LOW TEMPERATURE COATINGS WITH AQUEOUS FORMULATIONS FOR ORAL COLONIC DELIVERY OF THERMOLABILE MATERIALS

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ABSTRACT

Targeted oral delivery to the colon has previously been achieved with aqueous coatings of amylose and ethylcellulose applied at temperatures above 60°C. Such a high processing temperature may damage certain thermolabile drugs. Therefore, this study sets out to develop aqueous amylose-containing coating formulations which are suitable for use at temperatures below 37°C.

The coating materials used were aqueous coating dispersions of an experimental grade of amylose blended with each of two commercial brands of ethylcellulose. In general, aqueous coating dispersions applied at low temperatures have the increased risk of incomplete film formation due to insufficient energy to ensure fusion of the polymer. Also, aqueous amylose-butanol complex coating dispersion has the increased risk of retaining butanol within the film.

The quality of the film formed from the aqueous coating dispersions were assessed as cast isolated free films and as films sprayed onto pellets. Studies using free films showed that successful fusion of the films could be achieved using high quantities of effective plasticizers. The possible presence of residual butanol within the film was found to be minimal. The specific colon release performance of coating materials were assessed as spray-coats onto pellets containing 5-ASA and glucose pellets and tested in vitro. The ratio of ethylcellulose to amylose, the thickness of the films and the contents of plasticizer were all important influencing factors to ensuring the resistance of the spray-coats to premature drug release. Formulations containing (33.33 - 40)% of amylose, with (28 - 36)% of dibutyl sebacate as plasticizer, applied to 10% total weight gain, showed minimal 5-ASA release in the simulated upper gastrointestinal tract condition with significantly larger drug release in simulated colonic test conditions. However, these same coating formulations were not successful in preventing premature glucose release from the pellets. The success of these low temperature coating formulations appeared to be influenced by the solubility of the drug.
DEDICATION

With love and thanks to my father, mother and brother.

'...God said, “Let there be light,” and there was light. '  Genesis 1:3 (NIV)

........There is nothing impossible when you trust in God.
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<th>Full Form</th>
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<tr>
<td>5-ASA</td>
<td>5-Aminosalicylic acid</td>
</tr>
<tr>
<td>ASTM</td>
<td>American standards for testing of materials</td>
</tr>
<tr>
<td>DBS</td>
<td>Dibutyl sebacate</td>
</tr>
<tr>
<td>DICM</td>
<td>Differential interference contrast microscopy</td>
</tr>
<tr>
<td>DMA</td>
<td>Dynamic mechanical analysis</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulphoxide</td>
</tr>
<tr>
<td>DP</td>
<td>Degree of polymerisation</td>
</tr>
<tr>
<td>DSC</td>
<td>Differential scanning calorimetry</td>
</tr>
<tr>
<td>FID</td>
<td>Flame ionisation detector</td>
</tr>
<tr>
<td>GI</td>
<td>Gastrointestinal</td>
</tr>
<tr>
<td>GIT</td>
<td>Gastrointestinal tract</td>
</tr>
<tr>
<td>GLC</td>
<td>Gas-liquid chromatography</td>
</tr>
<tr>
<td>GOD</td>
<td>Glucose oxidase</td>
</tr>
<tr>
<td>HPLC</td>
<td>High performance liquid chromatography</td>
</tr>
<tr>
<td>HPMC</td>
<td>Hydroxypropyl methylcellulose</td>
</tr>
<tr>
<td>IR</td>
<td>Infra-red</td>
</tr>
<tr>
<td>MANOVA</td>
<td>Multivariate analysis of variance</td>
</tr>
<tr>
<td>MFFT</td>
<td>Minimum film forming temperature</td>
</tr>
<tr>
<td>MMC</td>
<td>Migrating motor complex</td>
</tr>
<tr>
<td>NADPH</td>
<td>Reduced nicotinamide adenine dinucleotide phosphate</td>
</tr>
<tr>
<td>RH</td>
<td>Relative humidity</td>
</tr>
<tr>
<td>RMS</td>
<td>Root mean square</td>
</tr>
<tr>
<td>SCFA</td>
<td>Short-chain fatty acids</td>
</tr>
<tr>
<td>SEM</td>
<td>Scanning electron microscopy</td>
</tr>
<tr>
<td>$T_g$</td>
<td>Glass transition temperature</td>
</tr>
<tr>
<td>TWG</td>
<td>Total weight gain</td>
</tr>
<tr>
<td>POD</td>
<td>Peroxidase</td>
</tr>
<tr>
<td>PTFE</td>
<td>Polytetrafluoroethylene</td>
</tr>
<tr>
<td>USNF</td>
<td>United States National Formulary</td>
</tr>
<tr>
<td>USP</td>
<td>United States Pharmacopeia</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet</td>
</tr>
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LIST OF SYMBOLS

C* Amylose coil overlap concentration
E' Mechanical real in phase, storage modulus
E'' Mechanical imaginary, out of phase, loss modulus
Mw Weight average molecular weight
T1 The average of temperatures measured at the beginning of each experiment
T2 The average of temperatures measured at the end of each experiment
Tav The average of temperatures T1 and T2 for the formulation
T2 Hotelling's multivariate test of significance
Tg The glass transition temperature
y % of cumulative drug released
r Solid weight ratio of Aquacoat to one part solid weight of amyllose
p % of plasticizer content calculated on solid weight of Aquacoat
twg % total weight gain
δ Phase angle
Chapter 1 General Introduction

1.1 General advantages of targeted release

Treatment of a localised diseased condition with drugs may be more effective when the administered drug concentrates at the affected site. Such localisation of drug following any routes of administration is known as drug targeting. Drug targeting may be conducted at different levels of specificity. It may be to a particular organ, first-order targeting eg. to the small intestines, following oral administration, or to a particular cell type within an organ, second-order targeting eg. to the M-cells residing in the Peyer's patch region of the small intestines. A third level of targeting is one where the target is a structure within a particular cell type residing in a particular organ (Wilding et al., 1994a).

Various advantages could be gained by drug targeting. Apart from maximising the therapeutic efficacy of a drug, maintaining the drug in its intact form as close as possible to the target site would minimise the incidence of undesired systemic adverse effects. It also allows a reduction of the required dose while achieving high therapeutic concentration at the diseased site (Rubistein, 1990).

1.2 Why targeted oral colonic delivery systems?

One of the organs that is currently of interest as a site for targeted oral delivery is the colon. A colon targeted drug delivery system would play an important therapeutic application in the treatment of disorders of the large intestine, such as irritable bowel syndrome, colitis, Crohn's disease, colon cancer and infectious diseases where it is necessary to attain a high concentration level of active agent in the affected areas. In addition, the large intestine or colon is attracting interest as a site where poorly-absorbed or endoenzyme degradable drug molecules may have an improved bioavailability. This region of the gastrointestinal tract (GIT) is recognized as having a somewhat less hostile environment than the stomach and the small intestine. As a consequence, drugs which are usually administered parenterally to avoid degradation in the upper GIT, may be
delivered by less invasive methods if targeted to this site. With the explosion of new peptide and protein products as therapeutic agents, utilizing the colon as a site for drug absorption is an attractive option. The colon has also been shown to be highly responsive to agents that enhance the absorption of poorly absorbed drugs when compared to the upper GIT (Muranishi, 1990, Hastewell et al, 1994, Ghandehari et al, 1995).

Rectal and oral delivery methods have been used for gaining access to this area. The rectal form is favoured over the oral form in the presence of nausea and vomiting, when the patient is unconscious, or in the presence of diseases in the upper GIT. It is also an easier route of drug administration to infants and children, especially if the oral form of the drug has an unpleasant taste. Drug contact with the digestive fluid is avoided in this route, hence, less drug degradation is likely to occur. However, this route of administration is not very effective. A high variability in the distribution and absorption of these rectal forms (suppositories and enemas) is observed. Suppositories are only effective in the rectum because of the confined spread to the rectum (Jay et al, 1985) and enema solutions and foams can only offer effective topical treatment to the sigmoid and descending colon (Hardy et al, 1986, Wilding et al, 1995). Absorption of the drugs are also poor and erratic across the rectal mucosa due to problems such as interruption of absorption by defecation, a limiting absorbing surface area, dissolution problems due to the small fluid content of the rectum and drug metabolism by microorganisms in the rectum. It is also not the most convenient or acceptable route of administration for most patients (Eppstein and Longenecker, 1988).

A more acceptable route of delivery would be oral delivery. An ideal oral colon-specific drug delivery system would be one which would not release its drug contents as it passes from the mouth, through the oesophagus, stomach and small intestines but delivers only in the colon. Such an oral delivery system has a greater potential for distribution along the entire colon compared to the rectal forms. This is very important for treating diseased states at the proximal and transverse sections of the colon which would not be reached by rectal forms. Oral delivery systems also have the advantage of the high residence time, thus, increasing total drug absorbed.
1.3 Gastrointestinal tract physiology and its relevance to drug delivery

1.3.1 General outline

An understanding of gastrointestinal physiology is essential if orally administered drugs are to be targeted rationally. When a drug is ingested orally, it passes through the mouth, the oesophagus, the stomach and the small intestines before reaching the large intestines (Fig 1.1). The effectiveness by which drugs can be delivered orally through the gastrointestinal tract is influenced by a number of key physiological variables such as transit time of the preparations through each of the major intestinal segments and the influences acting on the delivery systems in each segment during its residence there. It must be able to withstand the physiological environments in all these regions to arrive intact in the colon. Influences encountered include physical disintegration, intraluminal digestion, mucosal uptake, biotransformation and absorption. Intraluminal pH is also key for the uptake of many drugs. The drug may also interact with the other major constituents of the intraluminal milieu, food and digestive products (Phillips, 1993).

Transit time through the mouth is short hence, exposure to any adverse environment in the mouth is minimal. However, all patients should be advised that solid controlled release dosage forms are not to be chewed but swallowed whole.

1.3.2 Oesophageal motility and transit

The dosage form then passes through the oesophagus which connects the pharynx with the stomach. The oesophagus is not an absorbing or secreting organ. Therefore, its role in oral colonic drug delivery is small. Transit through the oesophagus is normally rapid, with liquids (~ 10 ml) and boluses of solids passing in 10 - 20 seconds after swallowing. Transit is accelerated by water and enhanced by gravity. Some care should be taken in ingestion of solids as the size and shape of the solid formulation can exert considerable influence on the efficiency with which the oesophagus moves solids to the stomach. There are serious clinical implications when solid formulations become lodged
Fig 1.1 The human gastrointestinal tract

- Liver
- Gallbladder
- Duodenum
- Transverse colon
- Ascending colon
- Ileum
- Cecum
- Vermiform appendix
- Stomach
- Pyloric sphincter
- Pancreas
- Ampulla of Vater
- Jejunum
- Descending colon
- Sigmoid colon
- Rectum
- Anus
in the oesophagus. Patients are generally advised to ingest medications with an adequate volume (100ml) of liquid, in the upright position if possible (Phillips, 1993, Evans, 1993).

1.3.3 Gastric motility, transit, pH, enzymatic and microbial considerations

Far more complex physiological influences act on a solid dosage form as it enters the stomach. One of the adverse events it experiences includes disruptions by powerful contractions in the antrum of the stomach. The stomach disperses any large particles into small particles to allow maximum opportunities for digestion. The pylorus then acts as a sieve retaining solids larger than several millimetres but letting very small solids and liquids to pass through to the small intestines. This continues until strong contractions empty large particles into the small intestines (Phillips, 1993).

Apart from physical disruption in the stomach, any large particles retained in the stomach are exposed to the gastric juice which contains hydrochloric acid and pepsin (endopeptidase). The gastric juice has a variable pH ranging from 1.1 - 3.0 in the fasted to 3.0 - 7.0 in the fed state. This can vary considerably on both intra- and inter-patient bases (Dressman et al, 1993, Russell et al., 1993). Enzyme digestion on a dosage form by host will also occur. Protein digestion is carried out in the stomach by pepsin. There is also preduodenal lipase which hydrolyses primarily ingested triglycerides. Acid non-enzymic hydrolysis of certain carbohydrates may also occur in the stomach.

In contrast, microbial enzymic digestion is minimal in the stomach since only small numbers of bacteria inhabit the stomach. This is primarily due to the low pH of the empty stomach. The microorganisms present are the acid-resistant bacteria such as lactobacilli, streptococci and yeasts (level 10^2 to 10^3 cfu/ml content). When the pH of the stomach is raised, bacteria from ingested food can proliferate up to 10^4 to 10^6 cfu/ml. As soon as the pH of the stomach content drops, the bacterial count drops. The acid environment of the stomach is the main barrier to bacteria entering the intestinal tract by mouth. There had been studies relating gastric pH with microbial counts in the stomach (Finegold et al, 1983).
The resident time of a solid dosage form in the stomach can vary from a few minutes to a few hours. Stomach motility is governed by two distinct physiological states; the fasted and fed state. The fasted gastric motility is governed by a rhythmic pattern of migrating motor complex (MMC) of quiescence (Phase I), irregular activity (Phase II) and intense burst of contractile activity (Phase III). Approximately every 90 minutes there is a brief (2 - 10 minutes) burst of intense muscle contraction which begins in the stomach and passes progressively along the small intestine into the distal ileum. This intense contractile event is followed by a period of relative quiescence and thereafter by a period of intermittent, mild to moderate motor activity, this then leads to another intense contractile burst (Kellow et al, 1986).

This regular cyclic pattern becomes disrupted when food is ingested and is replaced with a long period of irregular contractile activity with no apparent rhythm. The duration of this irregular contractile activity is governed by many factors including meal composition (especially lipid content)(Mojaverian et al, 1985), caloric load, particle size and density (Sugito et al, 1990).

These have serious implications on the transit of solid dosage forms. Small solids (a few millimetres in diameter or less) and liquids are believed to leave the stomach quickly but larger solids (of the size of medication capsules) require the presence of a phase III burst of strong contractions to empty from the stomach. In summary, the transit time is dependent on the size and type of dosage form, the phase of the interdigestive cycle when the dosage form is ingested as well as the time which the dosage form is ingested relative to meals and the contents of the meal (Davis et al, 1984a,b, Gruber et al, 1987, Bass, 1993, Evans, 1993). In practice, the transit time of a given dosage form through the stomach is highly variable from individual to individual and even at different times in an individual. It is not possible to predict the gastric residence time of any solid dosage form in a person.

1.3.4 Small intestinal motility, transit, pH, enzymatic and microbial considerations

The contents of the stomach are released through the pyloric sphincter into the
small intestines. The small intestine is about 5 - 8m in length and is divided into the duodenum (20 - 30 cm), jejunum (1.5 - 2.5 m) and ileum (2 - 3.5 m).

A dosage form which passes from the stomach to the duodenum experiences a marked pH change. The pH in the proximal duodenum is similar to that in the stomach but beyond the first few centimetres, the pH rises to ~7 (Evans et al, 1988, Russell et al, 1993, Phillips, 1993). This is due to bicarbonate secreted by the pancreas and the duodenal mucosa neutralising the acidic gastric contents. The neutrality continues essentially unchanged throughout the small intestines (Evans et al, 1988).

Apart from undergoing a marked pH change, the dosage form is also exposed to a cocktail of host enzymes. Pancreatic juice secreted into the lumen of the small intestines at the jejunum contains enzymes needed to maximise digestion and absorption efficiency. The enzymes present are trypsin, chymotrypsin, lipase, α-amylase, carboxypeptidase, elastase and phosphorylase A. In addition, there is bile secretion from the liver. Bile promotes efficient absorption of dietary fat from the gut lumen (micellar solubilisation) and is an important excretory pathway for degradation products. Finally, the terminal stages of digestion are accomplished by hydrolases and proteases located in the brush border cells of the intestinal mucosa. The digestive enzymes present in the epithelial cells are sucrase, maltase, lactase, aminotripeptidase and alkaline phosphatase. The enterocytes contain cytosolic peptidases and a broad specificity β-glucosidase and lysosomal hydrolytic and proteolytic enzymes. Any nutrients that are digested are absorbed through the mucosal surface which is greatly increased by submucosal folds (plicae circularis, folds of Kerckring), villi and crypts of Lieberkuehn.

In the proximal small intestines, the microfloral environment is similar to that in the stomach. Bacterial concentrations is 10³ to 10⁴ cfu/ml. Of note is the almost complete absence of coliforms and Bacteroides. There is very little bacterial enzyme digestions here. The bacteria do not grow well due to chemical factors such as bile juice and lysozyme and the physical factor, peristalsis, which tends to remove microflora at rates faster than they can reproduce. However, in the lower part of the small intestine, the number of bacteria increases due to the neutralization of the bowel content by intestinal
juices and the lowered transit speed. The bacterial count is about $10^5$ to $10^7$ cfu/ml content. The flora also more closely resembled colonic flora with higher counts of coliforms and *Bacteroides*. The terminal ileum appeared to be a "transitional" zone between relatively sterile upper small intestine and the colon with its rich bacterial population (Finegold *et al*, 1983a). These higher bacterial levels in the ileum can affect drug release and also the stability of peptide and protein drugs (Friend, 1991).

Normal motility of the small intestine in the fasting state is characterised by the cyclical appearance of the MMC which starts in the stomach and are propagated distally to the lower small bowel. After eating, the cyclic pattern is disrupted. However, transit through the small intestine is relatively constant. It generally takes about 3 hours for material to pass from the gastroduodenal junction to the ileocecal junction. Liquids, small solids and larger capsule sized units moved essentially at the same rates (Davis *et al*, 1986).

1.3.5 Ileocecal transit

The contents of the ileum then pass through the ileocecal junction which is located between the small and the large intestines. It acts as a mechanical valve preventing the retrograde flow from the colon into the small bowel as well as a functional sphincter regulating flow of material from the ileum to the colon. Contents pass from the distal ileum into the colon as boluses, separated by periods during which flow may be more like a steady trickle. The presence of food increases the motility in the ileocolonic region although such an increase in motility does not necessarily lead to the propulsion of material. The increase in segmental contractile activity would aid mixing and facilitates absorption (Price *et al*, 1993).

The ileocecal junction did not appear to differentiate between liquids and small solids although little or no information is available on the movement of larger solids (Phillips, 1993, Hammer *et al*, 1993). The regulation of the transit of material across the ileocecal junction may be a function of the whole ileocolonic region and not confined to a short sphincter zone (Nasmyth and Williams, 1985). Any dosage forms which have
been passed through the upper GIT at different rates tend to regroup at the ileocecal junction, irrespective of size and enter the colon as a bolus. It would appear that tablet size, in the range of 3 mm - 12 mm does not influence transfer across the ileocecal junction (Adkin et al, 1993).

The ileocecal junction also marks the distinct break from an essentially "sterile" environment to one that is rich in bacterial flora. The concentration of gut microflora rises by a few orders of magnitude upon reaching the colon (Friend, 1991). On the other hand, the luminal pH would drop by about one pH unit compared to the terminal ileum (Evans et al, 1988).

1.3.6 Colonic motility, transit, pH, enzymatic and microbial considerations and its relevance to drug delivery.

From the ileocecal junction, the oral dosage form empties into the large intestines. The large intestines is approximately 1.5m in length and is divided into three main parts: the colon, the rectum and the anal canal. The colon, in turn, is divided into the appendix, the caecum, the ascending, transverse, descending and sigmoid colon.

There are significant environmental changes experienced by the luminal content moving from the ileum to the large intestine. There is a significant pH drop from the small intestine (pH = 7.49) to the proximal colon (pH = 6.37) which then rises gradually through the mid colon (pH = 6.61) and distal colon (pH = 7.04)(Evans et al, 1988). The drop in pH in the lumen of the proximal colon is due to the greater digestive action of the large and diverse populations of anaerobic bacteria (Finegold et al, 1983a). These bacteria digest dietary residues such as resistant starch, non-starch polysaccharides, proteins and peptides (Cummings et al, 1989) into a variety of intermediates to short-chain fatty acids (SCFAs)(Cummings, 1981), ammonia (Macfarlane et al, 1986), H₂ and CO₂ and CH₄. It is these metabolites which lowers the pH of the luminal content. Measurements of bacterial metabolites in colon contents showed that SCFA concentrations are highest and the pH most acidic in the proximal colon (Macfarlane et al, 1992) due to the greater availability of fermentable substrate in the caecum and the
There are over 400 separate bacterial species in the colon, predominantly anaerobic. The highest bacteria concentration in the human colon is $10^{11} - 10^{12} \text{ cfu / ml}$ (Simon and Gorbach, 1984). The highest bacteria count are obtained in the stool specimens. Microbial counts in the transverse colon are, on the average, 2-3 logarithmic values lower than in stool samples; bacterial counts in the terminal ileum were even lower with relatively few anaerobes. These bacteria are found both in the lumen and on the mucosal surfaces with the faecal flora representing luminal flora and is approximately 30% - 60% of the dry weight of faeces (Banwell et al, 1981, Finegold et al, 1983a). Although most of the different bacterial families and genera of the gut flora are found in this region, some 30 - 40 species make up 99% of the bacterial mass (Drasar and Barrow, 1985). The most important anaerobic bacteria are *Bacteroides, Bifidobacterium, Eubacterium, Peptococcus, Peptostreptococcus, Ruminococcus, Propionibacterium, Veillonella* and *Clostridium* (Van den Mooter and Kinget, 1995).

There is remarkable similarity in colonic microbial flora between groups despite differences in diets and disease states of the Asian and Western populations studied (Finegold et al, 1983a). The colonic flora is also remarkably stable although the mechanisms that control the microflora in the large intestine is not fully understood. Freter (1983) proposed that the mechanisms of control are most likely to be due to the interplay of such factors as competition for limiting nutrient, the growth inhibitory actions of metabolites such as SCFAs and adhesion of indigenous species and strains to the gut wall to avoid invasion of ingested luminal microorganisms. Van den Mooter and Kinget (1995) has also reported that within broad limits, the composition of intestinal microflora is more or less constant with dietary factors playing little influence on its composition.

The major factors influencing the metabolic activity of the microflora are age (Rowland, 1988) and intake of drugs (Finegold et al, 1983b, Nord and Heimdahl, 1986). The intestinal microflora of newborn is different from that of a child most probably due to major diet change rather than to age. However, when comparing elderly to young
adults, the elderly subjects harboured fewer bifidobacteria but larger number of fungi and coliforms than younger subjects (Finegold et al., 1983a). Hence, age definitely has some influence. The ingestion of various antimicrobial agents had also been shown to affect the microbial flora, the extent of which is dependent on the type of antimicrobial agent used (Nord and Heimdahl, 1986).

Digestion by the host in the colon is low. There are relatively few host enzymes in the colon compared to the small intestines (Woodley, 1994). The primary source of digestion enzymes in the colon is exoenzymes from the microbial flora. The gut bacteria are capable of catalyzing a wide range of metabolic events. These reactions include hydrolysis (of glucosides, sulphates, amides, esters, nitrites and sulphonates), reduction (of double bonds, nitro groups, azo groups, aldehydes, sulphoxides, ketones, alcohols, N-oxides and arsonic acids), dealkylation, deamination, decarboxylation, heterolytic ring fission, nitrosamine amination, acetylation and esterification (Ashford and Fell, 1993). Such metabolic activities by microflora had been shown to significantly affect drug efficacy (Peppercorn and Goldman, 1972, Shamat, 1993). The bacteria biotransformation of drugs could lead to more active drugs (eg. azoreduction of sulphasalazine, a prodrug, to sulphapyridine and 5-aminosalicylic acid) or less active drugs (eg. reduction of digoxin to cardio-inactive metabolite, digoxin reduction products). It can also lead to greater adverse toxicity of a drug, eg. converting flucytosine to 5-fluorouracil. Normally, mammalian cells cannot convert flucytosine to 5-fluorouracil, only susceptible fungi has the required enzymic activity. Hence, it is a selective systemic antifungal drug. However, studies showed that biotransformation by colonic flora has led to the presence of 5-fluorouracil in healthy individuals (Shamat, 1993).

Unlike the stomach and small intestines whereby normal motility is characterised by the cyclical appearance of the migrating motor complex, there are no orderly motility patterns in the colon. Irregular contractions occur that are apparently randomly distributed in location and time. Low amplitude segmenting contractions coexist with contractions of higher amplitude that can be present simultaneously at points up to 10 cm apart but do not propagate proximally or distally, therefore, do not propel intraluminal contents over large distances. This segmenting pressure activity is probably responsible
for the mixing of bowel contents. Transit of luminal content is due to high amplitude (100 to 200 mm Hg) contractions, travelling distally over distances of at least 24 cm. These are termed high amplitude propagated contractions or giant migrating contractions and differ greatly from the segmenting pressure activity that normally predominates. The high amplitude contractions travel over relatively long segments of the colon and appear consecutively in the more distal bowel suggesting that this activity is coordinated and propulsive (Narducci et al, 1987, Jameson and Misiewicz, 1993).

Various factors affect this general motility pattern, such as food and circadian rhythm. In the fasted state, segmenting pressure activity is low and little transit of content is seen. After a meal, segmenting pressure activity increases and antegrade and retrograde transit of content occurs down a pressure gradient. However, giant migrating contractions which are associated with transit of contents over larger distances in a distal direction (Moreno Osset et al, 1989) occur infrequently between four and five times a day (Narducci et al, 1987). In addition to this basic motility pattern, the circadian rhythm of colonic activity is also important, with contractile activity reducing to virtually zero at night (Evans, 1993).

Predicting the transit rate through the colon is difficult as it is highly variable both among and within individuals. In general, transit through the colon is considerably slower than through the small intestines. Transit can be relatively short (2 - 3 hours in diarrhoea) or relatively long (> 100 hours in constipation)(Evans, 1993). Overall, mean transit time is 36 hours with an upper limit in healthy persons of 72 hours. This is roughly divided into one third of the time spent in each of the ascending and transverse colon, descending colon and sigmoid region and rectum (Phillips, 1993).

The transit through the colon is also affected by eating, diet and drugs (Barrow et al, 1991). Eating, as mentioned above, increases the motor activity in the colon but shows little increase in propulsive activity. Diets that are high in fibres, which lead to increase in faecal bulk, reduce the colonic transit times (Barrow et al, 1991) although studies involving meals with high carbohydrate, high fat and high protein contents showed no influence on the colonic transit (Price et al, 1993). Drugs such as codeine and loperamide increase colonic transit whereas lactulose decreases colonic transit times.
There are also other miscellaneous factors. Coffee, both regular and decaffeinated, increase motor response of the distal colon within 4 minutes of ingestion (Brown et al, 1990). The effect of stress on colonic motility is controversial. The general belief is that stress increases transit rates, however, the findings are often complicated by the prolonged gastric retention which occurs when an individual is stressed. Immobility leads to constipation and exercise is often recommended as therapy. However, increased exercise above normal appears to have no effect (Keeling and Martin, 1987). Transit time also appears to be shorter in men than in women (Lampe et al, 1993).

The vast array of factors affecting the colonic transit rate makes it difficult to predict the rate of transit of a pharmaceutical dosage form through the colon. Studies using various dosage forms had shown that transit of capsules through the proximal colon was independent of capsule density and size (Parker et al, 1988). However, when different tablet sizes were used, bigger tablets appeared to move faster than smaller ones. Tablet streaming in the colon appeared to be controlled by both tablet diameter and volume (Adkin et al, 1993). The authors suggested that smaller dosage forms would be better for targeting drugs to the ascending colon due to the relatively longer transit times compared with larger tablets. This was supported by the observation that when pellets and capsules were used, pellets appeared to move more slowly than capsules in the colon (Hardy et al, 1985). Transit of multiparticulate formulations also did not appear to be affected by disease states eg. active ulcerative colitis, although retrograde movement was observed quite frequently (Davis et al, 1991).

When a drug intended to exert systemic effects is successfully delivered to the colon, it would have to be absorbed to exert its pharmacological activity. The absorption power of the colon is lower than the small intestines due to the reduced surface area of the colon coupled with the poor diffusion of drugs in the high viscosity of the colonic content. This is compensated by the prolonged residence time in this region. The colon as a site for drug absorption had been carefully reviewed by Mrsny (1992a,b). There are many factors which can impede colonic drug absorption. These include enzymatic degradation of drugs by microbial flora, specific and non-specific drug binding, interaction between the negatively charged mucus layer and drug molecules and the physical barrier from the lipid bilayer of individual colonocytes and the occluding
There are two routes for colonic absorption, transcellular and paracellular. The principle route for colonic absorption appears to be transcellular rather than paracellular. The inter spaces are considerably smaller in the colon than in the duodenum or ileum, which may explain the apparent relative importance of the transcellular rather than paracellular absorption pathways in the colon. Unlike the small intestine, there are no documented active transporters for organic nutrients in the mature colon and most drugs rely on lipid solubility, the degree of ionisation of the drug, its size and the pH at the absorption site. Paracellular transport is only possible for relatively small, hydrophilic drugs which are membrane permeable in the colon. Larger compounds, e.g. D-xylene, had been shown to have very poor membrane permeabilities (Yuasa et al, 1995). Such insufficient permeabilities can be modified through the use of chemical enhancers e.g. chelating agents, non steroidal anti-inflammatory drugs, surfactants and mixed micelles, fatty acids and other substances (Hastewell et al, 1994, Van den Mooter and Kinget, 1995). However, caution should be exercised when using permeability enhancers as some may do so by inducing tissue damage (Ghandehari et al, 1995). There is also the risk of increasing absorption of potentially toxic or immunogenic substances from the GIT (Friend, 1991). Nevertheless, the findings that the colon is an absorptive organ is a significant and encouraging one for colonic drug targeting.

In theory, the best site for colon absorption of drugs is the caecum. This is the site with the lowest viscosity of content and greatest mixing action. Recent studies showed a higher absorption in the ascending colon compared to the transverse colon (Gilchrist et al, 1995). For drug delivery systems which rely on microbial metabolisms for specificity of drug release, this is also the site with the highest microbial activity (Macfarlane et al, 1992).

1.4 Approaches to colon-specific drug delivery

Various approaches have been used to target drugs to the large intestines. Below is a summary of some, and by no means all, of the approaches adopted. Special care has been taken to highlight the advantages and disadvantages of each system. The rationale
behind the development of each delivery system is also scrutinised.

There are three basic approaches to targeting drugs to the colon. They are based on time-controlled, pH-change or microbial biotransformation for release. There are also some attempts to merge two or more of these approaches in developing a delivery system.

1.4.1 Time-controlled release

With a time-controlled release, the dosage form has a pre-programmed lag phase before the onset of drug release. The lag-phase could be anything from 3 to 6 hours. While it is logical to assume that an oral colonic delivery system would have a lag phase between the time of swallowing and the time of drug release, it is very difficult to predict how long this lag phase is likely to be. As discussed earlier in Section 1.3, the transit time along the upper GIT is highly variable. Gastric transit has been shown to be anything between 0.5 to 9 hours. Its transit being dependent on fed or fasted conditions, on the size and type of dosage form, the phase of the interdigestive cycle when the dosage form is ingested as well as the time which the dosage form is ingested relative to meals and the contents of the meal (Davis et al, 1984a,b, Gruber et al, 1987, Bass, 1993, Evans, 1993). Small intestinal transit time is less variable but still has a margin of variation of one to two hours. If a relatively short lag-time is allowed, there is the possibility of a premature release of drug in the upper GIT. On the other hand, if a relatively long lag-time is allowed, there is the possibility of little to no release as the dosage form passes through the GIT. Hence, the reliability of a time-controlled drug delivery device to successfully target drug release to the colon is low.

Nevertheless, there are quite a few time-controlled systems which claimed by either in vitro or in vivo studies to target drugs to the colon. A thick insoluble coat is one way to achieve a lag phase. Pozzi et al (1994) developed a device called the Time Clock® which used a hydrophobic material and surfactant coating to delay onset of release. Takaya et al (1995) used gelatin capsules with an inner ethylcellulose coat to fill liquid recombinant human granulocyte colony-stimulating factor for colonic release. Others have used a swellable substance to slow diffusion of drug from the core by compression.
coating (Ishino et al, 1992). A third method is by using a drug container within an ethylcellulose capsule. This capsule has a swellable substance within it to create a pressure for the cap to fall out (Niwa et al, 1995) (Fig 1.2).

**Fig 1.2 Time controlled ethylcellulose capsule**

In time-controlled dosage forms, it is difficult to extrapolate *in vitro* results to *in vivo* performance. The stable, constant *in vitro* environment is a poor reflection of the much more variable *in vivo* conditions. It is hard to say how a reproducible dosage form would perform in a highly unreproducible *in vivo* environment (Ishino et al, 1992, Shameem et al, 1995). *In vivo* results in dogs, which have a very different GI make-up compared to man, also makes interpretation of the results difficult (Takaya et al, 1995, Niwa et al, 1995). In human studies, Pozzi et al, (1994) demonstrated the difficulties and variabilities encountered with a time-controlled drug delivery system. The Time Clock® delivery system administered 30 minutes after a light breakfast showed successful targeting of the dosage form to the proximal colon in most of the volunteers. However, when the same study was repeated by administering Time Clock® 30 minutes after a heavy breakfast, most of the drugs was released in the small intestines.
1.4.2 pH-controlled release

Another commonly used approach to target drug release to the colon is by utilizing a pH sensitive enteric coat. This coat remains intact at low pHs but dissolves at neutral pHs. The primary pH change that is often exploited is that between the stomach and the small intestine which affords the greatest pH change along the GIT in the fasted state (Evans et al., 1988). The pH difference between the small and the large intestine is only one pH unit which is too small to convey the specificity required for drug targeting (Ashford and Fell, 1993). It is assumed that the enteric coat would remain intact in the stomach but begins to dissolve as the pH rises in the remaining regions of the GIT, preferably in the distal small intestine or the colon. In general, exploiting pH changes for drug delivery is considered to be unreliable. This is because the pH in the stomach could rise to the values close to that of the small intestines due to ingestion of food (Russell et al., 1993, Dressman et al., 1993). Certain drugs are also known to affect it. Therefore, the coat has the risk of being disintegrated even within the stomach.

Nevertheless, enteric coatings have been used to protect the drugs from releasing in the stomach in oral colonic delivery. The advantage of using enteric coatings is that it has been used in the pharmaceutical environment before and is proven to be safe and easy to apply with conventional pharmaceutical coating technology. Ashford et al., (1993 b,c) and Watts et al (1994) have shown that it is possible to prevent premature release in the stomach and the small intestines by using very thick enteric coats although Ashford et al (1993b) pointed out that such a system afford poor site specificity in the colon due to the highly variable times and positions of drug release. The authors noted that late disintegration may reduce absorption as there is no back mixing of contents in the colon. Other novel attempts in utilising enteric coatings include synthesis of several methylated derivatives of Eudragit S which can have different water vapour permeabilities and stabilities as a function of pH (Peeters and Kinget, 1993).

