AN INVESTIGATION INTO COLONIC MUCOADHESION

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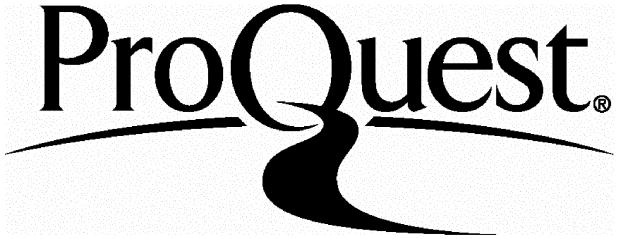
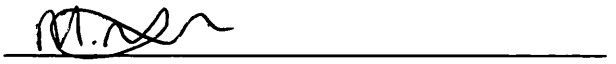


Plagiarism Statement

This thesis describes research conducted in the School of Pharmacy, University of London between 2003 and 2008 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 all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.



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List of abbreviations

ASA	Aminosalicylc acid
AR	Aspect Ratio
CAT	Caecum arrival time
CP 971	Carbomer Grade 971
CP 974	Carbomer Grade 974
CP 980	Carbomer Grade 980
CRT	Colon retention time
DC	Direct Compression
DSCG	Disodium cromoglycate
DTPA	Diethylenetriaminepenetaacetic acid
EC	Ethylcellulose
FDDS	Floating drug delivery systems
GE	Gastric emptying
GI	Gastrointestinal
GORD	Gastro-oesophageal reflux disease
GMS	Glycerol Mono Stearate
HBS	Hydrodynamically balanced system
HPC	Hydroxypropylcellulose
HPMC-AS	Hydroxypropylmethylcellulose acetate succinate
IBD	Irritable Bowel Disease
MCC	Microcrystalline cellulose
MMC	Migrating Myoelectric complex

MRI	Magnetic resonance imaging
PC AA-1	Polycarbophil Grade AA-1
PCDC	Pressure Controlled Colon Delivery Capsules
ROI	Region of interest
SCFA	Short Chain Fatty Acids
SEM	Scanning Electron Microscopy
SITT	Small intestine transit time
SMA	Shape memory alloy
TEC	Triethyl citrate
USP	United States Pharmacopeia

Chapter 1

Introduction

1. Introduction.

1.1. Gastrointestinal anatomy and physiology

The oral route is a safe, reliable and convenient method for the systemic and local administration of drugs using a variety of formulations including solutions, suspensions, tablets, capsules and pellets (Mayersohn, 2002). For drug delivery purposes the gastrointestinal tract (GI) can be divided into 5 main areas, the buccal cavity, oesophagus, stomach, small intestine and large intestine. The fate of drugs administered orally is dependent of the anatomy and physiology of the gastrointestinal tract and secretions such as enzymes which act on dosage forms immediately upon entering the buccal cavity. A diagram of the GI tract is shown in Figure 1.1.

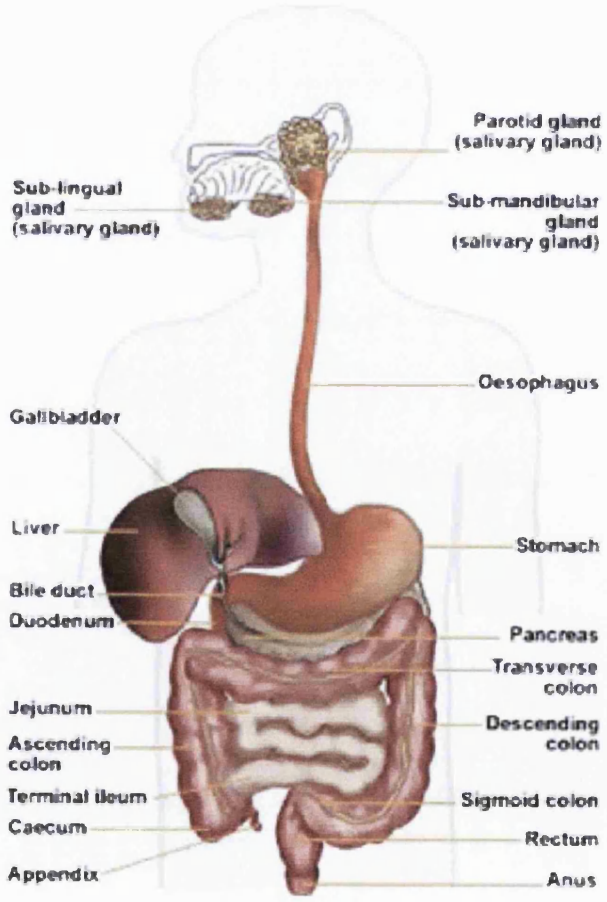


Figure 1.1 Diagram of the gastrointestinal tract
(www.thoughtfulhouse.org/images/awakefield-1150e.jpg, (18.01.2008)

Various strategies can be adopted to alter the release of drugs *in-vivo* to ensure they reach the intended site for delivery. Dosage forms which administer their drug to the stomach are termed immediate release, those which target more distal regions, modified release.

1.1.1. Mouth and oesophagus

In terms of digestion the primary purpose of the mouth is mastication of ingested foodstuffs. Immediately upon entering the mouth food, and orally administered drugs, are acted upon by amylase secreted from three pairs of salivary glands. Chewing of food aids in the digestion process by increasing the surface area on which secreted enzymes can act. The oesophagus extends from the mouth to the stomach and its primary function is to facilitate the passage of food from the mouth to the stomach by two forms of peristaltic movements. The first is a continuation of the movement started at upon swallowing and usually lasts 8-10 seconds before reaching the stomach. The second is a result of failure of the first wave to clear the oesophagus and will continue until the food has emptied into the stomach. At the distal end of the oesophagus, about 2-5 cm above its juncture with the stomach, the circular muscle acts as a sphincter and regulates entry into the stomach.

1.1.2. Stomach

The stomach lies in the upper part of the abdominal cavity, under the liver and diaphragm, in adults its usual capacity is 1.0 to 1.5 litres (Martini, 1995), however this can vary upon the intake of a large meal when the stomach can become distended. Other than acting as a reservoir for food, the stomach serves numerous functions. It regulates gastric pH; usually pH 1-2 ensuring the optimum working conditions for biological enzymes such as pepsin, controls and regulates the delivery of food via the pyloric sphincter to the small intestine, processes food into chyme, aiding subsequent absorption in the small intestine and finally, the acidic conditions sterilises gastric contents. The stomach contains two sphincter muscles which control the entry and exit of food. The cardiac sphincter controls the opening of the oesophagus to the stomach and the pyloric sphincter the opening of the stomach to the small intestine (Washington et al., 2001). Physiologically the stomach can be divided into three parts, the uppermost

region *fundus*, the main and largest section, *body*, and the lowermost region, *antrum*. These three regions have specific purposes, the *fundus* and body acting to reduce the increase in gastric pressure when food is taken in, whilst the *antrum* works to move gastric contents distally by contraction of the muscle wall towards the pyloric sphincter and out of the stomach. The wall of the stomach is comprised of several layers, mucosa, muscularis mucoasae, submucosa and two layers of muscle, the Inner Circular Muscle Layer and the Outer Longitudinal Muscle Layer. The mucosal surface, the outermost layer of the stomach wall is lined with simple columnar epithelium cells and gastric pits, leading to approximately four gastric glands each which contain two major secretory cells, chief cells and parietal cells which secret enzymes and hydrochloric acid respectively.

A protective layer of gastric mucus, secreted from goblet cells, lines the epithelium to assist the passage of food and prevent digestion of the wall by its own enzymes. Since this mucosal layer is in constant contact with gastric enzymes and the gastric contents it is continuously removed or digested, as such, it is also continuously being replaced. Mixing of food and gastric contents is achieved by the gastric muscle, the muscularis is comprised of three distinct sub layers of smooth muscle as well as longitudinal and circular muscles arranged in a crisscrossing pattern. This arrangement allows the stomach to contract strongly at many angles resulting in highly efficient mixing of gastric juices and contents.

In comparison to the rest of the gastrointestinal tract, absorption from the stomach is relatively limited. The stomach's function is mostly one of digestion rather than absorption, however, simple sugars, alcohols and some ionisable molecules such as aspirin can be absorbed

1.1.3. Small intestine

Distally from the stomach is the small intestine, which measures approximately 2.5 cm in diameter and 7-10 m in length and is greatly coiled. Along with the stomach, the small intestine has a role to play in the digestion of foodstuffs. In the small intestine food mixes with intestinal secretions, secreted from the Brunner's glands and intestinal cells. Intestinal contents are also mixed with pancreatic juices and bile secreted from the

liver. The small intestine is divided into three regions, the duodenum, jejunum and ileum. The duodenum is about 25 cm in length and is formed in a rough C shape. The point at which the intestine turns abruptly forward and downwards marks the start of the jejunum which continues for approximately 2.5 m until the ileum is reached which is about 3.5 m long. Although these regions are not distinct they differ in physiology, function and pH, increasing distally from pH 5-7.5 (Evans *et al.*, 1988).

As well as providing a suitable location for the mixing of chyme with digestive enzymes, the small intestine also has a role to play in absorption. It is well adapted to do so with an overall length of approximately 7-10 m, and structural modifications, such as villi and microvilli serving to increase surface area. Villi are projections from the mucosal layer of the small intestine which are each about 1 mm in height. Under microscopic examination the epithelial cells on the surface of the villi resemble a fine brush. This region, comprised of thousands of microvilli per cell is termed the brush border; intestinal digestive enzymes are produced with in this region, towards the top of the villi. Mucus secreting goblet cells are also found on the villi as well as crypts. Intestinal crypts are sites where regeneration of the mucosa occurs; as new cells are produced older ones are pushed up and out of the crypt towards the top of the villi where they are eventually shed.

The absorption of water is relatively constant throughout the small intestine, however a proximal to distal osmotic permeability gradient results in increase amounts of water moving back into the lumen, as such the permeability is greater in the duodenum than the jejunum which in turn is greater than the ileum. Approximately 2-3 litres of digestive fluids and enzymes are secreted in to the small intestine each day, as such the intestinal wall needs to have a rapid rate of turnover, renewal of the entire epithelium can occur in 6 days. Peristaltic waves project chyme distally through the small intestine towards the ileocecal valve where the contents empty into the colon (Pocock et al., 1999).

1.1.4. Large intestine

As with other areas of the gastrointestinal tract the large intestine can be described in the context of specific regions, caecum, ascending colon, transverse colon, descending

colon, sigmoid colon, rectum and anus. The caecum, the first section of the large intestine is 5 to 8 cm in length and separated from the ileum by the ileocecal valve which serves to limit the rate of passage of solids into the large intestine. Distally from the caecum is the colon which in turn is subdivided into three regions. The ascending lies in a vertical position to the right side of the abdomen and extends to the lower border of the liver. From the hepatic flexure to the splenic flexure runs the transverse colon, it passes horizontally across the abdomen, below the liver, stomach and spleen but above the ileum and terminates at a 90 degree angle, marking the start of the descending colon. As with the ascending colon, the descending colon lies vertical to the abdomen and leads to the sigmoid colon and rectum.

It is in the large intestine where fluids which remain after intestinal digestion are absorbed. Physiologically the small and large intestine possess many similarities; one important difference however, is the absence of villi in the large intestine. For this reason, despite the presence of similar enzymes, in comparison to the small intestine, the large intestine has a reduced level of metabolic activity at the intestinal wall due to the reduction of mucosal surface area. The colonic epithelium is comprised of a single layer of columnar cells lining the lumen, interrupted by the crypts of Lieberkuhn, responsible for the production of goblet and endocrine cells. In turn, goblets cell are responsible for the production of mucus which as with other areas of the gastrointestinal tract is an important factor in protecting the intestinal wall from abrasion from solid matter. In contrast to the stomach and small intestine, colonic mucus is also degraded by bacterial flora (Edwards, 1997).

One of the more important functions of the colon is the re-absorption of water. In addition to water, sodium is also reabsorbed in large quantities in the colon. About 500ml of water and a large amount of sodium chloride enter the caecum per day. This is removed via an osmotic gradient and active transport respectively (Rhoads et al., 1989). Approximately 200 ml of faeces is ejected per day from the 1500 ml of material which enters the colon (Martini, 1995). The main site of absorption of water is the ascending and transverse colon (Edwards, 1997) however this can be delayed by the presence of slowly fermentable dietary fibres. In contrast to the small intestine, the epithelium of the colon exhibits reduced permeability and allows water to be extracted against and large osmotic gradient.

1.2. Factors Affecting GI drug delivery

1.2.1. Gastrointestinal pH

Knowledge of gastrointestinal pH is of great importance in the field of drug delivery as not only can it be utilised to control the release of a dosage form, but also affects drug solubility and the microbial content of the region. Throughout the GI tract pH is variable, in general terms the stomach is the most acid region and some areas of the small intestine and large intestine the most alkaline. (Figure 1.2)

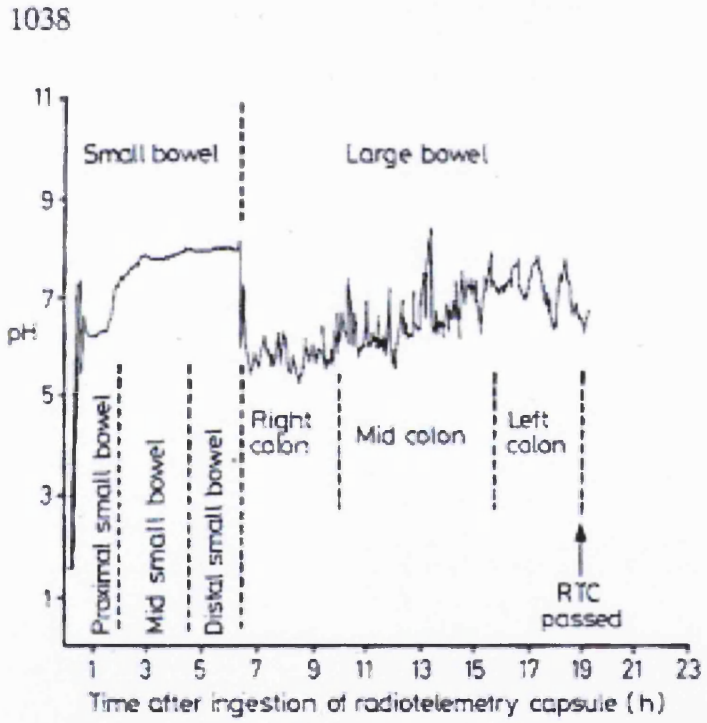


Figure 1.2 Gastrointestinal pH profile and position of the capsule in-vivo (Evans et al., 1988)

In the fasted state gastric pH ranges from pH 1-2.5, and increases upon ingestion of food, with the greatest increase measured rising from pH 1 to pH 6.5 (Hardy et al., 1987 and Dressman et al., 1990). Duration of the rise is however short lived, as the pH is greater than pH 5 for approximately 6 minutes but returns to pH 1.0 within an hour Hardy et al., (1987). More specific changes in gastric pH with food were observed by Dressman et al., (1990) who saw a rise above pH 4 for 73%, above pH 5 for 45% and

above pH 6 for 20% of the time with the peak pH usually occurring with in the first 5 minutes of eating.

Moving distally, from a gastric to intestinal environment is accompanied by increase in pH; this is as a result of the secretion of bicarbonate by Brunner's cells in the duodenum which helps to neutralise and buffer the low pH chyme from the stomach. As such, small intestine ranges from pH 4.4 - 9.8 with the average pH remaining above 6.0. The greatest increase in pH with in the small intestine is seen when contents move from the proximal to distal duodenum where a rise from pH 3.0 to pH 6.0 occurs (Hardy et al., 1987). Colonic pH is also variable and range from pH 4.9-7.7 in the caecum and 6.2-6.3 in the ascending colon (Hardy et al., 1987). A drop in pH is seen when progressing from the terminal ileum to the ascending colon (Evans et al., 1988) (Table 1.1), as a result of an increase in the number of microflora present within this region (Mackey et al., 1997). The resulting increase in metabolic activity produces greater amounts of short chain fatty acids (SCFA), additionally a reduction in bicarbonate secretions also occurs, both are responsible for the rising pH seen in the distal colon (Roediger, 1980).

Table 1.1 Mean small intestinal and colonic pH profiles (± S.D.) in normal ambulant human volunteers (Evans et al., 1988)

Region	**pH ± S.D.**
Proximal SI	6.63 ± 0.53
Mid SI	7.41 ± 0.36
Distal SI	7.49 ± 0.46
Right Colon	6.37 ± 0.58
Mid Colon	6.61 ± 0.83
Left Colon	7.04 ± 0.67

The stark decrease in intestinal pH seen at the ileocecal junction potentially presents a problem for drug delivery to the colon, specifically with pH dependent systems whose target pH range are usually between pH 6-7; if transit through the small intestine is rapid dosage forms may pass through the colon and be eliminated intact.

1.2.2. Gastrointestinal transit times

Gastrointestinal transit times are perhaps the most variable of all parameters that need to be overcome for successful drug delivery to the GI tract. Transit time of a dosage form is variable throughout the GI tract, however gastric emptying (GE) is particularly problematic, especially when compared to small intestinal transit times (Davis et al., 1986). The presence of food in particular has an effect on GE. In the fasted state the stomach exhibits a pattern of contractile activity known as the Migrating Myoelectric complex (MMC). Consisting of three phases, the final phase consists of 3-6 contractions which gradually increase in force, it is during this phase that the pylorus is relaxed allowing it to stretch to the maximum to facilitate the emptying of indigestible particles (Szurszewski, 1969). The MMC is particularly efficient at removing any stomach contents into the small intestine, however, its effect will vary depending on the physical form of the contents. For example, solutions will empty rapidly from the stomach and as such are not affected by the digestive state (Davis *et al.*, 1986). Consequentially food has a greater effect on GE of larger dosage forms and has been seen to significantly prolong gastric residence (Davis *et al.*, 1986 and (Sangekar et al., 1987).

The physical state of the dosage form is less important in determining the transit time through the small intestine, as is the presence of food (Davis et al., 1986 and (Khosla et al., 1989). Consequentially there is more agreement in the literature regarding a value for small intestinal transit, and is usually quoted as 3-4 hours. However, although the study by Davis et al., (1986) saw no significant differences (95% confidence) between the transit time of solutions, pellets and tablets, the longest and shortest times are highly varied at 9 hours (pellets) and 1.3 hours (tablets) suggesting that inter-subject variation should still be a concern.

The colonic transit of dosage forms is also highly variable. Times ranging from 17 to 72 hours have been reported (Hardy et al., 1985). Mixing of contents in the colon is known to occur (Wiggins et al., 1976). By delivering radio opaque pellets of different shape and X-raying passed stool samples Wiggins et al., (1976) determined that pellets administered on different days were able to mix within the colon of 83% of subjects. This effect, termed streaming, affects the transit of dosage forms in each region of the colon, with residence in the ascending and transverse colons greater (Adkin et al.,

1993). Surprisingly, the addition of dietary fibre does not affect the transit through the colon and stasis of dosage form is still observed at the ascending and transverse regions, particularly at both flexures (Price et al., 1993). Both size and volume of dosage forms affect their colonic transit times with larger forms transiting through faster (Adkin et al., 1993). Such a large variation in transit time can have a dramatic affect the arrival of drugs in the blood and have been seen to lead to differences in blood-plasma profiles for 4-aminosalicylc acid in two volunteers who experienced different transit times of a coated capsule designed for colonic delivery (Tuleu et al., 2002) (Figure 1.3).

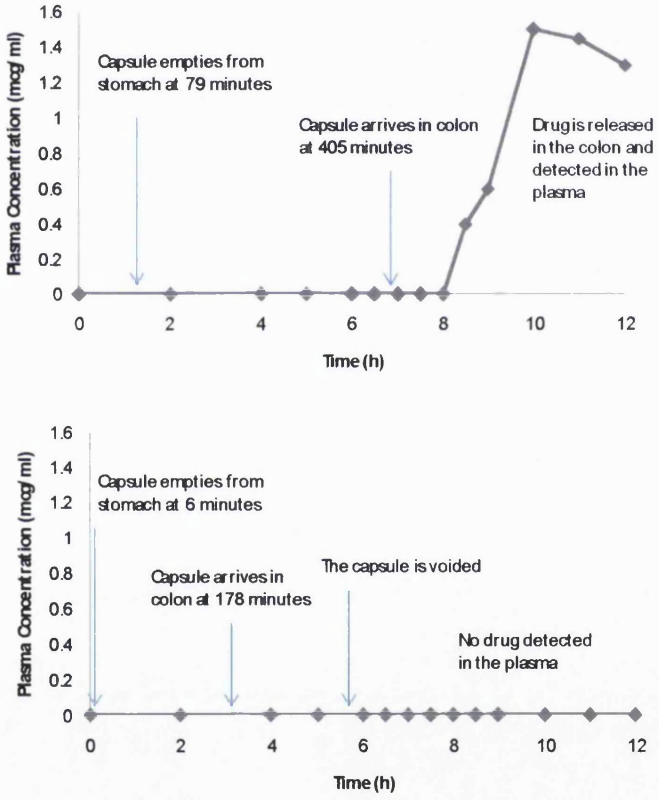


Figure 1.3 Plasma profiles for 4-ASA delivered from a coated capsule designed to target the colon for two different volunteers who experienced different gastrointestinal transit times. Adapted by McConnell et al., (2008) from data from Tuleu et al. (2002)

Variation of colonic transit is an important consideration, particularly for the delivery of poorly soluble drugs, understanding the effects of size and volume on transit can help investigators normalise transit time leasing to more reliable predictions of the behaviour of dosage forms in the human colon.

1.2.3. Gastrointestinal Fluid

Perhaps one of the most important factors regarding drug delivery to the gastrointestinal tract is the amount of fluid available to the dosage form to promote dissolution of the drug *in-vivo*. Low fluid volume is of particular importance if the drug is poorly soluble or if the system utilises swelling polymers to achieve controlled release or mucoadhesion. For years it has been generally accepted that the contents of the GI tract increase in viscosity distally due to re-absorption of water. Obtaining values for the amount of water present is of great importance particularly for colonic delivery systems as we would expect this region to have the least amount of fluid available within the GI tract. Attempts to quantify gastrointestinal fluid contents can be traced back to the mid 1950s (Gotch et al., 1956). At autopsy the investigators measured the amount fluid present in the stomach, small intestine and colon (caecum, ascending and half the transverse colons). The average total luminal fluid was found to be 407 mL (range 164-843), with the greatest amount of this located in the small intestine 50.6% (206 mL, range 60-352), the least in the large intestine 20.4% (83 mL, range 7-430) with the stomach containing the rest at 29.0% (118 mL, range 11-233).

Other researchers have estimated the water content of the human colon to be 187g from an original 220 g of wet content (Cummings et al., 1990). A more recent paper (Schiller et al., 2005) investigated the free fluid volume of the gastrointestinal tract using magnetic resonance imaging (MRI). Investigating 12 healthy volunteers, (6 male, 6 female) the study involved the administration of a non-disintegrating capsule (4.6mm diameter, 16.8mm length) dosage from containing 1, 2 or 3 pellets (2mm) of gelatine for ease of identification *in-vivo*. After 8 hours of fasting the subjects swallowed a capsule at three time points, 7 hours before imaging (containing 3 pellets), 4 hours before imaging (containing 2 pellets) and 1 hour before imaging (containing 1 pellet). MRI imaging was performed after the intravenous administration of 5 mg scopolamine hydrobromide to suppress intestinal motility. A summary of the results obtained can be seen in Table 1.2

*Table 1.2 Gastrointestinal fluid volumes determined by MRI under fed (1h after meal) and fasted conditions (Schiller et al., 2005) * values indicate total fluid volume of the stomach unbound water(free water) could not be distinguished.*

GI region	Free fluid volume (fed) mL ± S.D.	Free fluid volume (fasted) mL ± S.D.
Stomach	686* ± 93	45 ± 18
Small Intestine	54 ± 41	105 ± 72
Large Intestine	11 ± 26	13 ± 12

The values obtained by Schiller et al., (2005) are lower than expected, particularly for the small and large intestines. The study found that as expected the volume of water available in the stomach increased with the intake of a meal. In contrast the small intestinal fluid volumes decreased in the fed state, in the upper small intestine the investigators observed intestinal filling with a slurry of ingested food, however this was not seen in the distal small intestine. The intake of food did not appear to affect the fluid volume in the large intestine, however capsule distribution was affected. During fasting only 3 capsules (administered 7 h prior to imaging) were located with in this region. After the meal, 5 capsules that were ingested 4 h earlier and 12 capsules ingested 7 h pervious were located in the large intestine.

The study also looked at the distribution of fluids throughout the gastrointestinal tract and found that the fluid was not distributed evenly but in discrete pockets in the small and large intestines. Using three terms "surrounded by liquid", "partly surrounded by liquid" and "not in contact with liquid" the investigators sought to make links between this and capsule passage, the results can be seen in Table 1.3. In the stomach all of the delivered capsules were located surrounded by liquid and this is not affected by the administration of a meal. The investigators reported that after a meal as well as the fluid volume being reduced, there was an increase in the number of fluid pockets located in this region, however this of course meant that the fluid contained in each region was significantly less.

Table 1.3 Capsule distribution and environment during fasting and 1h post meal after consecutive ingestion of three capsules after 7, 4 and 1 hours (Schiller et al., 2005)

	Fasting			Number of capsules	Fed	
Application Time (h)	**Surrounded by liquid**	**Partly surrounded by liquid**	**Not in contact with liquid**	**Surrounded by liquid**	**Partly surrounded by liquid**	**Not in contact with liquid**
		Stomach			**Stomach**	
1	5	0	0	12	0	0
4	0	0	0	1	0	0
7	0	0	0	0	0	0
		Small Intestine			**Small Intestine**	
1	4	1	2	0	0	0
4	5	2	5	1	1	3
7	5	3	1	0	0	0
		Large Intestine			**Large Intestine**	
1	0	0	0	0	0	0
4	0	0	0	0	0	5
7	0	0	3	1	2	9

Within the large intestine the fluid regions were located predominantly in the caecum, ascending and descending colon regions and the number of pockets significantly increases in the fed state.

The findings by Schiller et al., (2005) are of great significance to oral drug delivery and go some way to explaining the differences observed when comparing *in-vivo* and *in-vitro* performances of drug delivery systems. A single unit system may be viewed with confidence regarding its interaction with fluid in the stomach; however availability of free fluid in the small and large intestine is less apparent and could cause difficulties when delivering drugs to these regions. Likewise the performance of a mucoadhesive in *in-vivo* will be greatly affected by these results, particularly in the small intestine. The results for the large intestine however may show some promise, since most of the fluid content is located in the proximal regions it would be possible, with the correct dosage form, to induce adhesion in the caecum or ascending colon regions limiting the passage of the dosage forms from the fluid pockets and into the transverse colon where less free water is available

1.3. Colonic drug delivery

In relation to the stomach and small intestine, colonic delivery systems are relatively recent innovations but have received more and more interest over the past 20 years. Complexities such as drug protection and since the colon has been generally considered a poor site for delivery due to low water content have meant that drug delivery to proximal regions of the GI tract has been less desirable. The need to treat local disorders such as irritable bowel syndrome, inflammatory bowel disease and carcinomas and its potential use as a site systemic delivery of drugs that are denatured in low pH values has spearheaded research to develop colonic delivery systems. An important consideration however for colonic delivery is gastric emptying and small intestine transit time. Investigating the transit of multiparticulate and single unit systems through the human gastrointestinal tract, Hardy et al, (1985) concluded that for optimal colonic delivery a drug should be retained within the delivery system for approximately 5 hours. To achieve this lag time several strategies have been employed.

1.3.1. pH sensitive approaches

The general rise in pH distally within the gastrointestinal tract has been exploited to deliver drugs to the colon. The first approach to this was in the application of Eudragit S (a methacrylic acid methyl methacrylate polymer) to capsules containing sulphasalazine for colonic release (Dew et al., 1982). Using X-ray to monitor transit of the capsules investigators found that after 12 hours, 29 of the 36 capsules delivered had reached the colon intact and 3 more were present but broken. Later studies using scintigraphy however show that *in-vivo* release of pH sensitive polymers was complicated and disintegration of tablets varied in both time (5-15 h) and location (ileum to hepatic flexure) and two tablets did not disintegrate at all, suggesting that in some of the subjects the pH of the ileocecal junction is not above pH 7 as expected (Ashford et al., 1993). This highlights a problem for systems of this nature, the inter-subject variation of pH and transit times can result in polymers never being exposed to pH values above their dissolution thresholds at all or for sufficient periods of time.

Although greater control over drug release *in-vivo* can be gained by varying the ratio of polymer in the coating and the coating level (Zahirul et al., 1999), better *in-vitro* dissolution tests in media more closely matching *iv-vivo* conditions may serve to more reliably predict the drug release and prevent these issues (Ibekwe et al., 2006).

1.3.2. Time dependent systems

Time dependent drug delivery is another approach to colonic targeting. Pulsed release formulations were devised to deliver captopril to the colon (Wilding et al., 1992). The device works by incorporating a lag time into the release to improve the chance of delivery in the distal regions. Pulsincap™ is composed of a water insoluble capsule with a water soluble cap; the drug is contained within the body of the capsule and sealed with a hydrogel polymer plug. In gastric juices the water soluble cap dissolves and the hydrogel swells. The hydrogel is specifically chosen for its gelation rate, therefore at a specific time after administration the swollen plug is ejected from the capsule body and the drug released (Binns et al., 1996). The device has been evaluated regards to its tolerability in humans and found it to be well tolerated. Further studies show strong correlation between *in-vivo* and *in-vitro* data of the Pulsincap™ with a 5 hour delay.

(Stevens et al., 2002). Although in one subject release was in the stomach, for all others drug release was observed in the small intestine, ileocaecal junction or ascending colon.

A multi-coated system combining a timed release function with a pH sensitive function has also been developed (Ishibashi et al., 1998). A hard gelatin capsule was coated three times, firstly with Eudragit® E100 sensitive at low pH up to pH 5, secondly with hydroxymethylcellulose (TC-S®) as a neutral water soluble polymer and thirdly with hydroxypropylmethlycellulose acetate succinate (HPMC®-AS) as an enteric polymer. The principle behind the system is that Eudragit polymer protects the system from gastric conditions but is dissolved in the proximal small intestine at pH values above 5.0. As it dissolved the water insoluble layer and the enteric layer limit the passage of fluid into the core of the capsule. Fluid ingress dissolves an organic acid within the core which as it leaches out dissolves the Eudragit coat. Dissolution studies showed that a predictable time release mechanism for colonic drug delivery could be obtained.

1.3.3. Bacterially triggered

Enzymatic action of bacteria on colonic contents is well known. Microorganisms inhabit most regions of the gastrointestinal tract with proximal regions such as the stomach, duodenum and upper ileum containing approximately 10^4 cfu per ml of intestinal fluid, most of which arrives via the mouth. *Streptococci*, aerobic *lactobacilli*, gram positive and fungi are the major constituents since they are generally resistant to low pH and can survive the stomach which would otherwise serve as a barrier to micro flora colonisation. Distally, an increase in microflora is seen with numbers in the region of 10^5- 10^8 cfu per ml of fluid observed towards the ileocaecal junction (Gorbach, 1971). Anaerobic micro organisms such as anaerobic bacteroides, anaerobic lactobacilli and clostridia increase in prevalence and, to date, over 400 different species which inhabit the colon have been identified, resulting in 10^{11} microorganisms per ml of fluid, equivalent to almost one third of the total dry weight of faeces in man (Mackey et al., 1997).

The metabolism of ammonia and bile salts are two processes which are undertaken by colonic bacteria, however the hydrolysis of glucosides and glucuronides, which are poorly metabolised by intestinal enzymes is by far the most prevalent. Metabolism can

be harmful, via the production of toxic metabolites, however they can also be exploited. For example, chloramphenicol (an antibacterial) and morphine are retained for longer periods in the systemic circulation due to hydrolysis by gut bacteria thus improving their respective therapeutic effects (Hill et al., 1975). Additionally, a large number of polysaccharides are degraded by colonic bacteria and as such may form the basis for a suitable carrier system. Early work (Ashford et al., 1993) evaluated the dissolution of a compressed tablet with a pectin coat in media both containing enzymes and absent of enzymes in conditions simulating mouth to colon transit. Drug release in the presence of enzymes was increased, but pectin alone did not provide adequate protection of the tablet core from proximal GI conditions. Later work (Wakerly et al., 1996) enhanced the system with the addition of a water insoluble component in the form of ethylcellulose (EC). By modifying the ratio of EC to pectin in a film coat sufficient protection for the drug in acid was provided as well as improved release in simulated intestinal conditions in the presence of enzymes. This was confirmed *in-vivo* by the use of gamma scintigraphy which showed the disintegration of 9 mm calcium pectinate and calcium pectinate/ guar gum tablets in the colon. Showing good correspondence with a previously run animal study (rat model) both tablet types released in the colon proving the suitability of this type of system for colonic targeting.

The fermentation of amylose, a plant polysaccharide from starch, in the colon has also been utilised for colonic drug delivery. Amylose and ethylcellulose coated pellets (1:4) have been shown to pass through the proximal GI tract to the caecum un-metabolised (Cummings et al., 1996). After arrival at the caecum a lag time of approximately 3 hours is seen until significant drug release occurs. Further investigation have confirmed protection of a model drug in ethylcellulose- amylose coated pellets for up to 12 hours in simulated gastric and intestinal conditions (Milojevic et al., 1996), and *in-vivo* studies have shown a mean colonic arrival time of 6 h for amylose coated capsules (Tuleu et al., 2002). More recently some researchers have begun assessing drug release from amylose systems in batch fermenters (Siew et al., 2004) and faecal slurries (McConnell et al., 2007) as novel approaches to *in-vitro* testing with more *in-vivo* like conditions.

Another group of compounds that have been exploited in this way are azo groups. The azo bond is reduced by colonic bacteria and can be exploited in much the same way as polysaccharides. Release here however is more dependent on the swelling of the

copolymers than the former due to shielding of the azo bond (Van den Mooter et al., 1994). Azo linking has also been used in the administration of pro-drugs. Sulphasalazine is used in the treatment of ulcerative colitis, it reaches the colon un-metabolised where colonic bacteria cleave the azo component leaving the two constituents parts, sulphapryridine and 5-aminosalicylic acid, it is the latter that has a therapeutic effect (Khan et al., 1977). A novel adaptation of this was adopted by Kakoulides et al., (1998) to deliver mucoadhesive polymers (azo-crosslinked polyacrylic acids) to the colon. Ex-vivo mucoadhesion studies were performed on rat stomach, proximal small intestine, distal small intestine and colon and the force needed to separate the polymers under test and the associated work of adhesion was measured, no *in-vivo* study was conducted.

1.3.4. Pressure controlled

A relatively novel method for drug delivery to the colon is the use of pressure controlled systems (Muraoka *et al.,* 1998). The driving force behind Pressure Controlled Colon Delivery Capsules (PCDC) is the physiological lumenal pressure (resulting from peristalsis) which is utilised to disintegrate the dosage form. Using Caffeine dissolved in PEG 400 and 1000 as a model drug and sealed in capsules Muraoka et al., (1998) were able to show that PCDCs could be successfully used to administer drugs to the colon. This method however was seen be unreliable and so its use has not been wide spread.

1.4. Approaches to altering gastrointestinal transit times to enhance drug delivery

The need to occasionally alter the normal transit time of dosage forms in the gastrointestinal tract has arisen from a need to be able to better predict the behaviour of the system *in-vivo.* Variable gastric emptying times and to a lesser extent variable small intestinal transit times have complicated the already difficult problem of modified release of drugs in the human intestinal tract. Delivery of poorly permeable, poorly soluble drugs may be enhanced by retarding the passage of the dosage form in the proximal GI tract. Additionally, treatment of localised disorders such as *Helicobacter pylori* will benefit from prolonged residence within the stomach. There are numerous factors which will affect the GE of a dosage form such as size, density, shape and food intake. Approaches to gastric retention attempt to exploit these factors to affect

emptying. In general gastro-retentive devices can be split into two types; those that use the stomach's physiology and those that work against it. Systems that float on the gastric contents or are intentionally large in size are examples of systems developed to utilise the stomach's own physiology to delay gastric emptying. Mucoadhesive, swelling and highly dense dosage forms are examples of systems which try to fight the normal processes to increase retention. Of all attempts to increase gastric residence, it is the floating devices that have had the most success.

Floating drug delivery systems (FDSS) are retained in the stomach due to, as the name suggests, their floating capabilities. In order to float the density of the system must be less than gastric contents, approximately 1.0g/ml (Singh et al., 2000). FDDS can be sub-divided into two main methods of working, effervescent and non effervescent. Non effervescent forms incorporate gel forming or highly swelling polymers such as cellulose and polysaccharides or matrix forming polymers such as polyacrylate and polystyrene. On contact with gastric fluids the polymers hydrate and swell, buoyancy is conferred to the system by air trapped with the gel matrix. An early example of a delivery system to utilise such a method is the Hydrodynamically balanced system (HBS) (Sheth et al., 1984). Effervescent systems incorporate carbon dioxide producing components such as citric or tartaric acid or sodium bicarbonate in a swellable polymer matrix. The released carbon dioxide is trapped by the gel layer and as before buoyancy is achieved (Stockwell et al., 1986).

A limitation to the effectiveness of floating systems is whether the stomach is in the fed or fasted state. Gastric emptying in the fasted state is more dependent on the activity of the MMC and the dosage form will empty during phase 3 activity. Therefore, instead of fighting the effects of the MMC the most efficient way to overcome the variability caused by food would be to consider this when designing the system (Whitehead et al., 1998). Subsequently, radiolabeled sodium alginate floating beads were administered in the fed state to 7 male volunteers and GE was monitored using gamma scintigraphy. The study concluded that the alginate beads exhibited a gastric residence time of over 5.5h significantly longer than the control which exhibited a mean onset emptying time of 1h.

Physical adhesion of the dosage form to intestinal or stomach mucosa is another approach to alter gastrointestinal transit times, this type of adhesion is termed mucoadhesion and its success is determined by the interaction of the adhesive polymer with mucin.

1.5. Mucoadhesion

1.5.1. Human mucosa and mucin.

Surfaces throughout the human body are covered with a layer of mucus; its function and purpose vary depending on the location. For example: within the respiratory tract it serves to protect the mucosal surface from foreign bodies and regulates ion transport and water balance; in the middle ear it assists with the clearance of cellular debris; and in the gastrointestinal tract the mucin layer serves to protect, lubricate, act as a diffusion barrier and regulate water movement. Mucus is secreted by surface epithelial cells which in the duodenum are Brunner's glands and goblet cells in the small and large intestines (Ganong, 1997).

This mucus blanket, serves to lubricate the GI tract, bind some bacteria and hold immunoglobulins so they can act on pathogens. The function of mucus to protect the mucosal epithelium is complicated by continuous erosion and mechanical damage by the passage of food, fecal material and auto digestion. A two component barrier consisting of the mucus gel and an underlying rapidly regenerating mucosal cell layer counters the continual erosion by replacement of the mucus. Irrespective of where it is formed mucus is comprised of up to 95% water, 0.5-5% lipids and glycoproteins, 0.5-1% mineral salts and 1% free proteins although its exact composition will vary depending on secretion site, function and disease state (Rathbone et al., 1991). The complex high molecular weight glycoproteins within the mucus layer are collectively termed mucins, of which, there are two forms, secretory mucin and membrane bound mucin. The secretory mucins are high molecular weight and form intramolecular disulfide bridges resulting in viscous gels. Membrane bound mucin, as the name suggests are attached to the plasma membrane via a hydrophobic domain and are unable to form disulfide bridges (Allen, 1981), (Strous et al., 1992).

Glycoproteins have a molecular weight ranging from 0.5- 20 MDa and are highly glycosylated, containing approximately 80% carbohydrates including N-acetylgalactosamine, N-acetylglucosamine, fucose, galactose, sialic acid and traces of mannose and sulphate. The oligosaccharide chains consist of 5-15 monomers, are moderately branched and attached to the protein core via O-glycosidic bonds. The remaining 20% is made up of the protein core, with a molecular mass of 200-500 KDa and is comprised of two regions. The glycosylated central region is formed from repeats of the amino acids serine, threonine and proline. The second region is located at the amino and carboxyl terminals is a region high in cysteine, it is here that the disulfide bridges are formed that resulting in the viscous gel (Bansil et al., 2006).

In addition to providing protection for the mucosal surface within the gastrointestinal tract the mucus layer also provides a region of unstirred fluid which can be utilised for drug absorption. However this can also be a hindrance as the mucin layer will act as a barrier by stabilising the water layer or by interactions with diffusing molecule, factors such as lipophillicity and molecular size playing an important role in the diffusion of drug through the mucus layer (Larhed et al., 1997). Other factors such as charge can have an impact on diffusion through the negatively charged mucin. It has been suggested that binding of non polar and polar drugs to intestinal mucus is a result of hydrophobic and other non specific interactions, with the binding occurring mainly at the non-glycosylated regions and other interactions, such as hydrogen bonding, taking place on the oligosaccharide chains. Additionally drug-protein interactions may occur due to the creation of a hydrophobic region created by the naked protein regions within the glycoprotein structure. (Khanvilkar et al., 2001).

The effect of disease on the mucus layer and mucins is an important consideration, especially when wishing to use mucoadhesive polymers to treat localised disorders. Ulcerative colitis for example causes a thinning of the mucus layer due to a depletion of goblet cells, in contrast with Crohn's disease no depletion is seen and as such the mucus layer is thicker (Pullen et al., 1994). The biochemistry of mucin is also affected in the diseased state. Patients with Irritable Bowel Disease (IBD) will have considerably shorter oligosaccharide chain length. In addition sulphation is reduced and sialyation is increased, there is also an increase in expression of carbohydrate structures such as galactose 1,3 N-acetylgalactosamine; these changes alter the viscoelastic properties of

the mucus which in turn alters the interactions with micro-organisms, electrolytes and defensive proteins reducing the effectiveness of its function (Shirazi et al., 2000).

1.5.2. Mechanism of Mucoadhesion

Within the literature the terms mucoadhesive and bioadhesive have been used interchangeably; bioadhesion generally applies to any material which binds, chemically or physically with a biological surface, mucoadhesion is a more specific term and relates to adhesion between the material and mucins or the mucus layer. Mucoadhesives polymers have a beneficial use in drug delivery as they increase the residence time of dosage forms as well as create intimate contact with the mucus layer which can enhance diffusion of the drug. For GI delivery mucoadhesive polymers can be exploited to delay the transit of the dosage form, for example to inhibit gastric emptying or in the case of this project promote increased residence within the colon. Park et al., (1984) categorised the mechanisms by which certain polymers act as bioadhesives into three types:

1. polymers that become sticky when placed in water and owe their bioadhesion to stickiness
2. polymers that adhere through non-specific, non-covalent interactions which are primarily electrostatic
3. polymers that bind to specific receptor sites in the cell surface

Mucoadhesives are examples of bioadhesives whose mode of action are primarily associated with type 1 and 2. Initiation of a mucoadhesive bond involves several steps, initially wetting of the polymer must occur, this will most likely result in swelling and the creation of a gel. If intimate contact between the gel layer and mucus layer occurs, interpenetration of the mucoadhesive polymer chains and the mucin will commence. Finally, weak chemical bonds form between the entangled chains.

There are two main stages in adhesion, the contact stage and the consolidation stage, the former being the creation of intimate contact, in the case of mucoadhesion it would be between the mucoadhesive polymer and the mucus layer, the latter the physicochemical interactions which strengthen the adhesive joint. In general there are six theories regarding adhesion:

- *electronic theory* proposes that upon contact of the two surfaces electron transfer occurs, the adhesion is caused by an electrical double layer at the interface.
- The involvement on interfacial energies is the basis of the *wetting theory*. Primarily applied to liquid systems it requires the liquid to spread onto the entire surface for adhesion to occur.
- The next theory is the *adsorption theory* and suggests that adhesion is the result of weak bonding forces such as van der Waals forces and hydrogen bonding.
- diffusion of one polymer chain with another and is termed the *diffusion theory*. The inter-diffusion is driven by concentration gradients and is affected by molecular chain lengths and mobility.
- *Mechanical theory* is concerned with adhesion resulting from interlocking of a liquid adhesive in to irregularities on a surface
- The final theory is the *fracture theory*, differing form the others it relates to the force required to cause detachment after adhesion

Mucoadhesion is a complex interaction and it is unlikely that one of these theories would completely explain it, therefore it should be viewed and a result of a combination of these theories (Smart, 2005). It has been shown that for strong mucoadhesion chemical and physical bonding is required; van der Waals interactions and hydrogen bonds are the most prevalent during mucin and polymer interactions (Mortazavi, 1995). Van der Waals forces are the result of a dipole-to-dipole or dipole-to-induced dipole interactions in polar molecules, hydrogen bonding comes about when a hydrogen atom is covalently bonded to a electronegative atom such as oxygen. This results in a slight positive charge carried by the hydrogen atom and causes attraction between it and other electronegative atoms. Although individually both types of bonding are weak, collectively, when produced over multiple sites across the polymer the resulting bond is substantial. Interpenetration of the polymers and mucins has also been shown to occur between mucin and polymer layers, providing a strong bond resulting in adhesion (Jabbari et al., 1993). Hydrogen bonding, van der Waals interactions and interpenetration of the polymer chains are all examples of the consolidation stage of adhesion. During mucoadhesion however, another component is involved which adds to

the consolidation and strengthens the adhesion. It has been suggested that water movement and dehydration of mucin have a role in mucoadhesion (Mortazavi et al., 1993) Transfer of water between mucoadhesive formulations and porcine gastric mucus was investigated. The adhesive and cohesive nature of the mucus gel was shown to increase as the water contend decreased, suggesting that the movement of water is an important factor of mucoadhesion.

1.5.3. Mucoadhesives in drug delivery.

Mucoadhesives have found use in drug delivery systems since the late 1970s when they were used in the field of ophthalmology to improve the efficiency of drug delivery systems. The use of mucoadhesives in ocular delivery was investigated when link between viscosity and contact time of a liquid preparation in the eye was determined (Patton et al., 1975). The clearance rate of a solution was dependent on its viscosity and the largest contributor to loss of drug in the eye was instilled solution drainage resulting in just 1-3% of the applied drug penetrating the cornea (Patton et al., 1976). Therefore mucoadhesive polymers were sought to increase the viscosity and induce longer contact of the delivery system with the cornea. Many polymers have been investigated to determine characteristic structural features required for mucoadhesion. Anionic polymers and those containing high numbers of hydrogen bonding groups (OH and COOH) were concluded to be most suitable for adhesion (Park et al., 1984), (Ch'ng et al., 1987) and (Peppas et al., 1985). Early investigations identified cross linked acrylic acids as good candidates for ocular adhesion (Hui et al., 1985). It was demonstrated that use of bioadhesives in this way increased the residence time of the in the eye resulting in a 4.2 fold increase in the bioavailability of progesterone. Further studies confirmed Hui and Robinson's work showing that bioadhesives, in particular cross linked acrylic acids or carbopols increased drug bioavailability when applied as solutions (Thermes et al., 1991), (Thermes et al., 1992), and (Gurtler et al., 1995).

The use of solid dosage forms and a move away from the exclusivity of acrylic acids came with chitosan microparticles to deliver acyclovir (Genta et al., 1997). More recent studies continued the investigation in to solid dosage forms with the delivery of 2 mm minitablets to healthy male volunteers (Weyenberg et al., 2006).

From their early use in ocular delivery, mucoadhesive polymers branched out to other areas of drug delivery, including the development of pessaries for treatment of genitourinary infections, (Ceschel et al., 2001), (Kast et al., 2002) and (Pavelic et al., 2005) and nasal (Soane et al., 1999), buccal (Khanna et al., 1996), and gastrointestinal tract targeting.

Within the GI tract, the potential of mucoadhesive polymers has been well exploited, with all the major regions becoming targets, as with any GI drug delivery the preferred route is orally, however there have been some investigations into the uses of mucoadhesive polymers for rectal drug delivery. The primary intention here, as well as increasing residence is to reduce the effect of first pass metabolism of drugs (Choi et al., 1998a) a further study, (Choi et al., 1998b) investigated effect of the addition of carbopol 974 and polycarbophil to suppositories. *In-vitro* and *in-vivo* investigations confirmed the conveyance of mucoadhesion to the formulations from the carbomers, furthermore, the authors reported that as little as 1% w/w of carbopol was needed to ensure the retention of the suppository in the rectum of the rabbit. The extensive exploitation of oral drug delivery means that oral delivery of mucoadhesives to even the most proximal regions of the GI tract may be possible. For example, drug delivery to the oesophagus is a comparatively under studied area, better understanding of mucoadhesive mechanisms and the types of polymers that interact with mucins has opened this region to investigation. Sodium alginates are natural polysaccharides composed of two monomeric sugar units α-L-guluronic acid and β-D-mannuronic acid and have been used in conjunction with a bicarbonate salt to produce a rafting system for gastro-retention for the treatment of gastro-oesophageal reflux disease (Washington et al., 2001).

Reapplication as a oesophageal mucoadhesive has shown some moderately promising results with 20% of a administered sodium alginate solution being retained on exercised porcine oesophageal mucosa, held on a mount at 60° to the vertical, after 30 minutes with washing with artificial saliva or deionised water (Batchelor et al., 2002). However, it should be noted that the tissue and the alginate solution were first held horizontally for a period of time to allow the mucoadhesive bond to form; it is unlikely that this amount of alginate would be retained were the mount to be held at an angle during application of the polymer. A later study (Smart et al., 2003) found slightly better

retention of radio labelled acrylic acids with 40% retention at 20 minutes. Differences in methodologies, specifically the elevation angle (30° and 60° for the latter and the former) and washing fluid (0.1M HCl and artificial saliva or deionised water both at 1ml/min), mean that the two studies are not directly comparable and this could account for the differences seen. So it is unclear if the additional retention is due to the polymer change or not. However Smart et al., (2003) did note that the distribution of the polymer and the relative retention on the two media tested (gastric and oesophageal mucosa) was affected by the polymer molecular weight and the mucosa. Proposed explanations for the effect of molecular weight were not given, however the difference in distribution on the mucosal surface was attributed to physiological and histological factors.

Mucoadhesion in the stomach has also been shown to be difficult, as with any adhesive system there is a need for intimate contact to be made, however, high levels of agitation and abrasions from food hinders this. As with floating devices, the effect of fed and fasted state will alter the effectiveness of the system, although in principle mucoadhesive systems would be able to resists the effect of the MMC, pH changes, location of adhesion and amount of mucin at the site of adhesion can affect its performance (Tobyn et al., 1995). As with other sites explored for mucoadhesion the most promising polymers for increased gastric retention are chitosan, and acrylic acids. Increased gastric retention of acrylic acids has been reported in rats (Longer et al., 1985), (Ch'ng et al., 1985) and (Harris et al., 1990). Longer et al., (1985) reported that 90% beads administered with polycarbophil polymer were retained in the stomach after 6 hours. Similarly Ch'ng et al., (1985) saw that after 24 hours 9.0 ± 3.2% of radiolabeled polycarbophil was retained compared with 8.3 ± 2.7% retained after 16 hours for a non-mucoadhesive control. Finally Harris at al., (1990b) saw ileocecal transit times 25% slower for polycarbophil formulations. Attempts to repeat this in dogs however have failed and no increased residence was observed for polycarbophil pellets compared to a non mucoadhesive control (Khosla et al., 1987), furthermore, studies in man seem to confirm the inability of carbomers to delay gastric emptying, (Harris et al., 1990) and (Cuna et al., 2001)

Ion exchange resins have been investigated and shown to have prolonged gastric residence in man (Burton et al., 1995). Investigations found that 20-25% of an ion exchange resin cholestyramine remained in the stomach for 5.5 h, further studies

showed as along side increased residence came the benefit of the ability to resist the effect of the MMC and remain in the stomach (Thairs et al., 1998). In an attempt to understand the reason for the apparent affinity for gastric mucosa Jackson et al., (2000) administered cholestyramine with a cationic exchange resin Amberlite IRP-69 to investigate the effect of surface charge on adhesion; these results supported work by Park and Robinson that greater mucoadhesion will be achieved using an anionic molecule.

Promising results have also been seen with chitosan microparticles, originally designed to deliver amoxicillin for the treatment of *H. pylori*, the " Stomach specific delivery" system was investigated (Shah et al., 1999) and (He et al., 1998). The former study concentrated more on drug release from the microparticles and so mucoadhesion was theorised but not measured, however the latter study, characterised the mechanism of adhesion. He et al., (1998) showed chitosan's potential by investigating its interactions with mucin, showing that mucoadhesion was dependent on charge and that strong interactions between the chitosan and mucin were present. Although the *in-vitro* performance of chitosan is promising, gastric retention *in-vivo* has been shown to be irreproducible (Sakkinen et al., 2006). Using gamma scintigraphy Sakkinen et al., observed a wide range of gastric emptying times in humans from 1.25-2.5 h for 45 and 90% chitosan loaded microcrystalline cellulose microparticles. Also investigating the small intestine as a potential site for mucoadhesion the study used, (in conjunction with scintigraphy images) the bioavailability of furosemide as a marker for retention, postulating that increased residence in the stomach or upper small intestine would increase the bioavailability of the drug due to its narrow absorption window. No increase in furosemide was seen in the blood plasma, also no delay in transit over the control was observed leading the authors to conclude that mucoadhesion had not occurred.

Other approaches to mucoadhesion in the small intestine have yielded similar results. Mucoadhesive microspheres of polyglycerol esters of fatty acids were prepared by spray drying tetaglycerol pentasterate and tetraglycerol monostearate and spray coated with carbopol 934 (Akiyama et al., 1995). *In-vivo* testing in male Sprague-Dawley rats demonstrated strong gastric retention with 78.3% of microparticles still present in the stomach after 1 hour compared with 20.7% remaining for a non-mucoadhesive control.

Small intestinal transit however was less promising, with both mucoadhesive and non-mucoadhesive formulations exhibiting the same transit times.

Enhancement of mucoadhesive polymers such as polyacrylic acids, chitosan and sodium alginates, is a new area of mucoadhesion research that is gaining popularity. The enhancement takes the form of the addition of a thio group to the main polymer back bone with the intention of creating a less generalised polymer/mucin interaction; as such these polymers have been dubbed second generation mucoadhesives. It is theorised that enhanced mucoadhesion is created by the polymers ability to form covalent bonds in conjunction with the non-covalent bonds seen with un-thiolated forms of the polymer (Bernkop-Schnurch et al., 2000). A disulfide bond is formed between thiomer and mucus by either oxidation of the thiol group or thiol/disulfide exchange reactions (Bernkop-Schnurch, 2005).

Comparisons between the first and second generation mucoadhesives gives some interesting results (Figure 1.4) When thiolated and non thiolated polyacrylic acids were investigated *in-vitro* the thiolated forms exhibited significantly greater mucoadhesion in low pH. The improved adhesion however is diminished at higher pH and disappears all together at pH 7-8, suggesting that although the new generation show increased promise for proximal GI tract mucoadhesion this is not the case for the distal GI tract.

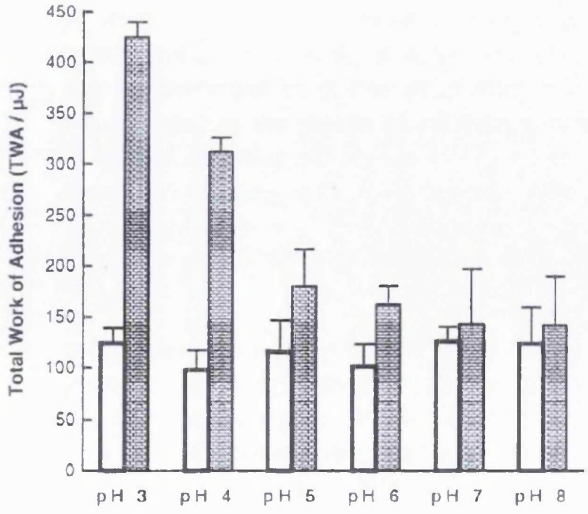


Figure 1.4 Comparison of work of adhesion for thiolated polyacrylic acid (Solid bars) with non-non-modified (open bars) polyacrylic acids (Guggi et al., 2004).

Assessment of mucoadhesive polymers in the distal gastrointestinal tract is an under researched area. Most investigations will use excised tissue to perform *in-vitro* investigations when designing adhesive systems specifically for the colon. For example, microparticles containing azo-crosslinked polyacrylic acid has shown positive *in-vitro* adhesion to colonic mucosa but has not been tested *in-vivo* (Kakoulides et al., 1998) Likewise, more recently, chitosan films for the treatment of colorectal cancer were investigated (Haupt et al., 2006). Although the degradation of the system and drug release were tested *in-vivo,* the mucoadhesion test was performed *in-vitro.* The lack of previous research into the *in-vivo* performance of mucoadhesive systems is likely due to the complexities of delivery, particularly with difficulties associated with limiting adhesion to just the distal intestine.

1.5.4. Rationale for the use of carbomers

The mucoadhesive polymers used within this study are a form of cross linked acrylic acid which are collectively known as carbomers. Carbomers are synthetic high molecular weight polymers of acrylic acid cross linked with either allylsucrose, allyl ethers of pentaerythritol or divinyl glycol (Figure 1.5)..

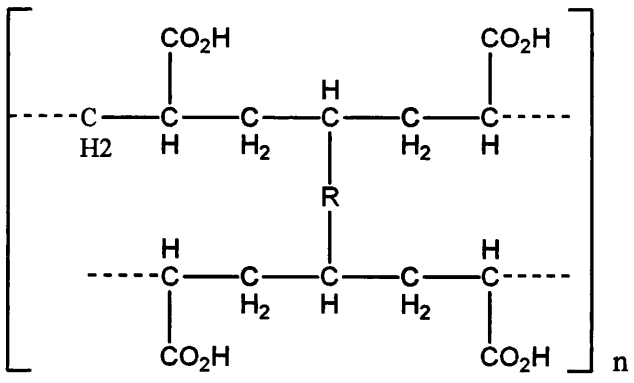


Figure 1.5 Structure of carbomers. R= allylsucrose, allyl ethers of pentaerythritol or divinyl glycol

Carbomers are white coloured, fluffy, acidic, hydroscopic powders. In water they disperse to form acidic colloidal dispersions (pH 2.7-3.5 for a 0.5 % w/v and 2.5-3.0 for a 1%w/v solution) of low viscosity which upon neutralisation will produce highly viscous gels. They are used commonly in industry as emulsifying agents, gelling agents,

suspending agents and tablet binders and more recently have undergone extensive investigation as potential mucoadhesives (Koleng et al., 2003). Carbomers are known mucoadhesives, as hydrophilic polymers they readily ionise at pHs above their pKa of 5.5 and form gels which are capable of interacting with the mucin. The effect of various formulation processes on the mucoadhesive properties are unknown

Mechanisms of mucoadhesion were discussed earlier; briefly it can be described as a physical or chemical bond between the polymer and mucin. Tensile testing of a variety of potential polymers by Smart et al., (1984), (Park et al., 1985), and Jackson et al., (2001) have shown carbomers to be a lead mucoadhesive material (Figure 1.6) As swellable anionic polymers carbomers are strong mucoadhesives (Park at al., 1984). Formation of a gel layer is fundamental to their mucoadhesive properties. As such the pH of the surrounding environment is important as this determines the degree of ionisation of the acrylic acid backbone (Ch'ng et al., 1985). Once ionized a carbomer gel layer will penetrate the mucus layer (Tamburic et al., 1997), where its mucoadhesive strength will be influenced by the extent of interpenetration between polymer and mucin, which itself is influenced by the expanding nature of the polymer network and the availability of suitable sites for hydrogen bonding (Leung et al., 1990) .

Four carbomer polymers were chosen for this study, three of which are identified by the commercial name Carbopol (CP), the fourth, polycarbophil (PC), these were CP 974 (PNF), CP 971 (PNF), CP 980 and PC AA-1 (USP) All the polymers used are intended for pharmaceutical applications, with CP 974, CP 971 and PC AA-1 specifically designed for oral mucosal mucoadhesion (Noveon, 2002), while CP 980 is a topical grade, is the most efficient thickener of all the grades has shown good performance as an ocular adhesive (Ceulemans et al., 2002)..

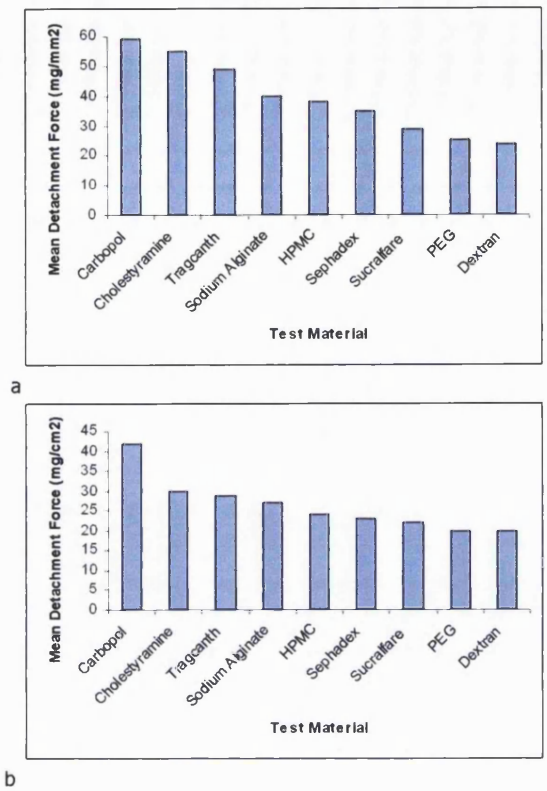


Figure 1.6 Summary results of Tensile testing of potential mucoadhesive polymers from a) human gastric mucosa and b) porcine gastric mucosa (Mean Peak Detachment Force (mg/mm) adapted from Jackson et al., (2001)

1.5.5. Scope of this research

The development of novel materials for distal GI drug delivery such as pH sensitive or bacterially degraded polymers have opened up the colon as a delivery site, particularly desirable for pH sensitive drugs, proteins or vaccines due to the near neutral pH. Unfortunately, the limited water content, most recently valued at 11 ± 26mL in the fed state and 13 ± 12mL in the fasted (Schiller *et. al.*, 2005) means that the absorption of such drugs are problematic. In principle, incorporation of mucoadhesive polymers to a dosage form will bind it physically and chemically with a mucin surface, retaining it wherever the interaction occurs. Although colonic transit times are much slower than small intestinal they are more variable (17-72 hours) (Hardy et al., 1985), normalisation of transit time by retention of the dosage form will increase the predictability of colonic transit and potentially increase bioavailability.

The colon is a more suitable site for the application of mucoadhesion compared to more proximal regions. Transit is slower and the region is generally less stirred. Additionally the mucus lining of the colon has been shown to be thicker in rats (Atuma C. et al., 2001), (Figure 1.7) than other areas of the GI tract, thus provided mucoadhesive polymers with a substantial layer with which to interact.

