addition there are other secretions from the various glands in stomach and small intestine, with an estimated total intrinsic fluid secretion of about 6 L per day (Davenport, 1982). However, much of these secretions are stimulated and the estimated basal fluid volume is 120 – 350 mL in the jejunum and iluern (Dillard et al., 1965).

More recently, Schiller et al. (2005) investigated the fluid volume and distribution along the gastrointestinal tract of 12 healthy subjects using water-sensitive magnetic resonance imaging methodology. Non-disintegrating capsules containing aqueous markers were administered to fasted subjects at three time points corresponding to 7, 4 and 1 hour before imaging. Each capsule was administered with 150 mL of water, and additional 100 mL water of water was administered at four specified intervals (total volume 850 mL). The study was repeated in the same volunteers after a seven day
wash-out period following ingestion of a standardised meal (volume of meal 900 mL, calorific value 803 Kcal) one hour before imaging. The free fluid volume in the intestine was low and most of the fluid was located in the distal intestine as small pockets (table 1-2).

Table 1-2 Gastrointestinal free fluid volume of human subjects measured by magnetic resonance imaging

(Adapted from Schiller et al., 2005)

<table>
<thead>
<tr>
<th>Gastrointestinal region</th>
<th>Fed state (mL)</th>
<th>Fasted state (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stomach</td>
<td>686 ± 93(^1)</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>

\(^1\) Value represents total volume of stomach contents, not only free fluid

Some estimates of fluid volume in the gastrointestinal tract have usually been based on estimated secretion volumes into the gastrointestinal lumen and fluid intake; although it does appear that the intestine maintains a relatively constant net fluid flux irrespective of intake and secretion volume. The results obtained in the studies described here were despite the ingestion of 850 mL of co-administered water during the study, in addition to the fluid associated with the meal.

The results also show that fluid was not uniformly distributed in the intestine, but instead existed in pockets. The non-disintegrating capsules was administered at different times to get a good distribution along the gastrointestinal tract, so as to enable determination of proportion of capsule in contact with fluid pocket and the result is shown in table 1-3.
Table 1-3  Location and contact of capsules with “free fluid” in 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>

Observed free fluid volume in the colon was very low at 13 ± 12 mL and 11 ± 26 mL in fasted and fed states respectively, but is perhaps unsurprising given the primary role of the colon in re-absorption of water and electrolytes. Previous estimate of the fluid content of the colon had referred to the total volume of wet contents of approximately 220 g (Cummings et al., 1990). However, the estimated fluid volume in these earlier reports may have included bound fluid, whereas the MRI method estimated free fluid volume.

The low fluid volume and the distribution as fluid pockets mainly in the distal small intestine and the extremely low fluid volume in the colon could have far-reaching implications on the performance of drug delivery systems for targeted drug release in the colon. In the context of the requirement for dissolution and diffusion of the active substance to the colonic mucosa once the dosage form arrives in the colon, the above results demands a critical re-evaluation of formulation strategies. Also there is a need to consider the fluid volume in the small and large intestines in design of biorelevant dissolution tests, particularly of poorly soluble drugs. It would have been interesting nevertheless to investigate whether there were any changes in contact of capsules with fluid in the short term.
1.3.3 Gastrointestinal transit

The gastrointestinal tract, particularly the stomach and small intestine, display two distinct patterns of contraction and transit, depending on the presence of food, referred to generally as the postprandial (fed) and inter-digestive (fasted) motility pattern. The gastrointestinal transit of an administered dosage form therefore depends on the prevailing motility pattern.

1.3.3.1 Inter-digestive motility pattern

The inter-digestive motility pattern is characterised by a cyclical series of contractions of varying intensity known as the migrating motor complex (MMC) (Szurszewski, 1969). The inter-digestive motility pattern is initiated approximately 2 hours after the stomach has been completely emptied of digestible materials and small particles, although it has been reported that the MMC returns to the jejunum earlier than this (Kellow et al., 1986).

The migrating motor complex moves from the stomach to the distal small intestine and is sub-divided, on the basis of frequency and intensity of contractions, into four phases (Code and Marlett, 1975). Phase I is a quiescent period lasting approximately 45-60 minutes and during which the stomach shows little or no motor activity. Phase II is characterised by intermittent contractions which gradually increase in frequency and intensity, and usually lasts about 30 minutes. Phase III lasts approximately 5 to 15 minutes and comprise intense and regular contractions. Phase III contractions of the MMC are able to clear most debris and indigestible materials from the gastric lumen and also move the contents of the small intestine distally, and are hence referred to as ‘house keeper waves’. Phase IV is a transition period during which the frequency and intensity of phase III contractions subside and lead to the quiescent phase. The cycle recurs every 90 to 120 minutes until it is disrupted by the ingestion of a meal which returns gastric motility to the fed state pattern. However, there is variability in the periodicity of the MMC cycle, with intervals of 30 minutes to over 3 hours having been reported (Kerlin and Phillips, 1982). Also there is variability in the involvement of the stomach and the distal intestine in the MMC cycle, with about 25% of MMC cycles commencing distally to the stomach, while the proximal jejunum has been
identified as the region of maximal occurrence of the MMC. There is even less involvement of the ileum in the MMC cycle with up to 80% of the cycles dying out before reaching the ileum, and only 2% reach the caecum (Kellow et al., 1986).

Phases I and II of the inter-digestive motility pattern lead mainly to mixing of gastric contents while gastric emptying of large indigestible particles and debris are thought to occur under the influence of phase III contractions (Kelly, 1980). Phase III contractions is widely believed to be responsible for small intestinal transit in the inter-digestive cycle, although some reports indicate that up to half of inter-digestive flow of intestinal luminal contents occur during phase II, with the rest occurring during phase III contractions (Kerlin et al., 1982).

1.3.3.2 Postprandial motility pattern

The postprandial motility pattern is initiated after ingestion of food and consists of slow peristaltic waves generated at the muscle pacemaker cells of the fundus and body, which increase in intensity as they move down towards the pylorus.

The rhythmic contractions of the proximal stomach ensure the homogenisation of ingested food while the stronger contractions of the antrum help to bring about size reduction of digestible solids and the expulsion force of the co-ordinated movement of resulting chyme into the duodenum. The postprandial motility pattern of the antrum consists of steady low amplitude contractions which occur at the rate of about 3-5 per minute and is sustained until the bulk of the ingested material is emptied from the stomach (Bueno and Fioramonti, 1993; Wynsberghe et al., 1996).

An important feature of postprandial gastric emptying pattern is the discriminatory handling of solid materials and liquids (Hinder and Kelly, 1977; Meyer et al, 1981; Feldman et al., 1984; Coupe et al., 1991). Gastric emptying of liquids and suspended particles occur at an exponential rate in response to the gastro-duodenal pressure gradient generated by contraction of the fundus (Kelly, 1980), although the exit of chyme into the duodenum is regulated by co-ordinated contractions of the antrum, pylorus and duodenum (Houghton et al., 1988). Large digestible solids are retained in
the stomach until the particle has been adequately reduced, and then emptied with chyme. Large indigestible solids on the other hand are retained in the stomach until return of the inter-digestive motility pattern. The mechanism of selective gastric emptying of solids and liquids is not yet very clear and a number of theories have been proposed. Hinder and Kelly (1977) proposed that selective emptying was due to restriction of pyloric outlet by simultaneous contraction of the pylorus as the postprandial peristaltic contractions reach the final segments of the antrum. However, the theory that selective gastric emptying of solids and liquids is due to narrow diameter of the pyloric passage has been contradicted and alternative theories have been proposed (Rao and Schulze-Delrieu, 1993).

Postprandial motility pattern of the small intestine is characterised by irregular occurrence of contractions similar to those observed in phase II of the MMC (Code and Marlett, 1975). The contractions are both segmental as well as peristaltic, although the peristaltic contractions are more frequent. Segmental contractions serve to mix the chyme with digestive juices and bring it in contact with absorptive surfaces, while peristaltic contractions propel chyme through the small intestine. Unlike the stomach, there is no difference in transit of solids and liquids through the small intestine. Postprandial flow of chyme through the small intestine is intermittent, but flow rate is reported to increase significantly in the postprandial phase with reported ileal flow rate of 4-6 mL/minute compared to a rate of 1-2 mL/minute in the inter-digestive state (Kerlin et al., 1982). There is a slow down in intestinal transit of chyme or other ingested materials in the terminal ileum, referred to as the ileal brake. The ileal brake has been attributed to the arrival of fat and protein in the ileal region and is believed to be a physiological feedback mechanism to optimise absorption.

Colonic motor activity is characterised by alternating periods of prolonged quiescence and short bursts of intense motor activity. The large intestine displays several motility patterns some of which occur in specific anatomical regions and include (Washington et al, 2001):

- Segmental contractions, occurring mainly in the proximal colon, and function mainly to mix the luminal contents of the colon with very little aboral movement.
• Peristaltic contractions which are propulsive in nature and move luminal contents through the ascending colon, hepatic flexure and the transverse colon.

• Retropulsive contractions have also been noted to occur in the ascending colon where it is believed to retard transit in order to maximise the reabsorptive role of the colon.

• Mass movement is a form of colonic motility which occurs rather infrequently at about 3-4 times a day. Mass movements are preceded by the loss of the haustral pattern of a segment of the colon, followed by very strong contractile wave developing proximal to each segment and propelling the entire content into the next segment. Mass movements are usually associated with the urge to defecate and are more common in the distal colon.

Unlike the stomach and small intestine, there is no marked inter-digestive or postprandial motor activity phase, although the phase III contractions of the MMC have occasionally been known to reach the caecum and is characterised by a burst of phasic pressure waves (Rao and Schulze-Delrieu, 1993). Also the ingestion of meals has been found to increase colonic motor activity (Ritchie, 1968; Snape et al., 1978) and this has been designated as ‘gastro-colonic response’ comprising a bimodal response at 30 and 60 minutes post cibum. It has been reported however, that although gastrocolonic response was associated with increased motor activity at the ileocaecal junction and ileum, these were mainly segmental contractions with little propulsive activity (Price et al., 1993).

1.3.3.3 Gastrointestinal transit of dosage forms

1.3.3.3.1 Gastric emptying

It can be deduced from the preceding section that gastric emptying time of an orally administered dosage form would depend on whether the stomach is in a fasted or fed state, as well as the type of dosage form - whether single-units, multiple-units or liquids.
Gastric emptying of liquid dosage forms is independent of the prevailing motility pattern and occurs at an exponential rate in response to gastro-duodenal pressure, although the gastric emptying time is likely to be dependent on the viscosity of gastric contents. For instance, Feldman et al. (1984) reported a mean gastric emptying time of 30 ± 2 minutes for a liquid drink ingested with a meal, compared to a mean gastric emptying time for radiolabelled water of 10 ± 2 minutes (Wilding et al., 1994).

In the postprandial motility phase, gastric emptying time of modified release solid dosage form will be primarily dependent on the size of the dosage unit. Results from gastric emptying studies in dogs were initially extrapolated to humans, indicating that solid dosage forms above a cut-off size of 2mm would be retained in the stomach until return of the inter-digestive cycle (Hinder and Kelly, 1977). Subsequent studies however, showed that tablets ranging in diameter from 3-12 mm (Davis et al., 1988; Khosla et al., 1989; Coupe et al., 1991) are able to empty from a fed stomach. Wilding and Davis (1996) conducted a meta-analysis of published studies and concluded that there is no cut-off size per se for gastric emptying of solid dosage forms. However, the results did indicate that gastric emptying becomes less predictable with increasing size of the dosage form. It is known that retention of large particles in the stomach for size reduction prior to gastric emptying is part of the digestive function of the gastrointestinal tract, therefore it is conceivable that the emptying of large single dosage forms from a fed stomach is a random fortuitous process, which could arise by the dosage form becoming trapped in the pyloric canal and being forced through to the small intestine during the next antral contraction (Khosla et al., 1989). Therefore, for large single dosage forms administered with food, gastric emptying time will depend on the time it takes for return of the inter-digestive motility pattern, which is in turn dependent on all the other variables affecting the gastric emptying of food such as calorific content of the meal.

Gastric emptying of dosage forms administered in the inter-digestive cycle is also potentially variable. For large dosage forms, the propulsive force for gastric emptying would be the phase III contractions of the migrating motor complex. Given that the frequency of the MMC is every 90-120 minutes, it follows then that the gastric emptying time could vary from a few minutes to 120 minutes, depending on the phase
of the MMC cycle of the subject at time of administration. The dependence of gastric emptying time during the inter-digestive cycle on phase III contractions of the MMC is supported by results of various studies in which the reported gastric emptying times have been within the upper interval of the MMC (Davis et al., 1986a; Khosla and Davis, 1989; Adkin et al., 1993; Wilding et al., 1993; Goto et al., 2004; Ofori-Kwakye et al., 2004). However, since the stomach is not always involved in every MMC cycle (Kellow et al., 1986), it could be up to several hours before arrival of a phase III cycle of the MMC. Coupe et al. (1991) reported the gastric retention of a large radiotelemetry capsule unit dosage form for several hours during which three phase III contractions of the MMC cycles occurred. Therefore gastric emptying could occasionally be delayed for extended periods as have been reported in a number of studies (Khosla and Davis, 1989; Ashford et al., 1993b).

Multiple-unit dosage forms are usually considered as more suitable single solid dosage forms, for purposes of achieving a more reproducible gastric emptying and intestinal transit pattern whether administered in the inter-digestive or the postprandial motility phase. While it is true that gastric emptying of multiple-unit systems are more reproducible than single-units, gastric emptying of pellets have also been reported to be random and delayed relative to co-administered fluid or meal (Khosla and Davis, 1987; Yuen et al., 1993; Coupe et al., 1993; Clarke et al., 1993, 1995).

The implication of gastric emptying time and food induced delay in gastric emptying is far reaching particularly for non-disintegrating enteric coated and modified release solid dosage forms. Enteric coated dosage forms are designed to disintegrate in the small intestine and so any delay in gastric emptying would also delay drug absorption. Another potential problem in the case of conventional enteric coated dosage forms is that depending on the constituents of the meal, gastric pH could rise to a level where the enteric coating becomes compromised. Also such delay could adversely affect the performance of non-disintegrating extended release dosage forms designed to release the drug gradually over time presumably in the absorptive regions of the small intestine.
1.3.3.3.2 Small intestinal transit

Although the small intestine displays two distinct motility patterns in the inter-digestive and postprandial phase, the transit of dosage forms through the small intestine have been reported to be similar in both motility cycles. Davis et al. (1986b) conducted a meta-analysis of small intestinal transit data of liquid, pellet and single dosage unit formulations, with a pooled total of 201 human subjects. The pooled population small intestinal mean ± SEM was reported as 3 ± 1 hours and was not significantly different for the different formulations comprising pellets, tablets and liquids. The mean small intestinal transit times reported by many investigations tend to agree with the mean result despite wide inter-subject variability in small intestinal transit. For instance Khosla and Davis (1989) reported a mean small intestinal transit time for 5 mm multi-unit tablets of 178 ± 58 minutes (approximately 3 ± 1 hour) but masked behind this mean is data range of 45 – 362 minutes. In contrast however, Ashford et al. (1993b) reported a mean small intestinal transit time of 6.5 ± 3.8 hours, suggesting that the reason most small intestinal transit data are similar to the mean value of 3 ± 1 hour is because investigators fail to include the delay period at the ileocaecal junction when calculating small intestinal transit time. Therefore, in line with other applications of statistical mean, the mean small intestinal transit time should prove useful as a guide but should be interpreted or applied with caution. For instance, a difference of two hours in small intestinal transit time can lead to a significant difference in performance of pH responsive dosage forms or to a significant difference in drug absorption. Also masked by the mean small intestinal transit time is the relative period of residence in the different regions of the small intestine which differ in pH, and could thus affect performance of enteric coated dosage forms. Other important aspects of small intestinal transit which are masked by the mean value but which could affect the performance of modified release dosage forms include the ileal brake mechanism involving a delayed transit across the ileum compared to other segments of the small intestine (Spiller et al., 1988), and delay in transit of materials across the ileocaecal junction (Phillips et al., 1988).
1.3.3.3 Colonie transit

Transit time through the colon is generally longer than in other compartments of the gastrointestinal tract, and is generally unpredictable with bursts of movement interspersed with variable periods of little motor activity. Transit time through the different segments of the colon in healthy adults is approximately 35 hours with transit time through each segment of 11.3, 11.4 and 12.4 hours for the right, left and rectosigmoid colon respectively (Metcalf et al., 1987).

Transit through the ascending and transverse colon has been observed to depend on the size of the dosage form (Hardy et al., 1985; Adkin et al., 1993; Abrahamsson et al., 1996). Adkin et al. (1993) reported that in addition to tablet diameter, the volume of the dosage form also affected transit through the ascending colon. In these studies, the larger sized dosage forms moved more rapidly ahead of the smaller units and the phenomenon has been referred to as streaming. In contrast, Watts et al. (1992), found similar residence times for 0.2 compared with 5mm and 8.4 mm particles in the ascending colon of healthy volunteers.

Colonic transit time is influenced by dietary fibre which has been found to increase luminal and hence faecal bulk as well as increase transit through the colon (Cummings et al., 1978). On the other hand regular addition of fibre to the diet exerts a normalising effect on colonic transit, increasing or decreasing transit where appropriate (Price et al., 1991).

The ascending colon is the region with the highest amount of fluid and so would be the preferred site for drug release for colon-targeted dosage forms. Rapid transit through the ascending colon and beyond can thus lead to poor performance of extended/delayed release dosage forms intended for drug release in the colon.

1.4 Drug delivery to the colon

Site specific oral drug delivery can help to overcome some of the limitations of conventional oral dosage forms, with drug release aimed at specific regions of the
gastrointestinal tract either for topical therapy of diseases affecting that site or to target drugs to regions whose physiology would optimise the bioavailability of the drug. One such gastrointestinal region to which drugs have been targeted is the colon.

1.4.1 Rationale for drug delivery to the colon

1.4.1.1 Topical treatment of diseases affecting the colon

The colon is susceptible to various diseases ranging in severity from constipation and diarrhoea due to altered colonic transit, to more debilitating inflammatory bowel disease (ulcerative colitis and Crohn's disease), spastic colon and irritable bowel syndrome, through to infections and colon carcinoma. Current systemic therapy for most of these colonic disorders are generally inefficient and improved therapeutics can be achieved through improved drug delivery strategies, which would ensure delivery of therapeutic moieties at the site of disease and thereby reducing the administered dose and associated adverse effects (Basit, 2005).

The most notable pathology of the colon for which improved therapeutics could be achieved by topical therapy is inflammatory bowel disease comprising ulcerative colitis and Crohn's disease. Crohn’s disease is largely an inflammatory condition of the distal small intestine although up to 30-40% of patients present with colonic involvement at diagnosis; whereas ulcerative colitis is entirely an inflammatory condition of the colonic mucosa (Both et al., 1983). Ulcerative colitis is a chronic condition characterised by periods of remission interspersed with acute flare-ups and given that the only effective cure is by procto-colectomy, the therapeutic goal is to achieve and maintain remission. Drugs used to treat acute ulcerative colitis include high doses of salicylates and corticosteroids. The use of corticosteroids in chronic therapy is limited by side-effects such as iatrogenic Cushing’s syndrome (Friend, 2005). Salicylates are generally preferred as first line therapy in mild to moderate active disease, although the effective dose is limited by the incidence of side effects (Gisbert et al., 2002). The chronic nature of inflammatory bowel disease would indicate a need for localised therapy in order to minimize side effects resulting from these drugs. Drug delivery to the colon has also been investigated for the local therapy
of other disorders such as infections (Hu et al., 1999) and Carcinoma (Jeong et al., 2001).

1.4.1.2 Site for absorption into the systemic circulation

The colon has also been investigated as a portal for systemic drug delivery, especially for drug molecules that are poorly absorbed or susceptible to degradation in the upper intestinal regions. One such group of molecules are the therapeutic peptides and proteins which are extensively denatured in the stomach by the secreted hydrochloric acid and are digested by the pepsin and intestinal peptidase enzymes. Peptide and protein drug have therefore been targeted to the colon due to reported lower levels of luminal and mucosal digestive enzymes. For instance, protease activity in the colon is estimated at 20 – 60 times less than in the small intestine (Gibson et al., 1989), thus highlighting the obvious advantages of targeting such molecules to the colon. Drug delivery to the colon for systemic actions is also considered beneficial when an intentional delay in absorption is required such as in the therapy of conditions that display a diurnal rhythm such as nocturnal asthma, angina and arthritis (chronotherapy) (Youan, 2004); in which case a dose taken at bed time would suffice for control of symptoms in the early morning.

A limitation to this approach is generally poor drug absorption from the colon. The colon lacks the well defined villi and microvilli as found in the small intestine and is thus limited in its absorptive capacity has limited absorptive capacity compared to the small intestine, essentially due to the considerably less surface area estimated at 1.3 m² compared to 200 m² for the small intestine (Faigle, 1993). Other identified barriers to systemic drug absorption include tight inter-cellular junctions and elevated levels of the P-glycoprotein efflux transporters. It should be stated however, that absorption is modestly enhanced by virtue of the prolonged residence time of materials in the colon (Hayton, 1980; Mrsny, 1992), and increased responsiveness to absorption enhancers (Kim et al., 1994; Swenson and Curatolo, 1992).

A further constraint to colonic drug delivery is the potential metabolism of certain drugs in the colonic lumen. For example, Basit et al. (2002) showed that some H₂-
antagonists, ranitidine and nizatidine, were degraded in the colonic lumen; thus highlighting the importance of assessing the stability of drug candidates in the colonic lumen.

1.4.2 Strategies for drug delivery to the colon

The anatomical position of the colon makes it relatively difficult for non-invasive access. Distal segments of the colon can be accessed by use of suppositories and enemas to varying degrees. However, the spread of suppositories is limited to the rectum (Jay et al., 1985) and that of administered enemas rarely extend beyond the descending colon (Hardy et al., 1986). Moreover, rectal dosage forms have low patient acceptability. Orally ingested dosage forms are therefore the preferred formulation choice.

Drug targeting to the colon via the oral route of administration has two functional requirements: Firstly, the robustness of form to prevent drug release in the upper gastrointestinal regions and secondly, sufficient sensitivity to the trigger mechanism to ensure prompt drug release in the colon. While it is not too difficult to formulate a dosage form that would remain intact on passage through the gastrointestinal tract, the challenge is to prevent opportunistic drug release and at the same time ensure sensitivity of the dosage form to the drug release mechanism. The design concept for colon-targeted drug delivery are therefore usually based on one or more select physiological features for which some degree of difference exists between the colon and the upper gastrointestinal regions. As would be expected, this has met with varying degrees of success owing primarily to the fact that the gastrointestinal tract is a continuum in which anatomical and physiological differences are not in many cases, sufficiently distinctive to enable colon specific drug delivery.

To date, several formulation strategies have been investigated and almost all are based on one or a combination, of four trigger factors for drug release in the colon: bacteria, time, pressure and pH.
1.4.2.1 Bacteria approach

The gastrointestinal tract is home to many species of micro organisms that contribute to its physiology and function, breaking down and metabolising ingested and endogenous materials (Harbelein and Friend, 1993). The microbial count is very low in the stomach due to the acidic pH, while the relatively rapid transit of materials through the small intestine also limits the microbial population in this region. In contrast, the higher pH, slower transit and abundance of substrates contribute to maintain a rich and diverse microbial population in the colon, estimated at up to $10^{11-12}$ CFU/mL and representing over 400 species (Moore and Holdeman., 1975); compared to approximately $10^4$ CFU/mL in the upper gastrointestinal regions (Simon and Gorbach., 1986). Some of the materials degradable by the colon bacteria include carbohydrate such as resistant starch, dietary fibre, chitin, polydextrose and amino sugars; as well some proteinaceous materials (McFarlane and Cummings, 1991).

This select feature of the colon have been exploited in the design of drug delivery dosage forms, by using materials which offer sufficient resistance to degradation in the upper intestinal regions, but are easily degraded by the target colonic bacterial enzyme. Several dosage forms have been developed based on this principle, either by use of bacterial enzyme degradable carrier materials in formulation of suitable dosage forms (formulation approach) and/or the use of pro-drugs containing enzyme degradable bonds (prodrug approach).

1.4.2.1.1 Pro drug approach

The use of pro drugs represents the earliest attempt at drug targeting to the colon, and usually consist of a drug molecule linked to either a microbial enzyme degradable carrier or linked using a microbial enzyme degradable bond. The prototype azo prodrug was sulfasalazine consisting of the active ingredient mesalazine linked by an azo bond (-N=N-) to a carrier molecule, sulphapyridine (Khan et al., 1977; Svartz, 1988). Although the active substance mesalazine is readily absorbed from the small intestine, the pro drug sulphasalazine is not absorbed until it reaches the colon where the azo bond is cleaved by bacterial azoreductase enzymes or according to alternate
theory, by a reducing reaction mediated by low molecular weight electron carriers such as NADPH (Scheline, 1973; Lloyd et al 1994). Investigations with sulphasalazine have shown that up to 85% of the administered dose is delivered intact to the colon where the bond is cleaved to release 5-ASA and sulphapyridine (Klotz 1985). Though not specifically developed as a colon targeted drug, it was soon discovered that the azo bond was cleaved in the caecum to yield 5-ASA and sulphapyridine, and logical testing of the components revealed that 5-ASA was pharmacologically equivalent to sulphasalazine. It was also found that the side effects were mainly due to the carrier moiety sulphapyridine, and this led to the development of alternative prodrugs of mesalazine that are devoid of sulphapyridine. Examples include olsalazine consisting of two molecules of mesalazine linked by an azo bond, and balsalazide) consisting of mesalazine azo-linked to an inert molecule. Olsalazine (Dipentum®) and balsalazide (Colazide®) are currently marketed in the UK for acute and maintenance therapy of ulcerative colitis (Qureshi and Cohen, 2005).

Several other pro drugs have been investigated such as the glycoside prodrugs (Friend and Chang, 1984, 1985), dextran prodrugs (Harboe et al., 1989; MacLeod et al., 1994); all with varying degrees of success in vitro or animal models (Sinha and Kumria, 2001). A key limitation to the pro-drug concept is that they are considered as new drug entities with the attendant regulatory requirement of extensive toxicological studies before testing can be conducted in man.

1.4.2.1.2 Formulation approach

Azo polymers

The azo bond principle has been extended to synthesis of azo-linked copolymers, which usually comprise a hydrophilic and hydrophobic polymer linked by an azo bond. Resulting azo polymers are then applied as conventional formulation materials. A typical example is the copolymer of polystyrene and hydroxyethyl methacrylate polymers cross-linked with divinyl azo benzene, and which yields water resistant films. Insulin and vasopressin loaded pellets have been film-coated with azo polymers and when administered to rats showed delayed effects somewhat akin to arrival in the colon though with some variability in the results (Saffran et al., 1986; Cheng et al.,
1994). Yet another variation to the theme is the synthesis of azo hydrogels (Brondsted and Kopecek, 1991, 1992), which rely partly on pH-triggered hydration of polymer in the upper intestine with subsequent azo reduction in the colon. However, drug release from hydrogels is still very slow, occurring over several days (Brondsted and Kopecek, 1992).

The major drawback with dosage forms based on the azo polymer concept is the fact that azo aromatic compounds are regarded as potential carcinogens and though it is not known if this is the case with azo polymers, the association constitutes significant safety concern and has prevented further investigation of the system in humans. Also there is evidence that azoreductase enzyme activity in humans is significantly reduced in active Crohn’s disease (Carrette et al., 1995).

**Polysaccharides**

To overcome safety concerns, naturally occurring polymeric materials such varying grades and complexities of polysaccharides have been investigated as potential carrier material for drug delivery to the colon. Candidate carrier materials are usually pre-approved formulations materials, have GRAS status or are constituents of human diet. Currently investigated formulation carrier materials include naturally occurring polysaccharides such as pectin, amylose, guar gum, xanthan gum, chitin and chitosan, although pectin and amylose are the most extensively investigated. These polysaccharides are specifically degraded in the colon by the bacterial enzymes and so should in principle provide colon-specific drug delivery. However, a common problem with polysaccharide carrier materials is their hydrophilic nature, which leads to uncontrolled swelling in most instances in the upper intestinal regions, resulting in premature drug release. To overcome this problem, they are usually either chemically modified or mixed with insoluble polymers to impart stability. Mixing of polysaccharides with insoluble polymers or chemical modification however, leads to reduced enzyme digestibility, effectively limiting ratio of insoluble polymer that can be added to control swelling.
Pectin

Pectin is a naturally occurring polysaccharide found in plant primary cell walls; with varying degrees of methyl ester substitution depending on the plant source and preparation method (Towle and Christensen, 1973). Pectin is largely indigestible in the upper intestinal regions, but is degraded by pectinase enzymes of the human colonic microflora (Salyers et al., 1977), and has thus been proposed as a carrier material for drug delivery to the colon (Rubinstein et al., 1993). Pectin, like most naturally occurring polysaccharides is very hydrophilic and less hydrophilic derivatives have been developed by formation of a low solubility calcium salt complex, calcium pectinate (Rubinstein et al., 1993).

The performance of calcium pectinate was evaluated in a matrix tablet formulation containing a poorly soluble drug, indomethacin (Rubinstein et al., 1993); and drug release was found to be higher in the presence of pectinolytic enzymes as well as rat caecal contents. However, matrix dosage forms would be unsuitable for very soluble drugs, and hence an outer compression coating was suggested. A calcium pectinate matrix tablet containing insulin, with a compression coating of calcium pectinate was evaluated in pancreatectomised dogs; the delay in response to the insulin was in the range of 5-8 hours claimed to correspond to transit time to the colon (Rubinstein et al., 1995).

Ashford et al. (1994) investigated the suitability of a compression-coated high methoxy derivative of pectin, and in vitro studies showed that 700 mg of pectin was required to prevent drug release proximal to the colon. Subsequent testing in humans showed a disintegration of the coated tablet in the distal gut, compression coating technology is difficult to scale up, and the amount of coating required to retard drug release is likely to lead to very large dosage forms.

Dosage forms formulated with mixtures of pectin or calcium pectinate and other polymers have been investigated, including compression coating of pectin-HPMC (Turkoglu and Urgulu, 2002), calcium pectinate and guar gum in a matrix dosage form (Adkin et al., 1997). Due to the limitations of compression coating and also of matrix formulations, interest has therefore shifted to combination of pectin and other insoluble
film-forming polymers. A mixture of ethylcellulose and pectin was applied as a film coating and evaluation of their performance in vivo led to selective drug release in the colon. Chitosan, a derivative of chitin – a naturally occurring component of the exoskeleton of crustaceans, has been mixed with pectin and HPMC to further improve the water resistance of the resulting mixed coating. Tablets coated with different ratios of pectin-chitosan-HPMC have been investigated in vivo in healthy male volunteers (Ofori-Kwakye et al., 2004). The tablets remained intact in the upper intestinal region, with drug release occurring when the tablets arrive in the colon. The mechanism for the improved drug delivery to the colon is believed to be as a result of the formation of polyelectrolyte complex between the chitosan and the pectin components.