1.4.3 Combined time- and pH-controlled release

There are also attempts to combine enteric coatings with other time-controlled
approaches to target drugs to the colon. The rationale here is that, transit time is most variable only during passage through the stomach. Small intestinal transit time is relatively constant. Therefore, if the enteric coating is used to protect the drug during its passage through the stomach, then a time-controlled method with a known lag phase can be used to protect premature drug release during its passage through the small intestine. In an ideal situation, this combined enteric coating and time-controlled approach should perform reasonably well. However, in the case of a raised gastric pH or/and a shorter or longer than anticipated small intestinal transit time, the release of drug may occur far from the targeted site. Quite a few factors would need to be fulfilled before the controlled release dosage form can target successfully to the colon.

A few novel attempts were made in this field. One of which is to enteric coat on an existing time-controlled delivery system eg enteric coating of the Time Clock® system to improve the chances of drug delivery to the colon (Wilding et al, 1994b). Various other researchers have also employed this bilayer coating technology onto standard drug cores with an enteric outer coat and an insoluble inner coat. Their choices of inner coats vary, ethylcellulose (Lin and Ayres, 1992) and high and low viscosity HPMC (Gazzaniga et al, 1995 a, b) have been used as inner coats. Ethylcellulose is a sustained release inner coat while HPMC retards release by swelling and erosion.

Another method involves using a single coat by mixing a sustained release coating polymer with an enteric polymer (Rao and Ritschel, 1995). A mixture of Eudragit NE30D and Eudragit S100 with cellulose acetate phthalate was used to show maximum colonic release with minimum premature release in upper GI conditions.

The enteric coat has also been used in combination with modified drug cores. A sustained release or matrix type drug core with beclomethasone dipropionate is formulated into a 25% HPMC core which is then enteric coated (Steed et al, 1994). A drug core can also be compressed coated with a swellable placebo fraction and coated with a semi-permeable membrane prior to an enteric coating. See Fig 1.3 (Quadros et al, 1995). The device is based on the principle that upon gastric emptying and the subsequent loss of the outer enteric coat, the placebo fraction would imbibe water
through the semi-permeable membrane to swell. The increased pressure as a result of such swelling would push the drug out through a drilled orifice in the dosage form. *In vivo* evaluation was with dogs, hence, some caution should be exercised in predicting its usefulness in man.

Fig 1.3 Diagrammatic cross-section of the colonic delivery system (Quadros *et al.*, 1995)

Another device which is developed along the same principles is the Pulsincap® system (McNeill and Stevens, 1990)(Rashid, 1990). This delivery system consists of an enteric coated capsule with a non-disintegrating hydrogel-plugged body and an enteric cap. The enteric coat and cap dissolves on entering the small intestine, exposing the hydrogel plug stoppering the body. This hydrogel plug then swells independently of pH and is eventually so swollen that it is ejected from the capsule body releasing its drug content (Fig 1.4).

All these devices rely heavily on the ideal gastric and small intestinal pH and the ideal small intestinal transit times to perform properly.
Fig 1.4 Pulsincap® system

ENTERIC-COATED PULSINCAP

ORAL ADMINISTRATION

Water soluble body

Water soluble cap

Acid soluble film coat

Drug formulation

Hydrogel plug

STOMACH EMPTYING

Cap dissolves in intestinal juice

Hydrogel plug expands in intestinal juice

Acid insoluble film coat dissolves in intestinal juice

INTESTINAL FLUID

Swollen expanded plug

Drug released in colon
1.4.4 Microbial enzyme degradation controlled release

One unique feature of the colon compared to other regions in the GIT is the presence of a large number and variety of bacteria (Fig 1.5). Enzymes produced by these bacteria are capable of metabolising substrates which escapes digestion in the upper GIT. It is this feature that is exploited for a truly colon-specific oral delivery system. These colon delivery systems would contain substrates which are not digested in the upper GIT, only in the colon. Unfortunately, most of these substrates in their natural states are soluble, swellable or poor film-formers. Further modifications are needed before they can be used. Below are examples of the attempts made to modify natural substrates to fulfil the requirements needed for colonic delivery.

Fig 1.5 Bacterial flora in the alimentary canal of the human (Mitsuoka, 1978)

There are basically two enzyme systems that are exploited for enzymatic drug release; the azoreductase enzyme system and the polysaccharidase enzyme system. These are the enzymes which are found most abundantly in the colon.
1.4.4.1 Utilisation of the azoreductase enzyme system in oral colonic drug delivery

Azo groups are thought to be reduced to amines by the azo reductase enzymes mediated event in the colon. Such reduction is colon specific. Therefore, azo containing compounds can be exploited for oral colonic delivery. There are very few naturally azo-bonded products that are medicinally useful. Most of the azo-bonded drug substances are synthetically produced. There are three ways in which an azo-bond can be used for colonic drug delivery. One method is to form prodrugs whereby the drug is azo-bonded to an inert material. A second method is to introduce azo cross-links into hydrogel matrices used as drug carriers. The third method is to introduce azo bonds into coating polymers.

The concept of an azo-bonded prodrug is not new. Sulphasalazine, which is 5-aminosalicylic acid (5-ASA) azo-bonded to a sulphapyridine moiety, has been used for a long time as a treatment for irritable bowel disease. 5-ASA was subsequently azo-bonded to various low molecular weight moieties with reduction of unwanted side-effects associated with sulphapyridine. Recent developments include azo bonding of 5-ASA to amino acids (Pellicciari et al, 1993). 5-ASA has to be azo-bonded as 5-ASA cannot be given orally in the treatment of colitis. It is unstable in the acidic medium of the stomach and is absorbed in the small intestine (Van den Mooter and Kinget, 1995).

Further development on this pro-drug concept was recently adopted by azo-bonding 5-ASA to a polymer. Brown et al, (1983) azo-bonded 5-ASA onto a water soluble polymer 7. The potential therapeutic advantages of 5-ASA bonded to polymer 7 include non-absorption / non-metabolism in the small intestine, direct 5-ASA release at the disease site and non-absorption / non-metabolism of the reduction-released carrier polymer. A similar approach was adopted by Schacht et al (1991) using a different polymer. Further modification to this approach was by azo-bonding the drug to a polymer backbone which contains a mucoadhesive moiety. The mucoadhesive moiety on the polymeric backbone complementarily binds to colonic mucosal lectins (Kopeček et al,1992, Kopečková et al, 1994). The rationale is that some bacteria eg. Shigella flexneri adhere to the colonic mucosa of guinea pigs and the binding is fucose and
glucose specific. Therefore, it is believed that such binding is colon specific. The authors claimed that having a mucoadhesive fraction in the polymer would increase its GI residence time to allow for maximum azo-reduction and absorption of 5-ASA in the diseased site. Rubinstein and Tirosi (1994) have shown in a rat study that the colon and the caecum are suitable locations for mucoadhesion due to the lower mucus turnover, lower sensitivity to mucus secretory stimulus and higher adherence properties to mucoadhesive materials than the stomach or the jejunum. However, other authors have claimed that lectin binding is often altered in colon diseased states (Rhodes et al, 1988). Colonic mucins in irritable bowel disease patients could also be different (Podolsky and Isselbacher, 1983, 1984). Furthermore, these bindings, being glucose and fucose specific could be inhibited by unbound fucose and glucose in the diet. In vivo studies so far had been conducted in healthy rats and guinea pigs which showed some specificity of binding towards colonic mucosa.

Despite the fact that all these polymeric prodrugs can deliver 5-ASA successfully to the colon, it has been argued that 5-ASA may not be the drug of choice for these systems (Van den Mooter and Kinget, 1995). The required dose for 5-ASA ranges from 0.5g up to 3g daily and since the drug makes only approximately 20% of the total weight of the prodrug a very large amount would need to be taken orally. It is not clear at this point whether any other drugs can be easily azo-bonded onto the polymers using this prodrug concept.

Other methods which are not drug-specific have also been developed. Hydrogels with azo-links is another possible drug carrier method for colonic delivery. In vitro experiments suggest that the there is biodegradation of the hydrogels (Yeh et al, 1994). However, these hydrogels are also pH-sensitive. The higher the pH, the greater the swelling. The rationale is that the polymer do not swell in the stomach, thus minimising premature drug release in the stomach. Upon leaving the stomach, the hydrogel would begin to swell. Maximum swelling is achieved in the colon exposing the azo bonds for reduction. This approach suffers the possibility of extensive premature drug release due to the swelling of the dosage form in the stomach in the event of a raised gastric pH. Its colonic specificity conferred by the azo bond is compromised by its pH dependence.
A third approach involves incorporating an azo-bond into a coating polymer. Selectivity, unlimited amount of drug incorporation and pH-independent profile are the major benefits of such coatings. This was first reported by Saffran et al (1986) who used a hydrophilic vinyl polymer, copolymers of styrene and hydroxyethylmethacrylate, cross-linked with azo aromatic groups, divinylazobenzene or substituted divinylazobenzenes, to successfully deliver peptide hormones in rats. Subsequently, other researchers have shown the possibilities of incorporating azo bonds within methacrylate coating copolymers, long chain copolymers of benzoic and hexadecanoic acids and polyurethanes (Kimura et al, 1992, Van den Mooter et al, 1992, Van den Mooter et al, 1993, Van den Mooter et al, 1994a,b, Cheng et al, 1994, Van den Mooter et al, 1995a,b, Samyn et al, 1995). These coatings showed varying degrees of success for colonic targeting. It was found that the degradation of the azo bond was not greatly influenced by the chain lengths of the azo aromatic group but by the hydrophilicity and swelling ability of the polymer (Van den Mooter et al, 1993, Van den Mooter et al, 1994b, Cheng et al, 1994). A hydophilic polymer with a high swelling ability is favoured for azo reduction. Such polymers afford poor site specificity as premature drug release from a swollen hydrophilic polymeric film is inevitable. It should also be noted that not all azo bonds incorporated into coating polymers are potentially useful for colonic delivery. Rao and Ritschel (1995) and Bauer and Kesselhut (1995) reported very poor drug release profiles when azo-coating polymers were used.

Although azo-containing polymers have shown some degrees of success in colon targeting, Lloyd et al (1994) have seriously questioned the validity of the findings to date. Most of the studies have failed to distinguish between the changes in macromolecular structure due to azo reduction to amino groups from that of other mechanisms eg. non-specific hydrolysis. The authors suggested that such distinction could only be made if enzyme degradations were carried out in anaerobic cultures representative of the colonic microflora and the degradation products analyzed by HPLC. Other methods of monitoring azo-reduction has since been developed (Soozandehfar et al, 1995). Kimura et al (1992) supported this view. They showed that azo aromatic groups were not reduced to amino groups within the polyurethane films. Instead the azo groups were reduced to hydrazo groups by colonic microflora without breaking the

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polymer backbone. Kimura et al (1992) suggested that a plausible mechanism for drug release from azo-coated dosage forms is the hydration of the film following conversion of azo bonds to hydrazo bonds. The hydrated films then form aqueous channels that allow drug dissolution through the film. There is no mechanical failure due to reduction of azo bonds to amino groups. There is also suggestion that time dependent release could have partially contributed to release shown in some of the above studies. Lloyds et al (1994) criticized the studies done by Saffran et al (1986) suggesting that the latter had failed to demonstrate that the devices had actually reached the large intestines let alone show that the peptides were released as a result of microbial degradation of the azopolymers. Previously, the authors (Lloyds et al, 1994) had also demonstrated that the release of drug from azopolymer coated capsules was time-dependent.

A better understanding of the azo-reduction process is also required. It is now believed that the azo-reduction process represents a non-enzymatic reduction by enzymatically generated flavins (Wilding et al, 1994a). Redox mediators, eg. low molecular weight electron carriers such as NADPH, act as electron shuttles between the intracellular enzyme and extracellular substrate. Therefore, reduction of azo bond is believed to be a general reaction caused by the redox potential of the colonic environment, rather than a specific reaction mediated by only a limited number of intestinal bacteria. Redox potential is an expression of total metabolic and bacterial activity in the colon and is believed to be insensitive to dietary changes. The mean redox potential in the proximal small intestine is -67±90, in the distal small intestine is -196±97 and the ascending colon is -415±72. Therefore, the microflora-induced changes in the redox potential can be used as a highly specific mechanism for targeting to the large bowel.

All these findings suggest that although azo polymers may still offer a means for colon specific drug delivery, some re-evaluations of current findings are needed. If azo-reduction can only be carried out in the presence of NADPH, then some degree of premature release due to the swelling of the hydrophillic polymer could not be avoided to allow better diffusion of the electron carriers within the polymer to mediate the reduction process. Future studies on the safety of these products are also needed.
1.4.4.2 Utilisation of the polysaccharidase and glycosidase enzyme system in oral colonic drug delivery

Biodegradable polysaccharides are also exploited for oral colonic delivery. These could be digested by exoenzymes produced by the colonic microflora. This was shown by the high polysaccharidase and glycosidase activities in various fractions of the human faeces (Englyst et al, 1987). Amylase, pectinase and xylanase were the major polysaccharide-hydrolysing enzymes detected, whilst α-L-arabinofuranosidase, β-D-xylosidase, β-D-galactosidase and β-D-glucosidase were the most active glycosidases. These enzymes are capable of digesting a whole host of materials not digested and absorbed in the upper GIT. Some of the substrates hydrolysed by bacterial glycosidases are listed below in Table 1.1 (Macfarlane et al, 1991).

In theory, all the substrates listed in Table 1.1, if prevented or resisted digestion in the upper GIT, could be utilised to provide the specificity required for oral colonic drug delivery. However, all of them do not naturally possess such qualities and have to be modified to comply to the requirements needed for drug delivery. Three general methods of modifications are (a) the formation of prodrugs, (b) the incorporation of the digestible fractions within inert polymers and (c) the incorporation of inert cross-linkers within digestible polymers.

In recent years, the concept of prodrugs for colonic drug targeting was tested with a series of steroid glycosides (Friend and Chang, 1985). Glycosides of drugs are larger and usually more hydrophilic than the drug themselves. These properties tend to reduce penetration across biological membranes. Orally administered drug glycoside that pass unabsorbed through the upper GIT then enters into the colon whereby the bacterial glycosidases release the hydrophobic drug by cleaving the glycosidic bond. The drug can then be absorbed from the colon. This confers the specificity required. It was found that the rate of enzymatic cleavage in the colon was dependent on both the type of drug and the nature of the aglycone (Friend and Chang, 1985). Subsequently, only dexamethasone-β-D-glucoside was developed further. The prodrug was tested in vivo in rats and in guinea pig models with irritable bowel disease and found to be effective.
Table 1.1 Substrates hydrolysed by glucosidases occurring in the human large intestine (Macfarlane et al, 1991)

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chitobiase</td>
<td>Fungal cell wall components</td>
</tr>
<tr>
<td>α-Glucosidase</td>
<td>Starch, dextrins, maltose, sucrose</td>
</tr>
<tr>
<td>β-Galactosidase</td>
<td>Lactose, arabinogalactan, mucins, galactomannans</td>
</tr>
<tr>
<td>β-Galacturonidase</td>
<td>Pectin</td>
</tr>
<tr>
<td>β-Glucosidase</td>
<td>Various plant glucosides (eg. cycasin, amylgdalin, iso-maltose, laminarin, β-glucans)</td>
</tr>
<tr>
<td>β-Mannosidase</td>
<td>Galactomannans</td>
</tr>
<tr>
<td>β-Glucuronidase</td>
<td>Various glucuronides (eg stilboesterol, benzo(a) pyrine glucuronide), chondroitin sulphate</td>
</tr>
<tr>
<td>α-Mannosidase</td>
<td>Unknown</td>
</tr>
<tr>
<td>Cellobiase</td>
<td>Small oligosaccharides, cellulose</td>
</tr>
<tr>
<td>α-Fucosidase</td>
<td>Glycoproteins</td>
</tr>
<tr>
<td>N-Acetyl-α-galactosaminidase</td>
<td>Glycoprotein</td>
</tr>
<tr>
<td>N-Acetyl-β-glucosaminidase</td>
<td>Mucins, bacterial cell wall components</td>
</tr>
<tr>
<td>α-Arabinofuranosidase</td>
<td>Arabinogalactan, xylans</td>
</tr>
<tr>
<td>α-Galactosidase</td>
<td>Galactomannans, melibiose, stachyose, raffinose</td>
</tr>
<tr>
<td>β-Xylosidase</td>
<td>Xylans</td>
</tr>
</tbody>
</table>

Therefore, prodrugs offered the use of lower doses of drugs to maintain efficacy while minimising side effects (Friend and Tozer, 1992). Although there were indications suggesting that the glycoside prodrug may be also susceptible to hydrolysis in the upper GIT (Tozer et al, 1991), the authors believed that the prodrugs would behave more favourably in man than in the animal models due to a lower levels of glucosidase activity in the small intestines of man and a comparable activity in the colon compared to guinea pigs (Friend and Tozer, 1992). Nevertheless, a second prodrug, dexamethasone-β-D-glucuronide was developed. The relatively lower level of glucuronidase in the upper GIT of animal models rendered greater stability to the prodrug. The sharp gradient of activity
between the small and the large intestines renders specificity (Haeberlin et al, 1993, Fedorak et al, 1995). Based on this model, other drug moieties, eg. budesonide and menthol, were attached to glucuronide. In vitro data suggested that colon specificity could be achieved in vivo (Friend, 1995).

A second model prodrug, developed by the same group of researchers, substituted glycosides / glucuronides with dextran as drug carrier. The drug, glucocorticoid, was ester-linked to dextran. The authors suggested that dextranases and esterases in the colon could degrade the conjugate, liberating drug at the colon. The stability of the prodrug conjugate was dependent on the choice of drug and the type of ester link (McLeod et al, 1993, 1994b). In vivo data in intragastric infusion fed rats showed that the drug was successfully liberated from its prodrug conjugate in the colon. There had not been any in vivo upper GIT stability data of the prodrugs (McLeod et al, 1994a). Both glucuronide conjugate and dextran conjugate prodrugs could prove to be useful oral colonic delivery models. Nevertheless, these prodrugs are drug-specific delivery systems. Not all drugs can be linked to dextran / glucose chain by ester bonds.

The second approach in the development of oral colonic drug delivery is by incorporation of digestible fractions within inert polymers. The digestible fractions may be chemically-bonded or physically mixed with the inert polymers.

Disaccharide-containing insoluble polymer matrices were formed by chemically bonding disaccharide side groups to the backbone of inert polymers. It was assumed that these matrices would remain intact as solid dosage forms in the stomach and the small intestines but eroded in the colon as a result of bacterial polysaccharidase degradation, thus, releasing its drug content only in the colon. However, it was shown that as the hydrophobicity of the polymer was increased to reduce the swelling of the polymer in water, enzyme permeation was limited, reducing glycosidic bond hydrolysis (Rubinstein, 1993, Sintov et al, 1993). Bauer and Kesselhut (1995) also encountered this problem when they used maltose as the biodegradable sequence in an inert polymer. The resulting copolymer showed low biodegradation profiles. This concept of incorporating disaccharides into a polymer to make it biodegradable is a good one, but, in practice, it is very difficult to formulate a polymer matrix that fulfils all requirements.
Bauer and Kesselhut (1995) claimed that the biodegradable sequence of a disaccharide was too small and cannot be attacked by the enzymes. Hence, they substituted maltose with ethylated oligogalactomannans in the inert polymer. Oligogalactomannans were partially ethylated to reduce its hydrophilicity whilst maintaining its digestibility. The resulting copolymer was shown to be stable in vitro in the upper GIT but biodegradable in the colon. This method of partially substituting a polysaccharide to reduce its hydrophilicity but maintaining its digestibility was also adopted in the development of pectin as a carrier for drug targeting to the colon (Ashford et al, 1993b, Ashford and Fell, 1993). High methoxy pectin, whereby 70% of the carboxylic acid groups on the basic galacturonic acid units of pectin were methoxylated, were found to reduce the solubility of naturally occurring pectin without compromising on this biodegradability profile. The high methoxy pectin when compression coated onto a tablet was able to prevent premature disintegration of the tablet in the upper GIT but disintegrated upon reaching the colon. This was successfully demonstrated both in vitro and in vivo. Another form of pectin which had been exploited for colonic delivery was calcium pectinate (Rubinstein et al, 1993, Rubinstein and Radai, 1995). Partially substituted dextrans were also synthesised as film formers for oral colonic delivery (Bauer and Kesselhut, 1995). After numerous attempts with different degrees of substitutions of acetyl-, caproyl-, stearoyl- and lauroyl-dextrans and with different molecular weights of dextrans, they finally found a range of substituted lauroyl-dextrans which were suitable.

Apart from chemical substitution of hydrophilic polymers to reduce premature release of drugs, physical mixture with non-biodegradable fractions could also be used to confer the mechanical support needed to withstand premature drug release in the upper GIT. Glassy amylose that are resistant to digestion in the upper GIT but digestible by colonic microflora was used to coat pellets for oral colonic drug delivery. On its own, glassy amylose swelled in the presence of water and could not prevent premature drug release. This disadvantage was overcome by using a physical mixture of ethylcellulose and amylose for coating. Ethylcellulose provides the mechanical support needed to prevent the swelling of amylose. The mixed polymer coated pellets when tested in vitro and in vivo showed no premature release in the upper GIT but rapid release in the colon due to enzyme digestion of the amylose fraction in the film (Milojevic et al, 1995, 1996a, b, c).
Biodegradable polymers could also be physically mixed with other coating polymers if its own film-forming properties are poor. For example, polygalactomannans were mixed with polymethacrylate (Lehman and Dreher, 1991), β-cyclodextrins were mixed with Eudragit RS® (Siefke et al, 1993) and inulin HP mixed with Eudragit RS® (Vervoort and Kinget, 1996) to form useful coatings for oral colonic delivery.

Instead of inert coating materials, enteric coatings have also been used with biodegradable polymers. Tohzaki et al (1995) used enteric coated chitosan capsules to target peptide drugs to the colon. In vivo studies in rat showed that the chitosan capsules survived the journey to the colon where its content was then released.

The third general method of development of oral colonic drug delivery is to chemically crosslink the digestible polymers. The purpose of cross-linking these polymers is to reduce the swelling of these polymers in water. Swelling would lead to release of drugs prior to entry into the colon. However, the extent of cross-linking needs to be controlled. If a polymer is too heavily cross-linked, it may take a very long time before the digestion of the polymer can occur. A balance needs to be struck between premature drug release and sufficient enzyme degradation.

Various polymers had been cross-linked and tested, in vitro and in vivo in animals, for their usefulness as drug carriers for oral colonic delivery. These included cross-linked chondroitin sulphate (Rubinstein et al, 1992, Sintov et al, 1995), cross-linked dextran hydrogels (Brønsted et al, 1995a,b, Hovgaard and Brønsted, 1995) and cross-linked galactomannans (Hirsch et al, 1995, Bauer and Kesselhut, 1995). Cross-linked chondroitin sulphate and galactomannans were developed as coating materials whereas cross-linked dextran was developed as a hydrogel matrix. The results suggested varying degrees of success. In addition to cross-linking density, the choice of drug used would also influence the rate of drug release (Brønsted et al, 1995a,b). Therefore, in developing a colonic drug delivery carrier with cross-linked polymers, the cross-linking density had to be constantly modified depending on the solubility of the drug.
Based on the findings shown above, it is clear that there are many approaches which could be exploited to successfully deliver drugs to the colon. Each method has its own advantages and disadvantages. The use of oral colonic drug delivery to replace rectal and parenteral administrations of drugs is anticipated in the near future. The oral route is by far a safer, more patient acceptable and more effective route of administration.

1.5 Introduction to the present study

This project is an extension of a previously successful oral colonic drug delivery project which used a coating containing glassy amylose and ethylcellulose for targeted drug delivery (Milojevic, 1993). As mentioned above, glassy amylose was used to provide the specificity for colonic targeting by being non-digestible in the upper GIT but digestible in the colon. Ethylcellulose provided the mechanical support to resist physical disruptions during transit in the upper GIT. In the previous project, the coating dispersion mixture was applied at 60°C. This temperature was chosen because it was believed to be the lowest temperature whereby successful film formation of glassy amylose and ethylcellulose could be guaranteed. This project focuses on investigating the possibilities of developing a coating polymer mixture which could be applied at lower temperatures, preferably no higher than the body temperature, 37°C.

There is a need for low temperature coating because recent developments suggest that quite a wide range of potential oral colonic delivery candidates may be thermolabile. These include peptides, proteins and health-promoting microorganisms. The recent advancement in delivering proteins and microorganisms as possible therapeutic agents are reviewed below.

An understanding of the film-forming mechanism of each polymer was needed to appreciate why 60°C was chosen as the operational temperature and how film-formation at a lower temperature may be possible.
15.1 Peptides and proteins as potential thermolabile candidates for oral colonic delivery

Traditionally, proteins and peptides are administered parenterally to overcome proteolytic degradation in the GIT. However, it may be possible to administer these orally if colonic drug targeting proves successful. The colon, with its reduced enzyme proteolytic activities compared to the small intestines, may be a potential site for the delivery and absorption of proteins and peptides (Woodley, 1994).

Chen (1992) and Rao and Ritschel (1995) reviewed some of the physiological aspects of the GIT and the physicochemical properties of the peptides that are involved in protein and peptide delivery. Physiological aspects such as reduced absorption surface areas, increased lumenal viscosity and moderate peptidase activity from the microbial flora in the colon could adversely affect peptide absorption. Nevertheless, the relatively constant pH and reduced endopeptidase activity with prolonged transit time works favourably for colonic delivery. The ideal physicochemical properties of peptides for colonic absorption were also considered. The peptide presented for absorption should be soluble, with its isoelectric point at a distant from the pH of the absorption site, stable and has reduced tendency for aggregation. Successful oral peptide administration can be achieved if two major issues are addressed adequately. One, the stability of the peptides in the colonic environment prior to absorption and two, the development of delivery systems which could overcome barriers to give reproducible and sufficient peptide absorption across the colonic mucosa. Various attempts were made to address these two issues.

There is a lot of controversy regarding the stability of peptides delivered to the colon. Saffran et al (1986) claimed success in delivery and absorption of insulin and vasopressin in the colon. Subsequently, other researchers had backed the claim that peptides were stable in the colon with various other model peptides (Antonin et al., 1992, Geary and Schlameus, 1993, Quadros et al, 1994, Takaya et al, 1995). However, some researchers had challenged this by showing that despite the lowered protease activity in the colon, some peptides were still significantly degraded (Langguth et al, 1994, Tohzaki et al, 1995, Rao and Ritschel, 1995, Bai, 1995, Bai et al, 1995). Nevertheless, such
enzymatic degradations of peptides could be limited with the concomitant administration of protease inhibitors. Various protease inhibitors and their relative usefulness to reduce peptide degradation had been shown (Tohzaki et al., 1995, Bai, 1995, Bai et al., 1995). Apart from protease inhibitors, enzyme degradation could also be limited with the use of prodrugs of peptides (Friis et al., 1996).

Once the stability of the peptides in the colon was ensured, their absorption in the colon could still be low. This could be due to physical barriers to absorption such as the presence of faecal material (Antonin et al., 1992), the insoluble nature of the drug (Takaya et al., 1995) or the low permeability of the colonic mucosa (Rubas et al., 1995). In order to overcome such barriers of absorption, it was proposed that the drug should be delivered in the soluble or microemulsion state (Takaya et al., 1995, Rao and Ritschel, 1995). This would overcome poor diffusion and dissolution problems due to the lower water content and higher luminal viscosity in the colon. In addition, absorption enhancers could also be used to increase paracellular absorption in the colon (Hastewell et al., 1994, Ghandehari et al., 1995).

All these suggest that oral protein and peptide delivery is possible when every aspect of delivery is carefully considered and controlled.

1.5.2 Health promoting microorganisms as potential thermolabile candidates for oral colonic delivery

Apart from peptides and proteins, health-promoting microorganisms may also be potential candidates for oral colonic delivery. There is currently much interest in increasing the numbers and activities of those health promoting bacteria in the colon. This is based on the belief that the bacterial species resident in the large intestine have some influence on the health of the host. These are divided into those, such as bifidobacteria and lactobacilli, which are thought to be beneficial to health, as well as those, such as hydrogen-sulphide-producing microorganisms, which are harmful. The aim of introducing health promoting health promoting microorganisms is to increase the healthy benefits of the resident microflora at the expense of the harmful species. Three
methods are being used to achieve this, using probiotics, prebiotics and synbiotics. Probiotics are the beneficial bacteria which are ingested directly (Fuller, 1989). Prebiotics are ingredients which can beneficially affect the host by selectively stimulating the growth and/or activity of one or a limited number of bacteria in the colon, that can improve the host health. Prebiotics cannot be digested in the upper GIT of the host only by bacterial activity in the colon (Gibson and Roberfoid, 1995). A further proposal is to use a combination of pro- and prebiotics known as synbiotics. Synbiotics are ingredients which are specifically fermented by live bacteria when they reach the colon (Gibson, 1996).

In pharmaceutical colonic delivery, it is the targeting of probiotics to the colon which is most attractive. The concept of probiotics and its ability to affect the gut microflora was tested in recent studies in Japan and the Netherlands with placebo controlled studies. Healthy volunteers were fed with *Lactobacillus casei* Shirota for 4 weeks. Although the acid-resistant microorganisms were not targeted specifically to the colon, both studies showed significant increase of *L. casei* Shirota counts in the wet faeces. Hence, it would appear possible to preferentially populate certain groups of bacteria using probiotics (Tanaka, 1996).

Probiotics may be useful as prophylaxis to various disease states because there is clear evidence of a controlled interaction between the gut commensal flora and the mucosal immune system (Perdigon *et al*, 1993). It may be possible to selectively increase the strains of those which show strong adjuvant/stimulatory activities (Collins, 1996). In addition, there are evidence to show that some intestinal microorganisms can produce substances with genotoxic, carcinogenic and tumour-promoting activity (Rowland, 1996). Probiotics provide a possible method to modulate the production of such harmful agents with health promoting ones (Aso *et al*, 1995, Lidbeck *et al*, 1992, Pool-Zobel *et al*, 1993, Reddy *et al*, 1993, Yokokura, 1989).

Probiotics have also been used to therapeutically treat disease or medical conditions. The choice of microorganisms so far had been restricted to acid-resistant strains due to the lack of a colon-specific targeting carrier. Nevertheless, the studies had
yielded favourable results. The microbial lactase activity had been successfully used to enable lactase deficient humans absorb lactose (Marteau et al, 1990). Clinical trials with freeze dried Saccharomyces boulardii 1g/dose had also shown that the probiotic could be used to prevent antibiotic associated diarrhoea / colitis due to Clostridium difficile (MacFarland et al, 1994, Surawicz et al, 1989). Lactobacillus casei had also been used to shorten the duration of the diarrhoea during rotavirus enteritis in children (Bellomo et al, 1980, Boudraa et al, 1990, Isolauri et al, 1991, Majamaa et al, 1995). There are other more controversial areas where the usefulness of probiotics are still being investigated such as the effect on cholesterol metabolism and the development of new oral vaccines (Marteau, 1996). Based on the above, targeting probiotics to the colon is a therapeutic necessity in the future.

1.5.3 Amylose

Oral colonic delivery of such potential thermolabile candidates requires special low temperature operational procedures. An understanding of the polymer behaviour of amylose was required to ensure that any changes in the operation temperature was not going to affect its useful functional behaviour of providing specificity for oral colon targeting.

1.5.3.1 The definition of amylose

Amylose is defined as that starch polysaccharide which binds 19.5% of its weight of iodine at 20°C (Banks and Greenwood, 1975). It forms the characteristic blue complex with iodine.

Amylose is a high molecular weight component of starch. It is made up of α-1,4-bonds (Fig 1.6). It is essentially linear, although evidence is now accumulating that some amyloses may contain a few very long branches (Whistler et al, 1984, Curá et al, 1995).
The value of $n$ can vary from anything like 300 to 3000. This variation is very much dependent on the source of amylose. Amylose is usually present as 20 to 35% of starch although breeders have managed to develop starches which contain no amylose (waxy type) or those with much higher amylose content (e.g. amylomaize with 50% - 85% amylose)(Biliaderis, 1991).

1.5.3.2 The formation of glassy amylose film

Amylose can be extracted from starch by an aqueous leaching process (Adkins and Greenwood, 1966, Ellis and Ring, 1985). The extraction process involves leaching an aqueous slurry of raw granular starch above its gelatinisation temperature. When heated above the gelatinisation temperature, the granular order is lost. The granules swell to many times their original size and amylose is preferentially solubilized. The solubilised amylose in water is separated from other insoluble granular starch fractions by centrifugation and filtration. The amylose solution is then further purified and stabilised with the addition of butan-1-ol. The final product is an aqueous amylose-butanol complex dispersion. The complex dispersion is used as a coating material. A schematic representation of how amylose in the form of an aqueous amylose-butanol complex dispersion is converted to glassy amylose is shown in Fig 1.7.
Glassy amylose would be successfully formed only when the preceding stages are well controlled. Therefore, an understanding of each stage is vital. The first stage involves the regeneration of amylose solution from amylose-butanol complex dispersion. The temperature required to melt the complex would depend on the nature of the complex i.e. crystalline ($T_m = 68^\circ C$) or amorphous ($T_m = 48^\circ C$)(Whittam et al, 1989). Generally, it is preferable to form amylose film from regenerated amylose solution as films cast from the dispersion are usually hazy in appearance rather than being completely clear (Wolff et al, 1951).

Once the metastable amylose solution is formed, depending on its concentration, as it cools, either a gel or a precipitate would form. Below a concentration known as the
coil overlap concentration, C*, a precipitate would form. Above C*, a gel would form. The value of C* is dependent on the linearity and degree of average polymerisation (Ellis and Ring, 1985). If gelation is favoured, as the amylose solution cools, the amylose chains reassociate or retrograde leading to irreversible phase separation of polymer rich and polymer deficient regions. In the continuous polymer rich phase, the molecular level reassociation of amylose chains results in the supermolecular formation of amylose filaments. These filaments are assemblies of many amylose chains. The filaments form an interpenetrating matrix structure i.e. a gel. (Miles et al, 1984, Miles et al, 1985, Müller et al, 1995).