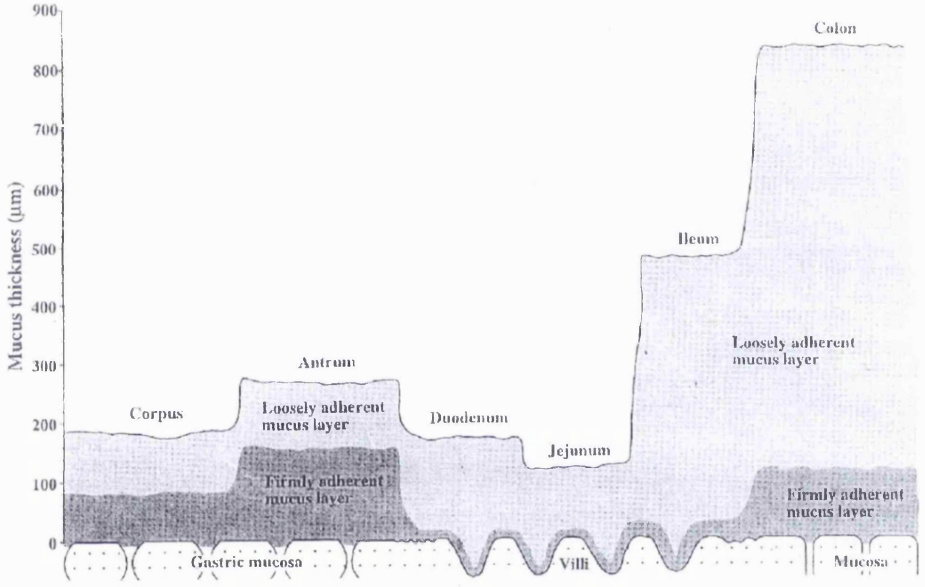


Figure 1.7 Mucin thickness in rat GI tract. Adapted from Atuma et. al.,(2001)

The loosely adherent mucin (secretory mucins) are high molecular weight and form intramolecular disulfide bridges resulting in viscous gels (Allen, 1981). It is with this layer that mucin/polymer interactions occur resulting in mucoadhesion. Additionally, within the colon the loosely adherent layer has been shown to be less affected by mucus secretory stimulus and as such the turnover is less in this region than any other in the GI tract (Lehr, 1991). As such the limiting step in prolonged mucoadhesion within the colon is likely to be mucin/polymer interactions and not mucin/ mucosa adherence.

1.6 Aims and Objectives

The aim of this work is two-fold: to assess the impact of dosage form formulation processes on the *in-vitro* mucoadhesion capabilities of carbomers and to determine whether carbomers polymers exhibit increase colonic residence *in-vivo*.

The objectives were therefore:

- To formulate a range of monolithic and multi-particulate dosage forms using carbomer polymers
- To assess the *in-vitro* mucoadhesion capabilities of these formulations
- To use gamma scintigraphic methods to determine the mucoadhesive capabilities of carbomers *in-vivo* using the dog model

Chapter 2

An assessment of monolithic mucoadhesive platforms

2.1 Overview

Since their initial use in ocular drug delivery (Patton et al., 1975) mucoadhesive polymers have been incorporated into many areas of drug delivery research. The choice of polymer used can be heavily dictated by the desired target region and its accompanying physiology, however the mechanism of the adhesion will largely be the same. As discussed in chapter 1, carbomers are established as mucoadhesive polymers and have responded well to *in-vitro* testing, however their *in-vivo* success is still a matter of debate. With the exception of azo cross linked acrylic acids (Kakoulides et al., 1998), few attempts to date have looked at *in-vitro* testing of orally delivered mucoadhesive systems to the colon. Certainly no attempts to evaluate the colonic mucoadhesion of any orally delivered polymer or formulated system *in-vivo* have been made. Additionally, studies tend to concentrate on testing of the polymers and not formulated systems.

Therefore, the work discussed in this section pertains to formulation and characterisation of monolithic dosage forms containing carbomers with a view to application in the colonic environment. Tablets film coated with carbomer aqueous solutions have been produced, as have two matrix formulations, the first prepared by direct compression, the second via wet granulation with an ethanol and water mix. As well as containing the polymers at a range of levels, the tablets produced needed to be of sufficient tensile strength to withstand manipulation and handling and they must have acceptable friability and dissolution profiles. As such they were suitably tested to ensure conformity. The effect of the grade of carbomer, carbomer loading and the effect of granulation have also been investigated, particularly with respect to tensile strength and drug release.

Previously discussed was the fact that within the literature the terms mucoadhesion and bioadhesion are used interchangeably and that bioadhesion generally applies to any material which binds, chemically or physically with a biological surface where as mucoadhesion is a more specific term and relates to adhesion between the material and mucins or the mucus layer (Park et al., 1984). The tests described within this chapter investigate the force required to detached the mucoadhesive platforms from an artificial surface (glass) and as such are measurements of adhesion, from mucin (mucoadhesion)

and their interaction with a mucus layer. The effect of carbomer grade, loading and the pH of the surrounding environment on adhesion will be investigated as will the effect of formulation of carbomer polymers into oral drug delivery platforms.

2.2 Monoliths

2.2.1 Tableting

Solid dosage forms have proven to be popular with patient groups and researchers alike; because of their ease of administration (for the former) and increased stability (for the latter) they are the most reliable and convenient method for systemic and local administration of drugs. Additionally, unlike emulsion and liquids, solid dosage forms are less likely to be subject to bacterial contamination. Commonly taking the form of granules, tablets or capsules, solid dosage forms have, until recently, been designed to release the active in the stomach or proximal small intestines, the invention of more sophisticated polymer technologies and greater understanding of *in-vivo* conditions has led to the development of modified release systems. To withstand and conform to industrial manufacturing processes these dosage forms are required to pass quality control tests, such as tensile strength, friability, content uniformity or disintegration.

The compaction of powders to make tablets was the earliest method of administering a solid oral dosage form and to date there are many forms of tablet compression machinery available, such as a single stroke press or a rotary press, however, whatever the machinery the compaction process usually follows the same steps:

- Lower punch drops allowing space for the particulate material to fill
- The upper punch descends, entering the fill space and confining the particles, continued lowering compacts the powder which aggregates and forms a cohesive mass
- The upper punch withdraws while the lower punch is simultaneously raised until it is flush with the die and the tablet is ejected

During this process a number of mechanisms occur which transforms the powder into a porous, coherent compact with defined shape. As the upper punch descends the particles are rearranged resulting in a closer packing structure, as the compaction load increases further movement of the particles is limited by either the packing characteristics or by

strong interparticulate friction. This leads to a reduction in volume accompanied by elastic or plastic deformation of the powder particles. Deformation leads to fragmentation of some particles which in turn find new positions within the compact further decreasing the volume, in turn, volume reduction results in particles being brought into close proximity with each other prompting the formation of interparticulate bonds. The bonding mechanisms involved can be classified into 5 types, i) solid bridges, ii) bonding due to movable liquids, iii) non-freely movable binder bridges, iv) attractions between solid particles and v) shape related bonding. Although all types are likely to contribute to the overall strength of the compact if the conditions are correct for their formation it is solid bridges, created by melting, crystallization, chemical reactions and hardening binders, and attraction between solid particles, molecular and electrostatic that are likely to be most important. Solid bridges that contribute to the overall strength of the compact can be defined as areas of real contact, as such their strength can be related to the bonding surface area, the effective surface area taking part in interparticulate attraction. Although not necessarily defined as areas of real contact, surface area is also important in determining the strength of molecular and electrostatic forces.

The area available for bonding is dependant on both the particle characteristics of the starting material and the changes caused by volume reduction, for example, fragmentation rather the plastic deformation is more efficient at producing large surface areas that promote interparticulate bonding. Conversely, materials which possess pronounced plasticity are effective means of creating large interparticulate attraction surface areas as they often possess small particles with high surface roughness which helps in promoting all types of the bonding mechanisms previously discussed. Therefore, materials which undergo a large degree of fragmentation can produce strong tablets due to the large number of contact points created during the compaction process. Less fragmenting materials however produce less contact points, as such, strong compacts will only be formed if strong attraction forces such as solid bridges could develop. Highly plastic materials can produce strong compacts due to an increase in the occurrence of weak distant forces (electrostatic) which significantly contribute to overall all tablet strength. Since the compaction of powder is a complex interplay between the occurrence of fragmentation and elastic and plastic deformation no one material is suitable for the production of tablets (Nystrom et al., 1996). As such, drug

particles are mixed with other excipients to aid the compaction process. These include: *Fillers or diluents* to make up most of the bulk in the tablet, *Lubricants,* easily deformable substances that provide a readily deformable film between the formulation and dies surfaces and prevents sticking of the powders to the punch and die surfaces. *Glidants*, to promote flow of powders to enhance die filling, *Disintegrants* to initiate break up of the tablets on contact with fluids serving to increase the surface area available for dissolution and *Flavours and colourants* to aid taste masking of bitter compounds and colour the tablets to aid in identification and patient compliance.

2.2.2 Direct compression

Direct compression is the simplest method of tablet manufacture but is however limited in that the number of pharmaceutical excipients that can be manipulated in this way is small. Direct compression involves dry mixing of powders to form a homogenous mix before tableting, powder particles must be large, possess good flow properties and the drug itself needs to be easily compactable. Since no heat or water is involved, direct compression can be useful in the tableting of drugs which are easily affected by temperature or moisture. In addition to this, tablets manufactured by direct compression tend to disintegrate into their primary particles which can lead to faster dissolution of drug due to increased surface area (Bolhuis et al., 2007). Direct compression is not however the most popular form of tablet production since differences in particle size and bulk density between the diluents and active can lead to inconsistent die filling and variation of results. As the largest component the diluent will have the most impact on the compaction of a direct compression tablet formulation, as such it is important to consider its properties. A good diluent would be cheap, possess narrow particle size distribution to limit segregation during storage or manufacturing process, it will be physiologically inert and be compatible with the active compound. Additionally it will possess good flow properties to ensure uniform die filling, have high bulk density and it should retain its structure after compression.

2.2.3 Granulation

Granulation is a process whereby the constituent powders are mixed to produce granules of uniform size and distribution, leading to enhanced flow and compaction properties, there are two forms, wet and dry. Dry granulation, involves mixing of powders under high shear and granules are made by either "slugging" or roller compression before sieving and compacting. Wet granulation involves mixing the primary powder particles with a fluid, commonly water, however, ethanol or isopropanol can also be used, to act as a binder to promote adhesion between the segregated powder particles to create granules.

By far the most commonly used process within the pharmaceutical industry, granulation is desirable for many reasons, it increases the size of the particulates and prevents segregation as granules are composed of all the primarily particles. This has the added advantage of improved flow characteristics as the size and surface properties in a homogeneous mix will be standardised. Granules are produced by promoting bonding between the particles within the mix, they must be strong enough to prevent the breakdown of the dried granules during handling and their strength is dependent on particle size, structure of the primary powder, moisture content and surface tension of any liquids added. Capillary and interfacial forces act on particles during the process, in the form of liquid bridges. Initially, immobile adsorbed surface liquid promotes particle adhesion by reducing surface imperfections and effective distance between particles, as the fluid volume increases its acts as a mobile liquid film. The liquid content in an agglomerate during wet granulation process has been defined and consists of four states, pendular, funicular, capillary and droplet with liquid content increasing from the pendular to capillary states. Nucleation, (particle to particle adhesion) will occur in the pendular state, further agitation increases the density of the mix to form the capillary state via the funicular state. Drying of the granules promotes the formation of solid bridges which are formed by powder deposited by the removal of liquid bridges. Sieving of the dry powder then serves to further reduce particle size distribution.

2.3 Film coating

Coating of pharmaceutical dosage forms has a long history. Traditionally sugar coats have been used for taste masking purposes and still to this day form one of the three main types of tablet coatings along with film and compression coating. From their taste masking origins tablet coatings have progressed to encompass a range of applications including drug protection from light, moisture or air, improving mechanical stability and more recently protection of the active from digestion and to enable modified release. The pharmaceutical dosage forms that can be considered as cores suitable for coating are varied, tablets, pellets, capsules and granules are the most predominant, however crystals and gums can also be coated. Regardless of the type of core, its suitability for coating is dependent on its hardness, shape, surface size, heat sensitivity and its tendency to react with the coating material. Of paramount importance in coating is the strength of the core as the dosage form needs to be strong enough to withstand the mechanical stress of the coating procedure. Resistance to water or solvents is another consideration to ensure the cores do not soften upon exposure to moisture. Shape of the core influences the film coating of the tablet in that for successful coating the cores need to be biconvex. Flat surfaces will stick together and the acute angle means the coat will not totally cover the surface edge, this is of particular significance when the coating purpose is modified release as this will promote leaking (Bauer et al., 1998).

During the film formation process two sets of forces operate between the film forming polymer and the tablet surface. The first, a cohesive force, occurs between the molecules of the film forming polymer, the second, adhesive between the polymer and the surface. For successful film formation high levels of cohesion must occur so that a strong bond is formed at the molecular level of the coating polymer. For the cohesive strength of a material to be high surfaces of the coating polymer must coalesce on contact (Voyutskii, 1963). Diffusion of molecules between and within film layers then occurs as the polymers are deposited in solution on the tablet surface or on an existing polymer layer. If sufficient cohesive force is achieved and diffusion and coalescence occurs the polymer structure will be restored to a uniform non-laminated matrix on the tablet surface. Processing factors such as contact temperature, contact pressure, coat thickness, coating solution concentration and viscosity can all influence the ability of the polymer to form cohesive forces (Forbes et al., 1958; Voyutskii et al., 1957).

Likewise, cohesion can also be affected by many formulation factors such as polymer chemistry, polymer structure, choice of solvent, plasticization and the addition of dispersed solids (Banker, 1966).

Polymer chemistry, in particular the shape of the molecules strongly influences cohesion as it determines the ability of the polymer strands to diffuse into one another and the strength of the resulting interacting areas. Regular structures will diffuse more readily than molecules with irregular stereochemistry; similarly branched molecules can exhibit stronger cohesion due to an anchoring effect in the diffusion layer (Voyutskii et al., 1957; Mark, 1942). The crystallinity of the polymer structure is also important; a highly ordered polymer represents the most cohesive structure available since the intermolecular forces which promote cohesion (hydrogen bonding) also promote crystallinity (Mandelkern, 1964). The choice of solvent used to deposit a film forming polymer on the tablet surface is dependent on the polymers solubility and both organic solvents and water can be used. The solvents convert the polymer into a homogenous, molecularly dispersed form (Gnamm et al., 1980). As the functional groups along the polymer chain become ionised in the solvent the charged groups repel each other and result in an un-stretching of the polymer chain. Simultaneously, molecules of the solvent interact with the charged groups of the polymer producing and increase in viscosity. The film formation process itself differs slightly depending on the solvent used, for solutions, the droplets of the spray spread on the surface and coalesce to a liquid film, evaporation of the solvent results in a thin deposit of solid material on the tablet surface. For dispersions the process is different, as the solvent evaporates the particles arrange themselves in the closest sphere packing, as drying continues remaining solvent is squeezed out and the particles coalesce (Bauer *et al.*, 1998).

Co-administered within the solvent along side the active polymer will be other excipients such as plasticisers. Plasticisers are added to modify physical properties of the polymer, specifically to reduce brittleness. In general they are high boiling point liquids with low molecular weight, which disperse homogenously in the solvent, and act by penetrating the chains of the film forming polymer. This reduces polymer to polymer interactions along the chain resulting in increased mobility of the polymer which in turn reduces the glass transition temperature (TG), and the film becomes plastic and able to coalesce (Banker, 1966).

2.4 Dissolution

Dissolution is a pharmacopoeial test used to gain an understanding of the rate at which the active compounds within a dosage form enters solution. Although the best test for this would be a suitable animal model and *in-vivo* study in man, this would be costly and immensely time consuming to use as an approach for high throughput screening of potential compounds. Therefore suitable alternatives have been sought to evaluate drug release *in-vitro*. Additionally, the need for such testing arose in the 1960's with reports of tablets which meet all the British Pharmacopoeial (B.P) requirements but fail to act as expected *in vivo*. The USP 24 currently lists 4 apparatus for testing of oral dosage forms in this manner;

USP apparatus 1- Basket apparatus

This consists of a closed rotating basket which is placed in a 1 L glass vessel which is also immersed in a water bath with a maintained temperature of 37 ± 0.5° C. The basket is attached to a shaft which keeps the dissolution medium under constant smooth rotation, the dosage form is placed in the basket before each test.

USP apparatus II – Paddle apparatus

USP II apparatus uses the same assembly as apparatus I, except the basket is replaced with a paddle in the form of a blade which provides the stirring motion. The dosage form is allowed to sink to the bottom of the containers before rotation commences. Floating dosage forms are placed in a sinker device, a small inert wire cage which sinks the dosage form to the bottom of the vessel. As with USP I apparatus the temperature is maintained at 37 ± 0.5° C in a water bath.

USP apparatus III – Reciprocating cylinder

The dosage form is placed in a set of glass cylinders (reciprocating cylinders) closed at the top and bottom with non-reactive screens. These cylinders are placed into a larger set of flat bottomed glass vessels within which the dissolution medium is retained. The reciprocating cylinders are attached to a motor and control device which can select and maintain the reciprocation rate at ± 5%. As with USP I and II apparatus the temperature is held at 37 ± 0.5° C in a water bath.

USP apparatus IV – Flow through cell

A transparent flow-through cell is mounted vertically into a reservoir which contains the dissolution medium. The medium is pumped through the flow through cell which contains a filter to ensure no non-dissolved particles escape. The apparatus is submerged in a water bath at 37 ± 0.5° C.

2.5 *In-vitro* mucoadhesive test methods

Early investigations into mucoadhesion sought the use of *in-vivo* models (Longer et al., 1985), for assessment of adhesive performance, however this was often subjective and identification of mechanisms of action were not possible. As a consequence *in-vitro* methods were needed to identify lead polymers and explain their binding actions. To date numerous methodologies have been devised, they can be divided into three main groups, those that measure detachment forces at the adhesive fracture joint, rheological methods (since the vast majority of mucoadhesive polymers are gel forming) and adhesion on a given substrate as a factor of time. It is important to note that within the literature there is no established method to test for mucoadhesion and none of the methods listed mimic (nor should they claim to) *in-vivo* conditions. Additionally, for all method types test parameters are first optimised to give the most consistent results before the test commences, as such internal controls are crucial for meaningful interpretation of results so trends can be compared as direct comparison of one set results from a particular test with another is not possible. Choice of test is dependent on the aims of the particular study and the type of formulation to be tested.

2.5.1 Measurement of adhesive fracture joint

This type of method concentrates on the measurement of a detachment force of a potential mucoadhesive from substrate (artificial, mucus or mucosa) in one of three ways, tensile testing (stress is applied perpendicular to the adhesive joint), shear testing (stress applied parallel to the adhesive joint) and peel testing (stress applied to a fine line at the edge of the joint) (Jackson et al., 2001).

One of the first approaches to measuring adhesive joint failure was the use of the Wilhelmey Plate method (Smart et al., 1984) (Figure 2.1). Effectively measuring the shear stress, a 1% aqueous solution of the polymer to be tested was produced and loaded onto a glass plate (C) by dipping it in the solution and allowing to dry. The platform (H) was then raised so as to immerse the glass slide in homogenised mucin (D) for 7 minutes after which time the platform was lowered once more. The maximum force needed to separate the slide from the mucin was recorded and expressed as a mean % adhesive force (i.e as a percent of the clean plate).

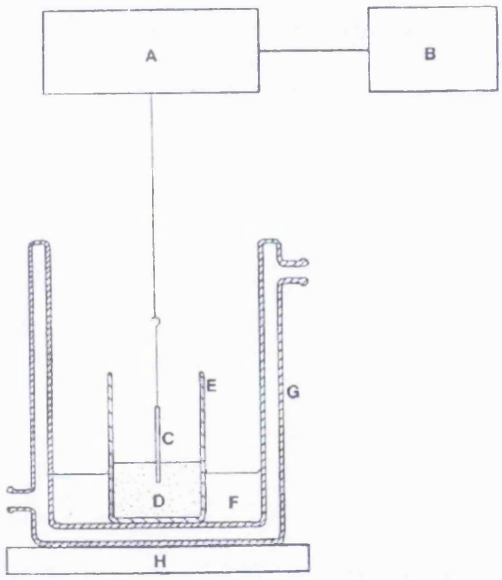


Figure 2.1 Diagram of an adapted Wilhelmy Plate apparatus. A = Microforce balance, B = Chart recorder, C = Glass plate D = 1mL homogenized mucus, E = Glass vial, F = Water, G = Water jacket at 20°C and H = Platform moving in a vertical direction (Smart et al.,1984),

Other similar studies are a variation of this theme; Leung et al., (1990) used a tenisometer to measure the tensile strength (mg/cm^2) of carbomer gels from a mucin surface. Varying parameters such as temperature, level of hydration and contact time the authors were able to draw some important conclusions about the mechanism of mucoadhesion and polymer mucin interactions.

Another adaptation to this form of methodology came in the addition of excised porcine and gastric mucosa (Jackson et al., 2001). Again looking at detachment force Jackson et al., investigated a range of polymers evaluating each for their mucoadhesive potential

base on the force per unit area available for adhesion (mg/mm^2) and compared the effect of substrate on the adhesive force.

The final adaptation to the Wilhelmy Plate method is the use of tensile testing apparatus to measure the work of adhesion. Total work of adhesion (TWA) is calculated as the area under the curve (AUC) of a force/ time graph (Guggi et al., 2004). A tenisometer was used to in conjunction with porcine intestinal mucosa to evaluate the work of adhesion of thiolated and non- thiolated polyacrylic acid polymers and their pH dependence.

Tensile testing is perhaps the one of the most popular methods, likely due to the fact that it is a direct measurement of adhesion. However, the application of force within these tests is particularly artificial as it is applied in one direction and only allows for a one time adhesion. *In-vivo* we would expect forces to act on the system from a multitude of angles as the dosage form collides with food or the wall of the GI tract and for the gel layer to erode. Also, *in-vivo*, it may be possible for the mucoadhesion to occur on more than one occasion, a factor not considered within the design of this type of test. That said, tensile testing is a good way to rapidly screen and rank potential formulations and, using this method, investigators have been able to obtain key insights into mucoadhesion and identify the most adhesive polymers.

2.5.2 Rheological measurements

These types of measurements involve the application of an oscillatory shear stress to a sample and the subsequent measurement of shear strain. If the response of gel systems to an oscillatory shear stress is different as the frequency changes, they are termed viscoelastic. At high frequencies the gel behaves like an elastic solid and as such once the stress is removed the material returns to is unstressed form. At low frequencies, viscous behaviour occurs and irreversible deformation is seen on application of shear stress. The energy stored and recovered per cycle of deformation is termed elastic modulus (G′) and represents the solid like aspect of viscoelastic material; conversely the energy lost per cycle is the viscous modulus (G″) and reflects the liquid like behaviour.

Analysis of these components can yield information on the structure of the sample, specifically rigidity and deformation of the system (Craig et al., 1994).

A correlation between the rheological behaviour of gels and adhesive properties of polymers has been seen (Tamburic et al., 1995). The peak detachment force of 2.5% w/v gels of Carbopol 934, 974 and polycarbophil AA-1 (neutralised and un-neutralised) from a 30% w/w mucin gel layer was determined along with G′ and G″. In doing this the authors showed that a greater force of detachment was seen from gels possessing a higher elastic component. Rheological evaluation is an indirect measurement of adhesion, as with tensile testing it has been shown to provide quantative data, particularly regarding polymer and mucus interactions and factors that can disrupt this interface. However, unlike tensile testing this method can not be used as easily for formulated dosage forms (e.g. tablet or pellets containing a mucoadhesive polymer) and as such is not as useful for screening potential systems.

2.5.3 Time dependent methods

Often used for liquids (Batchelor et al., 2002) or multiparticulate (Yohko et al., 1994) systems these forms of methods record the duration of adhesion, most commonly, from excised mucosa mounted on a rig and held at an angle (Figure 2.2).

Effectively a measure of elimination rather than adhesion this form of testing has been seen to show differences in adhesion arising from molecular weight differences, but can also show how retention of the adhesive system alters over time (Smart et al., 2003). This is the most subjective of the three test methods and as such little quantative data is gained from this test. It does however have the benefit of being able to assess initial adhesion in real time as unlike tensile testing or rheological methods the sample need not be prepared prior to testing (e.g held into contact with substrate). Additionally, the potential to alter more parameters of the test for example the pH of the washing fluid to simulated movement from one region of the GI tract to another is greater with this test method.

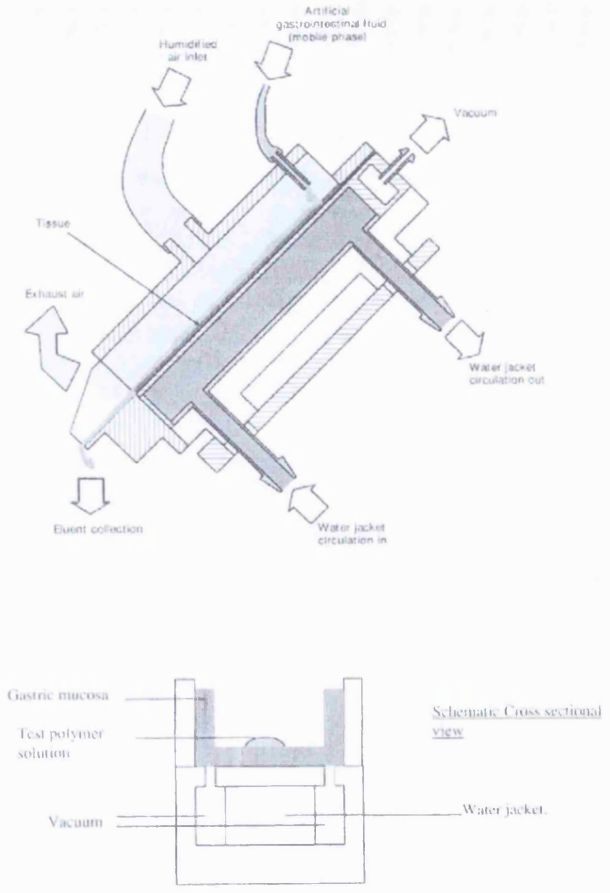


Figure 2.2 Diagram of mucosal retention apparatus (Smart et al., 2003)

2.6 Aims and objectives

The primary aim is to assess the potential of carbomer polymers as mucoadhesives, investigating the mechanism of mucoadhesion *in-vitro* and the role of mucin/ polymer interactions. Finally, to understand the effect of formulation of the polymers into deliverable monolithic dosage forms on their mucoadhesive properties.

Therefore the objectives are as follows

- Measurement of the force of detachment of compacts of 100% polymer from a glass surface to measure adhesion and from a mucin surface to assess mucoadhesion
- Investigate the role of mucin by altering the exposure time of the polymers prior to testing
- Formulate monolithic dosage forms and measure adhesion, mucoadhesion and the effect of mucin exposure

2.7 Materials

Paracetamol was supplied by Ellis and Everard, Essex, UK, it is a white, crystalline powder which is sparingly soluble (1 g/30 mL) in water and has a molecular weight of 151.17 g/mol.

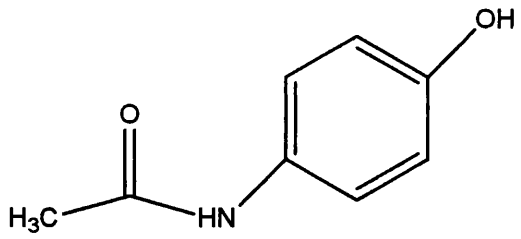


Figure 2.3 Structure of paracetamol

Carbopol 974, 980, 971 and polycarbophil AA-1 were received as gifts from Noveon, France. Lactose (Pharmatose - a wet granulation grade Lactose DC – a direct compression grade) and Glycerol monostearate (GMS) (Inwitor 900) were obtained from Huls AG, Marl, Germany. Triethyl citrate (TEC) was purchased from Lancaster

Synthesis, Lancaster UK. Magnesium Stearate, polysorbate 80 (Tween 80) and mucin (type II from porcine) was purchased from Sigma, St. Louis, MO. USA. Buffer salts and polyvinylpyrrolidone (PVP) was obtained from VWR International Ltd, Poole, UK. Where water is stated, de-ionised water was used.

2.8 Methods

2.8.1 Carbomer Film coating

Prior to coating, standard biconvex tablets (250 mg, 8 mm diameter, batch size 250 g) were produced as platforms for film formation. Paracetamol (8% equal to 20mg drug loading), lactose (86%) and PVP (5%) were added to a planetary mixer (Kenwood Chef KM200) and mixed together for 10 minutes with occasional stopping to remove material adhered to the side of the bowl. Deionised water, (approximately 8 g) was added drop wise under constant agitation until the powder mass was wet enough to sieve. The wet mixture was passed through a 500 μm and a 100 μm sieve and the granule fractions within this range were dried in an oven at 60° C. Once dry they were re-sieved (500-100 μm), weighed, and 1% (of total weight) of magnesium stearate was added and mixed for 5 minutes on a roller mixer.

Preparation of carbomer solutions

All coating solutions were aqueous and were formulated for all four of the carbomer grades, excipients were used in the quantities shown in Table 2.1. All mixing was performed at room temperature for 20 minutes with the exception of solution type C. Here the GMS and polysorbate (Tween 80) were mixed together with 30mL of water and heated to 85°C for 20 minutes to emulsify the GMS, it was allowed to cool to room temperature before the other components were added.

Table 2.1 Composition of film coating formulations (per 200 g of solution) Formulation type A: Carbomer only, B: Carbomer + plasticiser, C: Carbomer + plasticiser and anti-tack agent.

	Material weight (g)			
Formulation	**Carbomer**	**TEC**	**GMS**	**Tween 80**
A	2	0	0	0
B	2	0.2	0	0
C	2	0.2	0.01	0.004

Film coating was performed using a Strea-1 fluid bed film coater, the parameters are outlined in Table 2.2. The coating solutions, under constant stirring, were conveyed to the nozzle of the Strea-1, located at the bottom of the chamber, via a peristaltic pump. Coating was undertaken for predetermined time intervals (5, 10, 15 and 20 minutes) after which time coating was paused, the tablets were removed from the chamber, weighed and a small sample of approximately 20 tablets retained before coating was continued.

Table 2.2 Summary of operational parameters for the Strea-1 fluid bed film coater

Parameter	**Setting**
Inlet temp (°C)	60
Outlet temp (°C)	40
Atomisation pressure (bar)	0.2-0.4
Flow rate	1.0
Fan Capacity	15

2.8.1.1 Film Casting

Aqueous solutions of CP 974 (1%) with 0 %, 10 %, 50 % and 100% TEC (weight with respect to weight of solid content of CP 974) were made and 40ml of solution cast onto Teflon dishes (round, r= 4.5 mm). The films were dried at room temperature for 12 hours and then in an oven at 60° C for 4 hours. Visual assessments of the films were performed. Although due to their adhesive qualities films produced could not be

removed from the Teflon plate this method was used to establish the lead formulations to use in the Strea-1 fluid bed film coater.

2.8.2 Carbomer Matrix Tablets

2.8.2.1 Formulation of carbomer direct compression tablets

Lactose (DC), paracetamol and carbomer (0-20%) were added to a 500g jar (Table 2.3) and mixed for 20 minutes on a Turbula mixer, pausing frequently to remove any powders adhering to the side of the glass jar. After this time 2.5g (1% weight of total powder mix) of magnesium stearate was added and mixing was continued for a further 5 minutes on a roller mixer. The powder was transferred to the hopper of a Manestry F3 press and compressed into 250mg, 8mm concave tablets.

Compacts of 100% carbomer were produced as positive controls for tensile testing. Due to the poor flow properties of carbomer powders the compacts could not be produced in the conventional way. However, in the interest of standardisation of the test materials it was decided to produce compacts of as similar weight, dimensions and tablet properties as the matrix tablets as possible. As such, 250mg of carbomer powder was filled by hand into the die space of the Manestry F3 tablet press. To produce compacts of the required weight and thickness it was necessary to add the powder in four portions. After the addition of each quarter the upper punch was lowered by hand by completing one half turn of the cam wheel, this had the effect of compressing the powder and creating free space in the die for the next portion without raising the lower punch and expelling the powder. Once all of the powder was added to the die space the cam wheel of the press was turned through one complete revolution compacting the last portion of powder and raising the lower punch to allow retrieval of the newly formed compact.

Table 2.3 Formulation summary for 250mg tablets containing varying levels of carbomer

	Quantity (%)					
Material	**0 % carbomer**	**1 % carbomer**	**5 % carbomer**	**10 % carbomer**	**20 % carbomer**	**100 % carbomer**
paracetamol	8	8	8	8	8	0
lactose (DC)	91	90	86	81	71	0
Carbomer	0	1	5	10	20	100
Magnesium stearate	1	1	1	1	1	0

2.8.2.2 Formulation of Carbomer Granulated Tablets.

Granulated tablets (250 mg) were produced by the addition of paracetamol (8%), lactose (81%) (pharmatose), PVP (5%) and carbomer polymer (5%) to a planetary mixer mixing bowl and allowed to mix for 10 minutes. After this time the wetting solution (ethanol and water (75:25)) was added drop-wise as required. Mixing was paused periodically to remove any powders adhering to the side of the bowl and to test the consistency of the mix. Care was taken to ensure that the powder mix was exposed to air as little as possible to limit solvent evaporation. The resulting granules were sieved (500-100μm) and over sized and undersized granules were discarded. The granules were left at room temperature for 1 hour to allow the ethanol to evaporate and then placed in an oven at 60°C until weight loss was no longer observed. The remaining granules were weighed and 1% of the dry weight of magnesium stearate was added and mixed on a roller mixer for a further 5 minutes. The granules were compressed on a Manestry F3 tablet press using 8mm concave punch and die set.

2.8.3 In-vitro mucoadhesive testing

Preparation of mucin solution

Dried porcine stomach mucosa, 0.5g was added to 45.5 g of pH 6.8 phosphate buffer and mixed for 1 hour until all the mucin had dissolved. Mucin is approximately 95% water and 0.5- 5% lipid and glycoproteins (Rathbone et al., 1991). A 5% mucin solution

was initially used as this would be more representative of *in-vivo* conditions, however, when used the adhesive fracture joint was seen to be between the mucin solution and the glass dish and not the mucin and polymer.

The peak detachment force of the formulations prepared previously were determined using two tensile testing apparatus, the Texture Analyser (TA) (Stable Microsystems) and the Instron technical tester (Instron Ltd). Identical methods were used for both pieces of equipment and where possible test parameters were standardised.

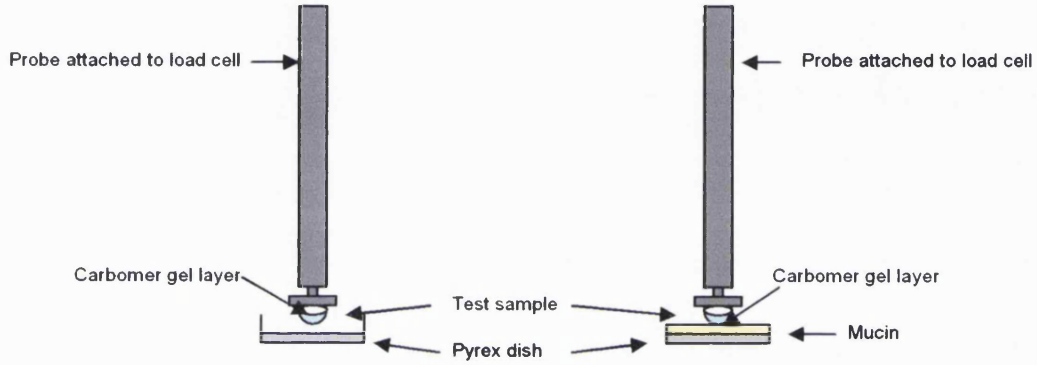


Figure 2.4 Diagrammatic representation of test set up for adhesion and mucoadhesion studies

The formulations under test were attached to the probe with adhesive tape which in turn was attached to a load cell. The probe was lowered sufficiently to submerge the sample in the hydration media (0.1M HCl or pH 6.8 phosphate buffer) for predetermined time periods of 1, 2 and 4 minutes. Post hydration the probe was raised and the vessel containing the buffer removed and replaced with an empty glass Pyrex dish or the same vessel containing 3mL of 1% mucin solution (n=5).

The data was analysed using parametric tests; results were analysed for significant differences using one-way analysis of variance (ANOVA), with post-hoc analysis using Tukey's test. All tests were carried out using SPSS Version 14.0 statistical software package.

2.8.3.1 Mucin Exposure

The samples were loaded as before and hydration of the sample under test was achieved by submersion in pH 6.8 phosphate buffer for 4 minutes The test procedure was as before, however the hold time of the probe in mucin was varied, the samples were exposed to the 1% mucin solution for 1, 2 and 4 minutes and the detachment force (N) and work of adhesion (mJ) were recorded.

2.8.4 Dissolution Testing

Drug release from the platforms was determined by USP II paddle dissolution apparatus (PTWS, Pharma Test. Hamburg, Germany). Tests were conducted in 900mL of dissolution media maintained at 37 ± 0.5 °C, with a paddle speed of 50 rpm. Dissolution medium was sampled automatically at 5 minute intervals by an in-line UV-Vis spectrophotometer (Cecil 2020, UK.) at a wavelength of 242nm. Sink conditions were maintained throughout the test since the paracetamol concentration was never greater than 10% of its saturation solubility.

2.8.4.1 Preparation of dissolution medium

Phosphate buffer pH 6.8

Potassium dihydrogen orthophosphate (6.8g) and 77mL of 0.2 M sodium hydroxide solution was added to 800L of water and mixed for 10 minutes. After this time the solution was added to a 1L volumetric flask and made to the mark. The pH was tested and adjusted to pH 6.8 with 1M sodium hydroxide

2.8.5 Tensile strength.

Ten tablets from each formulation were tested, the thickness of the tablets were individually measured before the samples were placed in an Erweka TBH 200 Tablet Tester. The test samples were placed individually in the jaws of the Erweka which closed them applying a constant force until the sample broke, the jaws retract and the force at breakage is recorded in Newtons. From the thickness and force measurements the tensile strength was then calculated (Equation 2.2).

$$\sigma = \frac{2 \times F}{\pi D t}$$

Where F is the crushing force in N, D is the diameter of the tablets and t is the thickness in mm

Equation 2.1 Determination of tensile strength

2.8.6 Measurement of powder flow

The angle of repose was measured as an indirect measurement of powder flow. Carbomer powder (5g) was dropped through a funnel onto a 20mm flat base, the height of the resulting cone of powder was measured using callipers (n=3). The angle of repose was then calculated (Equation 2.3).

$$\text{Tan } \alpha = \frac{2h}{D}$$

Where α = angle of repose, h = height of the cone and D is the diameter of the block (mm)

Equation 2.2 Calculation of angle of repose

2.8.7 Tap density

Sufficient amount of material was poured into a 250 ml measuring cylinder to achieve a fill volume of approximately 50 ml. The mass (M) and pour volume V_0 were recorded. The cylinder was placed on the adaptor of a Vankel Tap Density Meter (Varian Ltd., Oxford, UK). The volume of the material was recorded after 100, 200, 300, 400, 500 and 1000 taps. The volume of material was after 1000taps (V_t) was used to calculate the tap density. The % compressibility (Carr's index was calculated) (Equation 2.3)

$$\text{Compressibility (\%)} = \frac{V_t - V_0}{V_t}$$

Equation 2.3 Calculation of Carr's index

2.8.8 Imaging of gel formation on tablet surface

To visualise the formation of gel on the surface of a tablet a 5% CP 974 DC tablet was adhered to a pyrex dish with adhesive tape. The dish was filled with 50mL of pH 6.8 phosphate buffer, sufficient to submerge the tablet and images of the gel formation were taken at 0, 5, 10 and 20 minutes.

2.8.9 Scanning electron microscopy (SEM)

Samples were placed on SEM stubs and fixed using carbon discs before being gold coated using a EMITEC K 550 sputter coater for three minutes at 40mA. The samples were then transferred to a Philips XL20 Scanning Electron Microscope for imaging.

2.9 Results and Discussion

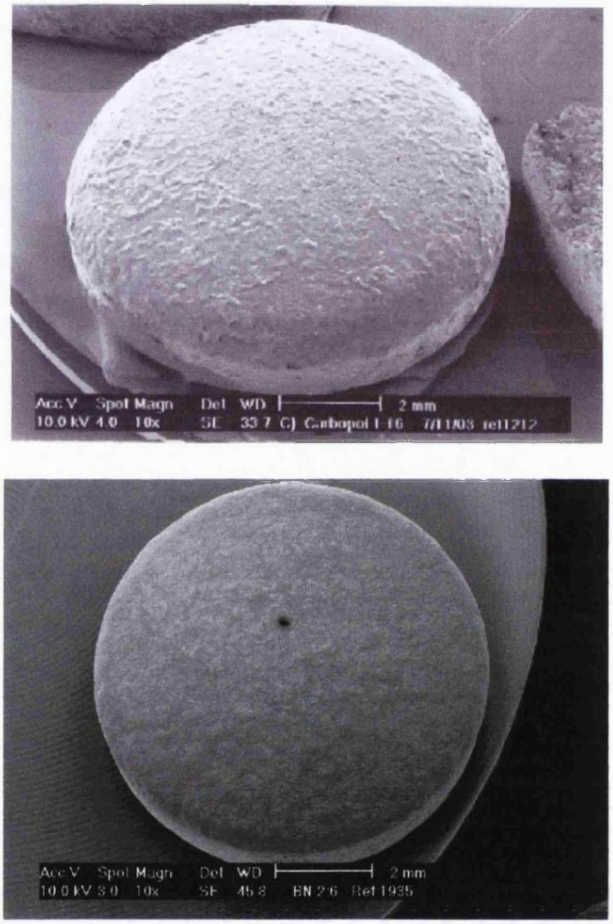
2.9.1 Carbomer Film coating

Initial film coating with carbomers proved to be problematic due to the gelling nature of polymers and the viscosity of their solutions which increased rapidly with concentrations above 1% w/w. Previous approaches to film coating with carbopol, for controlled release, found that an increase in viscosity of the solution caused an increase in geometric mean diameter of the spray mist (Muramatsu et al., 2000). It was found that the diameter remained small until the viscosity of the polymer solution reached 3000-6000 mPa, (a concentration greater than 1%w/w) and the solution was no longer suitable for coating, for this reason coating solutions within this study were set at 1% w/w level.

Attempts to film coat with carbomer solutions resulted in uneven distribution of the polymer on the tablet surface. The high viscosity of the solutions resulted in poor flow of the solution into the nozzle head which in turn was not easily atomised. As discussed earlier, for film formation to occur the solution must be allowed to spread across the surface of the core, this was not seen, instead the individual droplets of solution were randomly deposited on the surface (Figure 2.5). This unevenness caused cores to stick permanently together, a process known as twinning. As the tablets stick together the dynamic within the chamber is disrupted, as a result the tablet's movement in the chamber is reduced and they concentrate towards the bottom collecting solution which is not atomised, resulting in the eventual termination of the process.

It was decided that the addition of excipients that promoted film formation would help improve coating duration by facilitating distribution of the polymer on the tablet surface. A plasticiser, triethyl citrate (TEC) was added to the coating solution to promote film formation by interacting with the polymer chains increasing their mobility, which in turn lowers the Glass Transisition Temperature (T_G) of the polymer inducing a more plastic like behaviour and promoting greater diffusion of one polymer layer with another (Banker, 1965). Introduction of the plasticiser increased coating duration and improved reproducibility, however a substantial increase in weight compared to formulations without plasticiser was not seen, although visual inspection

(SEM) shows an improvement in polymer distribution on the surface of the tablet (Figure 2.5). The effect of plasticiser incorporation seemed to be greater for CP 974 and 980 grades, most likely due to the fact that these are more highly cross linked grades which possess more rigid structures in the first instance. Unfortunately however, agglomeration of the tablets still occurred and an overall weight gain was not improved.



a

b

Figure 2.5 SEM of Carbopol 974 coated tablet using a) 1% CP 974 solution at 30 minutes coating time, 0.8% weight increase and b) 1% CP974 + 10% TEC coated tablet at 20 minutes coating time, 0.6% weight increase. Magnification x 10

It was uncertain if the poor performance of the carbomer solutions was due to poor film formation or the adhesive properties of the polymers. To investigate the former films of CP 974 (the highest molecular weight of the four grades) were cast and visually assessed; the observations can be seen in Table 2.4. It was seen that although films were produced with as little as 10% plasticiser, holes and disruptions to the surface were seen, even with the incorporation of high levels of plasticiser (100% TEC, 1:1 ratio of CP to TEC). Additionally since the films adhesive qualities meant they could not be separated from the plate no mechanical tests could be performed.

Table 2.4 Film casting observations after casting films with CP 974 solution. A) no plasticiser, B) 10% plasticiser, C) 50% plasticiser, D) 100% plasticiser. Plasticiser expressed as % of solid weight of carbomer in solution

Formulation	Observation
A	Clumps of solid material deposited, no film formation
B	Film produced, rough surface with small holes apparent
C	Film produced, rough surface small holes apparent
D	Film produced, surface uneven with one half smooth the other rough with small holes

Conventionally, the effect of twinning can be reduced or eliminated by the addition of an anti tack agent such as glyceryl monostearate (GMS) or talc. Emulsified GMS was added to the formulation to act as a glidant to allow cores to smoothly pass over each other if they come into contact within the coating chamber. Addition of GMS showed some success as it allowed coating to proceed for longer time periods by delaying the onset of twinning, however, again a significant weight gain was not recorded.

A summary of weight gain and maximum coating times for all the solutions prepared and the effect of addition of TEC and GMS can be seen in Table 2.5

Table 2.5.Summary of weight gain (%) and coating duration times for 1% carbomer solutions prepared for film coating

Carbomer Grade	TEC (%)	GMS (%)	Post coating weight gain (%)	Coating duration (min)
974	0	0	0.56	10
980	0	0	0.18	15
971	0	0	0.31	20
AA-1	0	0	0.24	20
974	10	0	0.63	20
980	10	0	0.59	20
971	10	0	0.56	20
AA-1	10	0	0.35	20
974	10	5	0.63	40
980	10	5	1.03	40
971	10	5	0.55	40
AA-1	10	5	0.62	40

Once it had been established that film coating with carbomer polymers was possible and that anti tack excipients reduced agglomeration of core during the coating process an optimised formulation was sought. Again using CP 974, TEC levels were kept constant (10%) and two anti tack excipients utilised (GMS and talc) at varying amounts. The resulting weight increases and coating durations are shown in Table 2.6

Table 2.6 Weight gain (%) and coating duration for CP 974 + TEC and anti tack agents-GMS and Talc

Formulation				
TEC (%)	**GMS (%)**	**Talc (%)**	**Weight gain (%)**	**Coating duration (Min)**
10	0	0	0.63	20
10	5	0	0.61	20
10	50	0	0.91	30
10	0	5	0.76	30
10	0	50	0.92	50
10	0	100	1.46	50

Again, the addition of anti tack agents had some effect on the coating profiles however, twinning still occurred. The maximum coating duration was 50 minutes which produced the greatest weight gain of 1.46%, however since over 50% of the solids within the solution are non-mucoadhesive excipients, the actual amount of carbomer on the surface will be a lot lower.

Since the incorporation of plasticiser and anti-tack agents did not significantly improve the coating performance of the carbomer solutions a different approach was sought. Film casting showed that formation of films with carbomers was possible, however in practice coating with the solutions was not possible, it was proposed that this was due solely to their high viscosity. Theoretically, a reduction in solution viscosity should cause a reduction in spray droplet size which in turn would promote spreading of the solution on the core surface and promote coalescence. Since gel formation and hence viscosity of the polymers is dependent on dissociation of the carboxyl acid groups, acidification of the polymer solutions would lower the viscosity. Figure 2.6 shows the coating profile for two solutions of CP 974, the first with the pH adjusted to 1.9 (viscosity 7.78×10^{-3} Pa) with 5M HCL, the second, an unadjusted solution at pH 3.6 (viscosity 386 Pa). The drop in viscosity is caused by the degree of dissociation of the carboxylic acid groups in the polymer. Using the Henderson-Hasselbach equation the degree of dissociation can be calculated and is 6.5×10^{-10} and 3.27×10^{-8} for pH 1.9 and pH 3.6 solution respectively. At acidic pHs the polymer back bone is less ionised and as

such repulsion between the chain is reduced, resulting in a less viscous solution. Comparison of the two profiles shows that with the lower viscosity weight increase is initially greater than the unadjusted solution allowing a 1% weight increase in the first 5 minutes, after this time the increase in weight levels off and again after 20 minutes twinning occurs. The plateau seen at 1% level is potentially caused by loss of tablet core material as the low weight gain provides little protection from the mechanical forces involved in the coating process.

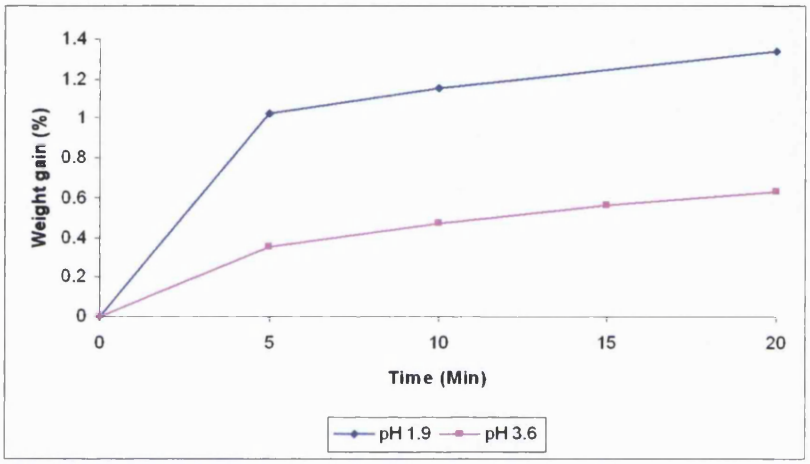


Figure 2.6 Comparison of coating profiles for CP 974 pH adjusted to 1.9 with 5M HCl and CP 974, unadjusted at pH 3.6.

Since production of mucoadhesive tablets in this fashion has not be undertaken previously it is not possible know the quantity of polymer required to obtain a suitably mucoadhesive tablet. A 1% polymer loading may be sufficient, however, it is reasonable to assume that were a greater weight gain achieved the resulting coat would at least be capable of producing a longer mucoadhesive affect if not a stronger one. Therefore, since it was not possible to film coat a tablet core with carbomer and achieve a weight gain of greater than 0.5-1% other strategies were investigated to increase the level of mucoadhesive polymer within the formulation.

2.9.2 Carbomer matrix tablets

Matrix systems were produced to increase carbomer content. Two approaches were adopted, direct compression of the powders and wet granulation. Powder flow is a key consideration for any formulation undergoing tablet formation; good flow results in

uniform die filling which in turn allows for uniform particle packing in the fill space and a constant volume to mass ratio, inducing low variability in tablet weight. Uneven powder flow can result in air becoming trapped with the powder which under some tableting conditions can lead to capping or lamination.

The ability of powders to flow freely is determined by the properties of their constituent particles, more specifically cohesion and adhesion. Although both can be considered to be the same phenomenon, the former is defined as interactions between similar surfaces such as the powder particles and the powder bed, the latter between two unlike surfaces such as the powder and machinery. Short range non-specific van der Waals forces are usually responsible for cohesive forces between particles, this is of particular concern with smaller particles. This is complicated by other attractive forces produced by surface tensional forces between adsorbed liquid layers at the particle surfaces and electrostatic forces (Bolhuis et al., 2007). Carbomers are flocculated powders with a particle size distribution of 2-7 μm, they are hydroscopic in nature, as such it would be expected that strong cohesive forces between the particles would result in poor powder flow. The angle of repose was measured for the four polymer grades and the results are shown in Table 2.7.

Table 2.7 Angle of repose of and Carr's index of carbomer powders

Carbomer Grade	Carr's index (%)	Angle of Repose (°)
974	23	50.95
980	22	36.12
971	25	45.93
AA-1	21	47.73

The angle of repose can be used as an indirect method of measuring powder flow, since it is dependent on interparticulate cohesion. If the cohesive forces between powders is high the measured angle will be high, likewise if the powders possess good flow properties the resulting angle will be less. Generally, powders with angle of repose greater than 50° will have poor flow characteristics, those with an angle of or close to

25° represent powders with good flow properties. Carr's index also gives an indication of powder flow properties, material with a Carr's index of 23-35 will exhibit poor flow. Having established the flow properties of the polymers, we can begin to explain the effect they will have on the tableting formulations. The greater the proportion of the powder mix given over to the carbomer polymers, the greater the effect this will have on the flow of the total mix, since the flow is poor, we can expect that powder mixes containing a greater amount of carbomer will also have poor flow. When tableting (direct compression) it was observed that achieving loading levels above 20% w/w carbomer was not possible as the increased carbomer levels resulted in poor and inconsistent die filling resulting from their poor flow properties. As such 100% carbomer compacts were not prepared in the conventional manner.

There are some steps that can be taken to improve powder flow such as altering particle size or distribution or particle shape, however these would need to be altered at the powder manufacturing stage and so were not viable here. The addition of more glident was investigated and formulations containing over 20% carbomer and up to 5% magnesium stearate were formulated, although tablets of sufficient weight uniformity and strength were still not possible to produce. Therefore a range of carbomer loading levels were chosen for the direct compression formulations with 20% as the maximum, these were 1, 5, 10 and 20%. The effect of the carbomer polymers on the properties of tablets produced was assessed by measurement of crushing strength, friability, thickness, tensile strength, weight uniformity and drug dissolution. Table 2.8 shows a summary of the parameters tested for each polymer grade at each loading level.

Carbomer loading on the strength of tablets can also be seen in Table 2.8, for most grades (PC AA-1 being the exception) the strength is reduced with a carbomer loading of 20 w/w. This can be linked to poor powder flow and inconsistent die filling. With three of the grades, CP 974, 980 and 971 an increase in tensile strength with an increase in polymer content from 1% to 5% was observed. This was not as expected, however it may be explained by the hydroscopic nature of the polymer and its known adhesive qualities. Carbomer solutions have been used as liquid binders in the past (Vilajato et al., 1986; Bayomi et al., 2001). The increase in tensile strength seen at 5% carbomer loading may be due to the polymer having a binding effect caused by a liquid layer on

the particle surface created by adsorption of moisture from the air. This layer will reduce the distance between particles within the mix which in turn increases the interparticulate bonding, since mechanical strength of the compact can be defined as a sum of all interparticulate attractions, we would then expect the tensile strength to increase.

Table 2.8 Summary of the effect of carbomer grade and loading level on tablet thickness, friability, crushing strength and tensile strength. Standard deviation shown in parentheses

	Carbomer grade and loading																			
	974					**980**					**971**					**AA-1**				
	1	**5**	**10**	**20**	**100**	**1**	**5**	**10**	**20**	**100**	**1**	**5**	**10**	**20**	**100**	**1**	**5**	**10**	**20**	**100**
Thickness (mm)	4.81 (0.14)	4.93 (0.08)	4.85 (0.04)	4.67 (0.07)	4.71 (1.17)	4.64 (0.20)	4.54 (0.04)	4.66 (0.02)	5.16 (0.03)	5.27 (0.13)	4.43 (0.04)	5.06 (0.11)	4.61 (0.04)	4.90 (0.08)	5.20 (0.56)	4.51 (0.02)	5.12 (0.06)	5.22 (0.03)	4.49 (0.10)	5.34 (0.01)
Friability (%)	0.38	0.43	0.19	0.22	2.90	0.27	0.11	0.21	0.18	5.10	0.35	0.29	0.28	1.45	3.70	0.28	0.77	0.69	0.16	9.1
Crushing strength (N)	91.5 (19.5)	139.8 (13.3)	142.2 (28.1)	101.8 (22.3)	100.6 (23.5)	100.7 (11.2)	174.8 (25.6)	113.8 (30.9)	71.5 (6.0)	90.3 (12.0)	83.8 (5.6)	149.8 (26.9)	185.3 (42.5)	60.2 (19.9)	99.3 (6.7)	85.8 (8.10)	84.9 (26.5)	97.8 (33.2)	107.0 (45.5)	78.0 (7.81)
Tensile strength (MPa)	1.51 (0.30)	1.80 (0.18)	2.25 (0.45)	1.73 (0.37)	1.66 (0.89)	1.73 (0.25)	2.45 (0.34)	1.94 (0.52)	1.10 (0.09)	1.36 (0.18)	1.51 (0.10)	1.88 (0.32)	3.12 (0.72	0.98 (0.32)	1.44 (0.86)	1.51 (0.14)	1.06 (0.53)	1.49 (0.50)	1.90 (0.78)	1.62 (0.11)

Granulation was sought as a way to improve the poor flow seen with the direct compression formulations as this increases particle size and reduces size distribution. Wet granulation with carbomers however is difficult since they form gels when exposed to water, additionally, their adhesive properties are attributed to their gel behaviour.

Wet granulation with carbopols and polycarbophils has been shown to be difficult (Prudat-Christiaens et al., 1996). Wet granulation with CP 971 and EX-55 (later renamed to CP 980) was undertaken using water, the investigators concluded that a carbomer loading above 10% was not possible as a sticky paste is produced. There are four states of moisture distribution during a granulation process. The first three, Pendular, Funicular and Capillary, are stages of progressive increases in the amount of fluid between particles, increasing from Pendular to Capillary states (Figure 2.7). If the droplet stage is reached the powder mix is too wet to granulate.

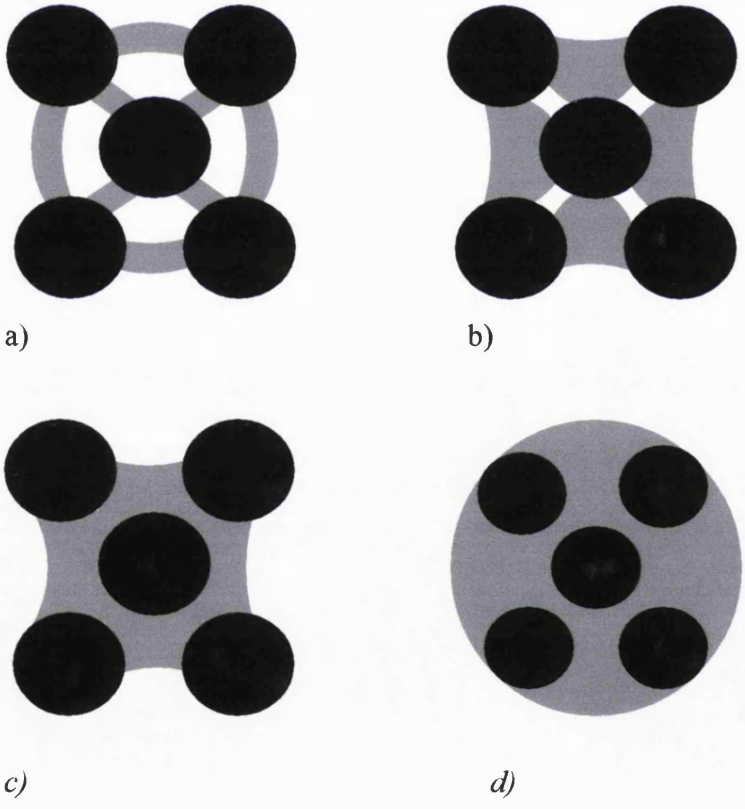


Figure 2.7 States of liquid content during wet granulation a) pendular state b) funicular c) capillary state d) droplet state. Adapted from Augsburger et al., 1997

For the production of strong granules, granulating fluid distribution needs to be in either the Funiclular or Capillary stages. Above 5% carbomer loading level a suspension of the constituent powders is formed, partly with the granulating fluid and also partly with the viscous gel resulting from the carbomer wetting (Prudat-Christiaens et al., 1996).

The purposed strategy was to granulate with ethanol, as this may be beneficial when using carbomers (Noveon, 2000). Granulation with a 75:25 ratio of ethanol (96%) and water proved to be possible and easier than with just water alone. The rationale for using an ethanol and water mix can be explained by the mechanism of gelling of the polymer. Carbomers are weak acids (pKa 5.5), in the presence of water the acid dissociates, ionising the resin with negative charges along the polymer chain (Figure 2.8). Repulsion between these charges causes an unfolding of the structure, while the cross linking results in a 3 dimensional matrix, finally resulting in a viscous gel (Sanz Taberner et al., 2002). In the presence of an ethanol and water mix the degree to which ionisation of the carboxyl group occurs is reduced as the hydroxyl group in ethanol will not under go reduction to form a H_3O^+ cation easily.

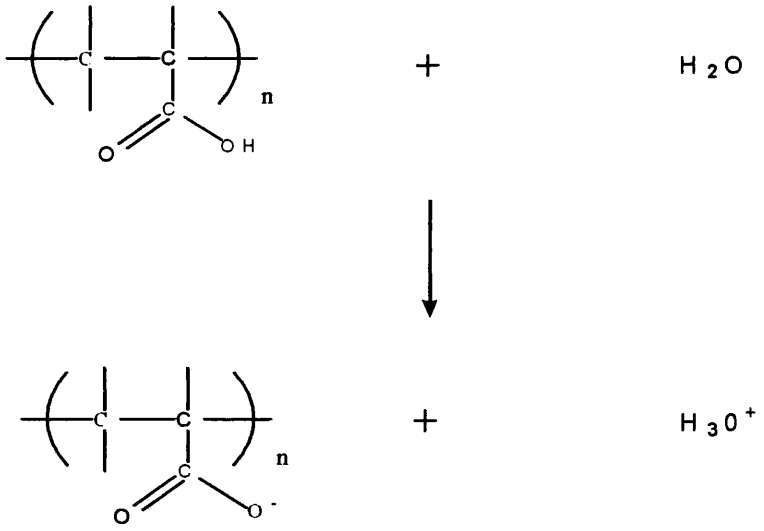


Figure 2.8 Ionisation of acrylic acid in aqueous conditions

However a maximum loading of only 5% w/w carbomer was achieved. Wet granulation of powders with ethanol is known to produce weaker more friable tablets. To understand this we must first discuss the mechanism of formation of bridges that occurs during the granulation process. Addition of liquid to the powder mix during granulation promotes the distribution of the binder throughout the mix and between the powder particles. When wet, powders are held together by a combination of surface tension

forces of the liquid/air interface and hydrostatic suction pressure in the liquid bridge. Solid bridges consisting of a combination of the binding polymer and constituent powder replaces the liquid bridges during the drying stage. Within an ethanol system, the constituent parts which form the solid bridges are different, solid bridges formed in the water system consisted of the both PVP and constituent powder particles, however, solid bridges formed in the ethanol system are formed from mostly the primary powder particles. This is due to the solubility of binders such as PVP in ethanol and the evaporation of ethanol which can occur before complete mixing is achieved (Bika et al., 2005).

The properties of the 5% carbomer granulated tablets can be seen in Table 2.9. Comparison of these data and those on Table 2.8 shows that the granulation process with ethanol did not affect the tensile strength or friability of the tablets as expected, therefore, manufacture of carbomer containing tablets in this way is viable. A carbomer loading of 5% was chosen as the only loading level for the granulated tablets. This was in most part due to the difficulties of increasing the content and successful mixing and sieving of the powders and partly as it provided a granulated formulation of equal carbomer loading as the strongest DC formulations.