The limitation with all pectin systems studied so far is the aqueous permeability of pectin with attendant excessive swelling in aqueous media and attempts to limit the swelling of pectin have either achieved limited success in control of swelling, or also limit the enzyme digestibility of the polymer, thus leading to rather slow drug release rate.

**Amylose**

Starch is a natural component of food and it is known that a fraction of starch resists digestion by pancreatic enzymes in the small intestine, but is however degraded in the large intestine (Englyst and Macfarlane, 1986). The glassy amorphous form of amylose is one of four types of resistant starch which escape digestion in the small intestine, but are biodegradable by a broad range of colonic bacterial enzymes (Salyers et al, 1977; Macfarlane and Englyst, 1986). Normal dietary starch are composed of 15-25% of amylose (Englyst and Macfarlane, 1987), though the proportion can be up to 70% in some special application high amylose starch such as Hylon VII® and Eurylon VII®.

Amylose, obtained in its glassy amorphous state has good film forming properties, and the possibility for its use as a film coating material to deliver drugs to the colon has been investigated (Milojevic et al., 1996). Amylose, like other polysaccharides, is very hydrophilic, necessitating the addition of an impermeable film forming polymer to control swelling. A matrix film coating formulation comprising one part amylose to four parts ethylcellulose was found to adequately retard drug release in the upper
intestinal regions. When exposed to colonic bacterial enzymes, the amylose component of the film is digested to create pores in the film coating through which the drug is released (Milojevic et al., 1996; Siew et al., 2004). The rate of drug release from the matrix film coating system was found to be dependent on the ratio of amylose to ethylcellulose and the coating thickness.

The colon specificity of amylose/ethylcellulose coated dosage forms has been investigated in a number of gamma scintigraphy studies in humans and good correlation between drug release and arrival of the dosage forms in the colon have been demonstrated (Cummings et al., 1996; Tuleu, et al., 2002; Basit et al., 2004). Amylose/ethylcellulose film coating systems form the basis for COLAL-PRED™ which comprises core pellets loaded with prednisolone metasulfobenzoate – a topical acting glucocorticoid. COLAL-PRED™ is currently under development as a new oral therapy for ulcerative colitis and has been evaluated successfully in phase II clinical trials in ulcerative colitis patients.

A limitation of the amylose/ethylcellulose film coating system is that the rate of drug release from the system is slow and considering current available information about free fluid volume in the colon, drug dissolution from such systems may well be erratic especially as transit through the colon may also be variable. Such problems could occur with the poorly soluble drugs.

1.4.2.2 Time approach

The concept of targeting drug release to the colon by a use of a delayed drug release formulation seems logical in principle, relying on data which suggest that small intestinal transit time is relatively constant at 3±1 hours (Davis et al., 1986a). A number of dosage forms have been formulated to release the drug load after a predetermined lag time to coincide with arrival of the dosage form in the colon.

The first of a number of the dosage forms that were designed to release the drug after a pre-determined lag time in the colon is the Pulsincap™ device which exists in several configurations (Rashid, 1990; McNeil and Stevens, 1990; McNeil et al., 1994). The early prototype comprised a water permeable hydrogel capsule containing a mixture of
the drug and an expanding material, and sealed with a hydrogel plug. Water permeates
the hydrogel wall, causing the internal chamber of the capsule to swell until the plug is
ejected by the internal pressure and thus release the drug load (Rashid, 1990). In vivo
performance of this configuration of the later named Pulsincap™ device system has
been investigated using captopril as model drug and a lag time of 5 hours (Wilding et
al., 1992). Drug release occurred in the colon in 6 out of 8 subjects while drug release
in the other two subjects occurred in the terminal ileum.

A latter modification of the device comprised an impermeable half capsule shell sealed
at one end with a hydrogel polymer plug. The hydrogel plug begins to swell on contact
with gastrointestinal fluid and slowly ejects from the capsule, with complete ejection
and release of the capsule contents after a pre-determined lag time (McNeil et al.,
1994). Thus the time it takes the hydrogel plug to hydrate and eject from the capsule
shell is the defining pre-programmed lag time before drug release and is dependent on
the composition and length of the hydrogel plug (Binns et al., 1993). The performance
of two Pulsincap™ devices with predetermined lag times of 5 and 6 hours was
investigated in fasted subjects using gamma scintigraphy, and results showed that the
position of the capsule at the time of drug release was highly variable ranging from the
stomach to the descending colon (Hebden et al., 1999). A configuration with a 5 hour
lag time has also been assessed for regional delivery of dofelitide in humans, and site
of drug release occurred from the stomach to the ascending colon (Stevens et al.,
2002). Further modifications have involved the use of erodible HPMC tablets in place
of the hydrogel plugs (Stevens et al., 1995, Ross et al., 2000, McConville et al., 2004).

It has been reported from a number of studies that drug absorption is often incomplete
following ejection of the plug which could be due to poor spread of the drug load from
the insoluble capsule or due to reduced absorption of the drug from the colon or simply
due to too little fluid for dissolution of the drug load, leading to impaction in the
capsule. Nevertheless, the device can be configured by attaching the drug formulation
to the back of the hydrogel, such that it gets pulled out on ejection (Hebden 1999).
Also the Pulsincap™ can be enteric coated to overcome the variability in site of drug
release caused by variable gastric emptying time; thus ensuring that hydration of the
plug or erosion of the tablet commence post gastric emptying.
The TIMECLOCK® is another dosage form that depends on transit time through the small intestine for delayed drug release (Pozzi et al., 1994), and comprises of a tablet core coated with mixture of a hydrophobic polymer and surfactant. The resulting coating erodes slowly and the drug contained in the coated core is released after a predetermined lag time which depends on the thickness of the coating. The TIMECLOCK® system has been evaluated in healthy volunteers and found to be reproducible in terms of the lag time before drug release, which occurred regardless of position of the tablet in the gastrointestinal tract (Pozzi et al., 1994). Due to the potential variability in gastric residence time, an outer enteric coating can be applied so that the time delay ‘clock’ is activated on dissolution of the enteric coating post gastric emptying (Wilding et al., 1994; Gazzaniga et al., 1994, 1995; Sangalli et al., 2004).

The main limitation of the time dependent systems however, is the intra- and inter-subject variability in small intestinal transit time which is evident in the variability in site of drug release seen in the in vivo study reports. Hence these systems are more suitably described as delayed release dosage forms, with potential application in chronotherapeutics.

1.4.2.3 Pressure approach

Intraluminal pressure is a consequence of gastrointestinal tract contractions and it is believed that due to a combination of haustral contractions and increased viscosity of contents, the intraluminal pressure is highest at the colon. Pressure-Controlled Delivery Capsule have been developed, which relies on the higher intraluminal pressure in the colon (Takaya et al, 1995). The Pressure-Controlled Delivery Capsule comprises a drug substance dispersed in a suppository base and coated with the water-insoluble polymer ethylcellulose. When ingested orally the suppository base liquefies at body temperature with a consequent increase in volume, forming a liquid filled balloon structure with an impermeable ethylcellulose wall. The integrity of the liquid filled balloon is maintained in the stomach and small intestine due to sufficient fluidity of luminal contents to dissipate the pressure on intestinal contractions. However, as the dosage form enters the large intestine, the pressure induced by haustral contractions
and augmented by increased intraluminal viscosity, leads to rupture of the ethylcellulose 'balloon' and subsequent drug release (Takaya et al., 1995, 1998). Hu et al. (1998) studied the performance of Pressure-Controlled Delivery Capsules in healthy volunteers and showed that the thickness of the ethylcellulose coating can be manipulated to affect the time and possibly position of drug release in vivo. In vivo evaluation of drug release from the dosage form was conducted by conventional pharmacokinetic analysis and the time to drug release was compared to published gastrointestinal transit data. The conclusion was that drug release occurred in the terminal ileum to ascending colon. However, this does not constitute adequate proof of concept, given the variability in residence time of dosage forms in different gastrointestinal regions.

Colonic drug delivery utilising the concept of gastrointestinal pressure has not been extensively investigated as have other approaches. The major limitation of the approach is the lack of data on intraluminal gastrointestinal pressure, particularly the values in the different gastrointestinal regions as well as inter- and intra-subject variability.

1.4.2.4 pH approach

Dosage forms that rely on gastrointestinal pH gradient as a trigger for drug release in the colon are based on the well established enteric coating technology (Chambliss, 1983), but employ polymers with higher dissolution pH threshold. The choice of polymers suitable for drug delivery to the colon is therefore limited by the requisite dissolution pH threshold which must be sufficiently high to prevent premature drug release in the small intestine. Though there are several enteric polymers (table 1-4), the choice of suitable polymers for ileo-colonic drug delivery is restricted to polymers with a dissolution pH threshold of \( \geq 7 \).
Table 1-4 pH-responsive polymers commonly used in enteric coated dosage forms

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Dissolution pH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cellulose acetate trimellitate</td>
<td>4.5</td>
</tr>
<tr>
<td>Hydroxypropyl methylcellulose phthalate 50</td>
<td>5.0</td>
</tr>
<tr>
<td>Hydroxypropyl methylcellulose phthalate 55</td>
<td>5.5</td>
</tr>
<tr>
<td>Hydroxypropyl methylcellulose acetate succinate L</td>
<td>5.5</td>
</tr>
<tr>
<td>Hydroxypropyl methylcellulose acetate succinate M</td>
<td>6.0</td>
</tr>
<tr>
<td>Poly(methacrylic acid, methyl methacrylate) 1:1 – Eudragit L</td>
<td>6.0</td>
</tr>
<tr>
<td>Poly(methacrylic acid, methyl methacrylate) 1:2 – Eudragit S</td>
<td>6.8</td>
</tr>
<tr>
<td>Poly (methacrylic acid, methyl methacrylate and methyl acrylate)</td>
<td>6.8 – 7.0</td>
</tr>
<tr>
<td>1:6.5:2.5 copolymer - Eudragit FS and Eudragit P4135</td>
<td></td>
</tr>
</tbody>
</table>

There are two enteric polymers with suitable dissolution pH threshold for drug delivery to the ileo-colonic region: the methacrylic acid and methyl methacrylate copolymer, Eudragit S and the recently introduced methacrylic acid, methyl acrylate and methyl methacrylate copolymer, Eudragit FS (Rohm Pharma, Darmstadt, Germany). Both polymers form films which are insoluble and relatively impermeable to aqueous media up to a pH threshold of 7.0 and so are usually applied as film coating onto solid dosage cores.

The first colon-targeted pH-responsive dosage form was developed by Dew et al. (1982) and consisted of a capsule dosage form coated with Eudragit S. Performance of the coated capsules was evaluated in humans and disintegration of the capsules occurred in the distal gut. Similar observations were reported following a subsequent study in ulcerative colitis patients (Dew et al., 1983). These results formed the basis for the development of Eudragit S-coated mesalazine tablets, marketed as Asacol® MR.
The investigations of Dew et al. (1982) which led to development of Asacol® MR were based on the physiological principle that gastrointestinal pH rose steadily from the low pH in the stomach and throughout the entire gastrointestinal tract, with the colon as the region of highest pH. However, it was subsequently reported that although intraluminal pH increased aborally from the stomach to the distal gut, the highest pH was observed in the ileoocaecal junction. In what has become the focus of consideration of applicability or otherwise of pH responsive systems for colonic drug delivery, the pH of the caecum and ascending colon was reported to be about one pH unit less than the terminal ileum (Evans et al, 1988; Fallingborg et al., 1998). The significance of these findings is that pH-responsive dosage forms such as Asacol® MR will be prone to disintegration in the terminal ileum or ileoocaecal junction where the pH is higher than dissolution pH threshold of the polymers. If however, the dosage form remains intact on passing into the caecum, there was concern that the pH of this region is insufficiently alkaline to effect the dissolution of the coating. In other words dosage forms formulated with Eudragit S could dissolve in the terminal ileum or not at all.

Ashford et al. (1993b) investigated the performance of Eudragit S-coated salicylic acid tablets in healthy volunteers using gamma scintigraphy. Results of the study showed variability in the site of disintegration of coated tablets, ranging from the terminal ileum to the splenic flexure, while some tablets did not disintegrate at all; and the time of disintegration of the tablets also varied considerably, ranging from 5 hours to more than 15 hours. Similar variability in the site of disintegration, and occasional failure to disintegrate, has been reported for the commercial product, Asacol® MR (Wilding, 1999; Sinha et al., 2003).

The reported variability in performance of dosage forms that rely on pH as a trigger for drug release have been attributed to inter-subject variability in pH and also in the case of ulcerative colitis patients, the lower intraluminal pH in the colon due to mucosal inflammation. There is currently some controversy over the influence of disease states, mainly inflammation of the colonic mucosa on the pH of the lumen. While some studies reported a fall in pH in the colon in some ulcerative colitis patients, particularly those in very active disease state (Fallingborg et al., 1993), others have found no
change and in some cases even a higher pH in patients than in healthy subjects (Press et al., 1998).

Different strategies aimed at improving the colon specificity of pH responsive dosage forms have been investigated. These include synthesis of methyl derivatives of Eudragit S (Peeters and Kinget, 1993) to raise the dissolution pH threshold and presumably prevent dissolution in the terminal ileum; although it seems, that subsequent dissolution in the colon was not given sufficient consideration. Khan et al. (1999) on the other hand proposed film coating mixtures of Eudragit S and another pH responsive polymer that dissolve at pH 6, Eudragit L. This combination would indeed still be subject to the same variable performance as was reported for Eudragit S-coated dosage forms. A dosage form comprising an injection moulded starch capsule which is coated with a mixture of Eudragit S and Eudragit L, such that the position of drug release in the gastrointestinal tract is controlled by the thickness of the coating which begins to dissolve in the small intestine, has been developed under the trademark TARGIT® (Watts and Illum, 1997). A success rate of more than 90% has been reported in phase I evaluations of TARGIT®, although it should be added that since this dosage form has a time-dependent feature, it is likely to be affected by delay at the ileocaecal junction.

Possibly in response to the reports of variability in performance of Eudragit S polymer coated systems, a copolymer of methacrylic acid, methyl methacrylate and ethyl acrylate, Eudragit FS 30D (an aqueous dispersion) was introduced (Rohm GmbH, Darmstadt, Germany), amid claims of improved drug carrier performance. Rudolph et al. (2001) conducted an in vitro evaluation of drug release from Eudragit FS coated 5-ASA pellets in comparison to the more established Eudragit S. They reported that onset of drug release was similar for both pH systems, but the dissolution rate was slower and more controlled for Eudragit FS-coated pellets. Evaluation of Eudragit FS in humans was conducted by film coating onto HPMC capsules filled with paracetamol (Cole et al., 2002). Coated capsules that showed an in vitro dissolution lag time of one hour at pH 6.8 was administered to healthy volunteers in a gamma scintigraphy study. Initial disintegration of the coated capsules occurred as early as 12 minutes post gastric emptying in one volunteers, varying in others from the mid small bowel (2 volunteers),
distal small bowel (2 volunteers) an ascending colon (3 volunteers). The investigators choice to proceed with a coating thickness yielding an in vitro lag time of one hour at pH 6.8 would appear to be an over estimation of the in vivo lag time usually observed with enteric polymers or a conservative interpretation of the gastrointestinal pH profile. Gupta et al. (2001) adopted a combined pH and time responsive approach, with a drug layered pellet dosage form, comprising a sub coat of a mixture of Eudragit RS and RL and a top coating with the new Eudragit FS. They showed in vitro that following dissolution of the outer Eudragit FS coating, the slowly permeable Eudragit RS and RL coating was able to sustain the release of loaded 5-ASA over 12 hours. The rationale and applicability of this approach would however, have to be considered in the context of the viscosity of the colonic lumen and limited fluid availability beyond the ascending colon. Nevertheless, the performance of multiunit dosage forms coated with Eudragit RL/RS and Eudragit FS polymers have been evaluated in humans, with favourable results (Bott et al., 2004). This delivery system is the basis for the proprietary drug delivery technology, EUDRACOL™.

Despite the much publicised inaccurate design principle and reports of inconsistent performance of pH responsive dosage forms for drug delivery to the distal gut, this approach holds particular attraction to formulation scientists not least due to the provenance of the approach, as an extension of enteric drug delivery technology. Therefore interest in the pH approach for drug delivery to the distal gut has remained strong buoyed by the fact also that several pH dependent dosage forms are currently marketed including Asacol® MR, Ipocol® and Mesren® MR, which have become clinically relevant drugs in the therapy of ulcerative colitis and Crohn’s colitis (Friend, 2005)

1.4.3 Colonic drug delivery or ileo-colonic drug delivery: changing concepts

A problem that applies to most of the formulation strategies that have been investigated for drug delivery to the colon is lack of specificity of drug release in the colon. In many cases, the reasons for this include the complexity of the gastrointestinal tract which makes it difficult to predict the behaviour of administered dosage forms, and also the fact that the gastrointestinal tract is a continuum for which there are few
features that are so markedly different between the colon and gastrointestinal tract. It is pertinent however, to re-evaluate the concept of colonic drug delivery in light of current knowledge of gastrointestinal tract physiology and behaviour of administered solid dosage forms therein.

Since the earlier attempts to achieve drug delivery to the colon, colon-specific drug release has remained the deciding principle of most dosage forms investigated. It is rather interesting to note that many investigators claim success even when drug release is observed to occur in the distal small intestine. Although it can be argued that drug release in the distal intestine such as in ileocaecal junction would lead to most of the released drug reaching the colon for local action or absorption, many of these studies are tied to the title or objective borrowed from the long established field of colonic drug delivery. If the studies are to be evaluated on this original concept, then the outcome will be a failure of the dosage form, despite the fact that the concept could actually work in practice. This is probably the reason for the clinical efficacy of dosage forms coated with pH responsive polymers in the therapy of ulcerative colitis and Crohn’s disease, despite the results of poor colon-specificity.

Three factors have been outlined which could potentially affect the performance of an orally administered solid dosage form, namely: gastrointestinal transit, fluid volume and pH. For pH responsive dosage forms for instance, the main barrier to achieving colon specific drug delivery include the high pH of the ileocaecal junction relative to the colon and the delayed transit across the ileocaecal junction. There is also a further constraint of low fluid volume and lower pH in the colon which can impair drug release beyond the ileocaecal junction. Therefore, given these constraints to colon-specific drug release, the probable optimal drug release site for pH responsive dosage forms is the ileo-colonic region, defined in this instance as the region encompassing the immediate region of the intestine either side of the ileocaecal junction. The kinetics of disintegrated tablets in this region of the gastrointestinal tract is usually followed by the aboral transit into the ascending colon. Therefore the concept adopted in this study is that of drug delivery to the ileo-colonic region.
1.4.4 Methods for assessment of ileo-colonic drug delivery systems

1.4.4.1 In vitro tests

In vitro test methods are often applied in the early stages of dosage form development, and are usually designed to mimic as much as possible the in vivo conditions to which the dosages form will be subjected. An important consideration in the choice of in vitro test method for a modified release dosage form is the need to prove the concept behind the design of the dosage form, in other words to prove that drug release is in response to the hypothesised physiological trigger. However, this is not always possible and in vitro tests can thus be based on an established correlation between a characteristic of the dosage form and its in vivo performance.

The most common in vitro test model for the assessment of solid oral dosage forms is dissolution test. In its generic form, dissolution testing measures drug release from the dosage form into the test media usually chosen to represent gastric and intestinal fluids. Dissolution tests at best suffice for rapidly disintegrating conventional solid dosage forms, while modified release dosage forms by their inherent time/site specific delivery feature, possess very diverse physicochemical requirements in order to meet the specified pharmacokinetic/therapeutic goal and which are rather difficult to adequately simulate in vitro (Khan, 1996). For instance an ideal dissolution test for a colonic drug delivery system should adequately simulate the prevailing pH, gastrointestinal secretions such as digestive enzymes, microbial population and associated enzymes, the hydrodynamics such as fluid volume changes and mixing intensity encountered during transit through the gastrointestinal tract. It is unsurprising therefore that there is as yet no dissolution testing that meets the criteria and it will indeed be near impossible to develop such a testing system. Despite the obvious limitations, dissolution testing still plays an important role during the development of oral colonic drug delivery dosage forms as long as the chosen test is able to discriminate between different formulations. Different in vitro tests have been adopted by investigators to assess the performance of different colonic drug delivery dosage forms. pH-responsive dosage forms for ileo-colonic drug delivery have been assessed using conventional dissolution apparatus, by sequential testing in media of different pH to simulate the pH along the gastrointestinal tract. However the major limitations
of in vitro test methods is two fold. Firstly, the dissolution media used usually represent only the pH condition with no consideration of other physicochemical properties of the gastrointestinal luminal fluid. Secondly, tests are usually conducted in media of different pH for specified periods to simulate residence of the dosage form in the different gastrointestinal regions, with testing at times conducted in just one buffer media. It is unsurprising therefore that there is often poor correlation between in vitro dissolution test and in vivo performance of pH-responsive dosage forms.

Conventional dissolution testing is inadequate in the assessment of bacterial enzyme responsive dosage forms, although it has at times been used to demonstrate the absence of drug release in the small intestine. On the other hand, simulating the colonic microbial environment in vitro is near impossible considering that the colon has over 400 microbial species, most of which are anaerobic. Several studies have used compendial phosphate buffer inoculated with freshly voided human faeces in a batch fermenter (Milojevic et al., 1996; Van den Mooter et al., 1993; Basit et al., 2004) but logical as this may seem, the microbial count of human faeces changes very rapidly once voided due to loss of viability of the obligatory anaerobes, is subject to variability and as such is not necessarily representative of the microbial flora population in the colon (Hawksworth et al., 1971). Caecal contents of animal models such as rabbits and pigs (Larsen et al., 1989), and rats (Rubinstein et al., 1993) have also been used in screening studies. Another approach involves the incubation of the dosage form in a modular fermenter with a pure bacterial culture, and examples include Bacteroides ovatus (Rubinstein and Radai, 1995). However the limitation to the use of a single or mixed bacterial culture is that while it provides some measure of control, it is not representative of the complex environment of the colonic lumen (Rubinstein et al., 1993). All these cell-based systems are complex, often requiring specialised equipment and analysis. Most investigators have therefore opted for dissolution testing in buffer media containing cell free enzymes of microbial origin such as amylase, pectinase and glucuronidase (Rubinstein et al., 1993, 1995; Basit et al., 2004; Ofori-Kwakye et al., 2004). The performance of these cell-free enzyme systems has been mixed, with reports of poor correlation with in vivo data, which is unsurprising given the disparity between the in vitro testing conditions and the prevailing in vivo conditions, especially
with regard to viscosity of contents of the colonic lumen vis-à-vis the dissolution media.

### 1.4.4.2 In vivo tests

The complexities of simulating the wide array of physiological and anatomical features of the human gastrointestinal conditions in an in vitro test, and poor correlation of results of current in vitro tests with in vivo results, has relegated in vitro tests to the task of screening different formulations. The ultimate performance test is where practical done by a study in humans.

Evaluation of dosage forms in animal models have also proved very common, especially in testing of dosage forms for which there are outstanding safety issues or where studies in humans is not considered relevant such as in formulation optimisation studies. However, the major limitation to the use of animal models to test orally administered colonic drug delivery dosage forms, is that the colon has the greatest interspecies difference of all the gastrointestinal regions. Also in contrast to immediate release dosage forms, the performance of ileo-colonic drug delivery dosage forms is affected by the transit time and conditions in the upper small intestinal regions and these conditions would be different in animal models.

Pharmacokinetic study in man is often employed to characterise the in vivo performance of dosage forms. The technique involves the monitoring of the drug in blood or other appropriate body fluid such as urine or saliva, over a set time post dose, providing information on the drug absorption-time profile. While sufficient for conventional immediate and modified release dosage forms, pharmacokinetic analysis does not provide adequate information on behaviour of the dosage form along the gastrointestinal tract, nor the site and possible mechanism for drug release.

Information relating to the transit and site of drug release from an orally administered dosage form can be provided by gamma scintigraphy studies. Gamma scintigraphy is an imaging modality originally applied in nuclear medicine, which enables the visualisation of the transit and disintegration behaviour of pharmaceutical under
normal physiological conditions. The technique involves the incorporation of a radionuclide into the dosage forms which enables it to be monitored using a gamma camera. Information on the site and rate of drug absorption can then be deduced by correlation of the time lapse scintigraphy images with pharmacokinetic measurements (Hardy et al., 1981; Wilding et al., 1991).

1.5 Scope and purpose of study

Despite the reported flaws in its concept and also of performance variability, pH responsive dosage forms designed for drug release in the ileo-colonic region have remained a focus for the development of drug delivery formulations in the therapy of ulcerative colitis. The key attraction of the pH approach is the simplicity and provenance of the technique, as an adaptation of the long-established enteric coating technology. Also the innovator pH-responsive dosage form, Asacol® MR is a clinically established treatment in the acute and maintenance therapy of ulcerative colitis and Crohn’s disease. Subsequent ethical generics, Mesren® MR and Ipocol are also approved for inflammatory bowel disease, and the established clinical efficacy of these products and simplicity of the technology suggest that similar products are likely to follow.

It is apparent therefore that there is a poor correlation between the drug delivery data such as pharmacokinetics and scintigraphy data and/or in vitro studies with actual clinical efficacy in patients (Friend, 2005). Many reports of inconsistency in performance of Eudragit S coated dosage forms have concluded that the reasons for failure of the dosage form have been low intraluminal pH. However, there is a lack of fundamental data on dissolution of these pH responsive systems.

The objectives of this study were:

I. To develop and assess the in vitro dissolution performance, including the factors affecting dissolution rate, of four variants of pH-responsive poly(meth)acrylate polymer systems.

II. To investigate the performance of the pH-responsive polymer systems in humans using gamma scintigraphy.
III. To correlate the in vivo performance of pH-responsive polymers with in situ gastrointestinal pH in healthy volunteers using gamma scintigraphy and a novel radiotelemetry pH capsule, and to investigate the influence of food on disintegration performance of the polymer systems.

In addition to evaluating the performance of the polymer systems as carrier materials for ileo-colonic drug delivery, this project aims also elucidate some fundamental aspects of the behaviour of the polymers, and also to try an establish a good in vitro / in vivo performance correlation.
CHAPTER 2: AN IN VITRO ASSESSMENT OF THE PERFORMANCE OF pH-RESPONSIVE POLYMER SYSTEMS FOR ILEO-COLONIC DRUG DELIVERY.
2.1 Introduction

The different approaches for drug delivery to the colon have been discussed in chapter one. The drug delivery concept that relies on differences in pH along the gastrointestinal tract was highlighted as an established formulation strategy for drug delivery to the ileo-colonic region. The range of polymers suitable as carriers for drug delivery to the ileo-colonic region limited by the requisite dissolution pH threshold, usually $\mathrm{pH} \geq 7.0$, to prevent drug release in the upper to mid small intestine. While the dissolution pH threshold would serve as a guide in the choice of candidate polymers for a particular application, the actual pH at which drug release occurs from a coated dosage form will depend on the additives in the coating formulation such as plasticizers and glidants, the coating thickness and the formulation of the core such as choice of disintegrant and solubility of drug substance.

Polymers whose dissolution is pH-dependent are mostly acrylic or cellulosic derivatives which contain ionising acid functional groups (table 1-4). Although some of the enteric polymers have been investigated as binders in matrix solid dosage forms, their most common mode of application is as film coatings onto solid dosage cores, from a solution of the polymer in alcohols, acetone and other organic solvents. There are currently three polymer variants whose dissolution pH thresholds are considered suitable for drug delivery to the distal gut: methacrylic acid and methyl methacrylate copolymer Eudragit S and methacrylic acid, methyl methacrylate and methyl acrylate copolymer Eudragit FS with its solid variant Eudragit P4135.

2.2 pH-responsive polymers for ileo-colonic drug delivery

2.2.1 Eudragit S

Eudragit S is available as a white free flowing spray-dried powder and is insoluble in water and dilute acids but soluble in some organic solvents. In common with other poly(meth)acrylate polymers, the pH-dependent dissolution of Eudragit S polymer and formed films is due to the free carboxylic functional groups of methacrylic acid. These acid functional groups are able to ionise in the pH range of $5 - 7$ to form salts and hence become soluble (Lehmann, 1997). The exact pH at which pH-responsive
Eudragit polymers dissolve depends on the ratio of methacrylic acid to methacrylate ester(s). The ratio of methacrylic acid: methyl methacrylate is 1:2 (27.6 – 30.7% methacrylic acid units) for Eudragit S and the dissolution pH threshold is quoted as ≥ 7.0 (Lehmann, 1997). The quoted acid value is 180 – 200 mg KOH per g of dry substance.

**Figure 1-1 Structure of Eudragit S**

![Structure of Eudragit S](image)

2.2.2 Eudragit FS and Eudragit P4135

Eudragit FS is available as a milky white colloidal aqueous dispersion containing 30% polymer solids, while Eudragit P4135 is available as granules. The chemical structure of Eudragit FS and P4135 are shown in figure 2-2. Both polymers have a methacrylic acid: methyl acrylate: methyl methacrylate ratio of 10:65:25 (Rohm Pharma product literature), with a methacrylic acid content of 10-12% on dry polymer substance and an acid value range of 60 – 80 mg KOH per g of dry polymer substance.

Eudragit P4135 is insoluble in water but soluble in a range of organic solvents. Both polymers are inherently very flexible polymers with a minimum film forming temperature (MFT) of 14°C which is probably also owing to the substantial proportion of the hydrophilic methyl acrylate ester. The inherent flexibility and low MFT of Eudragit FS and P4135 reduce the requirement for addition of plasticisers to the film coating formulation. This has implications not only from the point of view of
operational cost of materials and coating time, but also abates the impairment of coating performance since the film coat will contain less additives.

Figure 2-1 Structure of Eudragit FS and Eudragit P4135

\[
\begin{align*}
\text{CH}_3 & \quad \text{CH}_3 \\
\text{CH}_2 & \quad \text{C} \quad \text{CH}_2 \quad \text{CH} \quad \text{CH}_2 \quad \text{C} \\
\text{C} = \text{O} & \quad \text{C} = \text{O} \quad \text{C} = \text{O} \\
\text{OH} & \quad \text{OCH}_3 \quad \text{OCH}_3
\end{align*}
\]

methacrylic acid \quad \text{methyl acrylate} \quad \text{methyl methacrylate}

2.3 Film coating

Coating of dosage forms has been used in the past for such purposes as aesthetics and taste masking; but the advent of film coating and subsequent improvements in the process technologies have led to the use of coating to achieve more functional roles like stability improvement, and for enteric or other modified release properties. Film coating essentially involves a repeating cycle of application of very fine droplets of the coating material and drying, until the requisite thickness of the polymer film is obtained. The dynamics of the coating process can be summarised as follows (Mehta, 1997):

I. Formation of very fine droplets of the coating material using an appropriate delivery system usually a spray nozzle with a supply of pressurised air.