Upon further rapid drying of the gel, the amylose filaments pack more densely forming a glassy amylose film. The formation of any glassy material can be visualised as follows: as the solution forms a supercooled liquid, there is a simultaneous increase in viscosity, until at a temperature known as the glass-transition temperature, $T_g$, when the viscosity is around $10^{12}$ Pas, the liquid-like structure is "frozen in". The polymer chains in the liquid-like structure is not packed in a regular fashion, hence, a glassy, amorphous material is formed. The main determining factor of whether a glassy or crystalline material is formed is in the rate of drying. If a very slow drying rate is used, the polymers have sufficient time to reorganise in a regular fashion, hence, a crystalline material is formed. During the coating process where rapid drying is essential, only glassy amylose is likely to be formed.

1.5.3.3 The reasons for the specificity of glassy amylose for colonic delivery

Glassy amylose was chosen for oral colonic delivery because not all forms of amylose are resistant to digestion in the upper GIT. For example, amylose solution and amylose found in the starch granules are not necessarily resistant. Only retrograded amylose resist upper GI digestion (Englyst and Cummings, 1987, Ring et al, 1988, Leloup et al, 1992b). In retrogradation the free amylose chains are reassociated. The amylose chains becomes entangled and the intra- and inter- molecular hydrogen bondings of amylose (Fig 1.8 and 1.9) reduce its flexibility to fit into the active site of the enzymes making retrograded amylose resist digestion in the upper GIT (Englyst et al, 1992, Ring, 1995).
Apart from the inherent resistance of amylose due to inter- and intra-molecular hydrogen bonding, a second mechanism of resistance involving filaments of amylose was also proposed (Cairns et al, 1995, Ring, 1995). Amylose filaments are formed when a large number of reassociated amylose chains are assembled. These 20 nm wide filaments form a three dimensional network which can be viewed under SEM (Leloup et al, 1992a). As the degree of entanglement increases, the rate and extent to which the enzyme can diffuse into the core of the amylose substrate decreases. This leads to a further decrease in the hydrolysis of amylose. The schematic representation of this is shown in Fig 1.10.
Glassy amylose is a form of retrograded amylose which is believed to resist upper GIT digestion by both these mechanisms (Cairns et al, 1995). However, once the upper GIT resistant amylose is passed into the colon, it is digested by the bacterial microflora (Englyst and Cummings, 1987, Englyst and Macfarlane, 1986, Englyst et al, 1992). The end products are shown to be fatty acids and non toxic gases (Fig 1.11).

Fig 1.11 Consequences of the fermentation of carbohydrate in the human large intestine (Englyst and Cummings, 1987).

The amylose which escapes digestion in the upper GIT can be digested by the colonic microflora because bacterial cell-bound amylases are considerably more efficient than the pancreatic amylases in the initial stages of starch hydrolysis (Macfarlane and Englyst, 1986). The difference in efficiency of mammalian and microbial α-amylases could be explained in terms of the enzyme structure. All the α-amylases active sites have
two domains, a catalytic and a substrate binding domain. These domains are separate (Bertoft et al, 1993, MacGregor, 1993). Although all the α-amylases contain similar catalytic domains folded in a characteristic pattern known as \((\beta/\alpha)_6\)-barrel (Fig 1.12), they contain different substrate binding domains or subsites. For example, pig pancreas α-amylase would have five subsites (4-8 of Fig 1.13) while barley α-amylase may have as many as ten (0-9 of Fig 1.13)(MacGregor, 1993). It is differences in these subsites which give rise to subtle differences in the initial rates of enzyme hydrolysis and fermentation products.

Fig 1.12 Schematic representation of structural features of an α-amylase \((\beta/\alpha)_6\)-barrel domain. Loop 1 to 8 link the C-terminal ends of adjacent helices and carry amino acid residues that form the subsites of the enzyme active sites

Fig 1.13 Possible subsite structure of the active site of an enzyme of the α-amylase family. Each of subsites 0 to 9 can interact with one glucose residue of the substrate. The bond scission would occur between the glucose rings bound at subsites 6 and 7 glassy amylose films are formed from amylose-butanol complex dispersion.

In addition to its increased efficiency, there is an abundance of these enzymes in
the colon to aid digestion of resistant amylose. Macfarlane and Englyst (1986) have shown that there is a group of amylolytic bacteria, rather than just one species, which is responsible for the amylolytic activity (Table 1.2). These amylolytic bacteria make up to over 50% of the bacteria count of faeces (Table 1.3). Therefore, it is not surprising that the amylose could be digested in the colon. These amylolytic activities are not uniformly distributed along the colon but are highest in the caecum and ascending colon (Macfarlane et al., 1991, Macfarlane et al., 1992), allowing drug from amylose-coated dosage forms to be released all along the colon.

Table 1.2 Generic distribution of 120 starch-hydrolysing bacteria isolated from the faeces of six persons (Macfarlane and Englyst, 1986)

<table>
<thead>
<tr>
<th>Genus</th>
<th>No. of isolates</th>
<th>% Total amylolytic bacteria isolated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bifidobacterium</td>
<td>70</td>
<td>58.3</td>
</tr>
<tr>
<td>Bacteroides</td>
<td>22</td>
<td>18.3</td>
</tr>
<tr>
<td>Fusobacterium / Butyribrio</td>
<td>12</td>
<td>10.0</td>
</tr>
<tr>
<td>Unidentified Gram-variable rods</td>
<td>16</td>
<td>13.3</td>
</tr>
</tbody>
</table>

Table 1.3 Enumeration of amylolytic bacteria in faeces. Results are presented as range and mean of counts obtained from the stools of ten subjects (Macfarlane and Englyst, 1986)

<table>
<thead>
<tr>
<th>Bacterial counts/g faeces*</th>
<th>Amylolytic bacteria</th>
<th>Total anaerobic count</th>
<th>Mean % amylolytic bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range</td>
<td>Mean</td>
<td>Range</td>
<td>Mean</td>
</tr>
<tr>
<td>1.1 x 10^{10} - 3.3 x 10^{12}</td>
<td>5.0 x 10^{11}</td>
<td>5.5 x 10^{10} - 3.5 x 10^{12}</td>
<td>8.9 x 10^{11}</td>
</tr>
</tbody>
</table>

* Individual plate counts are the average of three determinations.
1.5.3.4 The heat requirement for the formation of glassy amylose

During the formation of glassy amylose, the highest input of heat energy occurs in the regeneration of the amylose solution from amylose-butanol complex dispersion. The aqueous amylose-complex dispersion has to be heated to ~70°C to melt the complex. Once amylose solution is formed, it has to be maintained at a high temperature to prevent it from solidifying. Hence, the coating material was sprayed at 60°C to prevent clogging of the spray nozzle. These steps would have to be omitted to achieve formulations suitable for low temperature coating.

1.5.4 Ethylcellulose

To overcome the problem of amylose swelling, ethylcellulose was added to the system. Ethylcellulose is an ethyl-substituted cellulose ether (Fig 1.14). The glucose units are joined by β-1,4 links. The ethoxyl substitution values of commercial products range from a DS of 2.2 to 2.6 ethoxyl groups per anhydrous glucose units. This corresponds to an ethoxyl content of 44.5 to >49% (Rekhi and Jambhekar, 1995). It is hydrophobic and has been successfully formulated into commercially available aqueous coating dispersions.

Fig 1.14 Ethylcellulose

1.5.4.1 The mechanisms of film formation from ethylcellulose coating dispersions

These commercially available aqueous coating dispersions contain discrete ethylcellulose spheres. Each of these ethylcellulose spheres, with less than 1 μm in...
diameter, contains hundreds of ethylcellulose chains. During deposition of these discrete spheres onto the surface, the water evaporates, the dispersion is condensed and the ethylcellulose particles become closely packed. If the condition favours coalescence, these particles would become deformed and fused together to form a film (Fig 1.15)(Onions, 1986a). The formation of a homogenous film from an aqueous dispersion of discrete ethylcellulose spheres is a complex, multistage process (Vanderhoff et al, 1973).

Fig 1.15 (a) Aqueous dispersion deposited on a surface (b) water evaporation (c) close contact spheres with water filling voids (d) water evaporation and polymer deformation (e) continuous coating

The initial stages of coalescence would only occur if there are various strong driving forces to overcome the inherent hardness of the ethylcellulose spheres and their repulsive electrostatic forces. Various models have been proposed to explain the driving forces which cause coalescence of these polymeric spheres to occur. These are reviewed in detail in various literature (Sheetz, 1965, Onions, 1986a, Bindschaedler et al, 1985). As more water is driven off the closely packed polymeric particles, a strong capillary pressure, located among interparticulate contact points, is generated. This pressure compresses the polymeric particles and forces them to fuse. How far particle fusion can proceed at this stage would depend on the rate of disappearance of the aqueous phase in addition to the surface tension and mechanical properties of the polymeric phase. After water is completely evaporated, the pressure caused by water-polymer interfacial tension
is replaced by air-polymer surface tension. This new pressure forces the polymers to fuse further, provided the polymeric particles are deformable. A dry, transparent, apparently continuous film is formed. This is generally accepted as the first stage of the process of coalescence.

The second stage, often considered to occur after the coating process, is a gradual completion of coalescence observed during ageing of the film. This process, which is time and temperature related, may be speeded up by a post coating high temperature treatment. This process of stabilisation of the film coat is known as curing (Goodhart et al, 1984, Onions, 1986b, Lafferty, 1992, Hutchings et al, 1994).

1.5.4.2 The heat requirement for the formation of ethylcellulose film

Both the first and the second stages of coalescence involves energy. Sheetz (1965) using the thermodynamic analysis of latex film formation theory, pointed out that the most important source of energy in ensuring particle fusion is the environmental heat energy. Heat is converted to useful work by evaporation of the water to overcome the repulsion forces to form a film. Satisfactory film formation depends on the prevailing temperature at which film formation is to take place. The minimum temperature below which the polymer particles do not possess sufficient energy to coalesce into a film is known as minimum film forming temperature (MFFT). MFFT is influenced by a number of factors, including the Tg of the polymer component. The relationship between the polymer Tg and the MFFT is complex as the MFFT is a property of the entire latex formulation and not just the polymer phase (Nicholson, 1985, 1989). One of the coating formulation additives shown to affect the MFFT is the plasticizer. Effective plasticizers tend to lower the MFFT of the coating dispersion. The MFFT of the ethylcellulose dispersions had been shown to be successfully lowered using various plasticizers to around 40°C (Goodhart et al, 1984, Aquacoat handbook, Laicher et al, 1995). Nevertheless, these plasticized ethylcellulose dispersions still required high coating temperatures, i.e. higher than 37°C to achieve complete coalescence (Yang and Ghebre-Sellasie, 1990, Parikh et al, 1993, Laicher et al, 1993, 1995).
Such additions of plasticizers to the coating formulations could influence their premature drug release profiles in the upper GIT (Lindholm et al, 1986, Li et al, 1990, Phuapradit et al, 1995). The influence of these plasticizers on the digestibility of the films also had not been investigated. Moreover, even if the application temperature is reduced, there is still the problem of curing. Milojevic (1993) showed that curing at 60°C for an hour was required to ensure long-term stability of the films. Therefore, low temperature coating provide the added challenge in the production of stable, continuous films.

1.6 Scope of present study

The scope of the present study is in developing aqueous coating formulations containing amylose and ethylcellulose which can be applied to solid dosage forms at temperatures below 37°C. When applied to a solid dosage form, these film-coats should be able to fulfil three criteria. First, they should be able to prevent any premature release of drug from the solid dosage form during its transit through the upper GIT. Second, when the solid dosage form reaches the colon, the amylose fraction within the films should be digested easily to give a rapid onset of drug release. Finally, the films should be stable, with particular reference to its drug release profile, on storage.
Chapter 2 Coating Materials

The two main coating polymers used were amylose and ethylcellulose. Various plasticizers were also used.

2.1 Amylose

Amylose in the form of an aqueous amylose-butanol complex dispersion was a ready to use coating material. Two sources of this complex dispersion were used in the experiments. One source of the complex dispersion was obtained from The Institute of Food Research, Norwich. The other was produced at The School of Pharmacy, University of London. Both sources were made by the same extraction procedure from the same batch of starch. In all subsequent experiments, the source of aqueous amylose-butanol complex was indicated.

Amylose in the form of a powdered material was also obtained. Powdered amylose is not a ready to use coating material. Some preliminary experiments to formulate the amylose powder into a ready to use aqueous coating material was done.

2.1.1 The production of aqueous amylose-butanol complex dispersion

Aqueous amylose-butanol complex dispersion was produced from smooth seeded pea starch, Nastar® (Cosucra, Belgium) by an aqueous leaching process developed by Adkins and Greenwood (1966). Smooth seeded pea starch was used because of its high amylose content (~35%), low gelation temperature (~70°C) and low swelling ratio at gelation temperature (Nastar® technical brochure). It had also been shown that amylose extracted from smooth seeded pea starch was purest compared with amylose extracted from other sources. The extraction process, done at The School of Pharmacy, University of London, used 30g of starch dispersed in 1200ml of cold distilled water in a conical flask. The powder was then thoroughly wetted by stirring or swirling the flask. The conical flask containing the starch slurry was then placed into a water bath set at 90°C for an hour. Oxygen-free nitrogen was bubbled through the hot slurry throughout this
process. Nitrogen prevents any oxidative degradation processes. The bubbling action also aids mixing of the starch powder with water.

During the initial stages of this extraction process, it is important that the concentration of the slurry and its temperature are well controlled. The concentration of the starch slurry is important because as the granules swell when the gelatinisation temperature is reached, there must be sufficient continuous water phase outside the granules to allow diffusion of amylose from the granules to occur. At high concentrations, the diffusion of amylose out of the swollen granules has been shown to be hindered. Amylose becomes solubilised within the aqueous phase inside the granule (Svegmark and Hermansson, 1993). The concentration of the slurry is dependent on the type of starch. Potato starch, for example, has a higher swelling ratio than cereal starch, leading to closer packing of the potato starch granules at lower concentration. A slurry concentration of < 3% w/v of pea starch was found to be successful for extracting amylose from it (Botham, 1993).

The slurry extraction temperature must also exceed the gelatinisation temperature to ensure that the starch granule is sufficiently swollen and amylose can be preferentially solubilised. Beyond the gelatinisation temperature, different temperatures would extract different molecular weight fractions of amylose, with different degrees of linearity and different gelation behaviours (Ellis and Ring, 1985). The higher the temperature is above the gelatinisation temperature, the higher is the molecular weight fraction and the lower is the degree of linearity of amylose extracted. This results in gels of lower shear modulus. For pea starch, the extraction temperature was set at 90°C to extract all high molecular weight fractions of linear amylose.

After an hour, the hot slurry was equally divided into six 250 ml centrifuge buckets and centrifuged at 2000 x g for 30 minutes in a MSE High Speed 18 centrifuge machine. Immediately after centrifuging, the supernatant was poured through a sintered glass filter no 2. A clear filtrate should result. The primary function of the centrifugation and filtration process was to separate the amylose solution from other insoluble components of starch.
Butan-1-ol was then added in excess to complex with amylose. The mixture was left overnight at 4°C. A white, fluffy amylose-butanol complex was formed. Butanol was added to stabilise the amylose in solution.

These purification and subsequent stabilisation of amylose, should be carried out as quickly as possible because amylose in solution is inherently unstable. The free amylose chains within the solution tend to reassociate. This process of reassociation of the free amylose chains is known as retrogradation. The rate of retrogradation would increase as the solution is cooled. In the presence of starch granules, amylose would associate to form an interpenetrating matrix trapping the swollen granules within it (Fig 2.1) (Biliaderis, 1991). In the absence of starch granules, amylose would still retrograde, forming either amylose gel or precipitates depending on the concentration of the solution (Ellis and Ring, 1985). Once this occurs, amylose could only be solubilised at temperatures above 160°C (Ring et al, 1987). Hence, care was taken to ensure that the amylose solution obtained after centrifugation and filtration was clear. Retrograded amylose would give a cloudy suspension.

Fig 2.1 A schematic representation of the process and structures observed during heating and storage of aqueous suspensions of granular starch (Biliaderis, 1991).

The aqueous complex dispersion was then concentrated by a second centrifugation process. The dispersion complex was centrifuged at 2000 x g for 30
minutes at ~10°C. The supernatant which contained excess butanol and water was discarded. The sediment, which was amylose-butan-1-ol complex, was resuspended in as little distilled water as possible. The concentration of this aqueous complex dispersion was measured by a loss of weight on drying process. The dry solid weight left after heating to dryness a carefully weighed portion of the aqueous amylose-butanol complex was taken as the amylose content of the dispersion. The concentration was expressed as % w/w. The aqueous amylose butan-1-ol complex dispersion was stable and would not retrograde on storage.

Once the dispersion was made, two simple tests were performed to ensure that amylose-butanol complex was formed. The first test involved placing ~10ml of the complex dispersion into a beaker and heating it to 90°C in a water bath under a continuous stream of nitrogen. The dispersion should turn clear. Failure to regenerate a clear solution would indicate that amylose had retrograded before butanol was added to form amylose-butanol complex.

The second test involved viewing a sample of the dispersion under low magnification of polarised light microscopy. Spherulitic complexes should be seen. In addition, there should be no birefringent starch granules (granule structures with the characteristic maltese cross) or presence of "ghost" granules (granule structures but without the maltese cross). The presence of such granular structures would indicate that the sample was contaminated with other starch components, such as amylopectin, which would be predominantly found in the granular shell.

All batches of amylose-butanol complex dispersions made at The School of Pharmacy passed these two tests. However, when used for spray-coating onto pellets, they clearly behaved differently from the dispersions supplied from The Institute of Food Research, Norwich (See Chapter 5). There was also batch to batch variations.
2.1.2 Investigation into the qualitative differences between the amylose-butanol complex produced at The School of Pharmacy, London and that at The Institute of Food Research, Norwich.

The complexes were analysed at The School of Pharmacy, London for its complex formation using the Differential Scanning Calorimetry (DSC), Perkin-Elmer DSC-7. The complexes were prepared by removing all excess water by centrifugation. The amount of water removed were known, so the aqueous content of the complex was known. The aqueous content in both cases were standardised. The complex was then carefully weighed into hermetically sealed aluminium pans and scanned between 10 and 150 °C at 10°C per minute. The DSC results indicated that the two samples were different (Fig 2.2). Based on the knowledge of the complex dissociation temperatures (Whittam et al, 1989), it would appear that the sample made at The School of Pharmacy, London was a mixture of amorphous and crystalline materials whereas the sample made at Norwich was only crystalline amylose-butanol complexes. This is because the amorphous complex dissociation temperature was ~50°C whereas the crystalline dissociation temperature was ~70°C. This interpretation of results was agreed by Dr R. L. Botham (Institute of Food Research, Norwich). Dr R.L. Botham also claimed that under microscopy, the amylose-butanol complex made at The School of Pharmacy, London showed predominantly amorphous structures compared to the samples made at Norwich which showed predominantly crystalline structures.

To confirm these results and to ensure that the samples were pure, the amylose purity and molecular size distribution were determined using iodine binding experiments and gel exclusion chromatography, respectively (All experiments were done at the Institute of Food Research, Norwich and the results were interpreted and communicated by Dr R.L. Botham). The molecular size distribution of samples from The School of Pharmacy was comparable to those made from The Institute of Food Research. However, the iodine binding test showed that the samples made at The School of Pharmacy bound more than 40% w/w of iodine compared to 19.5% w/w for the samples made at The Institute of Food Research samples. All results were reproducible. The iodine results were unusual. By definition, amylose binds 19.5% w/w of iodine and an iodine binding
Fig 2.2 Differential scanning calorimetry results of the two samples of amylose-butanol complexes. The top trace was obtained from the amylose-butanol complex made at The Institute of Food Research, Norwich, the bottom trace was obtained from the amylose-butanol complex made at The School of Pharmacy, London.

![Differential scanning calorimetry results](image)

value that is less than 19.5% indicates impurity. This is because all starch components bind 19.5% w/w of iodine or less. Amylopectin, the only other high molecular weight component of starch binds 0.2% w/w of iodine. The results indicate that a high molecular weight non-starch contaminant, $M_w = \sim 10^3$, must have been introduced accidentally during production.

The formation of amorphous rather than crystalline amylose-butanol complex could be explained by the different experimental conditions used at The School of Pharmacy compared to those at The Institute of Food Research. The batch size used for amylose production at The School of Pharmacy was much smaller than the batch size used at The Institute of Food Research. At The School of Pharmacy, the amylose solution was formed by centrifuging the starch slurry in 250ml containers whereas at The
Institute of Food Research, the amylose solution was formed from centrifuging the starch slurry in 500ml containers. Consequently, the amylose solution formed at The School of Pharmacy, after centrifugation and filtration and prior to butanol addition was significantly cooler (~25°C) than amylose solution formed at this stage at The Institute of Food Research (~50°C). When butanol was added to hot amylose solution at The Institute of Food Research and allowed to cool slowly to ~4°C, complex formation is slow, favouring crystalline complex formation. However, when butanol was added to a cool amylose solution at The School of Pharmacy, complex formation was fast favouring amorphous complex formation. Therefore, the problem was overcome by reheating the amylose solution obtained from centrifugation and filtration to 50°C prior to butanol addition. It was not possible to maintain the temperature of the centrifuge machine above 30°C due to an inbuilt cooling system. The mixture was then allowed to cool slowly in a fume cupboard to room temperature prior to being stored at 4°C overnight. More crystalline samples were observed under polarised light microscopy. Results were confirmed by Dr R.L. Botham.

The introduction of contaminant into the production of amylose-butanol complex cannot be explained. Double distilled water (pH = 4.5 compared to pH = 5.0 at The Institute of Food Research) was used. All tubings, glass ware and centrifuge containers were replaced with new ones. Glassware and centrifuge containers were also checked to ensure that no residual surfactants were left after the cleaning process. This was done by collecting the distilled water used to rinse the cleaned containers and analysing the water for iodine binding values. No iodine binding components were found. The whole extraction process was then repeated but without any starch to test if the contaminant was leached at high temperatures from the vessels used. Once again, no iodine-binding components were found. Finally, the extraction process was repeated with the same batch of starch but with a fresh batch of butan-1-ol. The amylose extracted still showed an iodine binding value of greater than 40% w/w (All iodine binding results conducted and communicated by Dr R.L. Botham).

This production problem was deemed to be beyond the scope of this project. Therefore, amylose-butanol complex dispersion was obtained from The Institute of Food
Research, Norwich. All batches supplied were tested with iodine binding test to ensure minimum batch to batch variation.

2.1.3 The properties of amylose powder produced from pullulanase-treated starch

Alternative sources of amylose were also investigated. A source of amylose powder was obtained from Avebe, Netherlands. This amylose was produced from potato starch. Unlike aqueous amylose-butanol complex dispersion, amylose powder is not a ready to use coating material. However, this source of amylose was attractive because no starch waste was generated in the production process. In the previous method, all non-amylose fractions were discarded as waste. The starch was pullulanase-treated and all the starch components were converted to amylose. To understand the basis of this new method of production and how it could possibly affect the film-forming properties of amylose, an understanding of all the starch components was necessary.

Starch is made up of two high molecular weight components, amylose and amylopectin, and some intermediate molecular weight materials (Banks and Greenwood, 1975, Biliaderis, 1991).

Amylopectin, like amylose, is made up of glucose units linked by $\alpha$-1,4 bonds but unlike amylose, it is not linear. They are branches linked through $\alpha$-1,6 bonds. These branches vary in length from $\sim$10 to $>100$ glucose units. On average, there is one branch point for every 20-25 main chain units (Fig 2.3).

The exact arrangement of chains within the amylopectin molecule is still unclear although current research favours the "cluster-model" in which short chains, which may be multiply branched, are arranged in clusters on longer chains that are themselves linked together (Fig 2.4)(Ring et al, 1987, Biliaderis, 1991). The chain length in the most abundant species by weight have degrees of polymerisation of 15 - 20 and 45 - 55. The molar ratio of short to long chains varies between 3:1 and 12:1, depending on the botanical source of the starch (Ring et al, 1987, Biliaderis, 1991, Ring, 1995). Amylopectin binds less than 0.2% w/w iodine at 20°C. It gives a reddish colour when
complexed with iodine (Banks and Greenwood, 1975).

Fig 2.3 Amylopectin

Fig 2.4 A generalised diagram demonstrating the cluster model structure of amylopectin (Biliaderis, 1991)

Starch also contains a substantial amount of intermediate materials (~5 - 10%). These may consist of linear chains with $50 < \text{DP} < 200$ and lightly branched molecules of low molecular weights ($<10^4$) with greater chain length and higher iodine binding capacities than that of normal amylopectin (Banks and Greenwood, 1975, Biliaderis, 1991).

Except for amylose, the other starch components are unsuitable for use as coating materials for oral colonic delivery for two reasons. One, they have poor film-forming abilities (Ring et al, 1987) and two, they are readily digestible in the upper GIT (Englyst and Cummings, 1987). However, by using pullulanase, a debranching enzyme, all the branched components of starch could be cleaved to linear glucose $\alpha$-1,4 linked chains.
Pullulanase specifically cleaves the $\alpha-(1,6)$ bonds but leaves all $\alpha-1,4$ bonds intact. Hence, the final product contains only linear $\alpha-(1,4)$ linked glucose units. By definition, these $\alpha-(1,4)$ linked glucose units which binds 19.5% w/w of iodine is amylose.

However, when the molecular weights of the amyloses produced by the aqueous leaching of pea starch and by pullulanase-treated potato starch were investigated by gel exclusion chromatography, the molecular weight distributions of the two amyloses were found to be very different. (Results communicated by Dr. R. L. Botham). The pea starch amylose showed a single broad peak chromatogram whereas the Avebe amylose showed multiple peaks in the chromatogram (Fig 2.5 and Fig 2.6). The molecular weights of the amylose based on the retention time peaks of the chromatograms are summarised in Table 2.1.

Table 2.1 Results of gel exclusion chromatography on amylose samples

<table>
<thead>
<tr>
<th>Sample</th>
<th>Retention time (minutes)</th>
<th>Molecular weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Avebe amylose</td>
<td>22.25</td>
<td>90151</td>
</tr>
<tr>
<td></td>
<td>25.69</td>
<td>9787</td>
</tr>
<tr>
<td></td>
<td>27.10</td>
<td>3946</td>
</tr>
<tr>
<td>Pea amylose</td>
<td>21.50</td>
<td>200000</td>
</tr>
</tbody>
</table>

The discrepancy in the molecular weight results of these two amylose samples could be explained by the differences in the method of production of amylose. The pea starch amylose was produced by leaching amylose found in the natural state in pea starch. The average degree of polymerisation of pea starch amylose is from 2000 to 3000 (Ring et al, 1985), hence, a very high molecular weight would be expected. In contrast, Avebe amylose was produced from a mixture of naturally occurring amylose and from cleaving branches of amylopectin. The naturally occurring amylose would give the highest molecular weight peak while the amylose produced from cleaving amylopectin branches would give the lower molecular weight peaks. Pullulanase debranched amylopectin had been shown to give characteristically bimodal distribution of molecular weight.
Fig 2.5 Gel exclusion chromatogram of pea starch amylose
Fig 2.6 Gel exclusion chromatogram of Avebe amylose
weight fractions with variations only in the number, average chain length and relative amounts of these fractions (Fig 2.7) (Biliaderis, 1991, Ring, 1995). Hence, the difference in the molecular weight profiles were observed.

Fig 2.7 Elution profile of a pullulanase-debranched amylopectin isolated from smooth pea starch (cv Triumph) on a BioGel P-10 column (2.4 x 96 cm), flow rate 16 ml/h. The minor peak, $V_o$, could represent an amylose impurity and (or) very long chains present as an integral part of the branched molecule liberated upon debranching (Biliaderis, 1991).

This difference in molecular weight fractions could result in differences in film-forming abilities of these two amyloses. Generally, it is believed that for a linear polymer, the higher the molecular weight, the better is its film-forming ability (Rowe, 1982, 1986, 1992). This was also shown to be true for amylose (Wolff et al., 1951). However, in this project, amylose was used with ethylcellulose. The good film-forming abilities of ethylcellulose could possibly compensate for any poor film-forming properties of this lower molecular weight amylose. Therefore, the suitability of this sample of amylose for film-coating was investigated further.
2.1.4 The formulation of amylose powder into an aqueous amylose coating material

To convert the amylose powder to an amylose coating material, three different approaches were tested. These included forming amylose-butanol complex dispersion, amylose gel dispersion and cold water soluble amylose.

To form aqueous amylose-butanol complex dispersion, amylose powder needs to be first converted to amylose solution. This was done by autoclaving a slurry of 4%w/w of amylose powder in solution (Model no AE89/148, Southtrim Autoclaves Ltd., UK). The autoclaving cycle used was the porous load cycle. The maximum temperature for this cycle was 134°C, held for 3 minutes. The entire cycle, including heating up and cooling down to 100 ±10°C, took 35 minutes. Although this resulted in the formation of amylose solution, the approach had several disadvantages. The high temperature used in the autoclaving procedure could potentially hydrolyse amylose reducing its degree of polymerisation (Banks and Greenwood, 1975). This was minimised by autoclaving the amylose in degassed water sealed under nitrogen. Degassing also served another purpose, the amylose powder was moistened, which increased the rate of solubilisation (Svegmark and Hermanssen, 1993). The shortest possible autoclaving cycle to achieve 134°C was also used. Another disadvantage was that the amylose solution was unstable and had to be used at very hot temperatures in order to avoid retrogradation. This was overcome by adding butanol to the hot amylose solution to complex with amylose. The final product was an aqueous amylose-butanol complex dispersion.

The initial step in the production of amylose gel dispersion was similar to the production of amylose-butanol dispersion in that it involved converting the amylose powder dispersion into an amylose solution by autoclaving. However, the hot amylose solution was then allowed to cool to a gel. This gel was suspended in water and blended with a Silverson homogeniser to form a 1% w/v gel dispersion which was then passed through a microfluidiser (Model 110s, Microfluidics Corp. USA) to form a submicron amylose gel dispersion in water. The gel particle size was sized using a nanosizer (Autosizer 2, Malvern Instruments, UK) which showed a normal distribution with a mean particle size of 0.5μm. The gel dispersion was then concentrated at 40°C using a
rotary evaporator. This gel dispersion was stable on storage i.e. no further gel formation or aggregation was seen. No preservatives were added.

The third approach was to form a readily cold water soluble amylose using a modified version of a patented technique (US Patent 3,086,890). Once again, the initial step involved converting the amylose powder dispersion into amylose solution by autoclaving. The amylose solution was then dried as quickly as possible ~80°C in a fan-assisted oven. The resultant retrograded amylose was pulverised using a small scale ball mill. However, the amylose powder formed failed to dissolve even at 80°C. (The patent claimed that the resulting powder should dissolve at 4°C).

The aqueous amylose-butanol complex dispersion was considered a much better coating material than the amylose gel dispersion for two reasons. The first reason is based on the film-forming mechanism. Amylose film is believed to be formed from the amylose-butanol complex dispersion via amylose solution. In solution, the amylose chains have the maximum ability to bridge whereas amylose film formed from the amylose gel dispersion was most likely to be by layering of the dried down gel particles. No bridging of individual gel pieces was likely. Hence, in theory, it would form a weaker film. Secondly, the amylose gel dispersion is more likely to support microbial growth. A choice of preservative would have to be included. This posed some difficulties. It was unclear what effect an added preservative would have on the film forming abilities of amylose. More importantly it was not clear if a preservative could interfere with the digestion properties of amylose in the colon. Therefore, the amylose powder, in the form of an aqueous amylose-butanol complex dispersion, was chosen as the formulation of choice.

2.2 Ethylcellulose

Two of the commercially available aqueous coating dispersions, Surelease® EA7100 (Colorcon, UK) and Aquacoat® ECD30 (FMC, USA), were investigated in this project.
These commercially available aqueous coating dispersions have been developed to overcome the hazards of using organic solvents. Both are pseudolatexes. Psedolatex is one of the two categories of aqueous coating dispersions, the other is known as the latex. Latexes and pseudolatexes differ only in their method of manufacture, the final products are virtually the same. A true latex is prepared by polymerisation of a monomer or monomer blend, normally emulsified in an aqueous medium with the aid of surfactants. The polymerisation process requires addition of an initiator, which may be chosen to function by free radical, anionic or cationic polymerisation mechanisms. In general, the particle size of the polymer produced by this way tends to be very small and there can be problems of toxicity associated with the presence of residual monomer. Pseudolatexes, on the other hand, are prepared from existing water-insoluble polymer. Surelease®EA7100 and Aquacoat®ECD30 are both prepared by the latter method and are deemed safer since there is no risk of residual amounts of free radicals within them (Onions, 1986a).

Pseudolatex dispersions also have the advantage of being low in viscosity while being high on polymer solids. The viscosity of a 40% pseudolatex dispersion of ethylcellulose is only 20cP, compared with 50cP for a 12% ethylcellulose solution of ethanol (Onions, 1986a). The low viscosities of pseudolatex dispersions bring further advantages such as reduced coating times, less water available to penetrate tablet surface, less water evaporation needed resulting in use of less heat, thus, indirectly protecting the drug from heat degradation.

Although Surelease® and Aquacoat® are both ethylcellulose pseudolatex dispersions, they differ in their contents and methods of manufacture. In manufacturing Surelease®, ethylcellulose (20cP, USNF), the plasticizer (dIBUTyl sebacate) and the stabilizer (oleic acid) are melted to create a homogenous mixture. The mixture is then diluted with ammoniated water to stabilise the dispersed polymer. The final product is a 25% w/w solids dispersion. Surelease is subject to some settlement over time but gentle to moderate agitation will redisperse the material. Surelease will foam if mixed vigorously but is not sensitive to high-shear mixing (Chang et al, 1987, Iyer et al, 1990, Moore, 1989, Surelease qualitative datasheet). The glass transition temperature of
Surelease is ~ 35°C (Iyer et al, 1990).

By comparison, Aquacoat is manufactured by dissolving ethylcellulose (10cPs, premium grade) in a water-immiscible organic solvent and then emulsifying in water in the presence of the anionic surfactant, sodium lauryl sulphate and the stabiliser, cetyl alcohol. After the crude emulsion is homogenised to generate submicron droplets, the organic solvent is evaporated under vacuum to form an aqueous pseudolatex. The final product is a 30% w/w solids dispersion. No plasticizer is incorporated in the pseudolatex during the manufacturing phase, the dispersion has to be plasticized prior to coating. The glass transition temperature of the Aquacoat is ~120°C (Onions, 1986b, Chang et al, 1987, Iyer, et al, 1990, Lafferty, 1992).