Table 2.9 Summary of tablet properties for granulated tablets containing 5% carbomer

	Carbomer Grade (5% Loading)			
	974	**980**	**971**	**AA-1**
Thickness (mm)	4.51 ± 0.11	4.61±0.087	4.96 ± 0.10	4.62 ± 0.05
Friability (%)	0.78	0.36	0.35	0.15
Crushing Strength (N)	102.3± 2.83	139.4 ± 3.50	110.7 ± 3.62	108.6 ± 3.57
Tensile Strength (MPa)	1.80 ± 0.06	2.41 ± 0.07	1.79 ± 0.06	1.87 ± 0.07

The three formulations produced allow for comparisons of the formulation techniques used and their affects on the carbomer polymer. Evaluation of test results from the coated systems and DC matrix tablets may provide important information regarding the effect of location of the mucoadhesive polymer within dosage form. For example a

1%w/w loading may be more mucoadhesive than a 5% w/w loading if all of the polymer is located at the surface of the tablet. Additionally, comparisons of the granulated and DC matrix formulations may help in understanding the consequences, if any, of pre-wetting the polymer prior to compaction.

2.9.3 Drug release

Drug release from the matrix tablets was investigated in dissolution media representative of the pH in the colonic environment (pH 6.8 phosphate buffer) and in the stomach (pH 1.2 HCl) to provide a comparison. During dissolution testing the ingress of fluid into the polymer invokes a change from a glassy to a rubbery state leading to swelling. As the individual polymer chains absorb water the glass transition temperature lowers and the end to end distance and radius of gyration of the polymer chains expands, this is evident macroscopically by the presence of a gel layer. The formation of this layer is controlled by the characteristics of the solvent fluid, in this instance, the dissolution media (Siepmann et al., 2002). Since carbomer polymers are weak acids with pKa 5.5 the rate and extent of gel formation is dependent on pH. At higher pHs the level of ionisation within the polymer backbone is increased leading to greater repulsion between polymer chains, and greater unfolding of the structure (Sanz Taberner 2002).

To help visualise the effect of the gelation on drug release images of a 5% CP 974 DC tablet were taken in situ after exposure to pH 6.8 phosphate buffer (Figure 2.9). During the dissolution process drug molecules leave the solid state and enter into solution, for this to occur fluid needs to diffuse in and fluid and drug diffuse out through out this layer. There are three possible mechanisms that can lead to proper release of drugs from polymeric matrices. The first is fickian release which results from the diffusion of the drug through the outside gel layer (Korsmeyer et al., 1983). The second, non-fickian or anomalous transport and the third, zero order or case II mechanism (Maroni et al., 1995).

The rate of release is dependent on the strength of the gel layer formed and is an interplay between diffusion of the drug through the swollen matrix and erosion of the gel layer. Gel erosion occurs at the outer surface of the layer where the polymer is

diluted to such an extent that the chains disentangle completely and the polymer loses its structural integrity (Maggi et al., 2002). If low viscosity gels are formed erosion of the polymer is principle in controlling drug release, conversely if high viscosity gels are formed then polymer dissolution is negligible and diffusion controls the process (Conti et al., 2007).

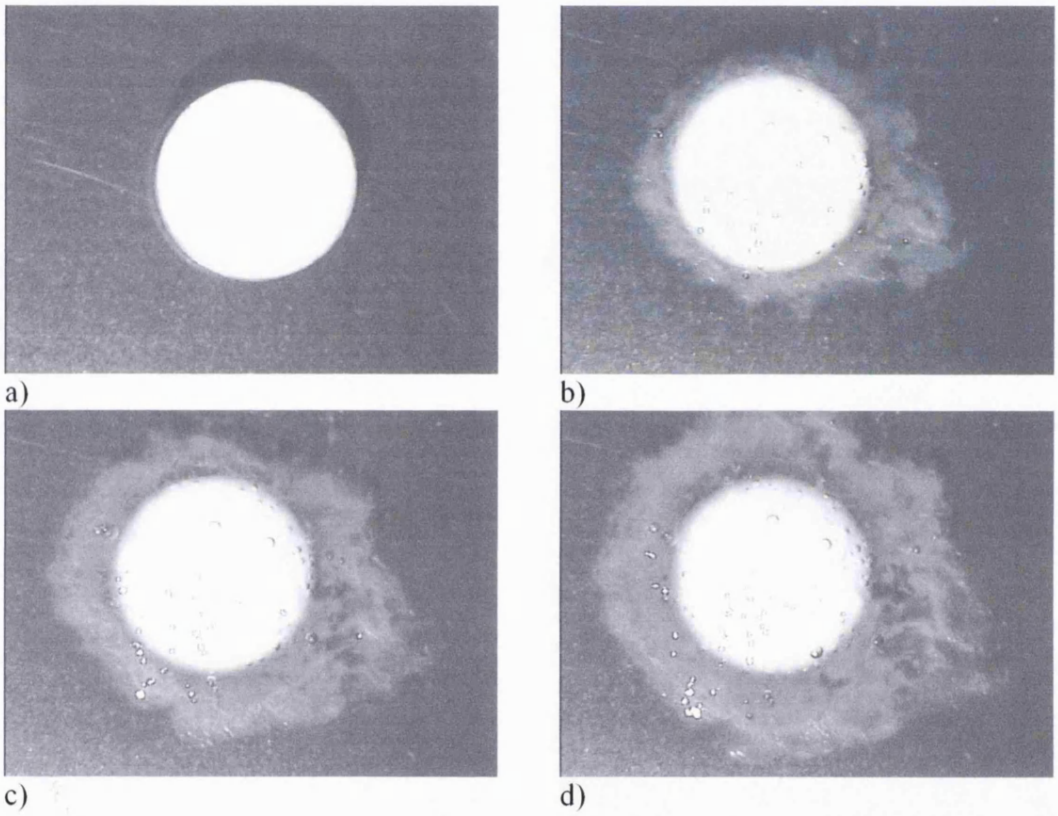
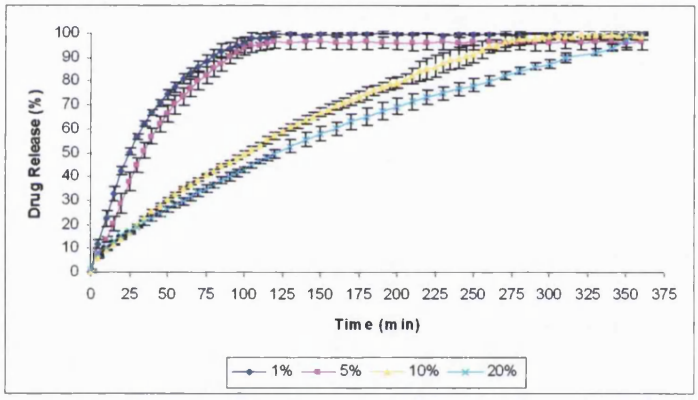


Figure 2.9 Visualisation of gel formation of carbomer polymer on the tablet surface for 5% CP 974 DC tablet. a) 5 minutes. b) 10 minutes. c) 15 minutes and d) 20 minutes in pH 6.8 phosphate buffer

Figure 2.10 shows the dissolution profile for CP 974 DC tablets in 0.1M HCl and pH 6.8 phosphate buffer and the effect of increasing carbomer loading. In both media the release of the drug is affected by the level of polymer present, with slower release at higher polymer loading levels. This suggests that at a polymer loading of 10% and 20% the gel layer formed is more viscous than those formed at lower carbomer loadings and as such drug release is limited by diffusion through this layer. At the lower loadings the gel layer is of sufficiently low viscosity to allow erosion of the gel layer to occur and as such rapid drug release. In pH 6.8 phosphate buffer while drug release for both 10% and

20% loaded tablets remain unchanged, 5% loaded tablets exhibit slower release than in acid. As discussed previously, the pH of the dissolution media affects the ionisation of the polymer chains. It is likely that the erosion of the gel layer, caused by dissolution of the polymer, in acid medium does not occur to the same extent in pH 6.8 phosphate buffer due to the increased ionisation and rapid solvation of the polymer. The result is a more prolonged drug release created by a more viscous gel layer as seen in Figure 2.10b. Conversely, for the 1% loaded tablets the release in buffer is more rapid than in acid, it is possible that the increased gelling here is not sufficient to effect the drug release particularly for a water soluble drug such as paracetamol and that the swelling of the carbomer may be having a disintegrating effect, resulting in the faster release.



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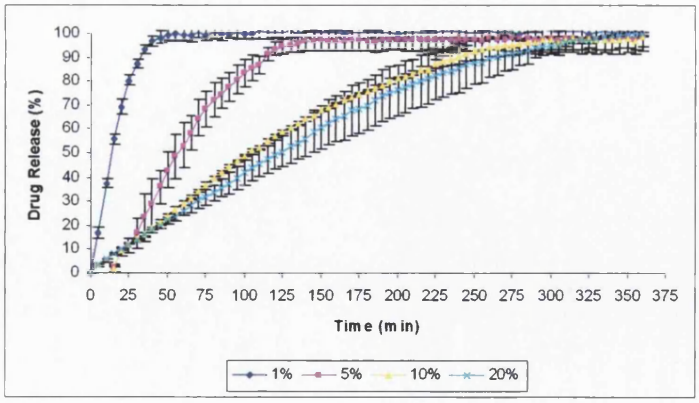
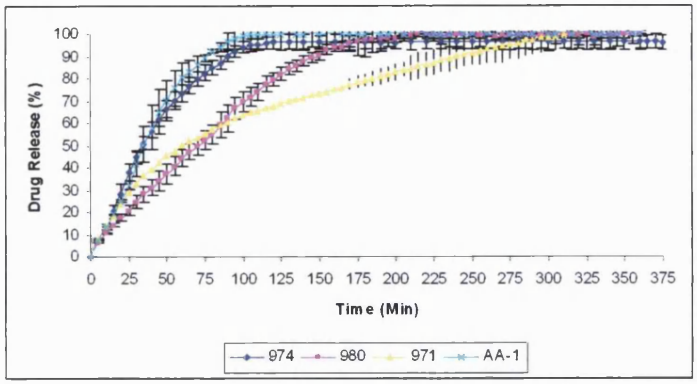


b)

Figure 2.10 Effect of carbomer loading on drug release from CP 974 DC tablets. a) 0.1 M HCl (pH 1.2). b) pH 6.8 phosphate buffer.(n=3)

The effect of the carbomer grade on drug release was also investigated in the same manner using direct compression tablets with a 5%w/w loading (Figure 2.11).



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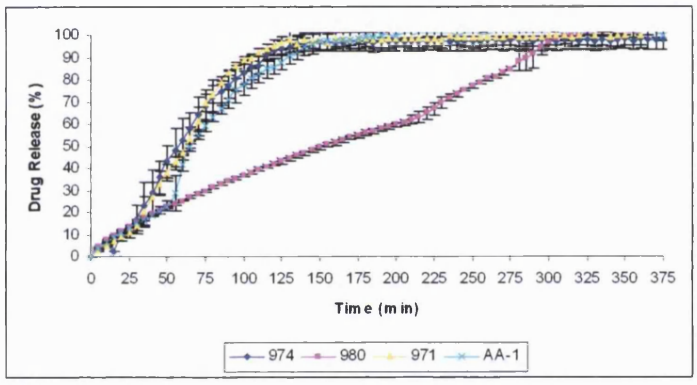


b)

Figure 2.11 Effect of carbomer grade on drug release for 5% loaded DC tablets. a) 0.1M HCl (pH 1.2). b) pH 6.8 phosphate buffer. (n=3)

Since drug release from hydrophilic matrices such as these are dependant on an interplay between gel formation and erosion, the rate and degree of gel formation are important in understanding the differences seen when different carbomer grades are used. The rate and extent of water up take by carbomer polymers is dependent on the type and number of hydrophilic groups in the polymer structure as well as pH of the media. Their rate of swelling is heavily dependent on molecular weight with an increase in swell rate accompanying and increase in molecular weight (Anlar et al., 1993). Conversely, the degree to which the polymers swell is inversely dependant on number of cross linkers (Flory, 1981).

However, since all carbomer grades possess the same basic polymer backbone (i.e same number of monomers) and differ only in the extent to which they are cross linked (for the homopolymers CP 974, 980 and 971) and the type of cross linker (PC AA-1) increases in molecular weight accompany increases in degree of cross linking, therefore distinctions between the effects of these two properties is not immediately evident. This can be seen in Figure 2.11a.

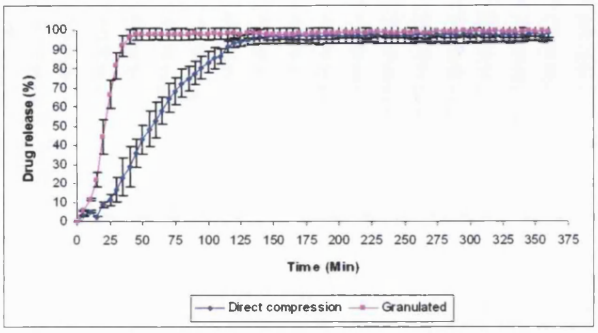
The slow drug release seen by CP 980 and CP 971 grades (Figure 2.11a) are both the result of the formation and diffusion of the drug through the gel layer. However, the reasons for the formation of the gel layer are different. As the least cross linked polymer CP 971 will undergo a greater degree of gel formation than the other grades. This is why the release of paracetamol in 0.1M HCl from tablets containing this grade is initially similar to CP 974 and PC AA-1 but slows to a prolonged release after a period of hydration in the dissolution media. In a similar fashion, as the highest molecular weight polymer CP 980 forms a gel layer at a greater rate than the other grades, as such the drug is unable to enter solution (as seen with CP 974 and PC AA-1) before the gel layer is formed. This explanation holds true when the drug release is investigated in pH 6.8 phosphate buffer (Figure 2.11b) where CP 980 expresses the slowest release of the four grades tested. However, CP 971 no longer exhibits a prolonged release profile. Explanation for this can be found in the balance between gel formation and erosion. For diffusion controlled release to occur the formation and erosion of the gel layer must reach equilibrium (Analar et al., 1993). As previously explained the level of ionisation of the polymers is greater in higher pH's which instigates greater gel formation. The faster release seen in pH 6.8 buffer suggests that this greater gel formation has created a more disperse gel layer where the rate of erosion is greater than formation, dissolving the polymer and promoting drug release.

However, if the rate and degree of gel formation were the only factors responsible for controlling drug release we would expect to see release from tablets containing CP 974 to be similar to those containing CP 980 as both grades are high molecular weight and highly cross linked. This is not the case, therefore other factors must be considered. The nature of gel formation is also important. Uniquely, CP 974 does not form a uniform gel layer, instead, the high level of cross linking ensures that initial gelation of the polymer

is non-uniform. This creates regions of varying microviscosities. Paracetamol, can escape the gel matrix and enter solution via the regions of low microviscosities before the gel layer becomes more uniform and thicker (Khan et al., 1998), and before diffusion through the gel layer becomes the rate limiting step.

The effect of granulation of the carbomers on drug release in simulated colonic pH (pH 6.8 phosphate buffer) can be seen in Figure 2.12. The formulations used for both types of tablet were identical (with the exception of granulation aids) and the dimensions (size and weight) and tensile strength of the tablets produced were the same. As such and differences in drug release could be attributed to either the granulation process directly or its affect on the polymers. No difference was observed in release between direct compression and granulated tablets in HCl (data not shown).

Granulation of the carbomers resulted in an increase in drug release for all grades except CP 971 and the sustained release properties seen with the DC formulations were no longer present. This is potentially of some concern as carbomers have proved to be useful in controlled release and have been shown to achieve zero order release kinetics of highly soluble drugs (Perez-Marcos et al., 1991a; Perez-Marcos et al., 1991b). The differences in behaviour of the carbomer polymers post granulation and inability to control drug release may be a result of water uptake which is known to be different for each grade (Anlar, 1993). During the granulation process exposure to the granulation fluid is likely to cause some uncoiling of the polymer chains (Sanz Taberner 2002). Subsequently, during the dissolution process those polymer chains that had suffered the most uncoiling are unlikely to form a viscous gel layer upon exposure to the dissolution medium. Additionally, the initially uncoiling during granulation maybe aiding the dissolution and erosion of the gel layer in pH 6.8 phosphate buffer. Evidence of this can be seen in Figures 2.12 a, b and d where the higher molecular weight grades, the grades that absorb water at the fastest rate are the most affected by the granulation process. This also helps to explain why CP 971 granulated tablets exhibit a longer release profile than their DC counter parts (Figure 2.12 c) as this grade will be less susceptible to uncoiling during the granulation process.



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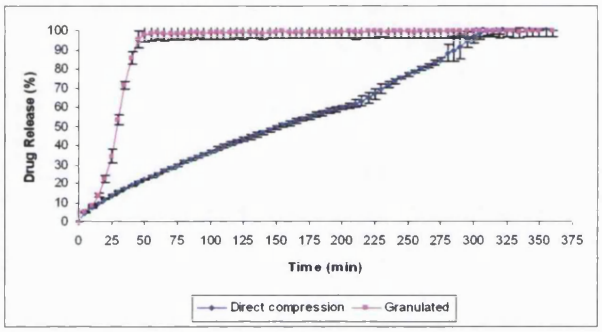

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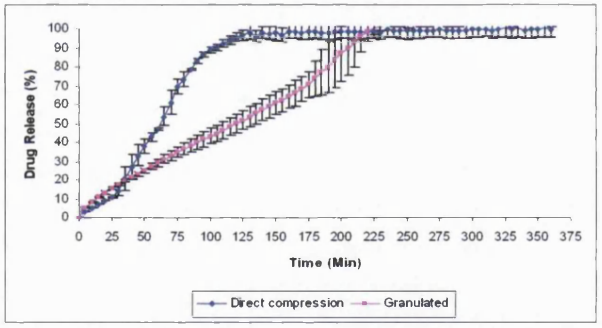

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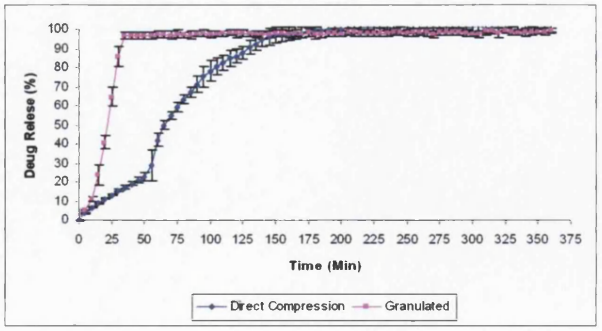


d)

Figure 2.12 Comparison of the effect of granulation on drug release in pH 6.8 phosphate buffer. a) CP 974 b) CP 980 c) CP 971 and d) PC AA-1. (n=3)

2.9.4 In-vitro evaluation of mucoadhesion

2.9.4.1 Comparison of two tensile test apparatus

Measurement of the peak detachment force is a measurement of adhesive joint failure and since the force applied to the sample is perpendicular to it, these tests are measuring the tensile stress of samples. To demonstrate the need for a standardised test for mucoadhesion two pieces of equipment were used and a comparison of the results obtained from each machine was undertaken. It was mentioned earlier that for all adhesion studies optimum conditions must be determined prior to testing, this was done for both sets of equipment. Testing was first conducted on the Texture Analyser (TA), subsequently, test parameters for the Instron similar to those of the TA was sought (Table 2.10).

Table 2.10 Summary of test parameters for determination for the Texture Analyser and the Instron technical tester.

	Texture Analyser (TA)	**Instron**
Load Cell (N)	5	100
Contact Force (N)	2	2
Probe retraction speed (mm/min)	60	10
Probe diameter (mm)	20	57

It was not possible to match all the test parameters of the two machines. Contact force was standardised, and set to a value that was sufficient to invoke contact between the two surfaces but which would not cause the sample to break. However, probe diameter, probe retraction speed and load cell could not be kept constant across the two machines. The differences in probe diameter were deemed not important since the probe itself never comes into contact with the test surface and the surface area of the test samples was kept constant. The force of the load cell was a parameter that could not be changed for both pieces of equipment as it is built into the TA, for the instron however it was possible and two loads cells were investigated, 100N and 2.5N. The 100N load cell was determined to be optimum since the capacity of the 2.5 N cell was not sufficient for all

samples tested, additionally minimum values obtained during testing were within the accuracy limits of the 100N cell. Both machines allowed for changes in the probe retraction speed; however these changes produced opposite effects. An increase in speed resulted in an increase in the detachment force for the TA but a decrease for the Instron. A possible reason for this is the load cell; with a higher maximum force capability a slower retraction speed was required for the Instron to allow the software to accurately record the data. With a much lower force capability the TA did not face this problem; as such the probe retraction speed could be set to its maximum setting. The hydration time was chosen specifically as time that would allow the carbomer polymers to gel on the surface of the tablet but at which significant disintegration of the tablet had not occurred.

To compare the TA and Instron data the peak detachment force from a glass surface (Method 2.8.3) for three samples was determined (100% CP 974, 5% CP 974 granulated tablet and 5% CP 974 DC tablet). It was found that direct comparisons of the two pieces of equipment could not be made as the force values obtained differed significantly, despite identical methods being employed. Discrepancies within the data, with the TA biased towards higher readings, were attributed to the previously mentioned differences in test parameters. Both machines did however rank the 3 samples in the same order with 100% CP 974 >5% CP 974 DC > 5% CP 974 granulated (Table 2.11).

Table 2.11 Detachment force from glass obtained from the Instron and TA after 2 min hydration in pH 6.8 phosphate buffer.

	Peak Detachment Force (N)	
Sample	Instron	Texture Analyser
100% Compact	1.82 ± 0.43	4.02 ± 0.38
5% DC	0.75 ± 0.06	1.02 ± 0.05
5% Granulated	0.63 ± 0.07	0.64 ± 0.04

Therefore, it was decided that two pieces of equipment could be used so long as no direct comparisons between them were made, instead the results and conclusions would

be based on observations of trends and with respect controls run in each particular test. Within this study the data will be generated from the Instron unless otherwise directed. The discrepancy with the data from the two machines highlights an additional problem faced when investigating mucoadhesion. The lack of any standard test makes it difficult for researchers to compare results and data, certainly arbitrary force values cannot be compared between different tests, due to inevitable discrepancies in test parameters. Additionally before comparisons could be made factors such as whether the sample should be a powder or compact, its surface area or any pre-treatment would have to be agreed upon and standardised. Until this is the case conclusions must be based on differences in trends and ranking order for any given data set.

2.9.4.2 Fundamental assessment of adhesion and mucoadhesion

The force of detachment from a glass surface (adhesion study), and the detachment force and work of adhesion from a layer of mucin (mucoadhesion study) and the effect of increased exposure to mucin (mucin exposure study) were investigated for the carbomer compacts using the Instron. The force of detachment was explained earlier as the force required to cause failure of the adhesive joint. The area under the curve of this graph is a measurement of force over time and as such is the work of adhesion (Figure 2.13.

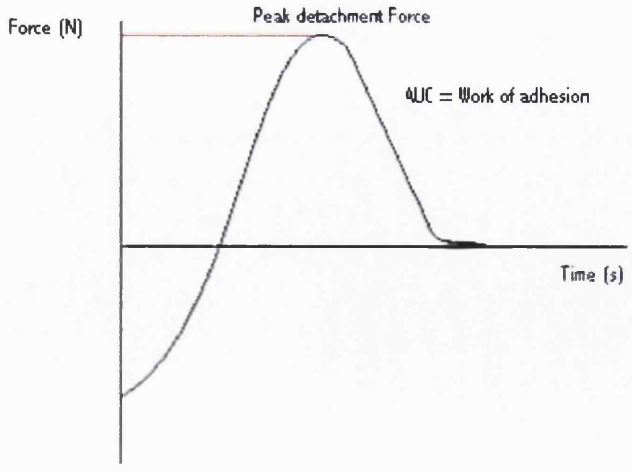


Figure 2.13 Typical force/time graph from tensile testing of mucoadhesive polymers

Detachment force of the compacts from a glass surface varies with time and is affected by the polymer grade (Figure 2.14). At 1 minute hydration in pH 6.8 phosphate buffer

all grades require a similar force to cause adhesive joint failure, however, after 4 minutes the results obtained align themselves roughly with the molecular weight and degree of cross linking.

The higher molecular weight grades, CP 980 and AA-1 express the greatest adhesion at 2.23 ± 0.07 N and 1.81 ± 0.05 N respectively and CP 974 and CP 971 the least. CP 971 and CP 974 expressed the same force of adhesion at 1.28 ± 0.06 N and 1.29 ± 0.06 N ($p<0.05$) and were significantly lower than the CP 980 and PC AA-1 grades. This is an unexpected result as CP 974 is listed as a high molecular weight and highly cross linked carbomer and as such we would expected to behave in a way similar to the CP 980 grade which is also high molecular weight and highly cross linked.

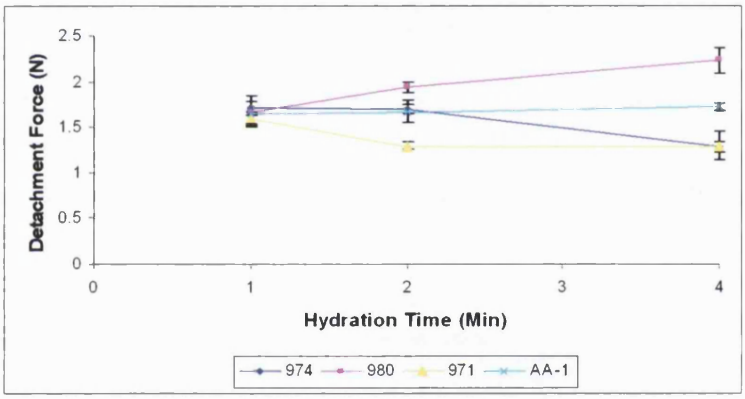


Figure 2.14 Comparison of peak detachment force for 100% carbomer compacts from a glass surface post hydration in pH 6.8 phosphate buffer. (n=5)

As the hydration time increases both CP 980 and PC AA-1 show an increase in detachment force conversely CP 971 exhibits a drop in force form 1 to 2 minutes and then remains constant from 2 to 4 minutes hydration. Finally the detachment force for CP 974 remains constant from 1 to 2 minutes and then drops from 2 to 4.

For an explanation of these observed behaviours and differences, it is important to first consider exactly what it is being tested. As stated earlier Park et al., (1984) categorised bioadhesion based on three mechanisms of action, the first being materials that become sticky when wet, since there is no mucin or biological material there is nothing for the carbomers to interact with in this test, therefore this test is one of simple adhesion of the viscous gels with the glass surface and therefore as with drug release, the results will be

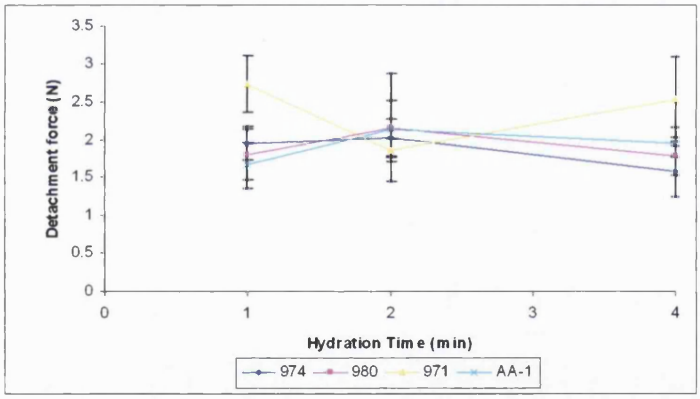
mostly be influenced by gel formation and as previously discussed, this is affected by molecular weight and degree of cross linking (Anlar, 1993). The drop in force for CP 971 from 1 to 2 minutes can be explained when considering the degree of gel formation. Containing a lesser amount of cross linker the extent of gelation for CP 971 is greater than other grades (Noveon, 2002a), the result of this being that the polymer gel contains more water, is more disperse and is less viscous resulting in the seen drop in adhesion. This is similar to the results seen previously with drug release from matrix tablets containing carbomers. Tablets containing the 971 grade were seen to have faster drug release at higher pH's due to greater expansion of the polymer chain and eventual dissolution of the polymer itself. The same is likely to be occurring here.

Similarly, as discussed previously CP 974's more rigid structure gives rise to a difference in the macro viscosity (viscosity of swollen carbomer particles) and micro viscosity (areas in between the particles) (BF Goodrich, 1991). These results suggest that when hydrated for 1 and 2 minutes the gel layer maybe forming with more regions of varying macro and micro viscosities, after 4 minutes in pH 6.8 phosphate buffer the gel layer is more uniform but one that is less adhesive. These results also suggest that CP 980 and PC AA-1's respective behaviour is somewhere between these two extremes, containing high and medium level of cross linking respectively. When initially hydrated, both take on water and swell, however after 2 minutes they reach an equilibrium where the gelation ceases or slows or where continued gel formation does not contribute to increased adhesion.

It has been reported extensively in the literature that hydrogen bonding (Mortazavi, 1995) and entanglement of the polymer and mucin is thought to pay a important role in mucoadhesion, (Jabbari et al., 1993; Leung et al., 1990; Smart, 2005). Figure 2.15a shows the detachment force and work of adhesion for compacts, after hydration in pH 6.8 phosphate buffer, from a mucin layer. It is interesting to note that within this test work of adhesion is only recorded for any sample when tested in the presence of mucin. The incorporation of the mucin alters the relative ranking of the four grades with respect to each other, suggesting that different mechanisms are involved. The most significant difference seen is that in contrast to the previous test it is CP 971 that expresses the greatest mucoadhesion at maximum hydration and not CP 980. Irrespective of hydration CP 974, CP 980 and PC AA-1 require the same force to detach them from the surface

($p>0.05$). At 1 and 4 minutes CP 971 requires a greater force to cause joint failure and remove it from the surface ($p<0.05$).

The work of adhesion for the four polymers (Area under the curve) (Figure 2.15b) shows greater deviation in their performance. At 1 minute hydration CP 974 and 971 exhibit the greatest and equal work of adhesion with 1.24 ± 0.20 mJ and 1.38 ± 0.21 mJ respectively ($p>0.05$) with PC AA-1 possessing the second greatest and CP 980 the least. As the level of hydration increases the work of adhesion of the four polymers alters uniquely for each grade, CP 980 remains constant while decreases are seen for both CP 974 and PC AA-1 until, at maximum hydration these three grades are equal at 0.64 ± 0.34 mJ, 0.35 ± 0.07 and 0.32 ± 0.11 mJ for CP 974, CP 980 and PC AA-1 respectively. The work of adhesion of CP 971 grade was greatest across all three time points and constant irrespective of hydration.



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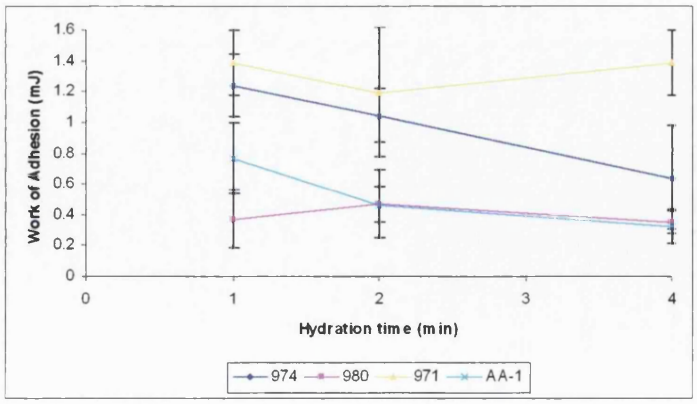


b

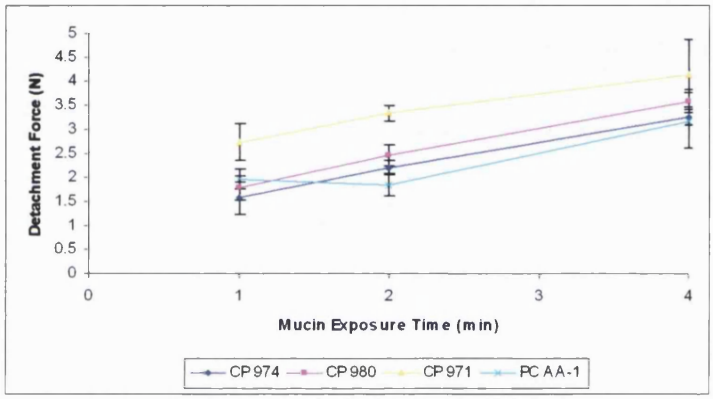
Figure 2.15 Comparison a) detachment force and b) work of adhesion for carbomer compacts from a 1% mucin layer after hydration in pH 6.8 phosphate buffer. (n=5)

Since significant work of adhesion was only recorded in the presence of mucin the assumption was made that it was a result of polymer/mucin interactions, furthermore since the work of adhesion for each grade alters in a different fashion with hydration time, the type of interaction or extent of interaction must be different for each grade. As discussed previously, both hydrogen bonding and interpenetration of the mucin and polymer contribute to mucoadhesion. The greater work of adhesion seen for the CP 971 grade is probably a result of greater interpenetration of mucus and the polymer made possible by the more expanded gel network which is known to occur for this grade. This implies then that the lower values for work of adhesion seen for CP 980 and PC AA-1 are caused by either reduced interpenetration, the effect of hydrogen bonding alone or a combination of both. The equal work of adhesion seen for CP 971 and CP 974 at 1 minute hydration is somewhat of an unusual result as the two grades represent the biggest differences in physical properties of all four grades and does not naturally follow the interpenetration hypothesis. However, this may again be explained by the non-uniform gelation known to occur with the 974 grade. At 1 minute hydration greater interpenetration of the polymers with mucin is possible through regions of low macroviscosity, as the hydration increases and these regions diminish, interpenetration is inhibited and the work of adhesion drops.

The final study conducted on the carbomer compacts investigated the effect of continued exposure to mucin. Interestingly a difference in detachment force is seen here (Figure 2.16), after 4 minutes hydration in pH 6.8 phosphate buffer and exposure to mucin for 4 minutes all grades exhibit the same force of detachment ($p>0.05$), with 3.38 $\pm$ 0.19, 3.60 $\pm$ 0.23, 4.14 $\pm$ 0.74 and 3.18 $\pm$ 0.58 N for CP 974, CP 980, CP 971 and PC AA-1 respectively. Another key difference is a general increase in detachment force alongside an increase in mucin exposure suggesting that continued exposure to mucin results in its further interactions with the polymers. This further supports the idea that the greater performance seen by CP 971 previously is due to its initial greater gelation and that this is short lived as the other grades catch up over time.

Although an increase in work of adhesion is seen for all grades, CP 971 requires a significantly greater amount, which as before can be attributed to greater interpenetration of the polymer and mucin. However, interestingly, the peak detachment force for all four grades at this time is equal, therefore, the increased interpenetration

that occurs with CP 971 compacts does not appear to result in an increase in its overall tensile strength. This suggests that CP 980, CP 974 and PC AA-1's mucoadhesive qualities are caused by other mechanisms. It is possible that hydrogen bonding is a more significant factor in determining strength of mucoadhesion for these grades, however it does not appear to contribute to work of adhesion so cannot be identified with this test method. One explanation for this may be increased competition for hydrogen bonding sites between the mucin and water. This may be of particular significance to the higher molecular weight grades as this will uptake water more rapidly than the lower molecular weight grades.



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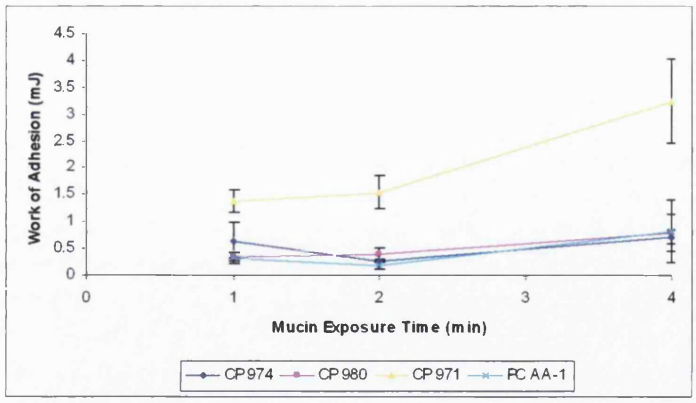


b

Figure 2.16 a) Detachment force and b) Work of adhesion for 100% carbomer compacts from mucin over a range of exposure times to mucin post 4 minute hydration in pH 6.8 phosphate buffer. (n=5)

A final possibility then is that interpenetration and hydrogen bonding are not the most significant contributors to the overall adhesion of the system. Increased water movement from mucin into gel layers has been shown to create an increase in adhesion

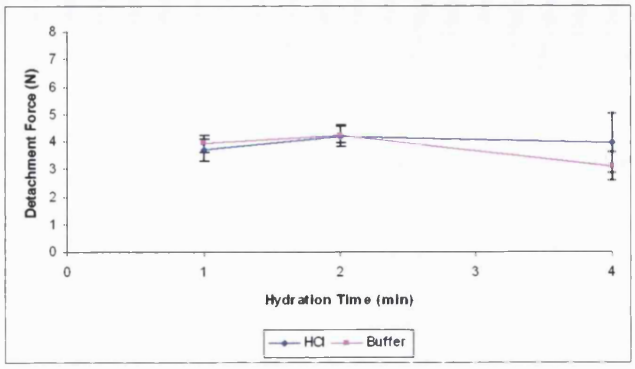
(Mortazavi et al., 1993). This may also explain why an increase in detachment force accompanies an increase in exposure to mucin.

2.9.4.3 Effect of pH on force of detachment

The pH of the environment to which the polymer is exposed can potentially affect its performance as ionisation of the acrylic acid backbone is important in gel formation (Leung et al., 1990). In the human G.I tract pH increases distally from 1.0 to 7.5, with colonic pH's ranging from pH 6.5- 7.5 (Evans et al., 1988). To understand the effect of pH on the polymers their detachment force from a glass surface was assessed using the TA in acid and compared with pH 6.8 phosphate buffer. A marked difference in adhesion behaviour of the four polymer grades in the two media can be seen in their ranking order, at 4 minutes hydration in 0.1 M HCl the order of strongest to weakest (peak detachment force) is CP971> CP974 = CP980> PCAA-1, compared with CP980> PCAA-1> CP974> CP971 in pH 6.8 phosphate buffer (Figure 3.7). After 4 minutes hydration CP 980 and PC AA-1 grades show stronger adhesion in pH 6.8 phosphate buffer, CP 974 exhibits equal adhesion in both media and CP 971 is stronger in acid than buffer.

The stronger adhesion in higher pH can be explained by the degree of ionisation of the polymer. At low pH less than 10% of the carbomer acid groups will be ionised (Noveon, 2002b), this affects the uncoiling of the polymer chain and subsequent formation of a gel layer which is fundamentally important to mucoadhesion as it allows polymer/mucin interactions and hydrogen bonding to occur. Even though in an in-vitro test such as this from an artificial surface, no polymer interactions or hydrogen bonding would occur, greater uncoiling of the polymer back bone results in a more viscous gel layer leading to stronger adhesion. This however is not true for CP 971 where a drop in adhesion seen with increased hydration. Again, as with drug release, these results can be interpreted with respect to rate and extent of gel formation. Unlike the other grades, in pH 6.8 buffer the CP 971 gel layer formed is simply too dispersed and prone to dissolution to provide a sufficiently viscous gel layer to be adhesive. As such this grade is most adhesive in acidic conditions.

a



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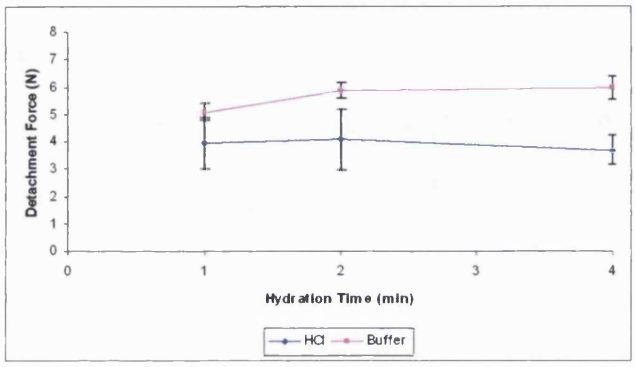

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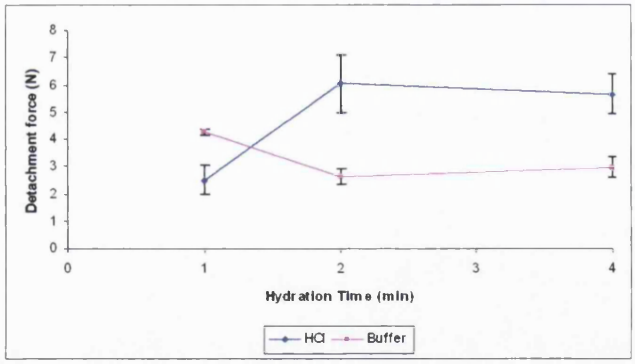

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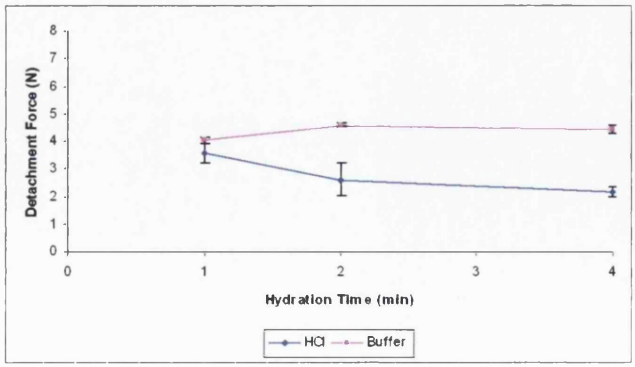


Figure 2.17 Detachment force over time in acid and pH 6.8 phosphate buffer for a) CP974, b) CP980, c) CP971 and d) PC AA-1 compacts. (n=5)

The differences between adhesion results in acidic and alkaline media may help to explain the confusion in the literature regarding the mucoadhesion of carbomer polymers particularly with regards to gastric retention. Studies in man have shown no retention of pellets containing PC AA-1 (Khosla et al.,1987) or capsules containing PC AA-1 or CP 934 (Harris et al., 1989) in the human stomach. However, animal studies in rats (Longer et al., 1985) showed increase gastric residence of PC AA-1 beads. In the fasted state, gastric pH measured between pH 1.0 and 2.5 for humans (Evans et al., 1988; Dressman et al., 1990; Russell et al., 1993) and between pH 3.1 and 5.1 in rats (Ward et al., 1987) Therefore, based on these results, we would expect to see greater uncoiling of the carbomer chains in higher pH's, in the rat stomach than the human, this would potentially allow for polymer and mucin interactions and hydrogen bonding to occur and increase gastric residence. Differences in fluid volume between the human and rat could also account for these results. A recent comparison the stomach fluid volumes for human and rat has determined that when normalised for body weight there is more water per Kg in the rat stomach than in man. (McConnell et al., 2008).

2.9.5 Effect of formulation on mucoadhesion

2.9.5.1 Carbomer film coated formulations

Film coating of carbomers onto the surface of a tablet showed limited success as the achievable weight gain was low (<1%). The adhesive properties of carbomer film coats were investigated after hydration in pH 6.8 phosphate buffer (Figure 2.18). At the minimum hydration time all grades show some adhesive properties compared with a non-mucoadhesive control, however this adhesion reduces as the hydration time increases. After 4 minutes hydration in pH 6.8 phosphate only CP 980 grade shows any significant adhesion from the control. The drop in detachment force seen as hydration time increases is likely to be caused by the removal of the gel layer from the tablet surface. This is either because the point at which the adhesive joint is broken is a weaker point than the joint between the gel and the glass, for example the gel layer is removed from the surface of the tablet and remains on the glass surface, or it is washed off in the buffer solution.

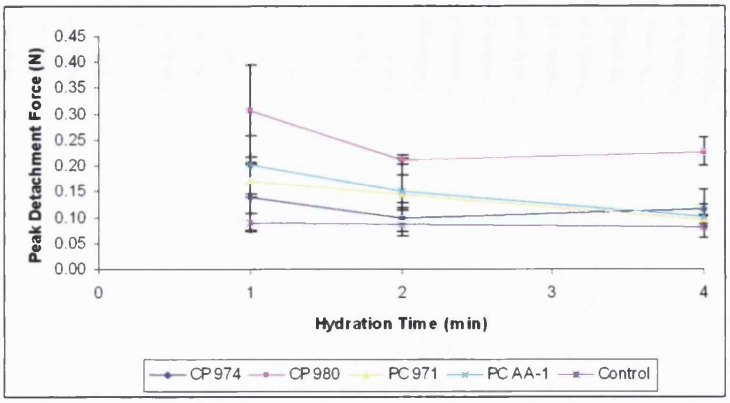


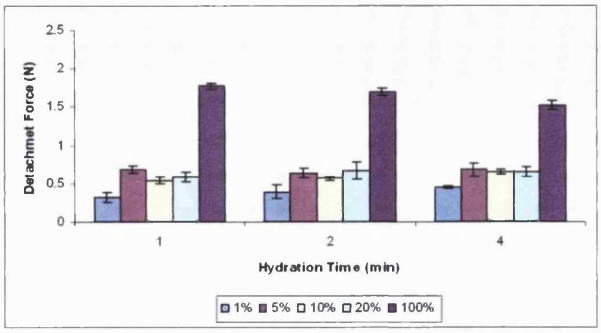
Figure 2.18 Detachment Force for CP 974, 980, 971 and PC AA-1 film coated tablets, containing 10% w/w TEC compared with an uncoated control, in 6.8 phosphate buffer (measured on the TA). (n=5)

Adhesion of the film coat to the tablet is dependent on penetration of the film into the core which is in turn influenced by molecular weight of the coating polymer, viscosity and spreading behaviour of the coating solution (Bauer Kurt et al., 1998). The hydroscopic nature of carbomers, poor spreading and high molecular weight means that it is unlikely to migrate into the tablet core and adhere, therefore upon hydration the gel layer is easily removed.

2.9.5.2 Matrix tablets

The effect of the level of carbomer within a formulation on adhesion was investigated using direct compression formulations at 1%, 5%, 10%, 20% and 100% (data shown previously) carbomer loading. An increase in carbomer loading gave unexpected results (Figure 2.19), whilst 1% and 100% loading resulted in the lowest and highest detachment force values respectively ($p<0.05$), no difference was observed with the 5% 10% and 20% loading. These results show that a large increase in carbomer loading is needed to significantly increase its adhesive properties. From a formulation perspective this is an important understanding, especially when considering the difficulties of manipulation of the polymers, achieving a suitable level of adhesion with the lowest possible carbomer loading is desirable.

The lack of any observable difference in 5%, 10% and 20% loading can be explained by the fast swelling ability of the carbopol polymer. In a short period of time the gel layer covers the surface of the tablet, it seems that even at relatively low levels such as 5%,



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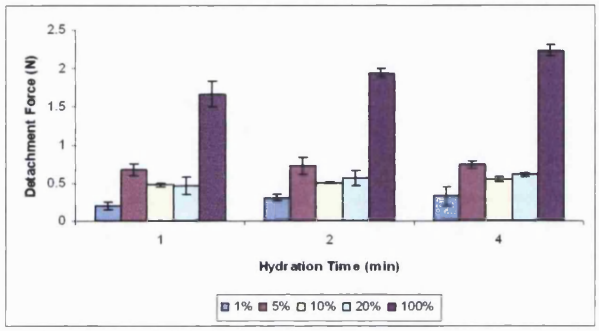

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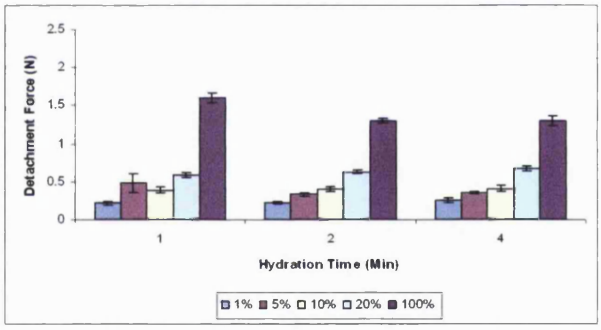

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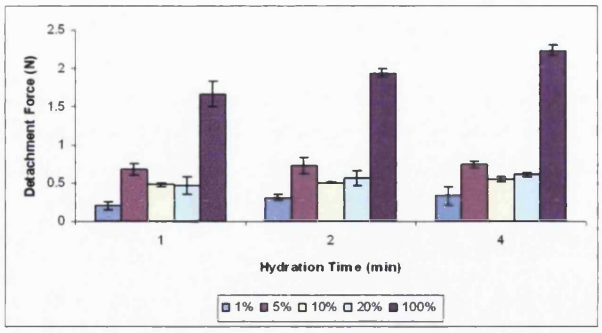

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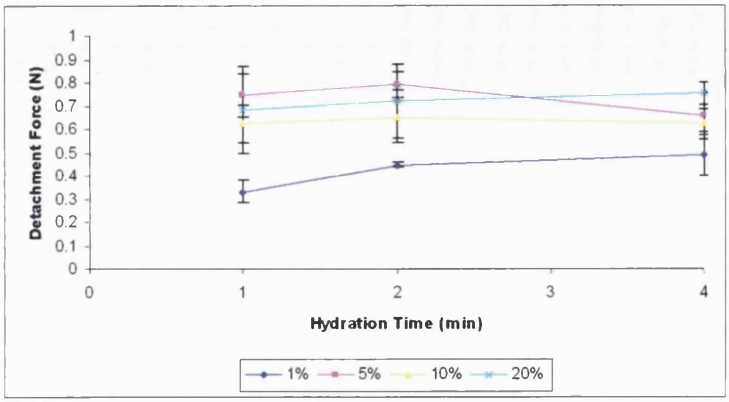
Figure 2.19 Detachment force from a glass surface of direct compression formulations after hydration in pH 6.8 phosphate buffer, a) CP 974, b) CP 980, c) CP 971, d) PC AA-1. (n=5)

this layer is sufficient enough to provide an adhesive force between the test sample and the glass plate. Although a higher loading of polymer would be expected to produce a thicker more viscous gel layer this appears to have no bearing on the force of detachment. This also explains why no significant rise in adhesion is observed when the formulations are hydrated for longer time periods. These results suggest that small changes in carbomer levels within a formulation will not affect the adhesion of the dosage form. However, a higher carbomer loading may have implications for their performance *in-vivo* as the presence of more carbomer polymer could result in more prolonged adhesion by the continued renewal of any eroded gel layers.

The effect of carbomer loading on polymer/mucin interactions was investigated with CP 974 grade (Figure 2.20). As before the peak detachment force for 5%, 10% and 20% loading are equal and the level of hydration does not alter this. One difference observed however is that for a loading of 1% the degree of hydration of the polymer does have an impact, as the hydration time increase from 1 to 4 minutes we observe an increase in detachment force. The work of adhesion is also affected by the carbomer loading. A carbomer loading of 5% showed significantly greater work of adhesion across all time points than 1%, 5% and 10% levels. It is probable that gel layer thickness is the reason for the differences in work of adhesion. In the pervious chapter we saw that a change in carbomer loading altered drug release with prolonged release observed with increased carbomer content. The same gel layer that inhibits drug release in 10% and 20% loaded tablets could be limiting polymer and mucin interactions.

If this were completely true however we would expect to see an increase in work of adhesion along side a decrease in carbomer content, whilst we do see this with a 5% loading it is not present at a 1% carbomer level. Therefore, polymer/ mucin interactions are dependent on the gel layers, where 10% and 20% loaded tablets can not undergo these interactions due to the layer being too thick, 5% loaded tablets can, however 1% loaded tablets can not since the gel layer maybe to be too disperse.



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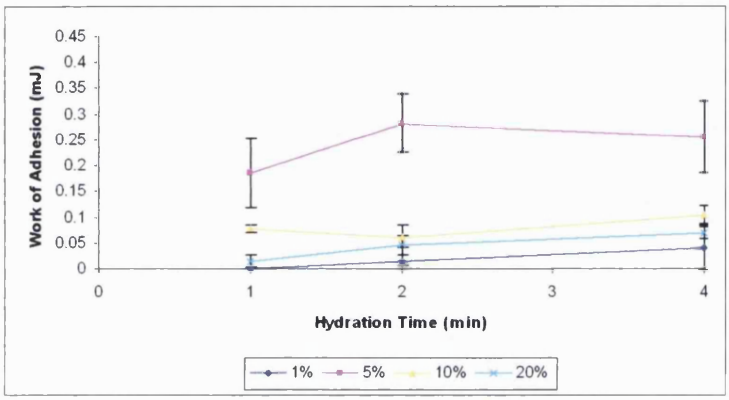

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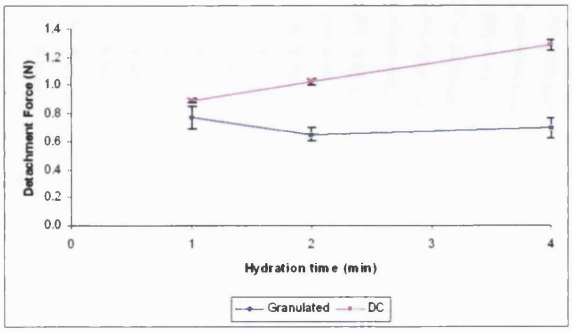
Figure 2.20 Effect of carbomer loading on a) detachment force and b) work of adhesion for CP 974 DC tablets from a mucin layer after hydration in pH 6.8 phosphate buffer. (n=5)

Wet granulation and subsequent tableting of the powder was performed and the adhesive properties of these tablets and their direct compression counterparts were investigated (Figure 2.21) (conducted using the Texture Analyser). In all cases the adhesive properties of the polymers is reduced when subjected to the granulation process. These results disagree with a previous study (Prudat-Christiaens et al., 1996) where no difference in adhesion was seen for CP 934, CP974 and EX55 (later renamed to CP 980) after granulation with either sodium carmellose or hypromellose. However work done by the authors shows that sodium carmellose and hypromellose powder alone expressed adhesive qualities comparable to the carbomer polymer, and no comparisons were made between DC and granulated tablets. An explanation for the decrease in adhesion seen here is pre-exposure to wetting fluid.



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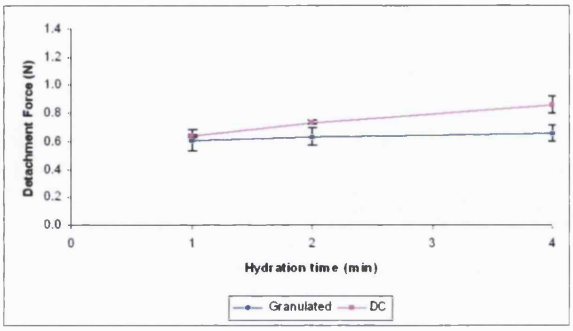

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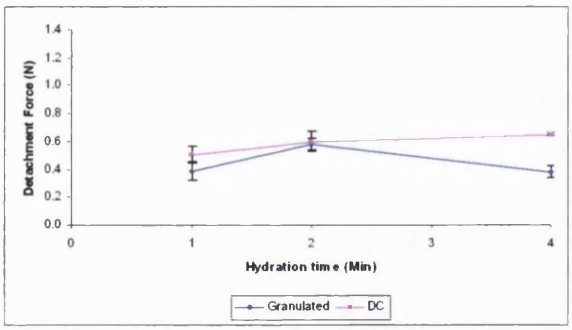

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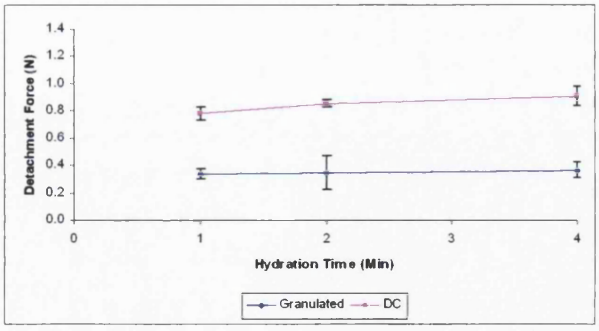

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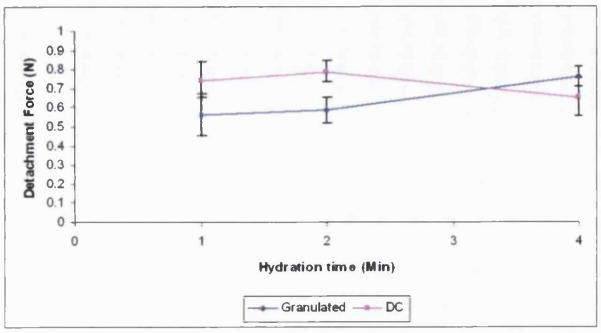
Figure 2.21 Comparison of detachment force for granulated and direct compression (5%) formulations from a glass surface after hydration in pH 6.8 phosphate buffer, a) CP 974, b) CP 980, c) CP 971 and d) PC AA-1)(Measured on the TA). (n=5)

In powder form the molecules of the carbomer polymers are strongly coiled into a spiral. Upon hydration and ionisation of the polymer chain the spiral unfolds producing the gel effect with increasing viscosity (Sanz Taberner et al., 2002). The reduced adhesion suggests that exposure to the ethanol and water mix during the granulation process causes the polymers to unwind and although when dried reverts to a unionised state there is no reason for the powders to reform their coiled form thus limiting gelling abilities when next ionised. The strengthening of the chains means that polymer/mucin entanglements are less likely or at least will be weakened. Additionally, when next exposed to fluid the uncoiled chains will be susceptible to dissolution as they can be more easily surrounded by solvent molecules and as such will reduce the mucoadhesive properties of the formulation.

As with the compacts the effect of mucin layer between the glass and the gel was investigated. As seen in other tests mucin has an effect on the detachment force (Figure 2.22a). For CP 974 and 980 the differences in detachment force between a granulated system and direct compression system which were evident in the previous test are no longer present, particularly at the 4 minute hydration mark. The equal detachment force seen for CP 974 and CP 980 DC and granulated tablets in mucin suggests that for these two grades uncoiling of the polymer and thus interpenetration of the chains and mucin is not the most important factor for mucoadhesion, this is confirmed by the work of adhesion which is significantly higher for the DC forms of both grades (Figure 2.22b).

The lower molecular weight grades however behave in a different manner. CP 971 shows greatest mucoadhesion of the two matrix systems post 4 minutes hydration in the granulated tablet ($p>0.05$), and zero work of adhesion was recorded for either tablet form. The latter suggests that little interpenetration is occurring. Due to the greater degree of swelling known to occur with this grade it was expected that CP 971 would be affected to a greater extent than the other grades by the granulation process and as such lower mucoadhesion would be seen. The increased force of detachment for the granulated system may be representative of a renewing of the gel layer after initial erosion caused by the hydration step in the method and aided by the granulation process This phenonom is not seen with the other grades as their increased cross linking makes them firstly less susceptible to erosion.



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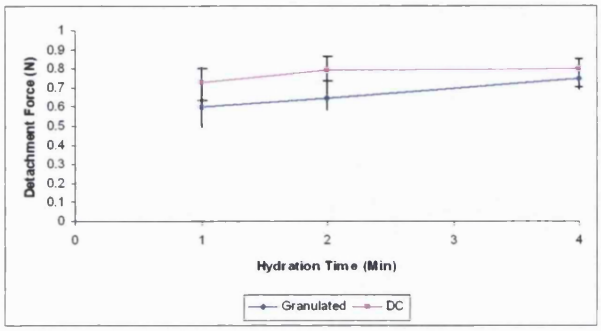

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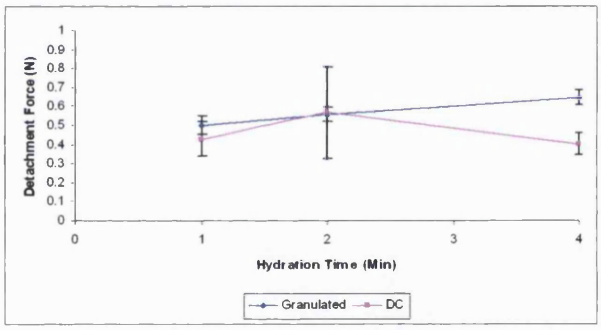

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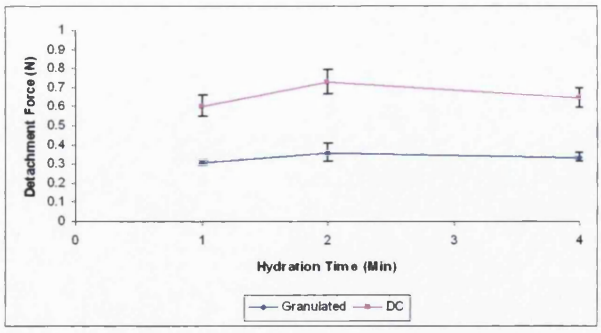

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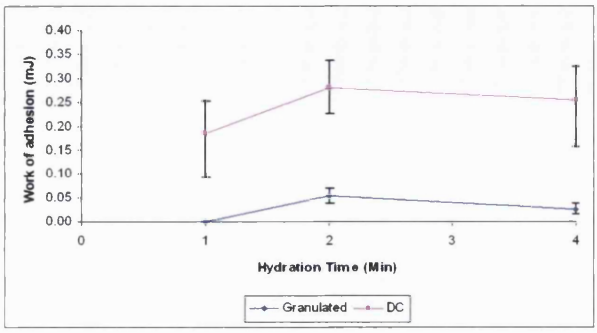
Figure 2.22a Comparison of detachment force for 5% granulated and direct compression formulations from a 1% mucin solution after hydration in pH 6.8 phosphate buffer, A) CP 974, b) CP 980, c) CP 971, d) PC AA-1. (n=5)



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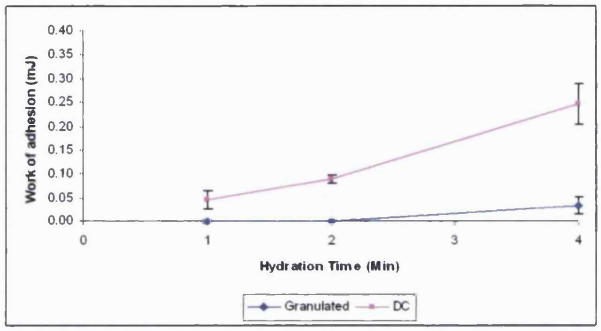

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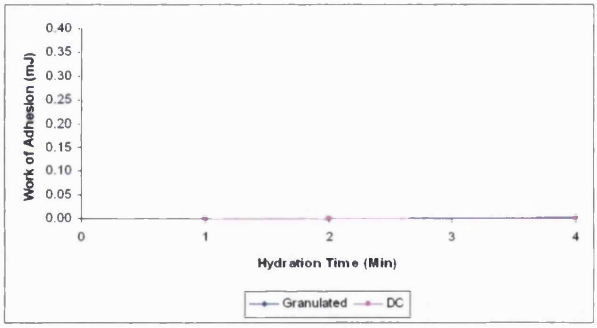

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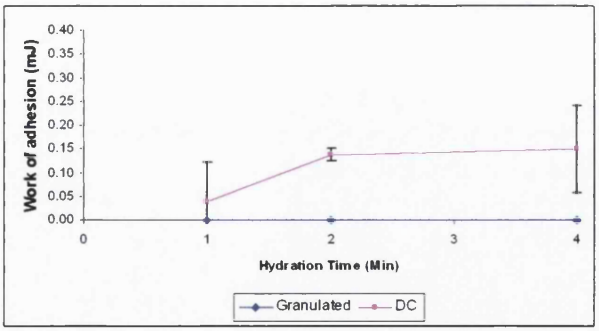

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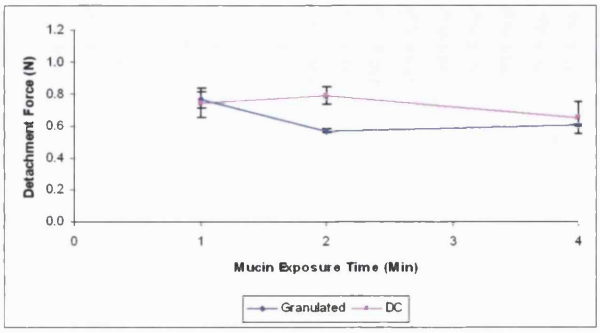
Figure 2.22b Work of adhesion from mucin for 5% granulated and direct compression tablets, from a 1% mucin solution after hydration in pH 6.8 phosphate buffer a) CP 974, b) CP980 and c) CP 971 and d) PC AA-1. n=5

Prolonged exposure to mucin is different for each grade (Figure 2.23a), while CP 974 and PC AA-1 show no change in trend between DC and granulated tablets, CP 980 and CP 971 tablets show greater detachment force ($p<0.05$) for DC tablets after exposure to mucin for 4 minutes. Work of adhesion (Figure 2.23b) is also seen to increase for three of the grades, CP 974, CP 971 and PC AA-1 for the DC tablets when exposed to mucin for longer time periods. Equal detachment forces for DC and granulated CP 974 combined with higher values for work of adhesion for the DC tablet suggest that prolonged mucoadhesion is not dependent on polymer and mucin interactions. In contract to this, the remaining grades all possess stronger detachment force and work of adhesion for the DC tablets implying that for prolonged mucoadhesion to occur these grades require polymer mucin interactions to take place.