II. Contact of formed droplets with the core.

III. Spreading of the droplet, coalescence of the polymer particles to form a thin film and adherence to the surface of the core.

IV. Evaporation of the solvent and completion of film formation.
The process stages outlined above occur repetitively over the duration of the coating, until the required coating thickness is attained. Therefore equilibrium has to be established between the spreading, adherence and coalescence of the coating material and rapid evaporation and drying to avoid penetration of the solvent into the core and prevent tablet agglomeration. This is facilitated by the optimisation of the coating parameters for each formulation as well as by choice of solvent and additives in the coating formulation. Film coating formulations consist of the polymer dissolved or dispersed in an appropriate vehicle, and usually contain one or more of the following additives.

I. **Plasticisers:** Plasticisers are generally added to polymer formulations to impart flexibility and decrease the brittleness of resulting films. They are thought to act by weakening intermolecular interactions in the polymer, thus allowing the polymeric molecules to flow more freely with a consequent increase in flexibility. Addition of plasticiser is particularly important in film coating with colloidal aqueous dispersions as they help to enhance the coalescence of polymer particles in the final phases of film formation (figure 2.3), by lowering the glass transition (Tg) and minimum film forming temperature (MFT). Plasticizers also increase the tensile strength and elongation-at-break (resistance to tear) of the resulting polymer film (O’Donnell and McGinity, 1997).

II. **Colorants:** Colorants are at times added to the coating formulation to improve the aesthetics of the dosage form and at times for purposes of identification.

III. **Glidants:** Glidants are added to improve the flow of the core and reduce tackiness or sticking of the cores to one another during the coating process. During the repeated polymer droplets deposition and drying, the polymer film can go through a tacky phase and lead to the sticking of cores and glidants are thus required to ensure that the core remain separated and flow independent of each other. Though these can be added as powders to the coating chamber at intervals during coating, the best practice is to include the glidant in the coating mixture.
There are a variety of techniques employed in film coating of solid dosage forms, but the fluidised bed coating equipment was used in this study and is generally preferred for aqueous coating systems possibly due to its drying efficiency (Mehta, 1997). The fluidised bed spray coating system is represented diagrammatically in figure 2-3 and comprises of a cylindrical chamber with a perforated plate at the base and a metal mesh lid.

*Figure 2-2 Diagrammatic representation of a fluidised bed film coating equipment*

(Adapted from Glatt Air Techniques, Inc., 1987).
Air is supplied into the chamber through the bottom of the column while the exhaust air is removed from the top of the chamber. The coating material is fed on to the path of a pressurised air system, causing atomisation of the liquid into fine droplets, while the inlet air from beneath the column causes fluidisation or agitation of the cores above the rising droplets aided also by the pressurised air jet of the atomiser. The top part of the coating chamber, referred to as the expansion chamber is usually of larger volume and so the air pressure is reduced in this region causing the cores to fall back into the chamber.

2.3.1 Aqueous vs. organic solvent coating

Polymers with pH dependent dissolution properties are usually insoluble in water and therefore applied as a solution in organic solvents. However, there has been a steady decline in the application of solvent coating systems, and currently the use of aqueous coating systems have become the norm rather than an exception wherever possible. The decline in use of organic solvent coating systems can be attributed to more stringent environmental regulations with regard to requirement for solvent recovery from the exhaust air of coating facilities. Similarly there is also the added cost of assuring operator safety (Hogan, 1995). In parallel, the advent of aqueous polymeric systems, coupled with improvements in coating technology such as greater drying efficiency which widen the scope for application of aqueous coating systems; have increased the attraction of aqueous coating systems. Other advantages of aqueous polymeric dispersions over organic solvent solutions is that viscosity of organic solution increases with increasing concentrations and molecular weight of the polymer, whereas the viscosity of aqueous polymeric dispersions is independent of the polymer molecular weight. Therefore aqueous polymeric dispersions with up to 30% solids and a low viscosity is commonly achieved (Wheatley and Steuernagel, 1997).

There is also an important difference in the mechanism of film formation between aqueous and solvent coating systems. Formation of films from organic solvent coating systems involves different states and dynamics of solvent loss from the surface of the formed droplets, with the film going through an intermediate tacky phase. The quality of films formed from organic solvents is dependent on interactions between the
polymer and the solvents as well as other properties of the solvent such as volatility. For aqueous polymeric dispersions however, the polymer exists as discrete particles in aqueous polymer dispersions and these come together as water is lost during drying, then deform and fuse together to form a continuous film (figure 2-3).

**Figure 2-3** Representation of film formation from aqueous dispersions

(Adapted from Wheatley and Steuernagel, 1997)

The key stages of film formation from aqueous polymer systems are as follows: In the first stage, drying occurs at a constant rate and particles move freely in the droplet film. In the second stage, the particles come into irreversible contact with one another and form a dense mass of spheres which start to coalesce. Further evaporation occurs through capillary channels between the deformed spheres, generating capillary forces and surface energy for coalescence of the particles and expulsion of any remaining...
water. However, complete coalescence of the colloidal particles into a homogenous film is often incomplete during coating, and further coalescence during storage can lead to changes in drug release from the coated cores. A curing step in the form of a thermal treatment is therefore usually recommended to ensure complete coalescence and film formation (Hogan, 1995; Wheatley and Steuernagel, 1997).

This difference in film formation underlines some vital difference in applications of aqueous and organic polymers. The deformation and coalescence of the colloidal particles of the aqueous polymer required a degree of softening of the polymer which is attained by use of significantly higher proportion of plasticizers compared to organic solvent systems.

There are concerns about the functional quality of film coatings from aqueous dispersions in comparison to the organic solvent systems. The process of preparation of aqueous systems of Eudragit S involves partial neutralisation of the carboxylic acid functional group, which could affect the functional enteric properties of resulting films. Another potential source of impaired function of aqueous polymer systems is the higher quantities of plasticizer that is required to achieve reasonable operating temperature and ensure complete film formation. There are currently few published reports comparing drug release from aqueous and organic film coating systems coating systems. Contrary to the above concern however, Garcia-Arieta et al. (1996) reported that thicker films of an organic polymer system (Eudragit L) was required to achieve enteric properties in comparison to the aqueous system (Eudragit L30D). Further evidence of lower permeability of films formed from aqueous latexes in comparison to organic solvent solutions has also been reported (Lehman, 1997).

2.4 **In vitro assessment of drug release**

The *in vitro* assessment of drug release from pH-responsive dosage forms is usually by sequential dissolution testing in compendial acid and near neutral pH buffer systems which represent the pH conditions in the stomach and small intestine respectively. Despite the limitations, dissolution testing still plays an important role during the development of oral dosage forms. Three different approaches towards the
development of a useful in vitro dissolution test have been proposed by Abrahamsson (2000) and are:

(a) To develop an in vitro test method that provides the best correlation to in vivo data irrespective of the physiological relevance of the test conditions.

(b) To develop an in vitro test method that fully models the physiological conditions in the gastrointestinal tract.

(c) To develop a formulation that provided release or dissolution that is insensitive to physiological conditions.

The most desirable of the aforementioned approaches would be the development of a fully physiologically relevant dissolution test. Owing to the multitude of approaches in development of modified release dosage forms, it is obvious therefore that any approach in developing a physiologically relevant dissolution test would have to be tailored to the factors that are deemed critical to drug release from each dosage form or formulation, although it would be near impossible to fully model the physiological conditions of the gastrointestinal tract.

For pH responsive dosage forms, some research efforts have been made in defining the physiological factors affecting drug release and most of these have been directed at the constitution of the dissolution test media. Compendial tests in the various national pharmacopoeias such as USP and BP as well as the Ph. Eur. for evaluation of drug release from enteric coated dosage forms recommend the use of dilute hydrochloric acid and simple two-phase phosphate buffer solutions, as representative of the pH condition in the stomach and the small intestine. For example, USP 24 recommends testing for two hours in 750 mL 0.1M HCl and then addition of 250 mL of 0.2M tribasic sodium phosphate and using 2M HCl or 2M NaOH to adjust pH to 6.8. Therefore the qualitative and quantitative composition of (compendial) phosphate buffers bear little resemblance to composition of the small intestinal luminal fluid (Table 2.1). From table 2.1, the predominant buffer component in human small intestine is bicarbonate.
Table 2-1 Electrolyte composition (mM) of the human jejunum and ileum

(Compiled from Banwell et al., 1971; Lindahl et al., 1997; Phillips and Giller, 1973)

<table>
<thead>
<tr>
<th>Electrolyte</th>
<th>Jejunum</th>
<th>Ileum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bicarbonate</td>
<td>8.2</td>
<td>30</td>
</tr>
<tr>
<td>Chloride</td>
<td>135</td>
<td>125</td>
</tr>
<tr>
<td>Potassium</td>
<td>4.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Sodium</td>
<td>142</td>
<td>140</td>
</tr>
<tr>
<td>Calcium</td>
<td>0.5</td>
<td>4.2</td>
</tr>
<tr>
<td>Magnesium</td>
<td>-</td>
<td>2.8</td>
</tr>
<tr>
<td>Ionic strength</td>
<td>0.139</td>
<td>-</td>
</tr>
</tbody>
</table>

The lack of resemblance of compendial dissolution media as well as dissolution test methods to physiological conditions can be attributed to the pharmaceutical industry’s application of dissolution testing mainly as quality control tool with a bias towards testing of the physical properties of solid dosage forms. The desired attributes of the dissolution tests were therefore to ensure complete drug release, reproducibility and robustness of the test and ease of automation; with the default choice of the simplest dissolution media possible (Dressman, 2000).

Recently however, interest has shifted towards the application of dissolution tests as a prediction of in vivo performance and subsequently, some biorelevant media have been proposed (Dressman et al., 1998) such as simulated gastric fluid with or without pepsin; the fasted state simulated small intestinal fluid (FASSIF) and fed state simulated small intestinal fluid (FESSIF). These dissolution test media were designed to elucidate the influence of bile salts and surfactants on drug release, particularly of poorly soluble drugs, with no representation of other gastrointestinal fluid components.

In addition to pH, other aspects of the dissolution media have been reported to affect drug release from enteric coated dosage forms and include: buffer capacity (Ashford et al., 1993a), ionic strength (Kararli et al., 1995) and constituent buffer salts (Chan et al., 2001). Another important aspect of dissolution testing of pH dependent solid dosage
forms for drug delivery to the ileo-colonic region is the simulation of the residence time of the dosage form in each segment of the gastrointestinal tract. For instance, the compendial dissolution tests recommend testing in dilute hydrochloric acid of two hours, followed by transfer to pH 6.8. In reality however, an administered dosage forms could reside in the stomach for only a few minutes to several hours depending on the motility cycle and dosage form size. Also in testing the dosage form in buffer media different researchers have been known to transfer it from the low pH media (pH 1.0 – 1.2) to pH 7.5, and this ignores the fate of the dosage form in the regions of intervening pH value. It is important therefore that the residence time of the dosage form in different gastrointestinal segment is considered in the design of dissolution test methods.

The objective of this phase of the study was to prepare disintegrating tablets coated with four variants of pH-responsive polymers:

- Eudragit S organic system
- Eudragit S aqueous dispersion
- Eudragit FS aqueous system
- Eudragit P4135 organic system

A comparative in vitro assessment of drug release from the polymer systems will be conducted, as well as elucidation of some factors that affect dissolution of the polymer systems. Dissolution will be tested in various media for the potential of achieving good in vivo correlation.

2.5 Materials

**Lactose** was obtained from Ellis and Everand, Essex, UK. Lactose was chosen as the diluent in order to help improve the dissolution of prednisolone from the tablet. **Polyvinyl Pyrrolidone and magnesium stearate** were purchased from Sigma Aldrich (Poole, UK). **Glyceryl monostearate (GMS)** was purchased as Imwitor 900® from Huls AG (Marl, Germany). **Triethyl citrate** was obtained from Alfa chemical (Bracknell, UK). **Eudragit S, Eudragit FS and Eudragit P4135** were obtained from Rohm Pharma (Darmstadt, Germany). All other materials were obtained from Sigma Aldrich (Poole, UK).
2.5.1. Rationale for model drug

Prednisolone Eur. Ph. was obtained from Sanofi Aventis Pharma SA (Nomainville, France). Prednisolone (figure 2-5) is a white hygroscopic powder with a molecular weigh of 360.4 Da and the solubility is about 1:1300 in water and 1:30 in ethanol (BP 2004).

*Figure 2-4 Chemical structure of prednisolone*

(Molecular weight: 360.4; Log P: 1.7)

Prednisolone is a corticosteroid indicated in acute and chronic inflammatory bowel conditions: ulcerative colitis and Crohn’s disease, usually at a daily dose of 20 – 40 mg daily. The use of prednisolone, as is the case also with other glucocorticoids, is limited by the side effects of iatrogenic Cushing’s syndrome. Rectal dosage forms of prednisolone are used to reduce the incidence of side effects by local delivery to the disease site but are less well received. Also enteric-coated formulations are used but these are designed to circumvent gastric irritation by the drug. Targeting drug release to the ileo-colonic region would therefore enable higher concentrations at the inflamed colonic mucosa and consequent reduction in therapeutic dose.

Furthermore, prednisolone is a neutral molecule with non-pH dependent solubility, and is therefore a suitable model drug for comparative evaluation of drug release from pH-responsive polymer coated dosage forms.
2.6 Methods

2.6.1 Determination of solubility of prednisolone

Prednisolone was added in excess to vials containing 10 mL of 0.1M HCL and pH 6.8 phosphate and the saturated solutions were agitated in a water bath maintained at 37°C for 72 hours. The solutions were then filtered through Whatman no. 6 filter paper to remove excess drug and the amount of prednisolone in solution was assayed by UV at 242 nm.

2.6.2 Preparation of prednisolone tablets

The tablets were manufactured by a wet granulation method. The ingredients were granulated in sub-batches of 300g in accordance with the formula in table 2.2.

Table 2-2 Formula for Prednisolone tablets

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>% w/w per tablet</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisolone</td>
<td>5%</td>
<td>Active</td>
</tr>
<tr>
<td>Polyvinyl Pyrrolidone (PVP)</td>
<td>5%</td>
<td>Binder</td>
</tr>
<tr>
<td>Lactose</td>
<td>89%</td>
<td>Filler/diluent</td>
</tr>
<tr>
<td>Magnesium stearate ¹</td>
<td>1%</td>
<td>Glidant</td>
</tr>
</tbody>
</table>

¹ Magnesium stearate was added to the dried granules and amount added is corrected for losses during granulation.

Prednisolone, lactose and polyvinyl pyrrolidone were individually sifted to remove any agglomerates. The powders were then weighed and added to the mixing bowl of a planetary mixer (model A707A, Kenwood, Hampshire, UK) and dry blended at medium speed for five minutes; followed by drop wise addition of de-ionised water (granulating fluid) under continued mixing until a moist coherent mass was obtained.

The moist granule blend was passed through a 710 μm mesh sieve onto a tared drying tray. The granules were evenly spread out on the tray, weighed and dried in an air
assisted oven at 60°C; turning over from time to time in the tray to ensure even drying and prevent possible solute migration. The granules were weighed at appropriate intervals to determine loss on drying and drying was continued until a loss on drying limit of ≤ 0.1%. The dried granules were then milled through nested sieves of size 250, 500, 710 and 1000 μm, using a mechanical sieve shaker. To ensure compliance with uniformity of weight and content of compressed tablets, dried granules in the narrow size range of 500-710μm were selected for compression. Magnesium stearate (1%) was then added to the granules in an amber glass jar and roller-mixed for approximately 3 minutes. 6 x 200 mg samples were removed from the final tabletting mixture and tested for prednisolone content by UV analysis.

The tabletting mixture was compressed using a single punch tabletting machine (Manesty, Speke, UK), fitted with a biconvex 8mm punch and die set (Holland, UK). The fill volume of the die was adjusted to yield tablets of 200 mg nominal mass and 10 mg active content (adjusted for the mean UV assay of prednisolone determined on the tabletting mixture). Tablets were sampled at intervals during tabletting and tested for the following in-process checks: appearance, uniformity of mass and crushing strength.

2.6.3 Characterisation of prednisolone tablets

Uniformity of mass

During tablet compression, the weight of each tablet is determined by shape and size distribution of the granules since the die is filled to a fixed volume. One of the aims of wet granulation is to produce granules of similar shape and size so as to limit the variability in tablet weight and hence content. To ensure the quality of the manufactured tablets therefore, twenty tablets were selected at random during tablet compression and weighed individually and the mean tablet weight was calculated.

Crushing strength / robustness

Tests were conducted to determine the optimum compression load in order to ensure that compressed tablets are able to pass the pharmacopoeia dissolution test, while also sufficiently robust to withstand the first few minutes during film coating without
chipping or breaking. A practical test for robustness was conducted by simulating the fluidisation and atomiser air turbulence in the coating column for 5 minutes and checking the tablets visually for signs of chipping at the edges. The optimum crushing strength range was found to be 50 – 70N and this was monitored as an in-process check during tablet compression, using a tablet crushing strength tester model CT40 (Engineering Systems, Nottingham, UK).

**Content uniformity test (uniformity of dosage unit)**

Thirty tablets were sampled at random during compression, out of which ten tablets were individually crushed and dissolved in 0.1M HCl. The resulting solution was then passed through a 0.45μm filter and the amount of prednisolone in the filtrate was measured by UV spectrophotometer at a wavelength of 242nm.

**Tablet friability**

Tablet friability measures the attrition of the tablets under conditions of simulated handling such as is obtained during packaging and handling, and is particularly essential to ensure that the tablet integrity will be maintained during coating. Friability was tested with a conventional friabilator (Erweka, Frankfurt, Germany). Twenty tablets randomly sampled during compression, were carefully de-dusted, weighed and put in the friabilator. Testing was conducted for 5 minutes, after which the tablets were weighed again and the weight loss calculated.

**2.6.4 Preparation of film coating formulations**

The basic formula for the film coating polymer systems are shown in table 2-3.
Table 2-3 Basic Formula for the polymer coating preparations

<table>
<thead>
<tr>
<th>Ingredient (g)</th>
<th>Eudragit S organic</th>
<th>Eudragit P 4135</th>
<th>Eudragit S aqueous</th>
<th>Eudragit FS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polymer</td>
<td>25</td>
<td>25</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>Water</td>
<td>-</td>
<td>-</td>
<td>277</td>
<td>35</td>
</tr>
<tr>
<td>96% Ethanol</td>
<td>350</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>1N ammonia</td>
<td>-</td>
<td>-</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>Acetone</td>
<td>-</td>
<td>285</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>-</td>
<td>190</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Triethyl citrate</td>
<td>2.5</td>
<td>-</td>
<td>25</td>
<td>-</td>
</tr>
<tr>
<td>Glyceryl monostearate</td>
<td>1.25</td>
<td>1.25 - 10</td>
<td>2.5</td>
<td>0.75</td>
</tr>
</tbody>
</table>
2.6.4.1 Glyceryl monostearate (GMS)

The requirement for inclusion of an anti-tack agent (glidant) in film coating preparations has been previously highlighted. Talc has been used as first choice glidant for Eudragit film coating polymer systems and was evaluated for compatibility during formulation development of the polymer systems. Good compatibility was demonstrated between talc and the Eudragit S solvent and aqueous coating systems. However, addition of talc to Eudragit FS coating preparation led to flocculation of the polymer substance. The certificate of analysis of the purchased talc showed that it contained several electrolyte ions which could have contributed to the problem, since the presence of electrolytes have been known to cause flocculation of dispersed polymer solids from aqueous dispersions.

Glyceryl monostearate (GMS) was identified as a potential glidant for Eudragit FS. However, to keep the formulation of the polymer systems as similar as possible, GMS was also tried as an alternative glidant in the Eudragit S organic solvent and aqueous systems, and drug release performance of resulting film coating compared with talc. Film coating preparations containing GMS were found to be more satisfactory, a fact that is attributed to the fact that more talc is required in the formulation than GMS for equivalent glidant effect. Therefore, GMS was used as a glidant in all the film coating preparations. Suitability of GMS as a glidant in Eudragit aqueous coating systems have previously been evaluated (Petereit et al., 1995). There are several grades of GMS but the grade that have been found to be most effective with Eudragit aqueous polymer systems is Imwitor 900® (Hüls AG, Marl, Germany), which contains mono- and di-glycerides, with 35-50% as monoglycerides. To function effectively, GMS has to be added as a fine dispersion, to the coating formulation (Petereit et al., 1995). The efficiency of GMS as a glidant in comparison to talc is such that as little as a tenth of the required quantity of talc is required for equivalent anti-tack effect, thus generally leading to better film coat efficiency. Other operational advantages of GMS include its colloidal range particle size, preventing sedimentation in tubes during film coating as well as reduced operational time.
Glyceryl monostearate was obtained from Huls AG (Marl, Germany) as Imwitor 900®, having the appearance of off white granules with a waxy appearance. An aqueous suspension of GMS was prepared by emulsification in water using polysorbate 80. Polysorbate (6 g) is dispersed in water, followed by addition of Glyceryl monostearate (15 g) and homogenisation using a high speed mixer for 15 minutes. After homogenisation, the suspension is heated to 70 °C and then allowed to cool under continuous stirring. GMS melts at 65 °C and forms a fine colloidal dispersion on cooling.

2.6.4.2 Eudragit S aqueous dispersion

Eudragit S aqueous dispersion was prepared by partial neutralisation of the acid functional group of Eudragit S according to the formula shown in table 2.3. Eudragit S was slowly added under high speed stirring to a beaker of water and stirring continued until no lumps are visible, followed by a drop-wise addition of 1N ammonia. Ammonia was added over 10 minutes and resulted in a change in appearance of the dispersion from a coarse dispersion to a milky latex appearance. Stirring was continued for one hour after which triethyl citrate (50% on dry polymer substance) was added and stirring continued for at least a further hour. GMS dispersion (5% on dry polymer) was added to the final coating dispersion as a glidant.

2.6.4.3 Eudragit S organic solution

Eudragit S 100 was dissolved in 96% ethanol under high speed stirring until a clear solution was obtained. Triethyl citrate (10% of dry polymer) was added as a plasticizer and GMS (5% of dry polymer) as a glidant.

2.6.4.4 Eudragit FS aqueous dispersion

Eudragit FS is commercially available as a 30% aqueous dispersion and was diluted to 15% dispersion with de-ionised water before use. GMS (5% of dry polymer) was added as a glidant, but due to the inherent flexible nature of the polymer and the low
minimum film forming temperature, no plasticizer was added to the film coating dispersion.

2.6.4.5 Eudragit P4135 organic solution

Eudragit P4135 granules were milled to fine particles using a coffee grinder and then dissolved in a solvent mixture of acetone and isopropyl alcohol (60:40). Eudragit P4135 is a variant of Eudragit FS and does not require the addition of plasticizer. GMS (up to 40% of dry polymer) was added as a glidant. In addition several other formulations were prepared including mixtures of solvents for example methylene chloride, methylene chloride + methanol, different ratios of water + ethanol; with polymer solid proportions ranging from 2% to 10%.

2.6.5 Film coating

The tablets were coated using a Strea-1 bottom spray fluidised bed spray coater (Aeromatic AG, Bubendorf, Switzerland) and process parameters were optimised for each polymer preparation. Tablets were introduced to the coating chamber and warmed under gentle air supply for five minutes before the pump and atomiser air were turned on, and the coating rate was generally slow with low air supply for the first 5-10 minutes before increasing to the normal rate so as to reduce attrition of the tablets.

Tablets were weighed before coating commenced and after appropriate intervals which are based on the calculated theoretical coating time and the increase in weight of the tablets due to film coating was calculated as the percentage weight gain. To assess the optimal polymer film thickness, the tablets were coated to several total weight gains by varying the coating time and hence the amount of coating applied. The amount of polymer substance applied is expressed as percent total weight gain.

After each coating run, tablets were fluidised for a further 10 minutes before checking the weight gain and then subsequently cured in a fan-assisted oven at 40°C for 24 hours. Several optimisation studies were conducted to determine the optimal curing conditions and duration, and curing in an air-assisted oven at 40 °C was found to lead
to satisfactory conditions with no further change in drug release on storage. Cured
tablets were stored in airtight containers until tested.

2.6.6 Scanning Electron Microscopy

The surface morphology and film thickness of the coated tablet was evaluated by
scanning electron microscopy of the coated tablets. The tablets were coated with gold
using a sputter coater (model K550, Emitech, Kent, UK) and scanned using a scanning
electron microscope (model XL20, Philips, Eindhoven, Holland). To measure the film
thickness, the tablet was cut before sputter coating, and film thickness measured from
the scanning electron micrograph.

2.6.7 Dissolution testing

Drug dissolution from uncoated tablets was evaluated in 900 mL of 0.1M HCl using
the same apparatus as described for the coated tablets.

Dissolution testing of coated tablets was by the pH change method whereby the tablets
were first tested in 0.1M HCl (pH 1.2) to represent pH conditions in the stomach, and
then transferred to near neutral buffer media (pH 6.8 – 7.4) representing intestinal pH
conditions. Compendial dissolution testing of enteric coated dosage forms
recommends testing in 0.1N HCl for 2 hours, but it is known that gastric emptying of
single-solid dosage forms can vary from immediately after administration up to 2
hours post dose. Pre-testing in 0.1N HCl was therefore conducted for 30, 60 and 120
minutes to represent varying gastric residence times. In the buffer phase, testing was
conducted at pH 6.8 – 7.4 for six hours to provide a comparison of performance
between the polymer systems.

2.6.7.1 Preparation of dissolution test media

A 0.1M HCl solution was prepared by a 50:1 dilution of 5M concentrated HCl with de-
onised water. The pH of the resulting dilute acid was measured as approximately pH
1.2.
Subsequent testing in buffer was conducted in three buffer systems: 0.067M mixed sodium and potassium phosphate buffer (Sorensen's buffer) and 0.05M potassium phosphate buffer, and a multi-electrolyte physiological buffer system (Hanks buffer). Sorensen's buffer and potassium phosphate buffer are compendial phosphate buffer systems commonly used in dissolution testing and the composition at each pH level is shown in table 2.4.

### Table 2-4 Concentrations of component salts of Sorensen's buffer and potassium buffer (g/5L)

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH</th>
<th>Na$_2$HPO$_4$.2H$_2$O</th>
<th>KH$_2$PO$_4$</th>
<th>NaOH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorensen's buffer</td>
<td>6.8</td>
<td>29.22</td>
<td>23.06</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>36.11</td>
<td>17.79</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>42.47</td>
<td>12.93</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>47.75</td>
<td>8.98</td>
<td>-</td>
</tr>
<tr>
<td>Potassium phosphate buffer</td>
<td>6.8</td>
<td>-</td>
<td>34.02</td>
<td>4.73</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>-</td>
<td>34.02</td>
<td>5.93</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>-</td>
<td>34.02</td>
<td>7</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>-</td>
<td>34.02</td>
<td>7.9</td>
</tr>
</tbody>
</table>

1 Sorensen's 1909, Geigy scientific tables; 2 Appendix 1D, BP 2007.

The buffers salts were weighed into a 5 L volumetric flask, then dissolved and made up to volume with de-ionised water. The pH of the resulting solution was checked and adjusted to ± 0.02 of the target pH if necessary using 2M HCl or NaOH. All buffers were freshly prepared and de-aerated before use.

A comparison of the ionic composition of the buffer media and small intestinal media (terminal ileum) is shown in table 2-5.
**Table 2-5** Electrolyte concentrations and properties of Sorensen's buffer, potassium phosphate buffer and Hanks buffer at pH 7.4 and small intestinal fluid

<table>
<thead>
<tr>
<th>Ions (mM)</th>
<th>Sorensen's buffer</th>
<th>Potassium phosphate buffer</th>
<th>Hanks buffer</th>
<th>Small intestinal fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Na⁺</td>
<td>107.3</td>
<td>39.5</td>
<td>141.7</td>
<td>140</td>
</tr>
<tr>
<td>K⁺</td>
<td>13.07</td>
<td>50</td>
<td>5.8</td>
<td>4.9</td>
</tr>
<tr>
<td>Cl⁻</td>
<td>-</td>
<td>-</td>
<td>142.9</td>
<td>125</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>-</td>
<td>-</td>
<td>1.3</td>
<td>4.2</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>-</td>
<td>-</td>
<td>0.8</td>
<td>2.8</td>
</tr>
<tr>
<td>HCO₃⁻</td>
<td>-</td>
<td>-</td>
<td>4.2</td>
<td>30</td>
</tr>
<tr>
<td>HPO₄²⁻</td>
<td>53.65</td>
<td>39.5</td>
<td>0.3</td>
<td>-</td>
</tr>
<tr>
<td>SO₄²⁻</td>
<td>-</td>
<td>-</td>
<td>0.8</td>
<td>-</td>
</tr>
<tr>
<td>H₂PO₄⁻</td>
<td>13.07</td>
<td>10.5</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>Osmolality (mOsm/Kg)</td>
<td>306</td>
<td>228</td>
<td>295</td>
<td>292</td>
</tr>
<tr>
<td>Ionic strength</td>
<td>0.174</td>
<td>0.129</td>
<td>0.155</td>
<td>0.139</td>
</tr>
<tr>
<td>Buffer capacity (Mmol/L/pH unit)</td>
<td>28.1</td>
<td>23.0</td>
<td>1.0</td>
<td>5</td>
</tr>
</tbody>
</table>
Though each buffer media was prepared to represent similar pH conditions, there are qualitative and quantitative differences between them with regard to the constituent buffer salts. Hanks buffer is a physiologically balanced salt that is normally used as a cell nutrient medium and has similar constituent salt as well as physicochemical properties compared to small intestinal fluid.

2.6.7.2 Dissolution test method

Drug release from the coated tablets was assessed by dissolution testing in accordance with the scheme shown in figure 2-5; using a USP II paddle dissolution apparatus (model PTWS, Pharma Test, Hainburg, Germany). Tests were conducted in 900 mL of dissolution media and the three maintained at 37.0 ± 0.5 °C, and the amount of drug dissolved in the test media was determined every 5 minutes by an in-line UV-Vis spectrophotometer (Cecil 2020, UK) at 242 nm.

Figure 2-5 In vitro dissolution scheme

The results were expressed as cumulative drug release vs. time and two numerical time points lag time and T50% were deduced from the dissolution profiles for purposes of comparing dissolution performance of the polymer systems. Lag time corresponds to
the time during which there is limited drug release from the test tablet, while T50% represents the time point corresponding to dissolution of 50% of the drug content. However, lag time and T50% are single time points on the dissolution curve and hence have the disadvantage of not providing information about the shape of the curve. Statistical moment theory which is usually applied in pharmacokinetic analysis and gastrointestinal transit measurements have also been used to describe the dissolution profiles (Sousa et al., 2002), by calculating the area under the curve (AUC) of % drug dissolved as a function of time and mean dissolution time (MDT). Similarly therefore, the AUC and MDT were calculated from the dissolution data of coated tablets (5% TWG) in different buffer media. While the AUC may be important for dissolution of immediate release dosage forms, it is not as important for the dissolution of the coated tablets in different media of varying pH; but the MDT could nevertheless highlight subtle differences that may exist in the shape of the dissolution profile.