2.3 Others

Dibutyl sebacate, Sigma, UK
Batch no: 40H0279

Diethyl citrate, Eudraflex®, Röhm Pharma, Germany
Batch no: 18-90762

Triacetin, Colorcon, UK
Batch no: 4490

Tributyl citrate, Croda Surfactants Ltd., UK
Batch no: 31/181

Acetyltributyl citrate, Reilly Chemicals, Belgium
Batch no: 930524

Glycerol, Sigma, UK
Batch no: 41H0083

PEG 400, Hoechst, Germany
Batch no: 106301906

Polypropylene glycol, Sigma, UK
Batch no: 129F0124

Tween 80, Merck, UK
Batch no: 3139220
Chapter 3 Free Film Study

3.1 Introduction

The possibility of forming films at low temperatures (i.e. < 37°C) using mixtures of aqueous amylose-butanol complex and ethylcellulose coating dispersions were investigated using isolated free films. This was done in stages. The first stage involved the study of low temperature film-forming behaviour of each polymeric dispersion. As mentioned previously in Chapter 1.5, both aqueous polymeric dispersions were recommended for use at temperatures higher than 37°C, their use at temperatures below 37°C had never been evaluated. Therefore, the minimum temperature at which each of these polymeric dispersions could form films were determined. Formulation variables which may contribute to this minimum temperature were also investigated. Once the film-forming abilities of each polymeric material was established, their combined polymeric behaviour was investigated.

3.1.1 Investigations of amylose films formed at temperatures below 37°C

One of the greatest concerns about forming amylose films below the complex melt temperature of 68°C was the possibility of having residual butanol within the films. Butan-1-ol could be present as unbound or chemically complexed to amylose. Unbound butanol within the film could be detected by leaching it out using a butanol solvent, e.g. methanol. Complexed butanol within the film could be detected by dissolving the film using an amylose solvent, e.g. dimethyl sulphoxide (DMSO). The methanol and DMSO solvents recovered from treating the amylose films could then be investigated for the presence of residual butanol by gas-liquid chromatography (GLC).

In the presence of water, an amylose film would swell. As the amylose film swells, the porosity of the film increases. This is undesirable and should be minimised as far as possible. There was some indirect evidence to suggest that the rate of swelling may be related to the concentration of the aqueous amylose-butanol complex dispersion from which the film was formed. Scanning electron microscopy results showed that
when more concentrated amylose-butanol complex dispersions were used to form gels, these gels contained higher densities of amylose filaments (Leloup et al, 1992a). A higher density of amylose filaments leads to a higher degree of filament entanglement. This natural cross-linking process was believed to reduce the swelling of the amylose film when these gels were dried to form films (Botham, 1993). Therefore, the swelling or porosity properties of amylose films formed from different concentrations of amylose-butanol complex dispersions were evaluated using a diffusion cell assembly. The optimum concentration for further experimentation was then selected.

3.1.2 Investigations of ethylcellulose films formed at temperatures below 37°C

Previously, the glass transition temperature, $T_g$, of ethylcellulose had been shown to be lowered with plasticizers to only $\sim(35 - 45)^\circ$C (Goodhart et al, 1984, Lippold et al, 1990, Iyer et al, 1990, Aquacoat handbook, Surelease datasheet). These temperatures, which are closely related to the film forming temperatures (Chapter 1.5), were considered too high for coating thermolabile products since the spray-coating temperature was recommended to be at least $(10 - 20)^\circ$C above the minimum film forming temperature (MFFT) (Lehman et al, 1989, Lippold et al, 1990, Laicher et al, 1993). Further lowering of the MFFT with more plasticizers would be needed. However, the Aquacoat handbook suggested that 20-24% of various plasticizers, based on Aquacoat solids, was the upper limits of plasticization. Above these limits, the excess plasticizers would be excluded from the Aquacoat films. No further improvement in coalescence was noted above these limits (Hutchings and Sakr, 1994). The validity of this claim was investigated. No additional plasticizers were recommended for use with Surelease.

Apart from the quantity, the type of plasticizer had also been shown to greatly affect the MFFT of ethylcellulose (Onions, 1986b, Bodmeier and Paeratakul, 1992, 1993). The most obvious criterion in choosing a plasticizer was considered to be the solubility of the plasticizer in the polymer (Onions, 1986a). When the polymer and plasticizer solubility parameter are the same, there is maximum interaction, hence, maximum chances of influencing the film-forming process (Entwistle and Rowe, 1979).
Nevertheless, the use of hydrophilic plasticizers with hydrophobic ethylcellulose had also been recorded (Aquacoat handbook, Bodmeier and Paeratakul, 1992, 1994b) Therefore, the interactions of a selection of commonly used hydrophilic and hydrophobic plasticizers with ethylcellulose were evaluated.

Finally, the mixing time (Lippold et al, 1990, Iyer et al, 1990, Bodmeier and Paeratakul, 1994c) and the order of introduction of plasticizers and other excipients into the pseudolatex coating dispersions have also been reported to be important. The most commonly used mixing time was 30 minutes. This relatively long period of mixing was required to enable the plasticizer to partition from the aqueous to the polymeric phase (Iyer et al, 1990, Aquacoat handbook). However, there was evidence to suggest that further diffusion of the plasticizer from the periphery to the core of the polymeric bead, followed by the complete solvation of the plasticizer within the polymeric beads could occur up to 12 hours post-mixing (Lippold et al., 1990). Contradictory orders of introduction of plasticizers and other coating excipients were also found. There were at least three known orders of introduction of plasticizers. The Aquacoat handbook and Bodmeier and Paeratakul (1994c) recommended that the plasticizer be added prior to addition of any other water-soluble excipients to the ethylcellulose dispersion. However, Iyer et al (1990) added water prior to addition of plasticizer to the ethylcellulose dispersion. Röhm Pharma, manufacturer of acrylic polymer coating dispersions, recommended mixing of the plasticizer with water, prior to addition into the coating dispersion (Röhm Pharma technical services, 1993).

Therefore, various plasticized formulations of ethylcellulose, containing different types and quantities of plasticizers, mixed in different orders of addition and with different mixing times were evaluated. The most efficient plasticizer is defined as the one that gives the best balance of end use properties for the amount incorporated. In this case, the end use is film formation of ethylcellulose dispersion at low temperature. Therefore, the formulation with the lowest MFFT was determined. These formulations were evaluated visually by casting films onto glass petri dishes or a MFFT bar. The former method merely indicated whether a MFFT close to the room temperature could be achieved. The method was not able to determine the specific MFFT for each formulation.
This method was employed as an initial screening process.

The MFFT test is a standard method for determining the minimum temperature at which latexes coalesce to form continuous films (ASTM D 2354-91). This test evaluates the performance of the entire latex and not just the film forming polymer. In this method, the MFFT was determined by visual observation of cracking or whitening in films that had dried over a MFFT bar having a controlled temperature gradient. The test is limited to latexes having minimum film formation temperatures below 25°C and should be determined using a standard apparatus (Fig 3.1). The apparatus is a stainless steel bar with a temperature gradient across it. This temperature gradient was created by immersing one end of the MFFT bar into a hot water bath and the other end into a cold water bath.

However, in this study, various modifications to the original design of the apparatus were done (Fig 3.2). The wide surface onto which the coating material was spread was divided into four equally sized surfaces. Commercially available MFFT bar, (Sheen/ICI minimum film forming temperature apparatus, Sheen Instruments Ltd., UK) had shown that such a wide surface was not needed. The advantage of having four surfaces was that it allowed four formulations to be tested simultaneously. The controls and the tests could then be subjected to identical testing conditions. The smaller surface for spreading the coating dispersion meant that the spreading process was easier to control and the overflow container was not needed. Uniform thickness of the coating material along the surfaces of the MFFT bar was achieved by leveling the bar prior to each experiment. The bar was levelled using the adjustable supports and spirit levels. More temperature sampling points than its original design were also added to the MFFT bar to increase accuracy.
Fig 3.1 The minimum film forming temperature bar according to ASTM D 2354-91 guidelines

Fig 3.2 The modified version of the minimum film forming temperature bar
Fig 3.3 A photograph of the minimum film forming temperature apparatus

Fig 3.4 A photograph of the adjustable spreader
3.1.3 Investigations of the combined amylose/ethylcellulose films formed at temperatures below 37°C

The polymer mixed films were evaluated for the minimum film forming temperature, spatial distribution and miscibility of its polymeric components and digestibility in simulated colonic conditions.

The minimum film forming temperatures of the polymeric mixtures were also evaluated using the MFFT bar. The range of temperatures at which mixed polymeric films could be formed was established.

The spatial distribution of amylose and its degree of miscibility with ethylcellulose within the mixed polymeric film was also investigated. An understanding of the spatial distribution of the polymers is important to confirm the proposed mechanism of drug release (Milojevic et al, 1996a). The proposed mechanism of drug release assumed that amylose forms domains which are continuous through the cross section of the films. When these amylose domains are digested in the colon, they would form pores which allow the drug within the core to be released. This proposed mechanism was based on previous observations of iodine-stained free films. When iodine stained free films were viewed under a light microscope, similar amylose domains were seen on the top and bottom surfaces of the amylose / ethylcellulose mixed films. Therefore, it was inferred that the amylose domains were continuous. Further studies on this were undertaken. Rather than just investigating the surface distribution of the amylose domains, spatial distribution of amylose domains and miscibility of amylose/ethylcellulose within the films were investigated. Light microscopy, differential interference contrast microscopy (DICM) and dynamic mechanical analysis (DMA) were done on isolated free films to try to gain a better understanding of the polymeric distributions within these films.

Light microscopy was done on the various levels of the iodine-stained films i.e. top, middle and bottom of the films. By focusing to different depths within the same area of the film, the iodine domain patterns across the films could be monitored.
DICM was then done on the unstained films. This additional microscopy technique was employed to ensure that optical artefacts had not been introduced accidentally by iodine staining. The amylose and ethylcellulose domains within the film could potentially be differentiated by DICM due to differences in phase contrast of these two polymers. DICM is a microscopy technique which uses beam splitting devices, the Wollaston prisms, to create phase contrast. The basic optical elements are shown in Fig 3.5. The first Wollaston prism (beam divider) is set at 45° with respect to the plane polariser and would split the polarised beam into the specimen beam and the reference beam. Differences in optical paths through the specimen introduce a phase shift between the two beams. When these two beams are reunited by the second Wollaston prism (beam combiner) which is reversed in orientation to the first, all optical differences, except for phase shift, are annulled (Slayter and Slayter, 1992). Since this phase shift is different for each material, two different components of a mixture could be differentiated. Amylose and ethylcellulose would show up as different colours under DICM.

Fig 3.5 The DICM: Basic optical elements and wave fronts at various points (Slayter and Slayter, 1992)
The extent of polymeric miscibility was then assessed by dynamic mechanical analysis (DMA). DMA was used to determine the glass transition temperature ($T_g$) of the polymeric blend. If the two polymers, with distinct $T_1$, $T_1$ and $T_2$, are immiscible, their resultant polymeric blend would show two glass-transition temperatures at $T_1$ and $T_2$, respectively. However, if the two polymers are completely miscible, then, the resultant polymeric blend would show just one $T_g$, $T_3$, whereby $T_3$ is in between $T_1$ and $T_2$. The level of miscibility detected by this technique is limited to a level of approximately 15 nm (Lafferty, 1992).

DMA was used to record the tan $\delta$ values and the storage moduli of the polymeric mixtures. An understanding of the DMA measuring technique is needed to appreciate the relevance of these results. In DMA, an oscillating sinusoidal loading pattern, eg. stress, is applied. This results in a sinusoidal strain pattern which follows the stress pattern with a phase lag. There would be a stress component which is in-phase with the rate of strain and a stress component which is out-of-phase with the rate of strain. These two components could then be expressed as storage modulus, $E'$ and loss modulus, $E''$. $E'$ is the ratio of the in-phase stress component over strain and $E''$ is the ratio of the out-of-phase stress component over strain. The relationship between $E''$ and $E'$ can be expressed as tan $\delta$, whereby,

$$\tan \delta = \frac{E''}{E'}$$

As the polymeric material passes through its $T_g$, the physical properties of the material changes from being glassy to being viscous. This physical change brings about an increase in the ratio of the $E''$ over $E'$ and a corresponding tan $\delta$ peak is seen. In summary, the $T_g$ is the temperature at which a tan $\delta$ peak is seen.

However, interpretation of results could be difficult as more than one tan $\delta$ peak is often seen with polymers. These loss transitions are termed $\alpha$, $\beta$ and $\gamma$ in decreasing order of temperature of appearance (Fig 3.6). The temperature at which $\alpha$-transition occurs which is associated with the major chain mobility, is considered the $T_g$. At this temperature, there is a sudden drop in the storage modulus. $\beta$ and $\gamma$ transitions which
are associated with side chains or localised motions in the main chains are usually not accompanied by great changes in modulus. Hence, by measuring both tan δ values and storage moduli, the T_g of the material can be determined.

Fig 3.6 Typical tan δ and storage modulus response of an amorphous polymer to a dynamic stress analysed at constant frequency (Lafferty, 1992)

Finally, the digestibility of the successfully formed mixed films were evaluated. In these mixed films, factors such as the increased presence of plasticizer and the presence of ethylcellulose could affect the digestibility of amylose. The increased presence of hydrophobic plasticizer within the mixed films formed at temperatures below 37°C could lead to a reduction or even cessation of microbial enzyme digestion. Therefore, the digestibility of these films were tested. In addition, the influence of varying the ethylcellulose to amylose ratios on the digestibility of the mixed film was also evaluated. The presence of ethylcellulose could have an effect on the distribution and orientation of amylose chain, hence affect its digestibility.

Unfortunately, there are no standard digestibility testing procedures. Various researchers had tried to use in vivo animal models as indicators to in vivo effects in man. However, Friend and Tozer (1992) had shown that most of the commonly used laboratory animals demonstrated rather different microbial enzyme activities in the colon.
compared to man. In short, *in vivo* results, other than in man, are poor indicators of the real situation. Therefore, various *in vitro* enzymatic experiments mimicking *in vivo* digestion conditions in man had been developed. These have been developed to various levels of complexities and can be broadly divided into cell-free and cell-containing enzyme systems.

The cell-free enzyme systems can be further divided into those with activities against selected substrates and those with activities against multiple substrates. The former, prepared from commercially available enzymes, are usually active against the tested substrate only e.g. dextranase used to test the digestion of dextran (Brønsted *et al*, 1995a) or pectinolytic enzymes used to test digestion of pectin (Rubinstein *et al*, 1993). These systems assume that the substrate digestion by these enzymes is a process independent of competition and inhibition by other enzymes naturally present in the colon. This assumption may not always be true. Therefore, more complex enzymatic models showing activities against multiple substrates have been developed. The enzymes were prepared from isolated rat caecal contents. The caecal contents were suspended in buffer, sonicated, filtered and centrifuged to obtain cell-free, enzyme-rich supernatant which may be freeze-dried until used (Brown *et al*, 1983, Brønsted and Kopeček, 1992). The advantage of using cell-free enzymatic systems are that they are relatively "clean" and easy to handle. However, they suffer the drawback of being representative of only extracellular enzymes, not cell-associated enzymes, present in the colon. Both extracellular and cell-associated enzymes are involved in substrate digestion in the colon (Salyers and Leedle, 1983, Macfarlane *et al*, 1991). In particular, amylose had been shown to be more effectively digested by cell-associated than cell-free amylases in the colon (Macfarlane and Englyst, 1986). Therefore, cell-free systems are considered poor digestion models for testing amylose.

Cell-containing testing systems, although more messy and more difficult to reproduce, are generally considered to be more representative of the enzymatic activities found in the colon. Both extracellular and cell-associated enzymes are present. Such testing systems can be developed from pure or mixed microbial cultures. Growth rates of pure cultures have been shown to be more difficult to maintain. Experiments using
pure *Bacteroides ovatus* batch culture, a human colonic anaerobe which hydrolyses pectin, showed that logarithmic growth rate could only be maintained for four hours (Rubinstein *et al.*, 1993). This was considered too short to mimic the transit time in the colon. In addition, there are no markers to suggest that the *in vitro* growth rates matched *in vivo* conditions in man. Mixed microbial continuous cultures, on the other hand, can be maintained for up to 336 hours (Allison *et al.*, 1989). Their growth rates can also be monitored by the rate of substrate fed into the system (Macfarlane *et al.*, 1992).

Unfortunately, there is no agreed standard source of culture material. Different sources of cultures would give rise to different digestion profiles (Kopečková *et al.*, 1994). The most commonly used culture sources have been rat caecal contents (Kopeček *et al.*, 1992, Kopečková *et al.*, 1994, Brønsted *et al.*, 1995a), guinea pig intestinal contents (Friend and Tozer, 1992) and human faeces (Van den Mooter *et al.*, 1992, Kopečková *et al.*, 1994, Friend, 1995, Milojevic *et al.*, 1996a). None of these cultures perfectly match the digestion activities in the human caecum, the targeted site for onset of drug release from oral colonic dosage forms. The best choice, based on cost and availability, is the human faeces. Macfarlane *et al.*, (1992) have shown that the rate of digestion from cultures taken from the human sigmoidal / rectum region was lower than the rate of digestion from cultures taken from the human caecum. Nevertheless, both cultures showed similar digestion products suggesting similar digestion abilities. Therefore, *in vitro* studies using human faeces can be considered reliable indicators for *in vivo* events (Macfarlane *et al.*, 1992).

The choice of culturing procedures was also considered. There are two known methods, batch and continuous cultures. In the continuous culture, the source of nutrients may be constantly replenished and the waste products selectively removed. This system mimics the constant change within the colon and may be set up as a multistage system to mimic the different regions of the colon. However, continuous culture is a very complex culturing procedure and is only useful for experiments lasting for over 24 hours. For experiments lasting 24 hours or less, batch cultures are more practical. Batch cultures are closed systems whereby the nutrients are not replenished and waste products not removed. Eventually, due to the lack of nutrients or inhibitory actions of waste products,
the logarithmic growth rate within the system can no longer be maintained. Such growth rates are generally unaffected in the first 24 hours of experiments (Macfarlane et al, 1993). In addition, batch cultures are cheaper, easier to set up and less substrate consuming (Macfarlane et al, 1993). Therefore, batch cultures using freshly voided human faeces was chosen as in vitro fermentation models for testing the digestion of the mixed films.

3.2 Materials

3.2.1 Coating materials

Two sources of amylose-butanol complex dispersion were used. One was supplied from The Institute of Food Research, Norwich. This complex was made from pea starch. The other aqueous complex dispersion was supplied from Avebe, Netherlands. This complex was made from potato amylose powder according to the method described in Chapter 2. In most experiments, only the amylose butanol complex obtained from The Institute of Food Research, Norwich was used. The limited supply of the amylose-butanol complex from Avebe, Netherlands restricted study. The latter source of amylose-butanol complex was only used in the digestion study.

All other coating materials used are listed in Chapter 2.

3.2.2 Gas-liquid chromatography reagents

Methanol of Super Purity Solvent grade from Merck, UK. Batch no 15250
Dimethyl sulphoxide of AnalaR grade from Merck, UK. Batch no 33187887880
Butan-1-ol of Super Purity Solvent grade from Merck, UK. Batch no 15287

3.2.3 Diffusion cell reagents

5N Hydrochloric acid, AnalaR grade, Merck, UK. Batch no 50065821
Sodium hydroxide pellets, AnalaR grade, Merck, UK. Batch no 050594H22S
Potassium orthophosphate, AnalaR grade, Merck, UK. Batch no A890225

3.2.4 Simulated colonic fermentation study reagents

All of general purpose reagent grade from Merck, UK.
Dipotassium hydrogen phosphate, $\text{K}_2\text{HPO}_4$ Batch no 302A604476
Potassium dihydrogen phosphate, $\text{KH}_2\text{PO}_4$ Batch no A890225551
Sodium chloride, $\text{NaCl}$ Batch no K20603432
Magnesium chloride, $\text{MgCl}_2.6\text{H}_2\text{O}$ Batch no TA576032449
Ferrous sulphate, $\text{FeSO}_4.7\text{H}_2\text{O}$ Batch no A843740522
Calcium chloride, $\text{CaCl}_2.2\text{H}_2\text{O}$ Batch no TA694532445

3.2.5 Miscellaneous

ConvoL solution, Merck, UK. Batch no 3972840M
Potassium carbonate, general purpose reagent grade, Merck, UK. Batch no K21928435530
3.3 Methods

3.3.1 Investigations of amylose films formed at temperatures below 37°C

3.3.1.1 The investigation of the possible presence of residual butanol within the amylose film using gas liquid chromatography (GLC)

The possible presence of butanol within the amylose film formed at temperatures below the complex melt temperature was investigated using gas liquid chromatography (GLC). The films were formed by pouring amylose-butanol complex dispersion (6% w/w) at room temperature into 8.5 cm diameter plastic petri dishes. They were then dried in a fan-assisted oven (Pickstone oven, serial no 16254) set at 30°C. Once dried, the films were cut into small pieces (0.5 x 0.5)cm$^2$ and soaked in methanol or dimethyl sulphoxide (DMSO). The films were ultrasonicated in sealed containers for 15 minutes. The solvents recovered from treating the films were then filtered prior to analysis. The standards were made up by dissolving butanol in methanol and in DMSO. They were also ultrasonicated in sealed containers and filtered prior to analysis.

The methanol and DMSO samples were then analyzed by GLC. A Philips Pye Unicam PU 4550 chromatograph was used. A Stabilwax® (Crossbonded Carbowax® PEG, Restek Corp., USA), 30m long column with an internal diameter of 0.53 mm and 0.5μm df (Column No 43486A) was used. The oven was set at an initial temperature of 40°C, held for 2 minutes then increased at a rate of 6°C / min to 230°C. The injector temperature was 120°C and the detector temperature set at 240°C. One μl of the sample was injected via the injection port and carried through the column by a carrier gas to the detector. Helium, the carrier gas, was fed at a rate of 2ml/min. The sample was detected with a flame ionisation detector (FID) supplied with 30ml/min of hydrogen and 300ml/min of air. The sample was fed into the FID at 40ml/min. Once detected, the signal was amplified with an ionisation amplifier. The amplifier sensitivity was 2 x 10$^{10}$ A f.s.d. The range switch was set at 10$^5$ and attenuation set at 4. The signal was recorded on a chart recorder (Kipp and Zonen BD40). Chart speed was 10mm/min, sensitivity was 10mV. All experiments were done in triplicates.
3.3.1.2 Investigation of the possible influence of the initial concentration of amylose-butanol complex dispersion on the porosity of the resultant film

Amylose free films, containing equal amounts of solids content, were made by pouring different concentrations of aqueous amylose complex dispersion onto 8.5 cm diameter plastic petri dishes. They were dried at ambient temperature (15 - 25)°C in Class 2 cleanroom environment to avoid dust contamination during the drying process. The formulations used are listed in Table 3.1.

Table 3.1 The formulations used to form amylose films from different amylose-butanol complex concentrations

<table>
<thead>
<tr>
<th>Concentration of dispersion, % w/w</th>
<th>Weight of solids, g</th>
<th>Weight of dispersion used per film cast, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>0.24</td>
<td>4.0</td>
</tr>
<tr>
<td>5</td>
<td>0.24</td>
<td>4.8</td>
</tr>
<tr>
<td>4</td>
<td>0.24</td>
<td>6.0</td>
</tr>
<tr>
<td>3</td>
<td>0.24</td>
<td>8.0</td>
</tr>
<tr>
<td>2</td>
<td>0.24</td>
<td>12.0</td>
</tr>
</tbody>
</table>

Prior to testing, the amylose films were maintained for at least 24 hours in a temperature- and humidity-controlled desiccator. Saturated potassium carbonate salt solution was used to maintain 44% RH within the desiccator stored at 20°C. The amylose films were cut into 25 mm diameter circular discs using a mould cutter. The thickness of each film was determined at five sites: North, South, East, West and central using a Mercer gauge Model 871. The average of 5 readings was taken as the thickness of the film.

Porosity tests were done using a cell diffusion assembly shown in Fig 3.7. The apparatus was immersed in a (37 ± 2)°C water bath. As the magnetic stirring base was liable to get very hot, the apparatus rested on a stand above the stirrer. The receiver
chamber (upper chamber) contained pH 7.34 phosphate buffer while the donor chamber (lower chamber) was filled with 0.1M HCl, pH 1.04. A 4.5mm diameter Russell combination pH electrode attached to a Jenway 3070 pH meter was used. The pH electrode was lowered to the same depth within the receiver chamber for each test. pH was read at specific time intervals. For each formulation, 3 films were tested.

Fig 3.7 Cell diffusion assembly

3.3.2 Investigations of ethylcellulose films formed at temperatures below 37°C

3.3.2.1 The methods used to evaluate the influence of plasticizer quantity, incorporation technique and type on the MFFT of Surelease®EA7100 and Aquacoat®ECD30

Two different methods were used to evaluate the influence of plasticizer type, quantity and incorporation technique on the MFFT of Surelease®EA7100 and Aquacoat®ECD30. The first was by pouring the dispersions onto glass petri dishes. Glass petri dishes replaced plastic petri dishes in this case because some of the plasticizers were found to react with the plastic. These were dried at ambient temperature (15 - 25)°C in Class 2 cleanroom environment and inspected visually. All visual observations were graded using a numerical grading system, shown in Table 3.2. They referred to the plasticization level needed at (20±5)°C.
Table 3.2 The numerical grading system used to evaluate the success of plasticization for formulations dried at (20±5)°C

<table>
<thead>
<tr>
<th>Number grading</th>
<th>Indication</th>
<th>Observations and Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Over-plasticization</td>
<td>A wrinkly, continuous film. The polymer is in a semi-solid state. Partial solidification gives rise to a wrinkled-surface appearance</td>
</tr>
<tr>
<td>2</td>
<td>Optimum plasticization</td>
<td>A smooth, clear, continuous film</td>
</tr>
<tr>
<td>3</td>
<td>Under-plasticization</td>
<td>A smooth, clear film with fine fissures. Slight increase in plasticization level is needed.</td>
</tr>
<tr>
<td>4</td>
<td>Under-plasticization</td>
<td>Clear, film fragments. Large increase in plasticization level is needed.</td>
</tr>
<tr>
<td>5</td>
<td>No plasticization</td>
<td>Opaque, powder compacts. Plasticization incorporation technique has failed completely.</td>
</tr>
</tbody>
</table>

The second method determined the specific MFFT for each formulation. The MFFT bar was used. The hot water bath on one end of the MFFT bar was set at (46±1)°C and the cold water bath on the other end was set at (3±1)°C. This created a temperature gradient of (32.5 ± 1.0)°C to (12 ± 1.0)°C across the spreading surface. The temperature gradient across the MFFT bar was allowed to equilibrate for at least two hours prior to use. The temperatures were measured at the beginning and at the end of each experiment using a Jenway 3070 digital-display thermometer. Polyethylene terephthalate films (Sellctape®) were placed on the spreading surface before casting the coating dispersions. The polyethylene terephthalate film not only permitted easier clean-up of the apparatus but also produced a semi-permanent record of the test (ASTM D2354-91). The apparatus was then levelled using the adjustable support and spirit levels. The plasticized dispersion formulations were spread using a purpose-built spreader (Fig 3.4) across the surface. Once spread, the film was allowed to form at room
humidity. After the film had dried, the lowest temperature on the bar which the film was still continuous was taken as the MFFT. If the film became discontinuous in between two temperature sampling points, then, the higher of the two sampled temperatures was taken to be the MFFT. All experiments were duplicated. The reported MFFT was an average of the four temperatures taken at the beginning and at the end of each duplicate experiment.

3.3.2.2 Investigations of the influence of plasticizer quantity, incorporation technique and type on the MFFT of Surelease®EA7100 and Aquacoat®ECD30

The quantity of plasticizer added was expressed as % w/w of the solids content of its ethylcellulose dispersion. In this study, this percentage was defined as:

\[
\% \text{ w/w of plasticizer} = \frac{\text{(the content of the added plasticizer)}}{\text{(the solid contents of the ethylcellulose dispersions)}} \times 100
\]

Surelease®EA7100 was taken as a 25% w/w solids dispersion; Aquacoat® ECD30 was taken as a 30% w/w solids dispersion. Based on this definition, the % w/w of plasticizer in all Surelease formulations was only reflective of the added plasticizer, not the true amount present. Even without additional plasticizer, Surelease already contained 24% w/w of dibutyl sebacate. Therefore, the quantities of plasticizer added to the Surelease dispersions were often much less than the quantities added to the Aquacoat dispersions.

Four different plasticizer incorporation techniques were evaluated. The first was by direct mixing of a plasticizer with an aqueous ethylcellulose dispersion using a magnetic stirrer. The plasticizer was mixed for half-an-hour, 24 hours and 72 hours. Thirty percent w/w of dibutyl sebacate was added to Aquacoat and 6% w/w of dibutyl sebacate was added to Surelease. The plasticized dispersions were cast in duplicate onto glass petri dishes.

The second technique involved the addition of Tween 80. The plasticizer and Tween 80 were mixed with a magnetic stirrer for 5 minutes. Then, ethylcellulose
dispersion was added and stirred for a further 30 minutes with a magnetic stirrer. After 30 minutes, water was added. The final formulation was then passed through a hand-operated homogeniser. Three mls of the final formulation was cast in duplicate onto glass petri dishes and dried under the conditions mentioned above. The formulations investigated are listed in Table 3.3. Aquacoat® ECD30 was plasticized with dibutyl sebacate, triethyl citrate and triacetin.

Table 3.3 The standard plasticized Aquacoat® ECD30 formulations investigated by casting onto glass petri dishes

<table>
<thead>
<tr>
<th>Aquacoat dispersion (g)</th>
<th>Plasticizer (g)</th>
<th>Plasticizer (% w/w)</th>
<th>Tween 80 (g)</th>
<th>Water (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>100</td>
<td>3</td>
<td>10</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>100</td>
<td>6</td>
<td>20</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>100</td>
<td>9</td>
<td>30</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>100</td>
<td>12</td>
<td>40</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>100</td>
<td>15</td>
<td>50</td>
<td>1</td>
<td>10</td>
</tr>
</tbody>
</table>

In the third technique, the required amount of plasticizer, Tween 80 and the pseudolatex were mixed with a high speed Silverson homogeniser mixer for 3 minutes. The formulations were left overnight in sealed containers. Any formulations which remained foamy after overnight storage were not investigated further. Otherwise, the formulations were evaluated on the MFFT apparatus. The basic formulations investigated are listed in Table 3.4. The plasticizers used were dibutyl sebacate, triethyl citrate, triacetin, acetyl tributyl citrate, tributyl citrate, polypropylene glycol, glycerol and polyethylene glycol 400.

In the final technique, Tween 80, the plasticizer and water were premixed into a crude emulsion prior to addition to ethylcellulose coating dispersions. A plasticizer emulsion was formed by mixing equal parts of plasticizer and water with ~0.1% of Tween 80 using a Silverson homogeniser mixture. The freshly mixed plasticizer emulsion was then added to the ethylcellulose coating dispersion by magnetic stirring for
Table 3.4 The ethylcellulose dispersions plasticized with Silverson mixer

<table>
<thead>
<tr>
<th>Product Name</th>
<th>Ethylcellulose dispersion (g)</th>
<th>Plasticizer (g)</th>
<th>Plasticizer (% w/w)</th>
<th>Tween 80 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surelease</td>
<td>10</td>
<td>0.00</td>
<td>0</td>
<td>0.000</td>
</tr>
<tr>
<td>Surelease</td>
<td>10</td>
<td>0.10</td>
<td>4</td>
<td>0.001</td>
</tr>
<tr>
<td>Surelease</td>
<td>10</td>
<td>0.20</td>
<td>8</td>
<td>0.001</td>
</tr>
<tr>
<td>Surelease</td>
<td>10</td>
<td>0.30</td>
<td>12</td>
<td>0.001</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>10</td>
<td>0.54</td>
<td>18</td>
<td>0.001</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>10</td>
<td>0.72</td>
<td>24</td>
<td>0.001</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>10</td>
<td>0.90</td>
<td>30</td>
<td>0.001</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>10</td>
<td>1.08</td>
<td>36</td>
<td>0.001</td>
</tr>
</tbody>
</table>

30 minutes. Preliminary studies suggested that 30 minutes mixing time was sufficient. Only Aquacoat®ECD30 formulations were investigated. Aquacoat®ECD30 was plasticized with dibutyl sebacate, triethyl citrate, triacetin, acetyl tributyl citrate, tributyl citrate, polypropylene glycol, glycerol and polyethylene glycol 400. The basic formulations are listed in Table 3.5.

Table 3.5 The Aquacoat®ECD30 dispersion plasticized with a plasticizer emulsion

<table>
<thead>
<tr>
<th>Aquacoat dispersion (g)</th>
<th>Plasticizer (% w/w)</th>
<th>Plasticizer (g)</th>
<th>Water (g)</th>
<th>Tween 80 (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>10</td>
<td>18</td>
<td>0.54</td>
<td>0.54</td>
<td>0.001</td>
</tr>
<tr>
<td>10</td>
<td>24</td>
<td>0.72</td>
<td>0.72</td>
<td>0.001</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>0.90</td>
<td>0.90</td>
<td>0.001</td>
</tr>
<tr>
<td>10</td>
<td>36</td>
<td>1.08</td>
<td>1.08</td>
<td>0.001</td>
</tr>
</tbody>
</table>
3.3.3 Investigations of the combined amylose/ethylcellulose films formed at temperatures below 37°C

3.3.3.1 Investigations on the MFFT of the mixed polymeric formulations

Ethylcellulose dispersion was first plasticized with the required quantity of plasticizer. Aquacoat® and Surelease® were plasticized using a different plasticization techniques. The plasticized ethylcellulose dispersion was then mixed with the aqueous amylose dispersion by stirring for 5 minutes with a magnetic stirrer. In all of the following formulations, the ratios of ethylcellulose to amylose referred to the polymer solid weights ratios, not the dispersions ratios. The final dispersion mixtures were then spread onto the MFFT bar surface.

Various temperature gradients were set across the MFFT bar surface. This was achieved by keeping the cold water bath set at (3±1)°C and varying the temperature of the hot water bath. Such variations were deemed necessary to avoid the MFFT being too close to either end of the MFFT bar. Accurate determination of MFFT was difficult when it occurred at the ends of the bar. Only formulations containing dibutyl sebacate were investigated. All experiments were done in duplicate. The formulations investigated are listed in Table 3.6.