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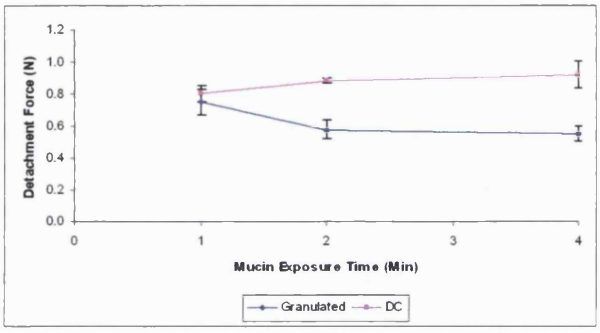

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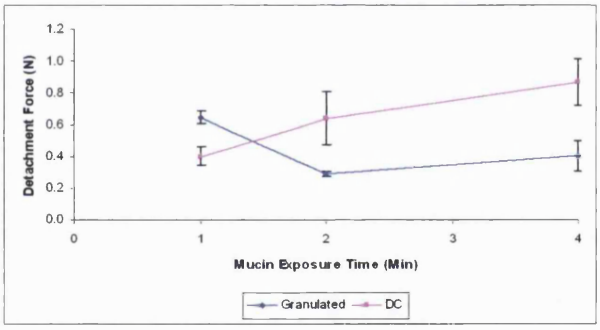

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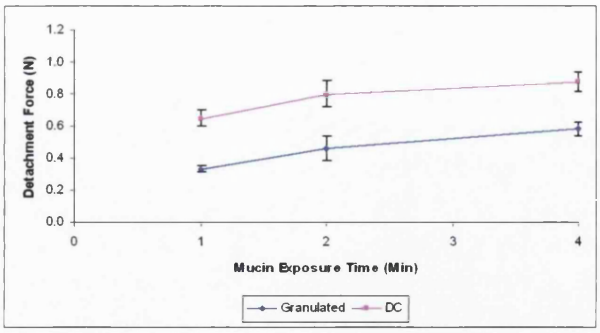

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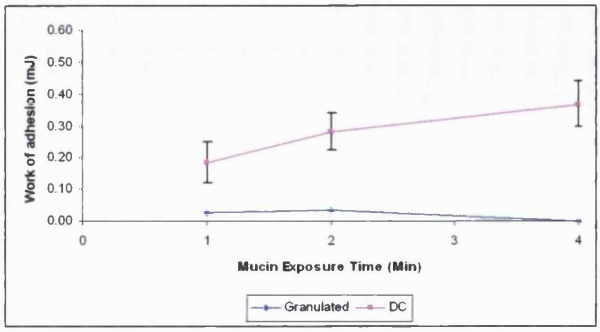
Figure 2.23a Comparison of detachment force for 5% granulated and direct compression tablets after 4 minutes hydration in pH 6.8 phosphate buffer and prolonged exposure to mucin (n=5). a) CP 974, b) CP 980, c) CP 971, d) PC AA-1. (n=5)



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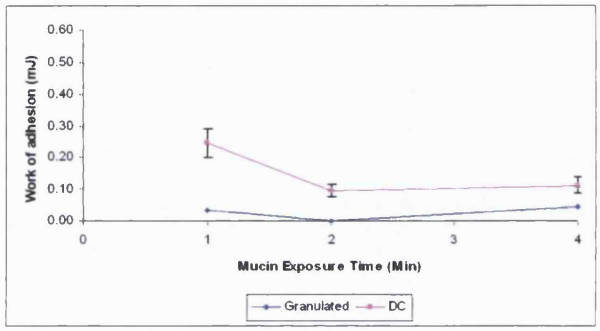

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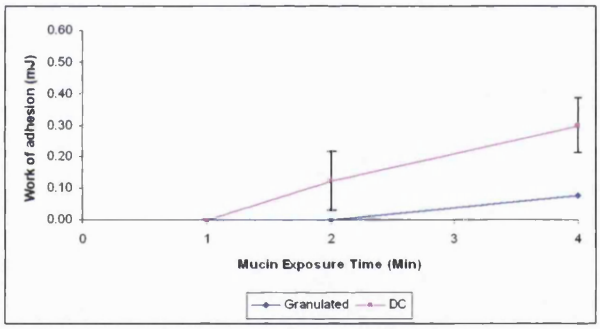

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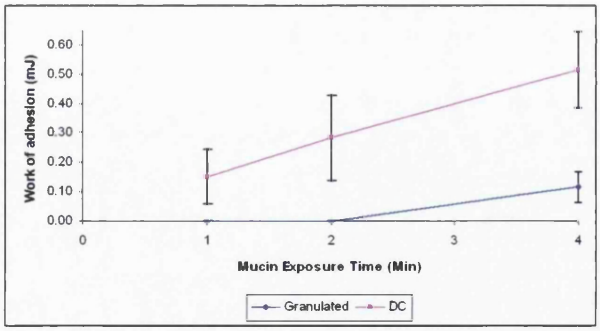

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Figure 2.23b Comparison of work of adhesion for 5% granulated and direct compression tablets after 4 minutes hydration in pH 6.8 phosphate buffer and prolonged exposure to mucin (n=5). a) CP 974, b) CP 980, c) CP 971, d) PC AA-1. (n=5)

Differences in detachment force and work of adhesion between DC and granulated tablets in the mucoadhesion and mucin exposure studies lead to the proposed mechanisms for mucoadhesion of each carbomer grade. Since the work of adhesion is a measurement of polymer and mucin interpenetration and granulated tablets exhibited little or no work we can conclude that interpenetration does not occur in the granulated systems, due to the uncoiled polymer network, therefore if granulated and DC tablets possess equal detachment forces other mechanisms other than interpenetration are likely to be involved:

- CP 974 – Detachment force for DC and granulated tablets in both the mucoadhesion and mucin exposure studies are equal. Combining this with the greater work of adhesion for the DC tablets in both tests leads to the suggestion that although polymer and mucin interpenetration will occur, initial and prolonged mucoadhesion is not reliant upon it and other mechanisms must be involved, these are likely to be either hydrogen bonding or mucin dehydration.

- CP 980 – From the mucoadhesion study, detachment forces for DC and granulated tablets are equal while the work of adhesion is greater for DC tablets, therefore, as with CP 974, this grade does not require polymer and mucin interactions to occur for maximum mucoadhesion. Other mechanisms (hydrogen bonding and water movement) must also be involved. However, during the mucin exposure study DC tablets exhibit a stronger force of detachment and work of adhesion than the granulated forms suggesting that optimal mucoadhesion will only occur if interpretation is allowed.

- CP 971 – Detachment force for granulated tablets was greater than DC in the mucoadhesion study and no work of adhesion was recorded for either. This implies that no interpenetration occurred, possibly due to the low carbomer loading, also explains the lower detachment force recorded for 5% DC tablets (compared with three other grades). Increased hydration and thus swelling in this instance is a hindrance to mucoadhesion as the gel layer is too disperse for meaningful interactions to occur quickly. However, after prolonged exposure to mucin the DC formulations express the greatest force

of detachment and work of adhesion, indicating that a strong adhesive joint establishes itself once interpenetration occurs. Therefore, for this grade initial and prolonged mucoadhesion requires mucin/ polymer entanglement.

- PC AA-1 – In both the mucoadhesion and exposure experiments granulated formulations showed a marked reduction in detachment force and work of adhesion compared to the DC formulations. Therefore, swelling of the polymer is vital for both initial and prolonged adhesion. Interestingly, during the mucin exposure study an increase in detachment force was observed for the granulated tablet from 1 to 4 minutes exposure, this was unique to this grade (an increase in work of adhesion was not seen). Unlike the other grades PC AA-1 is cross linked with divinyl glycol, not allylpentaerythritol, the latter processes numerous hydrogen bonding groups not present in the former. This could explain why this is the only grade that an increase in detachment force for the granulated tablet from 1 to 4 minutes exposure to the mucin is seen. Although these results suggest that for optimum mucoadhesion swelling of the polymer and entanglement with mucin are required, they also show that an increase in tensile strength upon exposure to mucin will occur that is not caused by entanglement.

Once characterised, successful delivery of these systems to the colon will require an additional step which is not considered within the scope of this work. The dosage form will need to be tailored to colonic delivery with the addition of a time delayed, enteric or bacteria activated coat. Subsequently the effect this addition has on the carbomer will need to be understood before a fully finalised colonic mucoadhesive system is produced.

2.10 Conclusion

The utilisation of two tensile testing apparatus highlighted a current problem with adhesion testing within the literature. It was seen that small differences in methods can produce a large difference in results and the force values obtained for a given sample are not consistent. Trends and relative order of adhesive capabilities were however the same. As such comparisons of current data with previous experimental data are not possible. For this area of research to develop a standard agreed upon test for mucoadhesion needs to be developed.

Adhesion and mucoadhesion testing of the carbomer polymers in compact form show that molecular weight and cross linking are important in determining mucoadhesive capabilities. More over, interactions between the polymers and a mucin solution were seen to be different for each grade. It was suggested that the work of adhesion measurement is an indicator of said polymer and mucin interactions. The lowest molecular weight polymer expressed the greatest work of adhesion suggesting that mucin and polymer interpenetration occurs to a greater extend for this grade. Exposure to mucin for extended periods of time uniformly caused an increase in detachment force but no increase in the work of adhesion. Only CP 971 continued to show an increase here, this implies that the work of adhesion is a representation of polymer and mucin entanglements and that these entanglements are not critical for adhesion for all grades.

pH of the environment surrounding the polymers was also seen to be important and affected the grades differently. In general the polymers were seen to be more adhesive in simulated colonic pH (6.8) than simulated gastric pH (1.2). The results obtained were used to offer and explanation to the discrepancies in the current literature regarding *in-vivo* testing, and highlighted the need for a suitable animal model to be selected.

Formulation of the polymers also had an effect on their adhesive and mucoadhesive behaviour. Increases in carbomer loading from 5-20% were seen to have no effect on adhesion but did affect polymer/mucin interactions. Testing of granulated tablets and comparison with their DC counterparts gave insights into the mechanism of mucoadhesion. Since the granulation process causes an initial unwinding of the polymer chain, little work of adhesion was seen for these tablets implying that little

interpenetration is occurring. From this it was concluded that, the mucoadhesive properties of CP 974 do not require polymer and mucin entanglement, CP 980 however can undergo initial mucoadhesion without interpenetration but stronger long term adhesion is seen when entanglement can occur. CP 971 is the most affected when inhibited from forming polymer and mucin interactions and, whilst PC AA-1 is also affected in this way, its mucoadhesion was seen to increase on prolonged exposure to mucin without the need for interpenetration. Drug release form the formulated systems was dependent on the carbomer grade and pH of the dissolution media with sustained release more achievable in pH 6.8 phosphate buffer at the higher loading levels. However, granulation of carbomers had an impact on this with three out of four grades losing their sustained release properties post granulation.

Chapter 3

An investigation into the feasibility of using multiparticulates as mucoadhesive platforms

3.1 Overview

Incorporation of carbomers into monolithic dosage forms provides the basis for a mucoadhesive colonic delivery system. However, due to limited water content (Schiller et al., 2005) the contents of the colon are highly viscous and faecal matter is packed much more densely than proximal regions of the gut. This combined with a potential "all or nothing effect" when delivering monoliths could prove problematic in providing opportunities for the carbomers to perform as intended *in-vivo*. Therefore it may be prudent to deliver a multiparticulate system to maximise the chance of contact of a dosage form with the wall of the colonic mucosa. Multiparticulate dosage forms containing the four carbomers were produced in the form of pellets (1mm) (**Section 1**) and microparticles (<100 μM) (**Section 2**). This chapter describes the formulation, optimisation, characterisation, drug release, adhesion and mucoadhesion testing of the multiparticulates. Two processes were evaluated to produce carbomer pellets, extrusion and spheronisation and hot melt extrusion. Microparticles were produced using a solvent evaporation technique.

Section 1 Pellets

3.2 Extrusion and Spheronisation

Extrusion and spheronisation is a process used widely to produce mutliparticulate dosage forms. The process contains several steps which are (i) dry mixing of ingredients, (ii) wet massing generally requiring the extrudable wet mass to be wetter than that needed for wet granulation, (iii) extrusion, (iv) spheronisation, (v) drying and finally, (vi) screening. Each process serves a specific purpose. For example, dry mixing and wet massing are necessary to produce a homogenous wet mass with sufficient plasticity to extrude, the properties of the extrudate are largely dependent on the plasticity and cohesiveness of the wet mass which is why the powders usually need to be wetter than when undergoing wet granulation. The next step, extrusion, is to produce rod shaped particles suitable for the following process of spheronisation; there are several types of extruders.

- Screw feed (including axial and radial), consisting of one or more helical screws which transport the material to the die at the opposite end. Sheering of the screw and friction of the material produces sufficient pressure to force the material through the die.

- Gravity feed extruders (cylinder or gear). Cylindrical extruders operate using two contra rotating cylinders, one of which contains equally spaced holes of identical diameter. The powder is fed between the cylinders and is forced through the holes and collected. Gear extruders are similar in that they operate by using two contra rotating gears with the dies between each tooth. The material falls between the cogs and is forced through the die on the opposing gear.

- Piston feed or ram extruders. These work on the principle of a pre-filled system. The material is packed into a cylindrical barrel with the die at one end. The material is forced through the die by the exertion of a constant load from a piston inserted into the barrel

During the spheronisation step, the rod shaped particles are spheronised by frictional forces caused by particle-particle and particle - equipment collisions. The drying process that follows is to form pellets of the desired moisture content and finally screening to produce uniform size distribution. A robust formulation should produce a high yield of pellets of the desired size distribution, uniform spherical shape, good flow properties, high strength, low friability, smooth surface and ease of coating.

One of the earliest methods of producing spherical pellets was with a ram extruder (Conine et al., 1970). Prior to extrusion the dry powders are mixed with a fluid, usually water, which serves as a binder but also increases the plasticity of the formulation. Early work with this process showed that the choice of excipients was critical to the production of spherical pellets, with the addition of microcrystalline cellulose enhancing the plasticity of the formulation and thus producing more spherical pellets (Reynolds, 1970)

3.3 Hot melt extrusion

The process of hot melt extrusion has been used extensively in the plastics industry since the mid-nineteenth century and has become increasing popular in the development of pharmaceutical dosage forms. The process involves pumping materials at high temperature towards a die to produce an extrudate of uniform shape and size. Hot melt extrusion offers some advantages over more conventional extrusion techniques since solvents and water are not necessary as the molten polymers act as thermal binders. As such, the polymers can be used to produce larger dosage forms without the need for compression.

The process equipment used for melt extrusion is categorised into two types, ram extruders and screw extruders. The former operates by utilising positive displacement of a ram with suitably high pressure so as to push the melt through a die to produce the desired shape. Prior to extrusion the excipients are placed into a heated cylinder causing them to soften (Crowley et al., 2007). The high pressures used mean that this technique is highly advantageous as it allows for the precision extrusion of highly valuable materials, however, limited melting capacity means that temperature uniformity of the extrudate is poor and the use of the heater cylinder means that the extrudate often has poor homogeneity. These drawbacks are however not present in the screw extrusion process due to the shear and stress forces and intense mixing involved. Screw extrusion equipment involves the use of either a single or a twin screw extruder. Both types of equipment offer advantages over the other, for example, twin screws have easier feeding mechanisms, high kneading and dispersing capacities, less tendency to over heat and shorter transit times. Single screw extruders are mechanically simpler and more cost effective (Repka et al., 2002).

As with traditional dosage forms the inclusion of certain excipients can impart specific properties to the hot melt extrudate. Most commonly used are plasticisers and carriers. Materials used as carriers must be thermodynamically stable and be processed at relatively low temperatures since most drugs are thermally sensitive. The matrix or carrier system used for hot melt extruded delivery systems is usually one or more "meltable" substances, often a polymer or low melting wax, into which the drug is embedded. The choice of carrier polymer is critical as it is this excipient which often

dictates the extrusion process parameters and as with any dosage forms can control the release of the active. Plasticisers are used to lower the processing temperatures of the system by decreasing the glass transition temperature and melt viscosity by increasing the free volume between the polymer chains (Aharoni, 1998).

The production of dosage forms using hot melt extrusion method has been investigated and documented in the literature. An investigation into the physiochemical properties and drug release mechanisms of ethylcellulose tablets prepared by melt extrusion has been undertaken (Crowley et al., 2004). Comparison of tablets produced in this manner with those prepared by direct compression showed extended release of guaifenesin for hot melt extruded tablets, which were shown to be less porous and had undergone a greater degree of densification than their direct compression (DC) counterparts. Other changes in the expected behaviour of the dosage form have been observed with the production of modified release tablets. Formulations containing either chitosan or xanthan gum as release modifiers were investigated and again compared with DC formulations (Fukuda et al., 2006). The investigators found that the melt extrusion process did not effect the release (compared with DC tablet) of chlorpheninramine maleate when either chitosan or xanthan gum were used separately but did when used together. They showed that such systems could provide pH and buffer species independent drug release. The differences seen compared to a DC formulation were attributed to water uptake differences with the two dosage forms. By observing the cross sectional morphologies the penetration of the dissolution media at 6 hours could be determined, and seen to be 100% for DC tablets and 72% for hot melt extruded tablets. Additional approaches to using melt extrusion to produce modified delivery dosage forms have been investigated by the production of pellets using ethylcellulose to provide sustained release (Follonier et al., 1994) and Eudragit polymers for both delayed release tablets (Bruce et al., 2005) and pellets (Young et al., 2002).

3.4 Carbomer pellets

The use of carbomer polymers to affect the GI transit of pellets has previously been studied (Khosla et al., 1987). Polycarbophil AA-1 was shown to delay the gastric emptying of pellets in the rat stomach. The carbomer however was not incorporated into

the pellet formulation but co-administered with radio labelled amberlite pellets in a capsule, as such retention of the pellets was reliant on the interaction between them and the carbomer gel *in-vivo*.

Incorporation of the polymers into the dosage forms themselves for modified release has shown some success (Neau et al., 1996, Neau et al., 2000). Evaluating the use of carbomers for controlled release, a ram extrusion process successfully produced Carbopol 974 pellets with up to 45% w/w polymer. With water as granulation fluid the investigators reported that to eliminate tack and to enable extrusion, strong electrolytes were added in the form of calcium chloride. The effect of carbopol and electrolyte level on tack was investigated by measuring the force required to separate wet powders from a stainless steel surface. The authors reported that increasing the carbopol content increased tack; however this was eliminated with a corresponding increase in electrolyte concentration. The effect of a strong electrolyte on carbomers is understood. As explained earlier, the unfolding of the polymer structure is caused by the repulsion of the negatively charged carboxyl groups on the polymer back bone, contact with a strong electrolyte neutralises the charge and reduces gelling (Kriwet et al., 1996). Further studies have quantified this and have shown the effect of addition of a strong electrolyte on the elastic moduli of carbopol gels. The elastic moduli of the system are seen to decrease upon the addition of 200mM solution of sodium chloride and calcium chloride (Bonner et al., 1997).

Although these studies successfully produced pellets with high levels of carbomer, they do not necessarily demonstrate a suitable way to manufacture pellets intended for mucoadhesive applications due to the effect of the electrolytes. As such, the limited number of studies focused on the production of carbomer pellets for bioadhesive purposes have omitted them from the formulation (Mezreb et al., 2004). Concentrating on the three process variables (extrusion speed, spheronizer speed and time) and one formulation variable (the amount of water) Carbopol 934 pellets were successfully produced with a maximum of 20% loading, however, although the aim of the study was the production of pellets for mucoadhesion, no adhesive tests on the manufactured pellets were undertaken so there is no way to ascertain if the process has affected the carbomers.

Within this current study the purpose was to produce mucoadhesive pellets; therefore the formulations investigated did not contain any electrolyte solutions. Instead the wetting of the powders was undertaken with an ethanol and water mix. However production of pellets with ethanol can present its own problems as the resulting pellets have been shown to be more porous and as such weaker and more friable (Berggren et al., 2001). Additionally, current literature regarding the mucoadhesive pellets is limited and most studies do not investigate the mucoadhesive properties of the fully formulated systems.

3.5 Aims

The primary aim of this section is the evaluation of the *in-vitro* mucoadhesive properties of carbomer containing pellets. Secondly to investigate a new extrusion process for the manufacture of carbomer dosage forms without the need for wet massing.

As such the objectives are as follows:

- To produce spherical pellets measuring 1000-1400 μm containing carbomer polymers without the use of anti-tack agents by a ram extrusion process and to assess the effect of ethanol and water solutions on the production of said pellets
- Investigate the use of hot melt extrusion as a means to produce dosage forms with higher carbomer loadings
- Evaluate the adhesive and mucoadhesive properties of the multiparticulate systems *in-vitro*

3.6 Materials

Carbopol 974, 980, 971 and polycarbophil AA-1 were received as gifts from Noveon, France. Lactose and paracetamol were supplied by Ellis and Everard, Essex, UK. Microcrystalline cellulose was obtained from FMC international, Little Island, Cork, Ireland. Hydroxypropylcellulose was purchased as Klucel EF, Honeywill and Stein, UK and Myvacet™ from Quest International, Zwijndrecht, Netherlands. Mucin was purchased from Sigma, St. Louis, MO. USA. Buffer salts were obtained from VWR International Ltd, Poole, UK. Where water is stated, de-ionised water was used.

3.7 Methods

3.7.1 Preparation of pellets by ram extrusion

Paracetamol, lactose, microcrystalline cellulose (MCC), and carbomer polymer (Table 3.1) were mixed in the appropriate amounts to produce a total batch of 50g in a planetary mixer for 20 minutes with frequent pauses to remove any powder adhering to the edges of the mixing bowl. The volume of wetting fluid, a mixture of ethanol and water, and the ratio of the two solvents was determined for each grade of carbomer in a two way optimisation study. The amount of wetting fluid added was investigated at 30, 40, 50, 60, 70 and 80% w/w of the dry weight of powders and the ratio of ethanol to water at 0:100, 25:75, 50:50, 75:25 and 100:0. Mixing was halted and the fluid was added in evenly distributed 5ml aliquots, across the power surface. Mixing was continued for 5 minutes before pausing for the addition of more fluid. Once all the fluid was added the lid was sealed to limit solvent loss and mixing continued for a further 10 minutes, after which the wet mass was placed in 25g samples in polyethylene bags and sealed.

Table 3.1 Formulation for the production of carbomer and control pellets

	Amount in Formulation (%)	
Material	**Control**	**Carbomer**
paracetamol	20.00	20.00
MCC	44.20	41.70
lactose	35.80	33.30
carbomer	0	5.00

The wet powder mass was packed tightly into a barrel, 2.54 cm diameter, 20.3 cm in length, to ensure little or no air remained in the barrel. A 1 mm diameter die was fitted to the end of the barrel and a piston inserted into the top. The piston, barrel and die arrangement were placed on the support of the MX50 ram extruder, under the cross head. The cross head was lowered a sufficient distance to allow contact between itself and the piston. The piston was forced downwards at 200 mm/min forcing the wet mass through the die.

The extrudate, collected at the bottom of the barrel, was spheronised using a 20.3 cm diameter spheroniser (GB Caleva Ltd., Sturminster Newton, UK), for 15 minutes for the control and 10 minutes for carbomer pellets with frequent stops to remove pellets adhering to the side of the spheroniser and to allow excess moisture to escape. Pellets were dried in air for 30 minutes to allow the ethanol to evaporate after which time they were placed in an oven at 60°C until dry. Size distribution of the pellets was performed using sieve analysis. 100 g of pellets were placed on a nest of British standard sieves, with a $\sqrt{2}$ progression ranging from 500 μm to 1700 μm, on a mechanical sieve shaker (Endecott Ltd., London, UK) for 10 minutes.

3.7.2 Drug release

Drug release from the platforms was determined by USP II paddle dissolution apparatus (PTWS, Pharma Test. Hamburg, Germany). Tests were conducted in 900 mL of dissolution medium maintained at 37 ± 0.5 °C, with a paddle speed of 100 rpm with 100mg of pellets. Dissolution medium was sampled automatically at 5 minute intervals by an in line UV-Vis spectrophotometer (Cecil 2020, UK.) at a wavelength of 242 nm.

3.7.3 Sphericity

Images of 50 pellets were taken, and the ratio of two diameters (perpendicular to each other) was calculated using image J analysis software.

3.7.4 Scanning electron microscopy (SEM)

Samples were placed on SEM stubs and fixed using carbon discs before being gold coated using an EMITEC K 550 sputter coater for three minutes at 40mA. The samples were then transferred to a Philips XL20 Scanning Electron Microscope for imaging.

3.7.5 Preparation of pellets by melt extrusion

The carrier polymers hydroxypropylcellulose (HPC) or ethylcellulose (EC) were mixed with carbomer powders at 10 % w/w by hand for approximately 5 minutes, total batch size 10 - 15g. When EC was used a plasticiser Myvacet^{TM} (mono glyceride) was

included at 5%w/w (5% w/w of carrier polymer). The Thermo Haake twin screw hot melt extruder was heated to the required temperature (Table 3.2) and the powder mix added to an opening at the base of the chamber above the screws. The powder passed through the opening, onto the screws, upon melting the powder mixes were forced down the increasingly narrowing screw barrel to a sample collection hole at the opposite end. The extrudate, originally soft and malleable, hardens within a few seconds at room temperature and was cut into pieces measuring 15cm for ease of collection. Extrudate of HPC and EC without carbomers were also produced in this way.

Table 3.2 Summary of conditions for the Thermo Haake twin screw extruder

	HPC + carbomer	**EC + carbomer**
Temperature (°C)	140	160
Speed (rpm)	50	100
Pressure (m Ncm^2)	40-60	40 -60

Ideally, pellets would then be formed by a continuous cutting process; however limitation of availability of this equipment meant that the extrudate itself underwent adhesion testing.

3.7.6 *In-vitro* mucoadhesion testing

Ram extruded pellets

The mucoadhesive potential of the pellets was assessed as in the previous chapter from a glass and mucin surface after hydration in pH 6.8 phosphate buffer. Exposure to mucin post hydration was also evaluated. Approximately 100 mg of pellets were attached via adhesive tape measuring 10 mm by 10 mm to the probe of the Instron.

Hot melt extrudate

Adhesion from a glass surface was evaluated for the hot melt extrudate using the texture analyser. Three sections of extrudate measuring 20 mm were fixed side by side to the probe of the TA. The test was conducted as detailed previously

Data was analysed using parametric tests; results were analysed for significant differences using one-way analysis of variance (ANOVA), with post-hoc analysis using Tukey's test. All tests were carried out using SPSS Version 14.0 statistical software package.

3.8 Results and Discussion

Previous methods of producing carbomer pellets have used the addition of strong electrolytes to limit tack; the negative effect of this is limitation of gel formation of the carbomer polymers which can potentially affect their adhesive properties. The purpose of this work was to investigate ways of producing carbomer pellets without these limitations. Previously, an ethanol and water mix was used in the granulation of carbomer tablets. Using a similar approach this section investigates the production of carbomer pellets extruded using a ram extrusion process and the effect of an ethanol and water mixture as the wetting fluid.

3.8.1 Ram extruded pellet manufacture

Initially pellets containing 100% carbomer were attempted, however this was seen to be unfeasible as a gel was formed and extrusion thus not possible. Therefore excipients such as microcrystalline cellulose were added to the formulation as an extrusion aid to improve the plasticity of the mix. A 5% loading of carbomer was initially chosen as this would produce pellets which could be compared to the granulated tablets made previously, however as with the tablets a loading greater than this could not be produced. The pellets were assessed with respect to their size, aspect ratio, visual assessment (SEM), drug release (dissolution) and adhesion and mucoadhesion properties.

It was theorised that as with granulation, wetting with an ethanol and water mix would reduce tack (compared to using water alone) and improve mixing and subsequent extrusion of powders. This study focused on the amount of fluid required to sufficiently wet powder formulations containing 5% w/w carbomer and the ratio of ethanol to water within the wetting fluid. As the highest and lowest molecular weight polymers respectively, CP 974 and CP 971 were initially investigated as it was assumed that these two grades would represent the upper and lower levels of required fluid. In all cases 50 g of powder was mixed and fluid added, however, it was not possible to extrude all formulations either due to rapid evaporation of ethanol and subsequent drying of the blend or due to the high level of tack resulting in difficulty during the spheronisation

process. Table 3.3 shows the effect of the solvent mixtures on pellet yield for 5% CP 974 pellets.

Table 3.3 Effect of solvent ratio and amount of wetting fluid on pellet yield from 5% CP 974 pellets produced by ram extrusion. Initial batch size 50g

	Amount of fluid (as percentage of dry weight)		
Ethanol:water	**40%**	**50%**	**60%**
100:0	0	74%	68%
75:25	69%	100%	42%
50:50	0	68%	23%
25:75	0	68%	47%
0:100	0	0	0

This study showed that the volume of fluid required and the ratio of ethanol to water affected the production of pellets. With the addition of 40% fluid the formulations were too dry to extrude (with the exception of a 75:25 ethanol to water ratio) likewise with 70% fluid (with the exception of 100% ethanol) the formulations were too tacky to spheronise resulting in a balling effect. The best results for this grade were seen when 50% fluid was used as extrusion was possible with all but one of the mixtures (100% water). The use of 60% fluid also produced extrudable material with 3 of the 4 mixtures (again the exception being 100% water), however the yields were lower. Once extruded, the extrudate was collected in polythene bags, placed below the ram head. At higher water contents the extrudate was too plastic and was observed to reform into a collective mass in the collection bag, resulting in less material available for extrusion.

3.8.1.1 Sieve analysis

Since the desired size of pellets is 1000-1400μm, sieve analysis was performed as a simple and practical way to determine the size distribution of the pellets and the effect of the ethanol and water ratio on this. Figure 3.1 shows the sieve fractions for the 7 formulations that were produced using CP 974.

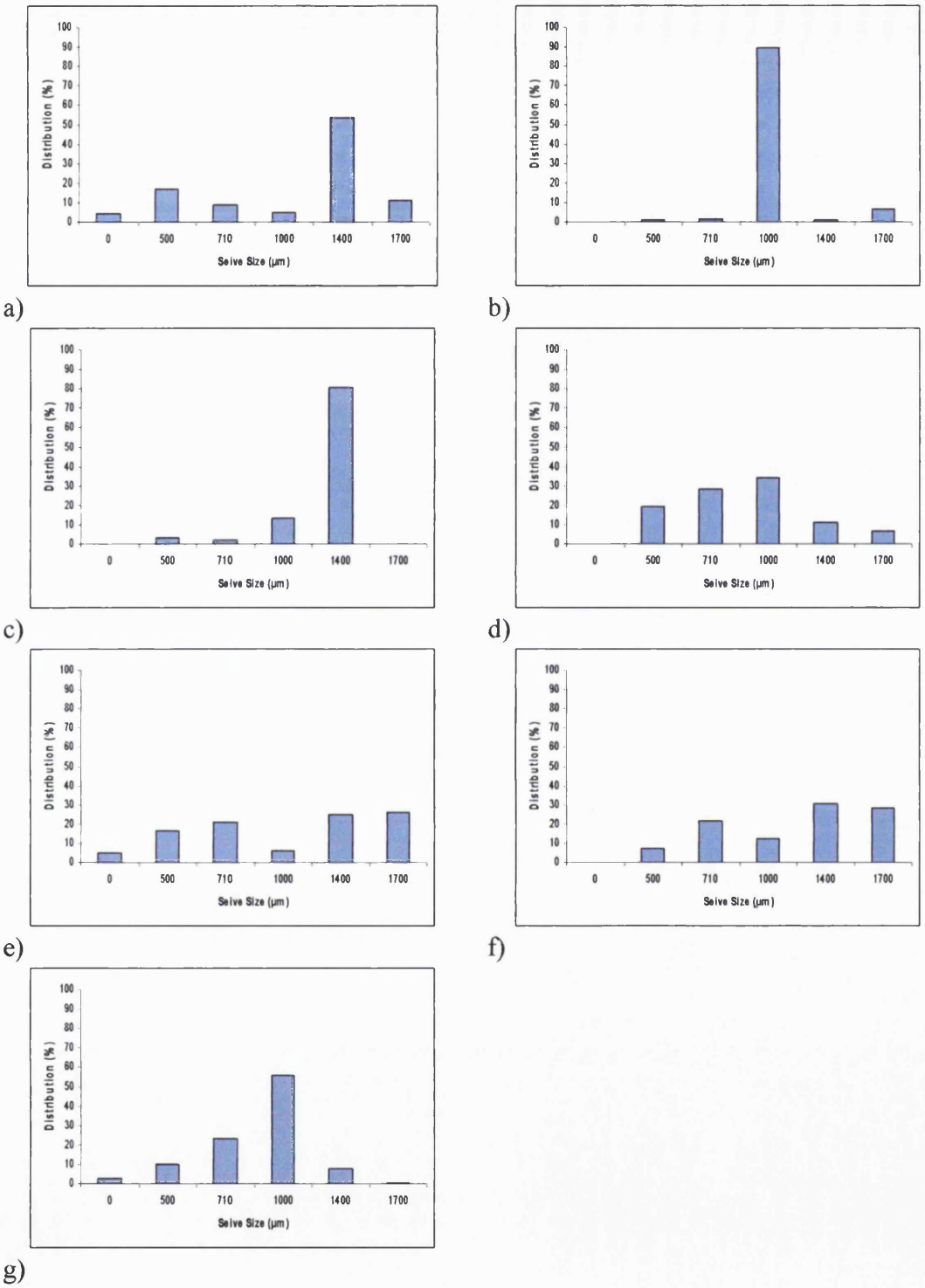


Figure 3.1 Size distribution of 5% CP 974 pellets produced with a range of ethanol and water mixtures, by ram extrusion ascertained by sieve fraction. a) 100 (ethanol) :0 (water) at 60% w/w (total dry weight of mix), b) 100:0 at 50% w/w, c) 75:25 at 60%w/w, d) 75:25 at 50% w/w, e) 50:50 at 60% w/w, f) 25:75 at 60% w/w and g) 25:75 at 50% w/w

None of the formulations produced pellets with a normally distributed size range, additionally for this grade it appears that ethanol alone as the granulation fluid produces narrower distribution (Figures 3.1 a and c). The addition of water to the fluid promotes production of pellets more widely distributed with a ratio of 25:75 ethanol to water at 50% w/w processing the greater number of pellets within the desired size range (Figure 3.1 d-g.).

The same study and analysis was performed on CP 971. Table 2.14 shows the yields produced from a 50 g batch containing 5%w/w carbomer powder. Firstly it is important to note that the minimum and maximum fluid content with this grade were 30 and 60% respectively not 40-70% as seen with CP 974, so we can already conclude that the less cross linked carbomer grades require less fluid content to extrude. We see similar trends with the yield as we did for CP 974 grade, again the addition of too much fluid (60%) results in a mass too tacky to extrude and again in general as the content of water in the granulation fluid increases the yield decreases.

Table 3.4 Effect of solvent ratio and amount of wetting fluid on pellet yield from 5% CP 971 pellet produced by ram extrusion. Initial batch size 50g

	Amount of fluid (as percentage of dry weight)		
Ethanol:water	**40%**	**50%**	**60%**
100:0	69%	81%	0
75:25	100%	65%	0
50:50	0	71%	0
25:75	0	56%	0
0:100	0	27%	0

Figure 3.2 shows the results of the sieve analysis. Previously, higher ethanol content of the granulation fluid produced narrower size distribution and larger pellets. With CP 971 the opposite is the case as it is an increase in water content not ethanol that leads to a greater number of oversized pellets.

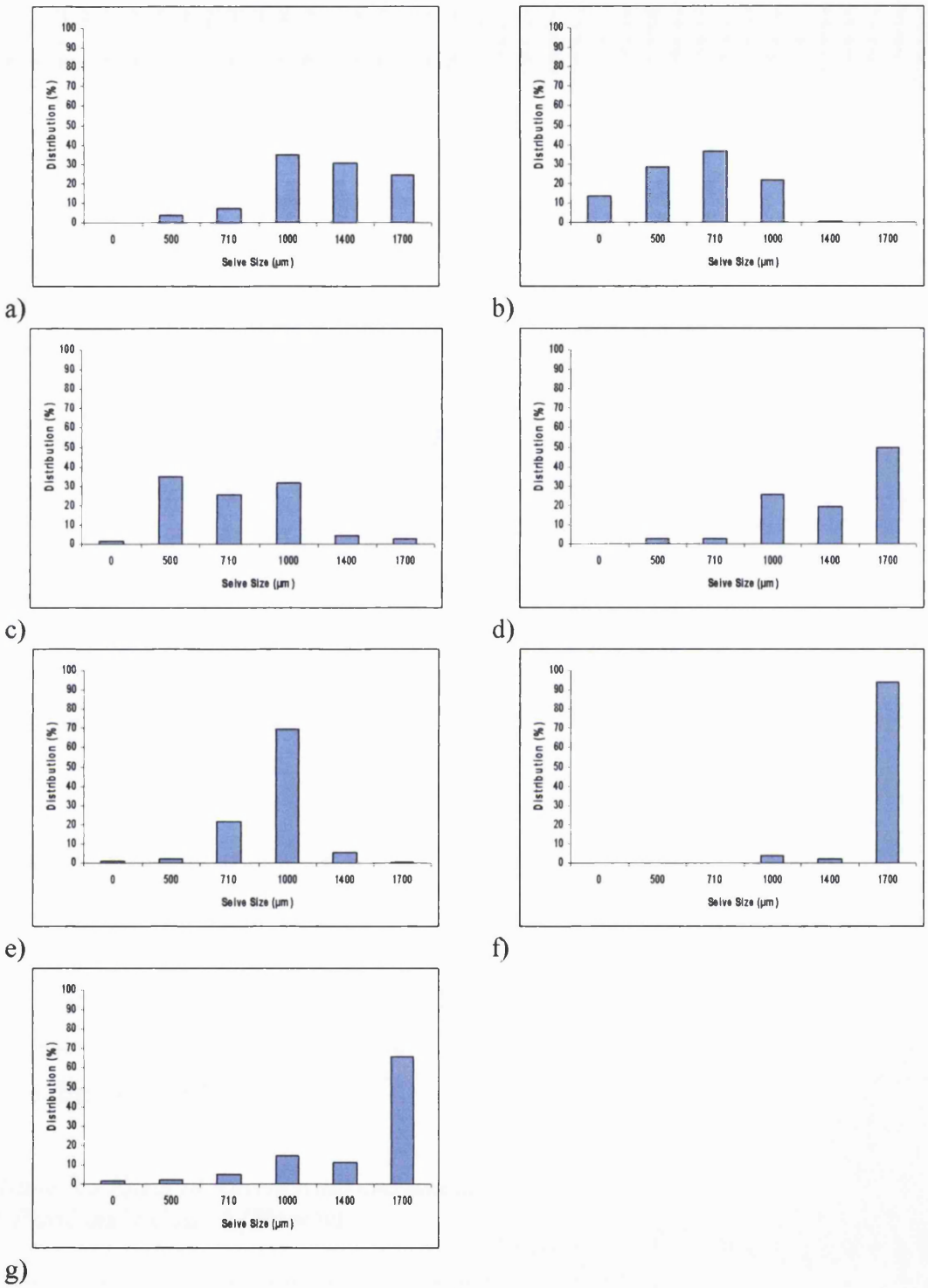


Figure 3.2 Distribution of 5% CP 971 pellets produced with a range of ethanol and water mixtures, by ram extrusion ascertained by sieve fraction. a) 100:0 at 50% w/w, b) 100:0 at 40% w/w, c) 75:25 at 50% w/w, d) 75:25 at 40%w/w, e) 50:50 at 50% w/w, f) 25:75 at 50% w/w and g) 0:100 at 50% w/w

The effect of the granulation fluid on the formation of pellets has been investigated previously in the literature; comparison of pellet properties such as porosity and densification and contraction of MCC pellets upon drying have been studied, comparing water to an ethanol and water (75:25) mixture (Berggren et al 2001). Pellets prepared using the ethanol and water mixture were shown to contract less upon drying than those prepared with water alone (40% and 54% respectively). This perhaps explains why larger pellets are obtained with CP 974 accompanying increases in ethanol in the granulation fluid. However, it does not explain why the converse is true for CP 971.

A mathematical model has been devised to explain the effect of granulation fluid during the drying process and has shown that the properties of the produced pellets are influenced by contraction driving and contraction counteracting forces (Dreu et al., 2005). Although the size of pellets specifically was not investigated, these findings can be used to provide an explanation for the sieve analysis results for CP 971. As the lowest molecular weight carbomer this grade has been shown to form more expansive gels than the other grades, particularly CP 974 (Noveon, 2000), undoubtedly this would serve to inhibit contraction driving forces resulting in the production of larger pellets.

The two remaining grades, CP 980 and PC AA-1, were also investigated, using values obtained from the optimisation study with CP 974 and 980 it was determined that 50% w/w of fluid would be added but the ethanol to water ratio would still be altered. For PC AA-1 an additional formulation was included using 60% fluid at a ratio of 50:50. Table 3.5 shows the formulations produced and the corresponding yields. Figures 3.3 and 3.4 show the sieve analysis.

Table 3.5 Effect of solvent ratio and amount of wetting fluid on ram extruded pellets of CP 980 and PC AA-1 (5% w/w)

	Pellet yield (%) using 50% w/w fluid	
Ethanol:water	CP 980	PC AA-1
75:25	71%	89%
50:50	44%	55%
25:75	69%	22%

As with CP 974 and 971 grades, the amount of wetting fluid required to formulate CP 980 and PC AA-1 pellets was consistently around 50-60%. The ratio of ethanol to water mirrored what was seen in the original optimisation study with the lower molecular weight polymer, PC AA-1 producing greater number of pellets of the desired size with less water present and vice versa for the higher.

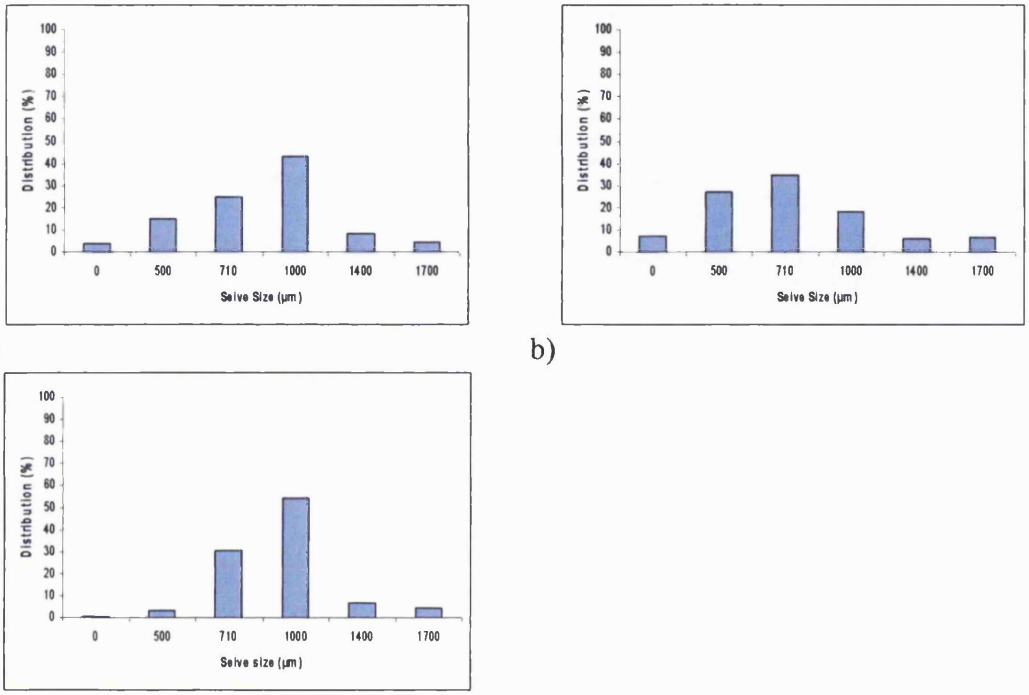


Figure 3.3 Distribution of 5% CP 980 pellets produced with a range of ethanol and water mixtures, by ram extrusion ascertained by sieve fraction. a) 75:25 at 50% w/w, b) 50:50 at 60%w/w, c) 25:75 at 50% w/w

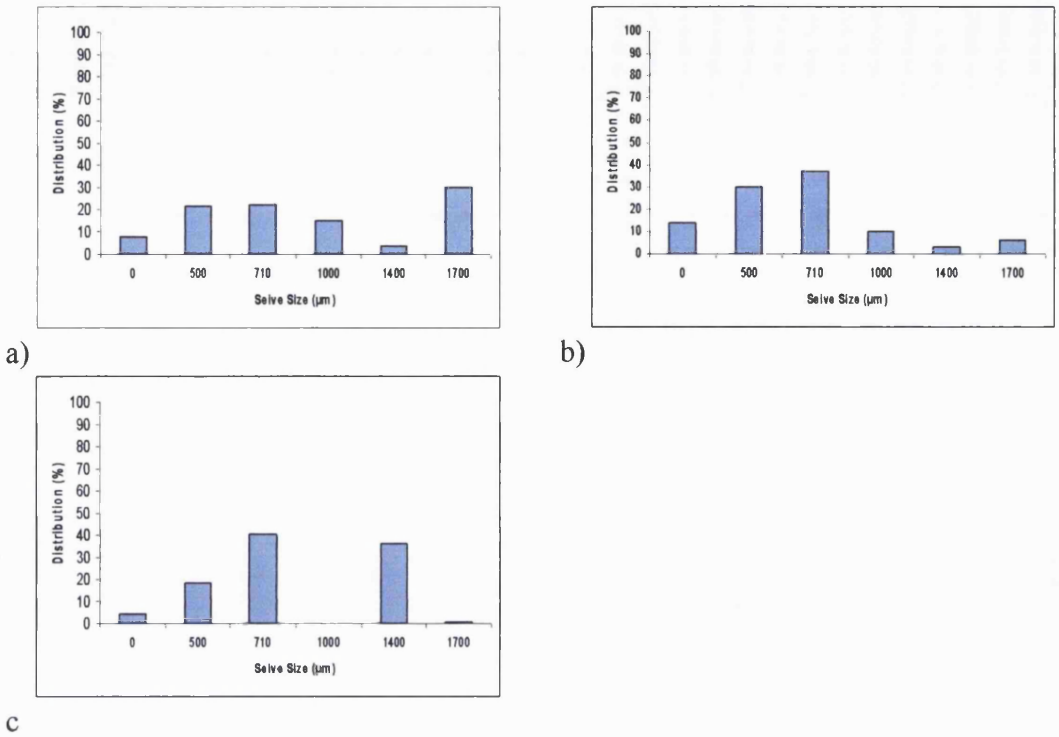


Figure 3.4 Distribution of 5% PC AA-1 pellets produced with a range of ethanol and water mixtures, by ram extrusion ascertained by sieve fraction. a) 75:25 at 50% w/w, b) 50:50 at 50% w/w, c) 25:75 at 50%w/w.

3.8.1.2 Sphericity and Aspect ratio (AR)

The uniformity of a dosage form is an important consideration in any production process. With tablets, the weight is the most critical factor as this directly influences drug content. With multiparticulate systems the weight of the individual unit is not as important as an increase or decrease of number of units which can be made where necessary to a delivery system to obtain the required dosage. The size and shapes however are important as these will affect both the movement of the dosage form in the GI tract and the release of the drug. Additionally, to achieve drug release in the distal GI tract the pellets need to be coated with a suitable polymer; this is more easily achieved with spherical pellets.

Within the literature most approaches to access shape have focused on a two-dimensional approach in that they evaluate one surface of the sphere and equate that to sphericity. Techniques such as "one plane critical stability" have been used to measure the sphericity of an object (Chapman et al., 1988). This measurement uses the theory

that an object at rest will move if the plane it is resting on is tilted to the point that angle of the plane is such that the centre of gravity of the particle will move out side that particle. The more elliptical the particle the harder it is for it to theoretically roll and so the greater the angle of the plane. Other techniques have focused on imaging pellets and measuring the ratio of two diameters (Lovgren et al., 1989 and Baert et al., 1992). Here images of the pellets are taken and the length, defined as this distance across the longest axis, and the width, defined as the distance across the particle on a line perpendicular to the midpoint of the longest axis, was measured and the ratio calculated. The ratio was expressed as percentage sphericity where 100% corresponds to the most spherical. Similarly, an aspect ratio (AR) was used by Baert et al., (1992) and was based on a 2D image of the particle. Elongation and roundness have also been used to define the shape of granules and pellets (Hellen et al., 1993).

The method used here was similar to those described in that a 2D image of the pellets was taken from which the width and length were measured using, image analysis software and the AR calculated (Table 3.6). More spherical pellets will have an AR of closer to 1.

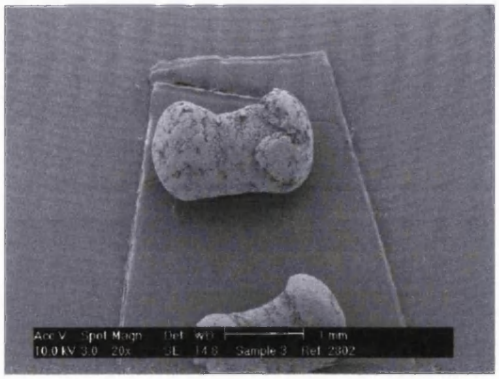
Table 3.6 Aspect ratio for lead formulation of pellets contain 5% carbomer w/w

	Formulation			
	100:0 at 50%	**75:25 at 50%**	**50:50 at 50%**	**25:75 at 50%**
974	0.50 ± 0.13	0.82 ± 0.10	0.90 ± 0.07	0.92 ± 0.04
971	0.78 ± 0.13	0.68 ±0.14	0.90 ± 0.08	0.91 ± 0.06

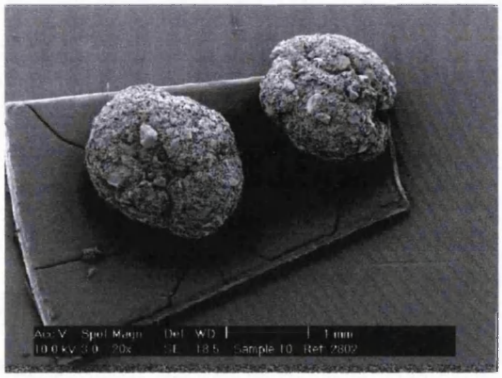
Again using CP 974 and CP 971 grades to optimise the formulation, the aspect ratios of pellets was assessed. The pellets were chosen based in high yields within the desired range (1000 μm – 1400μm).

For CP 974, 5 formulations were investigated. High yields (100% and 74%) and a large number of pellets of the relevant size were seen for the formulations prepared with 100% ethanol at 70% and 50% weight of the dry mass. However the aspect ratio for both is not ideal and visual assessment of the SEM (Figure 3.5) shows that the pellets

produced are dumbbell shaped and have rough surface morphology. As we increase the water content we find that the aspect ratio approaches 1(0.90 ± 0.07 and 0.92 ± 0.04 for 50:50 at 50% w/w and 25:75 at 50% w/w respectively) and the produced pellets appear more spherical under SEM. The same trend is observed for CP 971 pellets (Figure 3.6); we see an increase in sphericity with a decrease in ethanol content of the granulation fluid. The production of spheres is reliant on friction caused by particle to particle and particle to equipment collisions, the extrudate must remain moist and retain its plasticity, it can be seen here that higher levels of ethanol produce dryer extrudate which will not spheronise as easily as extrudate formed with higher ratios of water. Therefore a compromise must be made and the ethanol to water ratio must be such that a high yield of the desired size is achieved but the mix must retain its plasticity long enough to produce spherical pellets.



a)



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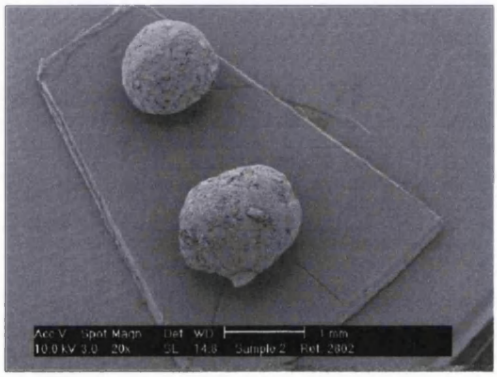

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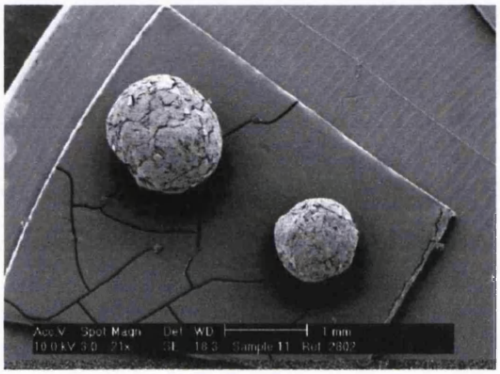

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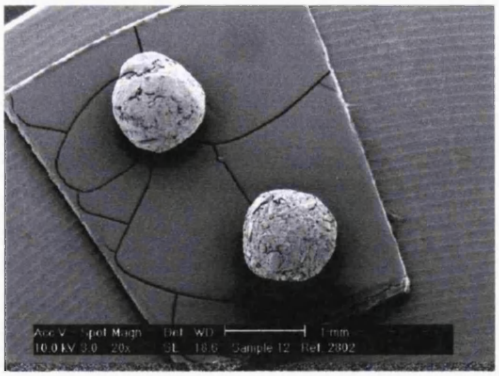


e)

Figure 3.5 SEM of CP 974 pellets prepared by ram extrusion with varying ethanol to water ratios and content. a) 100:0 Ethanol to water at 70% weight of dry mass, b) 100:0 Ethanol to water at 50% weight of dry mass, c) 75:25 Ethanol to water at 50% weight of dry mass, d) 50:50 Ethanol to water at 50% weight of dry mass, e) water 25:75 Ethanol to water at 50% weight of dry mass

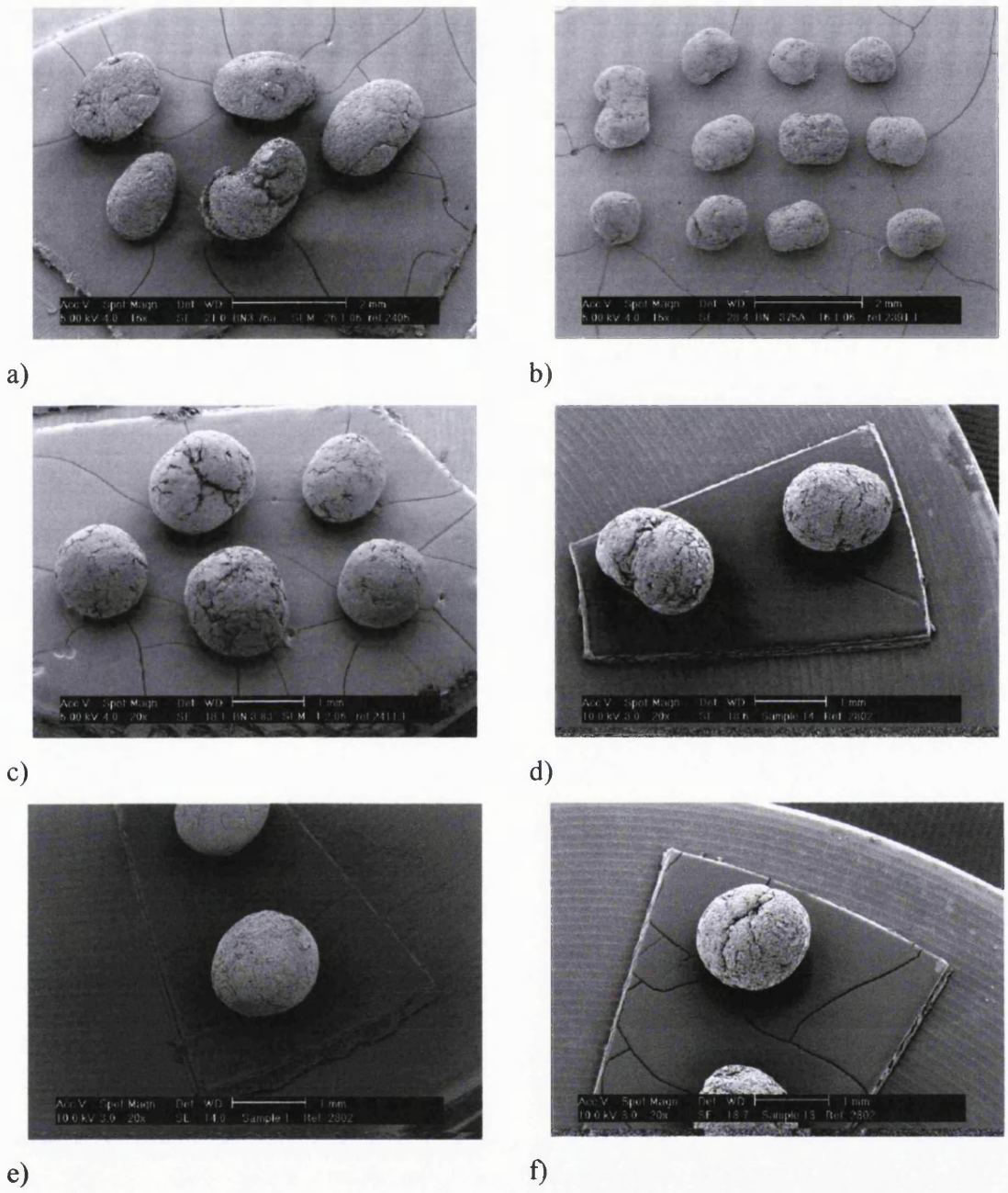


Figure 3.6 a) CP 971 100:0 ethanol to water at 50% weight of dry mass, b) CP 971 25:75 ethanol to water at 50% weight of dry mass, c) CP 971 Pellets 50:50 ethanol to water at 50% weight of dry mass, d) CP 971 25:75 ethanol to water at 50% weight of dry mass ,e) CP 980 25:74 ethanol to water at 50% weight of dry mass f)PC AA-1 50:50 ethanol to water at 50% weight of dry mass.

Previously discussed were some methods used to measure sphericity, however it is important to note that, although these methods go someway to quantifying the shape of pellets they may not be the most accurate. Firstly, the techniques use two-dimensional images from which to measure their respective functions and as such can not take the particle as a whole into account and secondly they also do not consider surface morphology or any irregularities. In an approach to clearly identify roundness and to incorporate the effect of the surface of the spheroids into the measurement, shape factor (e_R) was devised (Podczeck et al., 1994). Using a set of non-circular model figures and a mathematical model to calculate the e_R, the authors showed that shapes such as squares and triangles could have an aspect ratio approaching 1 however their e_R values would differ significantly, thus concluding that in some cases that e_R would be a more suitable tool than those based on 2D imaging.

It is for this reason that the final decision as to the optimum formulations for the pellets was based on sieve analysis, yields, aspect ratios and SEM observations collectively and not just the aspect ratios. Using the four parameters, it was possible to determine the optimum formulations for each grade of carbomer formulated. Table 3.7 shows the chosen ethanol-to-water ratios for each polymer grade, the corresponding yield, % remaining on the 1000μm sieve and the AR.

Table 3.7 *Optimisation of 5% carbomer pellets produced from ram extrusion*

Carbomer	Ethanol to water ratio and amount	Yield (%)	Quantity of pellets of the desired size (%)	AR
974	25:75 @ 50%	68	56	0.92 ± 0.04
980	25:75 @ 50%	69	54	0.93 ± 0.05
971	50:50 @ 50%	71	69	0.90 ± 0.08
AA-1	50:50 @ 60%	41	67	0.91 ± 0.06

For all grades the pellets produced had an AR between 0.90 and 0.93, the greatest yield was from CP 971 and the lowest for PC AA-1. Therefore, the ratios shown in Table 3.7 were considered to be producing the lead formulations and as such were used to investigate drug release and *in-vitro* mucoadhesion

3.8.1.3 Drug Release

Drug release from the pellets was investigated in simulated colonic pH at pH 6.8 phosphate buffer and simulated acid pH in 0.1 M HCl. Wet massing and extrusion of the carbomers has a similar effect on the release of paracetamol as granulation. Figures 3.7 and 3.8 shows the release of the paracetamol from 1 mm pellets in 0.1 M HCl and pH 6.8 phosphate buffers respectively. All pellet formulations show immediate release in both media and no difference is seen from the addition of carbomer polymers. Naturally we would expect to observe faster release from pellets than tablets due to the increased surface area and indeed this is the case, however in the previous chapter extended release of paracetamol in pH 6.8 phosphate buffer with 5% CP 971 granulated tablet was noted. This is not the case with the corresponding pellet formulation. There are two possible explanations for this, firstly the additional fluid required to achieve a sufficiently wet mass for extrusion has caused the polymer chain to uncoil to such an extent that the gel layer produced is no longer sufficient to act as a diffuse layer and retard drug release. Or, the combination of large surface area and highly soluble drug are the cause and the drug enters solution before the polymer is sufficiently hydrated to provide a diffusion barrier.