2.7 Results and discussion

2.7.1 Solubility of prednisolone

The intrinsic solubility of prednisolone in 0.1M HCL and pH 6.8 buffer solutions were 539.2 mg/L and 572.5 mg/L respectively. The results are in line with the solubility stated in the BP monograph and also a confirmation that the dissolution tests were conducted under sink conditions.

2.7.2 Uncoated tablet cores

Rapidly disintegrating biconvex tablets (8 mm diameter) containing 10 mg of prednisolone and having a nominal weight of 200 mg were successfully fabricated by the described wet granulation method. Drug release from the uncoated tablets was rapid and complete after 25 minutes in 0.1N hydrochloric acid (figure 2-6), complying with the BP requirement of drug dissolution from uncoated prednisolone tablets (≥ 70% drug release in 30 minutes). Results of other tests of the uncoated tablets are shown in table 2-6.
Table 2-6 *Characterisation data of uncoated prednisolone tablets*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Result</th>
<th>Compendial specification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crushing strength</td>
<td>59.4 ± 6.01 (50-70N)</td>
<td>-</td>
</tr>
<tr>
<td>Friability</td>
<td>0.28%</td>
<td>≤ 1% (Ph Eur)</td>
</tr>
<tr>
<td>Uniformity of weight</td>
<td>199.9 ± 2.4</td>
<td>Ph Eur 2</td>
</tr>
<tr>
<td></td>
<td>(196.7-205 mg)</td>
<td></td>
</tr>
<tr>
<td>Uniformity of content</td>
<td>AV: 0.28 (9.9±0.12)</td>
<td>AV &lt; 15</td>
</tr>
<tr>
<td>Dissolution</td>
<td>≥ 90% in 30 minutes</td>
<td>≥ 70% in 30 minutes (BP)</td>
</tr>
</tbody>
</table>

1 Mean ± STDEV (range)
2 Ph Eur requirement: Not more than two tablets deviate by more than 7.5% from the mean and none by more than 15%
3 AV = Acceptance value (mean ± STDEV).

The tablets therefore exhibit an adequate degree of uniformity of the active content (prednisolone) per dosage unit, as well as mass uniformity of the dosage units. In addition the tablets are considered sufficiently robust for the film coating application in a fluidised bed coater. The onset of drug release is sufficiently rapid, such that drug release from coated tablets can be assumed to be controlled by the film coating.

**Figure 2-6** Dissolution of prednisolone from uncoated tablets in 0.1N HCl
2.7.3 Coated tablets

The tablet cores were satisfactorily coated with Eudragit S (aqueous dispersion), Eudragit S (organic solution) and Eudragit FS (aqueous dispersion) to varying coating thicknesses corresponding to a range of 3% to 9% total weight gain (TWG).

However, considerable problems were encountered during film coating with Eudragit P4135. Excessive tackiness was observed to occur soon after coating was commenced which caused the cores to stick together. An intermediate tacky phase in the film formation process is a known issue with organic solvent polymer systems and is usually responsive to addition of glidants to prevent the cores sticking together, although the tackiness was excessive in this instance. A number of formulation and process variables were tried to improve the film coating process with Eudragit P4135 and are outlined as follows:

I. Dissolution in different solvents. The film formation behaviour and quality of the resulting film is partly determined by properties of the solvent for instance the evaporation rate, spread surface of droplets and solvent polymer interactions such as viscosity of the resulting solution. Eudragit P4135 was therefore dissolved in other solvents to ascertain if the film coating behaviour can be improved. Solvents used include: acetone + water, ethyl acetate, acetone + isopropyl alcohol, isopropyl alcohol, ethanol + water and methylene chloride. The solubility of the polymer varied in these solvents as did the viscosity of the resulting solutions.

II. Also various concentrations of polymer solids were prepared, ranging from 2 to 10% (at 10% and above, the viscosity of resulting solution was too high for use in film coating with fluidised bed coating). The idea being that by reducing the concentration of polymer substance in the solution, the extent of tackiness will also be reduced during the intermediate film-forming phase. However, the low concentrations not only led to very long coating time, but also to settling of the talc (when used as glidant) in the feeder tubes.

III. Use of different glidants in the film coating formulation including talc, GMS and magnesium stearate. These were added at various concentrations.
IV. In addition to variation in the film coating formulation, a number of process parameters were also varied in attempt to improve the coating process such as reducing the pump speed to reduce the rate of polymer application and increasing air flow volume to shorten the duration of the tacky phase. The in-let temperature was initially adjusted upwards in attempt to improve drying of the deposited polymer film and reduce duration of tacky phase. On the other hand, it was observed that the tackiness become excessive once the temperature in the column rises and moreover, the minimum film forming temperature of Eudragit P4135 is 14 °C and the glass transition temperature 48 °C; therefore lower temperature settings were also used to try and overcome the tackiness.

Out of all the formulations and process parameter combinations, the most significant polymer weight gain achieved with Eudragit P4135 was 5.7%, and this was achieved by use of a 4% solution in acetone + water (9: 1 ratio) and with addition of 40% GMS as glidant. No significant weight gain could be achieved with all the other formulation and process parameter combination before onset of excessive tackiness of the tablets. Interestingly, no such difficulties in coating were noted with Eudragit FS 30D, which is an aqueous dispersion variant of Eudragit P4135, and this is probably due to the different film forming processes exhibited by aqueous and organic solvent coating systems, as have been highlighted previously.

2.7.3.1 Simulated gastric pH

No drug release was observed from tablets coated with Eudragit S organic, Eudragit S aqueous and Eudragit FS for up to 6 hours in pH 1.2 HCl, thus confirming resistance of the polymer film coatings to gastric juice. In contrast, tablets coated with Eudragit P4135 were permeable in the acid media with 40% drug release occurring in 2 hours (Figure 2-7).
This can be attributed to the aforementioned difficulties in coating and the need for a high proportion of GMS in the formulation, which may have led to a weak and permeable film coating. Owing to the operational difficulties encountered during spray coating of Eudragit P4135 and significant drug release from the coated tablet in simulated gastric pH environment, further investigation of dissolution of Eudragit P4135 polymer system was discontinued. There have been reports in the literature describing the use of Eudragit P4135 for seal coating using cast films (Hu et al., 1999) and microencapsulation applications (Jeong et al., 2001). There is no published report on spray coating application, and the present study would suggest however, that its use as a spray coating polymer is limited.

2.7.3.2 Simulated intestinal pH

Drug release from coated tablets in buffer media occurred after a varying lag time during which there is no drug release. A period of rapid drug release associated with disintegration of the core then occurs which is believed to correspond to a dissolution of about 95% of the film coating (Ozturk et al., 1988). This is however, the typical
dissolution profile at the dissolution pH threshold, and the dissolution profile at lower or higher pH can be slightly different.

Drug dissolution from tablets coated to the various film thicknesses (range 3% - 9%) was investigated in Sorensen’s phosphate buffer. As would be expected, increasing the film coating thickness increases both the lag time and $T_{50\%}$ (figures 2-8 – 2-10).

**Figure 2-8** Dissolution of prednisolone from tablets coated with Eudragit FS in pH 7.2 Sorensen’s buffer following a 2h pre-soak in acid as a function of coating weight gain.
Figure 2-9 Dissolution of prednisolone from tablets coated with Eudragit S aqueous in pH 7.2 Sorensen's buffer following a 2h pre-soak in acid as a function of coating weight gain.

Figure 2-10 Dissolution of prednisolone from tablets Eudragit S organic in pH 7.2 Sorensen's buffer following a 2h pre-soak in acid as a function of coating weight gain.
Film coating thickness of approximately 3% of Eudragit S organic and Eudragit FS retarded drug release in pH 6.8 buffer for six hours, while a lag of approximately 4 hours in pH 6.8 was observed for 3% film thickness of Eudragit S aqueous. At film coating thickness corresponding to a total weight gain of approximately 5%, drug release from the three polymer systems was completely retarded in pH 6.8 buffer media for 6 hours. The effect of film coating thickness on drug release is as would be expected given that a thicker film is in simple terms implies that there is more polymer to dissolve prior to drug release. A coating weight gain of 5%, equivalent to a film thickness of approximately 84 ± 4 μm, was considered as providing optimal enteric properties for drug delivery to the ileo-colonic region. Comparison of drug release in the different buffer media was thus conducted with tablets coated to a 5% weight gain.

Also dissolution rate increased with increasing pH, with similar trend in potassium phosphate and Sorensen’s phosphate buffers (figures 2-11 – 2-13).

**Figure 2-11** Dissolution of prednisolone from tablets coated with Eudragit FS (5% TWG) in pH 6.8 to 7.4 Sorensen’s buffer media
Figure 2-12 Dissolution of prednisolone from tablets coated with Eudragit S aqueous (5% TWG) in pH 6.8 to 7.4 Sorensen’s buffer media.

Figure 2-13 Dissolution of prednisolone from tablets coated with Eudragit S organic (5% TWG) in pH 6.8 to 7.4 Sorensen’s buffer media.
The effect of increasing pH of dissolution media on dissolution rate is to be expected on the basis of the dissolution mechanism of the enteric polymers, with ionisation of the acid functional groups occurring more readily in media of higher pH. Also based on the observed data, the effective dissolution pH threshold of Eudragit S aqueous polymer coating is between pH 6.8 and 7.0, while corresponding range for tablets coated with Eudragit S organic and Eudragit FS is between pH 7.0 and 7.2. As was highlighted previously, there is not a precise dissolution pH threshold for enteric film coatings, but rather a pH range of up to one pH unit over which the film coating become increasingly permeable and prone to rupture. Therefore an enteric film coating could become hydrated and semi-permeable within this range. It is also possible that some dissolution from the core surface could occur with diffusion of the dissolved drug through the hydrated film coating into the bulk media. This would explain the relatively constant and zero order drug dissolution rate from Eudragit S organic and Eudragit FS polymer systems in pH 7.0 Sorensen’s buffer (figures 2-12 and 2-14).

From the foregoing, it is therefore apparent that drug release from enteric coated dosage forms will depend to some extent on the properties of the core constituents. For instance, the extent of drug release from a partially hydrated or semi-permeable film coating would depend on the solubility and even pKa of drug as well as other excipients in the core. In this instance however, prednisolone is a poorly soluble and neutral molecule, and hence would not compromise the integrity and performance of the polymer coating; and it is therefore believed that any observed effect is due to the performance of the film coating.

2.7.3.3 Influence of pre-testing in 0.1N HCl

Following the simulation of variable gastric residence time on subsequent drug release in intestinal fluid, drug release was quicker for tablets pre-tested in 0.1M HCl for 30 minutes compared to 120 minutes (Figures 2-14 and 2-15), and the same effect was observed for all the polymers systems. The slower dissolution rate for tablets tested in acid for two hours could be due to the ingress of low pH fluid into the film coat thus delaying the neutralising action of the alkaline buffer media on subsequent testing in near neutral pH buffer.
Figure 2-14 Dissolution of prednisolone from tablets coated with Eudragit S aqueous, Eudragit FS and Eudragit S organic (5% TWG) in pH 7.0 Sorensen’s buffer (post 2 hour pre-soak in 0.1N HCl).

Figure 2-15 Dissolution of prednisolone from tablets coated with Eudragit S aqueous, Eudragit FS and Eudragit S organic (5% TWG) in pH 7.0 Sorensen’s buffer after 30 minutes in 0.1N HCl
Moisture uptake studies were conducted to ascertain if acid media was taken up by the coated tablets during the pre-test in 0.1M HCl. Each coated tablet was weighed before and after soaking in acid for 30, 60 and 120 minutes. The observed results from the fluid uptake studies were variable, with slight increase in weight observed in some tablets but not in others. In particular, fluid uptake was consistently observed for tablets coated with Eudragit FS, some of which showed a change in shape around the edges after 120 minutes in 0.1M HCl. However, the amount of fluid uptake was considered negligible. Similar observations regarding fluid uptake by Eudragit S coated dosage forms have been reported by Ashford et al. (1993a) for Eudragit S coated salicylic acid cores at below 50 μm coating thickness. Fadda and Basit (2005) reported that no fluid uptake was observed following testing of Eudragit S coated 5-ASA tablets in 0.1M HCl for 2 hours. The formulations used in these studies were essentially different, possibly accounting for the different observations. It is possible nevertheless that if the fluid uptake is limited to the film coating, then it would be difficult to estimate unless by free film studies. Additionally, the observed effect could be due to some changes in the structure of the polymer coating caused by the acid media. Delay in drug release from salicylic acid tablets coated with cellulosic enteric polymers have been reported in pH 6.8 media following a gastric pre-soak in 0.1M HCl compared to testing without gastric pre-soak (Wu et al., 1997), which the authors also attributed to fluid uptake. It remains to be seen however; whether gastric residence will affect subsequent disintegration in line with this observation, although previous reports did not show any such effect (Ashford et al., 1993b).

2.7.3.4 Comparison of dissolution in different buffers

Drug dissolution was generally slower in potassium phosphate buffer compared to Sorensen’s buffer at pH 7.2 (figures 2-16 and 2-17). There was no drug release for six hours from Eudragit FS and Eudragit S organic coated tablets in pH 7.0 potassium phosphate buffer, and drug release was slow in pH 7.0 Sorensen’s buffer (figures 2-12 and 2.14).
Figure 2-16 Dissolution of prednisolone from tablets coated with Eudragit S aqueous, Eudragit FS and Eudragit S organic (5% TWG) in pH 7.2 potassium phosphate buffer.

Figure 2-17 Dissolution of prednisolone from tablets coated with Eudragit S (aqueous), Eudragit FS and Eudragit S (organic (5% TWG) in pH 7.2 Sorensen’s buffer.
Drug release from the three polymer systems was very rapid in pH 7.4 potassium phosphate and Sorensen’s phosphate buffers (figure 2-18), with no apparent difference in drug release rate between the polymer systems. In contrast however, drug release in pH 7.4 Hanks buffer media was considerably slower compared to the other buffers (figure 2-19). Additionally, drug release rate from the different polymer systems was markedly different in Hank’s buffer compared to pH 7.4 potassium phosphate and Sorensen’s buffers, despite the pH in both buffer systems being identical. The order of drug dissolution observed in Hank’s buffer is Eudragit S aqueous > Eudragit FS > Eudragit S organic. The considerably slower drug release in the physiological buffer is in agreement with Chan et al. (2001). Dissolution of Asacol® MR, a proprietary brand of Eudragit S-coated mesalazine tablets has also been reported to be slower in Krebs buffer another physiological buffer solution similar in composition to intestinal fluid (Fadda and Basit, 2005). The solubility of prednisolone in the test media did not differ markedly, therefore the difference in drug release observed between the phosphate buffers and Hank’s buffer could not have been due to differential solubility of prednisolone in the test media.

Figure 2-18 Dissolution of prednisolone from tablets coated with Eudragit S aqueous, Eudragit FS and Eudragit S organic (5% TWG) in pH 7.4 Sorensen’s buffer
Figure 2-19 Dissolution of prednisolone from tablets coated with Eudragit S aqueous, Eudragit FS and Eudragit S organic (5% TWG) in pH 7.4 physiological salt solution (Hank's buffer).

2.7.3.5 Comparative drug release from Eudragit S organic, Eudragit S aqueous and Eudragit FS polymer systems

Comparative drug release data (lag time and T_{50%}) for the three polymer systems (5% TWG) are shown in table 2.7, and MDT values in table 2.8 for comparison. However MDT values are similar and follow the same trend as the T_{50%} indicating therefore that T_{50%} is a suitable parameter in this instance.
Table 2-7 Lag time and $T_{50\%}$ (minutes) of the polymer coated tablets (5% TWG, coating thickness 84±4 μm) as a function of pH and dissolution media following a 2 hour exposure to acid.

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH</th>
<th>Eudragit S aqueous</th>
<th></th>
<th>Eudragit S organic</th>
<th></th>
<th>Eudragit FS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Lag time</td>
<td>$T_{50%}$</td>
<td>Lag time</td>
<td>$T_{50%}$</td>
<td>Lag time</td>
</tr>
<tr>
<td>Sorensen's</td>
<td>6.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>70</td>
<td>130</td>
<td>185</td>
<td>352</td>
<td>170</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>30</td>
<td>65</td>
<td>110</td>
<td>185</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>25</td>
<td>52</td>
<td>35</td>
<td>68</td>
<td>25</td>
</tr>
<tr>
<td>Potassium phosphate</td>
<td>6.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>175</td>
<td>247</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>50</td>
<td>97</td>
<td>150</td>
<td>252</td>
<td>70</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>35</td>
<td>57</td>
<td>65</td>
<td>112</td>
<td>45</td>
</tr>
<tr>
<td>Hanks</td>
<td>7.4</td>
<td>95</td>
<td>197</td>
<td>130</td>
<td>305</td>
<td>120</td>
</tr>
</tbody>
</table>
Table 2-8: Mean dissolution time, MDT (minutes) of prednisolone from tablets coated (5% TWG) with Eudragit S aqueous (aqu), Eudragit FS and Eudragit S organic (org), in different buffer systems

<table>
<thead>
<tr>
<th>Buffer</th>
<th>pH</th>
<th>Eudragit S (aqu)</th>
<th>Eudragit FS</th>
<th>Eudragit S (org)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sorensen's</td>
<td>6.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>129</td>
<td>251</td>
<td>298</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>64</td>
<td>154</td>
<td>176</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>52</td>
<td>56</td>
<td>69</td>
</tr>
<tr>
<td>Potassium phosphate</td>
<td>6.8</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>235</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>7.2</td>
<td>96</td>
<td>242</td>
<td>271</td>
</tr>
<tr>
<td></td>
<td>7.4</td>
<td>58</td>
<td>106</td>
<td>110</td>
</tr>
<tr>
<td>Hanks</td>
<td>7.4</td>
<td>187</td>
<td>231</td>
<td>278</td>
</tr>
</tbody>
</table>
The rate of drug release from the polymer systems is of the order Eudragit S aqueous > Eudragit FS > Eudragit S organic, and the trend is the same in all the test media. Faster drug release from tablets coated with Eudragit S aqueous dispersion compared to Eudragit S from an organic solution is in agreement with results reported by Rudolph et al (2001), and can be explained on the basis of knowledge of pH dependent dissolution mechanism of acrylic acid / acrylate ester copolymers. As previously highlighted, the carboxylic acid group of the methacrylic acid units ionise at near neutral pH leading to salt formation and dissolution of the polymer and the dissolution pH threshold of the polymer is dependent on the ratio of methacrylic acid to methyl methacrylate component. Therefore the faster dissolution of Eudragit S aqueous polymer system can be attributed to the preparation process of the aqueous polymeric dispersion, involving a partial neutralisation of the acid functional groups with ammonia. This would have led to partial salt formation (ammonium salt) and an inherently more readily soluble polymer coating.

Another possible reason for the faster drug release from Eudragit S aqueous polymer system is the high amount of additives in the film coating formulation. Due to the inherent brittleness of films resulting from aqueous dispersions coupled to the need for a considerably low MFT, the optimum quantity of plasticizer additive have been determined at 40% on polymer substance leading to MFT of 19 °C compared to 52 °C and 41 °C at 10 % and 30% TEC respectively (Lehmann, 1997). With addition of 40% TEC and 5% GMS therefore, the likely proportion of polymer substance in the final polymer coating is approximately 55%, and it has been shown by other reports that the permeability of polymer coating is increased with increasing proportion of plasticizer in the polymer coating (Fadda and Basit, 2005).

Drug release from Eudragit FS polymer system was also quicker than from Eudragit S organic, and is in contrast with Rudolph et al. (2001) who reported a slower dissolution from Eudragit FS compared to Eudragit S organic. According to available literature, Eudragit FS has a higher methacrylic acid to acrylate ester ratio in comparison to Eudragit S and so on the basis that dissolution is dependent on the acrylic acid: acrylate ester ratio should have a higher dissolution pH threshold. However, over a third of the ester component of Eudragit FS is the more hydrophilic and softer methyl
acrylate (Lehmann, 1997) and probably accounts for faster drug release than Eudragit S organic. Tablets coated with Eudragit FS aqueous dispersion is comparable to the Eudragit S organic coated dosage forms in terms of dissolution pH threshold. However, there are essential differences to the properties of the two polymer films. Eudragit FS polymer coating was observed to exhibit a pH dependent permeability to aqueous media, with some degree of swelling across the entire pH range used in the dissolution tests and culminating in the formation of a balloon-like structure around the tablet core prior to eventual drug release at pH > 7.0. This swelling has not been noted in previous studies with the Eudragit FS (Gupta et al., 2001, Rudolph et al., 2001), but this is probably because the authors used coated pellets, which would have made it difficult to detect. It is likely therefore that in the gastrointestinal tract, the Eudragit FS coated tablets would swell, with drug release commencing in more proximal region of the intestinal tract compared to the Eudragit S organic.

The difference between the results observed in this work and that of Rudolph et al. (2001) could be due to the different formulation used in their study. The authors used 50% talc and 10% triethyl citrate (on dry polymer) in Eudragit S organic coating preparation, whereas the only additive in the Eudragit FS formulation was used GMS (10% on dry polymer substance); and this is in comparison to 5% GMS and 10% TEC was used for Eudragit S organic and 5% GMS for Eudragit FS in the present study. Moreover, it was observed during the preformulation studies that drug release was delayed in the presence of GMS compared to talc and this informed the choice to employ talc as a glidant in all the polymer preparations, even though problems with flocculation of polymer solids was only experienced with inclusion of talc in the Eudragit FS formulation.

Cole et al. (2002) reported a more rapid onset of drug release from HPMC capsules coated with Eudragit FS, with drug release occurring after one hour in pH 6.8 phosphate buffer, which implies a much faster dissolution of Eudragit FS coating than was found in this study or in the results of Rudolph et al. (2001). It is known however, that drug release can occur by diffusion of dissolution media into the core and of the dissolved drug across hydrated polymer membrane, and also that this depends on the solubility of the drug in the core; there is a possibility therefore that drug release could occur at lower pH (around 6.8) if the core contains a very soluble drug and also
depending on the film coating formulation for instance if a plasticizer is included in the coating formulation. This could explain the more rapid dissolution especially given that the coated core in this case is a swellable HPMC capsule filled with a very soluble drug, and with the addition of plasticizer to the film coating formulation.

2.7.3.6 Factors affecting dissolution rate in different media

The observed difference in dissolution of the polymer systems in Hanks buffer compared to phosphate buffers is discussed in this section relative to the properties of a buffer solution that affect dissolution rate of a pH responsive polymer.

Ionic Strength

Drug release from Eudragit S coated dosage has been reported to be affected to a certain extent by ionic strength of the dissolution media (Spitael and Kinget, 1977; Ashford et al., 1993a; Kararli et al., 1996; Fadda and Basit, 2005). An increase in salt concentration of the buffer (and hence ionic strength) leads to a reduction in the thickness of the electrical double layer and reduction in repulsive forces between the carboxylic ions of the acid functional group (Zatz and Knowles., 1970). The changes in repulsions forces also lead to softening of the polymer with an increase in erosion of the polymer, hydration and diffusion across the film coating (Kararli et al., 1996). Rudolph et al. (2001) reported that increasing the ionic strength of the dissolution media did not affect the dissolution profile of Eudragit S coated pellets, however the dissolution test was conducted at a media pH of 6.5 which is well below the dissolution pH threshold of Eudragit S and it is no surprise therefore that no influence of ionic strength of the media were seen. Ionic strength of the various solutions has been calculated using the equation (Perry and Dempsey, 1974):

\[ IS = \frac{1}{2} \sum (C_i \cdot Z_i^2) \]

IS = ionic strength

\( C_i \) = concentration of each ionic component in moles/L.

\( Z_i \) = charge of ion of concentration \( C_i \).
The values derived for the three buffer media are 0.129, 0.155 and 0.174 for potassium phosphate buffer, Hank’s and Sorensen’s buffer respectively. Therefore while ionic strength of the buffers may explain the difference in drug release between potassium phosphate buffer and Sorensen’s buffer, the considerably slower dissolution in pH 7.4 Hank’s buffer cannot be attributed to the difference in ionic strength alone, since the ionic strength of Hank’s buffer is higher than for potassium phosphate buffer.

**Buffer capacity**

Another possible explanation for the difference in drug release between the compendial phosphate buffers and Hank’s buffer is the difference in buffer capacity of the three media. Buffer capacity of Hank’s buffer was determined as 1.0 mmol/L/pH which is closer to the value reported for the small intestine in the fasted state of 4 - 13 mmol/L/pH unit (Kalantzi et al., 2006; Moreno et al., 2006) compared to values of 28.1 and 23.0 mmol/L/pH unit for Sorensen’s buffer and potassium phosphate buffers respectively.

Although some authors had attributed changes in buffer concentration on dissolution of polymer films to the influence of ionic strength of the media, the observed effects was however as a result of changes to the buffer capacity too. Ozturk et al. (1988), has demonstrated the influence of pH of the core and of the buffer solution on dissolution of polymer film coatings, using aspirin as a model drug and polyvinyl acetate phthalate as model enteric polymer. The report showed that the core pH or presence of ionisable drugs in the core, affected the pH at the polymer diffusion layer around the polymer coating and also that pH in this polymer diffusion layer affected the rate of dissolution of the polymer film. Furthermore, the pH in the polymer layer can be different from the bulk pH and the prevailing pH depends on the buffer capacity of the bulk dissolution fluid. Similar influence of buffer capacity on dissolution of enteric polymers has also been demonstrated by Fadda and Basit (2005) on dissolution of Asacol®MR tablets, a proprietary brand of Eudragit S coated tablets. However, it is worth noting that the effect of buffer capacity on dissolution of enteric coated polymers is also dependent on the constitution of the coated core and pKa of the active substance. The studies cited above used ionisable drugs or weak acids in the cores, which have a tendency to lower the pH of the polymer dissolution layer. For instance,
5-ASA is an amphoteric drug molecule which can ionise at near neutral pH as dissolution/hydration of the coating commences, and lower the pH of the boundary layer. Thus the effect of buffer capacity of the dissolution media is likely to be exaggerated as the dissolution rate of 5-ASA itself and the ionisation and dissolution of the polymer coating will be retarded. The core used in this study however, contains a neutral drug (prednisolone), which is unlikely to affect the pH of the polymer dissolution layer.

Spitael and Kinget (1977) on the other hand, reported a direct linear relationship between the logarithm of the dissolution rate of enteric polymers and the pKa of the acidic component of the buffer salts, postulating therefore that increasing dissolution rate of the polymers in presence of different buffer salts is governed by the Brönsted catalysis law, with a resultant increase in dissolution rate of the polymer and the pKa of the conjugate acid with increasing concentration of the basic salt. The mechanism of Brönsted base catalysis can be explained using the following schematic sequence:

Scheme 1: The acid functional groups, R-COOH dissociate by proton transfer to the Brönsted base H$_2$O to form the conjugate base of the polymer (R-COO$^-$) and hydronium ions (H$_3$O$^+$).

$$\text{Scheme 1: } R-\text{C}^\text{O} \overset{\text{O} \cdots \text{O} \overset{\text{H}}{\text{H}}}{\text{H}} \rightarrow R-\text{C}^\text{O} \overset{\text{O}^-}{\text{H}} + H_3O^+$$

Scheme 2: The rate of proton transfer is increased in the presence of a basic salt A$^\Theta$, by increasing the affinity of the Brönsted base to accept a proton.

$$\text{Scheme 2: } R-\text{C}^\text{O} \overset{\text{O} \cdots \text{O} \overset{\text{H}}{\text{H}}}{\text{H}} + A^\Theta \rightarrow R-\text{C}^\text{O} \overset{\text{O}^-}{\text{H}} + H_3O^+ + A^\Theta$$

Therefore, although the pKa of the buffer is dependent on the concentration of buffer salts, it is however modulated by the ionic species present in the medium, since the study reported by Spitael and Kinget (1977) was conducted with different buffers at
same pH and buffer concentration. Fadda and Basit (2005) have also shown, following an investigation of dissolution of Asacol® MR in various dissolution media, that dissolution rate is not only dependent on the ionic strength of the buffer but also on the concentration of the conjugate base.

Therefore the observed slower dissolution of the coated tablets in the Hank’s buffer compared to the phosphate buffers can be attributed to a variety of differences between the buffers including ionic strength, buffer capacity and most importantly, the ionic composition of the buffer particularly of the base components.

2.7.4 Comparison of in vitro performance with published data in humans

It is indeed a generally agreed fact that the in vivo lag time before drug release from enteric coated dosage forms is significantly longer than is predicted by in vitro drug release tests performed in compendial phosphate buffers. In several gamma scintigraphy studies in human volunteers, coated dosage forms which exhibit rapid drug release in pH conditions representative of the proximal to mid small intestine, have been shown to stay intact in the terminal ileum for up to several hours (Ashford et al., 1993a, 1993b; Wilding, 1999; Sinha et al., 2003). From the drug release results in Hank’s buffer showing a delayed dissolution of coated tablets in the physiological buffer, it is reasonably conceivable that the disparity between in vitro and in vivo drug release characteristics of pH responsive polymer systems is attributable, inter alia, to the inadequate simulation of intestinal fluid by the in vitro dissolution media. It is to be noted also that pH 7.4 Sorensen’s buffer and potassium phosphate buffer did not discriminate between the different polymer coated dosage forms, as did the Hank’s buffer solution. The ability to discriminate between different formulations is an essential requirement of in vitro tests during formulation development, and it will be interesting to see if the polymer systems also show different dissolution performance in humans, in a manner similar to the prediction from tests in Hank’s buffer.
2.8 Conclusion

Drug release has been shown to be different for tablets coated with Eudragit S aqueous, Eudragit FS, and Eudragit S organic. While dissolution of Eudragit S aqueous coated tablets is likely to occur proximal to the ileo-colonic region, tablets coated with Eudragit S organic solution may however fail to dissolve at the physiological pH within the time frame of intestinal transit, especially in certain patient groups in whom intestinal pH is known to be lower. Tablets coated with the newer Eudragit FS polymer on the other hand, have been shown to exhibit a dissolution profile somewhat intermediate between early drug release from Eudragit S aqueous and much delayed drug release from Eudragit S organic coated tablets. In addition, Eudragit FS is available in an aqueous dispersion form and has shown desirable process advantages during film coating in this study, such as shorter film coating time due to the higher concentration of polymer substances in the dispersion and greater flexibility without requirement for plasticizer.