Table 3.6 The mixed polymeric formulations investigated for minimum film forming temperature

<table>
<thead>
<tr>
<th>Product name</th>
<th>Ethylcellulose: amylose ratio</th>
<th>Plasticizer (w/w)</th>
<th>Temperature of hot water bath (±1.0°C)</th>
<th>Temperature gradient across the bar surface (± 0.5°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquacoat</td>
<td>1:0</td>
<td>24</td>
<td>46</td>
<td>12.0 - 32.0</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>3:1</td>
<td>24</td>
<td>46</td>
<td>12.0 - 32.0</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>5:1</td>
<td>24</td>
<td>46</td>
<td>12.0 - 32.0</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>7:1</td>
<td>24</td>
<td>46</td>
<td>12.0 - 32.0</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>1:0</td>
<td>30</td>
<td>32</td>
<td>9.5 - 23.0</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>3:1</td>
<td>30</td>
<td>32</td>
<td>9.5 - 23.0</td>
</tr>
</tbody>
</table>
Table 3.6 (contd.)

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Ratio</th>
<th>Temperature</th>
<th>Time</th>
<th>Drying Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquacoat</td>
<td>5:1</td>
<td>30</td>
<td>32</td>
<td>9.5 - 23.0</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>7:1</td>
<td>30</td>
<td>32</td>
<td>9.5 - 23.0</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>1:0</td>
<td>36</td>
<td>20</td>
<td>7.5 - 16.0</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>3:1</td>
<td>36</td>
<td>20</td>
<td>7.5 - 16.0</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>5:1</td>
<td>36</td>
<td>20</td>
<td>7.5 - 16.0</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>7:1</td>
<td>36</td>
<td>20</td>
<td>7.5 - 16.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>1:0</td>
<td>0</td>
<td>45</td>
<td>12.0 - 32.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>3:1</td>
<td>0</td>
<td>45</td>
<td>12.0 - 32.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>5:1</td>
<td>0</td>
<td>45</td>
<td>12.0 - 32.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>7:1</td>
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<td>12.0 - 32.0</td>
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<td>Surelease</td>
<td>1:0</td>
<td>4</td>
<td>45</td>
<td>12.0 - 32.0</td>
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<tr>
<td>Surelease</td>
<td>3:1</td>
<td>4</td>
<td>45</td>
<td>12.0 - 32.0</td>
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<tr>
<td>Surelease</td>
<td>5:1</td>
<td>4</td>
<td>45</td>
<td>12.0 - 32.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>7:1</td>
<td>4</td>
<td>45</td>
<td>12.0 - 32.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>1:0</td>
<td>8</td>
<td>31</td>
<td>9.5 - 23.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>3:1</td>
<td>8</td>
<td>31</td>
<td>9.5 - 23.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>5:1</td>
<td>8</td>
<td>31</td>
<td>9.5 - 23.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>7:1</td>
<td>8</td>
<td>31</td>
<td>9.5 - 23.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>1:0</td>
<td>12</td>
<td>20</td>
<td>7.5 - 16.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>3:1</td>
<td>12</td>
<td>20</td>
<td>7.5 - 16.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>5:1</td>
<td>12</td>
<td>20</td>
<td>7.5 - 16.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>7:1</td>
<td>12</td>
<td>20</td>
<td>7.5 - 16.0</td>
</tr>
<tr>
<td>Amylose</td>
<td>0:1</td>
<td>0</td>
<td>20</td>
<td>7.5 - 16.0</td>
</tr>
</tbody>
</table>

3.3.3.2 Casting of the mixed polymer free films

The mixed polymeric formulations were prepared as described in 3.3.3.1. However, rather than spreading onto the MFFT bar, they were poured onto 8.5cm diameter PTFE petri dishes and dried at 35°C fan-assisted oven (Pickstone oven, serial no 16254). Custom-made PTFE petri dishes were used to enable easier removal of the films from the cast surface. The dried films were then stored for at least 24 hours in a
temperature- and humidity-controlled environment prior to further testings. Saturated potassium carbonate salt solution was used to maintain 44% RH within the desiccator stored at 20°C.

Although many mixed polymer formulations formed films at the drying temperature of 35°C, the films formed may be too brittle or too soft to be handled as free films. Surelease/amylose + 4% w/w dibutyl sebacate (DBS) and Aquacoat/amylose + 36% DBS, at various polymer ratios were considered the easiest to handle as free films. These formulations were used for further film studies.

3.3.3.3 Investigations of the spatial distribution and miscibility of amylose within the mixed films

The spatial distribution of the mixed polymeric films were investigated using light microscopy, differential interference contrast microscopy (DICM) and dynamic mechanical analysis (DMA).

Light microscopy investigations were done on the mixed film containing Surelease® EA7100 and amylose (5:1) with 4% additional dibutyl sebacate. The polymeric free films were cut into (3x3) cm² pieces of films and stained with freshly prepared iodine solution. The iodine solution was freshly prepared by diluting 50 ml of ConvoL concentrate (Iodine 0.5 mol l⁻¹) with 450 ml of distilled water resulting in 0.1N iodine solution (0.05 mol l⁻¹ = 12.69g per litre at 20°C). The stained mixed films were then washed with water to remove any excess iodine. This was viewed under a light microscope/camera (Nikon Microphot-FXA) set at 100x magnification. The images seen by focusing onto the top, middle and bottom layers of the same section of film was recorded onto Techpan 25 ASA black and white films.

Differential interference contrast microscopy (DICM) investigations were also done on the mixed film containing Surelease® EA7100 and amylose (5:1) with 4% additional dibutyl sebacate. The polymeric free film was embedded into an epoxy resin and sectioned into 3 µm thick sections with a rotary microtome (Reichert 2030). These were viewed under polarised light at 400x magnification using an Olympus BX 50
microscope attached with Wollaston prisms. The images were captured using a camera onto Kodak Gold 100ASA colour films.

DMA was carried out on a Perkin-Elmer DMA-7 Analyser. Surelease EA7100 +4%DBS films, amylose films and Surelease:amylose + 4%DBS (3:1), (5:1) and (7:1) mixed films were the only films investigated. Aquacoat + 36%DBS films and Aquacoat + 36% DBS mixed with amylose films were not analyzed as they were too fragile to be cut to size.

DMA is a highly sensitive technique which could detect very small thermal and mechanical changes. Such sensitivity meant that good sample preparation is imperative. Preliminary studies on sample preparation involved finding the optimum film thickness for analysis, the best cutting method which gave the least shear or edge effects and the most suitable mounting technique to attach the film sample to the measuring probe.

The free films were made as described in 3.3.3.2. In theory, thick films were preferred to thin films to minimise the effect of background noise during analysis. However, in practice, flat, thick films were very difficult to make. The very different rates of water evaporation from the top and the bottom of thick films often led to the formation of corrugated films. The thickest flat films which could be made reproducibly was (250 ± 50)μm thick.

There are various choices of measuring systems on the Perkin-Elmer DMA analyser. The extension mode is generally favoured for free film measurements. However, this mode was found to be very difficult to manage. The edge effects due to the cutting of the samples with a sharp knife, the poor alignment of the film between the top and the bottom probes, the ambiguity of the tightness of clamping between the jaws of the probes and the constant breakage of the films at Tg all contributed to errors in measurements. A much easier measuring system to manage, the parallel plates, replaced it. The samples for the parallel plates were cut using a manual cork-borer machine (Gallenkamp, Leicester) to reduce edge effects due to shearing. The samples required minimum mounting technique to be aligned.

Initially, stress scans were conducted on the various film samples to determine
the most suitable measuring conditions for measuring $T_g$ of the samples. The conditions for stress scan investigations are shown in Table 3.7.

The storage moduli and the linear viscoelastic regions of the amylose, Surelease+ 4% DBS and Surelease + 4% DBS:amylose (5:1) films were determined. Based on these results, the 15mm diameter stainless steel parallel plates for measuring samples with storage modulus of $(1 \times 10^3$ to $3 \times 10^6)$Pa was found to be optimum for measuring all these films (Perkin-Elmer DMA 7 measuring system selection table). The maximum operational dynamic force was 500mN.

After various attempts by trials and errors the optimum measuring conditions for measuring maximum tan $\delta$ values were found (Table 3.8).

All films were stored for 3 to 7 days at 20°C, 44%RH prior to testing. The tan $\delta$ and storage moduli were recorded over a range of temperatures investigated. Each film formulation was run in triplicate.

Table 3.7 The stress scan method used to determine the most suitable measuring conditions

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Measuring system: 15 mm diameter stainless steel parallel plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>15 mm diameter film disc</td>
</tr>
<tr>
<td>Environment</td>
<td>Purge gas: Helium, 0.5 bar</td>
</tr>
<tr>
<td></td>
<td>Cooling device: Perkin-Elmer CCA-7 set at -120°C, purged</td>
</tr>
<tr>
<td></td>
<td>with nitrogen at 5 bar</td>
</tr>
<tr>
<td></td>
<td>Coolant: Liquid nitrogen</td>
</tr>
<tr>
<td>Parameters</td>
<td>Mode: Stress scan</td>
</tr>
<tr>
<td></td>
<td>Temperature: 25°C</td>
</tr>
<tr>
<td></td>
<td>Frequency: 1 Hz</td>
</tr>
<tr>
<td></td>
<td>Static force: 2100mN</td>
</tr>
<tr>
<td></td>
<td>Scan rate: 20mN per minute</td>
</tr>
<tr>
<td></td>
<td>Scan range: 10 - 1000mN</td>
</tr>
<tr>
<td>Control</td>
<td>Static: Force</td>
</tr>
</tbody>
</table>
Table 3.8 The measuring conditions for determination of tan δ values of various films

<table>
<thead>
<tr>
<th>Instrument</th>
<th>Measuring system: 15 mm diameter stainless steel parallel plates</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>15 mm diameter film disc</td>
</tr>
<tr>
<td>Environment</td>
<td>Purge gas: Helium, 0.5 bar</td>
</tr>
<tr>
<td></td>
<td>Cooling device: Perkin-Elmer CCA7 set at -120°C, purged</td>
</tr>
<tr>
<td></td>
<td>with nitrogen gas set at 5 bar pressure</td>
</tr>
<tr>
<td></td>
<td>Coolant: Liquid nitrogen</td>
</tr>
<tr>
<td>Parameters</td>
<td>Mode: Temperature/time scan</td>
</tr>
<tr>
<td></td>
<td>Temperature range: -65°C to 100°C</td>
</tr>
<tr>
<td></td>
<td>Starting temperature held for: 10 minutes</td>
</tr>
<tr>
<td></td>
<td>Heating rate: 5°C/min</td>
</tr>
<tr>
<td></td>
<td>Frequency: 1 Hz</td>
</tr>
<tr>
<td></td>
<td>Static force: 2000mN</td>
</tr>
<tr>
<td></td>
<td>Dynamic force: 100mN</td>
</tr>
<tr>
<td>Control</td>
<td>Static: Force</td>
</tr>
<tr>
<td></td>
<td>Dynamic: Force</td>
</tr>
</tbody>
</table>

3.3.3.4 Investigations of the digestion of the mixed polymeric films

Cast and sprayed mixed polymeric free films were tested for digestion in the *in vitro* fermentation model. The cast films were formed by the method described in 3.3.3.2. The thickness of the films were kept as uniform as possible by casting mixed polymeric dispersions equivalent to a total 0.39g of dried solid weights in each case. This amount of solid weights gave the thinnest films which were strong enough to be handled.

Sprayed films were formed by spraying the dispersion formulations repeatedly onto a large piece of tin foil in a temperature controlled chamber set at 35°C. A table fan was housed within this chamber to increase the rate of drying of the dispersion. The dispersion was sprayed using a spray gun attached to a pressurised aerosol can. Comparatively large amounts of coating dispersions were required to form very thin
films due to loss on spraying. Hence, studies using sprayed films were limited.

All the films were stored for 7 days at 20°C and 44%RH before being cut into strips of approximately (3 x 1) cm². The strips of films were accurately weighed using a Sartorius 2001 MP2 balance, then placed in coded nylon mesh bags. The bag size was ~(2 x 8) cm², mesh size = ~(1 x 0.4) cm². The controls were incubated in phosphate buffers, the tests were incubated in faecal slurries.

The faecal slurries which contained (10-15)% w/w of freshly voided human faeces, was made up using the following recommended buffer solution (Silvester, 1993):

<table>
<thead>
<tr>
<th>Materials</th>
<th>g/L (in double deionised water)</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₂HPO₄</td>
<td>1.5</td>
</tr>
<tr>
<td>KH₂PO₄</td>
<td>1.5</td>
</tr>
<tr>
<td>NaCl</td>
<td>4.5</td>
</tr>
<tr>
<td>MgCl₂.6H₂O</td>
<td>0.5</td>
</tr>
<tr>
<td>FeSO₄.7H₂O</td>
<td>0.05</td>
</tr>
<tr>
<td>CaCl₂.2H₂O</td>
<td>0.15</td>
</tr>
</tbody>
</table>

The buffer was boiled for at least 15 minutes to aid the dissolution of the salts as well as the removal of oxygen. This buffer solution was then cooled in a water bath to 37°C. Nitrogen was constantly bubbled into the buffer solution throughout this cooling process. Half of the buffer solution was used as control solution. The other half was inoculated with faeces. The required quantity of faeces was weighed and added to the buffer solution. Homogenisation of the faeces in the buffer was done using a stomacher machine (Stomacher 3500, Colworth). The faecal slurry was then filtered through a 500 μm sieve to remove any fibrous materials which were not homogenised. A 100ml of this slurry was then filled into each test bottle. The films within the nylon-meshed bags were added and the bottles were sealed under positive nitrogen pressure using rubber stoppers and metal crimping. The bottles were then left unstirred in a 37°C incubator.

After either 6 or 24 hours of incubation, the films within the nylon-meshed bags were retrieved. The strips of films were carefully collected, washed with distilled water
and then dried between filter papers before being stored at 20°C, 44%RH for 7 days. After 7 days, the films were reweighed on a Sartorious 2001 MP2 balance. All film fragments were carefully washed and collected. Fragments as small as (0.2 x 0.2) cm² were retained due to adhesion onto the nylon mesh.

The formulations of the mixed polymeric films tested for digestion are listed in Table 3.9.

Table 3.9 Digestion study on the amylose-containing mixed polymeric films

<table>
<thead>
<tr>
<th>Film formulation [Sprayed (S)/Cast (C)]</th>
<th>Incubation time (hours)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Faecal slurry</td>
</tr>
<tr>
<td>Amylose (C)</td>
<td>6</td>
</tr>
<tr>
<td>Amylose (S)</td>
<td>6</td>
</tr>
<tr>
<td>Avebe* (C)</td>
<td>6</td>
</tr>
<tr>
<td>Surelease:amylose (1:1) + 4% DBS (C)</td>
<td>6</td>
</tr>
<tr>
<td>Surelease:amylose (3:1) + 4% DBS (C)</td>
<td>6</td>
</tr>
<tr>
<td>Surelease:amylose (5:1) + 4% DBS (C)</td>
<td>6</td>
</tr>
<tr>
<td>Surelease:amylose (5:1) + 4% DBS (S)</td>
<td>6</td>
</tr>
<tr>
<td>Surelease:Avebe* (3:1) + 4% DBS (C)</td>
<td>6</td>
</tr>
<tr>
<td>Aquacoat:amylose (1:1) + 36% DBS (C)</td>
<td>6</td>
</tr>
<tr>
<td>Aquacoat:amylose (3:1) + 36% DBS (C)</td>
<td>6</td>
</tr>
<tr>
<td>Aquacoat:amylose (5:1) + 36% DBS (C)</td>
<td>6</td>
</tr>
<tr>
<td>Aquacoat:amylose (5:1) + 36% DBS (S)</td>
<td>6</td>
</tr>
</tbody>
</table>

*Avebe refers to the amylose-butanol complex dispersion supplied from Avebe, Netherlands*
3.4 Results and Discussions

3.4.1 Investigations of amylose films formed at temperatures below 37°C

3.4.1.1 The investigation of the possible presence of residual butanol within the amylose film using gas liquid chromatography (GLC)

GLC was successfully used to resolve butanol dissolved in both methanol and DMSO. Methanol was detected at 1 minute 33 seconds, butan-1-ol at 4 minutes and 58 seconds and DMSO at 12 minutes. Discrete peaks for each material were seen in all cases (Fig 3.8 to 3.11).

Fig 3.8 Gas liquid chromatograph of methanol
Fig 3.9 Gas liquid chromatograph of butanol in methanol

Fig 3.10 Gas liquid chromatograph of dimethyl sulphoxide
Approximately 4% w/v of amylose films were leached with methanol. When methanol samples were analyzed by GLC, no butanol was detected (Fig 3.12). The GLC system should be sensitive enough to detect 0.1 v/v of butanol. Therefore, the results suggest that there is less than 2.5% of unbound butanol present in the amylose films.
Approximately 5% w/v of amylose films were dissolved in DMSO. When the DMSO samples were analyzed by GLC, again no butanol was detected (Fig 3.13). This GLC system should also be sensitive enough to detect 0.1 v/v of butanol. Therefore, the results suggest that there is less than 2.0% of butanol, either in the unbound or complexed form, present in the amylose films.

Fig 3.13 Gas liquid chromatograph of dimethyl sulfoxide sample containing 5% w/v of dissolved amylose film

The lack of detectable butanol suggests that butanol must have been dissociated from amylose-butanol complex below its melt temperature. There are two possible explanations as to how the dissociation of the complex could have occurred. As the film is formed, water is evaporated. The work done due to the evaporation of water may have been sufficient to replace the heat energy required to dissociate the amylose-butanol complex. Therefore, the complex has dissociated below its melting temperature.

There is evidence to suggest that in an aqueous amylose-butanol complex dispersion, butanol is constantly partitioning between the amylose and the aqueous phase. This partitioning process occurs even at room temperatures (Banks and Greenwood, 1975). As a film is formed, any butanol that has partitioned into the aqueous phase would
form a constant boiling solution and would be preferentially evaporated. The lack of butanol in the aqueous phase compared to the polymeric phase encourages further spontaneous dissociation of the complex. This continues until eventually, little to no butanol could be found in the system.

In conclusion, the possible presence of residual butanol within the amylose film was less than 2.5%.

3.4.1.2 Investigation of the possible influence of the initial concentration of amylose-butanol complex dispersion on the porosity of the resultant film

The pH within the receiver chamber of the diffusion cell was recorded over time. This was shown in Fig 3.14.

Fig 3.14

All the graphs in Fig 3.14 had a similar shape. The graphs could be divided into three sections as shown in Fig 3.15.
Section 1 showed very little pH change. This was considered to be the lag phase and most probably correlated to the rate of swelling of the film. When the swelling of the film had reached an equilibrium, the rate of diffusion across the film would have reached a maximum. This was shown in section 2 of the graph. Finally, as the pH of the receiver chamber approximated to the pH of the donor chamber, no further pH changes could be seen. This was shown in section 3 of the graph.

The two sections that were of interest for oral colonic drug delivery were sections 1 and 2. Section 1 gave an indication of the initial rate of swelling of the film, section 2 gave an indication of the porosity of the finally swollen film. For simplicity of analysis, section 1, the initial rate of swelling of the film, was taken to be the inverse of the time taken for the pH in the receiver chamber to drop from pH 7 to pH 6. The porosity of the swollen film was taken as the rate of change of pH from pH 6 to pH 2.5. The times taken for the respective pH changes are shown in Table 3.10. The rates of pH changes are shown in Fig 3.16 and 3.17.
Table 3.10 The rates of swelling and the porosities of the amylose films cast from different concentrations of amylose-butanol complex dispersions

<table>
<thead>
<tr>
<th>Concentration of amylose dispersion (% w/w)</th>
<th>pH change from 7 to 6</th>
<th>pH change from 6 to 2.5</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Time (minutes)</td>
<td>Time (minutes)</td>
</tr>
<tr>
<td>6</td>
<td>3.25 3.50 3.75</td>
<td>4.00 4.50 4.75</td>
</tr>
<tr>
<td>5</td>
<td>3.00 4.25 3.75</td>
<td>5.50 5.50 5.50</td>
</tr>
<tr>
<td>4</td>
<td>4.25 3.50 4.75</td>
<td>5.50 4.25 5.25</td>
</tr>
<tr>
<td>3</td>
<td>5.75 4.25 4.25</td>
<td>6.00 4.75 5.75</td>
</tr>
<tr>
<td>2</td>
<td>6.25 5.25 5.25</td>
<td>6.00 6.50 8.50</td>
</tr>
<tr>
<td>1</td>
<td>3.25 4.75 3.75</td>
<td>5.25 5.25 5.50</td>
</tr>
</tbody>
</table>

Fig 3.16

The relationship between the initial rate of swelling of amylose film and the concentration of the amylose butanol complex from which the films were formed.
From Figs 3.16 and 3.17, no correlations were seen between the parameters studied and the initial concentrations of the amylose-butanol complex dispersions used to form the films. More importantly, all the films swelled and were very porous. Nearly all the films reached the equilibrium pH in less than 10 minutes. Amylose would definitely have to be used in combination with ethylcellulose to form films strong enough to withstand premature drug release in the upper gastrointestinal tract.

For all future studies, the concentration of amylose-butanol complex dispersion used was fixed at 6% w/w. This was considered to be the most concentrated amylose-butanol complex dispersion that could be sprayed easily. A high concentration of amylose-butanol complex dispersion was favoured because there was less water present. A reduction in the amount of water needed to be driven off meant a reduction in the amount of energy required. This was especially important under low temperature coating conditions whereby the energy input was reduced.
3.4.2 Investigations of ethylcellulose films formed at temperatures below 37°C

3.4.2.1 Investigations of the influence of plasticizer quantity, incorporation technique and type on the MFFT of Surelease®EA7100 and Aquacoat®ECD30

The results of the first plasticizer incorporation technique i.e. direct mixing of the plasticizer with the ethylcellulose dispersions, are shown in Table 3.11.

Table 3.11 The influence of direct mixing of dibutyl sebacate (DBS) and the ethylcellulose dispersion

<table>
<thead>
<tr>
<th>Product name</th>
<th>% w/w of DBS</th>
<th>Mixing time (Hours)</th>
<th>Numerical grading</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquacoat</td>
<td>0</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>30</td>
<td>0.5</td>
<td>NR</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>30</td>
<td>24</td>
<td>NR</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>30</td>
<td>72</td>
<td>NR</td>
</tr>
<tr>
<td>Surelease</td>
<td>0</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>Surelease</td>
<td>6</td>
<td>0.5</td>
<td>1 and 2</td>
</tr>
<tr>
<td>Surelease</td>
<td>6</td>
<td>24</td>
<td>1 and 2</td>
</tr>
<tr>
<td>Surelease</td>
<td>6</td>
<td>72</td>
<td>1 and 2</td>
</tr>
</tbody>
</table>

NR = Not reproducible; 5 = No film formed; 4 = film fragments; 3 = film with fissures; 2 = good film; 1 = semi-solid film

In the case of Aquacoat, after mixing for half-an-hour and 24 hours, the oil droplets of DBS could still be seen floating on the surface of the dispersion. After mixing for 72 hours, some polymeric materials would stick to the magnetic stirring bar. When these formulations were cast onto glass petri dishes, no reproducible results were obtained.

In the case of Surelease, no oil droplets were seen floating on the surface of the dispersion after the various mixing times. However, the films formed from these dispersions were not homogenous in appearance. There appeared to be partially solidified
patches within a continuous clear film. This suggested that some chains of the polymers were plasticized more than others.

Overall, this incorporation technique was poor. Uniform plasticization of both of the ethylcellulose dispersions were not achieved.

To achieve uniform plasticization, Tween 80, a surfactant was added. This formulation was based on the recommendations of the Aquacoat handbook. Tween 80, Aquacoat and the plasticizer were mixed together for 30 minutes before water was added. In the formulation containing a hydrophobic plasticizer, i.e. dibutyl sebacate, oil droplets could be seen on the surface of the mixture. The final mixture was therefore, homogenised using a hand-operated homogeniser. This was done for hydrophilic and hydrophobic plasticizer formulations to maintain processing uniformity. The results are shown in Table 3.12.

<table>
<thead>
<tr>
<th>Plasticizer content (% w/w)</th>
<th>Numerical grading of film quality</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dibutyl sebacate</td>
</tr>
<tr>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>20</td>
<td>3</td>
</tr>
<tr>
<td>30</td>
<td>2</td>
</tr>
<tr>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>50</td>
<td>1</td>
</tr>
</tbody>
</table>

5 = No film formed; 4 = film fragments; 3 = film with fissures; 2 = good film; 1 = semi-solid film

The results suggested that it was possible to reduce the MFFT of Aquacoat to room temperature. Different quantities were needed for different plasticizers. However, poor plasticizer permanence was observed. Oily patches (in the case of dibutyl sebacate)
or tiny gas bubbles structures (more prominent in triacetin than in triethyl citrate formulations) were seen in the films. This suggested that as the polymers coalesce, the plasticizer was excluded from the film. Further improvements to the technique was required.

One way of improving the above formulations was by increasing the mixing speed. The magnetic stirrer was replaced with a high-shear mixer. The high shearing actions of the mixer allowed more intimate mixing between the plasticizer and the polymer. The high input of energy should also increase the rate of solvation of the plasticizer within the polymeric particles. However, a vacuum high shear mixer was not available, hence, a Silverson mixer was used. The mixing action drew a lot of air into the dispersions. The final mixtures had to be left overnight to allow the air bubbles to disperse. In the case of Surelease, no air bubbles were seen after overnight storage. In the case of Aquacoat, the thick layer of foam remained. Therefore, only plasticized Surelease formulations were evaluated on the MFFT bar. The results are shown in Table 3.13.

The results were reproducible. No oily patches or tiny gas bubble structures were seen suggesting that good plasticizer permanence may have been achieved. The MFFT was lowered by hydrophobic plasticizers only e.g. dibutyl sebacate, acetyl tributylcitrate and tributyl citrate; the hydrophilic plasticizers had little to no effect on the MFFT of Surelease. This confirmed the statement that in choosing a plasticizer, the most important criterion was its solubility (Onions, 1986a). This technique was successful in incorporating plasticizers into Surelease and should be used for further studies. All hydrophobic plasticizers tested, dibutyl sebacate, tributylcitrate and acetyl tributyl citrate, were the plasticizers which had been shown to be effective in lowering the MFFT of Surelease.
Table 3.13 The minimum film-forming temperatures (MFFT) of plasticized Surelease® EA7100 formulations as measured by MFFT bar.

<table>
<thead>
<tr>
<th>% w/w of plasticizer</th>
<th>MFFT (± 0.1°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dibutyl sebacate</td>
</tr>
<tr>
<td></td>
<td>$T_1$</td>
</tr>
<tr>
<td>0</td>
<td>&gt;</td>
</tr>
<tr>
<td></td>
<td>31.7</td>
</tr>
<tr>
<td>4</td>
<td>20.8</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>15.7</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>12.2</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3.13 (continued) The minimum film-forming temperatures (MFFT) of plasticized Surelease® EA7100 formulations as measured by MFFT bar.

<table>
<thead>
<tr>
<th>% w/w of plasticizer</th>
<th>MFFT (± 0.1°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tributyl citrate</td>
</tr>
<tr>
<td></td>
<td>$T_1$</td>
</tr>
<tr>
<td>0</td>
<td>&gt;</td>
</tr>
<tr>
<td></td>
<td>32.1</td>
</tr>
<tr>
<td>4</td>
<td>20.5</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>17.2</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>12</td>
<td>&lt;</td>
</tr>
<tr>
<td></td>
<td>11.9</td>
</tr>
</tbody>
</table>

$T_1$ and $T_2$ = The average of temperatures measured at the beginning and at the end of each experiment
$T_{av}$ = The average of temperatures $T_1$ and $T_2$ for the formulation

132
The fourth plasticizer incorporation technique was developed for Aquacoat formulations. This involved forming a plasticizer emulsion prior to addition to the dispersion. The plasticizer emulsion contained 50% of water. The presence of additional water would dilute the dispersion. This would increase spray coating time. Therefore, this technique was only used when the previous technique was not suitable. The results are shown in Table 3.14.

Using this technique, the Aquacoat results were reproducible and good plasticizer permanence was achieved. Therefore, this technique of incorporation of plasticizer into Aquacoat was used for further studies. Contrary to previous findings, the MFFT of Aquacoat was affected by selected hydrophobic and hydrophilic plasticizers. The success of hydrophilic plasticizers e.g. triethylcitrate and triacetin, in lowering the MFFT could be due to the influence of the other excipients within Aquacoat. The other excipients within Aquacoat, such as stabilizers and antifoaming agents, could have a cooperative plasticization action with triethyl citrate and triacetin on Aquacoat.

In summary, higher than 24% w/w of plasticizers could be incorporated into Aquacoat if a suitable plasticizer incorporation technique was used. Factors other than mutual solubility are involved in the interaction between the plasticizer and the polymer. These factors influence the lowering of the MFFT of the plasticized polymeric dispersions.

When the MFFT of plasticized Surelease formulations were compared with the MFFT of plasticized Aquacoat formulations, some common features were seen. In both cases, dibutyl sebacate, acetyl tributyl citrate and tributyl citrate were successful in lowering the MFFT. On the other hand, glycerol, polyethylene glycol 400 and polypropylene glycol were unsuccessful. The lowering of the MFFT of Surelease by dibutyl sebacate, acetyl tributyl citrate and tributyl citrate and the lowering of the MFFT of Aquacoat by dibutyl sebacate, acetyl tributyl citrate, tributyl citrate, triethyl citrate and triacetin all appeared to be directly proportional to the concentration of the added plasticizer.
Table 3.14 The minimum film-forming temperatures (MFFT) of plasticized Aquacoat®
ECD30 formulations as measured by MFFT bar.

<table>
<thead>
<tr>
<th>% w/w of plasticizer</th>
<th>MFFT (± 0.1°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Dibutyl sebacate</td>
</tr>
<tr>
<td></td>
<td>$T_1$</td>
</tr>
<tr>
<td>18</td>
<td>&gt;</td>
</tr>
<tr>
<td></td>
<td>33.3</td>
</tr>
<tr>
<td>24</td>
<td>23.4</td>
</tr>
<tr>
<td></td>
<td>14.6</td>
</tr>
<tr>
<td></td>
<td>12.5</td>
</tr>
<tr>
<td>36</td>
<td>&lt;</td>
</tr>
<tr>
<td></td>
<td>33.3</td>
</tr>
</tbody>
</table>

Table 3.14 (continued) The minimum film-forming temperatures (MFFT) of plasticized
Aquacoat® ECD30 formulations as measured by MFFT bar.

<table>
<thead>
<tr>
<th>% w/w of plasticizer</th>
<th>MFFT (± 0.1°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Tributyl citrate</td>
</tr>
<tr>
<td></td>
<td>$T_1$</td>
</tr>
<tr>
<td>0</td>
<td>&gt;</td>
</tr>
<tr>
<td></td>
<td>33.3</td>
</tr>
<tr>
<td>4</td>
<td>25.0</td>
</tr>
<tr>
<td></td>
<td>33.3</td>
</tr>
<tr>
<td>8</td>
<td>16.1</td>
</tr>
<tr>
<td></td>
<td>33.3</td>
</tr>
<tr>
<td>12</td>
<td>&lt;</td>
</tr>
<tr>
<td></td>
<td>12.6</td>
</tr>
</tbody>
</table>

$T_1$ and $T_2$ = The average of temperatures measured at the beginning and at the end of each experiment

$T_{av}$ = The average of temperatures $T_1$ and $T_2$ for the formulation

134
There were also some unique features in the Surelease and Aquacoat plasticized formulations. Triethyl citrate and triacetin were only successful in lowering the MFFT of Aquacoat and not that of Surelease. An explanation for the success of triethyl citrate and triacetin in the plasticization of Aquacoat, despite their hydrophilic nature, was suggested above. If this explanation is true, it followed that the apparent lack of success in Surelease would be due to the lack of the required plasticization aid. Different excipients are found in Surelease and Aquacoat (see Chapter 2.2). The pre-plasticized nature of the ethylcellulose in Surelease could also be a contributory factor. The hydrophobic plasticizer within ethylcellulose would naturally exclude any hydrophilic agents.

In conclusion, different plasticization techniques were needed for successful incorporation of plasticizer into Aquacoat and Surelease. The MFFT of both of the commercially available ethylcellulose dispersion had been successfully lowered to well below 25°C by using higher than recommended contents of plasticizers. The lowering of the MFFT was directly correlated to the quantity of the effective plasticizer present. Each ethylcellulose dispersion has its own unique range of effective plasticizers. For future studies, dibutyl sebacate was chosen for plasticizing both Aquacoat and Surelease. In the case of Surelease, dibutyl sebacate was chosen because it was already present in the formulation. Further addition of this plasticizer has the least potential of excipient compatibility problems. In the case of Aquacoat, dibutyl sebacate was the most effective plasticizer, hence, the obvious choice.