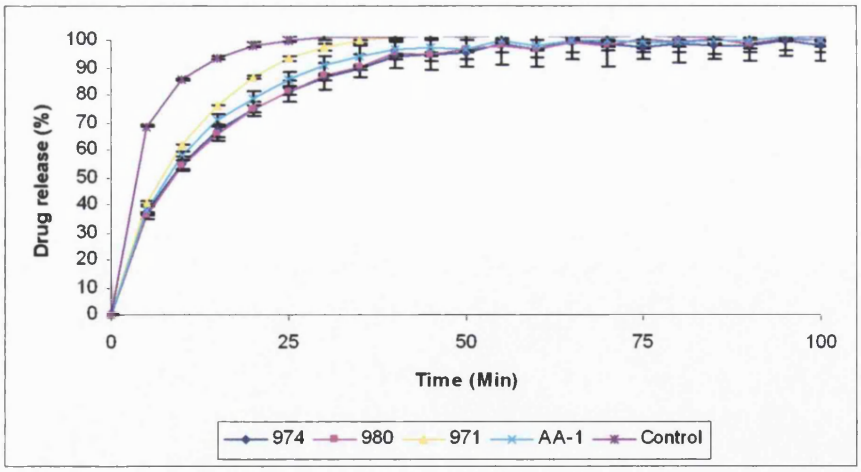


Figure 3.7 Drug release from 5% carbomer loaded pellets compared with a non carbomer containing control in 0.1 M HCL. (n=3)

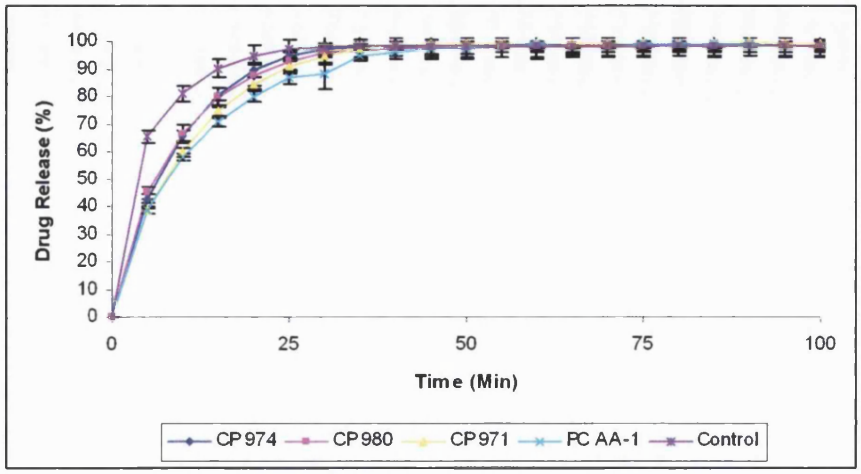


Figure 3.8 Drug release from 5% Carbomer loaded pellets compared with a non carbomer containing control in pH 6.8 phosphate buffer. (n=3)

3.8.1.4 *In-vitro* adhesion and mucoadhesion testing of ram extruded pellets

The adhesion, mucoadhesion and effect of exposure to mucin were also assessed for the ram extruded pellets using the Instron after hydration in pH 6.8 phosphate buffer. Direct comparison of these results with those obtained for tablets and compacts is not possible since surface area differences and weight differences (100 mg for pellets compared to 250mg for tablets and compacts) will affect the results. However as before trends can be observed and compared (Figure 3.9).

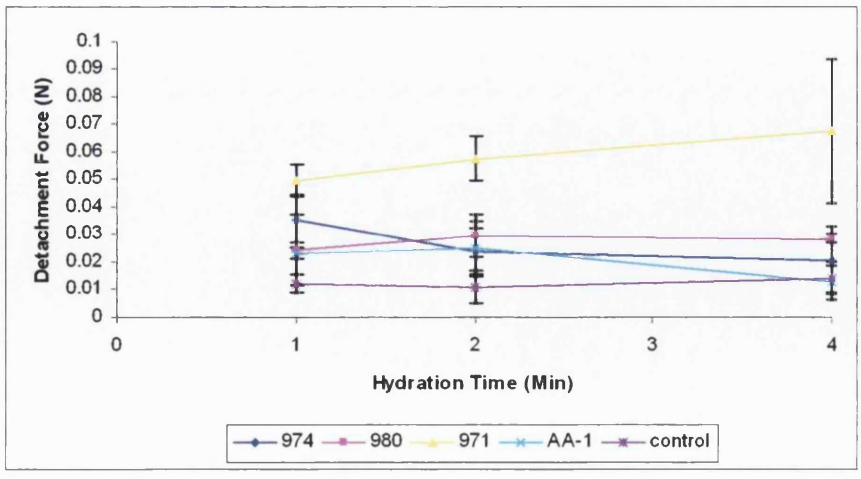


Figure 3.9 Force of detachment for carbomer pellets (5% w/w) and a non carbomer containing control produced by ram extrusion from a glass surface. (n=5)

After 4 minutes hydration in pH 6.8 phosphate buffer, CP 971 possesses the strongest force of detachment. Previously, with granulated tablets, it was seen that at this time point the higher molecular weight polymers exhibit stronger adhesion which was attributed to their ability to provide resistance to the uncoiling of the polymer chain as a result of their greater cross linking. This result suggests that the greater amounts of fluid required in the extrusion process has overcome the ability of the high molecular weight grades to resist deformation. The stronger adhesion enjoyed by CP971 is again likely due to its more rapid gel formation (Noveon 2002).

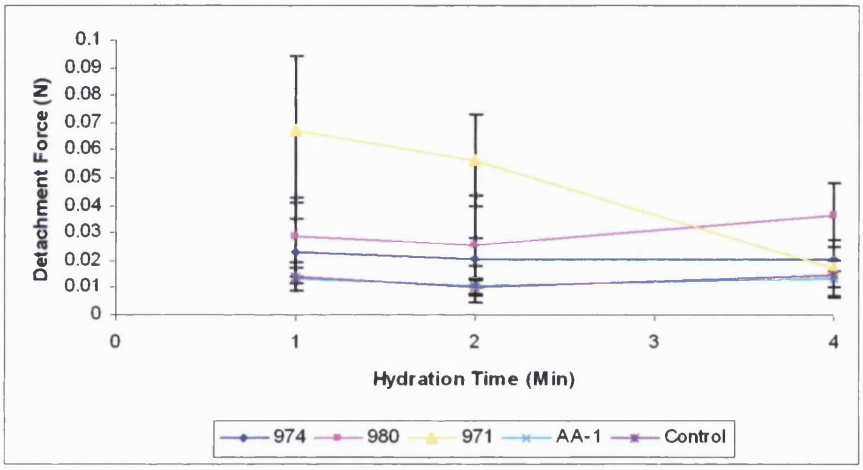


Figure 3.10 Force of detachment for carbomer pellets (5% w/w) and a non carbomer containing control produced by ram extrusion from a mucin layer. (n=5)

CP 974, 980 and AA-1 express equal force of detachment ($p<0.05$) at all time points with CP 971 requiring a greater force to cause adhesive joint failure at the lesser hydration points (1 and 2 minutes) (Figure 3.10). However at maximum hydration the peak detachment force is equal for all grades due to a reduction in the force needed for CP 971, it should be noted that the detachment force measured is only slightly greater than a non mucoadhesive control (0.015N ±0.005) and no measurable work of adhesion was detected. Again, pre-exposure of the carbomers to fluids, particularly at the volumes required for wet massing and extrusion has had a detrimental effect on their mucoadhesive properties. Continued exposure to the mucin solution does however improve the mucoadhesion of the pellets (Figure 3.11) with CP 971 and PC AA-1 both showing increases in adhesive force from 3 to 4 minutes and 1 to 2 minutes respectively. Processing conditions may be responsible for this as the wetting fluid used for these two grades contained less water than CP 974 and CP 980. However, the few

number of cross linkers may also be a reason for its continued mucoadhesive ability, as the less rigid structures still allow polymer to mucin interactions despite the initial uncoiling during the wet massing. Understanding the impact of formulation on the carbomers is important as previously reported attempts to produce and evaluate the mucoadhesion of carbomer pellets report tack measurements for powder blends but not fully formulated systems (Neau et al., 1996, and Gomez-Carracedo et al., 2001). These results suggest that formulation of carbomer powders in this way to make pellets will heavily influence their mucoadhesive properties.

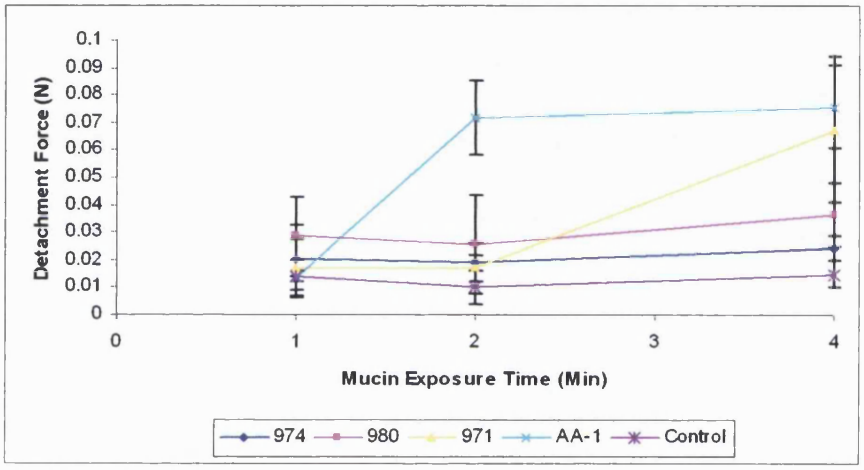


Figure 3.11 Detachment force for 5% carbomer pellets prepared by ram extrusion from and prolonged exposure to mucin after hydration in pH 6.8 phosphate buffer. ***(n=5)***

3.8.2 Melt extrusion

Using a mixture of ethanol and water it was possible to produce pellets via wet massing and ram extrusion which met the criteria for desirable pellets, however tack caused by the wetting of the carbomers meant that the maximum loading achieved was only 5% therefore, alternative methods of producing pellets or extrudate containing higher levels of carbomer were sought. Hot melt extrusion was investigated as an alternative to ram extrusion of the carbomer polymers. This process has a distinct advantage for processing carbomer polymers as the process is anhydrous, as such, a higher level of carbomer polymer can be incorporated into the dosage form and the previously seen affects on adhesion and mucoadhesion caused by pre-wetting may be eliminated. As the polymers are fed into the twin screw melt extrusion apparatus heat it conveyed to them causing them to melt, as it does so it enters the melting section of the apparatus. As more polymer melts heat dissipation induced form constant shearing becomes an

additional source of heat. Within the melting section of the apparatus the polymer exists in a molten form and a matrix is formed due to the polymer melt acting as a thermal binder (McGinity, 2004).

Attempts to extrude carbomer polymer alone not were successful as the polymer was seen to be discoloured at temperatures of approximately 200°C and no extrudate could be formed. As with all types of pharmaceutical process technologies the method is only suitable for materials which possess particular physiochemical properties. In this instance, melt extrusion is only possible for materials that express increased plastic behaviour upon melting and is dependent on the melting point and melt viscosity (a measurement of the rate of extrusion of a thermoplastic material) of the polymer. Therefore carrier polymers are needed to make up the bulk of the formulation, improve the thermo plasticity and act as a thermo binder. Hydroxypropylcellulose and ethylcellulose were chosen for this purpose. Both HPC and EC have been demonstrated to be a suitable materials for the melt extrusion process and can act as carrier systems, (Repka et al., 2000, Repka et al., 2001, Follonier et. al., 1994 and Crowley et al., 2007), additionally, since HPC is water soluble and EC water insoluble using these polymers makes it possible to assess whether hydrophobicity of the carrier affects adhesion.

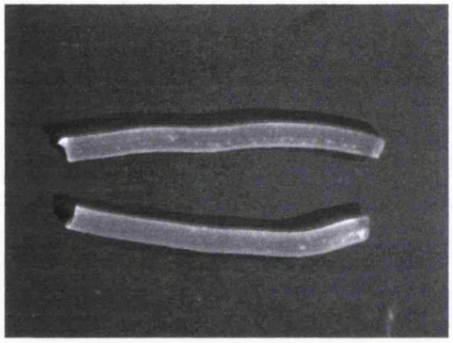
Table 3.8 Glass transition temperatures (Tg) for polymers used in the melt extrusion process.([a]Follonier et al., 1994)[b] (Noveon, 2002)

Polymer	T_g (°C)
HPC	130[a]
EC	133[a]
Carbomer	105[b]

Extrusion of both carrier polymers with carbomer loading up to 20% w/w was achieved and an example of the extrudate produced is shown in Figure 3.11. The higher carbomer loading achieved is a result of not needing to expose the carbomers to water. Carbomers being of high molecular weight (4000000 CP980) will not melt at the relatively low temperatures needed to extrude HPC and EC, as a result the produced extrudate is in effect a solid dispersion of carbomer within the carrier polymers. An example of the extrudate produced can be seen in Figure 3.12. Although not performed

during this study due to the unavailability of equipment the extrudate may be formed into spheres by a process of continuous cutting of the hot extrudate and immediate spheronisation in the conventional manner.

a)



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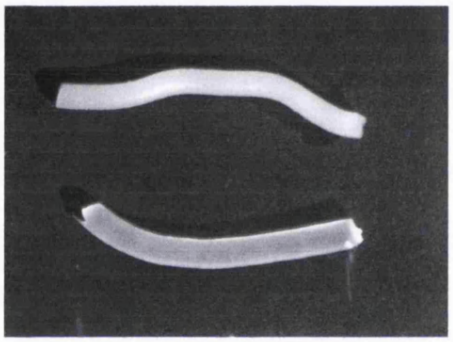

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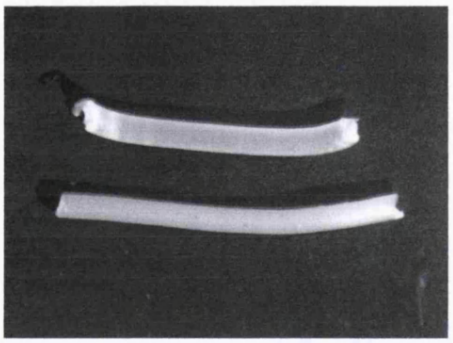
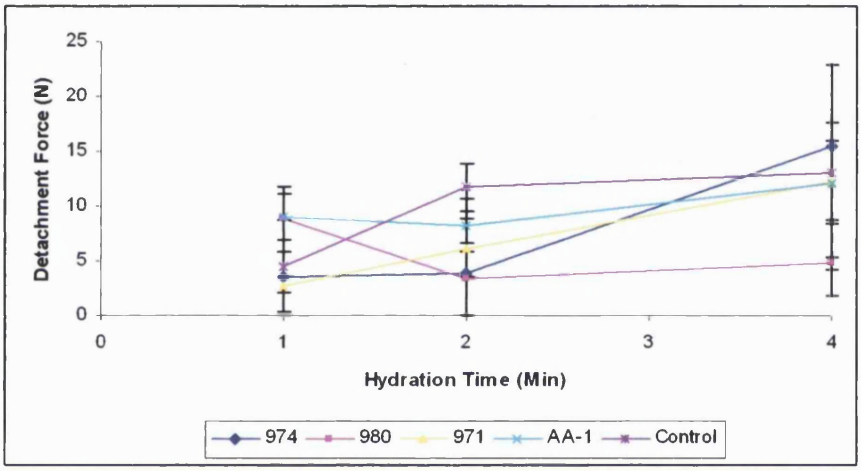


Figure 3.12 a) HPC (top) and EC (bottom), b) 10% CP 974 + HPC (top) and 10% CP 974 + EC (bottom)and c) 10% CP 971 + HPC (top) and 10% CP 971 + EC (bottom) prepared from hot melt extrusion

3.8.2.1 *In-vitro* adhesion testing

The creation of a solid dispersion had a detrimental affect on the ability of the polymer to under go adhesion. After exposure to pH 6.8 phosphate buffers all of the samples tested (10% carbomer loading level) using HPC as a carrier expressed adhesion (Figure

3.13). However the detachment force measured was not significantly different (95% confidence levels) from the non-carbomer containing HPC control. Interestingly ethylcellulose extrudate expressed no adhesion at all with and without the carbomer polymers. Although direct comparisons should not be made it is worthy of note that the detachment force measured for the HPC extrudate was higher than any of the previously tested systems. This may be a consequence of the test method as the surface area here is larger than previous tests, however HPC has been shown to be adhesive (Repka et al., 2001) and as such my be responsible for the greater adhesion. No increase in adhesion upon the addition of carbomer polymers is however unexpected.



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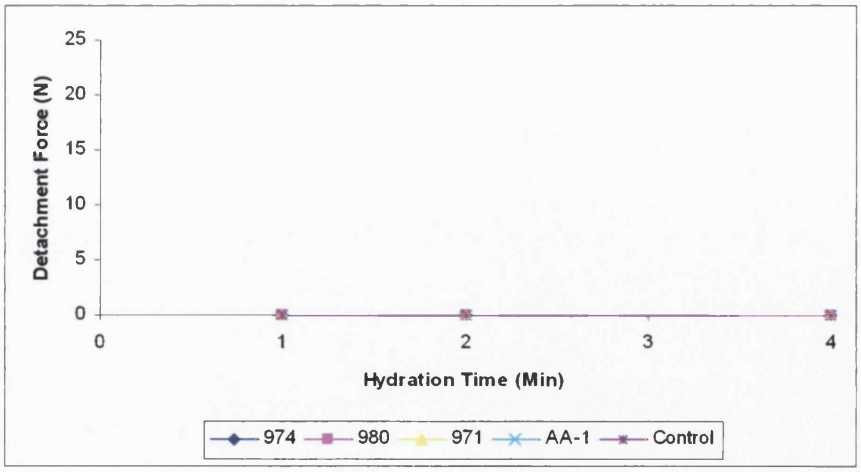


b

Figure 3.13 Adhesion of HPC hot melt extrudate a) HPC + 10% carbomer b) EC + 10% carbomer, from a glass surface after hydration in pH 6.8 phosphate buffer (Measured on the TA). (n=5)

A possible explanation for this is that since carbomers have no true melting point they are present within the extrudate as a solid dispersion and so are encapsulated within the carrier polymers and as such is not exposed to the hydration fluid during the test. Alternatively, hot melt extrusion has been shown to improve the bioavailability of drugs by the formation of a molecular dispersion (Forster et al., 2002, Kinoshita et al., 2002). If this is the case here, this may be inhibiting the gel formation of the carbomer polymers by reducing the number of carboxylic acid groups available for ionisation. These results however contradict those found by Repka et al., (2001) and Repka et al., (2000) who observed that the adhesion of HPC films is enhanced with the addition of carbomer polymers and so supports the solid dispersion hypothesis. The films prepared by Repka and coworkers would be thinner and possess a larger surface area than the extrudate produced here. As such permeation of fluids through the matrix polymer would be easier allowing for the hydration of the carbomers contained within. The negative result for ethylcellulose extrudate does not help to confirm the effect of hot melt on the carbomer polymers as the water insoluble EC meant that no value for adhesion could be determined.

Since the adhesion seen from the hot melt extrudate was the result of the addition of HPC and not carbomers testing of this system was ended at this point. However hot met extrusion is a promising technique for formulation of carbomer polymers as no water is involved. Careful consideration and further investigations into a suitable carrier molecule needs to be undertaken until the full potential of this technique for producing mucoadhesive carbomer pellets is fully understood

3.9 Summary

The production of carbomer pellets was not as straight forward as the production of tablets due to the high volumes of fluid needed to produce material plastic enough to undergo a ram extrusion process. The amount of fluid required and the ratio of ethanol to water was also dependent on the grade of carbomer with the two highest molecular weight grades (CP 974 and CP 980) requiring greater fractions of the fluid to contain water. In contrast, the lower molecular weight grades (CP 971 and PC AA-1) produce the best pellets when the levels of ethanol and water were equal. Problems associated

with exposure of the polymers to water meant that a maximum loading of 5% w/w was achieved. Drug release from the 5% pellets in pH 6.8 phosphate buffer was similar to that seen with the 5% granulated tablets and no extended release was seen when compared to a non carbomer containing control. The extended release observed for CP 971 granulated tablets was not seen in the corresponding pellets and this was attributed to the additional fluid needed causing further uncoiling of the polymer chain during mixing.

Adhesion testing showed that carbomer pellets possessed no immediate adhesive advantage over a non-mucoadhesive control; however this was not the case after exposure to a mucin solution for 4 minutes. At which time the lower molecular weight grades (CP 971 and PC AA-1) both expressed increased mucoadhesion over the control.

Hot melt extrusion proved to be a viable process for producing extrudates which contained higher carbomer content than was achievable with ram extrusion. Using HPC and EC as the matrix material carbomer level of 10% w/w was obtained. The formation of a molecular dispersion during the hot melt process; however appears to have eliminated the adhesive qualities of carbomers.

Section 2 Microparticles

3.10 Overview

The effect of surface area on the release of drugs from a delivery system is well known, since the surface area: volume ratio of a 100 μm particle is 10 times greater than a 1 mm pellet it is expected that drug release would be more rapid. In a colonic environment where free fluid available for dissolution is limited any increase in drug release is highly favourable. In addition to this it has been theorised that the mucoadhesive potential of particles *in-vivo* may be enhanced by their size, with smaller particles becoming trapped in the folds of the wall of the GI tract (Takeuchi et al., 2001). The most common method of microparticle production is coacervation/phase separation however other methods such as spray drying and emulsification/solvent evaporation have been used. Additionally with the development of pH sensitive polymers which dissolve at higher pH thresholds than conventional enterics (e.g. Eudragit S which dissolves at pH 7.0) drug delivery to the ileo-colonic region has become more achievable. Recently, particular success has been seen with the development of a universal method for the production of acrylic polymers, most notably, Eudragit S100 for drug delivery to the small intestine (Kendall, 2006). Eudragit S100 is poly methacrylic acid and as such possesses the same basic structure as carbomers which are formed of monomers of acrylic acid. Therefore it was thought that this method could be adapted to successfully produce microparticles from carbomer polymers.

3.11 Emulsification/Solvent Evaporation

Figure 3.14 shows a diagrammatic representation of the preparation of microparticles prepared by emulsification/ solvent evaporation. The polymers are dissolved in a suitable solvent; this internal phase is then added and emulsified in the continuous phase (in this study liquid paraffin) which causes discrete droplets to form. In order for microspheres to form the organic solvent must diffuse into the continuous phase and then evaporate at the oil/air interface, as the evaporation occurs the particles harden. Influences on the properties of the resultant microspheres created from using this method have been identified and include factors such as drug solubility, solvent type, diffusion rate, temperature, viscosity and drug loading (O'Donnell et al., 1997)

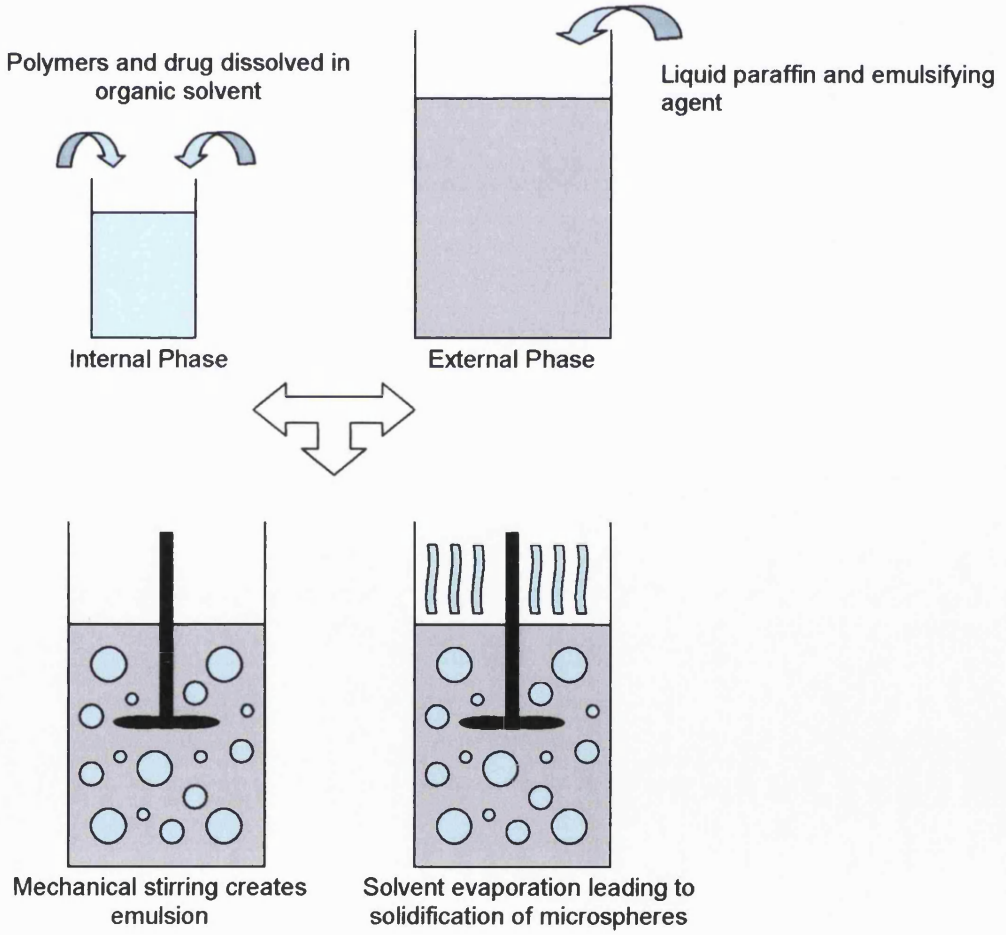


Figure 3.14 Diagrammatic representation of the preparation of microparticles by emulsification/ solvent evaporation method

The effect of polymer–to-drug ratio was seen to play a role in the mean size of Eudragit RS and terbutaline sulphate (a bronchodilator) particles with the mean size decreasing as the polymer to drug ratio increased (Kim et al., 1993). This was explained by the authors to be a result of a reduction in the viscosity of the internal phase. The selection of solvent for the internal phase has been shown to be of importance in both oil in water (o/w) and oil in oil (o/o) emulsifications (Bodmeier et al., 1998 and Bogataj et al., 1991). In both cases the miscibility of the solvent with the continuous phase affects the precipitation of the polymer and subsequent nucleation of the particles. The rate at which the solvent migrates into the continuous phase and evaporates is therefore of paramount importance.

3.12 Carbomer microparticles

Manufacture of carbomer microparticles has been investigated. Spray dried microspheres of Carbopol 934 (later replaced with CP 974NF) with disodium cromoglycate (DSCG) were prepared by Vidgren et al., 1992. Researchers found that although it was possible to produce the microspheres the morphology was irregular and shrunken compared with sodium carboxymethylcellulose and a comparative mucoadhesive and the DSCG control particles. Improved results were observed using a solvent emulsification/evaporation method to produce carbopol 907 microspheres for ocular adhesion (Durrani et al., 1995) and polycarbophil and chitosan microparticles in a hydroxpropylcellulose matrix (Bogataji et al., 1997). The aim was to compare a cationic and anionic mucoadhesive and evaluate how the charges influence adhesion on two mucosal surfaces, guinea pig bladder and intestinal mucosa. The study concluded that adhesion was dependent on the charge of the polymer and that polycarbophil as an anionic polymer was more suited to intestinal mucosal adhesion.

The effectiveness of microparticles as mucoadhesive platforms is still unclear and previous work on carbomer microparticles have been generally contradictory and tend to focus on attempts to increase gastric residence. Increased residence to gastric mucosa has been demonstrated with carbopol 934 microparticles produced with polyglycerol ester of fatty acid (PGEF) (Akiyama et al., 1995). Compared with PGEF particles 92.0% and 94.5 % retention of carbomer was observed on rat excised stomach and small intestine mucosa respectively after washing with fluid (pH1.2) compared with 5.5

and 0% of PGEF control particles. However this is not supported by *in-vivo* results, microparticles of amoxicillin loaded ion-exchange resin encapsulated in CP 934 did not improve gastric residence in Sprague Dawley rats (Cuna et al., 2001). It is interesting to note that results form this study are also contradictory to *in-vivo* studies using carbopol beads for gastric retention (Longer *et al.*, 1985), and suggests that the results are not only influenced by the *in-vivo* behaviour of the carbomers but other factors that effect gastric emptying, specifically in this instance particle size. This adds weight to the argument that mucoadhesive polymers are likely to be more effective in distal regions of the GI tract where less variables need to be considered.

Of course to successfully deliver dosage forms to the distal GI tract they must incorporate a delayed release function, for the previously discussed carbomer tablets and pellets this would need to be added at a later stage. However, the recent success seen with the production of Eudragit S100 microparticles using solvent emulsification/ evaporation technique (Kendall 2006) gave rise to the possibility of producing novel carbomer microparticles with an enteric functionality incorporated into the original formulation. Additionally, although particle formation is a complex process involving interactions between polymers/solvents/non-solvents/drug and surfactants it was rationalised that as Eudragits are methacrylic acids and carbomers are acrylic acids the technique may lend it self readily to carbomer polymers.

3.13 Aims

The primary aim is to formulate mucoadhesive microparticles for colon delivery.

Therefore the objectives are as follows:

- To assess the emulsification/ solvent evaporation method for its suitability to produce carbomer microparticles
- To test *in-vivo* the adhesion and mucoadhesion of microparticles containing carbomer and enteric polymers

3.14 Materials

Paracetamol was supplied by Ellis and Everard, Essex, UK. Prednisolone was obtained from Sanofi Aventis Pharma SA (Normanville France). Prednisolone is a white, crystalline powder with a molecular weight of 360.4., it has a solubility of 1 in 1300 in water and 1 in 30 in ethanol (B.P 2007) (Figure 3.15), as a neutral molecule its aqueous solubility is not influenced by pH, subsequently any differences seen in drug release can be attributed to the Eudragit or carbomer polymers.

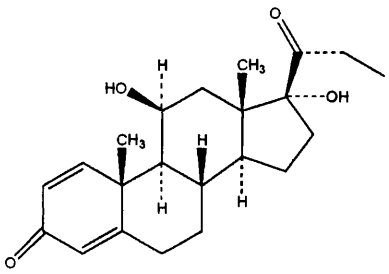


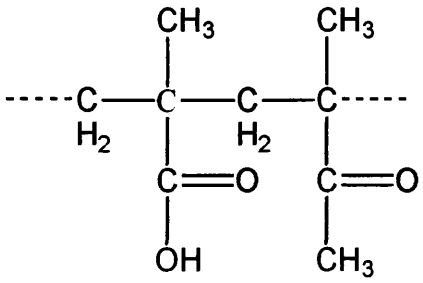
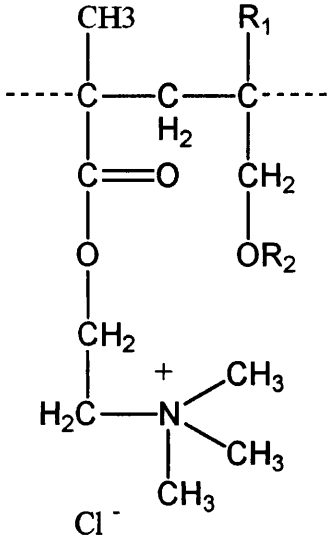
Figure 3.15 Chemical structure of prednisolone

Eudragit RS and S100 were gifts from Evonik (Darmstadt, Germany). Carbopol 974, 980, 971 and polycarbophil AA-1 were received as gifts from Noveon, France. Liquid paraffin B.P is supplied by JM loveridge Plc and Arlacel 85 was purchased from Sigma Aldrich, (Poole UK). All other reagents were of analytical grade and were used as received.

3.14.1 Eudragit polymers

Eudragit polymers are a range commercially available methyl methacrylate polymers utilised within the pharmaceutical industry, for taste marking and controlled drug delivery. The two Eudragit polymers used in this study are summarised in Table 3.9.

Table 3.9 Summary of Eudragit polymers used within this study

Polymer structure	Ratio (carboxylic acid to esters)	Solubility (pH)	Molecular Weight (Da)
	1:2	>7.0	135000
Eudragit S Poly methacrylic acid methacrylate			
	1:2:0.1	Water insoluble	150000
$R_1 = H, CH_3$ $R_2 = CH_3, C_2H_5$ **Eudragit RS** Poly ethyl acrylate, methylmethacylate, trimethly-ammonioethyl methacrylate			

Eudragit RS and S100 were utilised to add a pH resistant and water insoluble functionality respectively to the carbomer microparticles.

3.15 Methods

3.15.1 Preparation of carbomer microparticles by emulsification/ solvent evaporation

All microparticles were produced using the oil in oil emulsification solvent evaporation method.

The internal phase was prepared by the addition of the carbomer polymer to ethanol under constant stirring and allowing it to dissolve (Table 3.10). Separately, liquid paraffin was added to a 300ml beaker and stirred with a Heidolph RZR1 overhead stirrer fitted with a 3- blade propeller and calibrated to 1000rpm. Sorbitan sesquioelate (Arlacel 83) was added as an emulsifier and mixed for approximately 5 minutes to make the external phase. The internal phase was then added to the external phase to form an oil in oil emulsion. Stirring was continued for 12 hours after which time the microparticles were recovered by vacuum filtration through a Pyrex sintered glass funnel (pore size 4, 5-15 μm) and washed with three, 50ml aliquots of hexane.

Table 3.10 Formulation for the preparation of carbomer microparticles using a solvent/ evaporation technique

Material	Quantity
Carbomer	0.3 or 0.6 g
Ethanol	30 ml
Liquid paraffin	167 g
Arlacel 83	1.67 g

Two amendments were made to the method in an attempt to improve the resultant microparticles, they are as follows:

Solvent/evaporation and centrifugation method for the production of 100% carbomer microparticles

After 12 hours of mixing the emulsion was transferred to 50 ml centrifuge tubes and placed in a Sigma 3K30 laboratory centrifuge at 10,000 rpm for 25 minutes. The upper layer of paraffin was decanted leaving approximately 10 ml of fluid, within which the microparticles were retained. The particles were transferred to a stinted glass funnel, under vacuum, with a pipette, filtered and washed with three, 50 ml portions of hexane.

Acidification of the internal phase

Internal phase was prepared as in section before; the pH was lowered to pH 1.0 with the addition of 5M HCl to the solution. The internal and external phases were mixed and filtered as before.

3.15.2 Addition of co-polymers

Three microparticle formulations were prepared using the formulation outlined in Table 3.11. In all cases the external phase was prepared as detailed in the pervious section.

Table 3.11 Formulation of carbomer/Eudragit microparticles from a solvent emulsification/evaporation technique. a = carbomer polymer equal to 5% of the weight of Eudragit polymers. b = carbomer polymer equal to 10% of the weight of Eudragit polymers

	Microparticle formulation		
	S100	**S100/RS**	**RS**
Eudragit S100 (g)	3	1.5	0
Eudragit RS (g)	0	1.5	3
Carbomer (g)	0.15[a]/ 0.3[b]	0.15[a]/ 0.3[b]	0.15[a]/ 0.3[b]
Ethanol (mL)	30	20	20
Acetone (mL)	0	10	10
liquid paraffin (mL)	167	167	167
Arlacel 83 (mL)	1.67	1.67	1.67

Placebo microparticles as shown in Table 3.11 were produced for adhesion and mucoadhesion testing. Prednisolone and paracetamol were incorporated at a mass ratio of 1:30 for drug release profiling.

Eudragit S100 microparticles

Eudragit S100 and carbomer polymers were dissolved in 30 ml of ethanol and mixed for 1 hour, before being added to the external phase.

Eudragit RS/S100 microparticles

The internal phase was prepared in two stages. Eudragit S100 and carbomer polymers were added to 20 ml of ethanol and mixed for 1 hour. Eudragit RS was added to 10mL of acetone and mixed for 10 minutes in a sealed container. The two solutions were combined and mixed for a further 5 minutes before being added to the external phase.

Preparation of Eudragit RS microparticles.

Carbomer polymer was added to the ethanol and mixed for 1 hour. Eudragit RS was dissolved in the acetone and mixed for 10 minutes. The two solutions were mixed together and mixing continued for a further 5 minutes before being added to the external phase.

3.15.3 Encapsulation efficiency and drug release

Microparticles particles (30mg) were dissolved in 100 mL of methanol and placed in a sonic bath for 30 minutes. From this solution a 1 in 10 dilution to a solution of 10% methanol in 0.1M HCl was performed to precipitate out the Eudragit polymers. The solution was filtered and drug content read using a UV/Vis spectrophotometer at 247 nm and 242nm for prednisolone and paracetamol respectively.

Dissolution was performed under sink conditions in pH 6.8 phosphate buffer or with a pH change method using a USP II apparatus. Size 0 capsules were filled with 100 mg of particles (equivalent to 3 mg of prednisolone). Initially (for the pH change method) the particles were placed in 750 mL of 0.1M HCl for 2 hours with a paddle speed of 100 rpm. After 2 hours the pH of the media was increased to 7.4 with the addition of 250 mL of 2M Na_3PO_4.

3.15.4 *In-vitro* mucoadhesion testing

Adhesion, mucoadhesion and mucin exposure studies were performed as detailed previously. Approximately 100 mg of microparticles were fixed onto the probe of the instron using adhesive tape measuring 10mm x 10mm. The data were analysed using parametric tests; results were analysed for significant differences using one-way analysis of variance (ANOVA), with post-hoc analysis using Tukey's test. All tests were carried out using SPSS Version 14.0 statistical software package.

3.15.5 Scanning Electron microscopy

Samples were placed on SEM stubs and fixed using carbon discs before being gold coated using an EMITEC K 550 sputter coater for three minutes at 40mA. The samples were then transferred to a Philips XL20 Scanning Electron Microscope for imaging.

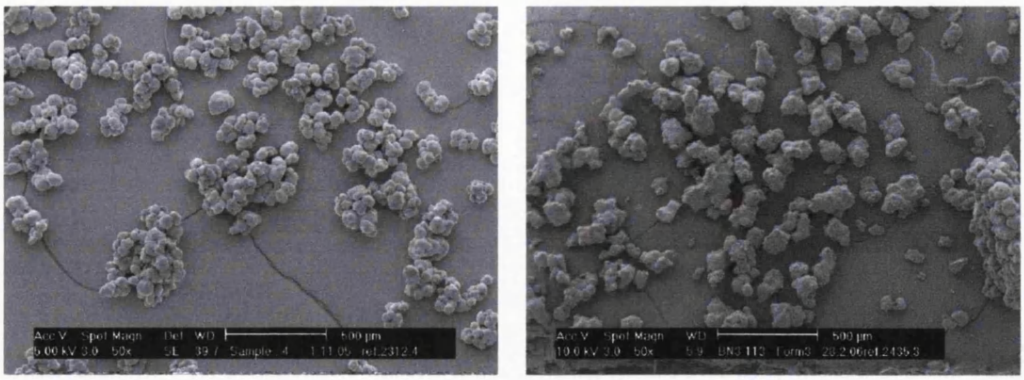
3.15.6 Particle Size analysis

Size distribution of the microparticles was determined using laser diffraction using a Malvern Mastersizer X fitted with a 45mm lens (Malvern Instruments Ltd., Malvern, UK). The microparticles were suspended in 1.5 mL of hexane in microcentrifuge tubes by vigorous vortex mixing for 30 seconds. The suspension was then added drop wise into the volume diffraction chamber which also contained hexane until an obscuration of 10-15% was achieved. The values for mean volume diameter at the 10%, 50% and 90% (DV10, DV50 and DV90) undersize were recorded.

3.16 Results and Discussion

3.16.1 Carbomer microparticles

The production of microparticles containing carbomers proved to be difficult, after washing with hexane the particles collected on the filter remained clumped together and were wet in appearance, additionally, the powder was not free flowing and drying over night in an oven did not improve this. SEMs of particles prepared from 1% and 2% solutions of carbopol 971 (Figure 3.16) show that to some degree particle formation had occurred, however the particles were aggregated and irregular. One of the most important factors in the production of microparticles in this was is the stability of the emulsion. The solvent evaporation process requires the formation of discrete droplets within the emulsion to form prior to the migration and evaporation of the solvent if this does not occur non uniform aggregated particles may be formed (O' Donnell et al., 1997). To ensure this was the case the emulsion was observed under a light microscope (Figure 3.17) and individual droplets were observed, as a consequence, this was ruled out as a possible cause of poor particle formation.



a B

Figure 3.16 a) CP 971 microparticles prepared by solvent evaporation, from a 1% w/v carbomer solution b) CP 971 microparticles prepared by solvent evaporation from a 2% w/v carbomer solution

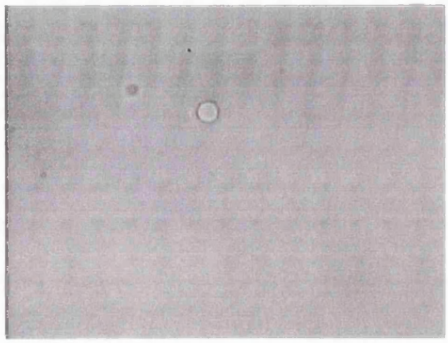
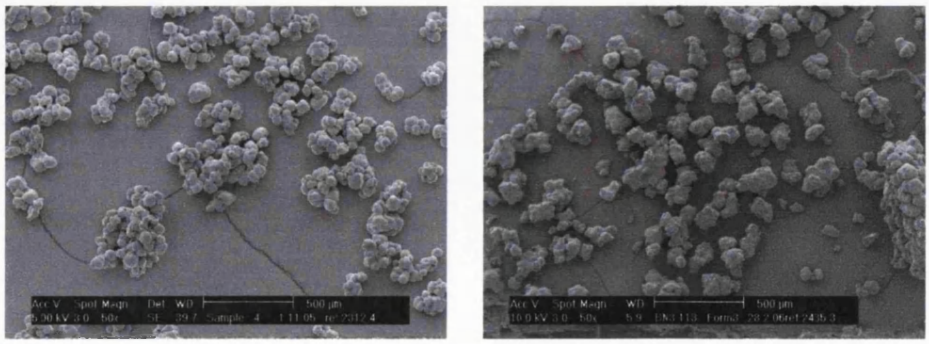


Figure 3.17 Discrete droplets of carbomer polymer observed under the light microscope

Since discrete particles can be seen in the emulsion it was hypothesised that gel formation caused by the addition of carbomer polymers to ethanol in the disperse phase, was the cause of agglomeration, in that it inhibits the removal of the paraffin when washed with hexane. To investigate this, the emulsion was centrifuged and the supernatant decanted prior to washing. Additionally, the viscosity of the internal phase will affect the size of particles produced (Kim et al., 1993), increased viscosity of the internal phase produces larger droplets which in turn increases the likelihood of collisions between droplets and decreases the likelihood of the collided droplets being re-dispersed (Pongpaibul et al., 1986). To resolve this, 5M HCl was added to the internal phase, lowering the viscosity in a similar process as seen previously during film coating of tablets, prior to incorporation into the external phase. SEMs of both particles produced can be seen in Figure 3.18, unfortunately no improvement in particle formation was observed



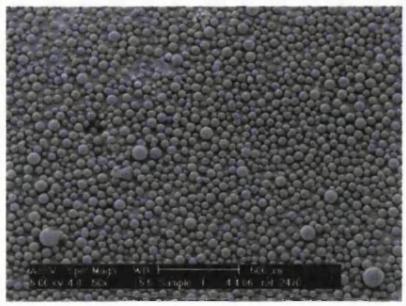
A b

Figure 3.18 a) CP 971 particles produced by solvent emulsification/evaporation and centrifugation, from a 1% solution b) CP 971 particles produced by acidification of the internal phase and solvent evaporation from a 1% solution

Although the high viscosity of carbomers in solution will certainly hinder particle formation due to collisions and large droplet formation, acidification of the internal phase did not significantly improve it. Another reason for poor particle formation may be the surfactant used. Production of microparticles is a complex interplay between solvent migration and subsequent evaporation and hardening of the particles due to polymer disposition. Therefore selection of surfactant is paramount in production of suitable microparticles as this stabilises the droplet within the emulsion by its positioning at the interface of the two phases, additionally different surfactants have been shown to produce particles of different yields and morphology (Watts et al., 1991).Two approaches could be adopted to investigate this, the first would be an optimisation study varying type and concentration of surfactant. The second is the incorporation of other polymers which are known to be more compatible with Arlacel 83. The latter strategy was adopted as this gave the additional benefit of being able to use enteric polymers which would improve the likelihood of the microparticles reaching the colon unaffected by physiological conditions.

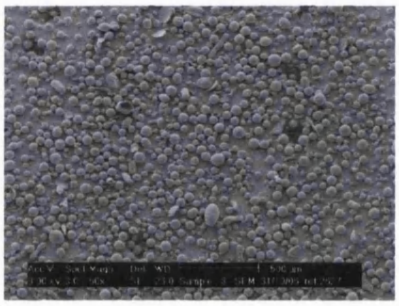
3.16.2 Eudragit and carbomer microparticles

In an attempt to develop the microparticle formulations further the decision was taken to incorporate other polymers to form the bulk of the particle, in a similar manner to the need to add MCC and lactose to produce ram extruded pellets and HPC or EC to extrude carbomers via hot melt extrusion. As previously mentioned Eudragit S100 microparticles have been successfully produced using a solvent emulsification evaporation method, adaptation of this method to incorporate carbomer polymers was undertaken with the intention that the addition of Eudragit S polymers would aid the formation of spherical particles as well as act as a controlled release platform. Two Eudragit polymers were chosen, Eudragit S100 due to its ability to lend itself easily to the process and its dissolution threshold of > pH 7.0 and Eudragit RS to act as a contrast since it is water insoluble. Figure 3.19 shows microparticles successfully produced using all three Eudragit combinations.



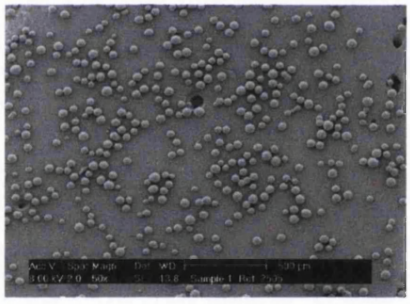
a

DV50 = 45.97μm



b

DV50 = 122.12μm



c

DV50 = 111.43μm

Figure 3.19 Microparticles prepared by solvent evaporation a) Eudragit S100, b) Eudragit RS, c) Eudragit S100/RS (1:1)

The addition of carbomer polymers at a 5%w/w and 10%w.w level however has a pronounced effect on the morphology of the particles produced. Figures 3.20, 3.21 and 3.22 show the effect of the addition of carbomer to the Eudragit polymers. The most dramatic difference is seen with Eudragit S100. The control particles produced were the smallest and most spherical of all three formulations; however, upon addition of carbomers more spherical and uniform particles are produced with Eudragit S100/RS. In general for all formulations carbomer containing particles are larger, less spherical than the controls and in some cases show signs of being hollow.

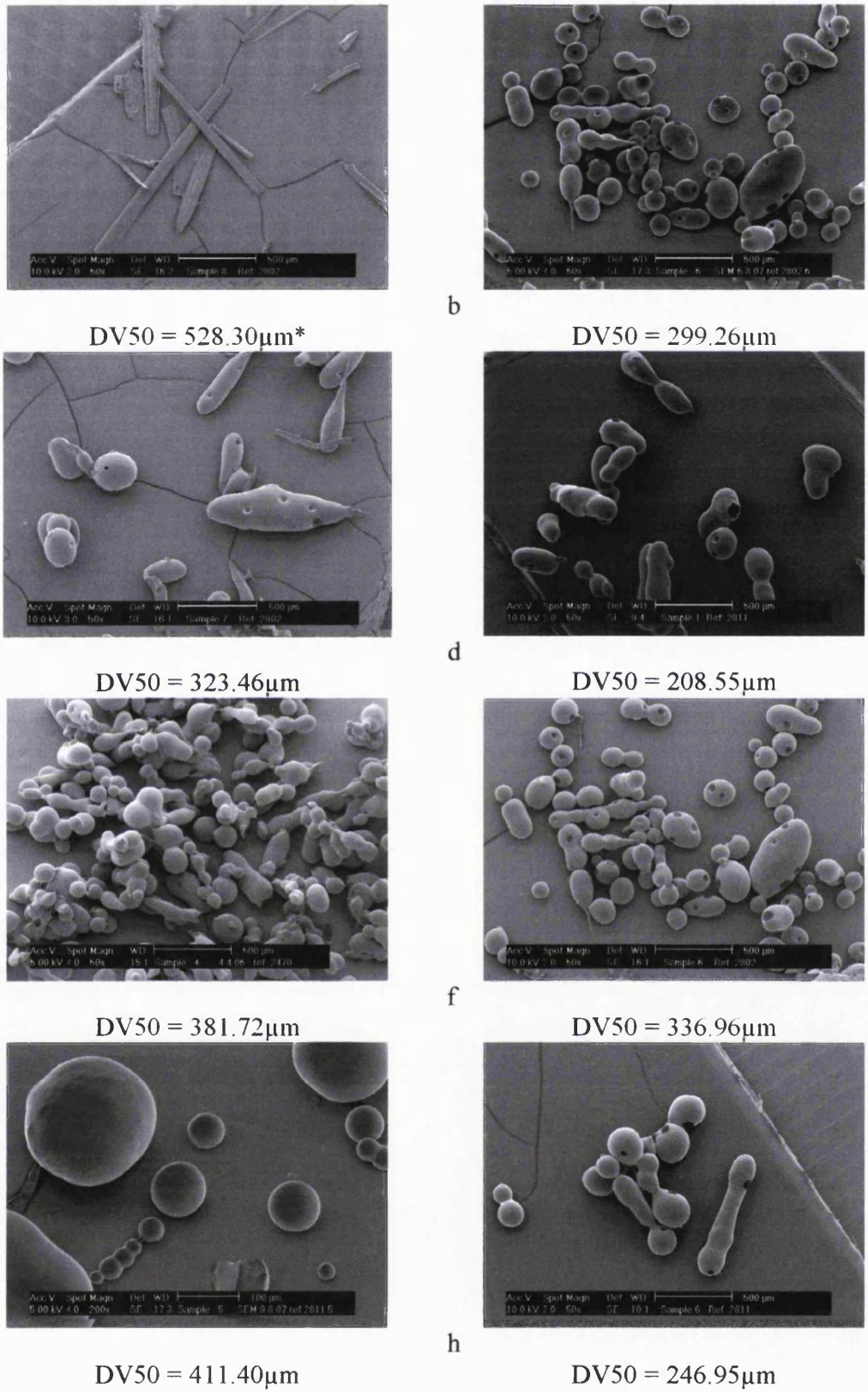
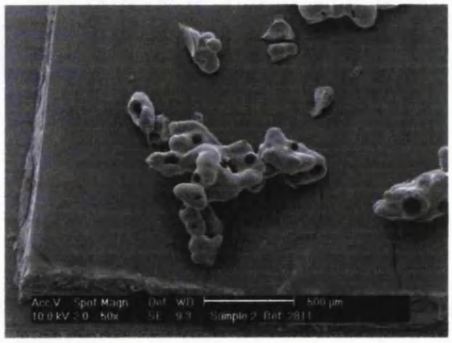


Figure 3.20 SEM of Eudragit S100 and carbomer microparticles a) 5% CP 974, b) 10% CP 974, c) 5% CP 980, d) 10% CP 980, e) 5% CP 971, f) 10% CP 971, g) 5% PC AA-1, h) 10% CP AA-1

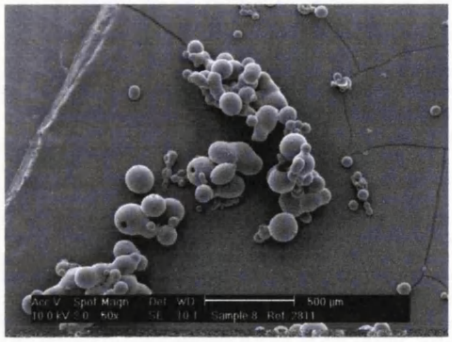
The incorporation of carbomers into particles containing Eudragit S100 is likely affecting the emulsion stability by changing the viscosity of the internal phase. The effect of viscosity on the size of particles was partly discussed earlier; an increase in viscosity produces an increase in particle size. If particles of the same size were desired a greater sheer force would be needed to be applied to disperse the internal phase, during this process however the sheer force is constant so larger particles are produced when carbomer polymers are added. Additionally the increase in polymer concentration inside the droplet, caused by the addition of carbomers, increases the volume occupied by the polymers upon solvent evaporation. This increase in droplet size will have an effect on agglomeration of the resultant particles, larger droplets within the emulsion increase the probability of two or more droplets coming into contact with one another and coalescing. Although the process is dynamic and particles are constantly coalescing and being dispersed by the stirrer simultaneously, as with particles produced with carbomer alone, increased viscosity caused by the presence of carbomers would be hindering the re-dispersion of coalesced particles and explains why Eudragit S100 and Carbomers microparticles are irregular and agglomerated.

For particles produced with Eudragit RS + carbomer and Eudragit S100/RS + carbomer the previous discussion is also true and increased viscosity of the internal phase has produced larger, more irregular and agglomerated particles. However, the solvent used to dissolve the polymers to create the internal phase is also a factor (Figure 3.20 and 3.21).



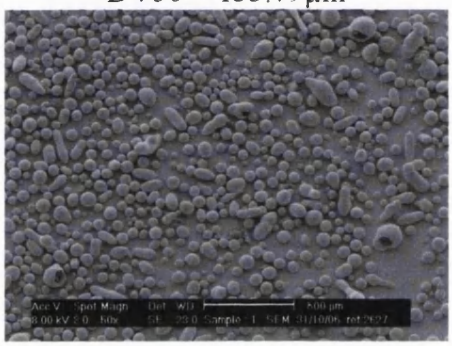
a

DV50 = 133.79µm



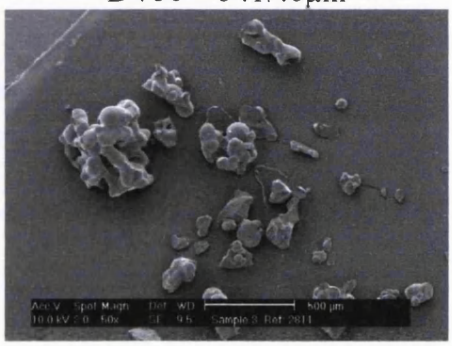
b

DV50 = 541.46µm



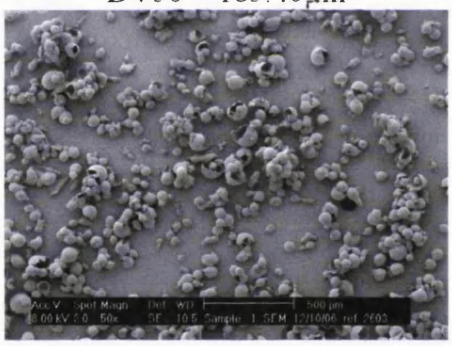
c

DV50 = 185.40µm



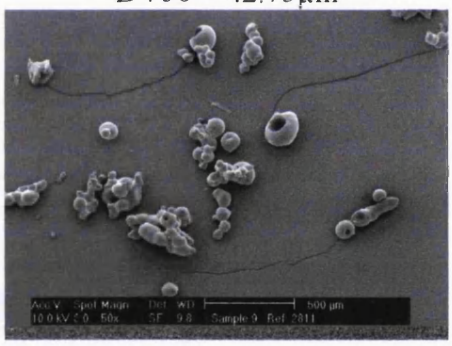
d

DV50 = 42.75µm



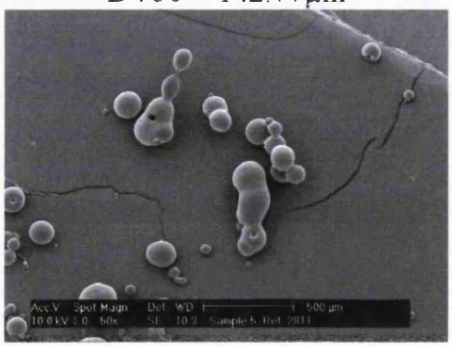
e

DV50 = 142.77µm



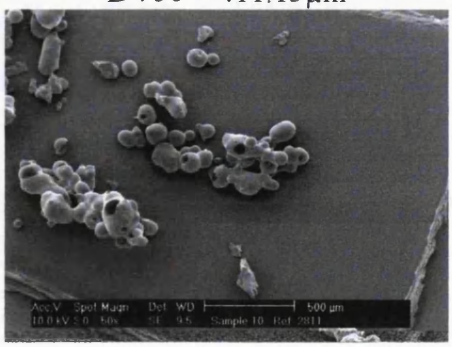
f

DV50 = 411.13µm



g

DV50 = 249.00µm



h

DV50 = 413.11µm

Figure 3.21 SEM of Eudragit RS and carbomer microparticles a) 5% CP 974, b) 10% CP 974, c) 5% CP 980, d) 10% CP 980, e) 5% CP 971, f) 10% CP 971, g) 5% PC AA-1, h) 10% CP AA-1

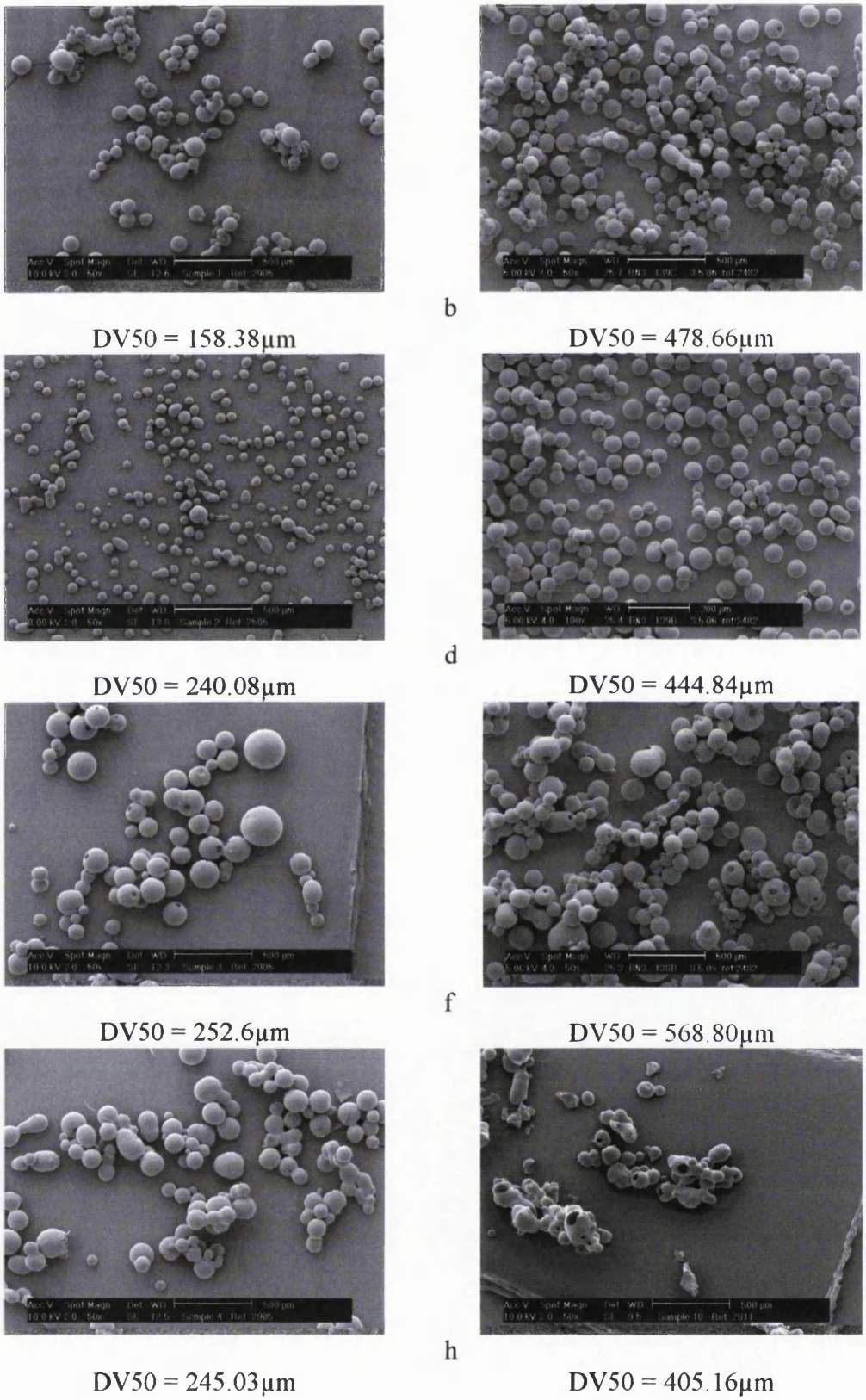
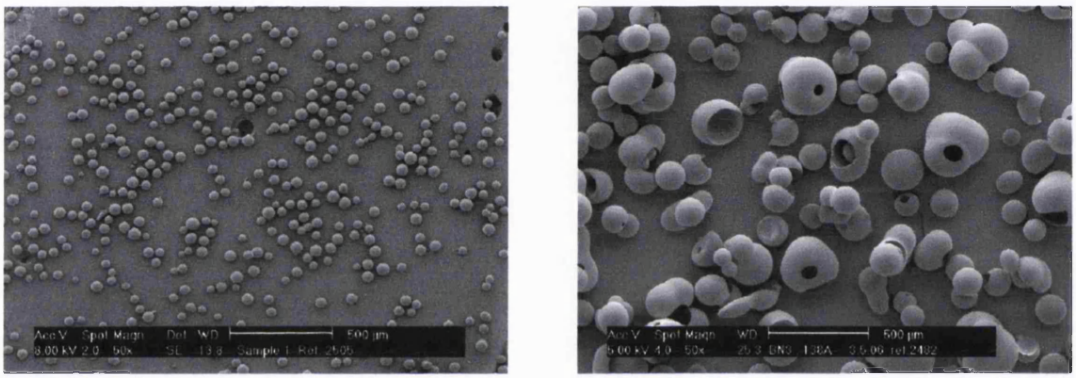


Figure 3.22 SEM of Eudragit S100/RS (1:1) and carbomer microparticles a) 5% CP 974, b) 10% CP 974, c) 5% CP 980, d) 10% CP 980, e) 5% CP 971, f) 10% CP 971, g) 5% PC AA-1, h) 10% PC AA-1

Solvent selection is an important consideration for the production of microparticles by solvent evaporation. Important factors that need to be considered prior to choosing a solvent are things such as its ability to dissolve the polymer and drug, and its immiscibility with the continuous phase (Watts et al., 1990). Acetone was needed to dissolve Eudragit RS, however increasing the acetone level in the dispersed phase produces particles that were more porous in appearance (Figure 3.23). Therefore it was decided to limit the acetone used as such, Eudragit S100 particles were produced using ethanol while the Eudragit RS and Eudragit S100 + RS microparticles used a dual solvent system comprised of ethanol and acetone (2:1).



a B

Figure 3.23 Eudragit S100 + RS microparticles prepared by solvent evaporation with a) ethanol to acetone ratio 2:1 and b) ethanol to acetone ratio 1:2

It was explained earlier that for a solvent evaporation technique to work effectively the organic solvent must migrate into the continuous phase and evaporate, moreover, key diffusional motions need to occur: solvent out, non-solvent in and (if present) drug out. The direction rate and magnitude of this depends on solvent/non-solvent/polymer/drug interactions (Bodmeier et al., 1987). Bodimeier et al., 1987 observed that for an o/w system the immiscibility of the organic solvent with water was critical to microparticle formation and that water miscible solvents such as acetone and dimethylsulfoxide did not form droplets. A similar reasoning can be applied to an o/o system; increased miscibility with the continuous phase will result in poorer quality particles.