In comparison to Eudragit S organic, drug release from Eudragit S (aqueous) coated tablets occurs much more readily in simulated intestinal pH media. The reason for this difference in drug release performance has been discussed, and suggest that Eudragit S aqueous systems prepared by partial neutralisation leads to films that show a faster dissolution rate than Eudragit S organic system.

The results also show that in addition to the intestinal pH conditions, drug release is additionally dependent on the ionic composition of the dissolution media, and must be considered alongside other aspects of in vitro drug release testing of enteric polymer coated dosage forms. The observed effects of different exposure times in acid on drug release, though not directly corroborated in published in vivo studies, highlights the error in the common practice of directly assessing dissolution of enteric dosage forms in buffers without prior exposure to acid.
CHAPTER 3: IN VIVO EVALUATION OF pH-RESPONSIVE POLYMER COATING SYSTEMS IN HEALTHY VOLUNTEERS BY GAMMA SCINTIGRAPHY
3.1. Introduction

The formulation development of three pH responsive polymer systems for ileo-colonic drug delivery: Eudragit S organic, Eudragit S aqueous and Eudragit FS; and subsequent assessment of in vitro performance by dissolution testing have been described in chapters 2. Drug release from the polymer coated systems was found to be very rapid and equivalent in pH 7.4 phosphate buffers. Interestingly, however, drug release was markedly slower in a physiological bicarbonate buffer (Hanks buffer) whose ionic composition was similar to that of small intestinal luminal fluids. Furthermore, the dissolution rate of the three polymer coated systems was also found to be different in the physiological buffer media in the following order: Eudragit S aqueous > Eudragit FS > Eudragit S organic.

However, the limitations of dissolution testing in predicting the in vivo performance of dosage forms, particularly modified release oral dosage forms, is all too well known (Khan, 1996) and has been highlighted in chapter 2. There is as yet no in vitro drug release evaluation method that satisfactorily predicts the performance of orally administered dosage forms in man, hence the truism that “the best model for man is man”.

The performance of dosage forms coated with Eudragit S organic have been evaluated in human subjects by several authors and many of the reports indicate that the site and time of drug release from the dosage forms are variable (Schroeder et al., 1987; Ashford et al., 1993b; Sciarretta et al., 1993; Watts and Illum, 1997; Wilding, 1999). Furthermore, investigations of gastrointestinal pH in healthy subjects (Bown et al., 1974; Fallingborg, 1989) reported a measure of inter-subject variability, while investigations in patients such as those with colitis have shown a reduction in intraluminal colonic pH (Raimundo et al., 1992; Fallingborg et al., 1993). Therefore the general consensus of most reviews (Watts and Illum, 1997; Rubinstein, 2005) and investigative reports (Ashford et al, 1993; Wilding, 1999) have been that pH cannot be used to reproducibly target drugs to the colon. The unreliability of dosage forms coated with Eudragit S for drug delivery to the distal intestinal regions have been attributed to a high dissolution pH threshold of the enteric polymer, Eudragit S, and lower
intraluminal pH in humans than was assumed in the formulation development of the dosage forms.

It has been known for some decades now that an aqueous polymeric dispersion of Eudragit S can be prepared (Lehmann, 1997; Rohm Pharma, 2001), yet the potential of Eudragit S aqueous polymer system has not been investigated as an alternative to the Eudragit S organic, especially given the environmental and safety incentives. One possible reason for this could be due to anecdotal evidence of greater permeability of film coatings applied from aqueous dispersions that have been formed by partial neutralisation with ammonia. In addition, the in situ formed aqueous latex, manufactured by emulsion polymerisation, Eudragit FS, is also now commercially available as an alternative to Eudragit S.

Following the in vitro evaluation of drug release performance of these polymer systems therefore, the aim of this study was to investigate the performance of the three pH responsive polymer systems in healthy human volunteers using the imaging modality of gamma scintigraphy.

3.2 In vivo assessment

Pharmacokinetic analysis has been the standard choice for the evaluation of drug release and absorption in man, but is challenged in the evaluation of delivery systems for site-specific drug release. Gamma scintigraphy has therefore emerged as a complimentary technique to pharmacokinetic analysis in assessing the performance of orally administered dosage forms and is of particular relevance in situations where it is important to monitor the transit behaviour and gastrointestinal site of drug release. The results can thus be considered along side traditional pharmacokinetic analysis of drug absorption to provide unequivocal proof of the performance of site specific targeted oral dosage forms.

3.2.2 Gamma scintigraphy

The technique of gamma scintigraphy has traditionally been employed in medical diagnostics, in which case a substance with a high affinity for the organ of interest is
radiolabelled with a gamma emitting radionuclide and administered to a subject. Subsequent distribution of the administered radiolabel can then be visualised with a gamma camera (Perkins, 1999). The gamma camera unit comprise the detector head and a software controlled acquisition, digitisation and data storage system (figure 3-1).

*Figure 3-1 Schematic diagram of a gamma camera and workstation (Perkins, 1999)*

Some cameras have two detector heads for simultaneous acquisition of posterior and anterior images in order to correct for lateral attenuation of activity counts. The detector is shielded by a collimator, essentially a lead plate containing numerous perforations which serve to channel radiated gamma photons from the source onto the detection crystal below it. The most common collimators are the parallel hole collimators which ensure that only radiated photons originating from the source directly in front of and hence parallel to the collimator are channelled onto the detector, keeping extraneous interference to a minimum. Immediately below the collimator is the sodium iodide crystal which absorbs the gamma photons, giving out a characteristic light pulse. The light pulse is then detected by the photomultiplier tubes attached to the crystal and whose outputs are connected to the image forming electronics, usually the computer and visual display unit. The image generated from the gamma camera is such that it is possible to visualise the point in space from which
the gamma radiation was emitted; making it possible therefore to follow the movement in space of a gamma source.

An adaptation of this vital medical imaging technique to study the in vivo fate of pharmaceutical dosage forms was first reported in 1976 (Alpsten et al., 1976; Casey et al., 1976). The underlying principles involve the incorporation of low gamma emitting substances into the dosage form as an unabsorbable complex and in such a way that the radioisotope is permanently associated with the dosage form in the course of transit through the gastrointestinal tract. Therefore making it possible to visualise the transit and behaviour of the dosage form in the gastrointestinal tract, and permitting the deduction of such important dosage form performance indicators as the transit and residence time in various anatomical regions as well as site of drug release in the gastrointestinal tract. Gamma scintigraphy has therefore led to advances in understanding of the fate of dosage forms in the gastrointestinal tract, and by integrating this technique with pharmacokinetic analysis, it is also possible to correlate transit time and/or observed disintegration of dosage forms with drug levels in biological fluids, providing unequivocal proof of the sites of drug release and of absorption in the gastrointestinal tract. Additionally, the advent of gamma scintigraphy has helped with general understanding of gastrointestinal physiology and interaction between gastrointestinal physiology and orally administered dosage forms. For instance, much of what is currently known about the gastrointestinal transit behaviour of various pharmaceutical dosage forms was derived by gamma scintigraphy studies. Due to the ability of the technique to provide in situ monitoring of gastrointestinal physiology and dosage form behaviour, it is also possible to monitor the effect of extraneous factors such as feeding and co-administered materials.

A major advantage of gamma imaging over the other radiological techniques such as X-ray is that following the administration of the radiolabelled preparation, subsequent image acquisition does not involve additional radiation dose to the subject. Serial or even dynamic images can therefore be acquired, permitting a more accurate analysis of the transit behaviour and site of drug release in the gastrointestinal tract, from administered dosage forms.
3.2.2.1 Radiolabelling of dosage forms

One of the limiting steps in gamma scintigraphy is the choice of an appropriate radiation source. The detection efficiency of the gamma camera is optimised in the gamma radiation energy of 100-200 KeV (Hardy and Wilson, 1981); hence it is essential that any potential radionuclide for gamma imaging have emission energy within this range. Other requisite properties of prospective radionuclides include absence of particle emission and a half life appropriate to the duration of the imaging procedure. Other important, albeit operational, factors include availability and cost of the radionuclide.

The most commonly used radionuclides for gamma imaging in pharmaceutical dosage form studies are the metal ion nuclides, technetium ($^{99m}$Tc) and indium ($^{111}$In). $^{99m}$Tc has a relatively short half-life of 6 hours, peak emission energy of 140KeV and is easily produced using portable generators. The relative short half life of $^{99m}$Tc limits its use to studies of short duration such as upper gastrointestinal tract transit studies. $^{111}$In on the other hand has a half life of 67 hours and an emission energy in the range of 173 – 247 KeV and since the half life is similar to whole gut transit time, it is mostly used in studies mapping transit through the entire gastrointestinal tract. $^{111}$In can only be produced in a cyclotron in special facilities, hence it is not as readily available as $^{99m}$Tc, limiting its use to studies of longer duration. The difference in peak photon energies of technetium ($^{99m}$Tc) and indium ($^{111}$In) have led to their simultaneous use in dual imaging studies, for instance in comparative evaluation of transit or drug release site of two dosage forms (Clarke et al., 1993).

A major assumption in gamma imaging is that the spatial location of the detected gamma photons is the same as the dosage form, and this will remain true if the radionuclide is unabsorbable and remains associated with the dosage form during transit through the gastrointestinal tract. Thus the first process in radiolabelling is usually to complex the radionuclide with an ion exchange ligand or chelating agent such as diethylenetriamine pentaacetic acid (DTPA), to prevent systemic absorption; and this is usually involves mixing the radionuclide with the chelating agent. Of critical importance however, is ensuring that the radionuclide maintains an association with the dosage form. There are two main approaches applied in conventional
radiolabelling of orally administered dosage forms. One of the methods involves the incorporation of the non-absorbable chelate of the radionuclide such as $^{99m}$Tc-DTPA and $^{111}$In-DTPA into the dosage form; while the other common method involves the radiolabelling of an ion-exchange resin which is then incorporated into the dosage form (Wilding et al., 1991). The former method is usually used for radiolabelling of aqueous liquid dosage forms, and involves the addition of the water soluble DTPA complex of the respective radionuclide. For radiolabelling of solid dosage forms on the other hand, the latter method is preferred as the radiolabelled resins remain within the dosage form.

The direct incorporation of radionuclides into solid dosage forms presents with particular challenges especially in the radiolabelling of complex modified release dosage forms which require special facilities and involve lengthy production techniques (Digenis and Sandefer, 1991). The lengthy and complex procedure of modified release systems usually precludes direct incorporation of radionuclides as this would lead to unacceptable period or extent of exposure of operators to radioactivity. Radiolabelling is therefore usually carried out as late as possible in the manufacturing process and in special production suites designated for handling of radioactive materials; often with scale down of the manufacturing process. The implication however is that in many cases, dosage forms manufactured in such special facilities under abridged processes to allow radiolabelling, may present with altered performance.

Various alternative methods of incorporating radioactive markers into finished dosage forms have therefore been applied, with varying degrees of robustness and efficacy. One of the methods includes drilling a hole in the middle of the dosage form and filling it with radiolabelled material and then resealing the hole with impermeable material (Sciaretta et al., 1993; Ofori-Kwakye et al., 2004). A common approach in radiolabelling of multiparticulate dosage forms such as pellets is to co-administer radiolabelled placebo pellets of similar size, with the assumption that the radiolabelled pellets would move through the gastrointestinal tract together with the rest of the dosage form (Basit et al., 2001).
A more elegant approach to radiolabelling of oral solid dosage forms, referred to as neutron activation technique, involves the incorporation of a non radioactive isotope into the dosage form during the manufacturing process. The dosage form containing the stable nuclide is then exposed to a high neutron flux just before the investigation, to derive the gamma emitting radionuclide (Parr et al., 1985; Digenis and Sanderfer, 1991). The most common stable isotopes used in the process are the lanthanide earth metals Samarium (Sm), Erbium (Er) and Ytterbium (Yb). These are available as stable isotopically enriched oxides and possess wide neutron capture cross sections which in addition to their isotopic enrichment lead to the generation of isotopes of high radionuclidic purity following a short exposure to a neutron flux. Neutron activation technique avoids many of the problems associated with the handling of radioactivity during the manufacturing process, reducing exposure time, but most importantly from a pharmaceutical point of view, permitting the radiolabelling of rather complex drug delivery dosage forms manufactured under GMP conditions. However, the wide adoption of this technique is limited by the availability of a nuclear reactor in proximity to the study facility. Also the inorganic complexes of the stable nuclide as well as the neutron activation process has a potential to alter the behaviour of the dosage forms such as disintegration time / tablet hardness, and integrity of enteric coating.

Whichever technique is used however, it is essential that it is validated to ensure that the process does not lead to differences in drug release performance of the radiolabelled dosage form, and also that association of the radiomarker with the dosage form is sufficiently robust to withstand transit and agitation factors in the gastrointestinal tract. Also if the study involves a visualisation of disintegration of the dosage form through the spread of radioactivity, then it is essential to ensure that the release of the marker is sensitive to the disintegration of the dosage form.

3.2.2.2 Limitations of gamma scintigraphy

A major constraint of the gamma scintigraphy methodology is that though the detection unit is able to provide the position of the source of radiation in space, this is displayed as a point source and no other anatomical detail is provided. The analysis of acquired scintiscans and deduction of transit behaviour and anatomical position of the
radioactive source therefore requires experience and is prone to error. Furthermore, since the gastrointestinal tract does not lie in a vertical plane, lateral movement of the gamma source would lead to attenuations of activity counts by body tissue and lead to errors in estimation of events such as gastric emptying and colonic entry. This complication can largely be overcome by calculating the geometric mean of the posterior and anterior detector counts, thus giving a value which is virtually independent of the changing source depth.

3.3 Scope of study

The primary objective of this phase of the study was to assess the performance of the three polymer coated systems: Eudragit S organic, Eudragit S aqueous and Eudragit FS in man using the technique of gamma scintigraphy. Further objectives include the assessment of intra-subject variability in performance of Eudragit FS as model pH-responsive polymer system; and correlation of the in vivo performance with in vitro dissolution of the polymers in phosphate buffers and the physiological bicarbonate solution.

3.4 Materials

Technetium-99m and Indium-111

$^{99mTc}$-DTPA and $^{111}$In-DTPA were both obtained daily from Amersham Radiopharmacy, Hammersmith Hospital, London. The reference activity of the nuclides were 20 MBq $^{111}$In in 0.2 mL and 400mBq $^{99mTc}$ in 0.4 mL respectively.

AquaCem®

AquaCem luting cement was received as a gift from DENTSPLY DeTrey GmbH (Konstanz, Germany). AquaCem is a white powder comprising polyacrylic acid and Calcium-sodium-fluoro-phosphoro aluminium silicate blend. Aquacem is a water miscible polymer powder which is used as a dental bonding agent in the repair of crowns and orthodontic bands, and so is safe for human application. On mixing with deionised water, a light paste is formed which dry in approximately 3-5 minutes to form an impervious solid.
Lactose was obtained from Ellis and Everand, Essex, UK. Polyvinyl Pyrrolidone and magnesium stearate were purchased from Sigma Aldrich (Poole, UK). Glyceryl monostearate (GMS) was purchased as Imwitor 900® from Huls AG (Marl, Germany). Triethyl citrate was obtained from Alfa chemical (Bracknell, UK). Eudragit S and Eudragit FS were obtained from Rohm Pharma (Darmstadt, Germany). All other materials were obtained from Sigma Aldrich (Poole, UK).

3.5 Methods

3.5.1 Manufacture and film coating of tablets

Rapidly disintegrating tablets were manufactured by wet granulation in accordance with the method previously described in chapter 2. The tablets had a nominal weight of 200 mg and measured 8 mm in diameter and 4.2 mm height. Following appropriate in vitro characterisation to assure the quality of the tablets, the tablets were film-coated with either Eudragit S dissolved in 96% ethanol, Eudragit S prepared as an aqueous dispersion or the newer aqueous based polymer system Eudragit FS. The manufacturing methods and process controls during the manufacture of the tablets and subsequent film coating process are in accordance with the methods previously described in chapter 2. Tablets were coated to approximately 5% total weight gain, corresponding to film coat thickness of 84 ± 4 µm.

3.5.2 Radiolabelling of coated tablets

The study was designed to administer two tablets on each study day, and hence each tablet would have to be radiolabelled with a different radioactive source to allow for simultaneous imaging by gamma scintigraphy. ⁱ¹¹In and ⁹⁹ᵐTc are two radioactive sources that have been successfully applied in dual gamma imaging studies, and so were chosen for use in radio-labelling of the film-coated tablets.

Radiolabelling of dosage forms has already been highlighted as a critical step in planning a gamma scintigraphy study of solid oral dosage forms. Direct incorporation of the radionuclide into the formulation during manufacture was considered unacceptable because manufacture of the tablet cores and film-coating with the
respective polymers followed by curing of the film is a lengthy process. Incorporating the radioactive sources into the core or the film coating formulation would therefore lead to unacceptable level of exposure of operator to the radioactivity. Moreover, the lengthy manufacturing process would have required very high initial loading of the radioactive source to allow for decay during manufacture, particularly of $^{99m}$Tc. In addition to the level of exposure of the operatives, there is a health and safety requirement for all the operations involving radioactive materials to be performed in a self contained facility suitable for handling of radioactive sources and the manufacturing involved so many different equipments that cannot be moved to a self contained radioactive handling area.

The application of neutron activation in radiolabelling of the coated tablets for this study was also considered. However, there have been published reports of changes in properties of Eudragit L and S free films (Ahrabi et al., 1999) following neutron activation. There has also been anecdotal evidence from our research laboratory, in which the integrity of a polymer coating was compromised after neutron activation. Another method was therefore used as described below.

A 1 mm diameter hole was precisely drilled in the middle of the coated tablets using an in-house crafted seating and a precision cut electric drill. The seating ensured that the hole was drilled at the same position on all the tablets. Drilled tablets showed no visible signs under magnification, of cracks or damage to the film coating.

Following receipt of $^{99m}$Tc- or $^{111}$In- DTPA on morning of the study, the total radioactivity in each vial was measured, and then the required activity was drawn up and added to a small quantity of lactose in a petri dish and left to dry in an oven at 60°C. When dry, the now radio-labelled lactose was pulverised and using a vibrating spatula, each radioactive source is filled into the hole drilled in the middle of the respective tablets to the required activity. The nominal radioactivity in each tablet at time of administration is 4.5 MBq of $^{99m}$Tc and 1.0 MBq of $^{111}$In. The hole was then sealed using AquaCem® bone cement mixed into a light paste with de-ionised water. Adequate care in the sealing process ensured that the spread of the bone cement was limited to only just beyond the diameter of the filled hole in the middle of the tablet.
The radiolabelling process was validated by comparative dissolution testing of intact coated tablets and coated tablets that have been drilled, filled with lactose and sealed with AquaCem® as described. The results showed that drug dissolution from the coated tablets was not adversely affected by the radiolabelling process.

3.5.3 In vitro imaging study

It was necessary to determine if the gamma imaging system could differentiate between tablets labelled with $^{99m}\text{Tc}$ and $^{111}\text{In}$ when placed in close proximity and at the nominal radiation dose. Tablets were loaded with either $^{99m}\text{Tc}$ or $^{111}\text{In}$ as described in the preceding section, and placed in a beaker of water so that they are adjacent to each other. The study showed that it was possible to image the two tablets labelled with $^{99m}\text{Tc}$ and $^{111}\text{In}$ simultaneously and in close proximity, with the energy windows of the gamma camera set at 126-150 KeV for $^{99m}\text{Tc}$ and 221-274 KeV for $^{111}\text{In}$.

3.5.4 Study protocol

The study protocol was submitted to and approved by the Committee on Ethics of Human Research of the East London and City Health Authority. Approval for administration of radioactive substance was granted by the Administration of Radioactive Substances Advisory Committee (ARSAC), Department of Health, London. The licensee for the radioactive substances administration was Dr. Neil Garvie, Consultant Radiologist, Radioisotope unit, Royal London Hospital. The study was conducted in accordance with the declarations of Helsinki (1965) and Tokyo (1975) and Venice (1983) revisions.

Eight healthy male volunteers (age 24-31 years, height 1.75-1.86 m and weight 72-84 kg) participated in the study. Health screening was carried out by means of a health questionnaire and all the volunteers declared themselves healthy with no acute or chronic medical condition, and none was taking any medications. Volunteers received a written 'Invitation to participate' clearly stating the aims and the inclusion and exclusion criteria for participation in the study. A written informed consent to participate in the study was obtained from the volunteers prior to commencement of the study.
Each volunteer completed two study days, receiving in randomised order, the following treatments with at least a 7 day wash-out period between treatments:

I. **Treatment 1**
   - Eudragit FS coated tablet
   - Eudragit S organic coated tablet

II. **Treatment 2**
   - Eudragit FS coated tablet
   - Eudragit S aqueous tablet

Eudragit FS was administered on two study days to assess the intra-subject variability in performance of pH-responsive polymer systems. Most studies often report the inter-subject variability in performance of pH-responsive systems, but there is little information available on the intra-subject variability in performance. It would have been preferable to investigate the intra-subject variability of the three polymer systems. However, this was not possible due to restriction on total dosage of radioactivity that can be administered. It was therefore decided to investigate the intra-subject variability for Eudragit FS since it is the newer polymer system that is being considered as an alternative to Eudragit S.

The study was conducted at the Wingate Institute for NeuroGastroenterology, Royal London Hospital, Whitechapel Road, London. Volunteers were requested to observe an overnight fast for at least 12 hours before the study, including abstinence from alcoholic beverages for a 24 hour period prior to the study; and adherence to the fasting schedule was confirmed with each volunteer before commencement of the study. A sealed point source of 0.5 MBq $^{99m}$Tc was taped to the most lateral part of the lower costal margin, to be used for accurate positioning of the volunteers between imaging and as an anatomical reference marker. The tablets were administered together with 150 mL of water radiolabelled with approximately 0.1MBq of $^{99m}$Tc to enable an outline of the stomach to be seen on the gamma camera.

Images were acquired using a single head camera, model GE Maxicamera 400AC, fitted with a medium energy parallel hole collimator. The energy windows were set at
126-150 KeV for $^{99m}$Tc and 221-274 KeV for $^{111}$In, permitting the two radioactive sources to be detected simultaneously. Sequential static anterior images were acquired over a 1 minute period at 10 minutes intervals for up to 12 hours, with volunteers standing in front of the detector. Usually there is a down-scatter of some radiation photons from $^{111}$In onto the $^{99m}$Tc window, which would require corrections to be made, in addition to the other main correction for movement of the radiation source (dosage form) along the lateral plane in the subject. However, since the radionuclide was localised in each tablet, and the aim of the study was to determine the site and time of tablet disintegration, no radiation counts were obtained from the images and also it was not necessary to acquire posterior images or to carry out corrections for down scatter and radioactive decay. Acquired images were displayed on a dual visual display unit with $^{99m}$Tc and $^{111}$In appearing in separate windows; enabling direct monitoring of major events such as gastric emptying, caecal entry and tablet disintegration. The images were however also stored on an online computer hard drive for subsequent more detailed image analysis after the study.

Volunteers were permitted to move away from the camera between image acquisitions, but were required to remain in an upright position and not undergo any form of physical exertion. Volunteers continued to fast and lunch comprising ham and cheese or chicken sandwich, a pack of crisps and orange juice was provided approximately 4 hours post dose. Water and orange juice were freely available after lunch.

3.5.5 Analysis of scintigraphy data

The series of acquired images for each volunteer was replayed on a computer and processed using the NucMed software (MicasX, Farnborough, UK). To correct for varying posture of the volunteers between images, regions of interest were drawn around the anatomical markers and used to motion correct the acquired images, using the NucMed software package.

Acquired images were analysed and various events of interest were deduced. These include gastric emptying, ileocaecal arrival, colonic arrival and tablet disintegration. These events were all expressed as time (minutes) post dose administration and also, to correct for variable gastric emptying times, as post gastric emptying time. Since the
images were not dynamic, the time of the various events were taken as the mean of the two time points either side of the event.

The inability of gamma scintigraphy to show the anatomy of the gastrointestinal tract has been discussed briefly in the previous section as a limitation to image analysis and deduction of transit behaviour of administered dosage forms. To reduce subjectivity, image analysis was undertaken independently by two investigators one of whom was blinded to the study and results of the two image analyses were compared. Both independent analyses reached similar interpretations of the events of interest.

Gastric emptying time (GET) was derived as the time from administration of the tablet till the tablet entered the small intestine. An outline of the stomach was shown by the $^{99m}$Tc-labelled water, and a region of interest was drawn around the stomach and used to confirm gastric emptying.

Colonic arrival time (CAT) is the time from administration of the tablets to confirmed entry of the tablet into the caecum, hence its designation in some literature as the oro-caecal transit time. Entry of the tablets into the caecum is almost always preceded by a period of relative stasis followed by a sudden upward movement of the tablets. It is also in most cases associated with a perceived location of the tablet behind the right iliac fossa relative to the anatomical markers. Also following the disintegration of the tablets and spread of radioactivity to highlight the anatomy of the colon, it was then possible to draw a region of interest around the colon and transpose this onto the motion corrected images; thus permitting the confirmation of colonic arrival.

Small intestinal transit time (SITT) the time period between gastric emptying and caecal arrival of the tablets, and is often an arithmetically derived function between the events of gastric emptying time and caecal arrival time. In this study however, transit through the small intestine has been broken down into transit through the upper small intestine and delay period at the ileocaecal junction designated as ileocaecal junction residence time (ICJRT). The reason for this format is that dosage forms are known to undergo varying periods of stasis at the ileocaecal junction which constitutes a significant proportion of the small intestinal transit time. Most importantly for pH responsive dosage forms, is the fact that the ileocaecal junction is the region of highest
pH of the gastrointestinal tract, such that the period of residence in this region could have direct impact on performance of pH responsive polymer coated systems.

Disintegration of the tablets leads to a spread in radioactivity, which can be visualised on the acquired scintigraphy images (scintiscans). The time of disintegration of the tablets and the location of the tablets when spread of radioactivity was first noticed were recorded.

### 3.6 Results and discussion

#### 3.6.1 Gastric emptying

Gastric emptying of the administered tablets was fairly rapid on both study days, with a mean gastric emptying time of 37 ± 19 (range 15 – 67) minutes and 35 ± 24 (range 10 – 60) minutes for Eudragit S organic and Eudragit FS respectively on treatment 1; then 25 ± 18 (range 11 – 65) minutes and 43 ± 34 (range 15 – 120) minutes for Eudragit S aqueous and Eudragit FS respectively on treatment 2. The values are in line with the generally expected gastric emptying time of less than 2 hours for dosage forms administered in the fasted state; and are also in general agreement with previously reported values for tablets of similar size (Davis et al., 1984; Khosla et al., 1989; Goto et al., 2004). The two co-administered tablets emptied from the stomach at the same time or on consecutive image acquisition times for most subjects and there was minimal intra-subject variability in gastric emptying, with the exception of subject number 4. Gastric emptying appeared considerably delayed in subject 4, with observed gastric emptying times of 55 minutes and 120 minutes on study day 1; and on study day 2, one of the co-administered tablets emptied at 21 minutes while the other tablet remained in the stomach for the duration of imaging of the study. The full transit and disintegration data for each polymer system are shown in tables 3.1a and 3.1b for treatment 1 and 3.2a and 3.2b for treatment 2.
<table>
<thead>
<tr>
<th>Subject</th>
<th>GET (minutes)</th>
<th>CAT (minutes)</th>
<th>USITT (minutes)</th>
<th>ICJRT (minutes)</th>
<th>DT (minutes)</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>274</td>
<td>138</td>
<td>100</td>
<td>327</td>
<td>AC</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>288</td>
<td>209</td>
<td>52</td>
<td>Intact</td>
<td>AC</td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>231</td>
<td>125</td>
<td>81</td>
<td>337</td>
<td>AC</td>
</tr>
<tr>
<td>4</td>
<td>55</td>
<td>-</td>
<td>152</td>
<td>225</td>
<td>432</td>
<td>ICJ</td>
</tr>
<tr>
<td>5</td>
<td>67</td>
<td>482</td>
<td>201</td>
<td>214</td>
<td>522</td>
<td>AC</td>
</tr>
<tr>
<td>6</td>
<td>15</td>
<td>192</td>
<td>131</td>
<td>46</td>
<td>Intact</td>
<td>AC</td>
</tr>
<tr>
<td>7</td>
<td>52</td>
<td>-</td>
<td>132</td>
<td>157</td>
<td>341</td>
<td>ICJ</td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>183</td>
<td>157</td>
<td>10</td>
<td>Intact</td>
<td>AC</td>
</tr>
<tr>
<td>Mean ± STDEV</td>
<td>37 ± 19</td>
<td>275 ± 110</td>
<td>156 ± 32</td>
<td>111 ± 80</td>
<td>392 ± 84</td>
<td></td>
</tr>
</tbody>
</table>

(GET Gastric emptying time; CAT caecal arrival time; USITT upper small intestinal transit time; ICJRT ileocaecal junction residence time; DT disintegration time; SD site of disintegration or location of intact tablets at time of final image; AC ascending colon; ICJ ileocaecal junction).
Table 3-2 Gastrointestinal transit and disintegration performance of Eudragit FS coated tablets.