3.4.3 Investigations of the combined amylose/ethylcellulose films formed at temperatures below 37°C

3.4.3.1 Investigations of the minimum film forming temperature (MFFT) of the mixed polymeric formulations

The results of the MFFT of the mixed polymeric formulations are shown in Table 3.15.
Table 3.15 The minimum film forming temperatures (MFFT) of the mixed polymeric formulations as measured by MFFT bar

<table>
<thead>
<tr>
<th>Product name</th>
<th>Ethylcellulose : amylose</th>
<th>Plasticizer (% w/w)</th>
<th>MFFT (± 0.1°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$T_1$</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>3:1</td>
<td>24</td>
<td>16.9</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>5:1</td>
<td>24</td>
<td>20.3</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>7:1</td>
<td>24</td>
<td>22.1</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>1:0</td>
<td>24</td>
<td>23.9</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>3:1</td>
<td>30</td>
<td>12.9</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>5:1</td>
<td>30</td>
<td>14.0</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>7:1</td>
<td>30</td>
<td>14.0</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>1:0</td>
<td>30</td>
<td>17.6</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>3:1</td>
<td>36</td>
<td>&lt;7.4</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>5:1</td>
<td>36</td>
<td>&lt;7.4</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>7:1</td>
<td>36</td>
<td>9.1</td>
</tr>
<tr>
<td>Aquacoat</td>
<td>1:0</td>
<td>36</td>
<td>9.1</td>
</tr>
<tr>
<td>Surelease</td>
<td>3:1</td>
<td>0</td>
<td>&lt;12.0</td>
</tr>
<tr>
<td>Surelease</td>
<td>5:1</td>
<td>0</td>
<td>24.7</td>
</tr>
<tr>
<td>Surelease</td>
<td>7:1</td>
<td>0</td>
<td>27.7</td>
</tr>
<tr>
<td>Surelease</td>
<td>1:0</td>
<td>0</td>
<td>&gt;31.8</td>
</tr>
<tr>
<td>Surelease</td>
<td>3:1</td>
<td>4</td>
<td>&lt;12.1</td>
</tr>
<tr>
<td>Surelease</td>
<td>5:1</td>
<td>4</td>
<td>&lt;12.1</td>
</tr>
<tr>
<td>Surelease</td>
<td>7:1</td>
<td>4</td>
<td>&lt;12.1</td>
</tr>
<tr>
<td>Surelease</td>
<td>1:0</td>
<td>4</td>
<td>24.2</td>
</tr>
<tr>
<td>Surelease</td>
<td>3:1</td>
<td>8</td>
<td>&lt;9.5</td>
</tr>
<tr>
<td>Surelease</td>
<td>5:1</td>
<td>8</td>
<td>&lt;9.5</td>
</tr>
<tr>
<td>Surelease</td>
<td>7:1</td>
<td>8</td>
<td>&lt;9.5</td>
</tr>
<tr>
<td>Surelease</td>
<td>1:0</td>
<td>8</td>
<td>15.3</td>
</tr>
</tbody>
</table>
Table 3.15 (continued)

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Surelease 3:1</th>
<th>Surelease 5:1</th>
<th>Surelease 7:1</th>
<th>Surelease 1:0</th>
<th>Amylose 0:1</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>12</td>
<td>&lt; 8.0</td>
<td>&lt; 8.0</td>
<td>&lt; 8.0</td>
<td>&lt; 7.5</td>
</tr>
<tr>
<td></td>
<td>&lt; 8.0</td>
<td>&lt; 8.0</td>
<td>&lt; 8.0</td>
<td>&lt; 8.0</td>
<td>&lt; 7.5</td>
</tr>
<tr>
<td></td>
<td>&lt; 8.0</td>
<td>&lt; 8.0</td>
<td>&lt; 8.0</td>
<td>&lt; 8.0</td>
<td>&lt; 7.5</td>
</tr>
</tbody>
</table>

$T_1$ and $T_2$ = The average of temperatures measured at the beginning and at the end of each experiment

$T_{av}$ = The average of temperatures $T_1$ and $T_2$ for the formulation

When amylose was added to ethylcellulose dispersions, the MFFT of the final mixed polymer dispersions appeared to be markedly lowered. The mechanism by which amylose lowers the MFFT of ethylcellulose is not understood. All the mixed formulations were now shown to have MFFTs of at least 10°C below 37°C. In theory, all of them would be suitable for spray-coating onto solid dosage forms at 37°C. However, not every film that could be formed would be suitable for oral colonic delivery. The film should be reasonably strong and flexible to withstand mechanical failures during its journey through the upper gastrointestinal tract. Such film qualities could only be tested when the formulations were spray-coated onto solid dosage forms.

In conclusion, although all the mixed formulations tested were likely to form films when being coated onto solid dosage forms at 37°C, their usefulness in an oral colonic delivery system need further evaluation.

3.4.3.2 Investigations of the spatial distributions of amylose within the mixed films

The iodine-stained mixed film was viewed under a light microscope. The results of focusing on the top, middle and bottom of the same section of film were shown in Fig 3.18 to 3.20.
Fig 3.18 The distribution pattern of the amylose-iodine complex as seen from the light microscope focused to the top of the iodine-stained amylose film

Fig 3.19 The distribution pattern of the amylose-iodine complex as seen from the light microscope focused to the middle of the iodine-stained amylose film
The results clearly showed that although amylose-iodine complexes were formed throughout the film, their domain patterns were different. Based on these observations, it was not clear if the amylose domains were actually continuous or discontinuous across the film. There is also concern that the results are not true reflections of the original distribution of amylose. This is because for the dark blue iodine-amylose complex to be formed, amylose would have to bind iodine within a highly specific six glucose units-per-turn-helical structure (Banks and Greenwood, 1975). The randomly orientated amylose chain within the glassy amylose would have to be reorganised into ordered helical structures. Such conformational changes in amylose chains could have had an effect on their original distribution pattern. Therefore, from these results the only conclusion that could be drawn was that amylose was widely distributed throughout the film. Their exact distribution pattern remained unclear.

Rather than viewing an iodine-stained film using light microscopy, an unstained film was viewed under differential interference contrast microscopy (DICM). Each component should have a unique colour. The results of sectioning and viewing of the mixed films under different focusing conditions are shown in Figs 3.21 and 3.22.
Resolution was poor. More than two colours, i.e. that of ethylcellulose and amylose, were seen. The extra colours could be due to excipients within the film or/and miscible blends of ethylcellulose and amylose.

The miscibility of amylose with ethylcellulose within the mixed film was therefore investigated using DMA. Initial determination of linearity using stress scans were done to ensure that all subsequent measurements were within the linear regions where Newton's and Hooke's laws apply (Figs 3.23 to 3.25).

Surelease +4%DBS films showed a complex tan $\delta$ profile (Fig 3.26). There are three peaks recorded at temperatures -37.5°C, 0°C and 50°C. Only the peak at 50°C was accompanied with a sudden drop in storage modulus. This was considered the $\alpha$-transition. The $\alpha$-peak temperature was taken to be the $T_g$ temperature. The other peaks were assigned to secondary transitions of $\beta$ and $\gamma$ in decreasing order of temperature of appearance.

Fig 3.21 The Surelease:amylose (5:1) + 4% DBS film viewed under the differential interference contrast microscope. Focus condition 1.
Fig 3.22 The Surelease:amylose (5:1) + 4% DBS film viewed under the differential interference contrast microscope. Focus condition 2.

Fig 3.23 Stress scan for Surelease+ 4% DBS

### Stress Scan

- **Strain (ε)**
  - 0.60
  - 0.50
  - 0.40
  - 0.35
  - 0.30
  - 0.25
  - 0.20
  - 0.15
  - 0.10
  - 0.05
  - 0.00

- **Storage Modulus (Pa x 10^9)**
- **Dynamic Stress (Pa x 10^7)**

**Dynamic Force (mN)**
- 200.0
- 400.0
- 600.0
- 800.0
- 1000.0
Fig 3.24 Stress scan for amylose

Fig 3.25 Stress scan for Surelease:amylose (5:1) +4% DBS
The $T_g$ was in good agreement with previously reported values for Surelease without additional plasticiser (Iyer et al., 1990). The $T_g$ generally vary a few degrees depending on the method of determination and the experimental conditions used.

Amylose films showed just one tan $\delta$ peak at -25°C and a rapidly increasing tan $\delta$ profile above 25°C (Fig 3.27). There was little change in the modulus of the material at -25°C and an increasing modulus profile. When the probe position was monitored, a sharp drop in the probe height was noted. This would suggest that the material had shrunk.

The tan $\delta$ peak at -25°C was not likely to correspond to the $T_g$ of amylose due to the lack of change in storage modulus. This peak was most likely to have been due to localised motions on the main chains. This is in good agreement with previously reported results which suggested that the $T_g$ of amylose was most likely to be above its thermal decomposition temperature (Scandola et al., 1991)(Orford et al., 1989). The position of this secondary relaxation peak would depend on the water content of the amylose film.
This peak had been reported to be between -85°C to -25°C in DMSO-based amylose films (Scandola et al., 1991).

The second broad tan δ rise about 25 °C was most likely to be due to water loss. This event was accompanied with the stiffening and shortening of the sample. Scandola et al. (1991) had shown that this is a common feature among hydrated polysaccharides in the temperature range where water mobilisation and evaporation can take place.

Fig 3.27 Temperature/Time scan for amylose

The tan δ profile of films containing blends of amylose and ethylcellulose are shown in Fig 3.28.
There were two prominent tan δ peaks in Surelease:amylose + 4% DBS (3:1) and (5:1) ratios at approximately -20°C and 55°C. The peak height at -20°C decreased relative to that at 55°C as the ratio of Surelease to amylose increased. This peak at -20°C disappeared altogether as the Surelease:amylose + 4% DBS ratio increased to (7:1). The tan δ profile for Surelease:amylose + 4% DBS (7:1) consisted of a tan δ peak at approximately 55°C with a shoulder peak at 0°C.

The peak at -20°C was most likely to be due to the secondary loss transition of the amylose component in the mixed film. The peaks at 0°C and 55°C were most likely to be due to the β and α loss transitions of Surelease in the mixed films. Within limits of experimental error, the temperatures at which these peaks appeared in the mixed film correlated very well to the temperature of appearance of the pure components. Therefore, the main chain components of amylose and Surelease are likely to be immiscible. However, it is not clear if there was some degree of miscibility between the side chains of ethylcellulose and amylose. The disappearance of the β and γ peaks of Surelease (i.e. at 0°C and -37.5°C, respectively) from the tan δ profile of the mixed polymer film could
be due to the "overlay" effect of the tan δ peak from amylose or it could be due to the shift of these secondary peaks as a result of partial miscibility of the side chains.

Amylose is largely immiscible with ethylcellulose and in theory, should be recognisable by the microbial enzyme as a digestion substrate. Therefore, the digestion profile of these mixed films were studied using simulated fermentation studies.

3.4.3.3 Investigations of the digestibility of the mixed polymeric films

All the free films in the fermentation experiment had different weights at the onset of the experiments. To make comparisons, the digestibility of the film was expressed as the percentage of film left after digestion. The percentage weight of film left, as shown in Table 3.16, was calculated as follows:

\[
\% \text{ of film left} = \frac{\text{final film weight}}{\text{initial film weight}} \times 100
\]

Amylose films formed by spraying or casting had comparable digestibility profiles. Therefore, cast films were considered valid models for testing the digestibility of eventually spray-coated films.

The percentage of film digested and the quantity of amylose present within the mixed Surelease/amylose film appeared to be related (Fig 3.29). As the amylose content of the film was increased, the degree of weight loss was also increased. This suggested that the digestible fraction within the film was most likely to be amylose. This was further confirmed by iodine tests. When films recovered from 24 hours of incubation in the fermentation studies were stained with iodine and viewed under light microscope, there were no regions within the Surelease:amylose (1:1) + 4% DBS film which turned dark blue indicating that the amylose fraction within the film had been digested away. This showed that the presence of increased hydrophobic plasticizer within this film had not prevented the digestion of amylose.
Table 3.16 The percentages of film left after digestion in \textit{in vitro} fermentation testing system

<table>
<thead>
<tr>
<th>Film formulation [Sprayed (S) or Cast (C)]</th>
<th>% Film left</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Test 6 hrs</td>
<td>Test 24 hrs</td>
<td>Control 6 hrs</td>
<td>Control 24 hrs</td>
</tr>
<tr>
<td>Amylose (C)</td>
<td>57.4</td>
<td>0.0</td>
<td>102.2</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Amylose (S)</td>
<td>32.2</td>
<td>0.0</td>
<td>95.7</td>
<td>76.6</td>
<td></td>
</tr>
<tr>
<td>Avebe* (C)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Surelease:amylose (1:1) + 4% DBS (C)</td>
<td>72.7</td>
<td>51.0</td>
<td>97.8</td>
<td>94.5</td>
<td></td>
</tr>
<tr>
<td>Surelease:amylose (3:1) + 4% DBS (C)</td>
<td>73.6</td>
<td>70.1</td>
<td>99.2</td>
<td>98.4</td>
<td></td>
</tr>
<tr>
<td>Surelease:amylose (5:1) + 4% DBS (C)</td>
<td>86.5</td>
<td>81.2</td>
<td>92.3</td>
<td>105.1</td>
<td></td>
</tr>
<tr>
<td>Surelease:amylose (5:1) + 4% DBS (S)</td>
<td>99.5</td>
<td>91.7</td>
<td>100.0</td>
<td>100.0</td>
<td></td>
</tr>
<tr>
<td>Surelease:Avebe* (3:1) + 4% DBS (C)</td>
<td>87.2</td>
<td>83.1</td>
<td>92.5</td>
<td>90.4</td>
<td></td>
</tr>
<tr>
<td>Aquacoat:amylose (1:1) + 36% DBS (C)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Aquacoat:amylose (3:1) + 36% DBS (C)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Aquacoat:amylose (5:1) + 36% DBS (C)</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td></td>
</tr>
<tr>
<td>Aquacoat:amylose (5:1) + 36% DBS (S)</td>
<td>94.9</td>
<td>88.2</td>
<td>95.7</td>
<td>94.6</td>
<td></td>
</tr>
</tbody>
</table>

* Avebe = amylose-butanol complex from Avebe, Netherlands
ND = Not done. Avebe --- No continuous Avebe films were produced. Only clear film fragments. Aquacoat --- Although continuous Aquacoat mixed films were produced they were too brittle to be removed from the PTFE plates. The continuous films cracked easily and no sufficiently large pieces of films could be obtained for testing.

When iodine was used to stain the Surelease:amylose (3:1) and (5:1) films which had undergone 24 hours of digestion however, some regions of the film still turned dark blue with iodine although such regions seemed less compared to those which had not undergone digestion. This would indicate that although amylose was still digestible when mixed with plasticized ethylcellulose, its rate of digestion was different. This change in the rate of digestion could be due to one or more of the following reasons.
The percentages of film left digested after 24 hours of incubation in faecal slurry as a function of the percentage of amylose present in Surelease/amylose mixed films.

![Graph showing the relationship between % of amylose present in the film and film loss. The line of best fit equation is y = 0.99x + 0.15 with r^2 = 0.996.]

The increased presence of ethylcellulose could result in amylose being less accessible to enzymatic attack because the amylose domains were no longer continuous through the cross-section of the film. The inaccessibility would be greatest when the ratio of ethylcellulose to amylose within the film was highest.

The presence of plasticizer could also have affected the rate of digestion of amylose. Most bacterial enzymes could not digest fats and oils and would not naturally be attracted to a non-substrate surface. Since the presence of dibutyl sebacate was directly proportional to the presence of ethylcellulose, the rate of digestion of amylose would decrease with the increasing ethylcellulose to amylose ratio. Further work, involving films which only differed in their plasticizer content, would have to be conducted to confirm this.

Thirdly, the rate of digestion of amylose could be affected by inhibition of swelling of amylose by ethylcellulose. The digestion of other polymers had been shown
to be affected when their maximum swelling abilities were reduced (Rubinstein and Gliko-Kabir, 1995).

The other important observation during this study was the inability of the amylose-butanol complex dispersion supplied by Avebe to form continuous films. This lack of continuous film structure was attributed to its low molecular weight fractions (Chapter 2.13). There were more low molecular weight fractions in the amylose from Avebe than in the from The Institute of Food Research, Norwich. Rowe (1980, 1981, 1982, 1986, 1992) has shown that there is a direct correlation between the molecular weight of a polymer and its film-forming properties. Better film-forming properties could be found in higher molecular weight polymers. Therefore, Avebe amylose was only considered for mixed film studies.

The amylose from Avebe, when mixed within a Surelease film, appeared to be digestible. When contained within the Surelease:amylose (3:1) + 4% DBS formulation, the digestion profiles of the two sources of amylose were similar. However, due to the limited results, it was not clear whether such similarities in digestion profiles would be true in all mixed polymer formulations.

The digestion of amylose within an Aquacoat/amylose mixed film could only be evaluated using sprayed films. Sprayed films, unlike cast films, showed greater experimentation errors. They tend to peel from their tin foil backing showing loss even in control experiments. In addition, the tin foil backing also reduced the rates of digestion by allowing digestion from only one side of the film. Nevertheless, the loss of film was greater in Aquacoat/amylose films incubated in the faecal slurries compared with those incubated in the phosphate buffer. This suggested that amylose within a highly plasticized Aquacoat film was digestible. The digestion rate was also comparable to Surelease:amylose (5:1) + 4%DBS sprayed film.

In conclusion, the amylose fractions within mixed films formed at temperature below 37°C were digestible. However, the rate of digestion of this amylose fraction decreased when the ratio of ethylcellulose to amylose increased. These observations were
true for mixed films formed from amylose-butanol complex supplied by The Institute of Food Research, Norwich and commercially available ethylcellulose dispersion, Surelease. When the source of amylose was changed to Avebe, Netherlands, and the source of ethylcellulose replaced by Aquacoat, no contrary findings were noted. However, based on the limited knowledge from this study, it was not possible to confirm that changes in the source of material would have no influence on digestion profiles of the formulations.

3.5 Conclusions

No butanol was detected by GLC in amylose films formed from amylose-butanol complex dispersion at 30°C. This would suggest that there was less than 2.5% of butanol present in the amylose film formed at this temperature.

Within the concentration range studied, i.e. 2% w/w to 6% w/w, no direct correlation could be shown between the degree of swelling of amylose film and the concentration of amylose-butanol complex dispersion used to form the films.

A second polymeric material, ethylcellulose was added to reduce the swelling of amylose. The commercially available aqueous ethylcellulose coating dispersions, Surelease®EA7100 and Aquacoat®ECD30, were shown to require additional plasticizer to form films at temperatures below 37°C. Different plasticizer incorporation techniques were needed to incorporate plasticizer into each of these aqueous ethylcellulose pseudolatices. The best plasticization technique for Surelease was by direct addition of plasticizer and Tween 80 with high-shear Silverson mixer. The effective plasticizers for Surelease were dibutyl sebacate, tributylcitrate and acetyl tributyl citrate. There was a direct correlation between the lowering of the minimum film forming temperature and the quantity of the effective plasticizer present. The best plasticization technique for Aquacoat was by mixing a crude plasticizer emulsion by low shear magnetic stirring for 30 minutes. The effective plasticizers were dibutyl sebacate, acetyl tributyl citrate, tributyl citrate, triethyl citrate and triacetin. A direct correlation was also shown between the lowering of the minimum film forming temperature and the quantity of the effective
plasticizer present.

Within the range studied, the mixed amylose/ethylcellulose films with additional dibutyl sebacate as plasticizer, were shown to have minimum film forming temperatures of no higher than 25°C. Therefore, it was possible to form mixed amylose/ethylcellulose films at temperatures below 37°C.

Investigations using light microscopy, DICM and DMA suggested that amylose formed immiscible domains within the mixed films. The domain pattern changes through the cross-section of the film and there was a possibility that they may become discontinuous. These amylose domains within the mixed film were digestible by human colonic bacteria when tested in *in vitro* fermentation systems. However, the rate of digestion decreased as the ratio of ethylcellulose to amylose increased.

The next stage of work would involve spray-coating these mixed polymeric coating formulations onto pellets at temperatures below 37°C.
Chapter 4: Preparation of 5-Aminosalicylic Acid and Glucose containing pellets by extrusion and spheronisation

4.1 Introduction

Pellets are spherical granules which can be produced by the extrusion and spheronisation processes. Pellets were chosen as the formulation of choice for oral colonic drug delivery for two reasons. First, its unique spherical shape, providing a smooth, minimum surface area is the easiest shape to achieve efficient, uniform coating. Second, pellets, a multiunit dosage form, is more suitable for targeted drug delivery as the risk of dose-dumping is equally subdivided (Bechgaard and Hegermann Nielsen, 1978, Follonier and Doelker, 1992). By the same token, the risk of incomplete release of the drug is also less likely to occur (Murthy, et al., 1983).

The technology of pellet production by the extrusion and spheronisation processes were reported in the pharmaceutical field as early as 1970 by Reynolds, Conine and Hadley. However, the science behind these processes are still being investigated. The four basic stages involved are formation of wet powder mass, high pressure extrusion, high speed spheronisation and drying (Newton, 1994, Vervaet et al., 1995). Various formulation and processing variables which are interdependable are involved in these processes (Pinto et al., 1992, Hasznos et al., 1992, Ku et al., 1993, Noché et al., 1994, Nesbitt, 1994, Blanqué et al., 1995, Sonaglio et al., 1995). Such complexity has made the prediction of the best pelleting formulation and production based on chemical and physical knowledge of the drug difficult. To date, the empirical approach of trial and error is still employed. In this project, to minimise trial and error experimentations, the processing variables were chosen based on literature knowledge, only formulation variables were studied. These are detailed below. First, the choice of model drugs and their effects on formulation variables are discussed.

4.1.1 Choice of model drugs

The pellet formulation contains two model drugs, glucose and 5-Aminosalicylic
acid (5-ASA). Glucose was chosen as this was the most likely model drug for future in vivo studies. In vivo studies involving $^{13}$C-labelled glucose has the advantage of being non-invasive. The metabolic product following ingestion, absorption and metabolism of $^{13}$C-glucose is the excretion of $^{12}$CO$_2$ in the breath, which can then be collected and measured using mass spectrometry (Schoeller et al, 1977, Milojevic, 1993, Milojevic et al, 1996b,c). However, glucose is not an easily detectable drug. In in vitro dissolution tests, it does not absorb uv, in in vitro fermentation tests, glucose could be confused with the end product of digestion of amylose. In view of these drawbacks, a second model drug, 5-ASA was introduced. It was hoped that once the system had been validated in vitro, 5-ASA in the formulation could be replaced with an inert excipient. This would minimise further pelletisation experimentations.

5-ASA was chosen as the second model drug because it readily absorbs uv and could be easily detected in in vitro dissolution and fermentation studies. It is an established drug for treating inflammatory bowel diseases (BNF, March 1996) and therefore is therapeutically logical to be targeted to the colon. Thirdly, previous work (Milojevic, 1993) suggested that it was likely to be stable against fermentation in the colon.

4.1.2 Choice of excipients and formulation variables to be investigated

The inherent properties of these model drugs have a great influence on the pelletisation process (Newton, 1990). It is rare to find a drug which naturally exhibit the right rheological properties to be extruded, the correct level of brittleness and plasticity to be cut up and shaped into pellets when spheronized. Often, these properties are achieved by the use of the right excipients. Knowledge of 5-ASA and glucose obtained so far (Milojevic, 1993) suggested that both drugs have very low plasticity. Therefore, a high level of the drugs within a pellet formulation would pose some difficulties. It was decided that a realistic maximum level of total drug content would be 60%, with each model drug contributing 30%. Microcrystalline cellulose (Avicel PH101) was chosen as the excipient to confer the required pelletisation properties to these drugs. Avicel PH101 had been shown to be able to form a wet powder mass with the appropriate rheological
characteristics i.e. a material which can be extruded to give a uniform product, yet is brittle enough to cut to uniform lengths on the spheronizer plate but still be plastic enough to round (Raines et al, 1989, Yuen, 1991, Pinto et al, 1992, Milojevic, 1993). This formed the basis of the dry powder mass to be investigated. Various other excipients could be added eg. lubricants and surfactants (Mesiha and Vallés, 1993) although their roles were not viewed to be of primary importance here.

Water was used to form a wet powder mass. The level of water was important. Too wet a powder mass would lead to subsequent agglomeration of pellets whereas too dry a powder mass would lead to subsequent fragmentation. The water level (Bains et al, 1991, Pinto et al, 1992, Haznos et al, 1992, Ku et al, 1993) and the water temperature (Ku et al, 1993) had been shown to be important in ensuring successful pelletisation. Although literature was abundant with the influence of water on the rheological properties of formulations for extrusion/spheronisation (Harrison et al, 1985a, Elbers et al, 1992, Shah et al, 1995), it was conspicuously void of any method of predicting the "right" water level needed for a given formulation. Therefore, water-level was identified as the formulation variable to be investigated in this project.

4.1.3 Choice of processing variables

As mentioned earlier, the formulation and processing variables are inter-related. In order to establish the influence of formulation variables, the operating conditions would have to be kept constant. Below is an account of how the important operating conditions were established.

Once a wet powder mass was formed, it would be extruded under pressure at a constant rate through a die. The rate of extrusion (Yuen, 1991, Pinto et al, 1992, Ku et al, 1993) and the length to radius ratio of the die (Harrison et al, 1985a, Fielden, 1987, Raines, 1990) were shown to influence the quality of pellets subsequently formed. A relatively high rate of extrusion, 400mm/min, was chosen for this project. A constant steady-state, which was a reflection of the uniformity of the extrudate formed, was easier to achieve with a high rate of extrusion. This was viewed as important especially when
two model drugs were involved. Care was also taken not to choose a die with short length to radius ratio. Harrison et al (1985a), Fieden (1987) and Raines (1990) identified that short length to radius ratio could give rise to rough and shark-skinned extrudates. Furthermore, Harrison et al, (1985b) warned that short length to radius rates fail to provide sufficient densification of pellets.

After the extrudates were formed, they were spheronised. Plate rotational speed (Woodruff et al, 1972, Chapman, 1985, Hasznos et al, 1992, Bataille et al, 1993) and spheronization time (Hasznos et al, 1992, Ku et al, 1993) were shown to be important. By increasing both spheronisation time and speed, the porousness and average pore diameter of the pellets formed would decrease resulting in smoother surfaced and harder pellets. All these were desirable pellet qualities for subsequent coating. Hence, a spheronisation time of 30 minutes and a spheronisation speed of 1000rpm were chosen.

Finally, the pellets formed were dried. There are three main methods employed for drying i.e. microwave, oven and fluidised bed drying. All these methods result in different qualities of pellets. Therefore, it was important to establish one method of drying. Microwave drying gave more porous pellets with higher average pore diameters compared to oven drying (Bataille et al, 1993) and is not the method of choice. Pellets formed from oven and fluidised bed drying were shown to have different dissolution profiles. There was a slightly slower dissolution profile from pellets dried by the fluidised bed technique (Dyer et al, 1994). Fluidised bed drying was chosen for its efficiency in drying.

Once all the operational conditions which had been shown to be important were set, the formulations for 5-ASA and glucose pellets were investigated.

4.2 Materials

5-Aminosalicylic acid (5-ASA) from Nobel Chemicals, Sweden. Batch no 27348/91
Dextrose anhydrous BP Ph Eur from BDH Merck, UK. Batch no 3099930
Avicel PH101 from FMC, USA. Batch no 0747 and Batch no 6240
4.3 Methods

Three formulations were investigated. They are listed in Table 4.1.

Table 4.1 Formulations of 5-ASA and glucose pellets

<table>
<thead>
<tr>
<th>Materials</th>
<th>Formulations (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>AG1</td>
</tr>
<tr>
<td>5-ASA</td>
<td>75</td>
</tr>
<tr>
<td>Dextrose</td>
<td>75</td>
</tr>
<tr>
<td>Avicel PH101</td>
<td>100</td>
</tr>
<tr>
<td>Water, deionised</td>
<td>87</td>
</tr>
</tbody>
</table>

Dry blending of the powders was carried out for 5 minutes using a Hobart planetary mixer with the speed set at 2. Then, deionised water was added. The wet mix was further blended for 10 minutes at the same mixing speed. In order to achieve a homogenous mix, care was taken to remove adherence of the mix to the sides of the mixing bowl periodically with a spatula.

The wet mass was extruded immediately after mixing. The wet mass (50-100)g was packed into a stainless steel barrel (2.54 cm internal diameter, approximately 20cm in length) and die (length 2mm, diameter 1mm) assembly. A piston, or ram, was inserted atop the powder mix into the stainless steel barrel. The whole assembly rested on a rigid metal C-piece. A load, driven by a physical testing instrument (J. J. Lloyd model MX50, UK), at a constant rate of 400mm/min was applied to the piston to extrude the material through the die. The displacement and load of the extrusion process were recorded by computer to produce a force-displacement profile.

The extrudates obtained were then processed in the spheroniser (GB Caleva Ltd., UK) using a 20.32 cm diameter radial plate rotating at 1000 rpm. The spheronisation time was set at a maximum of 30 minutes. The lid on the spheroniser chamber was periodically removed to avoid build up of excess moisture in the chamber.
The pellets obtained were then dried in a fluidised bed dryer (P.R.L. Engineering Ltd., Model No FDBL 70, UK) for 30 minutes at 40°C. After drying the pellets were sieved through a set of sieves, arranged in a $\sqrt{2}$ progression for 10 minutes, using a mechanical sieve shaker (Endecott Ltd., UK). The sieve fraction, (1.40 - 1.70) mm in diameter, was used for coating.

4.4 Results and Discussion

The observations were tabulated in Table 4.2 and the graphs of load against time of each formulation as computed by the physical testing instrument were shown in Figs 4.1 to 4.3.

The results suggested that the best formulation was AG1 which was the only formulation that had successfully produced any satisfactory pellets. The results demonstrated that the formulation was very sensitive to water.

Table 4.2 Results of each formulation of 5-ASA and glucose pellets

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Steady state force (kN)</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>AG1</td>
<td>10.7</td>
<td>Pellets were spherical. Yield in each size fraction was as follows: (1.0-1.4)mm diameter (11.3%); (1.4-1.7)mm diameter (59.4%); (1.7-2.0)mm diameter (29.3%)</td>
</tr>
<tr>
<td>AG2</td>
<td>6.5</td>
<td>Pellets agglomerated as soon as spherisation began. Most pellets were above 2mm in diameter.</td>
</tr>
<tr>
<td>AG3</td>
<td>10.7</td>
<td>Pellets were still dumbell in shape at the end of 30 minutes spherisation time.</td>
</tr>
</tbody>
</table>
Fig 4.1 The force against time graph for formulation AG1

Fig 4.2 The force against time graph of formulation AG2
The sensitivity of the formulations to water and its lack of plasticity could be partly explained by the poor water-retention property of 5-ASA. It was observed that during spheronisation, the build-up of water vapour within the spheronisation chamber was abnormally high. Sometimes, water could be seen dripping if the periodic removal of the lid was not done. The rapid loss of water would then lead to lack of plasticity of the wet mass. This in turn result in the long spheronisation time required. This was noticed by Milojevic (1993) in the development of a 5-ASA formulation and bentonite was added to the formulation to overcome the problem. In this case it was felt that since pellets were formed using the AG1 formulation, no additional excipients were necessary. However, the AG1 formulation was found to give lots of scale-up problems. When too large a batch was spheronised, the build up of water vapour was so rapid that the pellets agglomerated. This problem could not be overcome by leaving the lid off as the formulation then exhibited a lack of plasticity and would remained dumbell in shape after 30 minutes of spheronisation. Therefore, batches of 250g were made each time.
4.5 Conclusion

It was shown to be possible to make 5-ASA and glucose containing pellets. 250g batches were made each time to yield 60% 1.4-1.7 mm sieve fraction pellets. This was the size fraction used for spray-coating.
5.1 Introduction

In the development of a coated oral colonic delivery solid dosage form, 5-Aminosalicylic Acid (5-ASA) pellets were coated with amylose-containing coating formulations. The pellets were prepared as detailed in Chapter 4 and the coating formulations were developed based on findings in Chapter 3. The coated dosage forms were then tested in *in vitro* testing systems. A successful oral colonic delivery system is one which showed no release in the upper gastrointestinal tract (GIT) but rapid release in the colon.

The application of coat to the pellets was carried out by spraying. In effect, the film coat was made up by layering. Droplet formation, contact with the moving pellets, spreading, coalescence and evaporation are events which occur almost simultaneously during this process (Jones, 1994). The interplay of these events are better controlled in certain coating equipments than others. More importantly, the choice of coating equipment in this case should be one which has an efficient evaporation rate at low coating temperature. Therefore, special care was undertaken in the selection of the coating equipment to find one which was most efficient for low temperature coating.

5.1.1 Choice of coating equipment

Modern types of spray-coating equipment can be broadly divided into two main categories: rotating drum and fluidised beds (Arwidsson and Ruden, 1993). The rotating drum equipment is a modification of the coating pans. It is made of a cylindrical, perforated, rotating drum. Unlike conventional coating pans, which have low drying efficiency due to the drying air being able to contact only the upper surfaces of the core, the efficiency of the rotating drum is higher by optimising air-flow through the perforation drum. The heated air penetrates the bed of cores, greatly improving drying.
The spraying is usually concurrent with the air flow. This ensures rapid continuous drying without disruption of the spray-pattern. The movement of the cores are generated by the rotation action of the drum. As the drum rotates, the cores follow the drum up to a certain point and then "roll" downwards on top of the core bed. This gentle movement is useful for coating brittle material but not for coating small particles like pellets, as the low relative motion encourages agglomeration. Therefore, this equipment was not selected.

In the fluidised bed, the cores are moved more vigorously by moving air. This is more suited for small particles to avoid agglomeration. There are at least four types of equipment used in pharmaceutical industry, the Aeromatic, Wurster, Glatt and Hüttlin Kugelcoater (Pickard et al, 1974, Yang et al, 1992, Bueb et al, 1994, Arwidsson and Ruden, 1993). The basic principle in all these systems are the same. The cores are accelerated from the product container past the spray nozzle by air moving upwards through the core bed. As the fluidised core passed the spray nozzle, they are sprayed by the coating liquid. The coated cores then travel through the coating "zone" into the expansion chamber where drying takes place. The wider diameter in the drying "zone" permits deceleration of the cores. The cores then fall back into the product container and continue cycling throughout the duration of the process. The Wurster chamber, a cylindrical partition which is mounted in the centre and slightly above the gas distributor plate, can be used in combination with the Aeromatic (Yang et al, 1992) or the Glatt (Pickard et al, 1974) equipment. The main advantage of combining Wurster to these equipments is to provide better fluidisation of cores within the chamber.

There is also a choice of spraying mode. The cores within the chamber can be sprayed with one of the three spray modes, the top-spray or counter-current spraying, the bottom-spray or co-current spraying and the tangential-spray. Only certain Glatt models are capable of all three spray modes (Yang et al, 1992) and the Aeromatic is capable of top and bottom spray modes. The Wurster and Kugelcoater are essentially bottom-sprayed fluidisation equipments. Literature suggests that bottom and tangential spray modes are superior to top-spray mode in giving more even, reproducible results. There is very little difference between bottom and tangential spray modes (Yang et al, 1992,
Arwidsson and Ruden, 1993). The main reason for this is the distance between the bottom and tangential spraying device and the cores is short and well defined when coating occurs. This encourages even coating. On the other hand, the distance between the top-spray and the moving cores are variable. Therefore, the spray-droplet sizes as they impinge onto the moving cores vary resulting in an uneven coating.

The minimum batch sizes required for a Glatt machine is greater than an Aeromatic. Therefore a bottom-sprayed Aeromatic with a narrow product-containing chamber was chosen. A Hüttlin Kugelcoater was not available although in theory, it could also be used for small batch coating. A Wurster chamber was not used in combination with the Aeromatic because it was suggested that for small batches of cores, a narrow-coating chamber would be adequate to provide uniform movement of pellets in the bed and regular motion in the coating zone (Yang et al, 1992).

5.1.2 Consideration of processing variables

Once the choice of equipment was established, the processing variables such as air supply, temperature and spray-rate were considered. The three variables are interrelated in their contributions to the rate of evaporation of the coating application media which can significantly affect film formation. The rate of evaporation cannot be too high as it has been suggested that humidification can enhance film formation (Fukumori et al, 1993, Watano et al, 1993). Rapid drying minimises the time and thermal energy needed for the polymeric particles to deform and rearrange into films. In addition, it also causes electrostatic effects and greater loss of coatings. Conversely, low drying rate leads to agglomeration due to bridging between pellets.