Rapid migration of the solvent from one phase to another disrupts the action of the emulsifier. The emulsifier provides short term stability for suspended polymer droplets

until adequate solvent evaporation has occurred producing sufficient hardening of the polymer/drug droplets (Watts et al., 1990). In an o/o system the movement of the solvent has been shown to be dependent on its lipophillicity (Bogataj et al., 1991), acetone is more lipophillic than ethanol (dielectric constants of 20.7 and 24.3 respectively), therefore an increase in acetone in the dispersed phase will increase the precipitation of the polymer at the droplet surface resulting in more porous or hollow particles.

Eudragit S100/RS + carbomer microparticles (Figure 3.21) were consistently the most regular and spherical. The reason for this is not immediately clear, however, the solubility of the polymers in the solvents is likely to be an important factor (Watts et al., 1991). Although both ethanol and acetone were used as organic solvents with the Eudragit RS + carbomer particles, Eudragit RS is only soluble in acetone. Of the two solvents it is acetone that is likely to migrate into the continuous phase the quickest and may not allow sufficient time to pass for the surfactant to stabilise the droplets prior to Eudragit RS being deposited, as such the carbomer polymer plays a greater role in particle formation. However, with Eudragit RS and S100 + carbomer particles the S100, which is soluble in ethanol, may be able to form droplets due to the slower migration rate of the solvent into the continuous phase. Finally, the use of three polymers mean that there is an increase in concentration at the polymer droplet boundary promoting polymer precipitation, this maybe occurring at a sufficient rate as to form suitable particles before the solvent can migrate and evaporate.

3.16.3 Incorporation of a model drug and drug release

The incorporation of prednisolone into the particles was investigated. In previous dissolution studies with carbomer tablets and pellets the model drug chosen was paracetamol, however its high solubility and low molecular weight (1 in 30 mL and 150 Da) meant that the particles produced were leaky in acid. Subsequently a less soluble drug, prednisolone with a higher molecular weight was chosen as this is less likely to partition out of the particle and greater retention in 0.1M HCl was observed (Figure 3.24).

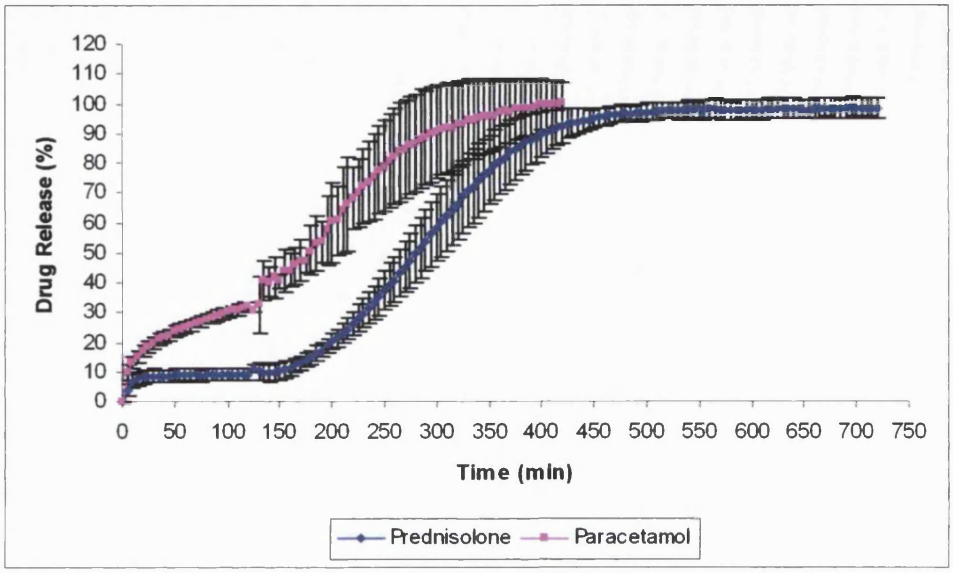


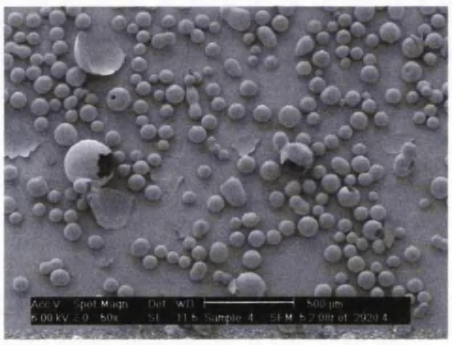
Figure 3.24 Release of paracetamol and prednisolone from Eudragit S100 + RS + 5% CP 980 microparticles using a pH change method 2 hours in 0.1 M HCl before pH is adjusted to pH 7.4. (n=3)

Drug release and effect of addition of carbomer polymer on said release was investigated using one of the carbomer grades. CP 980 was chosen as particles produced using this grade of polymer were consistently the most spherical and uniform (as observed by SEM). The encapsulation efficiencies of control and carbomer containing microparticles are shown in Table 3.12

Table 3.12 Encapsulation efficiency of prednisolone in Eudragit and carbomer microparticles

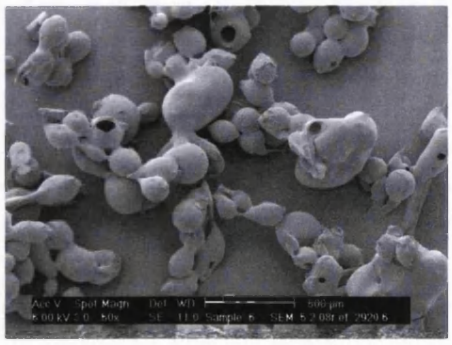
Formulation	Encapsulation Efficiency (%) ± S.D
Eudragit S100	86.19 ± 2.07
Eudragit S100 + 5% CP 980	92.12 ± 0.85
Eudragit RS	84.48 ± 0.65
Eudragit RS + 5% CP 980	80.93 ± 1.10
Eudragit RS + S100	89.02 ± 2.58
Eudragit RS + S100 + 5% CP 980	94.97 ± 0.47
Eudragit RS + S100 + 10% CP 980	88.67 ± 1.87

It is noteworthy that the encapsulation efficiency of prednisolone is affected by the carbomer polymer. In both the Eudragit S100 and RS + S100 addition of CP 980 increases drug loading, conversely for the Eudragit RS microparticles encapsulation efficiency decreases. The precise reason for this is unclear, however the structure of a particle is dependent on the interplay between the polymers, drug, solvent, emulsifier and continuous phase, therefore by altering one of these factors you can alter the composition of the microspheres. More specifically the encapsulation efficiency depends on the degree of partitioning of the drug out of the polymer solution phase and into the continuous phase (Watts *et al.*, 1990). Drug movement between phases is dependent on the rate of precipitation of the polymer, the faster the precipitation the greater the amount of drug encapsulated (Bodmeier et al., 1988). Addition of carbomer creates and increase in polymer concentration at the droplet boundary, as such precipitation is faster and diffusion of drug is retarded (Bodmeier el al., 1988). SEM images of the particles can be seen in Figure 3.25.



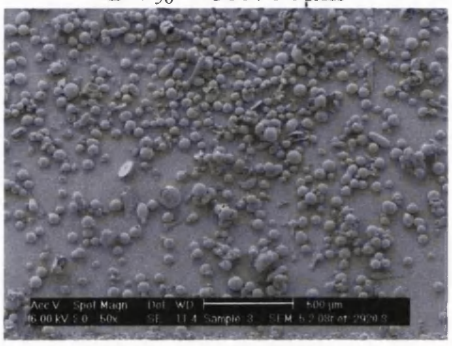
a)

DV_{50} = 185. 36µm



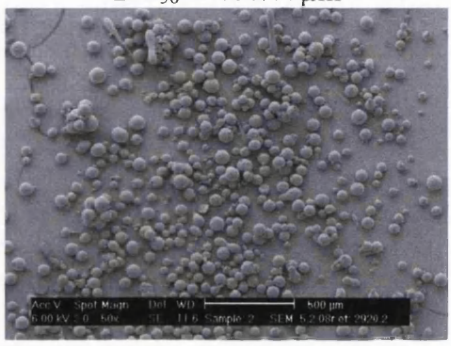
b

DV_{50} = 486.87µm



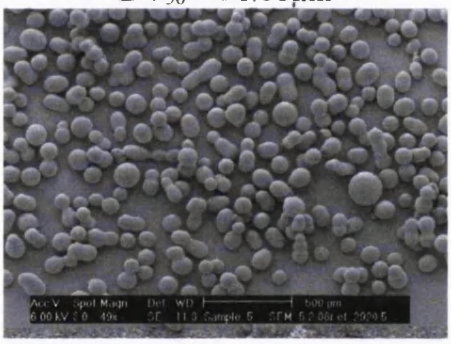
c

DV_{50} = 91.81µm



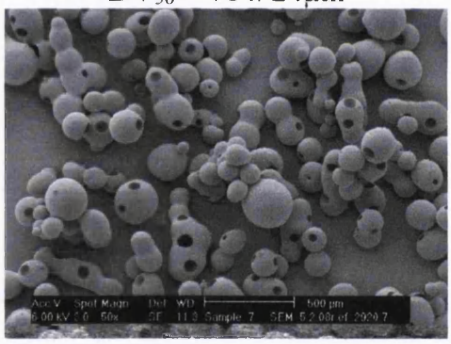
d

DV_{50} = 784. 24µm



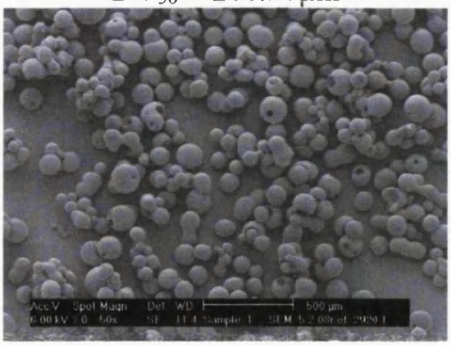
e

DV_{50} = 206.90µm



f

DV_{50} = 329.65µm

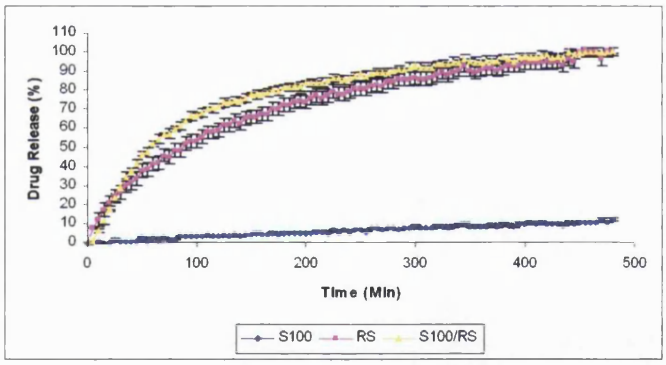


g

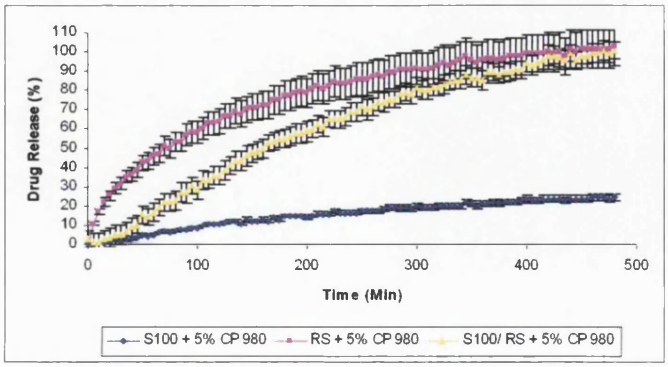
DV_{50} = 170.38µm

Figure 3.25 SEM of prednisolone loaded microspheres a) Eudragit S100, b) Eudragit S100 + 5% CP 980, c) Eudragit RS, d) Eudragit RS + 5% CP 980, e) Eudragit S100/RS (1:1), f) Eudragit S100/RS (1:1) + 5% CP 980 and g) Eudragit S100/RS (1:1) + 10% CP 980. 50 x magnifications

The addition of prednisolone to the microparticles increased their size in all cases except Eudragit RS and Eudragit S100/RS + 5% CP 980 where a decrease in DV_{50} was seen. An increase in particle size is expected with the addition of drug in the same way as it increases with increases in polymer concentration. An increase in concentration results in a high viscosity internal phase and larger particles. The addition of 10% CP 980 to Eudragit S100/RS (1:1) particles appears to have a stabilising effect and the microparticles produced are more spherical and smaller. Again this is likely to be a result of increase rate of polymer precipitation created by and increase on concentration of the continuous phase. Drug release from Eudragit S100 particles in pH 6.8 phosphate buffer (Figure 3.26) is as we would expect since its dissolution threshold is greater than pH 7.0. After 8 hours less than 10% of the encapsulated drug has entered solution. The addition of 5% CP980 increases this; 24% is released after 8 hours. Eudragit RS microparticles show no change in drug release in pH 6.8 phosphate buffer upon the addition of CP 980, Eudragit S100/RS microparticles do however. Drug release form Eudragit S100/RS + 5% CP 980 exhibits an extended release profile.



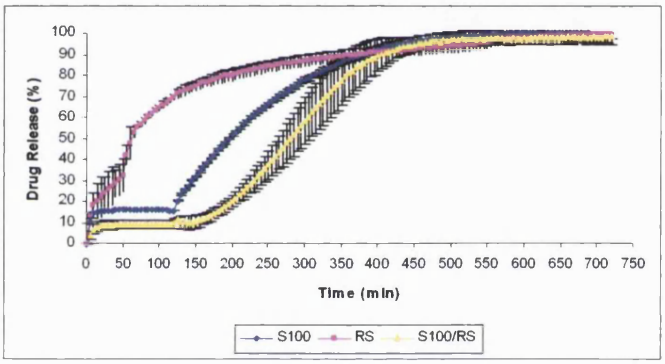
a



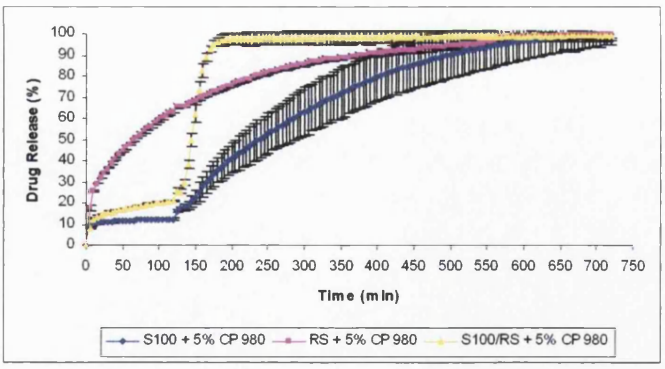
b

Figure 3.26 Release of prednisolone from a) control particles and b) Eudragit and carbomer containing particles, in pH 6.8 phosphate buffer. (n=3)

The release of prednisolone from the Eudragit only (control) formulations using a pH change method (Figure 3.27a) shows three distinct release profiles. Microparticles prepared with the water insoluble polymer Eudragit RS exhibit the most leakage of drug in acid with 69.7% loss after two hours compared with 15.7% and 9.4% loss for Eudragit S100 and Eudragit S100/RS (1:1) respectively. Although Eudragit RS is permeable to aqueous solution due to the presence of quaternary ammonium groups (Watts et al., 1991) it is not clear as to why drug release is greater than the other formulations, particularly the Eudragit RS and S100 particles. Size can be eliminated as a cause of this increased leakiness since the smallest of the three particles was produced using Eudragit S100 (DV50 = 45.97μm), instead a more likely explanation lies in the location of polymer/drug precipitation during particle formation



a



b

Figure 3.27 Release of prednisolone from a) control particles and b) Eudragit and carbomer containing particles, using a pH change method. Exposure to 0.1M HCl for 2 hours followed by an increase in pH to 7.4. (n=3)

Discussed earlier was the effect of solvent on the formation of particles and that the migration of acetone was the likely cause of the production of hollow or porous particles, this is also a likely cause of greater drug release in acid. However, acetone was also used in the Eudragit S100 + RS (1:1) particles and drug release in acid here was the least of the three, therefore additional mechanisms must be occurring in the release from the Eudragit S100 + RS (1:1) microparticles. Prednisolone is more soluble in ethanol than acetone, as is Eudragit S100, consequently drug/polymer (Eudragit S100) precipitation will occur more towards the core of the droplet, during formation, after the Eudragit RS has deposited on the surface. The result is a less porous particle with greater drug retention in acid.

The effect of addition of carbopol 980 to the microparticles is varied. Eudragit S100 particles appear to show no difference in drug release with or with out the presence of carbopol 980. However of the three formulations, Eudragit S100 + 5% CP 980 are the least spherical and uniform, so perhaps it is more prudent to discuss the addition of CP 980 on the RS and S100/RS (1:1) microparticles. With both formulations the addition of CP 980 promotes greater initial drug release (Figure 3.28), at 50 minutes in acid the Eudragit RS and RS + 5% CP 980 particles release 32.3% and 44.6% of drug respectively, at 60 minutes, due to a sudden increase in drug release form the RS particles, both formulations have released the same amount of drug (62.6% and 69.7% respectively) and continue to do so until 100% is released. Eudragit S100/RS (1:1) and Eudragit S100/RS + 5% CP 980 particles released 9.4% and 21.0% of their drug load after 120 min in acid respectively, however unlike the RS microparticles, Eudragit S100/RS (1:1) + 5% CP 980 continue to show an increased release over the control after the pH is adjusted to 7.4.

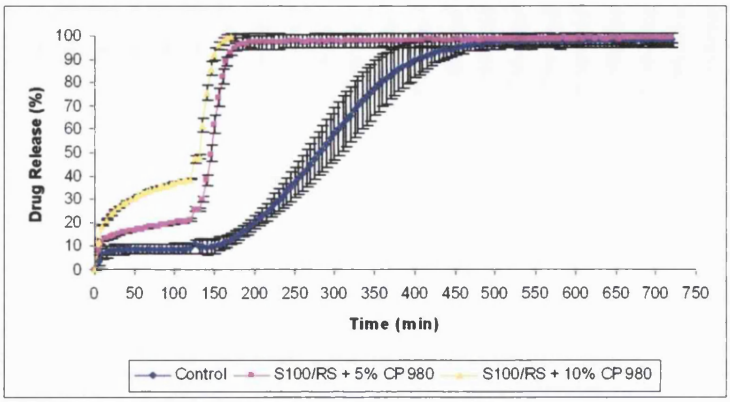
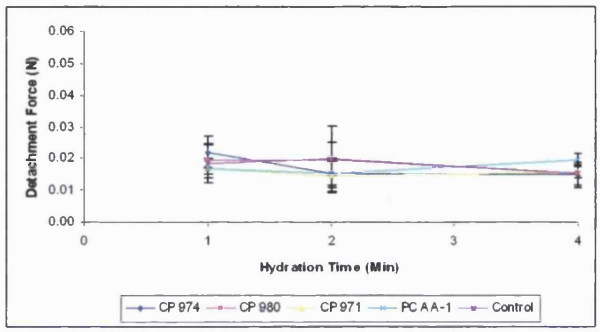


Figure 3.28 Effect of addition of 5% and 10% CP 980 on prednisolone release form Eudragit S100/RS (1:1) particles from pH change method. (n=3)

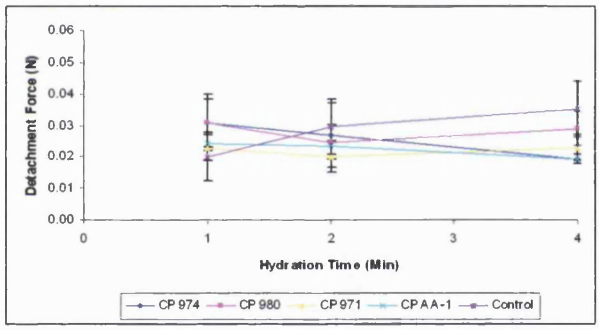
The reason for the increased release for the mucoadhesive particles is unclear; however we can speculate that the cause is location of precipitation of the carbomer polymer during particle formation. If this occurs at or towards the surface of the droplet then upon submersion in acid the polymer will hydrate creating channels through which the drug will diffuse. The hypothesis is supported by Figure 3.28 which shows that increasing the carbopol loading increases the release of prednisolone in acid.

3.16.4 *In-vitro* evaluation of mucoadhesion

Adhesion, mucoadhesion and mucin exposure experiments were conducted using all placebo Eudragit RS + S100 microparticles with the four carbomer grades at 5% and CP 980 at 5% and 10%. No increased mucoadhesion over the control was seen in any of the tests conducted (Figure 3.29).



a



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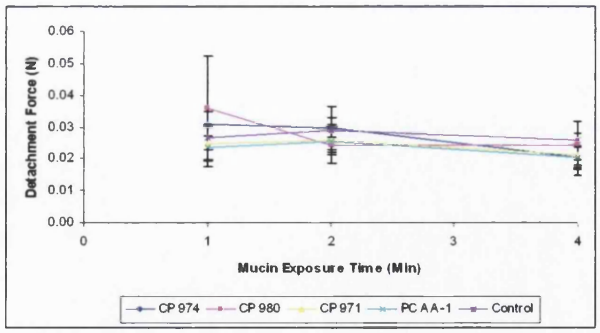


c

Figure 3.29 Comparison of detachment force for Eudragit S100/RS + carbomer microparticles, prepared by solvent evaporation, from, a)glass surface, b) a mucin layer c) Prolonged mucin exposure, compared with a non carbomer containing control after hydration in pH 6.8 phosphate buffer. (n=5)

A negative mucoadhesive reading suggests that either the carbomer polymer is not present in the particle or that it is present but inactivate. However, drug release from the particles was observed to be more rapid when the carbomer content was increased from 5% to 10% which was attributed to the increased carbomer loading. Links with the ability of the carbomer polymers to swell within a microparticle and its mucoadhesion have been shown (Kockisch et al., 2003). Studying carbopol 974 and polycarbophil AA-1 particles prepared by a water-in-oil emulsification solvent evaporation technique Kockish, et al., 2003 saw no increased detachment force from porcine oesophageal mucosa over a control formulation, however an increase in work of adhesion was recorded and prolonged retention over a control was also seen. The encapsulation of the carbomer polymers in the water insoluble Eudragit RS and pH soluble Eudragit S100 could be limiting polymer swelling and thus mucoadhesion. Upon hydration the carbomer polymer may be gelling but in isolation, in effect creating pools of gel which allows the drug to escape but which is not sufficiently extended from the particle surface to interact with the mucin.

3.17 Summary

The production of Eudragit and carbomer microparticles was achieved using a solvent evaporation process. Solvent selection was seen to be important in the production of non porous or hollow particles and the addition of carbomers was seen to affect the size and morphology of the microparticles produced. The former was explained by solvent migration and polymer precipitation at the droplet surface, the latter by the increase in viscosity of the dispersed phase upon addition of the carbomer polymers.

Due to a combination of the previously mentioned factors Eudragit S100 + RS (1:1) + carbomer proved to produce the most spherical and uniform particles. Drug release from 5% carbomer (CP980) loaded particles were investigated using a pH change method and compared against and non-carbomer containing control. It was hypothesised that release of the model drug prednisolone from the control particles was dependent on the location of polymer/drug precipitation during particle formation. The addition of carbomer polymer was shown to only affect drug release from one of the formulations, Eudragit S100/RS (1:1). This was attributed to two factors, firstly that these particles were the

only formulation where significant retention of drug in acid was seen, and secondly the location of the carbomer within the microparticle, suggesting that it was located towards the outer surface and thus providing, upon hydration, channels through which the drug could escape. This suggestion was reinforced by observing drug release from a 10% carbomer loaded microparticles which was subsequently leakier in acid than their 5% loaded counterparts.

3.18 Chapter 3 Conclusions

Multiparticulate systems containing carbomer polymers proved difficult to produce; the more complex manufacturing processes involved made manipulation of the polymers troublesome. However, acceptable carbomer pellets and microparticles were produced and tested. Hot melt extrusion was explored as a way to produce higher carbomer loaded systems since there is no need to use fluids. It was shown that higher loading of carbomer polymers could be incorporated into the formulation relatively easily but the adhesion of the system was dictated by the carrier molecule and the creation of a molecular dispersion eliminated its mucoadhesive capability. More investigation is needed to screen other thermo plastic polymers that might be suitable.

Drug release from ram extruded pellets show no prolonged release profile and their adhesion and mucoadhesion was not significantly different for any of the carbomer grades from a non–mucoadhesive control ($p>0.05$). As with granulated tablets, this was attributed to the addition of fluids at the preparation stage causing polymer chain uncoiling. Prolonged exposure to mucin however did yield some promising results and increased detachment forces were seen for the lower molecular weight polymers after exposure to a mucin solution for 4 minutes, however no measurable work of adhesion was observed. As with granulated tablets the exposure to fluid in the processing stages has affected the polymers ability to uncoil and thus form polymer/mucin entanglements. The fact that the force of adhesion increases when mucin exposure time increase is promising and suggests however that mucoadhesive pellets are a viable option.

The complexities of microparticle production with carbomers were even greater than that of pellets. With the solvent emulsification/solvent evaporation technique it was possible to produce spherical particles of all four carbomer grades, however the most promising were Eudragit S100/RS (1:1). Drug release form the particles in acid, pH 6.8 phosphate buffer and when a pH change method was used is dependent on the co-polymers and not the carbomers. Additionally, adhesion, mucoadhesion and exposure to mucin were all affected by the formulation process and none showed significant detachment forces over a control formulation ($p>0.05$). This is disappointing; the purpose of producing multiparticulate systems is to increase the likelihood of creating

intimate contact between the colon mucosa and the particles, the results from theses chapters suggest that current manufacturing procedures eliminate any mucoadhesive capabilities of carbomer polymers. The carbomer loading within the particles was however very low. As such the type of test performed here may not be the most suitable. Retention of particles on an exercised portion of GI tissue is a more popular method for testing microparticles. This method was avoided here though as it was hoped that more information would be gained from a tensile test method than a timed retention method. This still may be the case; however adaptations would need to be made to the current apparatus to allow for accurate determination of such low forces.

Chapter 4

In-vivo evaluation of carbomer mucoadhesion in the canine colon using Gamma Scintigraphy

4.1 Overview

Theoretically, successful mucoadhesion will occur *in-vivo* if intimate contact between the polymers and mucin can take place. As such, formulations likely to give a positive mucoadhesive result would need to be easily dispersed *in-vivo* to increase the chances of contact, additionally; they would need to be able to gel. In reality, however, this is complicated by the low fluid volume and high viscosity of the colon which will inhibit polymer gelation and thus mucoadhesion. Maximum dispersion requires the administration of a multiparticulate system, however in the previous chapter it was shown that the production of such systems significantly reduces the carbomer content and its mucoadhesive properties. Therefore the decision was made to administer carbomers in a powdered form with the expectation that they would act in a similar manner to a mutliparticulate system and spread throughout the colon from their original delivery site.

Practical considerations meant that only two of the four grades of carbomer tested previously were to be used. Current literature, as well as results shown previously were used to determine which two grades should be progressed forward. CP 980 and PC AA-1 were chosen as current data indicated that these grades represent the best chance of a positive mucoadhesive response. Both performed well in the *in-vitro* tests but due to their different molecular weights and cross linking agents, mucoadhesion was seen to be the result of different mechanisms. Carbopol 980 is a high molecular weight polymer cross linked with allylpentaerythritol, and polycarbophil AA-1 is a mid molecular weight polymer cross linked with divinyl glycol. Evidence from chapter 2 suggests that CP 980 is initially mucoadhesive without polymer/mucin entanglements but has greater tensile strength from interactions with mucin after prolonged exposure. In contrast PC-AA-1's mucoadhesion is far more dependent on polymer/mucin entanglements.

Using the canine as a model the InteliSite® companion device was used to administer the polymers, and ethylcellulose as a suitable non mucoadhesive, non gelling control, to the colon. The capsule was tracked using scintigraphy and was activated in the caecum in a 3 way cross over study, the retention time of the powders in 4 regions of interest (ROI), caecum, ascending colon, transverse colon and descending colon was determined as was the spread of powders in the ascending and transverse colons.

4.2 A comparison of human and canine anatomy and physiology

There are many anatomical, physiological and biochemical differences between the canine and human GI tracts, nevertheless, they are often the animal model of choice for *in-vivo* studies, particularly those focusing on transit. They are easier to handle than other species of similar size and have the advantage of being less expensive and time consuming than studies with human volunteers. Additionally, the dimensions of the dog GI tract and in some cases drug bioavailability are comparable to humans (Dressman, 1986). For example, stomach capacity in the dog has been shown to range from 400 – 500 mL to 1 L (Andersen, 1970), this is much closer to the capacity of the human stomach (1L) than other animals of similar size such as the pig or the monkey with 6-8 L and 0.1L respectively (Dressman et al., 1991). Gastric pH has also been shown to be similar in the fasted state with values of pH 1.7 and pH 1.5 in human and dog respectively, in the fed state however the observed pH in the human stomach is greater than that of the dog with values of 5.0 and 2.1 respectively (Dressman et al., 1991)

Despite the similar stomach volumes, gastric emptying (GE) times in dog and humans are different. This variation may be due to the size of the pyloric aperture which dictates the minimum size of a dosage form for retention. Khosla et al., (1990) showed that the cut off size for emptying of multiple units in the human stomach was 7-13 mm in diameter, this value was later revised to 11-13 mm (Davis et al., 1993). In canines this value has been recorded as 2-7mm (Dressman 1986). This has been confirmed by other studies (Aoyagi et al., 1992) which showed that the GE time of a 5.8 mm tablet in dogs was 0.8h compared with 1.2h in man. In contrast to this the GE time of 1 cm granules showed no differences. It is important to note however that in theory dosage forms of any size can empty fortuitously with food, the bigger the dosage form the more unlikely emptying with food will occur. Generally, even big dosage forms will leave with phase III MMC contractions. Therefore, the emptying times of a 5.8 mm tablet in dogs and humans may tell us nothing about pylorus size.

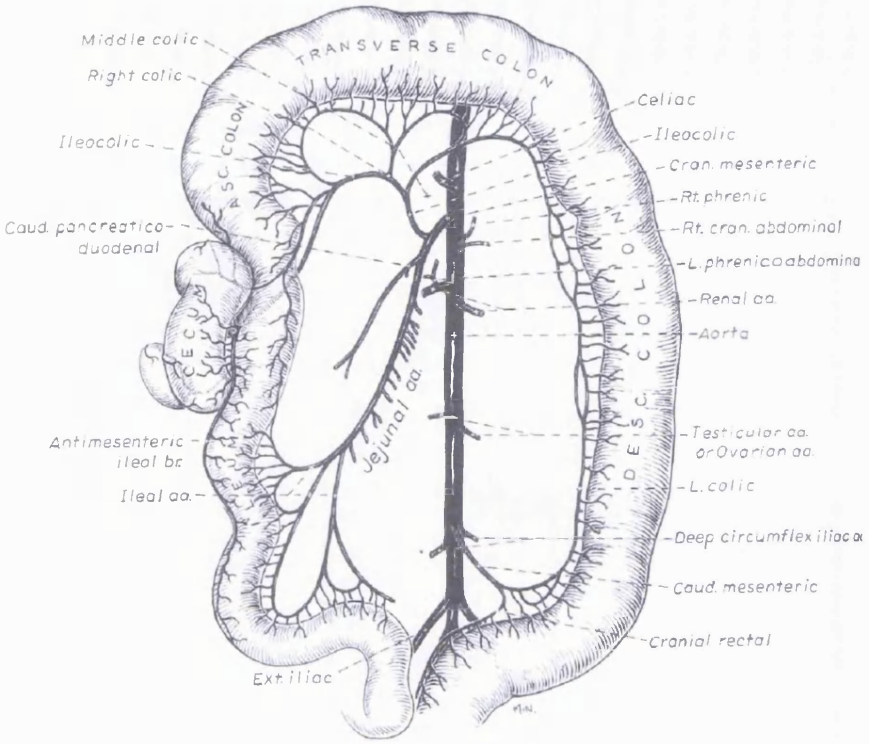


Figure 4.1 The dog colon Adapted from Dyce et al., 2002

Proceeding distally from the stomach the anatomical differences between humans and canine start to become apparent. In the dog the small intestine is approximately 225 to 290 cm in length with the first 25 cm the duodenum and the last 15 cm the ileum. In contrast the human small intestine is much longer, the human duodenum is approximately 25cm in length with both the jejunum and ileum measuring 300 cm each. The human colon length varies from 90 to 150cm and is made up from the ascending, transverse, descending and sigmoid regions. In the dog the sigmoid colon is not present. Additionally, the relative sizes of these regions also differ. The length of the ascending colon in humans measures 20cm, compared to only 5 cm in the dog. Although both possess a caecum, it is relatively undefined in the human and is continuous with the colon (Figure 4.1), however in the dog the caecum is a lateral appendage and is out of line with the direction of intestinal movement. Again however, despite these anatomical differences we do observe some physiological similarities. The pH range in the small intestine is 5 -7 in humans and 6.2-7.5 in dogs, similar overlaps are seen in the colon with pH range from 5.5 -7.5 and 6.4-7.0 in the human and dog respectively (Kararli, 1995)

4.2.1 Estimation of fluid content of the dog colon.

Within the literature experimental determinations of the fluid content of the canine GI tract are rare, and no data is available for the fluid content of the colonic regions. One study within the literature has measured the fluid content of the dog GI tract (Cizek, 1954). The authors quote total body weight of 5 male and female dogs both fed and fasted and the water in the gut as a percentage of body weight after evaporating GI contents to dryness. From these two reported figures we obtain a value of the volume of fluid in the dog gut as 418.19 mL in the fed state and 106.69 mL fasted as an average of 5 male dogs with an average weight of 5.61 Kg. Unfortunately the study quotes fluid volume of the GI tract as a whole so no values of fluid content of a specific region can be obtained. However, similar studies have been conducted in sheep and goat (Maloiy et al., 1980) and rat and mouse (McConnell et al., 2008) where fluid contents in particular regions of the GI tract have been determined.

Comparable values in man can be obtained by combining two previous studies. Gotch et al., (1957) reported values in the stomach (118 mL) and small intestine (206 mL) and Cummings et al., (1990) determined the fluid content of the human colon (187g). Combining these values (assuming 1 g = 1 mL) we obtain a value of 511 g of fluid for the human GI tract. Using these figures and those reported by Maloiy et al., (1980) and McConnell et al., (2008) the distribution of total GI fluid within the colon was calculated. Estimated values of the fluid content within the dog colon were then made (Table 4.1) based on the assumption that the distribution of fluid is the same as those quoted in each animal model, from this the colon fluid per unit body weight was also calculated.

*Table 4.1 Estimation of values of fluid volume in the dog colon based on current literature values of total GI water content and colonic water content in different models. a) Gotch et al., (1956), b) Cummings et al., (1990) c) Maloiy et al., (1980), and d) McConnell et al., (2008). * Assumption made that the average human body weight is 70Kg*

					Values for canine assuming the same fluid distribution	
Model	Total GI Fluid (g)	Colon Fluid (g)	Colon Fluid as % of Total	Colon Fluid g kg^{-1}	Colon Fluid g kg^{-1}	Colon Fluid (g)
Human [a, b]	511	187	36.60	2.67*	6.95	39.04
Sheep [c]	5320	490	9.21	18.14	1.75	9.83
Goat [c]	3380	410	10.70	17.08	2.03	11.41
Rat [d]	3.20	1.25	39.06	7.14	7.42	41.67
Mouse [d]	0.81	0.16	19.75	7.80	3.75	21.07

Of course the distribution of fluid within the dog is not likely to be the same as other animals, and the decision as to which of these models most closely resembles the dog is difficult. It may more closely resemble animals of similar body weight or similarities could be linked to diet. Certainly the dimensions of the large intestine of animals can be correlated with diet. In herbivores the large intestine is large and complex since they rely on microbial fermentation for digestion. Omnivores like pigs and humans have a substantial large intestine, but nothing like that seen in herbivores. Finally, carnivores such as dogs and cats have a simplistic and smaller large intestine (Dyce et al., 2002). Nevertheless estimations such as this can give a general idea of the content of fluid available within the dog colon. If the distribution of fluid is similar to humans then a favourable 5.98 g kg^{-1} of fluid is available in the dog colon, conversely if similar to sheep then a limiting 1.75 g kg^{-1} would be present

A final consideration is the estimation of the amount of fluid that is free fluid. Comparisons of the current literature values for free fluid (13 ± 12 mL Schiller et al., 2003) and total fluid (187 g (Cummings et al., 1990)) suggest that the amount of free

fluid within the human colon is approximately 14 times less than the total measured content. Extending this once more to the canine gives a value of 2.65 mL or 0.43 g Kg^{-1} of free fluid which would prove to be a substantial obstacle for drug delivery to the dog colon.

4.3 Principles of Scintigraphy

For many years the field of nuclear medicine has utilised scintigraphy to provide functionally based information of the bodies organs. Imaging of systems such as the cardiovascular system, urinary tract, skeletal system, gastrointestinal tract and identification of regions of infection or inflammation or the imaging of tumours can all be achieved with scintigraphy (Billington et al., 1992). In the mid 1960's physiologists began to use scintigraphic techniques to identify key physiological functions such as gastric emptying by incorporating a radioactive isotope (^{51}Cr) into a meal (Griffith et al., 1966). Realisation of the adaptability of this type of imaging to investigate the fate of dosage forms in the human GI tract came a decade later when the technique was used to investigate the disintegration of capsules (Digenis et al., 1976) and tablets *in-vivo* (Hunter et al., 1981). The popularity of the technique quickly spread and its applications broadened to include the determination of *in-vivo* dissolution rate of sustained release tablets (Daly et al., 1982) and the measurement of GI transit times (Christensen et al., 1985)

Scintigraphy or Single Photon Emission Computed Tomography (SPECT) is a technique that utilises radioactive decay to track a dosage form *in-vivo* or to identify physiological functionalities. Most elements have both stable and unstable isotopes, when these isotopes transform to a more stable form, either a charged particle is released from the nucleus or an electron is captured from one of the shelves surrounding the nucleus, this process is known as radioactive decay. The result is a change in the balance of the protons and neutrons in the element and the resulting nucleus is known as the daughter nucleus.

The radioactive decay from the parent nucleus to the daughter can take three forms, alpha (α) beta (β) and gamma (γ). Alpha decay is the emission of an α particle consisting of two protons and two neutrons and is identical to helium nuclei, the release

of an electron or positron (positive charged particle with the same mass as an electron) is β. Both alpha and beta radiation are accompanied by gamma radiation which are electromagnetic rays emitted from the daughter nuclei when in an excited state, this radiation is characteristic to the daughter nuclei and hence the parent element. It is this radiation that is detected in gamma scintigraphy (Short, 1995).

The detection efficiency of gamma camera is optimised within the gamma radiation energy range of 100-200KeV (Wilding *et al.,* 2001) as such radionuclides suitable for gamma imaging must have an emission energy within this range. The two most popular isotopes are the metal ion nuclides technetium (^{99m}Tc) and indium (^{111}In) with peak emission energies of 140Kev and 173-247KeV respectively. Half life of the isotopes is also an important consideration and needs to be suitable for the duration of the imaging procedure. For imaging of the upper GI tract ^{99m}Tc is most popular, as its low radiation dose and short half life (6 hours) are ideal for short duration studies. ^{111}In however has a half life of 67 hours and as such is more suitable to studies imaging the entire or distal regions of the GI tract. The differences in peak emission energies of the two radionuclide also make them suitable to be used together creating more detailed understandings and comparative information on the transit or drug release of two dosage forms simultaneously (Clarke et al., 1993).

Once the isotopes have been selected a method of radiolabelling the drug or dosage form in the study needs to be determined. The most conventional approaches select a non absorbable carrier, commonly chelating agents such as diethylenetriaminepenetaacetic acid (DTPA) to create ^{111}In-DTPA and ^{99}Tc-DTPA and incorporate this into the dosage form. Another approach is the use of an ion exchange resin which has the benefit of the radiolabel remaining with the device and its position *in-vivo* can be observed (Wilding et al., 2001). There are however limitations with this approach, usually the radiolabels have to be incorporated at a late stage to avoid contamination, the manufacturing processes have be scaled down due to the smaller amount of radiolabel being handled and the dosage forms need to be relatively simple. A second approach that helps overcome some of these problems is neutron-activation. This involves the incorporation of a small amount of non-radioactive isotope at the site of manufacture as oxides of erbium and samarium. The dosage form is then irradiated in a neutron source and converted to a γ emitting source (^{170}Er and ^{153}Sm).

During gamma imaging the radionuclide is detected by a sodium iodide crystal located in the head of the γ camera which emits a light pulse. Photomultiplier tubes are coupled to the crystal to detect the light pulses and the arrangement is cased in lead to shield the crystal from extraneous radiation and electrons, this is attached to a computer and visual display. A lead collimator directly in front of the crystal detects the γ-rays and focuses them into a detector (Wilding et al., 2001). Some important considerations which must be taken into account when using gamma imaging such as subtraction of background noise from natural isotopes, cosmic rays and the instrument must be performed before a reliable count from the source alone can be obtained. Additionally, during transit studies the isotope decay needs to be considered and count corrected for this. Finally, although the detection unit is able to provide the location of radiation source in space, no other anatomical information is provided, therefore anatomical positioning of the source requires extensive experience and knowledge of the anatomy and physiology of the test subject. Lateral movements of the source can also present difficulties as counts are attenuated by body tissue leading to errors in calculation of transit such as gastric emptying. However this is usually over come by calculating the geometric mean of the posterior and anterior detector counts.

4.4 Radio telemetric devices for delivery to the distal gastrointestinal tract.

Radio labelling of dosage forms and scintigraphy have given great insights into the performance of drug delivery system *in-vivo*, such as gastric emptying (Khosla et al., 1989), the effect food has on this (Sangekar et al., 1987), *in-vivo* dissolution rates (Daly et al., 1982), assessment of enteric coated dosage forms for small intestinal targeting (Wilding et al., 1994) and gastrointestinal drug absorption (Basit et al., 2004). To continue this trend and gain insights into drug absorption in the distal regions of the GI tract, the test materials have to arrive at the target region unaltered, as such alternatives to the formulation approach adopted by Wilding *et al.*, (1994) and Basit *et al.*, (2004) were sought. This prompted the creation of several engineering approaches.

The High Frequency (HF) capsule (Figure 4.2) was one of the first developments for such purposes. A latex balloon (into which the drug was placed) was held in a 2.5cm long polyurethane capsule with a diameter of 0.7 cm. Drug release was achieved upon the induction of a high frequency signal which burst the balloon and released the drug. The HF capsule has been successfully used to investigate the effects of regional absorption on drug plasma profiles (Harder et al., 1990 and Staib et al., 1990).

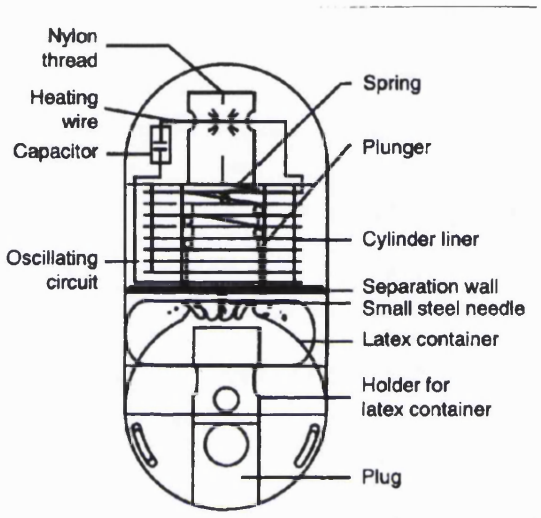


Figure 4.2 Schematic representation of the HF capsule (adapted from Wilding et al., 2001)

The device however had limitations for example x-rays were used to track the position of the capsule *in-vivo*, filling of the drug reservoir (the balloon) was reported to be difficult and only liquid preparations could be used. To overcome this a new device was conceived which was capable of delivering both powder and liquid formulations (Gardner et al., 1997). The InteliSite® capsule (Figure 4.3), is 35 mm in length and 10 mm in diameter. Its drug reservoir consists of two sleeves, an inner and outer, both incorporating six slots spaced around the circumference at 120° increments. Drug filling of the capsule is achieved through a port located at the bottom of the device.

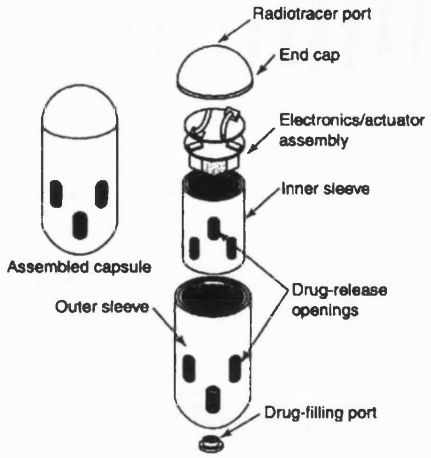


Figure 4.3 Schematic representation of the InteliSite® Device (adapted from Gardner et al., 1997)

An oscillating magnetic field produced from an external antenna induces activation of the device. Application of the magnetic field heats an aluminium thermal plate which in turn heats two Nitinol shape memory alloy (SMA) wires which have been pre-conditioned to straighten upon a change in temperature (40-43°C). This straightening provides torque causing the inner sleeve to rotate, aligning the slots and facilitating drug release (Gardner *et al.*, 1997). The InteliSite® device possess several benefits over the HF capsule, other than being more versatile and being able to deliver liquids and powders the addition of a 80 μL radiotracer port means that both capsule and dosage form could be traced independently with scintigraphy. Subsequent *in-vivo* testing of the device show that it is safe for human use and gastric emptying although slow was easily achievable and coincided with the large contractile wave of the MMC (Pithavala et al., 1998).

Delivery of solid dosage forms to the small intestine and colon have also been undertaken with the InteliSite® Device. Theophylline and frusemide immediate release tablets (split in half) were administered to healthy human volunteers and drug plasma profiles recorded (Clear et al., 2001). Successful activation of the device was seen in with only 1 failure however plasma profile showed differences depending on the site of activation. Theophylline was only detected in the plasma of 1 subject (n=7) when activated in the colon suggesting that release from the capsule was limited. Although this was not totally consistent with the amount of drug retained in the capsule (measured

on retrieval at 13.76%) the authors concluded the unaccounted drug must have been removed from the capsule at defecation and that the lower fluid volume in the colon meant that the current capsule design was not suitable for colonic delivery. Studies also showed that the capsule on occasion was leaky when containing liquid preparations and that release in the distal GI tract was problematic due to the decreased amounts of fluids within this region (Parr et al., 1999).

The Enterion capsule (Figure 4.4) is another non-invasive device designed for drug delivery. It was designed specifically to overcome the limitations of the InteliSite® Device. It is 32mm in length and has a drug reservoir of 1 ml and similarly to the InteliSite® contains space in the end cap for a radiotracer.

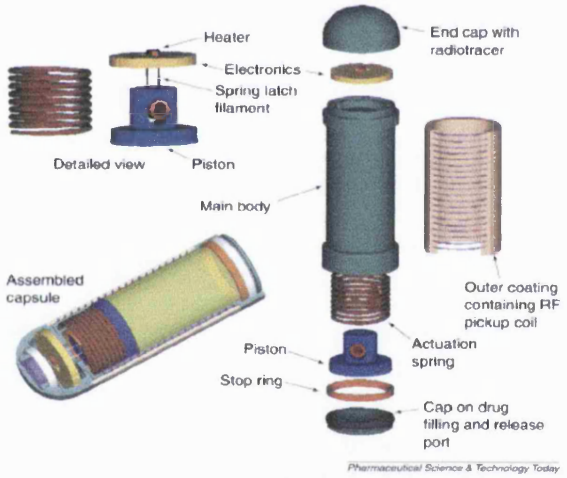


Figure 4.4 Schematic representation of the Enterion capsule (adapted from Wilding et al., 2000)

Activation of the capsule is again via heat transfer which upon the breaking of a small filament activates a spring loaded piston forcing out the drug (Wilding et al., 2000). Successful activation of the capsule and good release have been reported in the stomach, proximal small bowel, distal small bowel and ascending colon (Wilding *et al.*, 2000 and Oo et al., 2003).

The reports of leaking of the InteliSite® device and the success of release in the colon from the Enterion capsule lead to modification being made to the original InteliSite® device (Figure 4.5). The new device called the InteliSite® companion device consists of two main parts, the body and the cage. The body of the capsule measures approximately

3.5 cm in length and the cage fits inside it. The cage is spring loaded and is held in compression with two wire clips. The head of the capsule contains a 3mm diameter hole, through which loading of the dosage from can take place. As the next generation of the system the InteliSite® Companion device has been designed with improved release in mind. The main difference between the two devices is the method of release. Both operate by an increase in temperature and subsequent change in shape of a pair of resistor wires within the capsule, in the original InteliSite® this causes the inner sleeve of the capsule to rotate, aligning slots in the capsule and allowing release.

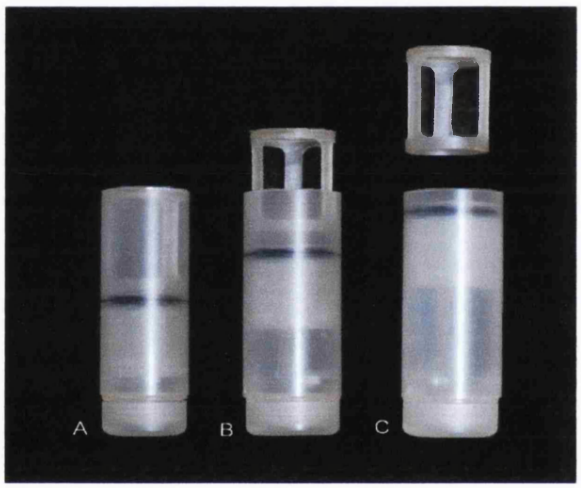


Figure 4.5 InteliSite® companion device. A) Device sealed with cage inside the body, B) Cage partly expelled from body, C) Cage fully released and expelled from the body.

Within the Companion device however, the deformation allows for the activation of a tightly coiled spring with propels the cage of the system out and away from the capsule body. To date there are no published studies with the InteliSite® companion device. The adaptations have been made with delivery of solids to the colon in mind, the cage design allows greater access of the drug or dosage form to the limited fluid in the region and the spring activation ensures complete separation of body and cage *in-vivo*.

4.5 Aims

The primary aim is to evaluate the suitability of a novel radiotelemetric device to deliver powders to the distal GI tract. Secondly it is to conduct a novel proof of principle investigation into the *in-vivo* mucoadhesive abilities of carbomer polymers within the colon.

Therefore the objectives are as follows

- Administration of two carbomer polymers (CP 980 and PC-AA-1) to the dog colon via the InteliSite® companion device
- To measure transit of the device *in-vivo* and assess its ability to release its contents in the dog colon
- Measurement of transit times of carbomer powders through the dog colon and comparison with an ethylcellulose control

4.6 Materials

Carbopol 980 and polycarbophil AA-1 were received as gifts from Noveon, France. Ethylcellulose was purchased from Mendall (Patterson NY U.S.A). ^{111}Indium was received from Photon imaging (NC, U.S.A.) in the form of indium chloride. The InteliSite® companion device was supplied by GlaxoSmithKline, (Moore Drive, NC, US.)

4.7 Methods

4.7.1 Preparation of ^{111} Indium radiolabel

An appropriate amount of ^{111} Indium chloride (equivalent to 75 μCi) liquid was added to 100mg of ethylcellulose powder in a glass vial. A heat gun was used to heat the mixture until all the liquid radiolabel had adsorbed onto the ethylcellulose. The dry powder was mixed by hand and the radioactivity measured and recorded.

4.7.2 Capsule preparation

The radiolabeled ethylcellulose (10 mg) was mixed with 150mg of the powders under test by hand for approximately 5 minutes. The powders were then transferred by hand to the InteliSite® companion capsule. The capsule was sealed and the amount of radiolabel determined using a dose calibrator. Approximately 2-4 mm of head space was left in the cage of the capsule after filling with carbomer powders, the small particle size of the ethylcellulose meant that more head space was left after filling for the control.

4.7.3 Study protocol

4.7.3.1 Animal diet/housing and water

Three male Beagle dogs aged 3-5 years, weighing 8-9 kg were used in the study. Prior to each study day the dogs were fasted for approximately 18 hours. Water was made available *ad libitum* through the study days and normal rations (200g PMI Canine 5006 Lab diet) were given to the dogs 4-6 hours after gastric emptying of the capsule had been observed. The dogs were housed in the imaging lab during the study days and overnight while they were radioactive. Once all radioactivity had been passed in the faeces the dogs were returned to the animal housing facility.

4.7.3.2 Dose administration and imaging

Ethical approval for the study was granted by the Institutional Animal Care and Use Committee and approval for the use of nuclear imaging was granted by GSK Radiation Safety Committee. The study was conducted as a randomised three-way cross over (Table 4.2). Each dog received two carbomer polymers and a control treatment, a total of 9 dosage arms, using the InteliSite® companion device.

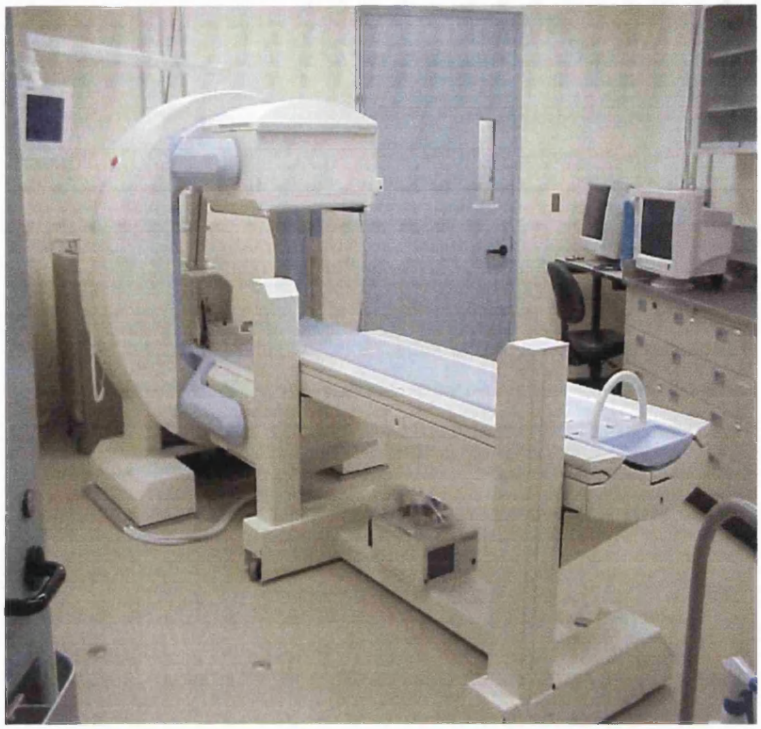
- Leg A = CP 980
- Leg B = PC AA-1
- Leg C = Ethylcellulose

Table 4.2 Overview of study design. A) CP 980 B) PC AA-1 and C) Ethylcellulose (negative control)

	Dosing Occasion 1	**Dosing Occasion 2**	**Dosing Occasion 3**
Dog 1	A	B	C
Dog 2	B	C	A
Dog 3	C	A	B

The animals were dosed orally (one capsule per dosing occasion), followed by 10 ml of sterile water given via syringe. Immediately after dosing each dog was placed under the gamma camera to await confirmation of the capsule's arrival at the stomach. Imaging was conducted using a Siemens eCam fixed 180° dual-head gamma camera. The camera was fitted with medium energy parallel-hole collimators and set to detect ^{111}Indium. Prior to dosing the left shoulder of each dog was shaved to affix a radiolabeled external body marker (fiducial) which allows the dog to be correctly re-aligned in the same position under the camera after rest breaks.

Imaging was continued until gastric emptying was observed. Further imaging was conducted approximately every 10 – 15 minutes (dependent on rest needs of the animal) until the InteliSite® companion device reached the caecum. Activation of the capsule was achieved remotely and imaging was continuous until activation could be confirmed. Imaging of the powders in the caecum, ascending colon and transverse colon was again continuous but allowed for one 10 minute break per hour of imaging for the dog's comfort. Due to the long transit times involved with the study, imaging of the descending colon was often performed late at night, as such imaging was less frequent at approximately 30 minute to 1hour intervals. Imaging was concluded when 100% of the activity was seen in the descending colon.



a

Figure 4.6 Siemens eCam dual-head gamma camera

4.7.4 Image analysis

Analysis was conducted using ScinWin^{TM} image analysis software to convert the scintigraphic image values, based on regions of interest (ROI) parameters, to numerical values. Prior to this the images were corrected for movement. The regions of interest were the caecum, ascending colon, transverse colon and descending colon. These values were corrected for isotope decay and background. Total mean colonic transit time, transit time in each ROI, RT_{50} (Retention Time of 50% or more of the powder) and RT_{10} (Retention time of 10% or more of the powder) were calculated. The data was analysed using non-parametric tests; results were analysed for significant differences using Kruskall-Wallis, with post-hoc analysis using Nemenyi's test. Kruskall-Wallis was carried out using SPSS Version 14.0 statistical software package, significance was stated where $p<0.05$.

4.7.5 Spread analysis

Using the ScinWin™ image analysis software the original ROIs for the ascending and transverse colons were re-analysed, sub dividing each region into three subsections. As before the values were corrected for decay and background and the relative amount of activity with respect to the total activity in each ROI was calculated for each sub section to determine the spread of the powder throughout the ROI.

4.8 Results and Discussion

The device was tracked through the canine GI tract using scintigraphy until the point of activation in the caecum, at this point only the powders could be detected. Transit times of the capsule through different regions of the GI tract were determined, gastric emptying (GE), small intestinal transit time (SITT), caecum arrival time (CAT), colon retention time (CRT) and the excretion time for 9 InteliSite® companion device capsules were calculated (Table 4.3). It should be noted that two capsules failed to operate as intended with one activating during handling, prior to administration, and the second failing to activate at all *in-vivo* (results excluded from data set). Activation of the device prior to administration to the animal is undoubtedly a manufacturing error with this particular capsule, the most likely cause being the temperature at which the SMA wires were pre-conditioned to straighten. Ideally this should be 40-43°C. Failure *in-vivo* has been seen previously with the InteliSite® companion devices predecessor the InteliSite® capsule (Clear *et al.*, 2001). Distance of the antenna and the device is important; the operating range of the antenna is 20 cm. It is likely that this failure was caused by the antenna signal not reaching the device, either due to distance between the two or occlusion from body tissue. The two failed activations were replaced with two new devices so a full data set could be obtained.

Table 4.3 GI transit times for the InteliSite® companion device in the dog

	Dog 1	Dog 2	Dog 3	Range	Mean ± S.D.
GE	1.82	1.36	0.30		
(Hours)	0.10	2.38	0.10	0.10-2.38	0.98 ± 0.75
	1.10	0.57	1.10		
SITT	1.07	1.95	2.27		
(Hours)	1.17	0.93	0.55	0.55-2.27	1.25 ±0.62
	2.02	0.77	0.57		
CAT	2.88	3.32	2.57		
(Hours)	1.27	3.32	0.65	0.65-3.32	2.24 ± 0.96
	3.12	1.33	1.67		
CRT	20.85	13.50	10.02		
(Hours)	23.63	15.10	24.31	10.02–30.81	20.38 ± 5.46
	20.02	15.00	30.81		
Excretion	23.73	16.83	23.58		
(Hours)	24.90	18.46	24.97	16.83-32.48	22.63 ± 5.00
	23.32	17.21	32.48		

Immediately post administration of the device the dogs were placed under the camera to confirm arrival of the dosage from in the stomach, in all cases this was a matter of a few seconds. Imaging was performed continuously until gastric emptying was observed, this was easily defined as the capsule moved up and to the left, immediately proceeding this the hot spots would drop lower than its pervious position in the stomach and a period of activity was seen (Figure 4.7). SITT could be calculated by subtracting the time interval between the device leaving the stomach and its arrival at the caecum. CAT was confirmed by exploiting the canine anatomy, since the dog caecum is out of line with the direction of intestinal movement a pause in transit is seen on arrival. CRT was determined as the difference between the CAT and the excretion time, which in turn was taken as the time interval between administration of the device and retrieval in the dog faeces.

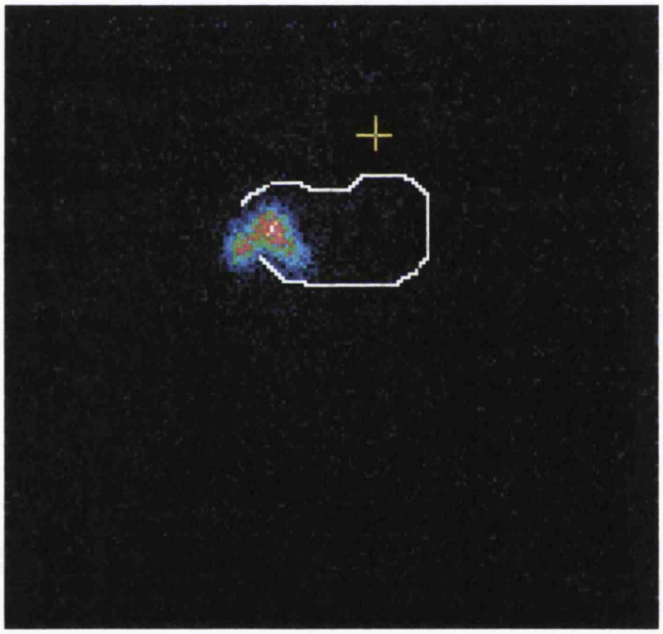


Figure 4.7 GE of the InteliSite® companion device dog 1. The stomach ROI has been added for clarity

As stated previously there is currently no published work regarding the use of the InteliSite® companion device, therefore comparisons will be made with respect to the original InteliSite® capsule. Gastric emptying recorded in this study is faster than seen for the original InteliSite® capsule where GE times of 3.17 hours and 3.62 hours in beagle dogs were recorded (Parr et al., 1999). The reason for these differences are unclear, however, factors affecting gastric emptying are known to be numerous. The

size of the dosage form will have an effect and the suggested minimum cut off size for GE in dogs is 2-7mm. Objects larger than this need to wait for the MMC before being emptied from the stomach. Indeed retention of the InteliSite® capsule in the human stomach has been shown to rely on the MMC to promote emptying (Pithavala et al., 1998). In the fasted state the pattern of motility is divided into three stages. The first is a stage of general inactivity, in the second phase, irregular contractions occur which increase in amplitude and frequency, when these contractions reach their peak the stomach empties, this is known as phase 3. The frequency of phase 3 activity is 113 ± 11 min in dogs (Dressman et al., 1986). The faster GE seen in this study could simply could be a result of "lucky timing" and administration occurring just prior to phase 3 motility. The orientation of the device to the pylorus, will also have an effect on emptying. If orientated in line with the pylorus then emptying is likely to be faster, consequently if originated perpendicular the reverse is likely to be the case.