<table>
<thead>
<tr>
<th>Subject</th>
<th>GET (minutes)</th>
<th>CAT</th>
<th>USITT</th>
<th>ICJRT (minutes)</th>
<th>DT</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>34</td>
<td>318</td>
<td>152</td>
<td>132</td>
<td>329</td>
<td>AC</td>
</tr>
<tr>
<td>2</td>
<td>50</td>
<td>186</td>
<td>136</td>
<td>-</td>
<td>Intact</td>
<td>DC</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
<td>199</td>
<td>128</td>
<td>56</td>
<td>222</td>
<td>AC</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Intact</td>
<td>ST</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
<td>385</td>
<td>149</td>
<td>171</td>
<td>424</td>
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</tr>
<tr>
<td>6</td>
<td>10</td>
<td>315</td>
<td>110</td>
<td>195</td>
<td>325</td>
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</tr>
<tr>
<td>7</td>
<td>60</td>
<td>-</td>
<td>109</td>
<td>179</td>
<td>348</td>
<td>ICJ</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
<td>274</td>
<td>199</td>
<td>64</td>
<td>355</td>
<td>AC</td>
</tr>
</tbody>
</table>

Mean ± STDEV: 35 ± 24, 280 ± 76, 140 ± 31, 133 ± 60, 334 ± 65

(GET Gastric emptying time; CAT caecal arrival time; USITT upper small intestinal transit time; ICJRT ileocaecal junction residence time; DT disintegration time; SD site of disintegration or location of intact tablets at time of final image; ST stomach; DC descending colon; AC ascending colon; ICJ ileocaecal junction.)
Table 3-3 Gastrointestinal transit and disintegration performance of Eudragit S aqueous tablets.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Eudragit S aqueous (Treatment 2)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GET (minutes)</td>
</tr>
<tr>
<td>1</td>
<td>34</td>
</tr>
<tr>
<td>2</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>15</td>
</tr>
<tr>
<td>4</td>
<td>21</td>
</tr>
<tr>
<td>5</td>
<td>65</td>
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<td>6</td>
<td>24</td>
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<td>7</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>11</td>
</tr>
<tr>
<td>Mean ± STDEV</td>
<td>25 ± 18</td>
</tr>
</tbody>
</table>

(GET Gastric emptying time; CAT caecal arrival time; USITT upper small intestinal transit time; ICJRT ileocaecal junction residence time; DT disintegration time; SD site of disintegration or location of intact tablets at time of final image; PSB proximal small bowel, MSB mid small bowel; DSB distal small bowel).
Table 3-4 Gastrointestinal transit and disintegration performance of Eudragit FS coated tablets.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Eudragit FS (Treatment 2)</th>
<th>GET (minutes)</th>
<th>CAT</th>
<th>USITT (minutes)</th>
<th>ICJRT</th>
<th>DT</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>47</td>
<td>274</td>
<td>127</td>
<td>100</td>
<td>338</td>
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<td>AC</td>
</tr>
<tr>
<td>2</td>
<td>27</td>
<td>-</td>
<td>142</td>
<td>76</td>
<td>245</td>
<td>ICJ</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>25</td>
<td>197</td>
<td>111</td>
<td>61</td>
<td>204</td>
<td>AC</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>120</td>
<td>-</td>
<td>112</td>
<td>200</td>
<td>432</td>
<td>ICJ</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>56</td>
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<td>150</td>
<td>60</td>
<td>266</td>
<td>ICJ</td>
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</tr>
<tr>
<td>6</td>
<td>15</td>
<td>315</td>
<td>131</td>
<td>169</td>
<td>377</td>
<td>AC</td>
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</tr>
<tr>
<td>7</td>
<td>39</td>
<td>-</td>
<td>152</td>
<td>81</td>
<td>272</td>
<td>ICJ</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>16</td>
<td>194</td>
<td>156</td>
<td>22</td>
<td>291</td>
<td>AC</td>
<td></td>
</tr>
<tr>
<td>Mean ± STDEV</td>
<td>43 ± 34</td>
<td>245 ± 60</td>
<td>135 ± 18</td>
<td>96 ± 60</td>
<td>303 ± 75</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(GET Gastric emptying time; CAT caecal arrival time; USITT upper small intestinal transit time; ICJRT ileocaecal junction residence time; DT disintegration time; SD site of disintegration or location of intact tablets at time of final image; AC ascending colon; ICJ ileocaecal junction).
Gastric emptying of dosage forms administered in the fasted state is believed to be brought about by phase III contractions of the MMC (Coupe et al., 1991). Gastric emptying time therefore depends on the frequency of the MMC cycle as well as the phase of the MMC in each subject at the time of dosage form administration; and could range from almost instantly following administration to up to 2 hours, assuming a 2 hour MMC cycle. The rapid gastric emptying times observed for most of the volunteers on the two study days does suggest however, that some of the tablets may have emptied from the stomach independent of phase III contractions of the MMC.

One reason for the variability in gastric emptying times usually reported is the observation that the frequency of the MMC cycle can vary between individuals from as frequent as every half hour to over three hours (Kerlin and Phillips, 1982), implying therefore that gastric emptying times could vary accordingly. Furthermore other authors have also reported extended gastric residence times of several hours for single solid dosage forms as was the case in subject number 4 (Khosla and Davis, 1990; Ashford et al., 1993b). Such protracted gastric residence of dosage forms could be due to the stomach not being involved in every MMC cycle (Kellow et al., 1986) or the inter-subject variability in frequency of the MMC. The implication being that it could be several hours before arrival of a phase III MMC contractions in the stomach. Even with arrival of the MMC phase III contractions, the tablet may well be trapped in the folds (rugae) of the proximal stomach and hence may fail to be cleared by several MMC cycles as was observed by Coupe et al. (1991), who also reported protracted delay in gastric emptying of a radiotelemetry device despite arrival of several MMC cycles in the same subjects. The explanation that the tablet may be trapped in the proximal gastric region is made more plausible by the fact that movement of the tablets was not observed on the scintiscans from the time of ingestion until end of imaging, even after ingestion of meals.

3.6.2 Small intestinal transit

Small intestinal transit data is shown in the modified format as upper small intestinal transit (USITT) and ileocaecal junction residence time (ICJRT) in tables 3.1a, 3.1b, 3.2a and 3.2b. The mean total small intestinal transit time for both study days are 246 ± 79 minutes (range 136 – 415 minutes) and 219 ± 59 minutes (range 178 – 300 minutes).
on study days 1 and 2 respectively. These mean values are in agreement with the pooled population mean of 3 ± 1 hours reported by Davis et al. (1986), but there was considerable inter-subject as well as intra-subject variability. It is generally accepted that small intestinal transit of dosage forms is constant around the quoted mean and is unaffected by other extrinsic factors such as feed status and type or size of dosage form. However, as was observed in this study, there is considerable variability in transit time which could hold important implications for drug release performance of pH-responsive dosage forms.

To gain a better insight on how small intestinal transit may impact on dissolution performance of pH-responsive polymer systems however, small intestinal transit has been sub-classified into upper small intestinal transit time (USITT) and ileocaecal junction residence time (ICJRT). This is necessary due to the variability in pH along the small intestine and the fact that the ileocaecal junction has been reported as the point of highest pH along the small intestine. This form of data presentation also has the advantage of providing some information on the transit behaviour of dosage forms which disintegrated at the ileocaecal junction. The mean USITT is 156 ± 32 (range 139 – 209) minutes for Eudragit S organic and 140 ± 31 (range 109 – 199) minutes for Eudragit FS on treatment 1; then 135 ± 18 (range 111 – 156 minutes) for Eudragit FS on treatment 2. Corresponding data for ICJRT was 111 ± 80 (range 10 – 225 minutes) for Eudragit S organic and 133 ± 60 (range 56 – 195 minutes) for Eudragit FS on treatment 1; then 96 ± 60 (range 22 – 200 minutes) for Eudragit FS in treatment 2.

It is seen from the data that there is less variability in upper small intestinal transit time compared to total transit time, while the ileocaecal junction residence time showed greater variability intra- and inter-subject variability. This suggests that the variability in ileocaecal junction residence time is responsible for variable small intestinal transit time, and supports the argument by Ashford et al. (1993b) that the absence of variability in reported mean small intestinal transit time data is because measurements of transit do not include the period of stasis at the ileocaecal junction. Also compared to the values for upper small intestinal transit, the data suggest that the ileocaecal junction residence time constitutes a considerable proportion of the small intestinal transit. In some subjects however, the tablets moved rapidly across the distal small intestine and into the ascending colon without a discernible delay in the ICJ. Widely
varying periods of ileocaecal stasis have been reported albeit for dosage forms of varying sizes (Malorva et al., 1987; Fallingborg et al., 1989; Ashford et al., 1993b; Goto et al., 2004; Ofori-Kwakye et al., 2004). The delay of dosage forms at the ileocaecal junction is in accordance with the perceived physiological role of the ICJ as a sphincter region controlling the entry of materials into the colon as well as retrograde flow of colonic contents into the small intestine (Philips et al., 1988; Spiller et al., 1987; Kerlin et al., 1983). In contrast, Goto et al. (2004) reported that no significant delay in transit across the ileocaecal junction was observed for 7.3 mm tablets, while Khosla and Davis (1989) also reported similar results for 5 mm tablets. The reports that no significant period of delay was observed at the ileocaecal junction are indicative of the considerable inter-subject variability in transit across the ileocaecal junction, which is somewhat akin to gastric emptying although less defined. Most importantly however, is the fact that many of the studies were not designed for quantitative determination of transit across the ileocaecal junction, as was the case in this study where some of the tablets disintegrated proximal to or at the ileocaecal junction.

There is evidence to suggest that transit across the ileocaecal junction is influenced by feeding via a physiological response mechanism termed the 'gastrocolonic response'; and could also be dependent on the size of the dosage (Spiller et al 1987; Kerlin et al 1983). Adkin et al. (1993) reported a significant reduction in ICJRT following consumption of lunch by volunteers, but on the other hand found no difference in ICJRT for dosage form in the size range 3 mm – 12 mm. Similar influence of meal consumption on transit across the ICJ has been reported (Price et al 1993; Spiller et al 1987). Due to the different location of the tablets at time lunch was consumed by the subjects, it was not possible to estimate the influence of meal consumption on transit across the ICJ. What is apparent however, is that small intestinal transit; particularly across the ICJ is increased immediately following consumption of lunch. Also observed is a rapid distal spread into the colon, of previously disintegrated tablets following meal consumption. This influence of meal consumption on transit across the ICJ was particularly pronounced in subject 2, in whom the tablet passed rapidly through the ileo-caecal junction and into the descending colon within 20 minutes after eating (see figure 3-2).
Figure 3-2 Three scintiscans showing the rapid transit of tablet through the ascending colon in one volunteer

(a) Eudragit FS coated tablet has just moved the ascending colon in subject 2 since the previous image, (b) The radioactivity trace across the transverse colon shows the tablets moving during the one minute image acquisition time, (c) Tablet located in the descending colon at the time of next scan At the time of the next scan

(a) Time of scan 14.29 hours, Lunch consumed 14.35 – 14.50 hours

(b) Time of scan 14.52 hours
Admittedly, this study was not designed to measure this potentially important transit behaviour, since some of the tablets disintegrated at or before reaching the ileocaecal junction, while consumption of lunch was not controlled to determine the effect of food on ileocaecal junction residence time.

The phenomenon of delay of flow of materials in the terminal ileum, with control of transit across the ileocaecal junction has long been confirmed. However, the importance of this physiological control mechanism on the performance of colon-targeted dosage forms has not been adequately elucidated. Also since gastrointestinal pH values have been reported to be highest in the distal small intestine, it is conceivable that drug release from pH-responsive dosage forms will be affected by transit through the distal small intestine. Ashford et al (1993b) however reported no influence of ICJ residence time on subsequent disintegration of pH responsive tablets.

An implication therefore of observed ICJRT is that small intestinal transit time which includes the period of transit through the ileocaecal junction into the ascending colon cannot be regarded as free of the influence of feeding and dosage form size. Indeed, it is conceivable that variability in duration of transit through the ileocaecal junction could account for the inter-individual variability in small intestinal transit time.
Representative scintiscans showing transit of coated tablets through the various segments of the gastrointestinal tract is shown in figure 3-3.

**Figure 3-3** Representative scintiscans showing disintegration of Eudragit S organic and Eudragit FS coated tablets in the ascending colon for volunteer 1.

3.6.3 Colonic transit

Caecal arrival time was only determined for intact tablets that entered the caecum at the time of the final image. The mean mouth to colon transit time is 275 ± 110 (range 183 – 482 minutes) and 280 ± 76 (range 186 – 385 minutes) for Eudragit S organic and Eudragit FS respectively in treatment 1; and 245 ± 60 (range 194 – 315 minutes) for Eudragit FS in treatment 2; and these are in broad agreement with previously reported data (Hardy et al., 1985; Davis et al., 1986). Transit of intact tablets through the ascending colon was relatively slow, with the exception of subject 2 in whom one of the administered tablets moved very rapidly across the ileocaecal junction and through the ascending and transverse colon after feeding. The mean mouth to colon transit
time is in agreement with previous reported values (Khosla et al., 1989; Adkin et al., 1993). Tablet disintegration in the ileocaecal junction or ascending colon led to distal spread of radioactivity through the ascending and transverse colon, although there were signs of accumulation of radioactivity at the hepatic and splenic flexures.

The study duration was not sufficiently long to enable a measure of transit through the colon. However, tablets that reached the colon remained in the ascending colon until disintegration or remained intact therein until the end of imaging. This is in agreement with reported slow transit of materials through the ascending colon (Adkin et al 1993; Kerlin et al 1983), but is in contrast to Ashford et al. (1993b) who reported a rapid transit (<2.1 hours) of tablets of similar size to this study through the ascending colon. The conflicting reports could however be as a result of the large inter-individual variability in colonic transit, also compounded by the fact that colonic transit is characterized by short bursts of movement with intervening periods of quiescence.

3.6.4 Tablet disintegration

The mean disintegration times are 392 ± 84 minutes (range 327 – 522 minutes) for Eudragit S (organic) and 131 ± 66 minutes (range 68 – 271 minutes) for Eudragit S aqueous. Mean disintegration time for Eudragit FS is 334 ± 65 minutes (range 222 – 424 minutes) and 303 ± 75 minutes (range 204 – 432 minutes) for treatments 1 and 2 respectively. To correct for varying periods of gastric residence, the gastric emptying times have been deducted and the corresponding mean disintegration times post gastric emptying are 345 ± 71 minutes (range 289 – 455 minutes) for Eudragit S (organic), 107 ± 52 minutes (range 55 - 158 minutes) for Eudragit S (aqueous), 260 ± 61 minutes (range 179 – 362 minutes) and 301 ± 54 minutes (range 207 – 359 minutes) for the two administrations of Eudragit FS.

The disintegration time of the coated tablets is deduced from the scintiscans as the time point at which a noticeable spread in radioactivity is observed from the tablets. All the tablets remained intact in the stomach as would be expected, including the Eudragit FS coated tablet which failed to empty from the stomach for the entire duration of imaging on the study day.
Disintegration of tablets coated with Eudragit S (organic) was observed in five subjects, three of which disintegrated in the colon and two in the ileocaecal junction. The three tablets which failed to disintegrate were located in the ascending colon at the end of imaging on the study day. The disintegration times observed in this study for tablets coated with Eudragit S organic are generally in agreement with mean disintegration times for similar coated tablets reported by Ashford et al. (1993b), albeit with greater inter-subject variability in their results. Other studies have reported similar variability in site of disintegration of dosage forms coated with Eudragit S organic system (Sinha et al., 2003; Wilding 1999). The dissolution pH threshold of Eudragit S is often quoted in the literature as 6.8 or 7.0 (Rohm Pharma, 1999, 2001), but results obtained from in vitro dissolution studies outlined in chapter 2, indicate that dissolution of Eudragit S organic polymer system is very slow in pH 7.0 Sorensen’s buffer with a lag time and T_{50\%} of 185 and 352 minutes respectively. In accordance with published reports of gastrointestinal pH values, disintegration of Eudragit S organic-coated tablets would be expected to occur in the mid small intestine to the terminal ileum. The failure of Eudragit S (organic) coated systems to disintegrate could therefore either be due to low intraluminal pH in those subjects or poor correlation of in vitro data with in vivo performance.

Disintegration of tablets coated with Eudragit S (aqueous) occurred in the proximal to mid small intestine of 7 subjects and in the distal small intestine of the remaining subject. The observed rapid disintegration of tablets coated with Eudragit S aqueous relative to Eudragit S organic coated tablets is in line with the in vitro dissolution test results of the two variants of Eudragit S polymer, in which the dissolution of tablets coated with Eudragit S aqueous was consistently quicker than Eudragit S organic coated tablets in all test media and pH conditions. Most importantly, dissolution of tablets coated with Eudragit S aqueous was observed to occur at lower pH with a lag time and T_{50\%} in pH 7.0 Sorensen’s phosphate buffer of 70 and 130 minutes compared to 185 and 352 minutes respectively for tablets coated with Eudragit S organic. Possible explanations for the observed difference in dissolution performance of the aqueous and organic solvent systems of Eudragit S have been discussed in chapter 2 and the observed difference in vivo dissolution performance can be attributed to the same factors.
Tablets coated with the newer Eudragit FS polymer disintegrated in all eight subjects on study day 1 and in six out of eight subjects on study day 2; and disintegration occurred mainly in the ileocaecal junction and ascending colon. In subject 4, the tablet resided in the stomach throughout the imaging period, remaining intact in the low pH environment of the stomach. In subject 2 on the other hand, the Eudragit FS coated tablet moved very rapidly from the terminal ileum across to the descending colon within 20 minutes of meal consumption (standard lunch); and given the reported lower pH of the colon and low fluid volume in the distal colon, it is unsurprising therefore that the tablet failed to disintegrate therein. Eudragit FS-coated tablets were administered to volunteers on two separate study days to evaluate the intra-subject variability in performance, and the results show that disintegration of the tablets are reproducible.

Disintegration of tablets coated with Eudragit FS occurred generally quicker than tablets coated with Eudragit S (organic), and this is accordance with the order of drug release observed in the in vitro dissolution studies. The essential difference between Eudragit FS and Eudragit S and their likely effect on dissolution performance have been highlighted in chapter 2. Also the disintegration of Eudragit FS coated tablets were less affected by small intestinal transit time and ICJ residence time; and unlike Eudragit S (organic) coated tablets, moderately rapid transit across the terminal ileum or short ICJ residence time as observed for subjects 3 and 8 did not prevent the tablet disintegrating in the ascending colon; and this is probably due to a combination of hydration of the film coating at pH > 6.8 such that eventual dissolution occurs more rapidly at the threshold pH medium than would Eudragit S coating and perhaps an intrinsic faster onset of dissolution of Eudragit FS.

Disintegration of Eudragit FS coated HPMC capsules have been reported to commence in more proximal regions of the gastrointestinal tract (Cole et al., 2002), compared to the observed disintegration sites in this study. However, the difference in results can be attributed to the differences in the formulation of the polymer coating system and nature of the core in the two studies. The film coating formulation used in the study by Cole et al. (2002) included triethyl citrate as plasticizer whereas no plasticizer was included in the formula for the present study; and triethyl citrate as plasticizer has been found to increase the dissolution rate of Eudragit S free films (Fadda and Basit, 2005),
and could have a similar influence on Eudragit FS. Also their core formulation comprised HPMC capsules, which is likely to contribute further to the difference in disintegration performance observed.

3.6.5 Correlation of in vitro dissolution with in vivo disintegration data

Dissolution testing of coated tablets in 0.1N HCl prior to testing in near neutral buffer media, showed that the period of exposure to acid media affected subsequent dissolution rate of the tablet in buffer media. However, no correlation was observed between gastric emptying time and subsequent disintegration time for the coated tablets. A review of other published data on transit and disintegration of dosage forms coated with pH-responsive polymer also do not show any relationship between gastric emptying time and subsequent disintegration in the intestine. This is unsurprising given the difference in residence of the tablets in different regions of varying pH along the small intestine; compared to the in vitro study in which everything was held constant other than exposure time to 0.1N HCl.

The results observed in this study do not correlate with results of in vitro dissolution tests of the polymer coated tablets in the commonly used compendial phosphate buffer media and also with published values for gastrointestinal pH (Evans et al., 1988; Raimundo et al., 1992; Press et al., 1998). In vitro dissolution of tablets coated with Eudragit S organic, Eudragit S aqueous and Eudragit FS in various media simulating the intestinal fluid pH conditions were tested and results are described in chapter 2. For instance, the T_{50%} of drug release from tablets coated with the different polymer systems in pH 7.4 Sorensen's phosphate buffer were 52, 55 and 68 minutes for Eudragit S aqueous, Eudragit FS and Eudragit S organic polymers respectively. In the physiological buffer on the other hand, drug release from the tablets was observed to be very slow, with T_{50%} in pH 7.4 Hanks buffer of 197, 245 and 305 minutes for Eudragit S aqueous, Eudragit FS and Eudragit S organic polymers respectively.

Reported values for gastrointestinal pH profile indicate that pH 7.4 is attained beyond the mid small bowel, and considered with results of in vitro dissolution testing of coated tablets in the compendial phosphate buffers at pH 7.4, it would be expected that the each of the administered tablets will disintegrate in the mid small intestine before
reaching the terminal ileum. Instead, the results show that tablets coated with Eudragit S (organic) and Eudragit FS can pass through the proximal and mid small intestine intact, with varying periods of stasis of the tablets at the ileocaecal junction. This indicates that a poor correlation exists between in vivo dissolution performance and in vitro dissolution of the polymers systems in compendial phosphate buffers. In contrast, the in vivo dissolution performance correlated relatively well with in vitro dissolution of the polymer systems in Hanks physiological buffer media, which as discussed in chapter 2, is similar in ionic composition to intestinal fluid.

Poor correlation of in vitro dissolution in compendial phosphate buffers and performance of pH-responsive solid dosage forms in humans has been reported in the past. For instance, Goto et al. (2004) studied three pH-responsive polymer coated formulations, employing pH transitions with Mcllvaine and phosphate buffers; the three formulations tested exhibited different in vitro disintegration profiles, however no significant differences were observed in the disintegration performance of the formulations in human subjects.

Poor correlation of in vitro dissolution with in vivo performance for enteric coated polymer systems is often reported with drug release in humans being several orders of magnitude slower than drug release evaluated in compendial dissolution media (Hardy et al., 1987). Such poor correlation is often attributed to difference in gastrointestinal transit or to variability in intestinal pH. Other factors that can affect the dissolution of enteric coated dosage forms in vivo include the non-uniform distribution of fluid in addition to low fluid volumes, and possibly low intraluminal pH relative to the dissolution threshold of the polymer. While all these possible variables mean that predicting the in vivo performance of an orally administered pH responsive dosage form using in vitro tests would indeed be difficult, in vitro/in vivo correlation can substantially improved by simulating the constituting electrolytes of the small intestinal fluid in the dissolution.

### 3.6.6 Effect of gastrointestinal transit on disintegration performance

Given the profile across the gastrointestinal tract as reported by many investigations (Evans et al., 1988, Fallingborg et al., 1998), it is conceivable that small intestinal
transit time, particularly the residence time of the dosage form in the terminal ileum or ileocaecal junction, could have an effect on dissolution performance of pH-responsive polymer systems. Gastrointestinal transit and disintegration data shown in tables 3.1a show that small intestinal transit of Eudragit S organic polymer system in subjects 6 and 8 were relatively rapid with relatively short periods of residence in the ICJ. Small intestinal transit was slower for subject number 2, but residence time of the tablet in the terminal ileum/ICJ was also relatively brief.

The apparent effect of ileocaecal junction residence time (ICJRT) on subsequent disintegration is to be expected on basis of known physiology of the gastrointestinal tract, as well as the dissolution behaviour of the polymer coating systems. Gastrointestinal pH is known to be highest in the ileocaecal junction, following which there is a drop in pH of up to one unit. On the other hand, it is known that tablets coated with Eudragit S organic dissolved after a lag time which was shown to be up to 130 minutes in pH 7.4 Hanks buffer. Therefore the longer the tablet is located in the ileocaecal junction with its associated high pH, the greater the chances that it would disintegrate on subsequent transit into the ascending colon, because though the pH in the ascending colon is below the dissolution threshold of the polymer, dissolution of the polymer coating would have commenced following the significant delay in the ileocaecal junction; thus leading to disintegration of the coating in the ascending colon. For tablets that move rapidly through the ileocaecal junction and into the ascending colon, the lower pH therein would be too low for dissolution of the polymer coating; hence the possible reason for failure of the two tablets coated with Eudragit S organic to disintegrate following relative short ICJRT. For tablets coated with Eudragit FS on the other hand, short residence time in the ileocaecal junction did not adversely affect subsequent drug release in the ascending colon as many more tablets disintegrated in the colon, including some subject in whom upper small intestinal transit and ileocaecal junction residence time was relatively rapid.

The effect of transit through the lower small intestine would be even greater in patients with ulcerative colitis, as the pH in the caecum and right colon of these patients have been shown to be lower than in healthy subjects (Raimundo et al., 1992; Fallingborg et al., 1993). The dissolution behaviour of Eudragit FS therefore shows some promise as a drug delivery carrier system in the therapy of ulcerative colitis and Crohn's colitis.
3.7 Conclusions

Tablets coated with Eudragit S aqueous dispersion disintegrated prematurely in the proximal to mid small intestine, probably owing to the preparation process of the aqueous dispersion, and which may have led to impaired enteric function compared to the parent polymer.

Tablets coated with Eudragit S organic solution failed to disintegrate in some volunteers although all the tablets reached the colon at the end of imaging. By sub-categorising small intestinal transit into upper small intestinal transit and ileocaecal junction residence time, an association was evident that ileocaecal junction residence time of those tablets that failed to disintegrate was more rapid compared to the other tablets. While it can be postulated therefore that the disintegration performance of Eudragit S organic polymer coated systems is influenced by ileocaecal junction residence time, it must be said however, that there are other factors that could also affect disintegration performance of a pH-responsive polymer system and these include intestinal fluid volume and luminal pH. However it is possible that other factors such as low pH in the small intestine or colon could have contributed to the observed failure of the tablets to disintegrate.

Eudragit FS coated tablets disintegrated in 14 out of the 16 administrations and the site of disintegration was mostly at the ileocaecal junction or ascending colon. Furthermore, Eudragit FS-coated tablets showed consistent intra-subject dissolution, from which it may be inferred albeit cautiously that day-to-day variability does not adversely affect the dissolution performance of this newer polymer system.

The data shown also indicate that aside from variability in pH, the dissolution performance variability of Eudragit S-coated dosage forms are likely to be affected by intestinal transit, and particularly transit across the ileocaecal junction.

It has been shown that in vivo dissolution performance of pH-responsive Eudragit polymers correlate well with in vitro dissolution in Hank’s media which is similar in
ionic composition to small intestinal fluid, implying therefore that it is indeed important therefore to adequately simulate the ionic composition of intestinal fluid during in vitro testing of enteric polymer coatings.
CHAPTER 4: INVESTIGATION OF THE INFLUENCE OF IN SITU GASTROINTESTINAL PH AND FOOD ON THE PERFORMANCE OF PH RESPONSIVE POLYMER SYSTEMS
4.1 Introduction

The dissolution performance of three pH responsive poly(meth)acrylate polymer systems was investigated in healthy subjects and according to results, described in chapter 3, the tablets coated with Eudragit S organic failed to disintegrate in three out of eight volunteers. Although all the tablets which failed to disintegrate had reached the ascending colon, there was some evidence that transit behaviour of the dosage form, particularly the residence time in the ileo-caecal junction (ileocaecal junction residence time, ICJRT), influenced the time and site of disintegration of the coated tablets. It is likely that the observed effect of intestinal transit on disintegration performance is due to the pH gradient along the gastrointestinal tract. There have been similar reports of considerable variability in performance of dosage forms based on Eudragit S organic, but the proffered explanations are equally speculative due to a lack of fundamental data about in situ factors affecting in vivo performance in man.

There are also some extrinsic factors which could affect the performance of pH-responsive dosage forms, one of which is the feed status of subject at time of ingestion of dosage form. The effect of food on dissolution of a proprietary Eudragit S coated tablet - Asacol®MR, has been investigated (Wilding, 1999), and results showed that time of disintegration as well as location in the gastrointestinal tract at which disintegration occur varied in the fasted and fed states. However, in vitro dissolution tests of Eudragit S coated tablets in media simulating the fed state showed similar dissolution performance of Eudragit S polymer systems in simulated fasted and fed dissolution media. Rudolph et al. (2001) also reported that there was no difference in dissolution of Eudragit S (aqueous and organic) and Eudragit FS polymer systems. However, it is not known whether the effect of food on disintegration performance is due to an effect on intestinal pH or transit behaviour.

Although the results described in chapter 3 showed that dissolution of Eudragit S was more variable in comparison to Eudragit FS, which taken together with many other reports of inconsistent performance of Eudragit S would prompt the same conclusion as Ashford et al. (1993b) that pH cannot be used to reliably target drug release to the colon; the importance of Eudragit S cannot be overlooked. Asacol® MR which is essentially a tablet formulation of mesalazine coated with Eudragit S is currently the
most prescribed orally administered mesalazine formulation, and currently has a 35% market share of all oral medicinal products prescribed in the therapy of inflammatory bowel disease; and it is pertinent to mention that inflammatory bowel disease constitute the only established therapeutic target for drug delivery to the colon. Also given that the pH-responsive approach to ileo-colonic drug delivery will continue to appeal to the pharmaceutical industry, it is likely that other products will be developed based on similar formulation principles as Asacol MR utilising Eudragit S as carrier materials.

Interestingly, given the long established history of Eudragit S formulations and the extent of use in clinical practice, of Asacol® MR and other pH responsive systems, there are still gaps in fundamental knowledge of the behaviour of these pH sensitive systems. For instance, although the basic principle for drug release from such systems is the response to pH, the influence of in situ gastrointestinal pH on performance and in vivo dissolution threshold of the polymer systems have not been definitely proven. Therefore assumptions that variable performance is caused by variability in intestinal pH, are made on the basis of existing information on typical gastrointestinal pH profile in healthy or diseased subjects.

The aim of the work described in this chapter was therefore, to investigate the relationship between in situ gastrointestinal pH and feed status on the in vivo performance of Eudragit S organic polymer systems.

4.2 Gastrointestinal pH

The gastrointestinal pH profile has been outlined in section 1.3.2. Gastrointestinal pH has been measured in the past for reasons of the perceived role of intraluminal pH as an indicator of disease states such as cystic fibrosis (Youngberg et al., 1984) and colonic neoplasia (Thornton, 1981) with investigations serving as epidemiological tools. Measurement of oesophageal and/or gastric pH is directly relevant in the diagnosis of gastro-oesophageal reflux disease and dyspepsia and constitutes perhaps the most common reason for in situ pH measurement in man, with a number of proprietary devices for ambulatory oesophageal or gastric pH monitoring (Branicki et al., 1982). pH in the small intestine and colon is also considered as indicative of active
disease in ulcerative colitis patients (Fallingborg et al., 1993), and more recently, there has been a lot of interest in the changes that occur in pathological conditions of the small intestine and how these may affect the performance of pH-responsive dosage forms (Fallingborg et al., 1993; Press et al., 1998, Ewe et al., 1999; Nugent et al., 2000).

The methods that have been applied to investigate pH of the intestinal lumen include: in vitro pH measurement of aspirated contents, pH catheters technique and the catheterless pH electrode commonly referred to as radiotelemetry technique.

4.2.1 In vitro method

Earlier investigations of gastrointestinal pH involved the aspiration of fluids from different segments of the gastrointestinal tract and in vitro measurement of the aspirated fluid under controlled conditions using standard glass pH electrodes. Although this approach has largely been superseded by more elegant and accurate methods, it is still used (Lindhal et al., 1997, Kalantzi et al., 2006), perhaps due to the high cost of the radiotelemetry system and perhaps also due to anecdotal evidence of technical difficulties with current pH systems. Other in vitro methods include pH measurements from organ bath preparations of resected gastrointestinal specimens and biopsies (McNeill et al., 1987). The major disadvantage of the in vitro techniques is that measurement is not in situ but rather of pooled samples and hence values can only be extrapolated with caution. For instance, Lindahl et al., 1997 found unusually high pH measurements from aspirated gastric fluid which was attributed by the authors to possible sampling of bolus of swallowed saliva.