In this case, a moderate air supply (fan capacity) was chosen to achieve the optimum drying rate. The spray-rate was set at a very low level as Milojevic (1993) showed that a lower spray rate was favoured in giving smoother, more even coating for amylose containing formulations.

Setting the coating temperature was more difficult. Although this project
primarily aims to investigate the effects of low temperature coating, Jones (1994) illustrated the danger of using too low a coating temperature. The author pointed out that if too low a coating temperature, eg 25°C, was used, the drying capacity could fluctuate greatly due to seasonal changes in the weather. The air supply would have to be adjusted on each coating trial depending on the weather. This is because the relative humidity within the chamber and that of the environment is low. On a hot, humid day, the humidity of the environment could be relatively higher than within the coating chamber therefore, very low drying capacity, or even condensation, would be seen. It was suggested that absolute humidity must be controlled to allow reproducible results when very low temperatures are used. Lehmann (1994) suggested dehumidification of incoming air when low coating temperatures were necessary. In addition, Lippold et al. (1989) and Lehmann (1989) suggested that good films could only be achieved if the coating temperature is at least (10-20)°C above the minimum film forming temperature (MFFT) of the coating material. Therefore, the coating temperature was set at 36°C, with a bed temperature of 32°C. This was considered as a temperature which was higher than atmospheric temperature at all seasons and above the MFFT of the formulations investigated but still low enough to coat thermolabile materials. The inlet air in all cases were not dehumidified nor was the absolute humidity of the laboratory controlled. In theory, a much lower coating temperature may be used if the equipment has a cooling device and a humidity-controlled device. Watano et al (1993) showed how humidity within the fluidised bed could be controlled using an IR moisture sensor. Glatt® has also developed a vacuum fluid bed particle coater (Glatt handbook, Wehrle, 1982) which could overcome humidity problems mentioned above.

5.1.3 Rationale of project design

The development of the coated oral colonic delivery solid dosage form was taken in three stages. The first stage involved an understanding of the formulation variables. This was studied using statistical analysis of variance. The second stage then involved developing coating formulations which could resist the premature release of drug in the upper GIT. The stability on storage and the reproducibility of coating of these formulations were also investigated. Thirdly, optimization of the release profiles of the
coated dosage forms were done. Two coating formulation systems were developed, the Surelease®EA7100/amylose system and the Aquacoat®ECD30/amylose system.

In the first and second stages of development, the resistance to drug release in the upper GIT of the coated solid dosage form could be tested using USP dissolution testing guidelines. In the third stage of development whereby the drug release profile in the colon was investigated, there were no well-known monographs or guidelines. Therefore, a colonic testing system, which had previously been used for testing digestion of free films (Chapter 3) was further optimised to test coated pellets.

5.2 Materials

The coating was applied onto pellets of (1.4 - 1.7) mm in diameter. These pellets, prepared as mentioned in Chapter 4, contained:

a) 30% of 5-ASA from Nobel Chemicals, Sweden. Batch no 27348/91
b) 30% of Dextrose Anhydrous BP from Merck, UK. Batch no 3099930
c) 40% of Avicel®PH101 from FMC Corporation, USA. Batch no 0747 and 6240

For the coating formulations, the followings were used:

a) Aqueous dispersion of amylose-butan-1-ol complex supplied by Institute of Food Research, Norwich and concentrated to 6% w/w at The School of Pharmacy prior to use.
b) Aqueous dispersion of amylose-butanol complex made and concentrated to 6% w/w at The School of Pharmacy
c) Surelease®EA7100 from Colorcon, USA. Batch no 600041-1
d) Aquacoat®ECD30 from FMC Corporation, USA. Batch no J3202
e) Tween 80, technical grade, from Merck, UK. Batch no 3139220

The coated pellets were tested in simulated gastric and small intestinal conditions using media made up of:
a) 5N Hydrochloric acid. AnalaR grade, Merck, UK. Batch no 50065821
b) Potassium dihydrogen orthophosphate, AnalaR grade, Merck, UK. Batch no A890225
c) Sodium hydroxide pellets, AnalaR grade, Merck, UK. Batch no 050594H225S
d) Pepsin from Sigma, UK. Potency 1:2500. Batch no 45H0867
e) Pancreatin from Sigma, UK. Potency equivalent to USP specification. Batch no 100H0124
f) Citric acid, general purpose grade, Merck, UK. Batch no 3863580M
g) Disodium phosphate, general purpose grade, Merck, UK. Batch no 5029200M

Stability studies were conducted in controlled relative humidity conditions using chemicals from Merck, UK:

a) Silica gel, technical grade, Merck, UK. Batch no 7019980N
b) Potassium carbonate, general purpose reagent grade, Merck, UK. Batch no K21928435530
c) Sodium nitrite, AnalaR grade, Merck, UK. Batch no C216466502

The coated pellets were tested in simulated colonic conditions using media made up according to the formula mentioned in Chapter 3 with the following chemicals from Merck, UK:

a) Dipotassium hydrogen phosphate, AnalaR grade, Batch no 302 A604476
b) Potassium dihydrogen phosphate, AnalaR grade, Batch no A890225 551
c) Sodium chloride, GPR grade, Batch no K20603432
d) Magnesium chloride, MgCl$_2$.6H$_2$O, GPR grade, Batch no TA576032
e) Ferrous sulphate, FeSO$_4$.7H$_2$O, GPR grade, Batch no A843740 522
f) Calcium chloride, CaCl$_2$.2H$_2$O, GPR grade, Batch no TA 694532 445
5.3 Methods

5.3.1 Preparation of aqueous coating formulations

The ethylcellulose coating dispersions were plasticized prior to mixing with amylose. The two commercially available ethylcellulose coating dispersions were plasticized with different techniques.

Surelease had been used with or without additional plasticizer. When Surelease was mixed with additional plasticizer, the required quantity of dibutyl sebacate (DBS) was added to Surelease and mixed with high shear mixing using a Silverson for 3 minutes. The mixture was covered and left overnight to remove the foam formed during mixing. The required quantity of plasticized Surelease dispersion was then mixed at room temperature, with amylose-BuOH complex dispersion using a low-speed magnetic stirrer. Stirring was maintained throughout coating.

Aquacoat could not be plasticized directly with the required quantity of plasticizer, instead it was plasticized with a plasticizer dispersion. The DBS plasticizer was made up using 50% DBS, 0.1% Tween 80 and 49.9% water (Röhm Pharma, 1993). The resulting mixture was mixed with Silverson mixture to give a crude emulsion. This had to be freshly prepared for two reasons. One, the plasticizer dispersion was not stable and oil and water separation was seen after one day's storage. This could be remixed by shaking the bottle by hand or passing it through the Silverson again. However, the oil turned rancid upon prolonged storage. Hence, it was found preferable to prepare the plasticiser dispersion freshly. Aquacoat was mixed with the plasticizer dispersion by magnetic stirring for 30 minutes prior to addition of amylose-BuOH complex dispersion. Stirring was maintained throughout coating. The process of plasticization of Aquacoat was carried out at room temperature.

All calculations in the coating formulations were the same as those discussed in Chapter 3 i.e. based on dry solids weight. All the percentages of DBS in the experiments were expressed as percentages of ethylcellulose dry polymer weight and not the total...
polymer weight of the system. This approach was adopted as DBS was an ethylcellulose plasticizer which was incorporated prior to addition of amylose. Examples of calculations from each formulation system are as follows:

**Surelease:amylose (5:1) + 4% DBS**

<table>
<thead>
<tr>
<th>Coating materials (% w/w)</th>
<th>Polymer solids (g)</th>
<th>Dispersions (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surelease (25% w/w)</td>
<td>5.0</td>
<td>20.0</td>
</tr>
<tr>
<td>Amylose (6% w/w)</td>
<td>1.0</td>
<td>16.7</td>
</tr>
<tr>
<td>DBS</td>
<td>0.2</td>
<td>0.2</td>
</tr>
</tbody>
</table>

**Aquacoat:amylose (5:1) + 36% DBS**

<table>
<thead>
<tr>
<th>Coating materials (% w/w)</th>
<th>Polymer solids (g)</th>
<th>Dispersions (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquacoat (30% w/w)</td>
<td>5.0</td>
<td>16.7</td>
</tr>
<tr>
<td>Amylose (6% w/w)</td>
<td>1.0</td>
<td>16.7</td>
</tr>
<tr>
<td>DBS (50% w/w)</td>
<td>1.8</td>
<td>3.6</td>
</tr>
</tbody>
</table>

5.3.2 Fluid bed coating

The coating process was undertaken in an Aeromatic AG Strea-1, fluidised bed bottom-sprayed coater (ACM Machinery, Tadley). Forty grammes batches of pellets were used each time, the pellets being prewarmed before coating. The conditions at the pre-warming stage were:

- Inlet temperature, °C = 35
- Outlet temperature, °C = 20-28
- Atomising air pressure, bar = 0.1
- Fluidisation air, units = 5
Once the outlet temperature was ~28°C (approximately 5 minutes), the coating solution was fed via a peristaltic pump into the bottom-sprayed nozzle. Coating conditions were:

- Inlet temperature, °C = 36
- Outlet temperature, °C = 32
- Atomising air pressure, bar = 0.1
- Fluidisation air, units = 13
- Spray rate, ml/minutes = 0.6-0.7 (Depending on the viscosity of the resultant mixture)
- Drying time, minutes = 30
- Curing time, minutes = 0

To determine the end point, its increase in pellet weight was checked. This increase in weight was expressed as percentage total weight gain (%TWG).

\[
\% \text{ TWG} = \frac{\text{weight increase}}{\text{Final weight of coated pellets}} \times 100
\]

5.3.3 Statistical analysis of variance

The pellets were spray-coated with a series of coating formulations to investigate the influence of various formulation variables. The Surelease / amylose system was studied using a two way analysis of variance. The two variables were coat thickness and the Surelease to amylose ratio. No additional plasticizer was incorporated. The MFFT results in Chapter 3 suggested that films could be readily formed at the chosen operational temperature. The experiments are listed in Table 5.1.

The Aquacoat / amylose system was studied using a three-way analysis of variance. The three variables were coat thickness, Aquacoat to amylose ratio and plasticizer quantity. Amylose-BuOH complex dispersion made at the School of Pharmacy was used for the statistical analysis of this system. The experiments are listed in Table 5.2.
Table 5.1 Pellets coated with the following coating formulations for Surelease / amylose two-way analysis of variance

<table>
<thead>
<tr>
<th>Surelease:amylose ratio</th>
<th>Thickness (% TWG)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.5:1</td>
<td>5</td>
</tr>
<tr>
<td>2.5:1</td>
<td>10</td>
</tr>
<tr>
<td>2.5:1</td>
<td>15</td>
</tr>
<tr>
<td>5:1</td>
<td>5</td>
</tr>
<tr>
<td>5:1</td>
<td>10</td>
</tr>
<tr>
<td>5:1</td>
<td>15</td>
</tr>
<tr>
<td>7:1</td>
<td>5</td>
</tr>
<tr>
<td>7:1</td>
<td>10</td>
</tr>
<tr>
<td>7:1</td>
<td>15</td>
</tr>
<tr>
<td>10:1</td>
<td>5</td>
</tr>
<tr>
<td>10:1</td>
<td>10</td>
</tr>
<tr>
<td>10:1</td>
<td>15</td>
</tr>
</tbody>
</table>
### Table 5.2 Coating formulations for Aquacoat / amylose, three way analysis of variance

<table>
<thead>
<tr>
<th>Aquacoat:amylose ratio</th>
<th>Thickness (% TWG)</th>
<th>Plasticizer content (% w/w of Aquacoat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:1</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>5:1</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>5:1</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>5:1</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>5:1</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>5:1</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>5:1</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>5:1</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>5:1</td>
<td>15</td>
<td>36</td>
</tr>
<tr>
<td>7:1</td>
<td>10</td>
<td>24</td>
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<tr>
<td>7:1</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>7:1</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>7:1</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>7:1</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>7:1</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>7:1</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>7:1</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>7:1</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>7:1</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>7:1</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>7:1</td>
<td>15</td>
<td>36</td>
</tr>
<tr>
<td>10:1</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>10:1</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>10:1</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>10:1</td>
<td>5</td>
<td>24</td>
</tr>
<tr>
<td>10:1</td>
<td>5</td>
<td>30</td>
</tr>
<tr>
<td>10:1</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>10:1</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>10:1</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>10:1</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>10:1</td>
<td>15</td>
<td>24</td>
</tr>
<tr>
<td>10:1</td>
<td>15</td>
<td>30</td>
</tr>
<tr>
<td>10:1</td>
<td>15</td>
<td>36</td>
</tr>
<tr>
<td>10:1</td>
<td>15</td>
<td>36</td>
</tr>
</tbody>
</table>
5.3.4 Stability on storage

The coated pellets were stored in desiccators maintained at different relative humidity environments and temperatures. The storage conditions investigated were as follows:

<table>
<thead>
<tr>
<th>Conditions</th>
<th>Temperature (°C)</th>
<th>Relative humidities (%)</th>
<th>Saturated salt solutions</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
<td>0</td>
<td>silica gel</td>
</tr>
<tr>
<td>2</td>
<td>20</td>
<td>44</td>
<td>potassium carbonate</td>
</tr>
<tr>
<td>3</td>
<td>20</td>
<td>66</td>
<td>sodium nitrite</td>
</tr>
<tr>
<td>4</td>
<td>4</td>
<td>44</td>
<td>potassium carbonate</td>
</tr>
<tr>
<td>5</td>
<td>37</td>
<td>44</td>
<td>potassium carbonate</td>
</tr>
</tbody>
</table>

5.3.5 In vitro dissolution studies

The coated pellets were tested using the paddle-stirred dissolution testing apparatus, PharmaTest model PTWS (Apparatebau, Germany). The quantity of pellets equivalent to 500mg of uncoated pellets were placed in 900ml of dissolution medium maintained at 37°C. The medium was stirred continuously at 100rpm. For the first 3 hours 0.1N HCl (pH 1.5) was used as dissolution medium. This was followed by 9 hours in phosphate buffer pH 7.2. At specific intervals, 3 ml samples were withdrawn by means of an automated sampler (PharmaTest, Apparatebau, Type PTFCl, Germany). The samples were then measured for absorbance using a UV-Vis spectrophotometer (Perkin-Elmer 554) at 302nm for 0.1N HCl and 332 nm for phosphate buffer.

The in vitro release of 5-ASA from the most acceptable coating formulation was further evaluated under the simulated gastrointestinal conditions as described in USP XXI. 900ml of freshly prepared simulated gastric fluid (0.1N HCl containing 0.32% w/v pepsin) was used as the dissolution media for the first three hours and then replaced with 900ml of freshly prepared simulated intestinal fluid (0.2M phosphate buffer containing 1% w/v pancreatin) for an additional nine hours. The samples were centrifuged and
filtered through 0.2 μm filters prior to measurement for absorbance.

5-ASA had previously been shown to absorb linearly in both the acid and phosphate buffers. The concentration of 5-ASA released from the coated pellets was calculated from the calibration curves.

The in vitro release of glucose from the most acceptable formulation was also evaluated as above. The dissolution media used were 0.1N HCl (pH 1.5) for 3 hours and phosphate buffer (pH 7.2) for 9 hours. The glucose concentration was measured using a Glucose-GOD PERID® diagnostic kit (Boehringer Mannheim, UK, Lenes, East Sussex). The technique uses the ammonium salt 2,2'-azino-di-[3-ethyl-benzothiazoline-(6)-sulphonic acid] (ABTS) as a redox indicator for the photometric determination of glucose following the enzymic action of glucose oxidase and peroxidase. The test solutions containing glucose was mixed with the GOD PERID reagent and incubated at 37°C for 15 minutes in which time the following reaction occurred:

\[
\text{GOD} \quad \text{glucose} + \text{O}_2 + \text{H}_2\text{O} \quad \rightarrow \quad \text{gluconate} + \text{H}_2\text{O}_2
\]

\[
\text{POD} \quad \text{H}_2\text{O}_2 + \text{ABTS} \quad \rightarrow \quad \text{coloured complex} + \text{H}_2\text{O}
\]

where: GOD is glucose oxidase

POD is peroxidase (catalyses oxidation in which molecular oxygen serves as an electron acceptor)

The resulting coloured complex absorbs UV light at 420nm and the absorbance in each medium was calibrated.

The in vitro conditions for testing release of 5-ASA from the coated pellets were also varied to investigate the influence of variation of the upper GIT conditions to premature release. The conditions tested were listed in Table 5.3.
Table 5.3 Conditions for testing the stability of the AG1 coated pellets in the upper gastrointestinal tract.

<table>
<thead>
<tr>
<th>Gastric pH</th>
<th>Gastric residence time (minutes)</th>
<th>Small intestinal pH</th>
<th>Small intestinal residence time (minutes)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.5</td>
<td>0</td>
<td>7.2</td>
<td>720</td>
</tr>
<tr>
<td>1.5</td>
<td>15</td>
<td>7.2</td>
<td>705</td>
</tr>
<tr>
<td>1.5</td>
<td>30</td>
<td>7.2</td>
<td>690</td>
</tr>
<tr>
<td>1.5</td>
<td>60</td>
<td>7.2</td>
<td>660</td>
</tr>
<tr>
<td>1.5</td>
<td>120</td>
<td>7.2</td>
<td>600</td>
</tr>
<tr>
<td>2.2</td>
<td>180</td>
<td>7.2</td>
<td>540</td>
</tr>
<tr>
<td>3.0</td>
<td>180</td>
<td>7.2</td>
<td>540</td>
</tr>
<tr>
<td>4.0</td>
<td>180</td>
<td>7.2</td>
<td>540</td>
</tr>
<tr>
<td>5.0</td>
<td>180</td>
<td>7.2</td>
<td>540</td>
</tr>
<tr>
<td>6.0</td>
<td>180</td>
<td>7.2</td>
<td>540</td>
</tr>
</tbody>
</table>

5.3.6 *In vitro* fermentation studies

The fermentation test system was a batch culture test system which had previously been used to test digestibility of free films. See 3.3.3.4. The system was tested to compare the release performance of uncoated pellets in the shaken and unshaken testing conditions. The volume of the fermentation medium and the quantity of pellets were also varied to investigate the optimum testing condition for 5-ASA within this medium. All experiments were done in duplicate.

Prior to the study of 5-ASA release from coated pellets in the colon, a study was done to investigate the stability of 5-ASA in faecal slurry. In this study, known amounts of 5-ASA powder was added to 100ml each of faecal slurry and phosphate buffer in anaerobically sealed bottles which were shaken under the same conditions as the testing procedure mentioned below. Samples were taken periodically and were treated in the same manner as the test samples. The content of 5-ASA in these samples was measured using high performance liquid chromatography by the Medicines Research Unit, University of Derby.
A known quantity of pellets was soaked in 100ml of 0.1N HCl for a maximum of 30 minutes, then transferred into simulated colonic medium in anaerobically sealed bottles. The bottles, sealed under positive N₂ pressure, were then laid horizontally in an incubator shaker (37°C) with the rotar arm speed of 100rpm throughout the experiment. 2.0ml samples were withdrawn from the bottles at specific times. These were then centrifuged for 5 minutes at 13 000 rpm. The clear supernatant was removed and centrifuged a second time at 13 000 rpm for 5 minutes. Lastly, the supernatant was filtered through 0.2μm pore size filters, frozen and sent to the Medicines Research Unit, University of Derby to be analyzed by HPLC at 300nm. HPLC was conducted using a Techsphere 5μm ODS 25 cm x 4.6 mm column, with Methanol:1% Acetic acid (10:90) as mobile phase. The flow rate was set at 1.5 ml/minute. To monitor the reproducibility of the new fermentation testing condition, the experiment with the uncoated pellets was repeated in each run. To ensure that each faecal slurry batch was active, two pieces of amylose cast films were incubated in 100ml for each batch of slurry used. The films should show digestion after 6 hours and be completely digested by 24 hours in an active slurry (See chapter 3).

5.3.7 Scanning electron microscopy (SEM)

SEM was employed to "visualise" the quality of the coated pellets. The pellets either whole, or halved were stuck to SEM stubs using carbon impregnated double sided tape. It was then sputter coated using an Emitech K550 sputter coated set at 25mA for 3 minutes. They were then scanned using Phillips XL20 SEM using low accelerating voltages usually 5 or 8 kV. Images were captured onto Kodak TMax 100 black and white film and printed on Ilford Multigrade.
5.4 Results

5.4.1 The Surelease® EA 7100 / amylose system

5.4.1.1 The statistical analysis of variance

When the Surelease to amylose ratio maintained constant, as the coat thickness was increased, the amount of drug released in dissolution tests was decreased (Figs 5.1 to 5.4). The effect of thickness was most marked when the Surelease to amylose ratio was lowest. Above a Surelease:amylose ratio of (7:1), the effect of thickness on dissolution was small.

Fig 5.1

Effect of coat thickness on the dissolution results of AG1 pellets coated with Surelease EA7100:amylose (2.5:1) at 35°C

- = uncoated
■ = 5% TWG
△ = 10% TWG
▼ = 15% TWG

% cumulative 5-ASA released

0 25 50 75 100 125

0 150 300 450 600 750

Time / minutes

- 0.1N HCl → --- phosphate buffer pH 7.2 → →
Fig 5.2

Effect of coat thickness on the dissolution results of AG1 pellets coated with Surelease EA7100:amylose (5:1) at 35°C

- • = uncoated
- ■ = 5% TWG
- △ = 10% TWG
- ▽ = 15% TWG

% cumulative 5-ASA released

0 25 50 75 100 125

0 150 300 450 600 750

0.1N HCl - - - phosphate buffer pH 7.2 ---

Time / minutes

Fig 5.3

Effect of coat thickness on the dissolution results of AG1 pellets coated with Surelease EA7100:amylose (7:1) at 35°C

- • = uncoated
- ■ = 5% TWG
- △ = 10% TWG
- ▽ = 15% TWG

% cumulative 5-ASA released

0 25 50 75 100 125

0 150 300 450 600 750

0.1N HCl - - - phosphate buffer pH 7.2 ---

Time / minutes

177
Similarly, by keeping coat thickness constant, it was shown that as the ratio of Surelease to amylose was increased, the amount of drug released in the dissolution test was decreased (Figs 5.5 to 5.7). The dissolution was retarded (i.e. < 5% in 12 hours) even with as low a coat thickness as TWG = 5% in some cases.
Fig 5.6

Effect of varying Surelease EA7100:amylose ratio on the dissolution results of AG1 pellets coated to 10% TWG thickness at 35°C

- = uncoated
- = 2.5 : 1
- = 7 : 1
- = 5 : 1
- = 10 : 1

Fig 5.7

Effect of varying Surelease EA7100:amylose ratio on the dissolution results of AG1 pellets coated to 15% TWG thickness at 35°C

- = uncoated
- = 2.5 : 1
- = 7 : 1
- = 5 : 1
- = 10 : 1
The dissolution results from all the 12 coating formulations could be represented in a contour graph (Fig 5.8).

Fig 5.8.

The results were analysed using multivariate analysis of variance (MANOVA). Hotelling's multivariate test of significance ($T^2$) was used. This statistical test is capable of analysing the entire dissolution profile, rather than just analysing it at specific sampling time points. Hotelling's $T^2$ values were approximated onto the F distribution and the results are quoted as a significance of F, (Table 5.4).

Table 5.4 The influence of ratio and coat thickness on the dissolution profile of AG1 pellets coated with Surelease/amylose mixed films analysed by MANOVA

<table>
<thead>
<tr>
<th>Factor</th>
<th>$T^2$</th>
<th>F</th>
<th>DF (H)</th>
<th>DF (E)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio</td>
<td>135.3</td>
<td>236.8</td>
<td>20</td>
<td>70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thickness</td>
<td>173.4</td>
<td>303.4</td>
<td>20</td>
<td>70</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interaction between ratio and thickness</td>
<td>142.2</td>
<td>122.7</td>
<td>40</td>
<td>138</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$T^2$ = Hotelling's value; F = Approximate F-distribution value; DF (H) = Hypothetical degrees of freedom; DF (E) = Error in the degrees of freedom; P = Probability value
MANOVA confirmed that, within the range of study, the influence of each of these variables was significant. More importantly, statistics highlighted that there was also a significant interaction between these two variables. This means that the effect of the change of one variable depends on the level of the second variable and vice versa. After an initial trial to fit the data to a linear model, which failed, non-linear equations linking the dissolution profile and the variables were generated empirically. No equation was chosen as all the equations generated empirically only provided a poor fit to the data.

5.4.1.2 Stability studies

The stability of AGI pellets coated with Surelease:amylose (5:1) TWG = 5% was investigated for stability on storage. One month stability studies showed a more rapid drug release compared to initial results. The stability of the coat was influenced by both temperature and humidity. Low temperature and high relative humidity (RH) were required to ensure stability (Figs 5.9 and 5.10).

SEM examination of the coat showed that there were drug crystals on the outer surfaces of the coat on storage (Fig 5.11). This would suggest that the coat may have cracked upon storage allowing drug migration to the outer surfaces. Drug migration was most serious in the batch stored at 0% RH.

Fig 5.9 Effect of storage temperature on the dissolution results of AG1 pellets coated with Surelease EA7100:amylose (5:1) TWG = 5% at 35°C

- = uncoated
- = initial results
- = 1 month, 0°C 44%RH
- = 1 month, 20°C 44%RH
- = 1 month, 37°C 44%RH
drug decomposed = 1 month, 37°C 44%RH

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5.4.1.3 Optimization of stability of coatings with additional plasticizers

The instability of the coat was thought to be due to incomplete film formation of the Surelease fraction. Surelease is an aqueous dispersion containing solid ethylcellulose in particulate form. To form a film, the polymer particles have to coalesce. Coalescence is promoted by softening of the polymer particles, which is promoted by using a high temperature and a suitable plasticizer. In this project, a low temperature is desirable and hence, the only way to improve film formation is by using a plasticizer, DBS.

Surelease/amylose with varying amounts of additional DBS, 0%, 4%, 8% and 12% were coated onto 5-ASA containing pellet cores. Dissolution results immediately after coating showed that there was little difference between the coats formed from the four formulations in terms of drug release. However, upon storage at ambient temperature and humidity, only formulations containing additional plasticizer were found to be stable (Figs 5.12 to 5.14). As little as 4% additional plasticizer was shown to be sufficient.
Fig 5.11 SEM showing drug migration to the outer surfaces of pellets coated with Surelease:amylose (5:1), TWG of 5% stored at:

a) 0% RH, 20°C,
b) 44% RH, 20°C, and
c) 78% RH, 20°C
Fig 5.12

Dissolution results to check the stability of AG1 pellets coated to 5% TWG with Surelease EA7100:amylose (5:1) at 35°C

- • = uncoated Amylose from Norwich 6/3/95A
- ■ = 5% TWG No plasticizer
- ▼ = 5% TWG 4% additional plasticizer
- ▲ = 5% TWG 8% additional plasticizer
- ♦ = 5% TWG 12% additional plasticizer

Open symbols = 1 month storage at 20°C, 44% RH

Fig 5.13

Dissolution results to check the stability of AG1 pellets coated to 5% TWG with Surelease EA7100:amylose (5:1) at 35°C

- • = uncoated Amylose from Norwich 6/3/95A
- ■ = 5% TWG No plasticizer
- ▼ = 5% TWG 4% additional plasticizer
- ▲ = 5% TWG 8% additional plasticizer
- ♦ = 5% TWG 12% additional plasticizer

Open symbols = 3 months storage at 20°C, 44% RH
5.4.1.4 The influence of simulated gastric environment on the drug dissolution of pellets coated with Surelease / amylose

Pellets coated with Surelease:amylose (5:1) + 4% DBS. TWG of 5% which had previously been shown to be stable for at least 3 months were then tested in fermentation studies. However, when tested under simulated colonic condition, the pellets were found to have burst open in both the tests and controls (> 20% released in 1 hour). This was an unexpected finding since the pellets had previously (i.e. within 7 days) been tested under simulated gastric and small intestinal conditions of 3 hours of acid (0.1N HCl) followed by 9 hours of phosphate buffer (pH 7.2) and found to release minimal amount of drug (< 10% released in 12 hours). There was certainly no signs of bursting in the pellets when tested under simulated upper gastrointestinal (GI) conditions.

The investigation was carried out to check if the coating was strengthened by the treatment of acid in the simulated gastric condition. A series of experiments were designed to account for the wide variation of reported gastric pH (1.0-5.5)(Russell et al, 1993, Evans et al, 1988) and emptying times (30 minutes-24 hours), (Mojaverian et al, 1985).
1985, Davis et al, 1984) within the population.

The results suggested that the exposure of AG1 coated pellets to acid prior to phosphate buffer pH 7.2 was crucial to reduce premature release of drug (Fig 5.15). The effect of acid on the coated pellets could be seen after a short exposure time of just 15 minutes. This would suggest that even in very quick gastric emptying times in vivo the coated pellets should be strong enough to avoid premature release in the upper GI.

**Fig 5.15**

The effect of different degrees of acidity on the coat was also investigated. It was shown that between pH 1.5 to 6.0, pretreatment with acid resulted in strengthening of the coat. There did not appear to be a specific pH, within the studied range, which had better strengthening effect than the rest (Fig 5.16). This meant that irregardless of gastric pH, which may fluctuate due to presence of food and medication, the coating should still be strong enough to withstand any premature release of drugs. Based on these findings, all coated pellets were soaked in acid for 30 minutes prior to in vitro fermentation studies.
5.4.1.5 In vitro fermentation studies

In vitro fermentation tests conditions were optimized using uncoated pellets. Two hundred and seventy three mg of uncoated AG1 pellets were tested in 60ml of colonic medium in anaerobically sealed bottles. This quantity of pellets and fermentation volume was chosen based on 80% of saturation level of 5-ASA. (5-ASA has a solubility in water of 1 in 500). The study showed that the system should be shaken to achieve a more rapid release of 5-ASA (Table 5.5).

The results also suggested that even in the shaken system, the uncoated pellets in the control were still releasing their contents much slower than expected. This was attributed to the possible saturation of the fermentation medium. A series of experiments suggested that the minimum quantity of fluid required to achieve a 100% release profile of drugs from 273mg of uncoated pellets within 6 hours was 480ml (Table 5.6). This was found to be prohibitively high for faecal slurry studies. Therefore, the quantity of pellets tested was reduced.

The optimum condition was found by increasing the quantity of the fermentation medium from 60ml to 100ml and reducing the quantity of pellets from 273mg to 50mg.
Table 5.5 The percentage of 5-ASA released from uncoated pellets, AG1, tested in colonic control and test media

<table>
<thead>
<tr>
<th>Time (Hour)</th>
<th>% of 5-ASA released</th>
<th>Unshaken system</th>
<th>Shaken system</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Test</td>
<td>Control</td>
</tr>
<tr>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>8.3</td>
<td>6.7</td>
<td>23.5</td>
</tr>
<tr>
<td>4</td>
<td>13.0</td>
<td>9.8</td>
<td>33.4</td>
</tr>
<tr>
<td>6</td>
<td>18.5</td>
<td>13.2</td>
<td>39.9</td>
</tr>
<tr>
<td>12</td>
<td>27.8</td>
<td>20.6</td>
<td>59.3</td>
</tr>
<tr>
<td>24</td>
<td>40.0</td>
<td>30.5</td>
<td>75.5</td>
</tr>
</tbody>
</table>

Table 5.6 The percentage of 5-ASA released from uncoated pellets, AG1, over time in different volumes of fluid

<table>
<thead>
<tr>
<th>Time (hour)</th>
<th>% of 5-ASA released</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>60ml</td>
</tr>
<tr>
<td>1</td>
<td>14.32</td>
</tr>
<tr>
<td>2</td>
<td>24.85</td>
</tr>
<tr>
<td>4</td>
<td>50.93</td>
</tr>
<tr>
<td>6</td>
<td>64.99</td>
</tr>
<tr>
<td>10</td>
<td>79.84</td>
</tr>
</tbody>
</table>

Prior to the study of 5-ASA release from coated pellets in the colon, equal amounts of 5-ASA powder was added to faecal slurry and phosphate buffer, the amounts subsequently detected by HPLC were not identical (Figure 5.17). Therefore, separate calibration curves were used to analyze the results in each media (Fig 5.18).
The amount of 5-ASA detected over time

- ■ = 50mg
- ▲ = 100mg
- ● = 150mg

Open symbols = phosphate buffer
Solid symbols = faecal slurry

Calibration curves to convert the amount of 5-ASA detected to the actual amount added

- ■ = 5-ASA in faecal slurry
- ▲ = 5-ASA in phosphate buffer

\[ y = 0.865020x + 2.3798 \]
\[ y = 0.653772x - 3.0303 \]
AG1 pellets coated with Surelease:amylose (5:1) + 4% DBS, TWG = 5% when tested under the optimum fermentation studies showed no greater release in the test compared to the control conditions (Fig 5.19).

5.4.1.6 Optimization of release from pellets coated with Surelease / amylose

To increase the release rate of the coated pellets, two approaches were adopted. The first was to reduce the thickness of the coating from TWG of 5% to 3%. This gave unacceptable amounts of premature release (Fig 5.20).