The mean small intestine transit time of the companion device (75 ± 37 min) is however more consistent with those found in previous studies in dogs. Parr *et al.*, (1999) recorded a time for the original InteliSite® capsule of 98 min (n=1). Both values are consistent with the previously reported value of small intestine transit times in dogs of 111 ± 17 (Dressman, 1986). Small intestinal transit is known to be less variable and less affected by fed state or type of dosage form. In man, times between 3-4 hours are regularly quoted (Davis et al., 1986), although as previously mentioned the shortest and longest times noted by this study are highly variable. However, these values have been confirmed by other studies where times ranging from, 196 to 238 min (value given as median value from data set), were recorded (Graff et al., 2000). Small intestinal transit times for InteliSite® device in man are also consistent with these figures at 258 ± 84 and 312 ± 90 minutes (Clear et al., 2001).

The point of activation within the current study was the caecum and arrival could be determined by a period of relatively little movement. As mentioned earlier the caecum in the dog is not in line with the passage of transit, therefore upon arrival the device will undergo a period of stillness, once this was confirmed (by continuous imaging) the device was activated. Determination of release of the contents proved to be problematic as burst release and dispersion throughout the region was not observed. Release of the powders could only be confirmed 5-10 minutes after the activation attempt (Figure

4.8a). Movement of the powders from the caecal region to the ascending colon was however much easier to determine, observers would notice the hot spot move to the left (of screen) and up as the powder moved into the ascending colon (Figure 4.8b). The limited dispersion of the powder observed upon activation was initially a cause for concern; subsequently the activity remaining in the capsule and body was measured after defecation. The initial activity of the capsule had been recorded and was corrected for decay, the amount of powder released from the capsule as a percentage of the total administered was determined (Table 4.4).

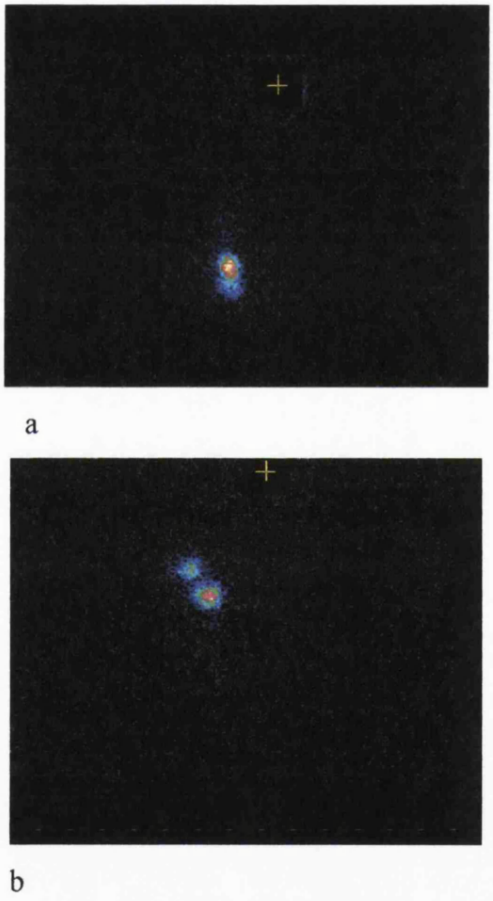


Figure 4.8 a) Release of powder from the InteliSite® Companion device 5 minutes after activation, b) Movement of the powders from caecum to ascending colon.

Table 4.4 Percentage of powder remaining in each capsule after successful activation and retrieval in faeces. N/D =Value not determined..

	Percentage of powder remaining in capsule after retrieval								
	Dog 1			Dog 2			Dog 3		
	EC	980	AA1	EC	980	AA1	EC	980	AA1
Cage	30.81	N/D	52.71	19.84	9.66	60.51	39.08	53.78	60.88
Body	4.4	N/D	6.2	9.92	4.83	3.02	3.31	2.2	3.04
Total	35.21	N/D	58.91	29.76	14.49	63.53	42.39	55.98	63.92

On all occasions upon recovery of the capsule in faeces the majority of powder remaining within was located in the cage and the cage and body were seen to be completely separated. Even though the open-faced design of the cage was not anticipated to be restrictive to polymer release, it appeared to be acting as a framework around which gelation of the polymers could occur. Ethylcellulose showed the greatest release from the device with 37.17 ± 4.57% of the powder remaining in the capsule on retrieval. Release of the carbomer powders however was more problematic, with the retention of CP 980 in the capsule being highly variable (35.14 ± 29.47%) and PC AA-1 generally high (62.12 ± 2.77%). The hydrophilic nature of the carbomer polymers can help to explain their poor release from the cage, as gelation of the polymers would have occurred upon contact with fluid or mucin. The difference in release of the polymers may be attributed to their molecular weight and the degree of crosslinking; polycarbophil AA-1 is less crosslinked and has a lower molecular weight than Carbopol 980 and as such can undergo greater hydration (Ch'ng et al., 1985). This hydration and swelling could occur within the inner chamber before the polymer can be released properly, and could contribute to the relatively poor release of polycarbophil AA-1 from the InteliSite® Companion device. Carbopol 980 also undergoes a degree of swelling, albeit to a lesser extent, and this may also contribute to its poor release.

This would not however account for the poor release of ethylcellulose, the hydrophobic nature of this polymer means that it is not expected to hydrate. It is likely that observed release is a consequence of the colonic environment, by nature of its function the contents of the colon are tightly packed and viscous and lack agitation. Studies in humans using the original InteliSite® capsule saw slow release of a radiolabeled liquid

when capsule activation occurred in the colon (Parr *et al.*, 1999) supporting the suggestion that the colonic environment is the limiting factor in release.

Powder release from the InteliSite® companion device is disappointing, as an engineered device one would expect to see close to 100% release from the capsule as there is no reliance on formulation aspects to deliver the powder. Additionally companion device is the next generation of the system and has been designed with improved release in the distal GI tract in mind. The poor dispersion of the powders was not improved as the powders moved through the colon. In most cases only one or two main hot spots were observed for the control and carbomers alike, however small regions of less intense radioactivity were seen. It was concluded that these regions were the result of deposits of the powder being left behind by the main bulk as it moved through the colon, an illustrative example is given in Figure 4.9.

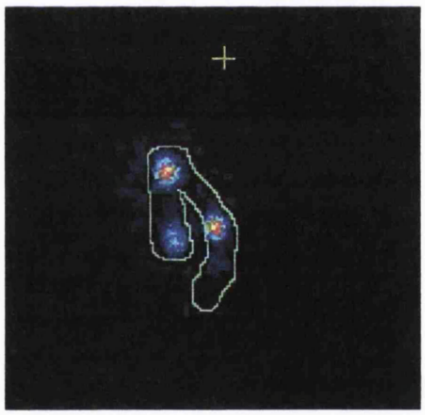
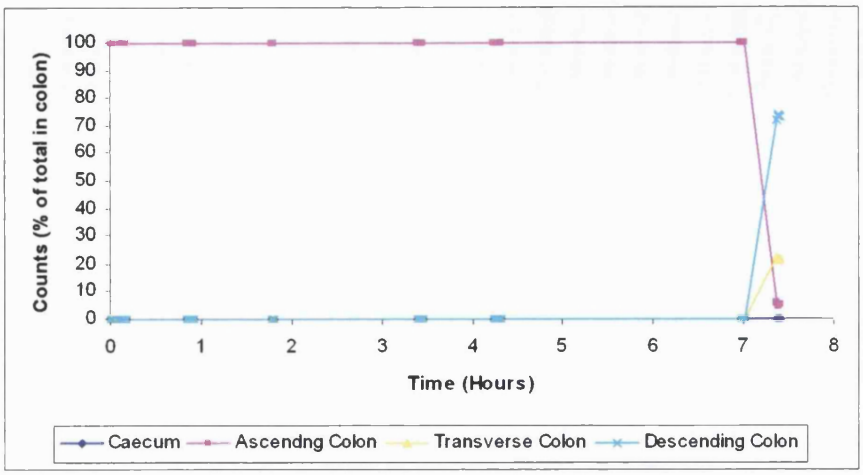


Figure 4.9 Scintigraphy images of CP980 3 hot spots in ascending, transverse, and descending colon

One immediate limitation of the InteliSite® companion device is the removal of head space for a second radioactive marker which was present in the original capsule. As such, once activated the position of the cage and body can not be ascertained. The region of least activity as seen in Figure 4.9 in the proximal ascending colon is likely to be free powder. The two remaining hotspots then are either the cage and body or the cage and a second amount of free powder which may or may not contain the body of the device.

Using the ScinWin^{TM} software the radioactive counts in the four ROI were determined and corrected for background and decay. As mentioned previously, identification of the ROI is partially subjective as the gamma camera provides no anatomical information, however determination of the each ROI can be achieved with an understanding of dog anatomy. As explained earlier the caecum was identified by a period of little activity especially when compared with the movement seen prior in the small intestine. The powders were deemed to be in the ascending colon from leaving the caecum (hot spot moved up and to the left of screen) until the a change in movement of the hot spot from an upwards direction to a diagonal downward direction was seen, at this point the hot spot were determined to be in the transverse colon. The end of the transverse colon was also identified by a change in the direction of movement, as the capsule enters the descending colon movement on the screen is seen to be from top to bottom, no longer diagonally.

An indication of the dispersion of the powders in the colon can be obtained by looking at the ratio of active counts over time in each ROI (4.10-4.12). If good dispersion is achieved a gradual decrease in counts in one region would accompany a gradual increase in counts in the next. In fact this is not seen and in most cases counts abruptly stop in one region and commence in the next.



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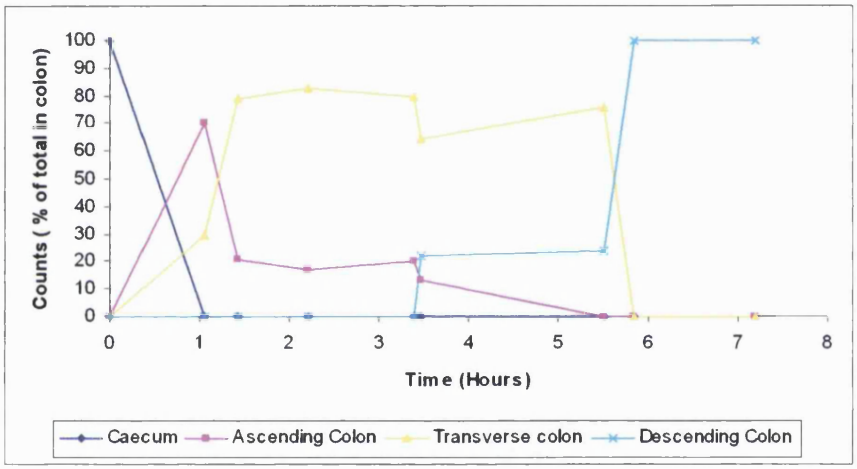

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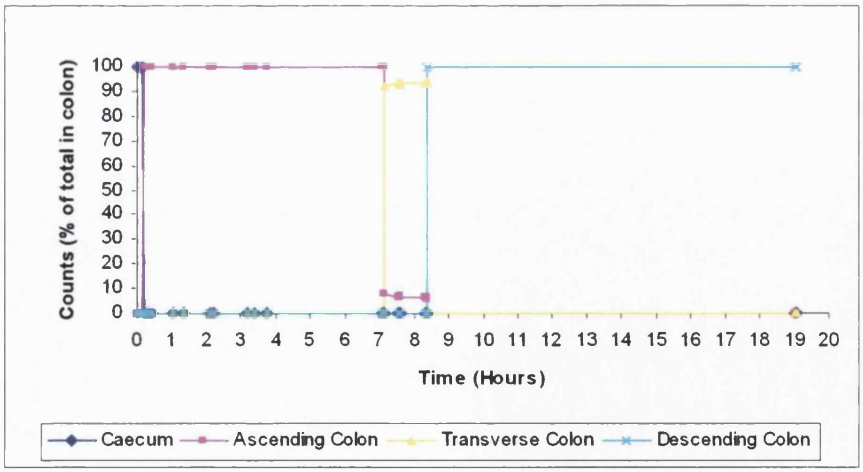
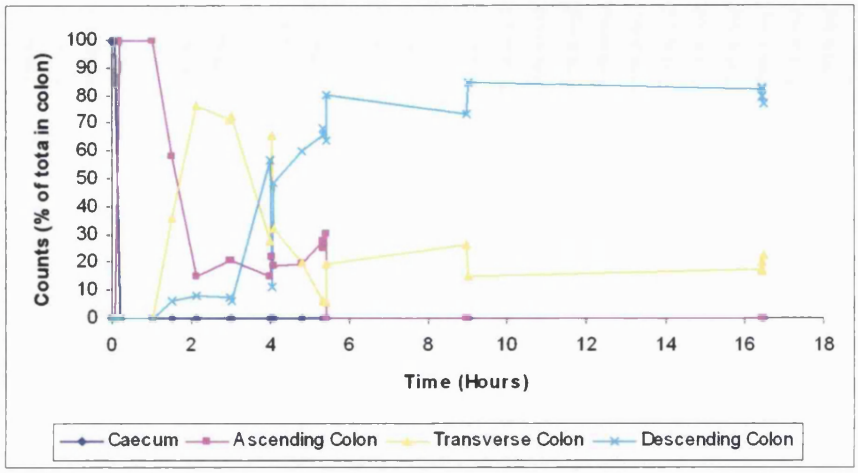


c)

Figure 4.10 Movement of ethylcellulose through the ROI a) dog 1 b) dog 2 c) dog 3



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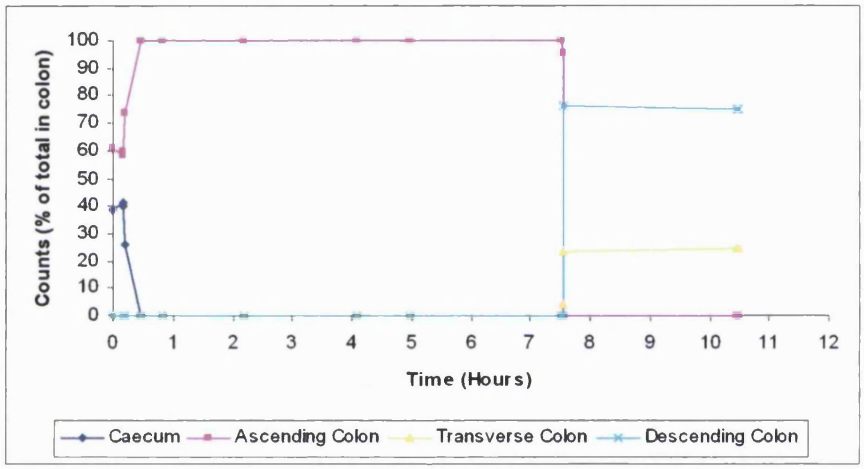

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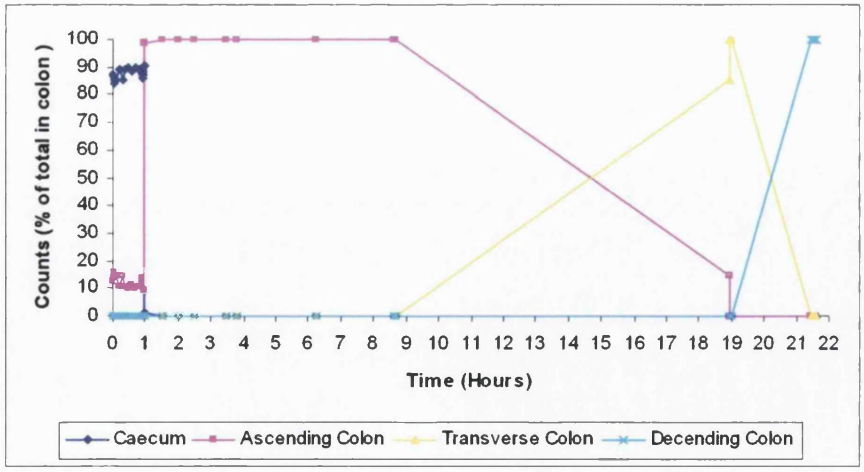
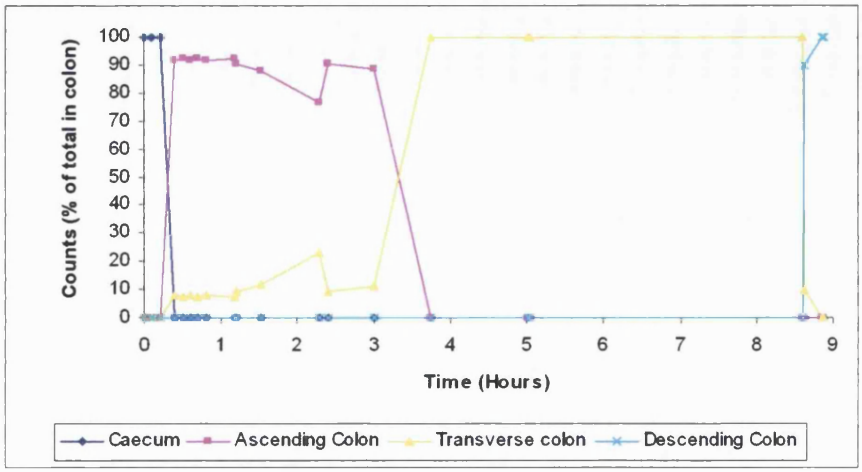
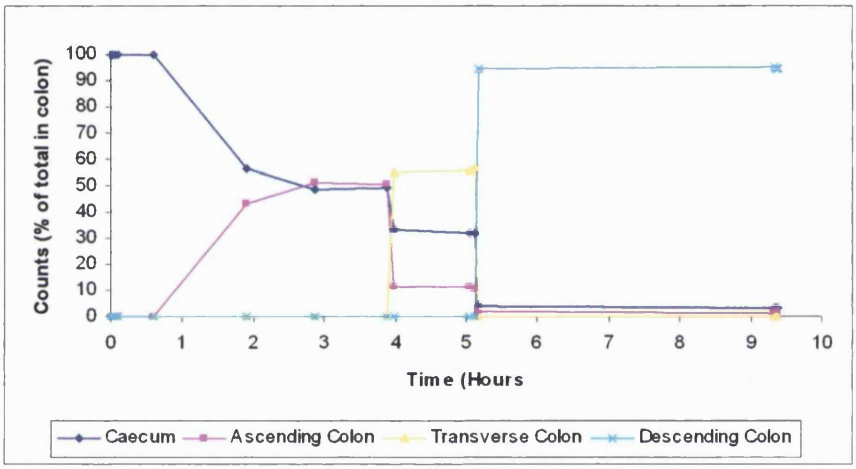


c)

Figure 4.11 Movement of CP 980 through the ROI a) dog 1 b) dog 2 c) dog 3



a)



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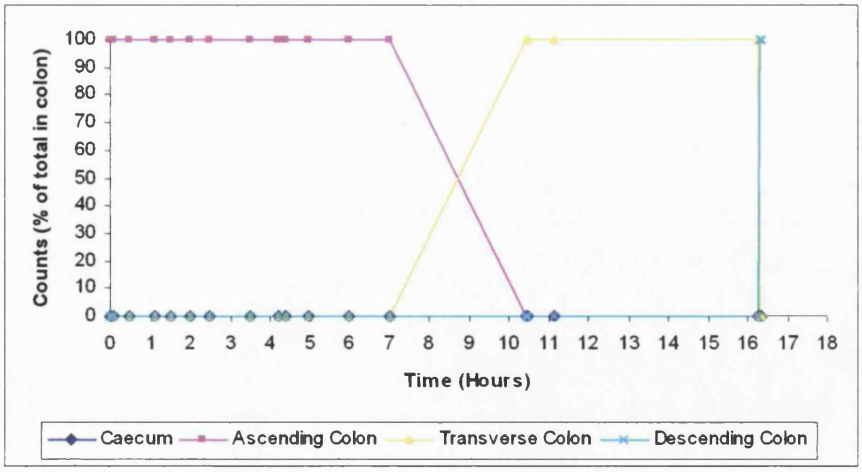


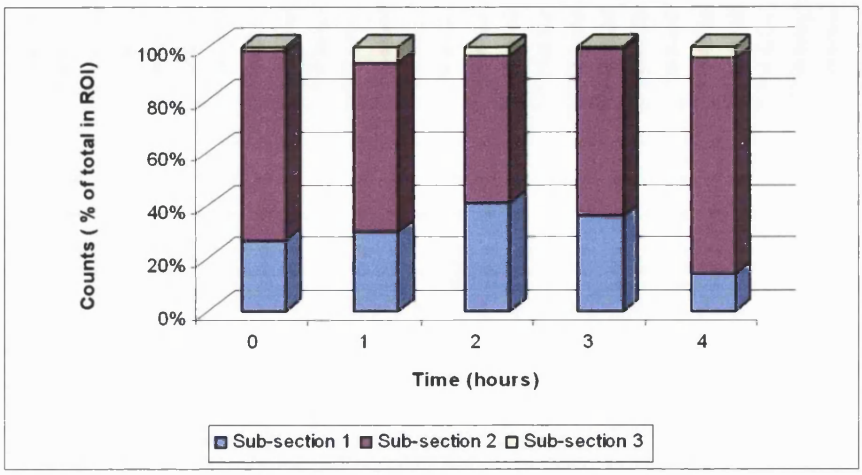
c)

Figure 4.12 Movement of PC AA-1 through the ROI a) dog 1 b) dog 2 c) dog 3

Due to the poor dispersion *in-vivo*, movement of the powders more closely resembled a monolithic system than a multiparticlulate additionally, movement and dispersion of the powders in the colon is no better for the control powder (ethylcellulose) than the carbomer polymers. In general, for all three polymers movement was for the most part as a single unit although some variation was seen to occur depending on the dog used with dog 3 consistently showing the least dispersion. It should be noted that the process of capsule activation in some instances caused powder to move from the caecum to the ascending colon resulting in activity counts in this region at time zero (Figures 4.11b, 4.11c). Also, some powders were present in the transverse colon at the point of defecation but were excreted at this time.

There is no information within current literature regarding the dispersion of powder, particularly those with mucoadhesive properties, in the distal GI tract. Dispersion of PC AA-1 solutions in the stomach have been investigated, although when compared to other polymers in the test (cholestyramine in particular) PC AA-1 expressed less dispersion, it was observed across the *Fundus*, *Body* and *Antrum* of the stomach, suggesting that a carbomer solution, at least, will disperse (Jackson et al., 2001). In light of the lack of data in the current literature it was decided to investigate the spread of the powders *in-vivo*.

Using the ScinWin^{TM} analysis software, the ascending and transverse colons were re-analysised, the ROI were sub divided into three smaller, equal regions. The caecum and descending colon were omitted. The caecum was excluded since the capsule was activated only after it had resided in this region for some time (on some imaging occasions up to an hour) so a true value of residence time of the powders could not be obtained. The descending colon was also omitted here as within this region any spreading that had been seen was observed to diminish as the hot spots collected in the distal descending colon to await a bowel movement additionally, the assumption was made that if spreading of the polymers were to occur it would have done so prior to entering the descending colon. The spread in the ascending colon can be seen in Figures 4.13-4.15.



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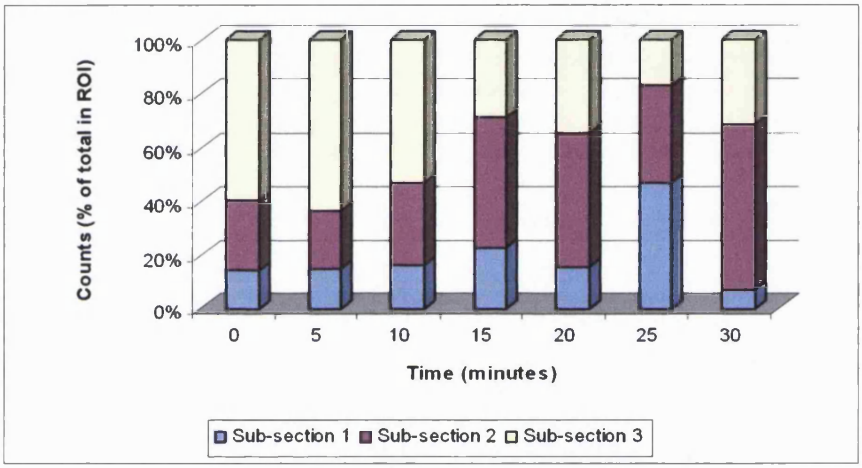

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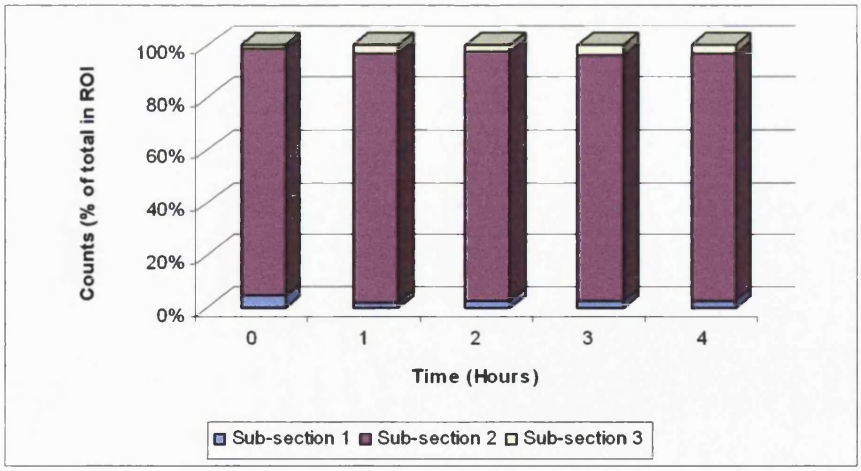
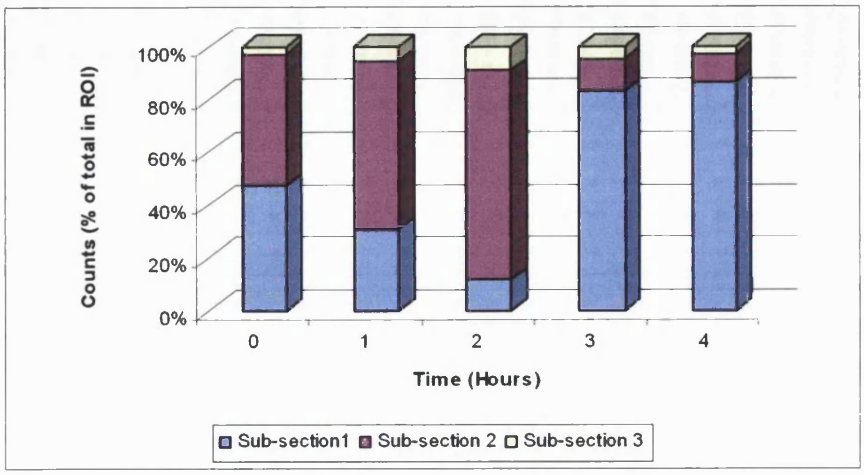


c)

Figure 4.13 Spread of Ethylcellulose in the ascending colon of the dog a) dog 1, b) dog 2, c) dog 3



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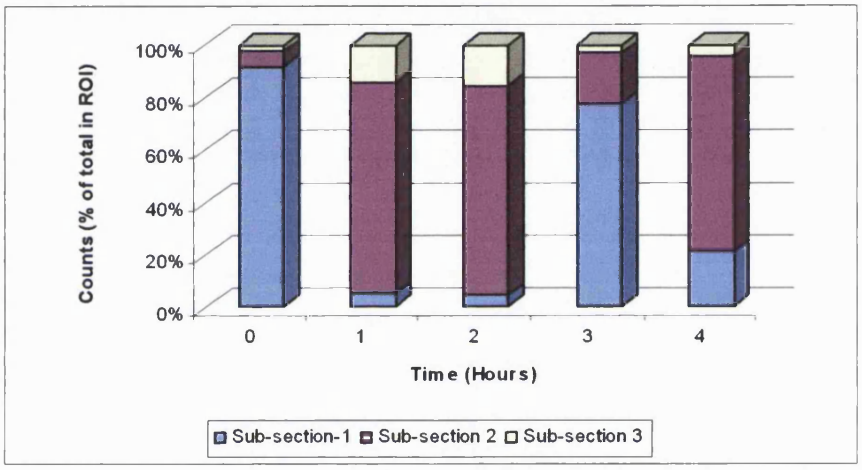

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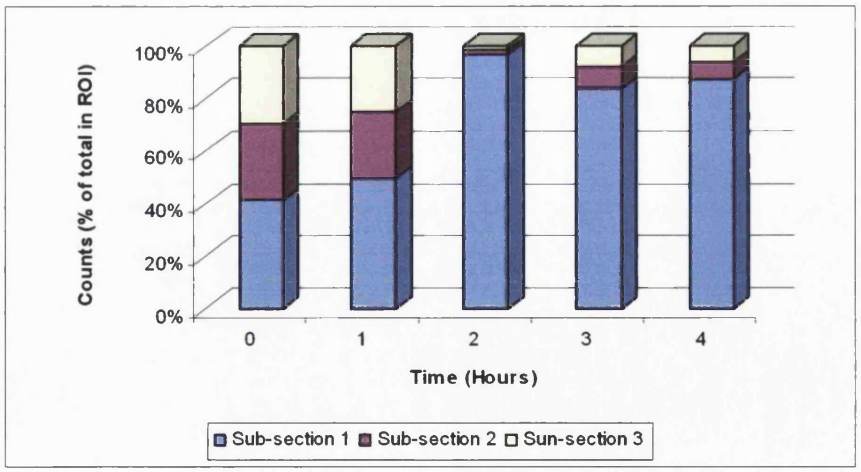
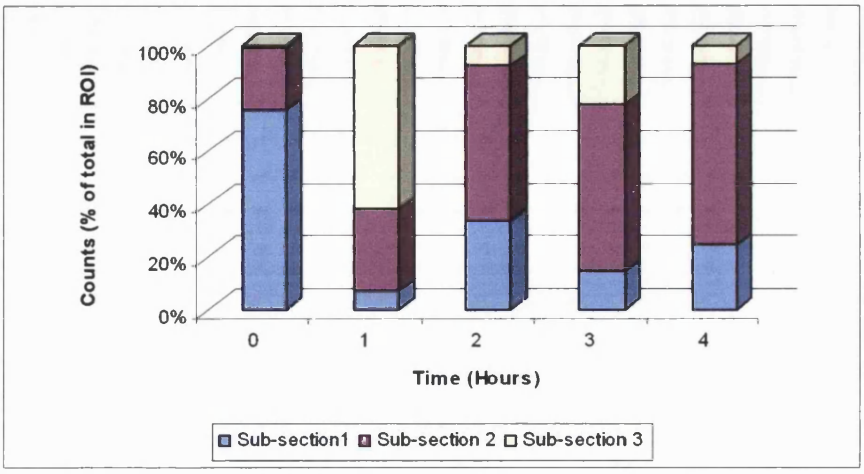
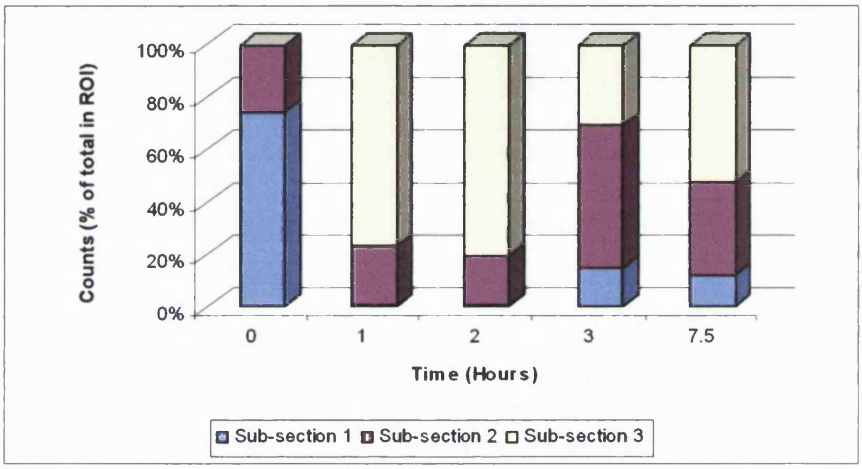


c)

Figure 4.14 Spread of CP 980 in the ascending colon of the dog a) dog 1, b) dog 2, c) dog 3



a)



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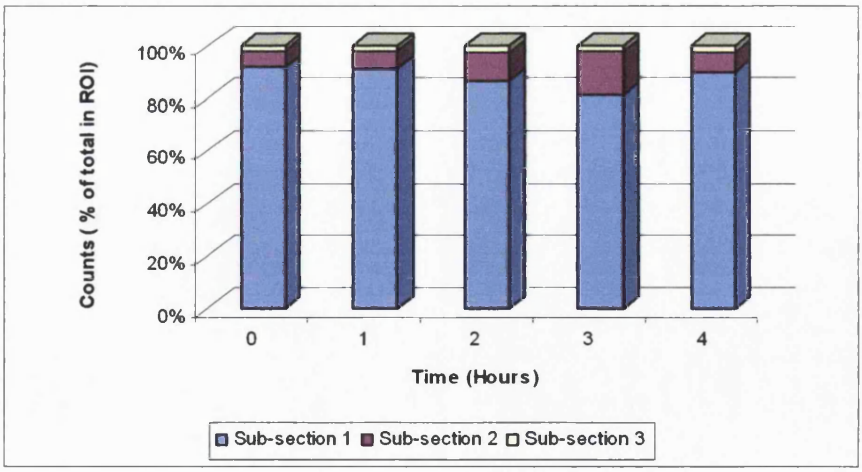


c)

Figure 4.15 Spread of PC AA-1 in the ascending colon of the dog a) dog 1, b) dog 2, c) dog 3

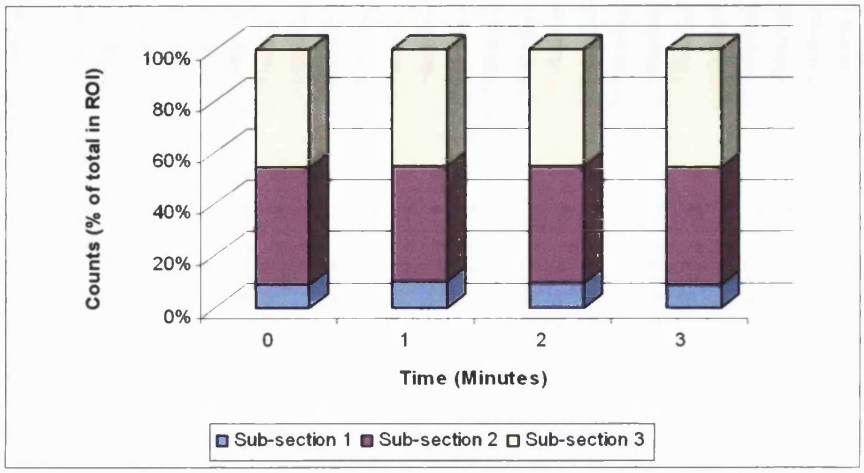
The spread of the powders was calculated in the ascending colon for the first 4 hours (where available), for the three powders. Time 0 is the spread of the powder upon entering the region. Counts are relative to the total counts in the ascending colon at the time of sampling, therefore from these graphs no conclusions can be drawn regarding the amount of polymer in each subsection.

After activation in the caecum the movement and spread of the three powders within the ascending colon is limited, if an even distribution of the powder within each ROI had occurred we would expect to see an equal amount across the 3 sub-sections, this is not the case. From Figures 4.13 to 4.15 we can see that the three powders behave differently from each other and are highly variable. Ethylcellulose in 2 out of 3 of the dogs (dog 1 and dog 3) moves rapidly into sub-section 2 of the ascending colon and the majority of the powder resides here for the first 4 hours. An exception to this is seen in dog 2 which shows the best example of dispersion, however the residence time here is much shorter than the other dogs. This suggests a couple of possibilities, either, in this instance the spreading of the powder facilitated rapid movement through the region, or that as matter of physiological variability the contents of the colon in this animal were not as packed as dog 1 and dog 3 so the powder was allowed to disperse and move through rapidly. An additional important note here is that the CRT and the excretion time were shorter for this dog during this leg of the study than any of the others.

Unlike ethylcellulose, CP 980 powder concentrated itself for, the most part, in the first sub-section. Dog 3 show the best example of this (Figure 4.14c). Here we see at time 0 the powder is spread across all parts of the ascending colon, after 2 hours, there is an increase in the amount residing in section 1, which is caused by more powder entering the region from the caecum. Greater spread is seen in dogs 1 and 2 and is limited mostly to sub-sections 1 and 2. For PC AA-1 the picture is not as clear. In dog 1 we observed that the powders were spread across the ascending colon (Figure 4.15a), after 2 hours the majority was in sub-section 2 however, approximately 20-40% were still seen in both sub-section 1 and 3. In dog 2 we see a similar pattern, however most powder is now in the more mid and distal parts of the ascending colon (sub-sections 2 and 3). Finally for dog 3, 80-90% of the powder within the ascending colon spends the first 4 hours in the most proximal section (sub-section 1).

Within the transverse colon residence times varied greatly, additionally due to practical considerations (images were needed to be taken over night) imaging was not performed at the same time points for all dogs. Figure 4.15 to 4.18 show the spread of the powder in the transverse colon, as mentioned it was not possible to consistently image at the same time intervals, therefore the times chosen were those that best represent the spread.

Dog 1 exhibits the best spread for ethylcellulose, however once again the residence time is short, only 3 minutes in this case. For dogs 2 and 3 the residence time was longer, 30 and 50 minutes respectively, but the distribution was reduced and concentrated in sub-section 1 and 2 respectively. For the CP 980 leg we see similar distribution in dog 2 as we saw in dog 1 for the ethylcellulose, the powder is evenly dispersed but the residence time is very short. In dog 1 and 3 the spread is concentrated more in the mid and distal sub sections (1 and 3), in dog 3 it is concentrated in the latter. Finally for the PC AA-1 leg we observe the majority of the powder to be held in the proximal section (sub-section 1) of the transverse colon in all three dogs, also the residence time in dog 3 is significantly greater than that seen of any other legs (10 hours).



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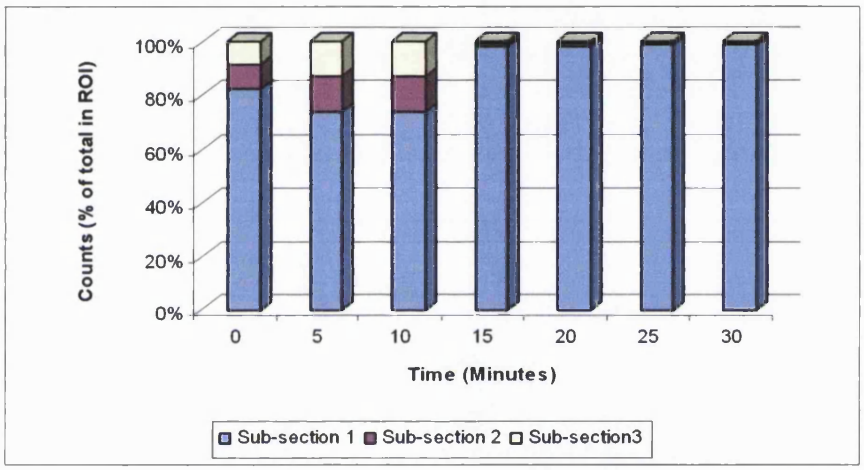

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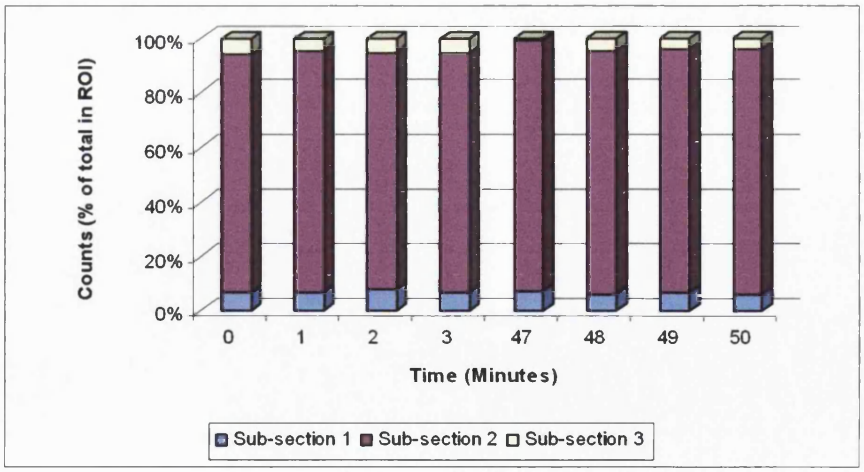

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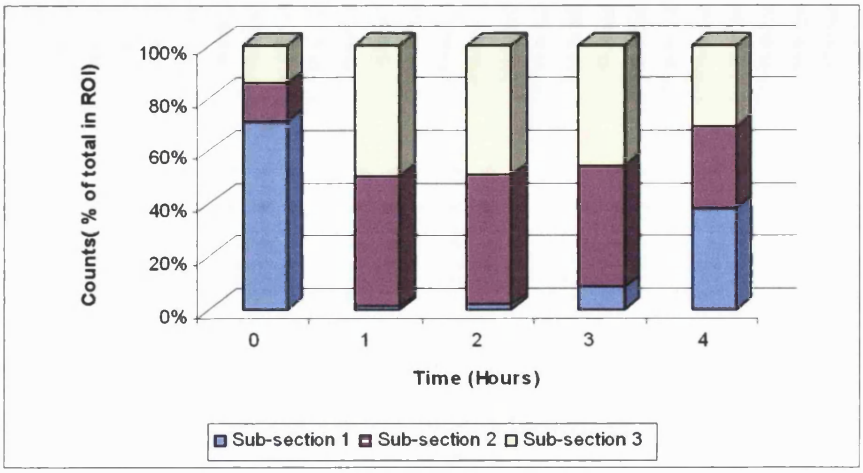
Figure 4.16 Spread of ethylcellulose in the transverse colon a) dog 1, b) dog 2, c) dog 3



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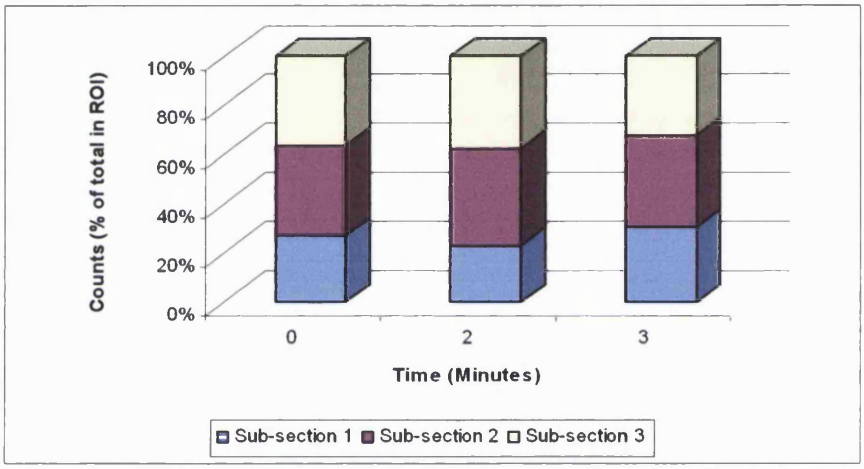

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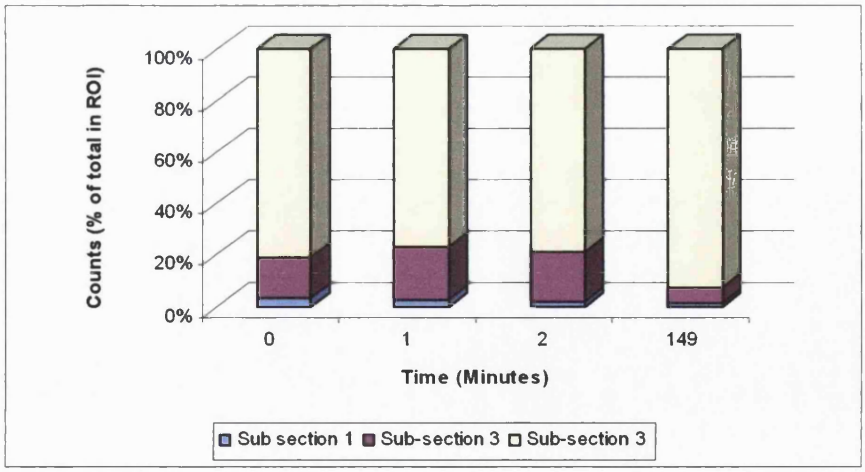
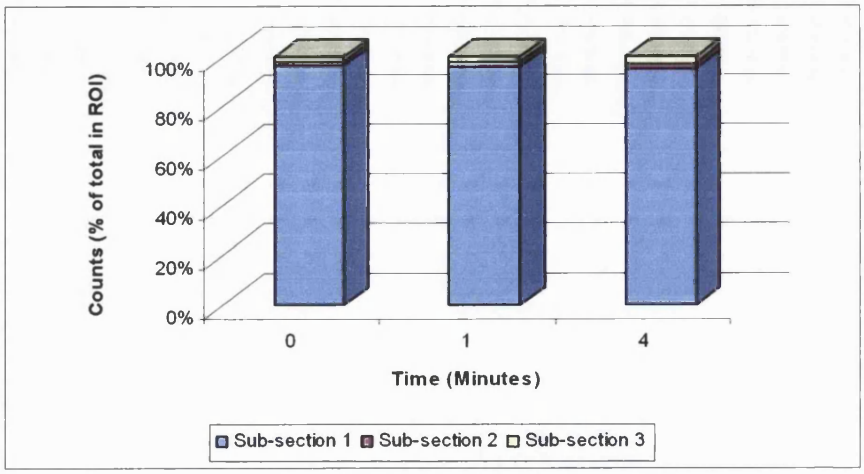


c)

Figure 4.17 Spread of CP 980 in the transverse colon a) dog 1, b) dog 2, c) dog 3



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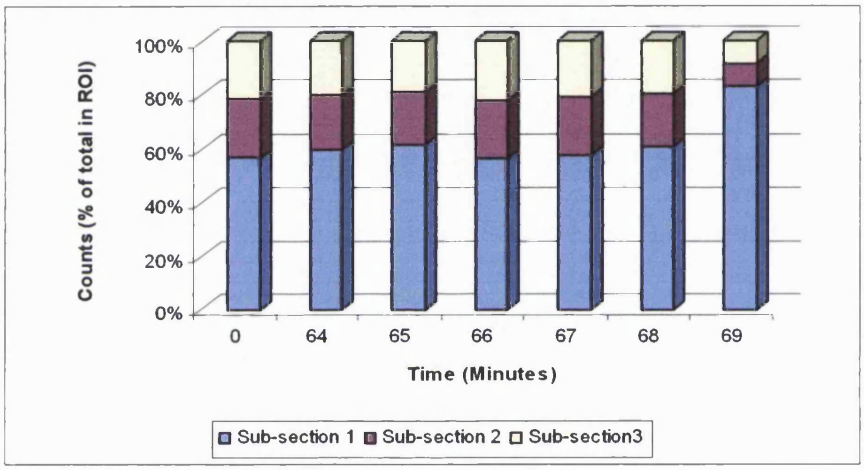

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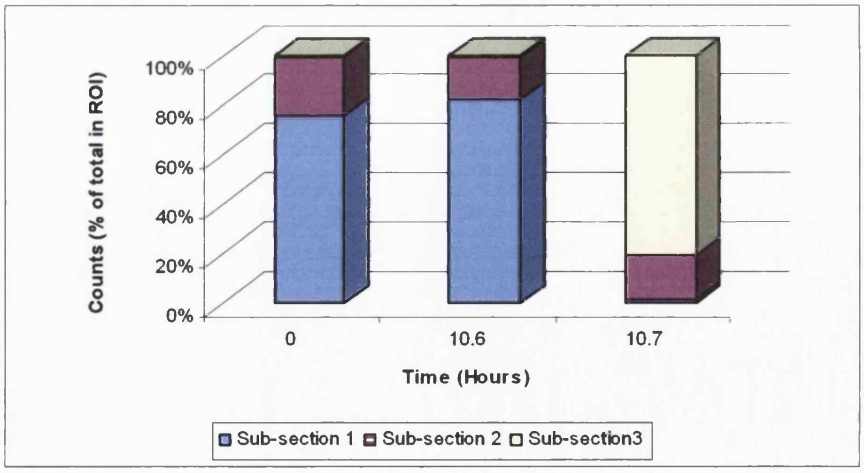


c)

Figure 4.18 Spread of PC AA-1 in the transverse colon a) dog 1, b) dog 2, c) dog 3

Lack of spread can potentially be damaging to any mucoadhesive system, particularly one charged with prolonging retention in the distal GI tract. Limited dispersion decreases the likelihood of contact between the mucoadhesive and the colonic mucosa, increasing the chances of an all or nothing effect and the dosage form being excreted without adhesion. Release of the powders from the device was seen to be a problem; however, upon retrieval the vast majority of the polymer remaining in the device was located in and around the cage. This is not ideal, however, it does not mean that the polymers would not be unable to act were they to come into contact with the colonic mucosa. The cage of the device is acting as a frame work around which the carbomers gel, as it passes through the colon small amounts of polymer are deposited and retained in each region. As such, the mucoadhesive potential of the carbomers can still be assessed.

To adequately determine transit times three values were investigated, the retention time of 50% or more of the polymer (RT_{50}), retention time of 10% or more of the polymer (RT_{10}), and total transit time. RT_{50} and RT_{10} values and total transit times were calculated for both the ascending and transverse colons separately. Additionally the total transit time from the caecum (post capsule activation) to the descending colon (time taken for 100% of counts to reside in the descending colon) was determined.

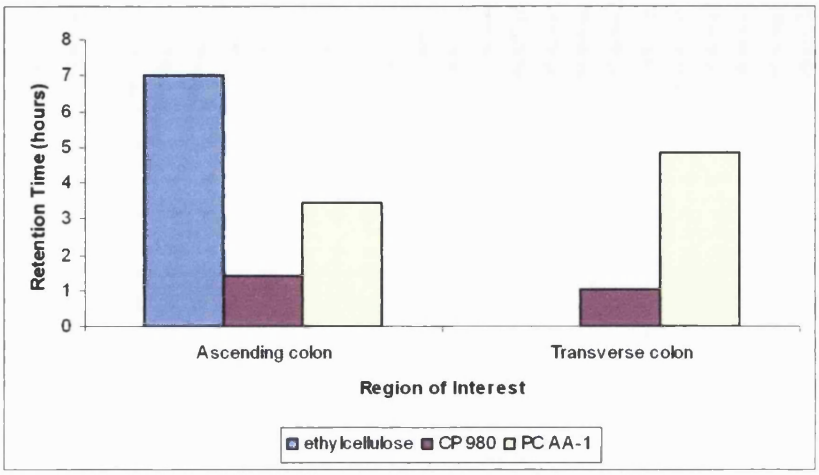
The mean total transit times in the ascending and transverse colon (Table 4.5) show no statistical differences (95% confidence) between either carbomer polymer and the control ($p<0.05$). However the small sample size and high degree of variation means that statistical determination is difficult, therefore the results were evaluated and will be discussed for each dog separately. In the ascending colon of Dog 1 neither carbomer polymer is seen to possess longer transit times than the control. However in the same ROI in dog 2 the transit time of CP 980 and PC AA-1 are more than double that of the control. In dog 3, in the ascending colon, we see that again CP 980 is retained, in this case the transit time is 10 hours greater than the control.

Table 4.5 Transit times of ethylcellulose and carbomer powders in the ascending and transverse colon of the dog

	Ascending colon transit time (hours)				Transverse colon transit time (hours)			
	Dog 1	**Dog 2**	**Dog 3**	**Mean ± S.D**	**Dog 1**	**Dog 2**	**Dog 3**	**Mean ± S.D**
Ethylcellulose	7.42	3.42	8.23	6.36 ± 2.57	0.05	5.50	1.23	2.26 ± 2.87
CP 980	5.20	7.55	18.97	10.57 ± 7.57	15.31	2.93	0.07	6.10 ± 8.10
PC AA-1	3.42	7.48	7.03	5.98 ± 2.23	8.41	1.15	5.81	5.12 ± 3.68

The transverse colon shows similar trends, in dog 1 both carbomers are retained, in dog 2 neither are retained and in dog 3 PC AA-1 exhibits a longer transit than the control. Interestingly, in looking at these results on a dog by dog basis we see that prolonged residence of both carbomer polymers is observed in all 3 dogs, if prolonged retention does not occur in the ascending colon we will see the carbomers retained in the transverse colon.

The RT_{50} and RT_{10} values also show trends. In dog 1 the retention time in the ascending colon for the carbomer polymers is less than the control at the 50% level, however, the transit of the ethylcellulose is particularly rapid in the transverse colon and as such both carbomer polymers remain in this ROI for longer with PC AA-1 possessing the greatest retention. In dog 2 (Figure 4.19b) prolonged residence is seen for both carbomer polymers at the 50% level in the ascending colon with CP 980 remaining in this region for the longest time. In the transverse colon however CP980 is not retained and PC AA-1 is only retained for approximately 1 hour. Finally dog 3, all three powders remain in the ascending colon for similar periods of time and all are greater than 6 hours. CP 980 is not retained in the transverse colon; however, PC AA-1 is and shows a retention time greater than the control. At the 10% level (Figure 4.20) a different picture emerges In dog 1 in the ascending colon no prolonged retention of either carbomer is seen when compared with ethylcellulose, however, both carbomer polymers are retained in the transverse colon with CP 980 possessing the greatest residence time. In dog 2 greater retention of CP 980 is seen in the ascending colon and no retention of the carbomers over the control is observed for the transverse colon. Finally at the 10% level in dog 3 CP 980 expresses greater retention in the ascending colon than the other two powders but is not retained in the transverse colon, PC AA-1 is retained here. Combining the RT_{50} and RT_{10} transit times it is possible to identify which ROI each polymer is distributed when prolonged residence is seen. Where longer transit times for CP 980 are observed it is due to 10% or more of the polymer residing in the region, this tended to be the ascending colon (2 in 3 dogs). PC AA-1 is seen to be retained in the transverse colon longer than the control and this retention is due to 50% or more of the polymer residing there (2 in 3 dogs).



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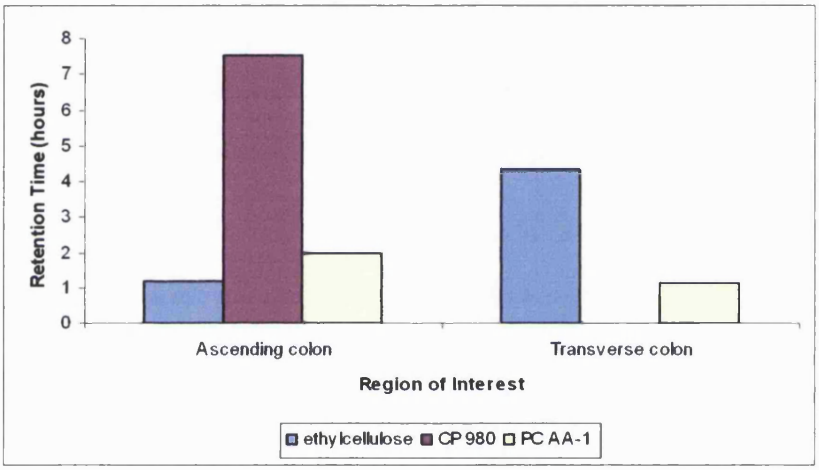

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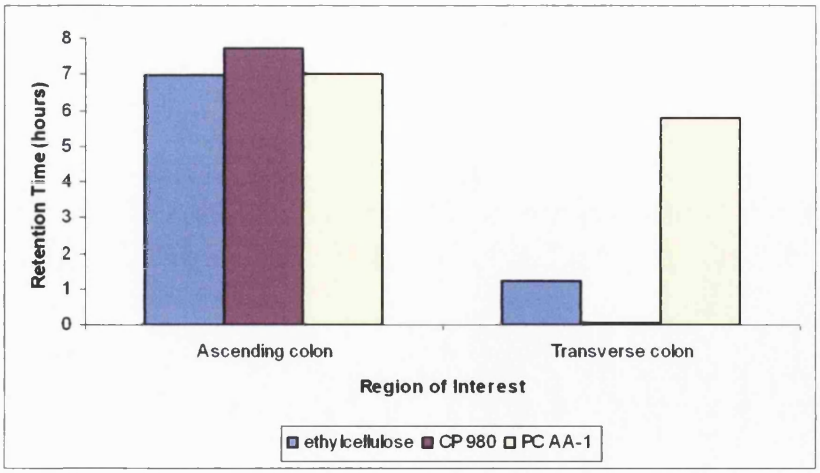
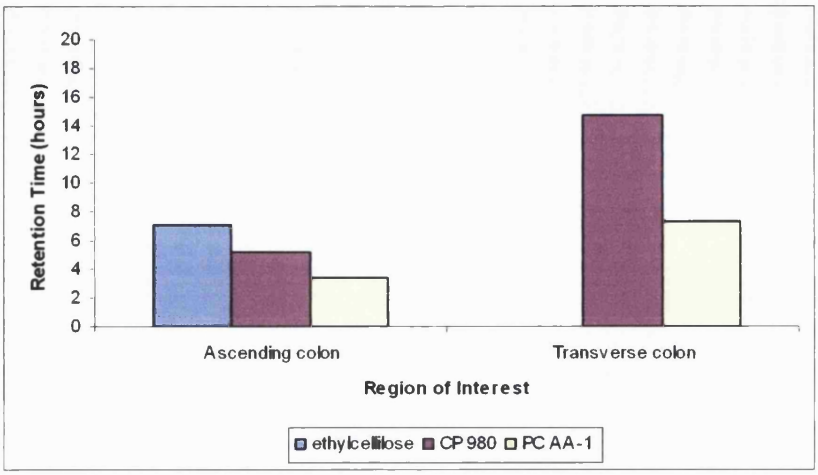


c

Figure 4.19 RT_{50} values in the ascending and transverse colons for a) dog 1, b) dog 2 and c) dog 3



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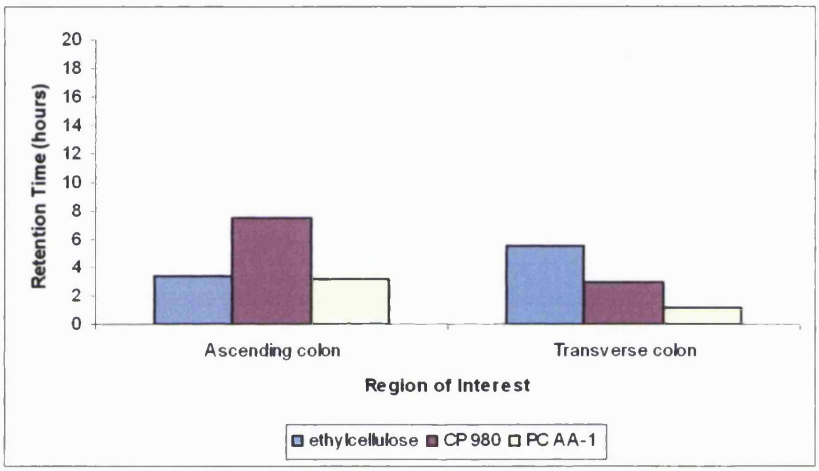

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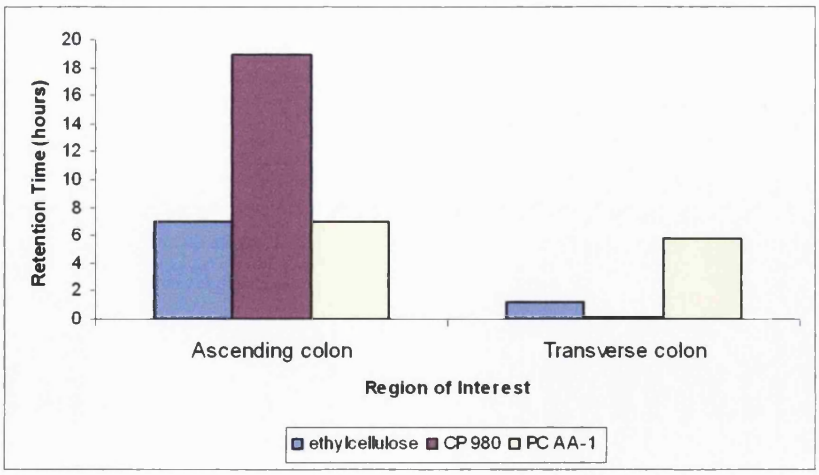

c

Figure 4.20 RT_{10} values in the ascending and transverse colons for a) dog 1, b) dog 2 and c) dog 3

Total mean transit of the powders post activation from the caecum to descending colon can be seen in Figure 4.21. Again the transit times through the descending colon were omitted since the powders were observed to reside here until defecation, as such retention in this region is dependent of the dog's bowel movements and not mucoadhesion.

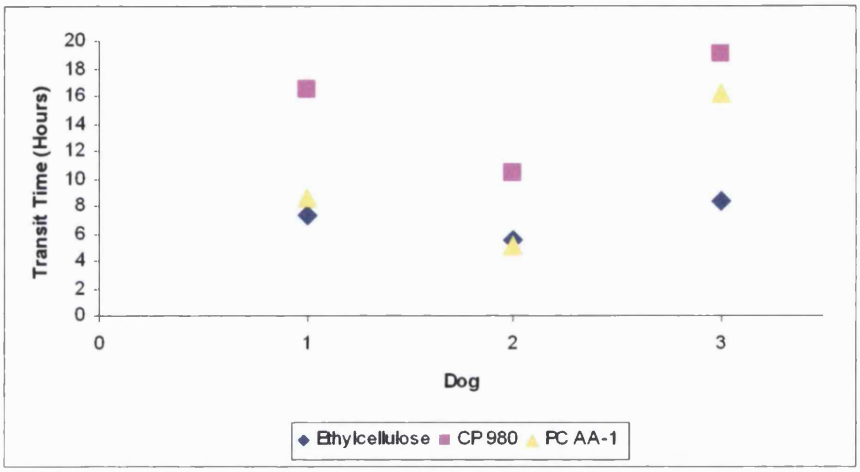

Figure 4.21 Total transit time (post activation of capsule) from caecum to descending colon as a average of the 3 dogs. Mean large intestinal transit times (n=3)

Total mean transit time post activation shows that CP 980 is retained for longer in the dog colon (15.33 ± 4.39 hours) than PC AA-1 (10.02 ± 7.50 hours) and ethylcellulose (7.11 ± 1.45 hours). Statistically these results are significant, and suggest that the CP 980 grade of the carbomer polymers is displaying mucoadhesive behaviour leading to prolonged transit in the canine colon. However, as discussed previously, a large proportion of the polycarbophil AA-1 was retained in the cage of the device and this may have affected the results. In dog 3, the InteliSite® Companion device released similar amounts of Carbopol 980 and polycarbophil AA-1 (56% and 63% respectively). In this case, both polymers exhibited increased retention in the colon. However, in dog 2 substantially higher amounts of Carbopol 980 were released, perhaps contributing to the higher retention seen in this dog. It can be surmised that had the entire polymer mass been fully released in each case, improved colonic retention might have been observed with polycarbophil AA-1.