4.2.2 pH catheter method

pH catheters usually consist of pH electrodes mounted at specific intervals along the axis of a tube which is lowered through the mouth or nasal passage and can be manipulated to any position in the intestine up to the ascending colon. The position of the tube and mounted electrodes is usually confirmed by fluoroscopy and the measured pH data is stored by a digitrapper connected to the catheter. One such system is the Bravo Digitrapper pH catheter (Medtronic Inc., USA). A variant of the pH catheter
method comprises a pH electrode connected to wires and which is lowered intranasally or orally into the region of interest. The wires are taped to the face of the subject to hold the electrode in place at the desired section of the gastrointestinal tract and are connected to a data logger which may be worn by the volunteer.

The advantage of this technique is that it can provide simultaneous continuous measurement at different locations in the intestine. Potential disadvantages however include the discomfort caused to the patients or subjects, and the fact that insertion of the tubes could affect gastrointestinal secretions which may in turn lead to a change in luminal pH.

4.2.3 pH sensitive radiotelemetry method

This approach involves the use of pH sensitive devices which comprise a pH and reference electrodes as well as radiofrequency transmitters encased in an ingestible capsule body. The capsules are usually of a size that is suitable for oral ingestion by subjects to enable in situ measurement of luminal pH as the capsule transits through the gastrointestinal tract. The measured pH is transmitted as radio signal to an external receiver unit or detector.

The major attraction of the pH sensitive radiotelemetry capsules is that it is non-invasive and so unlikely to contribute to artefacts in pH readings as would be the case with nasogastric pH catheters for instance. From a pharmaceutical point of view however, this approach to pH measurement provides a valuable tool for applications such as transit studies and most relevantly to pH responsive dosage forms, the capsule can actually simulate the transit behaviour of an orally administered dosage form and thus measure the in situ pH environment encountered by the dosage forms along the gastrointestinal tract.

Several radiotelemetry capsules have been applied in measurement of gastrointestinal pH (Kunz et al., 1971; Meldrum et al., 1972; Bown et al., 1974), but many of these had problems associated with drift in measured pH, data loss due to directional nature of the radio signals and short battery life. There are two commonly used radiotelemetry pH capsules that have been applied in ‘free fall’ measurement of gastrointestinal pH:
Heidelberg pH capsules (Noeller, 1962; Heidelberg Inc, New York, USA) and the remote control pH sensitive radiocapsule (Colson et al., 1981; Remote Control Systems, London).

The Heidelberg system pH capsule measures approximately 7.1 mm x 15.4 mm and comprises of a polyacrylate capsule body enclosing a pH sensitive and reference electrodes, internal battery and a radiotransmitter (figure 4.3). It however has a short battery life (approximately 6 hours) and so has generally been used only for the monitoring of gastric and small intestinal pH (Youngberg et al., 1984; Dressman et al., 1990; Russell et al., 1993). There are two models of the Heidelberg in current use: a previous model which comprised a detector belt that was worn by subjects and which was hard wired directly to the receiver unit. The system has since been updated and now consists of a pH sensitive capsule, wireless transceiver enclosed in a medallion worn by the subject and which digitises the pH data and transmits it to the computer system. The Heidelberg pH capsule system is primarily a device marketed for measuring gastric and upper intestinal pH hence the short battery life, and is therefore unsuitable for measurement of whole gut pH.

The remote control pH capsule comprises of a 26 x 7.6 mm glass capsule enclosing a reference and pH sensitive glass electrode, internal mercury battery and a radio transmitter. The pH signal is transmitted every 6 – 60 seconds and is detected by an aerial band worn by the subject around the abdomen and which is connected to a portable solid state data recorder (Colson et al., 1981). The Remote Control Systems pH capsule had a much longer battery life of approximately 60-90 days and unlike the Heidelberg system therefore, was suited for whole gut pH measurement.

There are some problems that are generic to radiotelemetry pH capsule systems such as:

I. Identifying the position of the capsule in the gastrointestinal tract in ‘free fall’ mode. Although knowledge about the marked pH change between the stomach and small intestine can be used to decide whether the capsule is in either region, it is difficult thereafter to detect the position of the capsule in the intestine. Investigators have thus used various methods for this purpose, including the use of a hand held signal detector to detect the position of
strongest signal using an anatomical reference map of the abdomen (Evans et al., 1988) and using tether of fixed length (Dressman et al., 1990). A more robust method however is to radiolabel the capsule and visualise its transit by gamma scintigraphy (Hardy et al., 1987).

II. Data loss is a common problem with radiotelemetry pH and has been observed to be as much as 75% in some investigations (Evans et al., 1988; Press et al., 1998). This problem is believed to be due to a unidirectional nature of the radio signals.

III. A drift in measured pH is a common problem of pH capsule systems and it is good practice to recover the capsules at the end of the study to recalibrate it and correct for drift (Wise et al., 2004).

More importantly, production of the Remote control pH capsule system has since been discontinued. A new pH-sensitive radiotelemetry capsule – Bravo pH system (Medtronics Inc., USA) has been recently introduced. Bravo pH system is a wireless pH capsule and system monitor that is marketed and used for study of oesophageal reflux episodes by suction attachment to the oesophageal mucosa. The radiotelemetry capsule measures the pH and transmits the data by radiotelemetry to pager sized receiver (figure 4.3) which can be worn on the waistline. The internal battery of the capsule has an effective life of five days, although the receiver software is only able to store data for two days. The Bravo pH capsule system may be suitable for use in measuring whole gut pH, although it is marketed for and has only been used so far in the measurement of oesophageal pH.

4.3 Effect of food on intestinal transit and dissolution performance of pH responsive polymer systems

Food is one of a number of extrinsic factors that can affect the performance of oral dosage forms and the influence of food on gastrointestinal transit of oral dosage forms and pH has been highlighted in chapter one. The effect of food on in vivo dissolution of Eudragit S polymer system has not been extensively investigated, although a report by Wilding (1999) showed that complete drug dissolution from a proprietary Eudragit S-coated tablet (Asacol\textsuperscript{®}MR) was faster when administered to fed subjects compared
to fasted subjects. The observed influence of feeding on dissolution of Eudragit S organic polymer system is conceivable on basis of the known effects of food on gastrointestinal physiology such as change in transit behaviour, gastrointestinal pH and ionic strength/buffer capacity of intestinal fluid.

Typical inter-individual variability in gastrointestinal transit have been highlighted following the results in chapter 3 which indicated that dissolution of pH responsive polymers could be affected by the residence time of the dosage form in the distal segment of the intestine especially at the ileocaecal junction. Small intestinal transit has been shown to display different motility patterns in the fed and fasted state as does the stomach. Transit through the small intestine in the fasted state is believed to be under the influence of the MMC with an estimated transit rate of about 4.7 cm/minute (Kerlin and Phillips, 1983). Kaus et al. (1986) measured the transit rate of non-disintegrating perspex capsules through the small intestine, and transit through the duodenum was found to be too rapid and could not be measured; whereas transit through the rest of the small intestine was in the range 4.2 - 5.6 cm per minute. Therefore given the estimated length of small intestine in man of 5-6 m, a solid dosage form is likely to arrive at the colon in just 1.5 - 2.5 hrs; but results of transit studies show that small intestinal transit time is in most cases longer, possibly as a result of varying periods of delay in transit across the ileocaecal junction as was shown by the results described in chapter 3. Feeding leads to a disruption of intestinal MMC to give a postprandial pattern of motility comprising irregular contractions similar those observed in phase II of the MMC in dogs (Code and Marlett, 1975). It is believed that the greatest flow of digesta occurs during the postprandial disruption of the MMC (Buena and Fioramonti, 1993) and propulsion through the small intestine has been reported to be most predictable in the early to mid-postprandial phase. Therefore it is conceivable that feeding can affect small intestinal transit time, which in turn would affect the disintegration performance of Eudragit S-coated dosage forms.

Protocols for investigations of dosage form performance usually consider the fed state as ingestion of the dosage form immediately after a standard meal, and the fasted state as ingestion of the dosage form after an overnight fast. The rationale for these extremes of fed and fasted states have occasionally been questioned with deviations to the sequence of eating and ingesting the dosage form. For instance, O'Reilly et al. (1987)
studied the gastric emptying of pellets administered 10 minutes before, mid way and 10 minutes after a meal, and the results were different when compared to standard fed and fasted state regimens.

Digenis et al. (1990) reported the results of a study in which enteric coated erythromycin pellets were administered to one set of volunteers in the fasted state and similarly to another group of volunteers but with a standard breakfast given 30 minutes post dose. The results showed that transit through the upper intestinal region, particularly the jejunum, was more rapid in the group of volunteers that received a meal 30 minutes post dose with disintegration of the pellets occurring in more distal regions of the small intestine compared to the completely fasted volunteers. These results were corroborated by the pharmacokinetic profile of erythromycin which showed a faster onset of drug absorption with earlier $T_{\text{max}}$ but lower $C_{\text{max}}$ which was attributed to less absorption due to shorter intestinal residence time for the group that received a meal 30 minutes post dose. This study was emphasised by the authors in a number of subsequent review articles as evidence that small intestinal time can be potentially influenced by food, contrary to earlier reports.

The objectives of the study described in this chapter include the measurement of intraluminal gastrointestinal pH in healthy volunteers using a novel radiotelemetry pH capsule (Bravo pH system), including an assessment of intra-subject variability in pH; and the correlation of measured in situ gastrointestinal pH with disintegration performance simultaneously administered Eudragit S organic coated polymer system. Furthermore, the effect of different feed status on transit behaviour and dissolution performance of Eudragit S organic polymer coated system will be measured with the tablets given in the fasted state, 30 minutes before a meal and immediately after food. Eudragit S was selected as a model enteric polymer for this study due to its clinical relevance and contrasting reports of variability in dissolution performance of the polymer system.
4.4 Materials

_Technetium-99m and Indium-111_

$^{99m}$Tc-DTPA and $^{111}$In-DTPA were both obtained daily from Amersham Radiopharmacy, Hammersmith Hospital, London. The reference activity of the nuclides were 20 MBq $^{111}$In in 0.2 mL and 400 mBq $^{99m}$Tc in 0.4 mL respectively.

_AquaCem®_

AquaCem luting cement was received as a gift from DENTSPLY DeTrey GmbH (Konstanz, Germany). AquaCem is a white powder comprising polyacrylic acid and Calcium-sodium-fluoro-phosphoro aluminium silicate blend. Aquacem is a water miscible polymer powder which is used as a dental bonding agent for the cementation of crowns and orthodontic bands, and so is safe for human application. On mixing with deionised water, a light paste is formed which dried approximately 3-5 minutes to form an impervious solid seal.

_Lactose_ was obtained from Ellis and Everand, Essex, UK. _Prednisolone_ was obtained from Sanofi-Aventis Pharma (Nomainville, France). _Polyvinyl Pyrrolidone and magnesium stearate_ were purchased from Sigma Aldrich (Poole, UK). _Glyceryl monostearate (GMS)_ was purchased as Imwitor 900® from Huls AG (Marl, Germany). _Triethyl citrate_ was obtained from Alfa chemical (Bracknell, UK). _Eudragit S 100_ was obtained from Rohm Pharma (Darmstadt, Germany). All other materials were obtained from Sigma Aldrich (Poole, UK).

4.4.1 Bravo pH system

The Bravo pH system is a novel radiotelemetry pH monitoring system and is available in a pre-packaged delivery device for attachment to oesophageal mucosa (figure 4.1). The pH capsule has an effective pH measuring range of 0.50 – 9.0 and is oblong in shape, measuring 6 x 25 mm (width x length). The capsule body is made of epoxy.
enclosing the antimony pH and reference electrodes which protrude slightly at the
distal end of the capsule, the radiofrequency transmitter and internal battery. The
capsule has a recess at the opposite end from the electrodes, which serves for applying
suction to enable attachment to the mucosa.

The pH data signals are transmitted by radiotelemetry to a pager-sized receiver (7 x 10
x 3 cm, weight 150g) worn on the waistline by the subjects, although the optimal
distance for signal transmission from the pH capsule to the receiver has been found to
be up to 3 feet. Each capsule is supplied with a unique identifier number which is
encoded on to the receiver before the study and ensures that there is no data
interference between when two subjects are in close proximity. The pH capsule
samples the pH of the media every 6 seconds but transmits the data every 12 seconds
to the receiver. The capsule simultaneously transmits the identifier code along with the
pH data thus ensuring that the pH data is received only by the receiver unit that has
been encoded with the identifier number. This aspect of the study was validated before
the study to ensure that two pH studies can be conducted simultaneously without
interference. Two capsules were encoded onto separate receivers and then placed side
by side in media of different pH to detect any interference between transmitted data,
and no data interference was observed.

Bravo pH system has until now only been used in the monitoring of oesophageal pH
by attachment to the mucosa (Pandolfino et al., 2005). The capsule is supplied in an
integral delivery device (figure 4.1) which consists of an end housing containing the
capsule and a tubular silicone coated flexible tube connecting the end housing to the
handle of the delivery device which is designed for oral or nasal placement. A well is
located on the superior-lateral aspect of the capsule and by applying suction of up to
600 mmHg via the handle, mucosal tissue is sucked into the well which is pinned
down by the activation button. The activation button also serves to release the capsule
from the housing. The capsule transmits data in this mode and falls off with time and is
passed out in faeces.

However, the aim of this study was to monitor whole gut pH during gastrointestinal
transit of the capsule (free fall mode) and at the same time monitor the transit of the pH
capsule by gamma scintigraphy, which involved incorporation of radiolabel into the
capsule. As this was a novel application of the Bravo pH system, involving the detachment and swallowing of the capsule by volunteers, it was necessary to validate every aspect of the study to ensure suitability for purpose.

The capsule was swallowed by one subject to test the feasibility of monitoring whole gut pH by the system. This study showed that data transmission by the capsule was adequate for the intended purpose with minimal data loss. An in vitro study was also carried out to test compatibility of electronic circuitries of the pH capsule and gamma camera, and the results showed that the pH capsule could be radiolabelled and visualised by gamma scintigraphy with no interference with transmitted pH data.

The pH receivers, calibration buffers, calibration modules and computer system pre-installed with Polygram Net® Software for processing of the data were kindly donated by Synectics Ltd, Herts., UK.

**Figure 4-1** Bravo pH capsule and integral delivery system for intranasal or oral placement and attachment of capsule to oesophageal and mucosa Close-up view of capsule attachment to the delivery device
Figure 4-2 Bravo pH capsule detached from delivery device

Reference electrode

pH probe

transmitter

battery

Figure 4-3 Picture of Bravo pH receiver and pH sensitive capsule along side the Heidelberg pH capsule (orange capsule with black cap)
4.5 Methods

4.5.1 Manufacture and radiolabelling of Eudragit S organic coated tablets

Rapidly disintegrating tablets were fabricated and film-coated with Eudragit S dissolved in 96% ethanol in accordance with the methods and process controls described in chapter 2. Tablets coated with Eudragit S organic were radiolabelled with $^{99m}$Tc on the morning of the study using the methods already described in chapter 3. The nominal activity of the tablets at time of administration was 3 MBq.

4.5.2 Radiolabelling of pH capsule

The capsules were received still attached to the delivery device as shown in figure 4.1, with the electrodes immersed in saline solution and were detached from the delivery device by pressing down and turning the activator button. The pH capsules were then radiolabelled by adaptation of the method previously described in chapter 3. $^{111}$Indium-DTPA was added to a small quantity of lactose and dried in the oven. The dried radiolabelled lactose was pulverised and the required activity filled into the inbuilt well located at one end of the capsule. The well was then sealed with bone cement paste. The electrodes of the detached capsules were left immersed in the saline solution during radiolabelling. The radiolabelling process was validated and did not affect the accuracy of pH data acquisition and transmission.

4.5.3 Calibration of the pH capsule

The capsule was calibrated immediately before commencement of the study using pH 1.07 and 7.01 calibration buffers in accordance with the supplied instructions for the pH capsules. During calibration the identifier number on each capsule was encoded onto the pH receiver.

4.5.4 Study protocol

The study protocol was submitted to and approved by the Committee on Ethics of Human Research of the East London and City Health Authority. Approval for
administration of radioactive substance was granted by the Administration of Radioactive Substances Advisory Committee (ARSAC), Department of Health, London. The licensee for administration of radioactive substances was Dr. Neil Garvie, Consultant Radiologist, Radioisotope Unit, Royal London Hospital. The study was conducted in accordance with the declarations of Helsinki (1965) and Tokyo (1975) and Venice (1983) revisions.

Eight healthy adult male volunteers (age 22 – 34 years) took part in the study. Health screening was carried out by means of a health questionnaire and all the volunteers declared themselves healthy with no acute or chronic medical condition, and none was taking any medications. Each volunteer received a written ‘Invitation to participate’ clearly stating the aims of the study and the inclusion and exclusion criteria for participation in the study; and a written informed consent was obtained prior to the study.

Each volunteer completed three study days, receiving in randomised order, the following treatments with at least a 7 day wash-out period between treatments:

**Treatment 1: Fasted**

Eudragit S organic coated tablet and Bravo pH capsule were ingested following an overnight fast.

**Treatment 2: Pre-feed**

Eudragit S organic coated tablet and Bravo pH capsule were ingested after an overnight fast followed by a standard breakfast 30 minutes post dose.

**Treatment 3: Fed**

Eudragit S coated tablet was ingested immediately after a standard breakfast.

The standard breakfast ingested in the pre-feed and fed states comprised the following: 30g cornflakes, 100 mL semi-skimmed milk, 2 slices of toasted brown bread, 5g margarine and 150 mL orange juice.
4.5.5 Intra-subject variability in pH

The pH capsule was administered on two different occasions following an overnight fast to one volunteer in order to provide an indication of intra-subject variability in measured pH. Furthermore, administration of the capsule on the fasted and pre-feed study days would also provide general indication of intra-subject variability of measured gastrointestinal pH.

The study was conducted at the Wingate Institute for NeuroGastroenterology, Royal London Hospital, Whitechapel Road, London. Volunteers were requested to observe an overnight fast for at least 12 hours before the study. The study was commenced at 09.00 hours and on arrival each morning, adherence to the fasting schedule was confirmed with each volunteer, then a sealed point source of 0.5 MBq $^{99}$Tc was taped to the most lateral part of the lower costal margin, and used for correction of posture between imaging and as anatomical reference markers. Volunteers swallowed the tablet and pH capsule with 150 mL of water and the pH monitoring was commenced once the capsule was swallowed. All the capsules were easily swallowed by the patients as confirmed by the registered low pH corresponding to the known pH of the stomach.

Images were acquired using a single head camera, model GE Maxicamera 400AC, fitted with a medium energy parallel hole collimator. The energy windows were set at 126-150 KeV for $^{99m}$Tc and 221-274 KeV for $^{111}$In, permitting the two radioactive sources to be detected simultaneously and visualised on a dual display monitor. Sequential static anterior images were acquired over a 1 minute period at 10 minutes intervals for up to 12 hours, with volunteers standing in front of the detector. Acquired images were displayed on a dual display unit with $^{99m}$Tc and $^{111}$In appearing in separate displays. The images were stored on an online computer hard drive for subsequent image analysis after the study.

Volunteers were permitted to move away from the camera between imaging but were required to remain in an upright position and not undergo any form of physical exertion. The pH receivers were also worn on the waistline though effective detection range had already been confirmed to be up to 3 feet apart. With exception of the pre-
feed treatment, volunteers continued to fast until a standard lunch comprising ham and cheese or chicken sandwich, a pack of crisps and orange juice was provided approximately 4 hours post dose. Water was freely available after lunch. Events during the imaging study for instance image acquisition time; timing and details of meals consumed were recorded in a study events log. Each administered capsule is single-use only but volunteers were requested to recover the capsule from the faeces where possible. Recovered capsules were washed and then recalibrated at pH 1.07 and 7.01 to assess data drift during the study.

4.6 Results and discussion

4.6.1 Gastrointestinal transit and disintegration performance

The gastrointestinal transit and the site and time of disintegration of the coated tablets observed for each arm of the study are shown in tables 4.1 – 4.3.
Table 4-1 Gastrointestinal transit and disintegration performance of Eudragit S coated tablets: Treatment 1 (Fasted).

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>GET (minutes)</th>
<th>USITT (minutes)</th>
<th>ICJRT (minutes)</th>
<th>CAT (minutes)</th>
<th>DT (minutes)</th>
<th>LOCATION</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>68</td>
<td>99</td>
<td>81</td>
<td>-</td>
<td>248</td>
<td>ICJ</td>
</tr>
<tr>
<td>2</td>
<td>36</td>
<td>143</td>
<td>10</td>
<td>179</td>
<td>220</td>
<td>AC</td>
</tr>
<tr>
<td>3</td>
<td>79</td>
<td>107</td>
<td>88</td>
<td>266</td>
<td>462</td>
<td>AC</td>
</tr>
<tr>
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<td>38</td>
<td>64</td>
<td>199</td>
<td>300</td>
<td>440</td>
<td>AC</td>
</tr>
<tr>
<td>5</td>
<td>15</td>
<td>155</td>
<td>43</td>
<td>213</td>
<td>Intact</td>
<td>AC</td>
</tr>
<tr>
<td>6</td>
<td>164</td>
<td>97</td>
<td>83</td>
<td>286</td>
<td>444</td>
<td>AC</td>
</tr>
<tr>
<td>7</td>
<td>64</td>
<td>193</td>
<td>10</td>
<td>257</td>
<td>292</td>
<td>AC</td>
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<tr>
<td>8</td>
<td>43</td>
<td>251</td>
<td>76</td>
<td>370</td>
<td>515</td>
<td>AC</td>
</tr>
<tr>
<td>MEAN ± STDEV</td>
<td>63 ± 46</td>
<td>139 ± 61</td>
<td>74 ± 60</td>
<td>278 ± 71</td>
<td>374 ± 117</td>
<td></td>
</tr>
</tbody>
</table>

(GET Gastric emptying time; CAT caecal arrival time; USITT upper small intestinal transit time; ICJRT ileocaecal junction residence time; DT disintegration time; SD site of disintegration or location of intact tablets at time of final image; AC ascending colon; ICJ ileocaecal junction).
Table 4-2 Gastrointestinal transit and disintegration performance of Eudragit S coated tablets: treatment 2 (Pre-feed).

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>GET (minutes)</th>
<th>USITT (minutes)</th>
<th>ICJRT (minutes)</th>
<th>CAT (minutes)</th>
<th>DT (minutes)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
<tr>
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<td>132</td>
<td>-</td>
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<tr>
<td>2</td>
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<td>57</td>
<td>239</td>
<td>-</td>
<td>296</td>
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<tr>
<td>3</td>
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<td>180</td>
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<td>461</td>
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<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>Intact</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>16</td>
<td>119</td>
<td>10</td>
<td>145</td>
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<td>AC</td>
</tr>
<tr>
<td>6</td>
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<td>26</td>
<td>399</td>
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<td>AC</td>
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<tr>
<td>7</td>
<td>37</td>
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<td>202</td>
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<tr>
<td>8</td>
<td>54</td>
<td>71</td>
<td>140</td>
<td>-</td>
<td>211</td>
<td>ICJ</td>
</tr>
</tbody>
</table>

**MEAN ± STDEV** 100 ± 109 106 ± 43 95 ± 81 354 ± 181 229 ± 38

(GET Gastric emptying time; CAT caecal arrival time; USITT upper small intestinal transit time; ICJRT ileocaecal junction residence time; DT disintegration time; SD site of disintegration or location of intact tablets at time of final image; AC ascending colon; ICJ ileocaecal junction)
### Table 4-3 Gastrointestinal transit and disintegration performance of Eudragit S coated tablets: treatment 3 (Fed).

<table>
<thead>
<tr>
<th>SUBJECT</th>
<th>GET (minutes)</th>
<th>USITT (minutes)</th>
<th>ICJRT (minutes)</th>
<th>CAT (minutes)</th>
<th>DT (minutes)</th>
<th>Location</th>
</tr>
</thead>
<tbody>
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<td>55</td>
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<td>117</td>
<td>ICJ</td>
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<td>-</td>
<td>117</td>
<td>ICJ</td>
</tr>
<tr>
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<td>Intact</td>
<td>AC</td>
</tr>
<tr>
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<td>83</td>
<td>355</td>
<td>Intact</td>
<td>AC</td>
</tr>
<tr>
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<td>205</td>
<td>155</td>
<td>49</td>
<td>409</td>
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</tr>
<tr>
<td>7</td>
<td>119</td>
<td>84</td>
<td>97</td>
<td>300</td>
<td>Intact</td>
<td>AC</td>
</tr>
<tr>
<td>8</td>
<td>219</td>
<td>258</td>
<td>-</td>
<td>-</td>
<td>258</td>
<td>TI</td>
</tr>
</tbody>
</table>

**MEAN ± STDEV**

173 ± 94 145 ± 78 81 ± 40 389 ± 82 208 ± 88

(GET Gastric emptying time; CAT caecal arrival time; USITT upper small intestinal transit time; ICJRT ileocaecal junction residence time; DT disintegration time; SD site of disintegration or location of intact tablets at time of final image; AC ascending colon; ICJ ileocaecal junction; TI Terminal ileum)
The mean gastric emptying times were 63 ± 46 (range 15 – 164), 99 ± 109 (16 – 245) and 173 ± 94 (range 20 – 273) minutes for the fasted, pre-feed and fed states respectively.

Gastric emptying of the tablets administered in the fasted state mostly occurred within the expected range of 0 – 120 minutes for dosage forms administered in the fasted state and cleared under the influence of phase III MMC contractions (Davis et al., 1986; Cole et al., 2002; Ofori-Kwakye et al., 2004), with the exception of subject 6 who showed a gastric emptying time of 164 minutes.

Gastric emptying of tablets administered in the pre-feed state was highly variable due to the fact that some tablets had emptied before the meal was given, with subsequent delay in gastric emptying of some of the remaining tablets. Following the administration of food, gastric emptying still occurred within 30 minutes in 3 subjects in the pre-feed treatment and in 1 subject in the fed treatment, while gastric emptying of all the other tablets were delayed for approximately 2 – 4 hours, possibly corresponding to the time it takes to return of the inter-digestive cycle. The results are therefore in agreement with the concept of delayed gastric emptying of solid dosage forms during the postprandial motility pattern, and also demonstrate the random nature of the gastric emptying process. Gastric emptying was observed in 3 subjects soon after food was consumed and this is in line with other reports, indicating that gastric emptying of large solid dosage forms from a fed stomach is largely a random process (Davis et al., 1984; Davis et al., 1988; Coupe et al., 1991a).

It is important to note that the shape of the dosage form could affect gastric emptying, particularly through a contracted pylorus in the fed state. There are also differences in the kind of meals ingested, and these make it difficult to compare results directly. The various arguments about gastric emptying of dosage forms in the fed and fasted states, and other extrinsic factors that affect gastric emptying time have been outlined in chapter 1.

O’Reilly et al. (1987) reported that pellets administered 10 minutes prior to a meal emptied faster than when administered mid way through or 10 minutes after a meal. However, the difference in the results of this study with that of O’Reilly et al. is
probably due to the known different emptying patterns of multiparticulates and larger single solid dosage forms. Brown et al. (1992) reported that single solid dosage forms usually reside at the base of the greater curvature of the stomach post administration until emptied by the phase II or III contractions of the MMC, and hence subsequent feeding as was the case in this study is likely to lead to more random gastric emptying pattern. In their study, they showed by ultrasonography that administration of chickpeas with a liquid broth led to an increase in size of the greater curvature of the stomach with the antrum becoming funnel-shaped. The chick peas initially resided at the base of the greater curvature and as gastric emptying of the broth progressed, the antrum reverted to a tubular conformation and the chickpeas were propelled towards the pyloric outlet by the antral contractions.

Small intestinal transit was estimated as the difference between caecal arrival time and gastric emptying time; hence it was only possible to estimate small intestinal transit time for intact tablets reaching the colon at end of imaging. The estimated small intestinal transit times are 215 ± 74, 178 ± 45, and 216 ± 66 minutes for tablets administered in the fasted, pre-feed and fed conditions respectively. There appears to be a difference in small intestinal transit of tablets administered in the pre-feed conditions in comparison to the standard fed and fasted, but on the other hand the values are in agreement with the commonly reported population mean of 3-4 ± 1 hour (Davis et al., 1986). In line with the proposal in chapter 3 with regard to format for presentation of intestinal transit data, the USITT and ICJRT have been derived and are shown in tables 4.1 – 4.3 respectively. The data indicate that transit through the upper small intestine (USITT) was more rapid in the pre-feed state which was 106 ± 43 minutes compared to 139 ± 61 and 145 ± 78 minutes for fasted and fed states respectively. There was considerable delay in transit across the ileocaecal junction in all three treatments, although the delay was greater in the pre-feed treatment than the fed and fasted states.
Figure 4-4 Set of scintiscans for subject 6 showing transit of Eudragit S coated tablet and pH capsule through the gastrointestinal tract and subsequent disintegration in the colon.
Figure 4-5: Representative scintiscans (subject 3) showing the transit of Eudragit S coated tablet and pH capsule through the gastrointestinal tract and subsequent disintegration of coated tablets in the colon.
There was no defined trend in small intestinal transit between the treatments. Although estimated small intestinal transit time would indicate a slightly faster transit for tablets administered in the pre-feed conditions, the transit data show that the tablets which had emptied from the stomach before or soon after feeding mostly disintegrated in the colon. During the study, there was some evidence of increased movement of the tablets during scanning, but it would appear from subsequent image analysis that the increased motility did not result in significant aboral transit. Digenis et al. (1990) reported that increased transit was observed for the tablets which had emptied at time of meal administration. Similarly in this study, transit through the upper small intestine was slightly more rapid in those subjects where the tablet had emptied before food, although this was counteracted by a longer delay at the ileo-caecal junction.

Tablet disintegration occurred in 7 out of 8 subjects in the fasted state, but was more variable, occurring in 5 out of 8 subjects in the pre-feed and fed states. Mean disintegration times were 374 ± 117, 229 ± 38 and 208 ± 88 for the fasted, pre-feed and fed states respectively. Disintegration of coated tablets administered in the fasted state was observed in the ascending colon in 6 out of 7 subjects. Disintegration site for tablets administered in the fed and pre-feed conditions was mainly at the ileocaecal junction. The more rapid disintegration of tablets in the fed state is in line with the findings of Wilding (1999) who also reported a more rapid disintegration time for a proprietary Eudragit S coated tablet (Asacol®) when administered with food compared to fasted condition.