The behaviour of the polymers *in-vivo* can be explained by their molecular weights, cross linking and results obtained in chapter 2. Mucoadhesion is complex and is the result of several simultaneous interactions (Smart, 2005) such as interpenetration of the polymers and mucin (Jabbari et al., 1993), chemical and physical bonding (van der Waals interactions and hydrogen bonds) (Mortazavi, 1995) and water movement and dehydration of mucin (Mortazavi et al., 1993). There is currently no data regarding the mucoadhesion of these polymers in the colon, however, there is evidence to suggest that Carbopol 980 might have a stronger mucoadhesive potential than polycarbophil AA-1. In vitro testing of mucoadhesion (adhesion time and work of adhesion) to small intestinal porcine mucosa at pH 7 ranked Carbopol 980 as being more mucoadhesive than polycarbophil AA-1 (Grabovac et al., 2005). These data are supported by the results in chapter 2 (detachment from mucin at pH 6.8) and suggests that increasing the molecular mass of the polymer leads to higher internal cohesion of the molecule, and a consequent increase in mucoadhesion. The degree of crosslinking is also an integral factor; crosslinking reduces the dissolution rate of hydrophilic chains in an aqueous environment providing greater cohesion (Tobyn et al., 1997). Polycarbophil AA-1, with less crosslinkers can hydrate more and so has less internal cohesion. In addition, Carbopol 980 with its higher molecular weight possesses more potential bonding sites for chemical and physical bonding and has less hydrophobic crosslinkers than polycarbophil AA-1. Previous studies have shown that polymers possessing fewer hydrophobic groups adhere more strongly to mucin and epithelial tissue (Ch'ng et al., 1985). These described mechanisms could account for the increased retention demonstrated by the Carbopol 980 polymer in the dog colon.

These results suggest that mucoadhesion occurs within the dog colon. Similarities of the dog and human colon such as pH means that in extrapolating these results to man, we may expect to see a similar outcome. However differences in fluid volume and comparative sizes will complicate matters. Additionally free fluid in the human colon has been estimated to be exceptionally low and may not be sufficient to activate the carbomer polymers. The swell kinetics of CP 974 and PC AA-1 have been investigated *in-vivo* in the canine duodenum and ileum (Slovin et al., 1997). Two important findings of this study are that the polymers swell more

in the more distal regions and secondly the fluid for this swelling is provided by the mucin layer. Therefore volume of free fluid may not be important for mucoadhesion and factors such as pH, contact with the colonic mucosa and erosion of the polymer gel layer may be more significant in determining a mucoadhesive response. The greater length of the human ascending colon, 20 cm compared with 5 cm in the dog, (Kararli et al., 1995) may also be an advantage allowing more time for polymer gel formation and thus mucoadhesion.

4.9 Conclusion

The InteliSite® companion device was successfully administered and activated in the dog colon in 9 of 11 attempts. The two fails were attributed to oversensitivity of the SMA wires and the inability for the transmitter signal to reach the device *in-vivo* due to shielding from body tissue. GE of the device was seen to be the most variable of all transit times but is consistent with current literature, as were small intestinal transit times which were less variable.

Upon retrieval of the device the body and cage were observed to be completely separated in all 9 successful activations. Release of the powders from the InteliSite® companion device was poor with 37.17 ± 4.57 %, 35.14 ± 29.47% and 62.12 ± 2.77% of ethylcellulose, CP 980 and PC AA-1 remaining within the capsule until excretion. The poor release of the ethylcellulose led to the conclusion that the limiting factor here is the colonic environment, its contents are too packed and viscous to allow release and subsequent dispersion of the powder. The current design of the InteliSite® companion device however does not allow for this, particularly for water insoluble or swelling polymers. Removal of the cage from the design of the device may aid release as this will eliminate the need for free fluid to enter the frame work of the cage to come into contact with the administered drug or powders.

Dispersion of the powders *in-vivo,* was also seen to be limited and was investigated within the ascending and transverse colon. Greater residence times were seen for the carbomer powders over a control so the limited dispersion of the polymers did not eliminate the carbomers mucoadhesive properties all together.

CP 980 was observed to be retained at the 10% or greater level in the ascending colon in 2 out of 3 dogs and PC AA-1 in 2 out of 3 dogs in the transverse colon at 10% or greater and 50% or greater levels. Additionally and perhaps crucially, the total mean transit time post activation from caecum to descending colon for CP 980 was seen to be greater and statistically significant than that of the ethylcellulose control. This important finding strongly suggests that gel forming polymers are retained in the dog colon longer than non-gel forming polymers and that this retention is likely to be a consequence of the mucoadhesive properties of said polymers.

Chapter 5

General Discussion and Future Work

5.1 General discussion and conclusions

Drug delivery to the distal regions of the GI tract is fraught with complications ranging from provision of suitable protection for the active compound from stomach pH to metabolism of the drug by colonic microflora. Perhaps the most prevalent challenge however is the variability of GI transit times. It seems that from the moment a dosage form is swallowed its arrival time at any particular region is highly unpredictable. Inter-subject variations in gastric emptying, small intestinal transit time and colonic transit time have all been recorded within the literature and there are inherent clinical implications of this; drastic differences in bioavailability and dose timing with modified release systems have been reported.

Normalisation of transit times or retention times has clear clinical benefits. Theoretically it could improve site-specificity of drug release and improve and harmonise variability. Additionally it may help in the treatment of localised disorders such as ulcerative colitis or Crohn's disease. Retention of dosage forms within the ascending and transverse colonic regions may serve to improve drug bioavailability whilst simultaneously standardising colonic transit times. Further benefits of this approach stem from the decreasing presence of water in the distal regions which makes drug dissolution more challenging; keeping the dosage form in the more proximal colonic regions should minimise the complications of this issue to some extent. Mucoadhesion could be employed to facilitate this retention in the colon. The lower mucus turnover, thicker mucin layer and general unstirred environment of the colon may make it more suitable for this kind of approach than other sites along the GI tract.

This project was therefore concerned with the feasibility of using mucoadhesives within the colon and, as such, a range of monolithic (tablets) and multiparticulate (pellets and microparticles) dosage forms were formulated containing a known mucoadhesive polymer (carbomers). The effect of the formulation process on the mucoadhesive polymers has been neglected in the literature, with studies focussing mainly on the polymer itself.

The polymer was assessed first in three studies, to understand the mucoadhesive mechanisms at play for each of the grades examined. *In-vitro* tensile testing of the

polymers showed that molecular weight and degree of cross linking were of paramount importance in determining their adhesive and mucoadhesive qualities. Interactions of the polymers with a mucin solution was different for all four of the carbomer grades tested and showed that the lower molecular weight polymers underwent greater interactions with mucin than higher molecular weight grades. This did not however necessarily increase their overall mucoadhesive capabilities. The effect of pH was also seen to be crucial and results obtained here help to explain some discrepancies in the literature regarding *in-vivo* gastric retention of carbomer formulations and highlight the need for a suitable *in-vivo* animal model.

Tablets, pellets and microparticles were prepared, to establish their effects on the mucoadhesive process. Comparisons of tablets produced via a direct compression method with a wet granulation method helped to show differences in the mechanisms of mucoadhesion for each grade of carbomer tested and their reliance on polymer/mucin interpenetration. Formulation of carbomer polymers into dosage forms was seen to affect their *in-vitro* adhesion and mucoadhesion. Detachment force from a glass surface and a mucin surface were seen to be reduced by the granulation process for monolithic systems, additionally both pellet and microparticle formulations showed no increase in force from their respective non-mucoadhesive controls. Prolonged exposure to a mucin solution did however improve the mucoadhesion of granulated tablets and two of the pellet formulations (CP 971 and PC AA-1). This suggests that formulation has an impact on mucoadhesion in the short term but not in the long term. Drug release was also affected by formulation, where extended release profiles were observed for direct compression tablets it was not seen for granulated tablets or pellets.

There are currently no studies listed in the literature investigating colonic mucoadhesion *in-vivo*. Therefore, a proof of principle *in-vivo* study was conducted in beagle dogs using gamma scintigraphy to monitor the transit of two of the carbomer polymers (CP 980 and PC AA-1) and an ethylcellulose control. Delivery of the polymers to the ileo-caecal junction (ICJ) was achieved using a novel delivery device, the InteliSite® companion. Transit of the device to the ICJ was consistent with times reported for the device's predecessor the InteliSite® capsule. Release of the powders in the caecum was suboptimal and was attributed to the high viscosity and low fluid volume of the region. Spreading of the powders throughout the colon did not occur; instead transit was similar

to what one would expect for a single unit system. The retention time of 50% or more of the powders and 10% or more of the powders in the ascending and transverse colon regions were evaluated, as was the total transit time in both regions. In 2 of the 3 dogs transit of CP 980 through the ascending colon for 10% or more of the polymer was longer than the control. Additionally, transit of PC AA-1 in 2 of the 3 dogs was longer at the 10% or more and 50% or more levels in the transverse colon. Finally, mean total transit time form the point of activation to the descending colon was seen to be greater for both carbomer polymers over the control, with CP 980 possessing the longest transit times.

In summary: formulation of carbomer polymers was seen to affect their mucoadhesive properties, specifically polymer to mucin entanglements, limiting there initial mucoadhesive capabilities. However after exposure to mucin this limitation diminished. This is an important understanding for the practical use of carbomers as mucoadhesives, particularly in the colon. It is not unreasonable to assume that contact with the colonic mucosa may only occur once, particularly for a monolith, therefore any lag time in the dosage form's ability to interact with mucin may result in the system being eliminated prior to an adhesive joint being formed.

In reality *in-vivo* mucoadhesion is a complicated process and many factors such as pH of the region, fluid volume and erosion of the gel layer physically and chemically will affect retention time. Never the less *in-vivo* results are promising, and analysis of transit times on a dog by dog basis shows retention of the carbomers in the colon is a real possibility.

5.2 Future work

- Reviews of the literature regarding mucoadhesion show an array of tests each with its own parameters and unique results. Development of a standardised test is important for this field of research to allow researchers to compare results. Standard values for surface area under test, contact times, load cell and test surface need to be established. This should probably take the form of a tensile test and could be combined with an standardised rheology method

- The results from the current *in-vivo* study warrant further investigations. This would include increasing the sample size in an attempt to limit variation within the results. The study would be extended to man and combined with a second study to help understand the level of carbomer polymer required within a formulation to invoke a mucoadhesive response *in-vivo*. It could initially be performed using the polymers in the powdered form at various ratios of carbomer polymer with a diluent

- One of the aims of this current research was to assess the effect of formulating carbomer polymers into deliverable dosage forms. To target the colon the systems produced here would need to be enhanced with an enteric/ water insoluble/ bacteria degraded polymer coating. *In-vitro* assessment of the effect this has on mucoadhesion would be ascertained, as would the occurrence of interactions of the carbomer polymer with the coat

References

References

Adkin, D. A., Davis, S. S., Sparrow, R. A., & Wilding, I. R. 1993, "Colonic transit of different sized tablets in healthy subjects", *Journal of Controlled Release*, vol. 23, pp. 147-156.

Aharoni S., M. 1998, "Increased glass transition temperature in motionally constrained semi crystalline polymers", *Polymers for Advanced Technologies*, vol. 9, no. 3, pp. 169-201.

Akiyama, Y., Ngahara, N., Kashihara, T., Hirai, S., & Toguchi, H. 1995, "In Vitro and in-vivo Evaluation of Mucoadhesive Microspheres Prepared for the Gastrointestinal Tract using Polyglycerol Ester of fatty Acids and a poly(acrylic acid) derivative", *Pharmaceutical Research*, vol. 12, no. 3, pp. 397-405.

Allen, A. 1981, "Structure and Function of Gastrointestinal Mucus," in *Physiology of the Gastrointestinal Tract*, Johnson L.R., ed., Raven Press, New York, pp. 617-639.

Andersen, A. C., 1970, "Digestive System," in *Beagle Dog as an Experimental Animal*, Anderson A. C., & Good L. S., eds., Iowa State University Press, pp. 226-231.

Anlar S., Capan Y., & Hincal A.A. 1993, "Physico-chemical and bioadhesive properties of polyacrylic polymers", *Pharmazie*, vol. 48, no. 4, pp. 285-287.

Aoyagi, N., Ogata, H., Kaniwa, N., Uchiyama, M., Yasuda, Y., & Tanioka, Y. 1992, "Gastric Emptying of Tablets and Granules in Humans, Dogs, Pigs and Stomach-Emptying-controlled Rabbits", *Journal of Pharmaceutical Sciences*, vol. 81, no. 12, pp. 1170-1174.

Ashford, M., Fell, J. T., Attwood, D., Sharma, H. L., & Woodhead, P. 1993, "An Evaluation of Pectin as a Carrier for Drug Targeting to the Colon", *Journal of Controlled Release*, vol. 26, pp. 213-220.

Atuma C., Strugala V., Allen A., & Holm L. 2001, "The adherent gastrointestinal mucus gel layer: thickness and physical state in vivo", *American Journal of Physiology Gastrointestinal Liver Physiology*, vol. 280, p. G922-G929.

Augsburger, L. L. & Vuppala, M. K. 1997, "Theory of granulation," in *Handbook of pharmaceutical granulation technology*, Parikh D.M., ed., New York, pp. 7-24.

Baert, L., Fanara, D., Remon, P., & Massart, D. 1992, "Correlation of extrusion forces, raw materials and sphere characteristics", *Journal of Pharmacy and Pharmacology*, vol. 44, pp. 676-678.

Banker, G. 1966, "Film coating theory and practice", *Journal of Pharmaceutical Sciences*, vol. 55, no. 1, pp. 81-89.

Bansil, R. & Turner, B. S., 2006, "Mucin structure, aggregation, physiological functions and biomedical applications", *Current Opinion in Colloid & Interface Science*, vol. 11, no. 2-3, pp. 164-170.

Basit, A. W., Podczeck, F., Newton, J. M., Waddington, W. A., Ell, P. J., & Lacey, L. F. 2004, "The use of formulation technology to assess regional gastrointestinal drug absorption in humans", *European Journal of Pharmaceutical Sciences*, vol. 21, pp. 179-189.

Batchelor, H. K., Banning, D., Dettmar, P. W., Hampson, F. C., Jolliffe, I. G., & Craig, D. Q. M. 2002, "An in vitro mucosal model for prediction of the bioadhesion of alginate solutions to the oesophagus", *International Journal of Pharmaceutics*, vol. 238, no. 1-2, pp. 123-132.

Bauer Kurt, H., Lehmann Klaus, Osterwald Hermann, P., & Rothgang Gerhart 1998, "Testing of coated Pharmaceutical Dosage Forms," in *Coated Pharmaceutical Dosage Forms*, medpharm GmbH Scientific Publishers, Stuttgart, pp. 185-203.

Bauer, K. H., Lehmann, K., Osterwald, H. P., & Rothgang, G. 1998, *Coated Pharmaceutical Dosage Forms* CRC press, Stuttgart.

Bayomi, M. A., Al-Auwayeh, S. A., & El-Helw, A. R. M. 2001, "Excipent-excipent interaction in the design of sustained-release Theophylline tablets: In-vitro and in-vivo evaluation", *Drug Development and Industrial Pharmacy*, vol. 27, no. 6, pp. 499-506.

Berggren, J. & Alderborn., G. 2001, "Drying behaviour of two sets of microcrystalline cellulose pellets", *International Journal of Pharmaceutics*, vol. 219, no. 1-2, pp. 113-126.

Bernkop-Schnurch, A., Schwarz, V., & Steininger S. 2000, "Polymers with thiol groups: a new generation of mucoadhesive polymers?" *Pharmaceutical Research*, vol. 16, no. 6, pp. 876-881.

Bernkop-Schnurch, A. 2005, "Thiomers: A new generation of mucoadhesive polymer", *Advanced Drug Delivery Reviews*, vol. 57, no. 1569, p. 1582.

BF Goodrich 1991, *High Performance Polymers for Pharmaceuticals: Controlled Release Tablets and Capsules* 17.

Bika, D., Tardos, G. I., Panmai, S., Farber, L., & Michaels, J. 2005, "Strength and morphology of solid bridges in dry granules of pharmaceutical powders", *Powder Technology*, vol. 150, no. 2, pp. 104-116.

Billington, D., Jayson, G. G., & Maltby, P. J. 1992, "Radioisotopes in nuclear medicine," in *Radioisotopes*, First edn, Graham J.M. & Billington D., eds., Bios Scientific Publishers, Oxford, pp. 123-141.

Binns, J., Stevens, H. N. E., McEwen, J., Pritchard, G., Brewer, F. M., Clarke, A., Johnson, E. S., & McMillan, I. 1996, "The tolerability of multiple oral doses of Pulsincap(TM) capsules in healthy volunteers", *Journal of Controlled Release*, vol. 38, no. 2-3, pp. 151-158.

Bodmeier, R. & McGinity J.W. 1987, "Solvent selection in the preparation of poly(DL-lactide) microspheres prepared by the solvent evaporation method", *International Journal of Pharmaceutics*, vol. 43, pp. 179-186.

Bogataj, M., Mrhar, A., Kristl, A., & Kozjek, F. 1991, "Eudragit E microspheres containing bacampicillin prepared by solvent removal methods", *Journal of microencapsulation*, vol. 8, no. 3, pp. 401-406.

Bogataji, M. & Mrhar, A. 1997, "Mucoadhesion of polycarbophil and chitosan microspheres on isolated Guinea pig vesicle and intestinal mucosa", *Journal of Controlled Release*, vol. 48, no. 3, pp. 340-342.

Bolhuis, G. K., & Chowhan, Z. T., 2007, "Materials for direct compression," in *Pharmaceutical Powder Compaction Technology*, First edn, Alderborn G. & Nystrom C., eds., Marcel Dekker Inc, NewYork, pp. 419-500.

Bonner, M. C., Jones, D. S., & Woolfson A.D., 1997, "Rheological characterisation of carbopol gels as electrically conducting drug delivery interfaces", *Journal of Pharmacy and Pharmacology*, vol. 49, no. Suppl. 4, p. 74.

Bruce, L. D., Shah, N. H., Waseem Malick, A., Infeld, M. H., & McGinity, J. W., 2005, "Properties of hot-melt extruded tablet formulations for the colonic delivery of 5-aminosalicylic acid", *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 59, no. 1, pp. 85-97.

Burton, S., Washington, N., Steele, R. J. C., Musson, R., & Feely, L. 1995, "Intragastric Distribution of Ion-exchange Resins: a drug Delivery System for the Topical Treatment of Gastric Mucosa", *Journal of Pharmacy and Pharmacology*, vol. 47, pp. 901-906.

Ceschel, G. C., Maffei, P., Ronchi, S. L., & Rossi, S. 2001, "Development of a Mucoadhesive dosage form for vaginal administration", *Drug development and Industrial Pharmacy*, vol. 27, no. 6, pp. 541-547.

Ceulemans, J. & Ludwig, A. 2002, "Optimisation of carbomer viscous eye drops: an in vitro experimental design approach using rheological techniques", *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 54, no. 1, pp. 41-50.

Ch'ng, H. S., Park, H., Kelly, P., & Robinson, J. R. 1985, "Bioadhesive Polymers as Platforms for Oral Controlled Drug Delivery II. Synthesis and Evaluation of some Swelling, Water-Insoluble Bioadhesive Polymers", *Journal of Pharmaceutical Sciences*, vol. 74, no. 4, pp. 399-405.

Chapman, S. R., Rowe, R. C., & Newton, J. M. 1988, "Characterisation of the sphericity of particles by the one plane critical stability", *Journal of Pharmacy and Pharmacology*, vol. 40, pp. 503-505.

Choi, H. G., Jung, J. H., Ryu, J. M., Yoon, S. J., Oh, Y. K., & Kim, C. K. 1998a, "Development of in situ-gelling and mucoadhesive acetaminophen liquid suppository", *International Journal of Pharmaceutics*, vol. 165, no. 1, pp. 33-44.

Choi, H. G., Oh, Y. K., & Kim, C. K. 1998b, "In situ gelling and mucoadhesive liquid suppository containing acetaminophen: enhanced bioavailability", *International Journal of Pharmaceutics*, vol. 165, no. 1, pp. 23-32.

Christensen, F. N., Davis, S. S., Hardy, J. G., Taylor, M. J., Whalley, D. R., & Wilson, C. G. 1985, "The use of gamma scintigraphy to follow the gastrointestinal transit of pharmaceutical formulations", *Journal of Pharmacy and Pharmacology*, vol. 37, pp. 91-95.

Cizek, L. J. 1954, "Total water content of laboratory animals with special reference to volume of fluid within the lumen of the gastrointestinal tract", *American Journal of Physiology*, vol. 179, pp. 104-110.

Clarke, G. M., Newton, J. M., & Short, M. D. 1993, "Gastrointestinal transit of pellets of differing size and density", *International Journal of Pharmaceutics*, vol. 100, pp. 81-92.

Clear, N. J., Milton, A., Humphrey, M., Henry, B. T., Wulff, M., Nichols, D. J., Anziano, R. J., & Wilding, I. 2001, "Evaluation of the Intelisite capsule to deliver Theophylline and frusemide tablets to the small intestine and colon", *European Journal of Pharmaceutical Sciences*, vol. 13, no. 4, pp. 375-384.

Conine, J. W. & Hadley, J. R. 1970, "Production of small solid pharmaceutical spheres", *Drug Cosmetic Industry*, vol. 106, no. 4, pp. 38-41.

Conti S., Maggi L., Segale L., Ochoa Machiste E., Conte U., Grenier P., & Vergnault G. 2007, "Matrices containing NaCMC and HPCMC 1. Dissolution performance characterization", *International Journal of Pharmaceutics*, vol. 333, pp. 136-142.

Craig, D. Q. M., Tamburic, S., Buckton, G., & Newton, J. M. 1994, "An investigation into the structure and properties of Carbopol 934 gels using dielectric spectroscopy and oscillatory rheometry", *Journal of Controlled Release*, vol. 30, pp. 213-223.

Crowley, M., Schroeder, B., Fredersdorf, A., Obara, S., Talarico, M., Kucera, S., & McGinity, J. W. 2004, "Physicochemical properties and mechanism of drug release from ethyl cellulose matrix tablets prepared by direct compression and hot-melt extrusion", *International Journal of Pharmaceutics*, vol. 269, no. 2, pp. 509-522.

Crowley, M., Zhang, F., Repka, M. A., Thumma, S., Upadhye, S. B., Kumar, S., McGinity, J. W., & Martin, C. 2007, "Pharmaceutical applications of Hot-melt extrusion: Part 1", *Drug Development and Industrial Pharmacy*, vol. 33, no. 9, pp. 909-926.

Cummings J.H., Banwell J.G., & Segal N. 1990, "The amount and composition of large bowel contents", *Gastroenterology*, vol. 80, no. 2, p. A408.

Cummings, J. H., Milojevic, S., Harding, M., Coward, W. A., Gibson, G. R., Botham, R. L., Ring, S. G., Wraight, E. P., Stockham, M. A., Allwood, M. C., & Newton, J. M. 1996, "In vivo studies of amylose and ethylcellulose coated [C-13] glucose microspheres as a model for drug delivery to the colon", *Journal of Controlled Release*, vol. 40, no. 1-2, pp. 123-131.

Cuna, M., Alonso, M. J., & Torres, D. 2001b, "Preparation and in vivo evaluation of mucoadhesive microparticles containing amoxicillin-resin complexes for drug delivery to the gastric mucosa", *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 51, no. 3, pp. 199-205.

Daly, P. B., Davis, S. S., Frier, M., Hardy, J. G., Kennerley, J. W., & Wilson, C. G. 1982, "Scintigraphic assessment of the in vivo dissolution rate of a sustained release tablet", *International Journal of Pharmaceutics*, vol. 10, no. 1, pp. 17-24.

Davis, S. S., Hardy, J. G., & Fara, J. W. 1986, "Transit of pharmaceutical dosage forms through the small intestine", *Gut*, vol. 27, pp. 886-892.

Davis, S. S., Wilding, E. A., & Wilding, I. R. 1993, "Gastrointestinal transit of a matrix tablet formulation: comparison of canine and human data", *International Journal of Pharmaceutics*, vol. 94, no. 1-3, pp. 235-238.

Dew, M. J., Hughes, P. J., Lee, M. G., Evans, B. K., & Rhodes, J. 1982, "An oral preparation to release drugs in the human colon", *British Journal of Clinical Pharmacy*, vol. 14, pp. 405-408.

Digenis, G. A., Casey, D. L., Beihn, R. M., & Shambhu, M. B. 1976, "Method for Monitoring hard Gelatine Capsule Disintegration Times in Humans using External Scintigraphy", *Journal of Pharmaceutical Sciences*, vol. 65, no. 9, pp. 1412-1413.

Dressman, J. B. 1986, "Comparison of Canine and Human Gastrointestinal Physiology", *Pharmaceutical Research*, vol. 3, no. 3, pp. 123-131.

Dressman, J. B., Berardi, R. R., Dermentzoglou, L. C., Russell, T. L., Schmaltz, S. P., Barnett, J. K., & Jarvenpaa, K. M. 1990, "Upper gastrointestinal (GI) pH in young, healthy men and women", *Pharmaceutical Research*, vol. 7, pp. 756-776.

Dressman, J. B. & Yamada, K. 1991, "Animal Models for oral drug absorption," in *Pharmaceutical Bioequivalence*, Welling P. & Tse F.L., eds., Dekker, New York, pp. 235-266.

Dreu, R., Sirca, J., Pintye-Hodi, K., Burjan, T., Planinsek, O., & Srcic, S. 2005, "Physicochemical properties of granulating liquids and their influence on microcrystalline cellulose pellets obtained by extrusion-spheronisation technology", *International Journal of Pharmaceutics*, vol. 291, no. 1-2, pp. 99-111.

Durrani, M. J., Farr, S. J., & Kellaway, I. W. 1995, "Precorneal clearance of mucoadhesive microspheres from the rabbit eye", *Journal of Pharmacy and Pharmacology*, vol. 47, pp. 481-584.

Dyce, K. M., Sack, W. O., & Wensing, C. J. G. 2002, "The digestive apparatus," in *Textbook of Veterinary Anatomy*, 3 edn, Kersay R. & LeMelledo D., eds., Saunders, Philadelphia, pp. 100-147.

Edwards, C. 1997, "Physiology of the colorectal barrier", *Advanced Drug Delivery Reviews*, vol. 28, pp. 173-198.

Evans, D. F., Pye, G., Bramley, R., Clark, A. G., Dyson, T. J., & Hardcastle, J. D. 1988, "Measurement of gastrointestinal pH profiles in normal ambulant human subjects", *Gut*, vol. 29, pp. 1035-1041.

Flory P.J. 1981, *Principles of polymer chemistry* Cornell University Press, Ithaca, N.Y.

Follonier, N., Doelker, E., & Cole, E. T. 1994, "Evaluation of hot melt extrusion as a new technique for the production of polymer based pellets for sustained release capsules containing high loading of freely soluble drugs", *Drug Development and Industrial Pharmacy*, vol. 20, no. 8, pp. 1323-1339.

Forster, A., Hempenstall, J., & Rades, T. 2002, "Characterizations of glass solutions of poorly water-soluble drugs produced by melt extrusion with hydrophilic amorphous polymers", *Journal of Pharmacy and Pharmacology*, vol. 53, no. 3, pp. 243-250.

Forbes W.G & McLeod L.A 1958, *Transactions of the Institution for the Rubber Industry*, vol. 154, pp. 34-35.

Fukuda, M., Peppas, N. A., & McGinity, J. W. 2006, "Properties of sustained release hot-melt extruded tablets containing chitosan and xanthan gum", *International Journal of Pharmaceutics*, vol. 310, no. 1-2, pp. 90-100.

Ganong, W. F. 1997, "Regulation of Gastrointestinal Function," in *Review of Medical Physiology*, Eitheenth edn, Butler J.P., Suver A.M, & Langan C, eds., Appleton and Lange, pp. 448-479.

Gardner, D., Casper, R., Leith, F., & Wilding, I. 1997, "Non-invasive methodology for assessing regional absorption from the gastrointestinal tract", *Pharmaceutical technology Europe*, vol. 9, no. 6, pp. 46-53.

Genta, I., Conti, B., Perugini, P., Pavanetto, F., Spadaro, A., & Puglis, I. G. 1997, "Bioadhesives microparticles of Ophthalmic administration of acyclovir", *Journal of Pharmacy and Pharmacology*, vol. 49, no. 8, pp. 737-742.

Gnamm H & Fuchs O 1980, *Physical principles and properties of solutions of low and high molecular weight compounds*, 8th Ed, Wiss Verlagsges, Stuttgard.

Gomez-Carracedo, A., Alvarez-Lorenzo, C., Gomez-Amoza, J. L., Martinez-Pacheco, R., Souto, C., & Concheiro, A. 2001, "Extrusion-spheronisation of blends of Carbopol 934 and microcrystalline cellulose", *Drug Development and Industrial Pharmacy*, vol. 27, no. 5, pp. 381-391.

Gorbach, S. 1971, "Intestinal Micro flora", *Gastroenterology*, vol. 6, pp. 1110-1129.

Gotch, F., Nadell, J., & Edelman, I. S. 1956, "Gastrointestinal water and electrolytes. IV. The Equilibration of deuterium oxide in gastrointestinal contents and the proportion of total body water (T.B.W.) in the gastrointestinal tract", *The Journal of Clinical Investigation*, vol. 36, no. 2, pp. 289-296.

Grabovac, V., Guggi, D. and Bernkop-Schnürch, G. Comparison of the mucoadhesive properties of various polymers. Advanced Drug Delivery Reviews. 57:1713-1723 (2005)

Graff, J., Brinch, K., & Madsen, J. L. 2000, "Simplified scintigraphic methods fro measuring gastrointestinal transit times", *Clinical Physiology*, vol. 20, no. 4, pp. 262-266.

Griffith, G. H., Owen, G. M., Kirkman, S., & Shields, R. 1966, "Measurement of rate of gastric emptying using chromium 51", *The Lancet*, vol. 51, no. 1, pp. 1244-1255.

Guggi, A., Marschutz, M. K., & Bernkop-Schnurch, A. 2004a, "Matrix tablets based on thiolated poly (acrylic acid): pH dependant variation in disintegration and mucoadhesion", *International Journal of Pharmaceutics*, vol. 274, pp. 97-105.

Gurtler, F., Kaltsatos, V., Boisrame, B., Deleforge, J., Gex-fabry, M., Balant, L. P., & Gurny, R. 1995, "Ocular Availability of gentamicin in Small Animals After Topical Administration of a Conventional Eye Drop Solution and a novel Long Acting Bioadhesive Ophthalmic Drug Insert", *Pharmaceutical Research*, vol. 12, no. 11, pp. 1791-1795.

Harder, S., Fuhr , U., Beerman, D., & Staib, A. H. 1990, "Ciprofloxacin absorption in different regions of the human gastrointestinal tract. Investigations with the HF capsule", *British Journal of Clinical Pharmacology*, vol. 30, no. 1, pp. 35-39.

Hardy, J. G., Wilson, C. G., & Wood, E. 1985, "Drug Delivery to the Proximal Colon", *Journal of Pharmacy and Pharmacology*, vol. 37, no. 874, p. 877.

Hardy, J. G., Evans, D. F., Clark, A. G., Tonnesen, H. H., & Gamst, O. N. 1987, "Evaluation of an Enteric Coated Naproxen Tablet Using Gamma Scintigraphy and pH Monitoring", *International Journal of Pharmaceutics*, vol. 37, pp. 245-250.

Harris, D., Fell, J. T., Sharma, H. L., & Taylor, D. C. 1990, "GI transit of potential bioadhesive formulations in man: A scintigraphic study", *Journal of Controlled Release*, vol. 12, no. 1, pp. 45-53.

Haupt, S., Zioni, T., Gati, I., Kleinstern, J., & Rubinstein, A. 2006, "Luminal delivery and dosing considerations of local celecoxib administration to colorectal cancer", *European Journal of Pharmaceutical Sciences*, vol. 28, no. 3, pp. 204-211.

He, P., Davis, S. S., & Illum, L. 1998, "In vitro evaluation of the mucoadhesive properties of chitosan microspheres", *International Journal of Pharmaceutics*, vol. 166, no. 1, pp. 75-88.

Hellen, L. & Yliruusi, J. 1993, "Process variables of instant granulator and spheroniser. III. Shape ad shape distributions of pellets", *International Journal of Pharmaceutics*, vol. 96, pp. 217-223.

Hill, M. J. & Drasar, B. S. 1975, "The Normal Colonic Bacterial Flora", *Gut*, vol. 16, pp. 318-323.

Hui, H. W. & Robinson, J. R. 1985, "Ocular delivery of progesterone using a bioadhesive polymer", *International Journal of Pharmaceutics*, vol. 26, no. 3, pp. 203-213.

Hunter, E., Fell J.T., & Sharma, H. 1981, "A Comparison of the behaviour of a tablet and capsule formulations in-vivo", *Journal of Pharmacy and Pharmacology*, vol. 33, pp. 617-618.

Ibekwe, V., Fang, L., Fadda, H. M., Khela, M. K., Evans, D. F., Parsons, G. E., & Basit, A. W. 2006, "An investigation into in-vivo performance variability of pH responsive

polymers for ileo-colonic drug delivery using gamma scintigraphy in humans", *Journal of Pharmaceutical Sciences*, vol. 95, no. 12, pp. 2760-2766.

Ishibashi, T., Hatano, H., Kobayeshi, M., Mizobe, M., & Yoshino, H. 1998, "Design and evaluation of a new capsule-type dosage form for the colon targeted delivery of drugs", *International Journal of Pharmaceutics*, vol. 168, pp. 31-40.

Jabbari, E., Wisniewski, N., & Peppas, N. A. 1993a, "Evidence of mucoadhesion by chain interpenetration at a poly (acrylic acid)/mucin interface using ATR-FTIR spectroscopy", *Journal of Controlled Release*, vol. 26, no. 2, pp. 99-108.

Jackson, S. J., Bush, D., Washington, N., & Perkins, A. C. 2000, "Effect of resin surface charge on gastric mucoadhesion and residence of cholestyramine", *International Journal of Pharmaceutics*, vol. 205, no. 1-2, pp. 173-181.

Jackson, S. J., Bush, D., & Perkins, A. C. 2001, "Comparative scintigraphic assessment of the intragastric distribution and residence of cholestyramine, Carbopol 934P and sucralfate", *International Journal of Pharmaceutics*, vol. 212, no. 1, pp. 55-62.

Kakoulides, E. P., Smart, J. D., & Tsibouklis, J. 1998, "Azocrosslinked poly(acrylic acid) for colonic delivery and adhesion specificity: in vitro degradation and preliminary ex vivo bioadhesion studies", *Journal of Controlled Release*, vol. 54, no. 1, pp. 95-109.

Kararli, T. 1995, "Comparison of Gastrointestinal Anatomy, Physiology and Biochemistry of Humans and commonly used Laboratory Animals", *Biopharmaceutics and Drug Disposition*, vol. 16, pp. 351-380.

Kast, C. E., Valenta, C., Leopold, M., & Bernkop-Schnurch, A. 2002, "Design and in vitro evaluation of a novel bioadhesive vaginal drug delivery system for clotrimazole", *Journal of Controlled Release*, vol. 81, no. 3, pp. 347-354.

Kendall, R. A. 2006, *Novel pH sensitive microparticles for site specific drug delivery to the gastrointestinal tract.*

Khan, A. K., Piris, J., & Truelove, S. C. 1977, "An experiment to determine the Active therapeutic Moiety of Sulphasalazine", *The Lancet*, vol. 11, pp. 892-895.

Khanna, R., Agarwal, S. P., & Ahuja, A. 1996, "Preparation and Evaluation of Bioerodable Buccal Tablets containing clotrimazole", *International Journal of Pharmaceutics*, vol. 138, no. 1, pp. 67-73.

Khanvilkar, K., Donovan, M. D., & Flanagan, D. R. 2001, "Drug transfer through mucus", *Advanced Drug Delivery Reviews*, vol. 48, no. 2-3, pp. 173-193.

Khosla, R. & Davis, S. S. 1987, "The Effect of polycarbophil on the gastric emptying of pellets", *Journal of Pharmacy and Pharmacology*, vol. 39, pp. 47-49.

Khosla, R., Feely, L., & Davis, S. S. 1989, "Gastrointestinal transit of non-disintegrating tablets in fed subjects", *International Journal of Pharmaceutics*, vol. 53, pp. 107-117.

Kim, C. K., Kim, M. J., & Oh, K. H. 1993, "Preparation and evaluation of sustained release microspheres of terbutaline sulfate", *International Journal of Pharmaceutics*, vol. 106, pp. 213-219.

Kinoshita, M., Baba, K., Nagayasu, A., Yamabe, K., Shimooka, T., & Takeichi, Y. 2002, "Improvement of solubility and oral bioavailability of a poorly water-soluble drug, TAS-301, by its melt-adsorption on a porous calcium silicate." *Journal of Pharmaceutical Sciences*, vol. 91, no. 2, pp. 362-370.

Kockisch, S., Rees, G. D., Young, S. A., Tsibouklis, J., & Smart, J. D. 2003, "Polymeric microparticles of drug delivery to the oral cavity: An in-vitro evaluation of mucoadhesive potential", *Journal of Pharmaceutical Sciences*, vol. 92, no. 8, pp. 1614-1623.

Koleng, J. J., McGinity, J. W., & Wilber, W. R. 2003, "Carbomers," in *Handbook of Pharmaceutical Excipients*, 4 edn, Rowe R.C, Sheskey P.J, & Weller P.J, eds., Pharmaceutical Press, Oxford, pp. 89-92.

Korsmeyer R.W., Gurny R., Doelker E., Buri P., & Peppas N.A. 1983, "Mechanisms of solute release from porous hydrophilic polymers", *International Journal of Pharmaceutics* no. 15, pp. 25-35.

Kriwet, B. & Kissel, T. 1996, "Interactions between bioadhesive poly (acrylic acid) and calcium ions", *International Journal of Pharmaceutics*, vol. 127, no. 2, pp. 135-145.

Larhed, A. W., Artursson, P., Grasjo, J., & Bjork, E. 1997, "Diffusion of Drugs in Native and purified Gastrointestinal Mucus", *Journal of Pharmaceutical Sciences*, vol. 86, no. 6, pp. 660-665.

Lehr, C. M. 1991, "An estimate of turnover time of intestinal mucus gel layer in the rat in situ loop", *International Journal of Pharmaceutics*, vol. 60, pp. 235-240.

Leung, S. H. & Robinson, J. R. 1990a, "Polymer structure features contributing to mucoadhesion. II", *Journal of Controlled Release*, vol. 12, no. 3, pp. 187-194.

Longer, M. A., Ch'ng, H. S., & Robinson, J. R. 1985, "Bioadhesive Polymers as Platforms for Oral Controlled Drug Delivery III: Oral Delivery of Chlorothiazide Using Bioadhesive Polymers.", *Journal of Pharmaceutical Sciences*, vol. 74, no. 4, pp. 406-411.

Lovgren, K. & Lundberg, P. J. 1989, "Determination of sphericity of pellets prepared by extrusion/ spheronisation and the impact of some process parameters", *Drug Development and Industrial Pharmacy*, vol. 15, no. 14-16, pp. 2375-2392.

Mackey, M., Phillips, J., & Hastewell, J. 1997, "Peptide Drug Delivery. Colonic and Rectal Absorption", *Advanced Drug Delivery Reviews*, vol. 28, pp. 253-273.

Maggi L., Segale L., Torre M.L., Ochoa Machiste E., & Conte U. 2002, "Dissolution behaviour of hydrophilic matrix tablets containing two different polyethylene oxides (PEOs) for the controlled release of a water soluble Drug. Dimensionality Study. *Biomaterials*, vol. 23, no. 1113, p. 1119.

Maloiy, G. M. O. & Clemens, E. T. 1980, "Gastrointestinal osmolality electrolyte and organic acid composition in five species of east African herbivorous mammals", *Journal of Animal Science*, vol. 51, no. 4, pp. 917-924.

Mandelkern, L. 1964, *Crystallization of polymers*, eds., Book Company, New York, pp 1-12

Mark H. 1942, Industrial Engineering Chemistry Intern Education 34, pp.1343.

Maroni A. & Ghebre-Sellassie I. 1995, "Application of poly(oxyethylene) homopolymers in sustained release solid formulations", *Drug Development and Industrial Pharmacy*, vol. 21, pp. 1411-1428.Martini, F. H. 1995, *Fundamentals of anatomy and physiology* Prentice Hall, New Jersey.

Martini, F. H. 1995, *Fundamentals of anatomy and physiology* Prentice Hall, New Jersey.

Mayersohn, M. 2002, "Principles of Drug Absorption," in *Modern Pharmaceutics*, G. S. Banker & C. T. Rhosdes, eds., Marcel Dekker, New York, pp. 21-73.

McConnell, E. L., Tutas, J., Mohamed, M. A. M., Banning, D., & Basit, A. W. 2007, "Colonic drug delivery using amylose films: the role of aqueous ethylcellulose dispersions in controlling drug release", *Cellulose*, vol. 14, no. 1, pp. 25-34.

McConnell, E. L., Basit, A. W., & Murdan, S. 2008, "Measurements of rat and mouse gastrointestinal pH, fluid and lymphoid tissue, and implications for in-vivo experiments", *Journal of Pharmacy and Pharmacology*, vol. 60, pp. 63-70.

McConnell, E. L., Fadda, H. M., & Basit, A. W. 2008,"Gut Instincts: Perspectives on physiology and intestinal delivery". International Journal of Pharmaceutics, In press.

McGinity J.W 2004, "Hot-Melt Extrusion as a Pharmaceutical Process", *AAPS News Magazine*, vol. March, pp. 21-25.

Mezreb, N., Charrueau, C., Boy, P., & Chaumeil, J. C. 2004, "Production of carbopol 974 and carbopol 971 pellets by extrusion-spheronization:Optimization of the processing parameters and water content", *Drug Development and Industrial Pharmacy*, vol. 30, no. 5, pp. 481-490.

Milojevic, S., Newton, J. M., Cummings, J. H., Gibson , G. R., Botham, R. L., Ring, S. G., Stockham, M. A., & Ilwood, M. C. 1996, "Amylose as a coating for drug delivery to the colon: Preparation and in vitro evaluation using 5-aminosalicylic acid pellets", *Journal of Controlled Release*, vol. 38, no. 1, pp. 75-84.

Mortazavi, S. A. & Smart, J. D. 1993, "An investigation into the role of water movement and mucus gel dehydration in mucoadhesion", *Journal of Controlled Release*, vol. 25, no. 3, pp. 197-203.

Mortazavi, S. A. 1995, "An in vitro assessment of mucus/mucoadhesive interactions", *International Journal of Pharmaceutics*, vol. 124, no. 2, pp. 173-182.

Muramatsu, M., Kanada, K., Nishida, A., Ouchi, K., Saito, N., Yoshida, M., Shimoaka, A., Ozeki, T., Yuasa, H., & Kanaya, Y. 2000, "Application of Carbopol(R) to controlled release preparations I. Carbopol(R) as a novel coating material", *International Journal of Pharmaceutics*, vol. 199, no. 1, pp. 77-83.

Neau, S. H., Chow, M. Y., & Durrani, M. J. 1996, "Fabrication and characterization of extruded and spheronized beads containing carbopol(R) 974P, NF resin", *International Journal of Pharmaceutics*, vol. 131, no. 1, pp. 47-55.

Neau, S. H., Chow, M. Y., Hileman, G. A., Durrani, M. J., Gheyas, F., & Evans, D. F. 2000, "Formulation and process considerations for beads containing Carbopol 974 NF resin made by extrusion-spheronisation", *International Journal of Pharmaceutics*, vol. 199, no. 2, pp. 129-140.

Noveon 2000, Technical manual: *Controlled Release Tablets and Capsules* 17.

Noveon 2002, Technical manual: *Bioadhesion* 16.

Nystrom C. & Karehill P. 1996, "Intermolecular Bonding Forces," in *Pharmaceutical Powder Compaction Technology*, Alderborn G. & Nystrom C., eds., Marcel Dekker, New York, pp. 55-133.

O'Donnell, P. B. & McGinity, J. W. 1997, "Preparation of microspheres by solvent evaporation technique", *Advanced Drug Delivery Reviews*, vol. 28, pp. 25-42.

Oo, C., Snell, P., Barrett, J., Dorr, A., Liu, B., & Wilding, I. R. 2003, "Pharmacokinetics and delivery of the anti-influenza prodrug oseltamivir to the small intestine and colon using site-specific delivery capsules", *International Journal of Pharmaceutics*, vol. 257, no. 297, p. 299.

Park, K. & Robinson, J. R. 1984, "Bioadhesive polymers as platforms for oral-controlled drug delivery: method to study bioadhesion", *International Journal of Pharmaceutics*, vol. 19, no. 2, pp. 107-127.

Park, H. & Robinson, J. R. 1985, "Physic-chemical properties of water insoluble polymers important to mucin/epithelial adhesion", *Journal of Controlled Release*, vol. 2, pp. 47-57.

Parr A., Sandefer E.P., Wissel P., McCartney M., McClain C., Ryo U., & Digenis G.A. 1999, "Evaluation of the feasibility and use of a prototype drug delivery capsule (RDDC) for non-invasive regional drug absorption studies in the GI tract of man and Beagle dog", *Pharmaceutical Research*, vol. 16, no. 2, pp. 266-271.

Patton, T. F. & Robinson, J. R. 1975, "Ocular evaluation of Polyvinyl alcohol Vehicle in Rabbits", *Journal of Pharmaceutical Sciences*, vol. 64, no. 8, pp. 1312-1316.

Patton, T. F. & Robinson, J. R. 1976, "Quantitative Precorneal Disposition of Topically Applied Pilcarpine Nitrate in Rabbits Eyes.", *Journal of Pharmaceutical Sciences*, vol. 65, no. 9, pp. 1295-1301.

Pavelic, Z., Skalko-Basnet, N., Filipovic-Grcic, J., Martinac, A., & Jalsenjak, I. 2005, "Development and in vitro evaluation of a liposomal vaginal delivery system for acyclovir", *Journal of Controlled Release*, vol. 106, no. 1-2, pp. 34-43.

Peppas, N. A. & Buri, P. A. 1985, "Surface, interfacial and molecular aspects of polymer bioadhesion on soft tissue", *Journal of Controlled Release*, vol. 2, pp. 257-275.

Perez-Marcos, B., Iglesias, R., Gomez-Amoza, J. L., Martinez-Pacheco, R., Souto, C., & Concheiro, A. 1991a, "Mechanical and drug-release properties of atenolol-Carbomer hydrophilic matrix tablets", *Journal of Controlled Release*, vol. 17, no. 3, pp. 267-276.

Perez-Marcos, B., Gutierrez, C., Gomez-Amoza, J., Martinez-Pacheco, R., Souto, C., & Concheiro, A. 1991b, "Usefulness of certain varieties of Carbomer in the formulation of hydrophilic furosemide matrices", *International Journal of Pharmaceutics*, vol. 67, no. 2, pp. 113-121.

Pithavala, Y. K., Heizer, W. D., Parr, A., O'Conner-Semmes, R. L., & Brouwer, K. L. R. 1998, "Use of the InteliSite® capsule to study Ranitidine absorption from various sites within the Human Intestinal Tract", *Pharmaceutical Research*, vol. 15, no. 12, pp. 1869-1875.

Pocock, G. & Richards, C. G. 1999, *Human Physiology: The basics of medicine* Oxford Press, Oxford.

Podczeck, F. & Newton, J. M. 1994, "A shape factor to characterise the quality of spheroids", *Journal of Pharmacy and Pharmacology*, vol. 46, no. 82, p. 85.

Pongpaibul, Y. & Whitworth, C. W. 1986, "Preparation and in vitro dissolution characteristics of propanolol microcapsules", *International Journal of Pharmaceutics*, vol. 33, pp. 243-248.

Price, J. M. C., Davis, S. S., & Wilding, I. R. 1993, "Characterization of colonic transit of non-disintegrating tablets in healthy subjects", *Digestive Diseases and Sciences*, vol. 38, no. 6, pp. 1015-1021.

Prudat-Christiaens, C., Arnaud, P., Allain, P., & Chaumeil, J. C. 1996, "Aminophylline bioadhesive tablets attempted by wet granulation", *International Journal of Pharmaceutics*, vol. 141, no. 1-2, pp. 109-116.

Pullen, R. D., Thomas, G. A., & Rhodes, M. 1994, "Thickness of adherent mucus gel on colonic mucosa in humans and its relevance to colitis", *Gut* no. 35, pp. 353-359.

Rathbone, M. J. & Hadgraft, J. 1991, "Absorption of drugs from the human oral cavity", *International Journal of Pharmaceutics*, vol. 74, no. 1, pp. 9-24.

Repka, M. A. & McGinity, J. W. 2000, "Physical-mechanical, moisture absorption and bioadhesive properties of hydroxypropylcellulose hot-melt extruded films", *Biomaterials*, vol. 21, no. 14, pp. 1509-1517.

Repka, M. A. & McGinity, J. W. 2001, "Bioadhesive properties of hydroxypropylcellulose topical films produced by hot-melt extrusion", *Journal of Controlled Release*, vol. 70, no. 3, pp. 341-351.

Repka M.A, McGinity J.W, Zhang F, & Koleng J.J 2002, "Hot-melt extrusion technology," in *Encyclopaedia of pharmaceutical technology*, 2nd ed, Swarbrick J & Boylan J, eds., Marcel Dekker, New York, pp. 203-266.

Reynolds, A. D. 1970, "A new technique for the production of spherical particles", *Manufacturing Chemistry Aerosol News*, vol. 41, no. 6, pp. 40-44.

Rhoads, R. & Planzer, R. 1989, *Human Physiology* Saunders college publishing, Oxford.

Roediger, W. E. 1980, "The colonic epithelium in ulcerative colitis: an energy deficiency disease?" *Lancet*, vol. 2, pp. 712-715.

Russell, T. L., Berardi, R. R., Barnett, J. K., Dermentzoglou, L. C., Jarvenpaa, K. M., Schmaltz, S. P., & Dressman, J. 1993, "Upper gastrointestinal pH in seventy-nine healthy, elderly North American men and women.", *Pharmaceutical Research* no. 10, pp. 187-196.

Sakkinen, M., Marvola, J., Kanerva, H., Lindevall, K., Ahonen, A., & Marvola, M. 2006, "Are chitosan formulations mucoadhesive in the human small intestine? An evaluation based on gamma scintigraphy", *International Journal of Pharmaceutics*, vol. 307, pp. 285-291.

Sangekar, S., Vadino, W. A., Chaudry, I., Parr, A., Beihn, R., & Digenis, G. 1987, "Evaluation of the effect of food and specific gravity of tablets on gastric retention time", *International Journal of Pharmaceutics*, vol. 35, pp. 187-191.

Sanz Taberner, T., Martin-Villodre, A., Pla-Delfina, J. M., & Herraez, J. V. 2002, "Consistency of Carbopol 971-P NF gels and influence of soluble and cross-linked PVP", *International Journal of Pharmaceutics*, vol. 233, no. 1-2, pp. 43-50.

Schiller, C., Frohlich, C., P., Giessmann, T., Siegmund.W., Monnikess, H., Hosten, N., & Weitschies, W. 2005, "Intestinal fluid volumes and transit of dosage forms as assesses by magnetic resonance imaging", *Alimentary Pharmacology and Therapeutics*, vol. 22, pp. 971-979.

Shah, S., Qaqiah, R., Patel, V., & Amiji, M. 1999, "Evaluation of the Factors Influencing Stomach-specific Delivery of Antibacterial Agents for Helicobacter pylori infection", *Journal of Pharmacy and Pharmacology*, vol. 51, pp. 667-672.

Sheth, P. R. & Tossounian, J. 1984, "The hydrodynamically balanced system (HBS): a novel drug delivery system for oral use", *Drug development and Industrial Pharmacy*, vol. 10, pp. 313-339.

Shirazi, T., Longman, R. J., Corfield, A. P., & Probert, C. S. J. 2000, "Mucins and inflammatory bowel disease", *Postgraduate Medical Journal*, vol. 76, pp. 473-478.

Short, M. D. 1995, "Basic Principles of Radionuclide Physics," in *Textbook of Radio pharmacy*, Charles B.Sampson, ed., Gorden and Breach Publishers, pp. 1-6.

Siepmann J., Streubel A., & Peppas N.A. 2002, "Understanding and predicting drug delivery from hydrophilic matrix tablets using the "sequential layer" model", *Pharmaceutical Research*, vol. 19, pp. 306-314.

Siew, L. F., Man, S. M., Newton, J. M., & Basit, A. W. 2004, "Amylose formulations for drug delivery to the colon: a comparison of two fermentation models to assess colonic targeting performance in vitro", *International Journal of Pharmaceutics*, vol. 273, no. 1-2, pp. 129-134.

Singh, B. N. & Kim, K. H. 2000, "Floating drug delivery systems: an approach to oral controlled drug delivery via gastric retention", *Journal of Controlled Release*, vol. 63, no. 3, pp. 235-259.

Slovin, E. M. & Robinson, J. R. 1997, "In-vivo swell kinetics of a series of hydrogel polymers in the cannulated gastrointestinal tract of the canine", *International Journal of Pharmaceutics*, vol. 155, no. 53, p. 63.

Smart, J. D., Kellaway, I. W., & Worthington H.E.C. 1984, "An In-vitro Investigation of Mucosa-adhesive Materials for use in Controlled Drug Delivery", *Journal of Pharmacy and Pharmacology*, vol. 36, pp. 295-299.

Smart, J. D., Riley, R. G., Tsibouklis, J., Young, S. A., Hampson, F. C., Davis, J. A., Kelly, G., Dettmar, P. W., & Wilber, W. R. 2003, "The retention of 14C-labelled poly(acrylic acids) on gastric and oesophageal mucosa: an in vitro study", *European Journal of Pharmaceutical Sciences*, vol. 20, no. 1, pp. 83-90.

Smart, J. D. 2005, "The basics and underlying mechanisms of mucoadhesion", *Advanced Drug Delivery Reviews*, vol. 57, no. 11, pp. 1556-1568.

Soane, R. J., Frier, M., Perkins, A. C., Jones, N. S., Davis, S. S., & Illum, L. 1999, "Evaluation of the clearance characteristics of bioadhesive systems in humans", *International Journal of Pharmaceutics*, vol. 178, no. 1, pp. 55-65.

Staib, A. H., Harder, S., Fuhr, U., Liermann, D., Becker, D., Schollnhammer, G., & Kummel, B. 1990, "Absorption of the psychotropic agent ipsapirone-HCl along the human gastrointestinal tract. An HF capsule study", *European Journal of Pharmacology*, vol. 183, no. 2, pp. 393-394.

Stevens, H. N. E., Wilson, C. G., Welling, P. G., Bakhshaee, M., Binns, J. S., Perkins, A. C., Frier, M., Blackshaw, E. P., Frame, M. W., Nichols, D. J., Humphrey, M. J., & Wicks, S. R. 2002, "Evaluation of Pulsincap(TM) to provide regional delivery of dofetilide to the human GI tract", *International Journal of Pharmaceutics*, vol. 236, no. 1-2, pp. 27-34.

Stockwell, A. F., Davis, S. S., & Walker, S. E. 1986, "In vitro evaluation of alginate gel systems as sustained release drug delivery systems", *Journal of Controlled Release*, vol. 3, no. 1-4, pp. 167-175.

Strous, G. J. & Dekker J 1992, "Mucin-type glycoproteins", *Critical Reviews in Biochemistry and Molecular Biology*, vol. 27, pp. 57-92.

Szurszewski, J. H. 1969, "A migrating electric complex of canine small intestine", *American Journal of Physiology*, vol. 217, pp. 1757-1763.

Takeuchi, H., Yamamoto, H., & Kawashima, O. 2001, "Mucoadhesive nanoparticulate systems for peptide drug delivery", *Advanced Drug Delivery Reviews*, vol. 47, no. 1, pp. 39-54.

Tamburic, S. & Craig, D. Q. M. 1995, "An investigation into the rheological, dielectric and mucoadhesive properties of poly(acrylic acid) gel systems", *Journal of Controlled Release*, vol. 37, pp. 59-68.

Tamburic, S. & Craig, D. Q. M. 1997, "A comparison of different in vitro methods for measuring mucoadhesive performance", *European Journal of Pharmaceutical Sciences*, vol. 44, pp. 159-167.

Thairs, S., Ruck, S., Jackson, S. J., Steele, R. J. C., Feely, L. C., Washington, C., & Washington, N. 1998, "Effect of dose size, food and surface coating on the gastric residence and distribution of an ion exchange resin", *International Journal of Pharmaceutics*, vol. 176, no. 1, pp. 47-53.

Thermes, F., Rozier, A., Plazonne, T. B., & Grove, J. 1991, "Bioadhesion: The effect of polyacrylic acid on the ocular bioavailability of timolol", *International Journal of Pharmaceutics*, vol. 81, pp. 59-65.

Thermes, F., Grove, J., Rozier, A., Plazonnet, B., Constancis, A., Bunel, C., & Vairon, J. P. 1992, "Mucoadhesion of Copolymers and Mixtures containing polyacrylic acid", *Pharmaceutical Research*, vol. 9, no. 12, pp. 1563-1567.

Tobyn, M. J., Johnson, M. J., & Dettmar, P. W. 1995, "Factors affecting In-vitro Gastric Mucoadhesion 1. Test conditions and Instrumental parameters", *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 41, no. 4, pp. 235-241.

Tobyn, M. J., Johnson, J. R. & Dettmar, P. W. 1997. Factors affecting in vitro gastric mucoadhesion IV. Influence of tablet excipients, surfactants and salts on the observed mucoadhesion of polymers. European Journal of Pharmaceutics and Biopharmaceutics. 43:65-71.

Tuleu, C., Basit, A. W., Waddington, W. A., Ell, P. J., & Newton, J. M. 2002, "Colonic delivery of 4-aminosalicylic acid using amylose-ethylcellulose-coated

hydroxypropylmethylcellulose capsules", *Alimentary Pharmacology and Therapeutics*, vol. 16, no. 10, pp. 1771-1779.

Van den Mooter, G., Samyn, C., & Kinget, R. 1994, "The Relation Between Swelling Properties and Enzymatic Degradation of Azo Polymers designed of Colon Specific Drug Delivery", *Pharmaceutical Research*, vol. 11, no. 12, pp. 1737-1741.

Vidgren, P., Vidgren, M., Arppe, J., Hakuli, T., Laine, E., & Paronen, P. 1992, "In-vivo evaluation of spray-dried mucoadhesive microspheres for nasal administration", *Drug Development and Industrial Pharmacy*, vol. 18, no. 5, pp. 581-597.

Vilajato, J. L., Concheiro, A., Seijo, B., & Viana, B. 1986, "Aging of nitrofurantoin tablets containing Carbopol 934 as a binder", *International Journal of Pharmaceutics*, vol. 30, no. 2-3, pp. 229-236.

Voyutskii S.S 1963, *"Autoadhesion and adhesion of high polymers."* InterScience Publishers, New York N.Y.

Voyutskii S.S & Zamazii L.A 1957, *Rubber Chemistry Technology* no. 30, pp. 544-545.

Wakerly, Z., Fell, J. T., Attwood, D., & Parkins, D. 1996, "Pectin and ethylcellulose Film Coating Formulations for Colonic Drug Delivery", *Pharmaceutical Research*, vol. 13, no. 8, pp. 1210-1212.

Ward, F. W. & Coates, M. E. 1987, "Gastrointestinal pH measurements in rats: influence of the microbial flora, diet and fasting", *Laboratory Animals*, vol. 21, pp. 216-222.

Washington, N., Washington, C., & Wilson, C. G. 2001, *Physiological Pharmaceutics: Barriers to Drug Absorption* Taylor and Francis, London.

Watts, P. J., Davies, M. C., & Melia, C. D. 1990, "Microencapsulation using emulsification/solvent evaporation: An overview of techniques and applications", *Critical Reviews in Therapeutic Drug Carrier Systems*, vol. 7, no. 3, pp. 235-259.

Weyenberg, W., Bozdag, S., Foreman, P., Remon, J. P., & Ludwig, A. 2006, "Characterization and in vivo evaluation of ocular minitablets prepared with different

bioadhesive Carbopol-starch components", *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 62, no. 2, pp. 202-209.

Whitehead, L., Fell, J. T., Collett, J. H., Sharma, H. L., & Smith, A.-M. 1998, "Floating dosage forms: an in vivo study demonstrating prolonged gastric retention", *Journal of Controlled Release*, vol. 55, no. 1, pp. 3-12.

Wiggins, H. S. & Cummings, J. H. 1976, "Evidence for the mixing of residue in the human gut", *Gut*, vol. 17, no. 1007, p. 1011.

Wilding, I. R., Coupe, A. J., & Davis, S. S. 2001, "The role of gamma scintigraphy in oral drug delivery", *Advanced Drug Delivery Reviews*, vol. 46, pp. 103-124.

Wilding, I. R., Davis, S. S., Bakhshaee, M., Stevens, H. N. E., Sparrow, R. A., & Brennan, J. 1992, "Gastrointestinal Transit and Systemic Absorption of Captopril from a Pulsed-Release Formulation", *Pharmaceutical Research*, vol. 9, no. 5, pp. 654-657.

Wilding, I. R., Hirst, P., & Connor, A. 2000, "Development of a new engineering-based capsule for human drug absorption studies", *Pharmaceutical Science Today*, vol. 3, no. 11, pp. 385-392.

Yohko, A., Naoki, N., Toshio, K., Shinichiro, H., & Hajime, T. 1994, "In vitro and in-vivo Evaluation of mucoadhesive microspheres prepared for the gastrointestinal tract using Polyglycerol esters of fatty acids and a poly(acrylic acid) derivative", *Pharmaceutical Research*, vol. 12, no. 3, pp. 397-405.

Young, C. R., Koleng, J. J., & McGinity, J. W. 2002, "Production of spherical pellets by a hot-melt extrusion and spheronisation process", *International Journal of Pharmaceutics*, vol. 242, no. 1-2, pp. 87-92.

Zahirul, M., Khan, I., Prebeg, Z., & Kurakoivic, N. 1999, "A pH Dependant Colon Targeted delivery System using Methacrylic acid Copolymers. I. Manipulation of Drug Release using Eudragit L100-55 and Eudragit S100 Combinations", *Journal of Controlled Release*, vol. 58, no. 215, p. 222.