The tablets in the fed and pre-feed states had a significantly delayed gastric emptying and most also disintegrated after a shorter residence time in the small intestine. The possible mechanisms for the observed rapid disintegration include elevated gastric pH with delayed gastric emptying in the fed and pre-feed states, and effect of increased viscosity of intestinal contents and motility. It would be recalled that size reduction of gastric contents is brought about by the postprandial antral contractions and the propulsion and retropulsion of gastric contents to and from the pyloric sphincter. It is conceivable therefore that the protracted gastric residence of the tablets in the fed and pre-feed states could have contributed in some measure to a weakening effect on the polymer coating.
Other factors that could lead to a more rapid disintegration of the tablets include the increase in buffer capacity and osmolality which occur after food. It has been reported that the resting buffer capacity in human intestinal fluid is about 5.6 mmol/L/pH to about 18 – 30 mmol/L/pH after food (Kalantzi et al., 2006; Perez et al., 2006). This is expected given that the buffer salt in human fluid is bicarbonate which is known to have a low buffering capacity (Perry and Dempsey, 1974). In the discussion of the results of in vitro dissolution testing of pH responsive polymers that the higher buffer capacity of the phosphate buffers contributed to a more rapid drug release from the coated tablets. Hence the possible increase in buffer capacity of the intestinal fluid arising from ingested food could have also resulted in a faster disintegration time of the coated tablet. However, the buffer capacity of the small intestine is reported to be tightly controlled by pancreatic secretions and so is likely to return to basal values quickly (Hörter and Dressman, 2001). Unlike the return of pH to basal values after elevation by food, no information was found on return of buffer capacity to basal value after food, although this is likely to vary with the constitution of the meal. The meal that was given in this study is a light breakfast meal and it is not thought that it can sustain the buffer capacity at a higher level for long. Although the result for pH is discussed ahead, suffice to mention at this point that a sustained rise in gastric pH was not observed after food.

Other possible reasons for the more rapid disintegration include the gastric and intestinal secretions accompanying the fed state; and although drug release from the coated tablets was found to be similar in media simulating the fed and fasted states, it could well be a case of inadequate simulation of the fed state secretions in the tested dissolution media. However, the failure of some tablets to disintegrate shows that the dissolution of Eudragit S polymer systems could be influenced by several factors in vivo.

4.6.2 Gastrointestinal pH

The gastrointestinal transit data for the pH capsules administered are shown in tables 4.4 and 4.5.
Table 4-4 Gastrointestinal transit of pH capsule (Pre-feed treatment)

<table>
<thead>
<tr>
<th>Subject</th>
<th>GET (minutes)</th>
<th>USITT (minutes)</th>
<th>ICJRT</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>80</td>
<td>280</td>
<td>170</td>
<td>530</td>
</tr>
<tr>
<td>2</td>
<td>26</td>
<td>99</td>
<td>355</td>
<td>480</td>
</tr>
<tr>
<td>3</td>
<td>301</td>
<td>76</td>
<td>235</td>
<td>612</td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>258</td>
<td>252</td>
<td>150</td>
<td>660</td>
</tr>
<tr>
<td>7</td>
<td>34</td>
<td>175</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>264</td>
<td>138</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

Mean ± STDEV 160 ± 127 170 ± 82 227 ± 92 570 ± 81

Table 4-5 Gastrointestinal transit (minutes) of pH capsule (fasted treatment)

<table>
<thead>
<tr>
<th>Subject</th>
<th>GET (minutes)</th>
<th>USITT (minutes)</th>
<th>ICJRT</th>
<th>CAT</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>69</td>
<td>222</td>
<td>84</td>
<td>375</td>
</tr>
<tr>
<td>2</td>
<td>40</td>
<td>85</td>
<td>98</td>
<td>223</td>
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<tr>
<td>3</td>
<td>83</td>
<td>207</td>
<td>23</td>
<td>313</td>
</tr>
<tr>
<td>4</td>
<td>75</td>
<td>285</td>
<td>132</td>
<td>492</td>
</tr>
<tr>
<td>5</td>
<td>30</td>
<td>250</td>
<td>212</td>
<td>492</td>
</tr>
<tr>
<td>6</td>
<td>78</td>
<td>100</td>
<td>-</td>
<td>178</td>
</tr>
<tr>
<td>7</td>
<td>76</td>
<td>399</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>43</td>
<td>251</td>
<td>304</td>
<td>598</td>
</tr>
</tbody>
</table>

Mean ± STDEV 62 ± 21 225 ± 100 142 ± 100 381 ± 154

The mean gastric emptying time of radiotelemetry pH capsule was 66 ± 17 (range 40 – 83) minutes and 160 ± 127 (range 26 – 301) minutes in the fasted and pre-feed treatments respectively. The gastric emptying time in the fasted treatment is
broadly similar to data obtained for the 8 mm tablets in the fasted treatment and thus in line with the expected clearance time for large single dosage forms by phase III of the MMC cycle. In the pre-feed treatment, gastric emptying of the radiotelemetry capsules was considerably delayed and more variable. The capsules emptied in two volunteers just before food at 30 minutes post dose and within an hour after food in another subject. Gastric emptying of the capsule did not occur in subject 4 for the entire duration of the study but the gastrointestinal profile was still obtained as the study was set for 48 hours. The observed gastric emptying data for the radiotelemetry capsule is in line with previously reported results (Mojaverian et al., 1989; Coupe et al., 1991b).

Following gastric emptying, transit through the small intestine was normal but entry into the colon was delayed at the ileo-caecal junction, and in a number of volunteers the pH capsule did not reach the caecum at the end of imaging. Therefore it was not possible to obtain the pH of the colonic lumen in some of the volunteers. Although a period of stasis at the ileocaecal junction was also observed for the coated tablets, the stagnation of the capsule is considered a particular prolonged and could be associated with the shape of the capsule, as similar observations have been reported with other radiotelemetry capsules including the remote control capsule (Ewe et al., 1999) and Heidelberg capsule (Mojaverian et al., 1989) and a pressure sensitive radiotelemetry device (Coupe et al., 1991).

Data loss was minimal with a mean total signal loss of 5% (range: 2 – 15%). Most of the data loss occurred while the capsule was in the colon, which is to be expected given the consistency of the colonic lumen. Data loss during whole gut pH measurements is a known problem with radiotelemetry pH capsules and up to 75% data have been previously reported (Evans et al., 1988; Press et al., 1998). The causes of data loss is usually due to poor signal emission from the capsule as well as poor reception by the detector aerials owing to the directional nature of the radio frequency emissions (Branicki et al., 1982), such that the receiver have to be worn around the abdomen with an integral solid state recorder. The Bravo pH capsule and receiver system on the other hand provide were found to have an optimum data reception range of 3 feet and have an inbuilt audible data loss signal.
The measured pH data were downloaded onto computer using Polygram Net software®. The data were subsequently converted to ASCII format to allow data processing using own brand computer. The pH value in the different segment of the gastrointestinal tract was expressed as ‘mean ± standard deviation’. The segments for which data have been reported include:

I. **Stomach**: The gastric residence of the pH capsule is determined by both a sudden rise in the measured pH values as the capsule enters the more alkaline duodenum, and is also confirmed by the acquired gamma scintiscan.

II. **Small intestine**: Unlike the stomach and the colon which are discrete organs it was not possible to determine the specific location of the pH capsule in the loops of the small intestine. The methods applied by Evans et al. (1988) was adapted to divide the small intestinal residence time of the capsules into the proximal small bowel, mid small bowel and distal small bowel.

III. **Colon**: It was relatively easy to identify entry of the capsule into colon characterised by a period of stagnation at the right iliac fossa followed by an upward movement and movement across the abdomen from the right to the left side.

The full gastrointestinal pH profiles as measured by the Bravo pH capsule system following administration of the capsule in the pre-feed and fasted states are shown in tables 4.4 and 4.5, with the transit data of the capsules in tables 4.6 and 4.7. Individual pH traces are also shown for each volunteer in the fasted and pre-feed states in figures 4.6 to 4.20.
Figure 4-6 Gastrointestinal pH profile for subject 1 (fasted)
Figure 4-7 Gastrointestinal pH profile for subject 1 (pre-feed)
Figure 4-8 Gastrointestinal pH profile for subject 2 (fasted)
Figure 4-9 Gastrointestinal pH profile for subject 2 (pre-feed)
Figure 4-10 Gastrointestinal pH profile for subject 3 (fasted)
Figure 4-11 Gastrointestinal pH profile for subject 3 (pre-feed)
Figure 4-12 Gastrointestinal pH profile for subject 4 (fasted)
Figure 4-13 Gastrointestinal pH profile for subject 4 (Pre-feed)
Figure 4-14 Gastrointestinal pH profile for subject 5 (fasted)
Figure 4-15 Gastrointestinal pH profile for subject 6 (fasted)
Figure 4-16 Gastrointestinal pH profile for subject 6 (pre-feed)
**Figure 4-17** Gastrointestinal pH profile for subject 7 (fasted)
Figure 4-18 Gastrointestinal pH profile for subject 7 (pre-feed)
Figure 4-19 Gastrointestinal pH profile for subject 8 (fasted)
Figure 4-20 Gastrointestinal pH profile for subject 8 (pre-feed)
Table 4-6 Gastrointestinal pH - fasted treatment.

<table>
<thead>
<tr>
<th>Subject</th>
<th>Stomach</th>
<th>PSB</th>
<th>MSB</th>
<th>DSB</th>
<th>Right Colon</th>
<th>Left colon</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.7 ± 0.3</td>
<td>6.5 ± 0.2</td>
<td>7.3 ± 0.4</td>
<td>7.5 ± 0.1</td>
<td>7.6 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1.8 ± 0.3</td>
<td>6.0 ± 0.4</td>
<td>7.0 ± 0.8</td>
<td>7.2 ± 0.1</td>
<td>7.1 ± 0.3</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>0.8 ± 0.3</td>
<td>6.2 ± 0.8</td>
<td>6.7 ± 0.3</td>
<td>7.6 ± 0.2</td>
<td>7.1 ± 0.15</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.3 ± 1.2</td>
<td>7 ± 0.4</td>
<td>6.3 ± 0.5</td>
<td>6.5 ± 0.2</td>
<td>6.5 ± 0.2</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>1.2 ± 0.1</td>
<td>6.4 ± 0.3</td>
<td>6.8 ± 0.2</td>
<td>7.3 ± 0.3</td>
<td>6.2 ± 0.1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>1.7 ± 0.9</td>
<td>6.8 ± 0.5</td>
<td>6.7 ± 0.72</td>
<td>7.8 ± 0.32</td>
<td>5.5 ± 0.7</td>
<td>7.78 ± 0.7</td>
</tr>
<tr>
<td>7</td>
<td>1.2 ± 0.2</td>
<td>6.7 ± 0.5</td>
<td>6.7 ± 0.7</td>
<td>7.7 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>1.1 ± 0.2</td>
<td>6.2 ± 1.2</td>
<td>6.7 ± 1.2</td>
<td>7.7 ± 0.9</td>
<td>5.8 ± 0.2</td>
<td></td>
</tr>
</tbody>
</table>

(PSB: proximal small bowel; MSB: mid small bowel; DSB: distal small bowel)
<table>
<thead>
<tr>
<th>Subject</th>
<th>pH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Stomach</td>
</tr>
<tr>
<td>1</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>1.3 ± 0.5</td>
</tr>
<tr>
<td>3</td>
<td>1.1 ± 0.7</td>
</tr>
<tr>
<td>4</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>1.5 ± 0.6</td>
</tr>
<tr>
<td>7</td>
<td>3.1 ± 1.1</td>
</tr>
<tr>
<td>8</td>
<td>1.7 ± 0.6</td>
</tr>
</tbody>
</table>

(PSB: proximal small bowel; MSB: mid small bowel; DSB: distal small bowel).
The measured pH in the stomach was in the range of 1.0 – 2.4 and 1.0 – 3.0 in the fasted and pre-feed states respectively. Meal administration only led to a transient rise in pH in some subjects due to the buffering effect of food but the pH generally remained below 4.0. Other authors have reported a median fasting pH of 1.7 (range 1.4-2.1), increasing to a median 5.0 (range 4.3-5.4) after meal ingestion for healthy subjects (Dressman et al., 1990; Russell et al 1993). The measured pH of the stomach is therefore in agreement with previously reported data. Though it is primarily dependent on acid secretion, the luminal pH of the stomach is also affected by gastric contents. Food neutralises the gastric acid leading to an increase in gastric pH, though the magnitude and duration of this change due to food ingestion is dependent on the nature of the meal; with the buffering effect of protein being higher than an isocaloric carbohydrate meal for example (Richardson et al., 1976). Also liquid meals have stronger buffering effect than mixed phase meals, though the return to basal pH is quicker due to the more rapid emptying of liquids (Richardson et al 1976). The meal used in this investigation was a light breakfast and hence it did not lead to any sustained rise in gastric pH. Also because the capsule was ingested before the meal, its resting position in the stomach would have been at the base of the greater curvature and hence would undergo little mixing with the subsequently ingested food before emptying.

Gastric emptying of the capsules is marked by a sustained steep rise in pH as the capsule enters the more alkaline lumen of the duodenum and this was usually followed by a slight dip in pH as the capsule transit distally. The peak pH attained while the capsule resides at the ileocaecal junction which is also characterised by a period of relatively stable measured pH. The measured pH values in the proximal, mid and distal small intestine are in broad agreement with other investigations of luminal pH in healthy subjects (Bown et al., 1974; Press et al., 1998; Ewe et al., 1999).

On moving across the ileocaecal junction into the caecum, a fall in measured pH was observed in some subjects, while in other volunteers the pH remained more or less the same as in the distal small bowel. Where a fall in pH was observed, the change was gradual. This is in contrast to the studies cited above which have all very sharp drop of 1 or more pH unit as the pH capsule enters the caecum. The pH then rises gradually again as the capsule moves aborally in the colon and the values in the colon were also
generally higher than was previously reported. This contrast in measured pH values could be due to the different electrodes used in the Remote control systems pH capsule and the Bravo pH system (Evans, 1993).

Feeding did not affect the measured pH valued, except in the proximal small intestine which showed a slightly lower pH profile in the pre-feed treatment compared to the fasted treatment. A similar result was observed by Kalantzi et al. (2006) on measuring the pH of samples aspirated from the duodenum in healthy subjects. This could be due to the incoming low pH chyme from the stomach.

Intra-subject variability

The gastrointestinal pH profile for one subject that ingested the pH capsule on two different occasions in the fasted state, with no intervening change in diet, are shown in figures 4-21 and 4-22. The results show that there is a significant level of intra- as well as inter-subject variability in measured pH, although this would be expected given the heterogeneous environment of the gastrointestinal tract. Hence a more important indicator therefore would be whether the pH rises up to the dissolution pH threshold of the common pH responsive polymers for ileo-colonic drug delivery.
Figure 4-21 Gastrointestinal pH profile for subject 9 (assessment of intra-subject variability)
Figure 4-22 Gastrointestinal pH profile for subject 9 (for assessment of intra-subject variability)
4.6.3 Correlation of intestinal pH with disintegration performance

Failure of pH responsive polymer coated dosage forms to disintegrate has always been attributed to low intestinal pH in the subjects relative to the dissolution pH threshold. However, there has been no investigation of this claim and the aim of this study was to investigate this link for the first time.

The pH along the gastrointestinal tract was sufficient for the dissolution of the coated tablets in most volunteers. The measured small intestinal pH in all the volunteers was generally above 7.0, while the value in the ascending colon was also found to be above 7.0 in many of the volunteers. The pH is therefore above the dissolution threshold for pH responsive polymers and where the dosage form fail to dissolve in the small intestine, the pH of the colon could prove sufficient to effect drug release, subject to other limitations in the colon. However in order to determine if pH was responsible for failure of the tablets to disintegrate in some subjects, it is necessary to consider the transit behaviour of each tablet and the measured intestinal pH for subjects in whom the tablet did not disintegrate.

Collated transit and disintegration data for the tablets and the measured intestinal pH for each volunteer are shown in tables 4-8 and 4-9. Interestingly, the tablet did not disintegrate in subject 5 for the three treatments, indicating therefore that there must be an underlying reason. The gastrointestinal profile for fasted treatment 1 show that pH was generally below 7.0 in the proximal intestine but rises to above pH 7.0 in the distal intestine. However, although the pH reading in the ileocaecal junction was steadily above pH 7.0, the transit of the tablet through this region is also fairly rapid at 43 minutes and the pH of the colon was below pH 7.0, thus probably accounting for failure of the tablet to disintegrate. In the pre-feed state, subject 5 declined to swallow the capsule, but it is seen from the transit data for the tablet that ileocaecal junction residence time was less than 10 minutes, suggesting that if the profile was similar to treatment 1, then a combination of low pH and rapid transit through the intestine can be assumed to be cause for failure of the tablets to disintegrate.
The tablet failed to disintegrate in subject 3 in the pre-feed treatment and this can also be attributed to a combined effect of pH and of relatively short residence time in the ileocaecal junction. The mean intestinal pH in subject 3 was generally below pH 7 which is considered as the threshold dissolution pH of Eudragit S, and the pH was only above 7.0 for about one hour before entry of the capsule into the caecum. If we consider the transit of the tablet however, the period of residence in this region of highest pH was only about 36 minutes. Furthermore the pH of the colon in subject 3 is also below 7 for the first hours in the ascending colon.

The other failed tablet in the pre-feed state was due to the protracted gastric residence of the tablet in subject 4 for the duration of the study. In the fed state, the tablets did not disintegrate in subjects 4 and 7 (and subject 5), but the pH capsule was not included in the fed treatment.

It can be concluded therefore that the disintegration of Eudragit S organic-coated tablets is affected by gastrointestinal media pH as well the residence time of tablets in the distal small intestine, especially at ileocaecal junction where there is distinct period of stasis for most dosage forms including multiparticulates. Thus the tablets could still dissolve in volunteers where the pH is low if the delay at the ileocaecal junction is sufficient, as was observed for volunteer 8 in the fasted treatment where the pH is below 7.0 but the tablets showed a reasonably long transit period through the small intestine, and dissolving eventually in the ascending colon. Thus for subjects in whom pH is average or below average (of pH 7.0), then transit through the distal intestine is important to the performance of polymer coated system. For subjects with sufficiently high pH however, disintegration performance was not as dependent on transit, such as was observed for subjects 2 and 7 in the fasted treatment.

It is likely that the fluid volume available for dissolution of the film coating will play a role in the performance of the dosage form, given the recent report of the existence of free fluid in pockets, mainly in the distal intestine, thus emphasising transit through the distal intestine as critical to the performance of pH polymer coated systems.
Table 4-8 Correlation of disintegration performance of Eudragit S coated tablets with gastrointestinal transit and pH (fasted treatment)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Regional pH</th>
<th>Intestinal transit of Eudragit S coated tablets (minutes)</th>
<th>Disintegration data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSB</td>
<td>MSB</td>
<td>DSB</td>
</tr>
<tr>
<td>1</td>
<td>6.5± 0.2</td>
<td>7.3 ± 0.4</td>
<td>7.5 ±0.1</td>
</tr>
<tr>
<td>2</td>
<td>6.0 ± 0.4</td>
<td>7.0 ±0.8</td>
<td>7.2 ±0.1</td>
</tr>
<tr>
<td>3</td>
<td>6.2 ± 0.8</td>
<td>6.7 ±0.3</td>
<td>7.6 ±0.2</td>
</tr>
<tr>
<td>4</td>
<td>7 ± 0.4</td>
<td>6.3 ±0.5</td>
<td>6.5 ±0.2</td>
</tr>
<tr>
<td>5</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>6.8 ± 0.5</td>
<td>6.7 ±0.72</td>
<td>7.8 ±0.32</td>
</tr>
<tr>
<td>7</td>
<td>6.7 ± 0.5</td>
<td>6.7 ±0.7</td>
<td>7.7 ±0.2</td>
</tr>
<tr>
<td>8</td>
<td>6.2 ± 1.2</td>
<td>6.7 ±1.2</td>
<td>7.7 ±0.9</td>
</tr>
</tbody>
</table>
Table 4-9 Correlation of disintegration performance of Eudragit S coated tablets with gastrointestinal transit and pH (pre-feed treatment)

<table>
<thead>
<tr>
<th>Subject</th>
<th>Regional pH</th>
<th>Intestinal transit of Eudragit S coated tablets (minutes)</th>
<th>Disintegration data</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PSB</td>
<td>MSB</td>
<td>DSB</td>
</tr>
<tr>
<td>1</td>
<td>6.3 ± 0.3</td>
<td>7 ± 0.5</td>
<td>7.7 ± 0.3</td>
</tr>
<tr>
<td>2</td>
<td>5.9 ± 0.6</td>
<td>6.6 ± 1.6</td>
<td>7.5 ± 0.6</td>
</tr>
<tr>
<td>3</td>
<td>6.2 ± 0.7</td>
<td>6.2 ± 0.8</td>
<td>6.9 ± 0.3</td>
</tr>
<tr>
<td>4</td>
<td>6.6 ± 0.4</td>
<td>7.5 ± 0.2</td>
<td>7.6 ± 0.8</td>
</tr>
<tr>
<td>5</td>
<td>6.6 ± 1.4</td>
<td>6.6 ± 1.2</td>
<td>7 ± 0.2</td>
</tr>
<tr>
<td>6</td>
<td>6.3 ± 0.5</td>
<td>6.3 ± 0.4</td>
<td>7.8 ± 0.3</td>
</tr>
<tr>
<td>7</td>
<td>6.2 ± 0.6</td>
<td>6.8 ± 0.2</td>
<td>7.1 ± 0.03</td>
</tr>
<tr>
<td>8</td>
<td>6.4 ± 0.6</td>
<td>6.6 ± 0.4</td>
<td>7.6 ± 0.2</td>
</tr>
</tbody>
</table>

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It is pertinent to point out that the pH capsule in most instances did not travel together with the tablets. Nevertheless the temporal difference in their transit is not expected to lead to a change in the measured pH along the gastrointestinal tract. Another potential limitation in the measured pH is that the pH capsule measured the pH of the fluid or luminal content surrounding the pH sensing electrodes, therefore extrapolation of measured values to the entire region of the gastrointestinal tract is based on an assumption of homogeneity in luminal contents. This is more so in the colon where the consistency of the luminal contents and less motor activity could lead to a heterogeneous lumen, which could give false reading.

4.7 Conclusion

The results confirm that both the time and location of drug release from pH responsive dosage forms is influenced by the feed status, being more reproducible in the fasted state. The study also shows that meal ingestion soon after a dose affects the transit and drug release from tablets coated with Eudragit S. This study was not appropriate to measure this transit behaviour and this should be investigated further using perhaps, indigestible dosage forms. Though it is unclear how the disintegration time of Eudragit coated tablets is influenced by food, it could have practical implications relative to dosage instructions; and so requires further investigations.

This study is further evidence that the gastrointestinal pH can provide suitable trigger for drug release in the distal gut. There is significant variability in the measured pH value between individuals and within subjects on repeat administration of the pH capsule. The pH across the gastrointestinal tract is generally above the dissolution threshold of pH responsive dosage forms, suggesting that where drug release fails, this could be due to other factors that affect dissolution such as fluid volume and transit time across the gut. The study has also demonstrated that the Bravo pH capsule system is a robust and reliable system for the measurement of gastrointestinal pH.
CHAPTER 5: GENERAL DISCUSSIONS, CONCLUSIONS AND FUTURE WORK
5.1 **General discussion and conclusion**

The pH-dependent approach for drug delivery to the ileo-colonic region is the formulation concept for a number of currently approved products for therapy of inflammatory bowel disease including Asacol®MR and associated generic products. The site of drug release from dosage forms that are based on this concept are reported to vary considerably due to inherent variability in gastrointestinal pH. However, there are few published data on the fundamental in vitro and in vivo drug release from pH-responsive dosage forms.

The objectives of this study were to assess the in vitro and in vivo dissolution performance of four pH responsive polymer systems comprising the aqueous dispersion and organic solvent systems of Eudragit S and a newer polymer system Eudragit FS / Eudragit P4135, and then to investigate the relationship between in situ pH and food on the performance of Eudragit S organic as a model polymer system.

Rapidly disintegrating prednisolone tablets were fabricated and coated with Eudragit S aqueous dispersion, Eudragit S organic solvent system (ethanol), Eudragit FS aqueous dispersion and Eudragit P4135 organic solvent system respectively. Significant problems were encountered during film coating with Eudragit P4135 due to excessive tackiness, resulting in the cores sticking together during coating. Several different film coating preparations were tried as well as various adjustments to the process parameters, but the problem remained. Therefore further work with the polymer was discontinued, and the application of the polymer for film coating may be limited due to this problem of excessive thermoplasticity.

Comparative in vitro dissolution testing of the coated tablets was conducted in various media simulating the pH conditions of the intestinal tract but of different ionic composition. Tested media included compendial phosphate buffer media and a physiological buffer solution, Hanks buffer. Drug release rate was found to increase with increasing pH of the buffer solution and at pH 7.4, drug dissolution from the coated tablets was very rapid and similar for each polymer system in the compendial phosphate buffers. However, in bicarbonate buffer, a physiological media similar in ionic composition to the small intestine, drug release from the polymer systems was
found to be markedly delayed in comparison to compendial phosphate media at pH 7.4 and also differed in the following order: Eudragit S aqueous > Eudragit FS > Eudragit S organic. Incidentally, Hanks buffer was similar in ionic composition, osmolality and buffer capacity to intestinal fluid, compared to the compendial phosphate buffers which comprised simple two phase sodium or sodium/potassium buffer systems.

The disintegration performance of each polymer coated system was subsequently investigated in healthy subjects by gamma scintigraphy. Tablets coated with Eudragit S aqueous disintegrated prematurely in the proximal to mid small intestine. Tablets coated with Eudragit S organic solvent system showed variable performance with disintegration in 5 out of 8 subjects. Eudragit FS coated system which was administered on two separate occasions to assess reproducibility of performance, disintegrated in 14 out of 16 administrations. It was found also that intestinal transit of the tablets particularly through the ileocaecal junction affected dissolution performance in certain instances.

It is generally accepted that there is a poor correlation between in vitro and in vivo dissolution of pH responsive polymers, following observations that dosage forms which show rapid in vitro dissolution then subsequently dissolve in vivo with a lag of several hours while residing in the distal small intestine where the pH is up to 7.4. When predicting in vivo performance from in vitro dissolution data therefore, it is common to assume a lag time of some orders of magnitude in vivo compared to in vitro results. From the results observed in vivo however, the lag time was found to correlate well with in vitro dissolution of the tablets in bicarbonate buffer.

Due to the importance of Eudragit S organic system, being the film coating agent in a number of commercial products, and owing to the performance variability, further investigation was carried out in healthy volunteers to correlate disintegration performance of Eudragit S organic coated tablets with in situ gastrointestinal pH and also to investigate the effect of food on performance. The coated tablets were co-administered with a novel radiotelemetry pH capsule to healthy volunteers under different feed status. The disintegration time of Eudragit S-coated tablets was found to be more rapid when administered with food compared to the fasted treatment. This effect was not due to the influence of food on gastric emptying time as the values were
normalised for variable gastric emptying time. Also as opposed to the standard fed and fasted state treatment, the pre-feed treatment in which food was administered 30 minutes post dose also showed a faster disintegration time relative to the fasted treatment. Thus making it important that fasted and fed conditions are clearly defined for these polymer systems. The effect of food on disintegration performance is thought to be due to a change in buffer capacity in the intestinal lumen, which has been shown to increase significantly after food, as well as other intestinal secretions.

There was a correlation between pH and disintegration of the tablets as shown by the failure of the tablets to disintegrate in some subjects in whom the measured pH was lower than the mean pH of the other subjects. Where the pH was lower than average, transit time became very important to disintegration, and a slower intestinal transit particularly through the distal intestine would allow sufficient time for the tablet to dissolve.

Intestinal transit time of the tablets was found to have a marked influence on disintegration of pH responsive polymer systems. This effect is believed to be related to the time it takes for the polymer coating to ionise and dissolve. The tablets were observed to be delayed at the ileocaecal junction for varying periods and since the ileocaecal junction is the region of highest pH, the period for which the tablet resides there was found to affect the disintegration of the tablets subsequently or therein. Therefore, it is proposed that in contrast to reporting of total small intestinal transit time, it is more relevant, for pH-responsive dosage forms at least, to report the upper small intestinal transit time (USITT) and the ileocaecal junction residence time (ICJRT).

The effect of disease conditions which affect pH is likely to also affect the performance of these pH polymer systems. Although other conditions such as cystic fibrosis have been shown to lead to lower intestinal pH (Youngberg et al, 1987), the focus is nevertheless on the influence of mucosal inflammation of Crohn's and ulcerative colitis on intestinal and colonic pH and hence on the performance of the pH-based drug delivery systems used in their treatment. The evidence of the influence of mucosal inflammation and other disease processes associated with inflammatory bowel disease on pH has been contradictory, While some investigators claim that pH is
reduced (Raimundo et al., 1992; Fallingborg et al., 1993), others suggest that pH is not reduced and may indeed be higher than in healthy subjects (Press et al., 1998; Ewe et al., 1999). However, other reports indicate that luminal pH is indeed lower in the diseased patients, but that the effect is not sufficient to affect the performance of pH responsive systems (Nugent et al., 2000). It is vital nevertheless, that arguments and results of these investigations are considered in the formulation design of delivery systems for the specific disease condition. It does seem that the main effect of inflammation is reduction of luminal pH in the colon, whereas the dissolution of pH responsive polymer systems appears to be influenced to a greater extent by intestinal pH. Moreover, the changes that occur in the colon due to disease conditions further supports the proposal that the aim of drug delivery should be to target the ileo-colonic region.

Overall, the result showed that Eudragit FS coated system is more suitable for ileo-colonic drug delivery compared to Eudragit S, and also showed adequate reproducibility of performance. Furthermore, the importance of pH to the performance of the polymers has been demonstrated both in vitro and in vivo, although intestinal transit has also been shown to affect performance. Co-administration with food as well as feeding up to 30 minutes post dose markedly increases the disintegration time of coated tablets. The in vivo disintegration results correlate well with dissolution data in Hanks buffer which is important in formulation screening for prediction of in vivo performance.

5.2 Future work

Despite the variability in performance of Eudragit S organic-coated tablets reported in other studies as well as in this study, dosage forms that rely on the pH of the distal gut for drug release will continue to appeal to formulation scientists due to its provenance and simple technological requirement. Moreover, Asacol® MR already has the largest market share of the established IBD therapies and it would be expected that similar dosage forms will continue to be developed. Further studies are nevertheless considered important:
I. The studies reported indicate that transit was a major factor affecting performance. Transit of single solid dosage forms is known to be random and subject to variability, particularly with regard to gastric emptying, transit across the ileo-colonic junction, and they also show faster transit through the colon. The advantages of the multi-particulate include more reproducible gastrointestinal transit across the aforementioned region, and in addition the in vivo dissolution of polymer coated dosage forms may present with less variability due to the disperse nature especially in light of the information that intestinal fluid exists in pockets. Therefore investigations of dissolution performance of the polymers using multi-particulate dosage forms are required.

II. There is a need for further fundamental investigations of factors both intrinsic to the polymer systems as well as extrinsic formulation factors, which affect their drug carrier performance, such as nature of plasticisers and other additives.

III. The in vivo disintegration performance of pH responsive systems should be investigated in disease patients following on from reports of reduced luminal pH in patients with ulcerative colitis and Crohn’s colitis.

IV. The number of subjects in the reported studies is small; therefore larger sample studies would be necessary to validate the observed results.
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