The second approach was to increase the amylose fraction in the mixed polymer formulation. Premature release of drug from coated pellets was compensated for by using a thicker coat. The fraction of amylose in the film tested ranges from 0 to 100%. The coated pellets were tested for premature release of 5-ASA in in vitro dissolution tests as detailed in 5.3.5 and for release in in vitro fermentation studies as detailed in 5.3.6. The results were summarised in Figs 5.21 and 5.22 for upper GI release and in Figs 5.23 to 5.28 for colonic release. Pellets coated with Surelease:amylose (1.5:1) + 4% DBS, TWG = 10% showed very much higher release in the test compared
Fig 5.20

Effect of coat thickness on the dissolution results of AG1 pellets coated with Surelease EA7100:amylose (5:1) at 35°C

- = uncoated
△ = 3% TWG 4% additional plasticizer
□ = 5% TWG 4% additional plasticizer

Fig 5.21

Effect of Surelease EA7100:amylose ratio containing 4% additional dibutyl sebacate on dissolution of AG1 pellets coated to 10% TWG thickness

- = uncoated
■ = 100% amylose
▼ = 100% Surelease
● = 1 : 1
▲ = 1.5 : 1
○ = 2 : 1
△ = 3 : 1
▼ = 4 : 1

Amylose from Norwich (14/7/95)
Fig 5.22

Dissolution results of AG1 pellets coated at 35°C to TWG = 10%

% of 5-ASA released in 12 hours vs % of amylose in Surelease/amylose mixtures

Fig 5.23

In vitro digestibility study of drug release from AG1 pellets

■ = uncoated pellets
▲ = Amylose per se TWG=10%
Solid symbols = slurry test
Open symbols = phosphate control

% cumulative of 5-ASA released vs Time / hours
Fig 5.24

In vitro digestibility study of drug release from AG1 pellets

■ = uncoated pellets
△ = Surelease: Amylose (1:1) TWG=10%
Solid symbols = slurry test
Open symbols = phosphate control

Fig 5.25

In vitro digestibility study of drug release from AG1 pellets

■ = uncoated pellets
△ = Surelease: Amylose (1.5:1) TWG=10%
Solid symbols = slurry test
Open symbols = phosphate control
**Fig 5.26**

In vitro digestibility study of drug release from AG1 pellets

- ■ = uncoated pellets
- ▲ = Surelease:Amylose (2:1) TWG=10%
- Solid symbols = slurry test
- Open symbols = phosphate control

**Time / hours**

---

**Fig 5.27**

In vitro digestibility study of drug release from AG1 pellets

- ■ = uncoated pellets
- ▲ = Surelease:Amylose (3:1) TWG=10%
- Solid symbols = slurry test
- Open symbols = phosphate control

**Time / hours**
The pellets recovered at the end of the fermentation studies were viewed under SEM. These are shown in Figs 5.29 to 5.34.
Fig 5.29 Twenty four hours of incubation of AG1 pellets coated with only amylose at 35°C to TWG of 10%. a) whole pellet; b) pellet surface; c) cross-section of pellet; d) close-up of cross-section of pellet surface

In phosphate buffer

In faecal slurry

196
Fig 5.30 Twenty four hours of incubation of AG1 pellets coated at 35°C with Surelease: amylose (1:1) + 4% DBS, TWG of 10%. a) whole pellet; b) pellet surface; c) cross-section of pellet; d) close-up of cross-section of pellet surface

In phosphate buffer

In faecal slurry
Fig 5.31 Twenty four hours of incubation of AG1 pellets coated at 35°C with Surelease: amylose (1.5:1) + 4% DBS, TWG of 10%. a) whole pellet; b) pellet surface; c) cross-section of pellet; d) close-up of cross-section of pellet surface

In phosphate buffer

In faecal slurry
Fig 5.32 Twenty four hours of incubation of AG1 pellets coated at 35°C with Surelease: amylose (2:1) + 4% DBS, TWG of 10%. a) whole pellet; b) pellet surface; c) cross-section of pellet; d) close-up of cross-section of pellet surface

In phosphate buffer

In faecal slurry
Fig 5.33 Twenty four hours of incubation of AG1 pellets coated at 35°C with Surelease: amylose (3:1) + 4% DBS, TWG of 10%. a) whole pellet; b) pellet surface; c) cross-section of pellet; d) close-up of cross-section of pellet surface

In phosphate buffer

![Image a)](image1)

![Image b)](image2)

![Image c)](image3)

![Image d)](image4)

In faecal slurry

![Image a)](image5)

![Image b)](image6)

![Image c)](image7)

![Image d)](image8)
Fig 5.34 Twenty four hours of incubation of AG1 pellets coated at 35°C with Surelease: amylose (4:1) + 4% DBS, TWG of 10%. a) whole pellet; b) pellet surface; c) cross-section of pellet; d) close-up of cross-section of pellet surface

In phosphate buffer

In faecal slurry
5.4.1.7 The enzymatic dissolution test

AG1 pellets coated with Surelease:amylose (1.5:1) + 4% DBS, TWG = 10% were further tested for premature release in simulated gastric (0.1N HCl + 0.32%w/v pepsin) and simulated small intestinal condition (1.0% pancreatin in pH 7.2 phosphate buffer). The results are shown in Fig 5.35. There appeared to be greater release of 5-ASA from the coated pellets under these new conditions.

Fig 5.35

Dissolution results of Surelease EA7100:amylose (1.5:1) + 4% dibutyl sebacate on dissolution of AG1 pellets coated to 10% TWG thickness

5.4.1.8 Glucose release

The premature release of glucose from AG1 pellets coated with Surelease:amylose (1.5:1) + 4% DBS, TWG = 10% was also investigated using in vitro dissolution test detailed in 5.3.5. The release from the pellets is shown in Fig 5.36.
5.4.2 Reproducibility of performance of coating materials

When the pellets AG1 were coated again with Surelease:amylose (5:1), TWG of 5%, the results were found to be different (Fig 5.37).
This difference was due to the difference in the source of amylose-butanol complex used (Fig 5.38). The sample of amylose-butanol complex made in Norwich were found to give consistent results from batch to batch whereas there was significant batch variation in the amylose made at The School of Pharmacy. Therefore, it was decided that only amylose-butanol complex made at Norwich would be used in future studies.

Fig 5.38

The statistical analysis of variance and subsequent optimisation experiments of AG1 pellets coated with Surelease/amylose were not affected as the pellets were coated with amylose prepared from Norwich. However, the statistical analysis of variance study of AG1 pellets coated with Aquacoat/amylose as shown below was done with amylose-butanol complex made at The School of Pharmacy. Therefore, the results can be viewed as trend indicators and will not be used to define the limits of the system.
5.4.3 The Aquacoat® ECD30 / amylose system

5.4.3.1 The statistical analysis of variance

Twenty seven coating formulations with varying Aquacoat to amylose ratio, coat thickness and plasticizer quantity were studied. The parameter that had not previously been shown was the quantity of plasticizer incorporated. For a given film thickness and a fixed Aquacoat to amylose ratio, as the quantity of plasticizer increased, the amount of drug release in the dissolution test decreased Figs 5.39 to 5.47).

Fig 5.39

![Diagram showing effect of varying plasticizer content on Aquacoat ECD30:amylose (5:1) on dissolution of AG1 pellets coated to 5% TWG thickness.](image-url)
Fig 5.40

Effect of varying plasticizer content on Aquacoat ECD30:amylose ratio (5:1) on dissolution of AG1 pellets coated to 10% TWG thickness

- = uncoated
■ = 24%w/w calculated on Aquacoat solids
● = 30%w/w calculated on Aquacoat solids
▽ = 36%w/w calculated on Aquacoat solids

% cumulative 5-ASA released

Time / minutes

0.1N HCl ➔ phosphate buffer pH 7.2 ➔

Fig 5.41

Effect of varying plasticizer content on Aquacoat ECD30:amylose ratio (5:1) on dissolution of AG1 pellets coated to 15% TWG thickness

- = uncoated
■ = 24%w/w calculated on Aquacoat solids
● = 30%w/w calculated on Aquacoat solids
▽ = 36%w/w calculated on Aquacoat solids

% cumulative 5-ASA released

Time / minutes

0.1N HCl ➔ phosphate buffer pH 7.2 ➔
Fig 5.42

Effect of varying plasticizer content on Aquacoat ECD30:amylose ratio (7:1) on dissolution of AGI pellets coated to 5% TWG thickness

- ■ = 24%w/w calculated on Aquacoat solids
- ♦ = 30%w/w calculated on Aquacoat solids
- ▼ = 36%w/w calculated on Aquacoat solids

% cumulative 5-ASA released

0 25 50 75 100 125

0 150 300 450 600 750

-0.1N HCl ← phosphate buffer pH 7.2 →

Time / minutes

Fig 5.43

Effect of varying plasticizer content on Aquacoat ECD30:amylose ratio (7:1) on dissolution of AGI pellets coated to 10% TWG thickness

- ■ = 24%w/w calculated on Aquacoat solids
- ♦ = 30%w/w calculated on Aquacoat solids
- ▼ = 36%w/w calculated on Aquacoat solids

% cumulative 5-ASA released

0 25 50 75 100 125

0 150 300 450 600 750

-0.1N HCl ← phosphate buffer pH 7.2 →

Time / minutes
**Fig 5.44**

Effect of varying plasticizer content on Aquacoat ECD30:amylose ratio (7:1) on dissolution of AG1 pellets coated to 15% TWG thickness

- • = uncoated
- □ = 24%w/w calculated on Aquacoat solids
- ♦ = 30%w/w calculated on Aquacoat solids
- ◄ = 36%w/w calculated on Aquacoat solids

% cumulative 5-ASA released

<table>
<thead>
<tr>
<th>Time / minutes</th>
<th>0</th>
<th>150</th>
<th>300</th>
<th>450</th>
<th>600</th>
<th>750</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1N HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate buffer pH 7.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Fig 5.45**

Effect of varying plasticizer content on Aquacoat ECD30:amylose (10:1) on dissolution of AG1 pellets coated to 5% TWG thickness

- • = uncoated
- □ = 24%w/w calculated on Aquacoat solids
- ♦ = 30%w/w calculated on Aquacoat solids
- ◄ = 36%w/w calculated on Aquacoat solids

% cumulative 5-ASA released

<table>
<thead>
<tr>
<th>Time / minutes</th>
<th>0</th>
<th>150</th>
<th>300</th>
<th>450</th>
<th>600</th>
<th>750</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1N HCl</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phosphate buffer pH 7.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Fig 5.46

Effect of varying plasticizer content on Aquacoat ECD30:amylose ratio (10:1) on dissolution of AG1 pellets coated to 10% TWG thickness

- ◦ = uncoated
- □ = 24%w/w calculated on Aquacoat solids
- ▲ = 30%w/w calculated on Aquacoat solids
- ▽ = 36%w/w calculated on Aquacoat solids

Time / minutes

0.1N HCl → phosphate buffer pH 7.2

Fig 5.47

Effect of varying plasticizer content on Aquacoat ECD30:amylose ratio (10:1) on dissolution of AG1 pellets coated to 15% TWG thickness

- ◦ = uncoated
- □ = 24%w/w calculated on Aquacoat solids
- ▲ = 30%w/w calculated on Aquacoat solids
- ▽ = 36%w/w calculated on Aquacoat solids

Time / minutes

0.1N HCl → phosphate buffer pH 7.2
The trend of results for varying Aquacoat to amylose ratio and coat thickness were very similar to what was shown previously in the Surelease/amylose system. As the coat thickness was increased, the amount of drug released in the dissolution test decreased (Fig 5.48).

![Fig 5.48](image)

As the ratio of Aquacoat to amylose was increased, the amount of drug released in the dissolution test decreased (Fig 5.49).

![Fig 5.49](image)
The results from all the 27 coating formulations could be represented in three contour graphs which gives a range in which each variable could operate to provide a dissolution profile which would potentially be useful for oral colonic delivery (Fig 5.50 to 5.52).

Fig 5.50

Dissolution results of AG1 coated with Aquacoat and amylose mixture plasticised with 24% dibutyl sebacate

Fig 5.51

Dissolution results of AG1 coated with Aquacoat and amylose mixture plasticised with 30% dibutyl sebacate
The results were analysed using multivariate analysis of variance (MANOVA). Hotelling’s multivariate test of significance ($T^2$) was used. Hotelling’s $T^2$ value was approximated onto the F-distribution and the results were quoted as significance of F, (Table 5.7).

By applying the Hotellings multivariate measure of significance in the analysis of variance, it was shown that the influence of each variable on the dissolution profile was significant. However, these three influences were shown to interact. This meant that the effect of each variable on the dissolution process depends on the level of the other two variables. Simple linear equations involving the main factors and the interaction terms failed to model the relationship. Non-linear equations were then achieved empirically.
Table 5.7 The influence of ratio, coat thickness and plasticizer content on the dissolution profile of AGI pellets coated with Aquacoat/amylose mixed films as analysed by MANOVA

<table>
<thead>
<tr>
<th>Factor</th>
<th>$T^2$</th>
<th>$F$</th>
<th>DF (H)</th>
<th>DF (E)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ratio</td>
<td>172.3</td>
<td>1076.8</td>
<td>20</td>
<td>250</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Thickness</td>
<td>326.9</td>
<td>2042.9</td>
<td>20</td>
<td>250</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Plasticizer content</td>
<td>68.1</td>
<td>425.4</td>
<td>20</td>
<td>250</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interaction between ratio and thickness</td>
<td>151.6</td>
<td>471.9</td>
<td>40</td>
<td>498</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interaction between ratio and plasticizer content</td>
<td>21.5</td>
<td>66.8</td>
<td>40</td>
<td>498</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interaction between plasticizer content and thickness</td>
<td>65.7</td>
<td>204.5</td>
<td>40</td>
<td>498</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Interaction among ratio, plasticizer content and thickness</td>
<td>78.9</td>
<td>122.6</td>
<td>80</td>
<td>994</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

$T^2$ = Hotelling's value; $F$ = Approximate $F$ value; DF(H) = Hypothetical degrees of freedom; DF(E) = Error in degrees of freedom; P = Probability level

A series of empirical equations were obtained and the equation which best fitted the 27 sets of data (Root Mean Square in residual analysis : RMS = 122.15) was found to be:

\[
y = -13.133(r) - 4.480(p) + 665.271(1/twg) - 0.491(r)(twg) + 0.033 (r)(p)(twg) + 145.046
\]

where  
- $y$ = % of cumulative drug released in 12 hours i.e. 3 hours in 0.1N HCl followed by 9 hours in phosphate buffer pH 7.2.
- $r$ = the solid weight ratio of Aquacoat to one part solid weight of amylose
- $p$ = % of plasticizer content calculated on solid weight of Aquacoat
- $twg$ = % total weight gain
5.4.3.2 Stability studies

The range of formulations shown in Table 5.8 were stored at 20°C at 0% RH, 44% RH and 78% RH to establish whether the three variables in the formulation had any influence on the stability of the coat.

Table 5.8 The 5-ASA pellets, AG1, coated with various Aquacoat / amylose formulations were placed on stability studies

<table>
<thead>
<tr>
<th>Aquacoat : Amylose ratio</th>
<th>Thickness (% TWG)</th>
<th>Plasticizer content (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:1</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>7:1</td>
<td>5</td>
<td>36</td>
</tr>
<tr>
<td>7:1</td>
<td>10</td>
<td>36</td>
</tr>
<tr>
<td>7:1</td>
<td>15</td>
<td>36</td>
</tr>
<tr>
<td>7:1</td>
<td>10</td>
<td>24</td>
</tr>
<tr>
<td>7:1</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td>10:1</td>
<td>10</td>
<td>36</td>
</tr>
</tbody>
</table>

By varying the coat thickness of a given formulation, it was shown that the thinnest coat, 5% was unstable on 1 month storage at 20°C, 0% and 44% RH but stable at 20°C, 78% RH (Fig 5.53 to Fig 5.55). On the other hand, coat thicknesses of 10% and 15% were shown to be stable at all the investigated storage conditions. Coat thickness had an influence on the coat stability.
Fig 5.53

Effect of varying coat thickness on Aquacoat ECD30:amylose (7:1) with 36% dibutyl sebacate on dissolution of AG1 coated pellets

- • = uncoated
- ■ = 5%
- ♦ = 10%
- ▼ = 15%

Open symbols = 1 month storage at 20°C, 0%RH

% cumulative 5-ASA released

0 150 300 450 600 750
0 25 50 75 100
0.1N HCl → --- phosphate buffer pH 7.2 ---→
Time / minutes

Fig 5.54

Effect of varying coat thickness on Aquacoat ECD30:amylose (7:1) with 36% dibutyl sebacate on dissolution of AG1 coated pellets

- • = uncoated
- ■ = 5%
- ♦ = 10%
- ▼ = 15%

Open symbols = 1 month storage at 20°C, 44%RH

% cumulative 5-ASA released

0 150 300 450 600 750
0 25 50 75 100
0.1N HCl → --- phosphate buffer pH 7.2 ---→
Time / minutes

215
By varying the quantity of plasticizer within a given formulation, it was shown that this variable also influenced coat stability. The influence of plasticizer was most marked at low humidity (Fig 5.56 to 5.58).
Fig 5.57

Effect of varying plasticizer content on Aquacoat ECD30:amylose ratio (7:1) on dissolution of AG1 pellets coated to 10% TWG thickness

- = uncoated
■ = 24%w/w calculated on Aquacoat solids
♦ = 30%w/w calculated on Aquacoat solids
▼ = 36%w/w calculated on Aquacoat solids
Open symbols = 1 month storage at 20°C, 44%RH

% cumulative 5-ASA released

0 0.1N HCl phosphate buffer pH 7.2 750

Time / minutes

Fig 5.58

Effect of varying plasticizer content on Aquacoat ECD30:amylose ratio (7:1) on dissolution of AG1 pellets coated to 10% TWG thickness

■ = 24%w/w calculated on Aquacoat solids
♦ = 30%w/w calculated on Aquacoat solids
▼ = 36%w/w calculated on Aquacoat solids
Open symbols = 1 month storage at 20°C, 78%RH

% cumulative 5-ASA released

0 0.1N HCl phosphate buffer pH 7.2 750

Time / minutes
When the ratio of ethylcellulose to amylose was varied from 5:1 to 10:1, the coat was stable only when stored at relative humidities of 44% and above (Fig 5.59 to 5.61).

**Fig 5.59**

Effect of varying Aquacoat ECD30:amylose ratio containing 36% dibutyl sebacate on dissolution of AG1 pellets coated to 10% TWG thickness

- ● = uncoated
- ■ = 5 : 1
- ● = 7 : 1
- ♦ = 10 : 1

Open symbol = 3 months storage at 20°C, 0%RH

**Fig 5.60**

Effect of varying Aquacoat ECD30:amylose ratio containing 36% dibutyl sebacate on dissolution of AG1 pellets coated to 10% TWG thickness

- ● = uncoated
- ■ = 5 : 1
- ● = 7 : 1
- ♦ = 10 : 1

Open symbol = 3 months storage at 20°C, 44%RH
5.4.3.3 Reproducibility of performance of coating materials

In 5.4.2, it was found that the amylose-butanol complex dispersion made at The School of Pharmacy was not reproducible from batch to batch. The results for the study of Aquacoat / amylose system analysed by using analysis of variance was done using amylose-butanol complex made at The School of Pharmacy. Therefore, it was possible that the actual results would not be reproducible. However, it was believed that the trends generated by the statistical analysis was still valid.

Based on the knowledge gained from this previous study, it was decided that the most stable Aquacoat / amylose formulations were those containing 36% w/w DBS. Five crucial experiments containing this level of plasticizer were repeated with the new batch of amylose-butanol complex from Norwich (Table 5.9). The two variables investigated were solid polymer ratio and coat thickness.
Table 5.9 Aquacoat and amylose coating formulations (+36% w/w DBS), coated onto AG1 pellets were repeated with amylose-butanol complex supplied by Norwich.

<table>
<thead>
<tr>
<th>Aquacoat:amylose ratio</th>
<th>Total weight gain, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>5:1</td>
<td>10</td>
</tr>
<tr>
<td>7:1</td>
<td>5</td>
</tr>
<tr>
<td>7:1</td>
<td>10</td>
</tr>
<tr>
<td>7:1</td>
<td>15</td>
</tr>
<tr>
<td>10:1</td>
<td>10</td>
</tr>
</tbody>
</table>

When compared with previous coatings from the same formulations, these coatings, formed with amylose-butanol complex from Norwich, appeared to be better at preventing premature release of 5-ASA. The stability profiles were similar to those shown previously. They were shown to be stable for six months (Fig 5.62 and 5.63).

Fig 5.62

Effect of varying coat thickness of Aquacoat ECD30:amylose (7:1) with 36% dibutyl sebacate on dissolution of AG1 coated pellets

- = uncoated
- = 5%
- = 10%
- = 15%

Open symbols = 6 months storage at 20°C, 44%RH
Fig 5.63

Effect of varying Aquacoat ECD30:amylose ratio containing 36% dibutyl sebacate on dissolution of AG1 pellets coated to 10% TWG thickness

- = uncoated Amylose from Norwich 6/3/95A
■ = 5 : 1
♦ = 7 : 1
△ = 10 : 1
Open symbol = 6 months storage at 20°C, 44%RH

5.4.3.4 In vitro fermentation study

The coating with the lowest ratio of Aquacoat:amylose which appeared to protect the pellet from premature release of 5-ASA was tested in fermentation study. The results showed no greater release compared to the control (Fig 5.64). Further optimization of the system was therefore required.

Fig 5.64

In vitro digestibility study of drug release from AG1 pellets
■ = uncoated pellets
△ = Aquacoat:amylose (5:1) TWG=10%
Solid symbols = slurry test
Open symbols = phosphate buffer
5.4.3.5 Optimisation of release from pellets coated with Aquacoat / amylose

To increase the release rate of the coated pellets, the amylose fraction in the mixed polymer formulation was increased. The fraction of amylose in the film tested ranges from 0 to 100%. The coated pellets were tested for premature release of 5-ASA in \textit{in vitro} dissolution tests as detailed in 5.3.5. The results were summarised in Fig 5.65 and 5.66. These were then tested for release in \textit{in vitro} fermentation studies detailed in 5.3.6. The results were summarised in Fig 5.67. Pellets coated with Aquacoat:amylose (2:1) + 36% DBS, TWG = 10% showed the optimum balance of minimal premature drug release in the upper GIT with sufficiently large drug release in the colon.

Fig 5.65

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure5.65.png}
\caption{Effect of Aquacoat:amylose ratio containing 36% dibutyl sebacate on dissolution of AG1 pellets coated to 10% TWG thickness}
\label{fig:5.65}
\end{figure}
Fig 5.66

Dissolution results of AG1 pellets coated at 35°C to TWG = 10%

% of 5-ASA released in 12 hours

% of amylose in Aquacoat/amylose mixtures

Fig 5.67

The fermentation results of AG1 coated with Aquacoat / amylose + 36% dibutyl sebacate, TWG = 10%

- = Aquacoat:amylose (1.5:1)
△ = Aquacoat:amylose (2:1)
♦ = Aquacoat:amylose (3:1)
Solid symbols = fermentation slurry
Open symbols = buffer

The pellets recovered at the end of the fermentation studies were viewed under SEM. These are shown in Figs 5.68 to 5.70
Fig 5.68 Twenty four hours of incubation of AG1 pellets coated at 35°C with Aquacoat:amylose (3:2) + 36% DBS, TWG of 10%. a) whole pellets; b) pellet surface; c) cross-section of pellet; d) close-up of cross-section of pellet surface.

In phosphate buffer

In faecal slurry
Fig 5.69 Twenty four hours of incubation of AG1 pellets coated at 35°C with Aquacoat:amylose (2:1) + 36% DBS, TWG of 10%. a) whole pellets; b) pellet surface; c) cross-section of pellet; d) close-up of cross-section of pellet surface

In phosphate buffer

In faecal slurry
Fig 5.70 Twenty four hours of incubation of AG1 pellets coated at 35°C with Aquacoat:amylose (3:1) + 36% DBS, TWG of 10%. a) whole pellets; b) pellet surface; c) cross-section of pellet; d) close-up of cross-section of pellet surface

In phosphate buffer

In faecal slurry
5.4.3.6 The enzymatic dissolution test

AG1 pellets coated with Aquacoat:amylose (2:1) + 36% DBS, TWG = 10% were further tested for premature release in simulated gastric and simulated small intestinal conditions as described in 5.3.5. The results are shown in Fig 5.71. No greater release of 5-ASA from the coated pellets were detected under these new conditions.

Fig 5.71

5.4.3.7 Glucose release

The premature release of glucose from AG1 pellets coated with Aquacoat:amylose (2:1) + 36% DBS, TWG = 10% was also investigated using in vitro dissolution test detailed in 5.3.5. The release from the pellets is shown in Fig 5.72.
Fig 5.72

Dissolution results of glucose from AG1 pellets coated to TWG = 10% with Aquacoat:amylose (2:1) + 36% DBS at 35°C

- = 5-ASA release from coated pellets
■ = glucose release from coated pellets

% cumulative drug released

0 25 50 75 100 125

0 150 300 450 600 750

Time / minutes

0.1N HCl phosphate buffer pH 7.2
5.5 Discussion

To aid interpretation of the results obtained, an understanding of the mechanisms of film-formation and of drug release from these formulations would be useful.

In theory, the mechanism of film-formation from sprayed film is similar to the mechanism of film-formation from cast films. Therefore, the wet sintering theory, the capillary pressure theory and the dry sintering theory would still apply (Onions, 1986a). However, films formed by spraying had been shown to be different from cast films (Bindshaedler et al, 1987, Lafferty, 1993, Narisawa et al, 1993 and 1994). The primary reason being a sprayed film is not made from one complete film but a collection of many layers of films. Therefore, to form a strong and stable sprayed film, the polymeric particles within each spray droplet need to fuse successfully, not just with each other but also with polymeric particles from other droplets applied subsequently. In theory, this can be achieved by maintaining a very slow evaporation rate to allow for the drops on the coated surface to intertwine over the border between drops. In practice, this is not feasible, as too slow an evaporation rate would lead to sticking and agglomeration. Therefore, in practice, the spray droplets are relatively dry before the next spray droplet is applied. A certain degree of incomplete fusion is to be expected. This problem is usually overcome by using a high operational temperature to encourage rapid polymeric fusion during coating i.e. greater wet sintering and capillary pressure effects and by applying a post coating heat treatment called curing i.e. greater dry sintering effect (Goodhart et al, 1984, Hutchings et al, 1994, Bodmeier and Paeratakul, 1994a). Based on this understanding, the disadvantages of this coating system were identified and hopefully minimised by manipulation of formulation variables. The first problem is that in the absence of heat, incomplete fusion would have to be overcome by other means. There are two other heat-free methods for encouraging fusion --- addition of plasticizers and photocuring. The addition of plasticizers allow greater molecular movements at a given temperature. This would hopefully encourage fusion. The second and relatively novel technique is photocuring whereby cross-linking of polymer is promoted by the use of uv irradiation (Wang et al, 1995). This technique involves the addition of a cross-linking agent which could potentially affect the digestibility of amylose. In addition, this
technique had only been used in solvent free systems. Therefore, the latter approach was not adopted.

Even if better polymeric fusion could be encouraged with the use of plasticizer, there is a second problem. This is the presence of two volatile agents in the coating formulation --- butanol and water. Unlike the "hot" coating system previously developed whereby butanol was removed prior to spraying (Milojevic, 1993), in the "cold" coating system, butanol was not removed prior to spraying. (36°C is below complex dissociation temperature of 70°C, Whittam et al, 1989). Butanol and water, owing to their different boiling points and heat capacities have different evaporation rates. Narisawa et al, (1993 and 1994) had shown that when a coating formulation contained different volatile solvents, porous films often result. A gel would essentially be formed during the evaporation of the first solvent. As the second solvent evaporated from this gel, pores would result. The presence of butanol complexed with amylose could not be omitted, hence, there would always be a potential of a porous film. This effect was minimised by applying a relatively thicker coat. A thicker coat should minimise the chances of having any pores which span the entire cross section of the film allowing premature drug release. Therefore, the original coating formulation developed by Milojevic (1993) was modified to include more plasticizers and was applied to a greater thickness to compensate for poorer film forming properties at lower temperature.

The quality of the resulting sprayed films were evaluated based on their performances in in vitro dissolution tests. Dissolution tests measure the rate of drug release from the coated pellets. The mechanisms of drug release are discussed briefly here to show how the rate of drug release indicates the quality of sprayed films.

Drugs from coated pellets could be released by one or more of the four mechanisms --- solution / diffusion through the continuous plasticized phase, diffusion through plasticizer channels, diffusion through aqueous pores and osmotically driven release (Dressman et al, 1994).

Solution/diffusion through the continuous plasticized polymer phase assumes that
a homogenous film is formed. The migration of drug through the film is due to a cooperate movement of drug and polymer. Solution/diffusion through plasticizer channels would occur when the plasticizer is not uniformly distributed in the coating polymer and forms patched channels through which the drug preferentially diffuses. Diffusion through aqueous pores assumes that the coating is not homogeneous or continuous but is punctuated with pores. Finally, the osmotically driven release assumes that release is brought on by an osmotic pressure difference between the core materials and the release environment. When the quality of the sprayed films is evaluated by dissolution tests, it is assumed that release by the first two mechanisms, solution/diffusion through the continuous plasticized phase and through plasticizer channels, does not occur. This is because the first mechanism assumes a homogeneous film which is usually only formed from organic solvent systems and the second mechanism assumes the localization of plasticizer phase which represents an extreme condition (Ozturk et al, 1990). In any case, the plasticizer used is a viscous material which would not encourage preferential diffusion of drug.

Therefore, the primary mechanisms of release from the coated pellets were considered to be through aqueous pores and osmotic release. Aqueous pores could be due to swelling of amylose, a porous film structure or incomplete fusion of film. The formation of such pores or cracks would then lead to a weak film which has low osmotic resistance. In general, a low rate of release is an indication of a strong, low porosity, good film. A high rate of release is an indication of a weak, porous, poor film.

Once good quality sprayed-films which would not prematurely release their contents in the upper GIT were successfully formed, they were evaluated for their release profiles in in vitro fermentation studies. The measurement of drug release in the fermentation studies is an indirect measurement of the digestion of amylose. The studies assume that if the drug was not released in the control but was released in the test, then, the drug was released due to diffusion through pores left by digestion of the amylose fraction of the film. SEM was then used to provide some visual assessment of the digestion of amylose.
The results from the two coating formulation systems, the Surelease / amylose and the Aquacoat / amylose systems, will be discussed separately.

5.5.1 Surelease / amylose system

5.5.1.1 The statistical analysis of variance

Surelease was added to the formulation primarily to curb the swelling of amylose. As the quantity of Surelease in the film was increased, the amount of 5-ASA released prematurely decreased. This followed logically from the argument that as the quantity of Surelease in the film was increased, the swelling of amylose was decreased. This decrease in swelling of amylose proportionately decreased the number of aqueous pores through which 5-ASA could diffuse through, hence reducing the surface available for permeation. Donbrow and Friedman (1975) and Guo (1994) had shown how hydrophillic materials within a hydrophobic film could act as aqueous pores to allow diffusion of drug.

As the thickness of the film was increased, the rate of 5-ASA released was also decreased. The increase in thickness proportionately increased the distance through which the drug had to diffuse to be released. Hence, as the distance through which the drug had to diffuse was increased, the time needed for diffusion through the film was increased. The increase in thickness also increased the collection of tiny sprayed films, hence, increased the strength of each ill-formed film. As discussed earlier, thicker films also reduce the chances of a pore forming across the cross section of a film. All these decrease the release rate.

Statistical analysis also showed that there was an interaction between the two variables, ratio of Surelease and amylose and thickness of the film. This would suggest that for a given thickness of film, apart from the distance and strength of the film, the tortuosity of the amylose channel dictates how quickly 5-ASA could diffuse through the film. Hence, the two variables interact. From a formulation point of view, it was important to bear in mind that, adjusting any variable, however small, would affect the
performance of the film quite significantly due to the interplay of factors.

5.5.1.2 Stability studies

Although it was possible to form Surelease / amylose bipolymeric films without additional plasticizers, these films were subsequently found to be unstable. See 5.4.1.3. The instability of the film was believed to be due to the incomplete fusion of coalesced ethylcellulose. These coalesced particles initially formed a barrier, however, upon storage, points of weak fusion would rebound to their discrete forms causing the film to crack. The effect was most significant at low humidity as water, the general plasticizer to the film was removed. The instability was not believed to be caused by incomplete fusion of amylose as amylose had a very low minimum film forming temperature, see Chapter 3. This instability was successfully addressed by specifically plasticizing ethylcellulose. This correlated well with the findings of Bodmeier and Paeratakul (1994a) which showed that high plasticizer content could overcome poor fusion due to lack of curing.

5.5.1.3 The influence of simulated gastric environment on the drug dissolution of pellets coated with Surelease / amylose.

When each polymer itself was used to coat AG1 pellets and tested in dissolution media, the coatings were found to be pH independent in their performance. See Fig 5.73. However, when the pellets were coated with mixed polymeric films, the film coat was found to be "weaker" without prior treatment in acid, see Fig 5.15. It was not clear why this happened. However, this finding dictated that for all fermentation studies, prior exposure of the coated pellets to acid was necessary to avoid achieving false positive results. In humans, all oral colonic delivery systems would undergo prior exposure to acid in the stomach. The experiments showed that a short exposure time of 15 minutes was sufficient to strengthen the film coat. It also showed that as long as the acid pH was between 1.5 and 6.0, the strengthening effect would be achieved, see Fig 5.16. This would cover most gastric residence times and environments found within the population (Davis et al, 1984a, Mojaverian et al, 1985, Evans et al, 1988, Russell et al, 1993).
5.5.1.4 In vitro fermentation studies

In vitro fermentation studies using batch culture were shown to be active against resistant amylose as all amylose free films were digested after 24 hours. However, the formulation Surelease:amylose (5:1) + 4% DBS, TWG = 5%, which most closely correlated to the previously successful hot-coating mixed film (Milojevic, 1993) did not show any release in this in vitro test system. Therefore, further optimization was required.

5.5.1.5 Optimization of release from pellets coated with Surelease / amylose

The thickness of the film could not be reduced without greatly compromising loss of drug due to premature release in the upper GIT. Therefore, the ratio of the amylose in the mixed film was increased to encourage greater digestion in the colon. For a given thickness, there appeared to be a crucial ratio at which there would be enough resistance to prevent premature drug release in the upper GIT and still allowed sufficiently fast onset of release in the colon. SEM showed that digestion of amylose had occurred at all
ratios of amylose for the film thickness, TWG of 10%. However, when the ratio of amylose in the film was high (e.g. amylose itself or amylose:Surelease (1:1)), the release of 5-ASA by diffusion through the amylose fraction in the control (i.e. premature release due to mechanical failure) was equivalent or greater than release of 5-ASA through the pores left by amylose digestion in the tests (i.e. actual release in the colon due to digestion). On the other hand, when the ratio of amylose within the film was low (e.g. amylose:Surelease (1:3) or (1:4)), there appeared to be no 5-ASA released although surface digestion of amylose was seen using SEM. This could be due to either the high tortuosity of the amylose pores preventing easy diffusion of drug or the absence of an amylose-channel through the surface to the core of the coated pellets due to the low amount of amylose present.

At the optimum condition, when there was sufficient ratio of amylose to allow easy digestion of amylose without greatly compromising on premature release (i.e. amylose:Surelease (1:1.5) or (1:2)), there appeared to be greater 5-ASA release in the test compared to the controls. The onset of release in this case appeared to be dictated by the ratio of amylose to Surelease.

5.5.1.6. The enzymatic dissolution test mimicking the enzymic environment in the upper GIT

The better of the two formulations, Surelease:amylose (1.5:1) + 4% DBS, TWG of 10%, was tested for resistance in upper GIT using in vitro simulated gastric and small intestinal conditions with enzymes. The release profile in the gastric condition with or without enzymes was similar. This was not surprising as most of the gastric enzymes would have little effect on any of the components of the films. The release profile in the small intestinal condition showed differences between the enzyme-free and enzyme-containing test. There appeared to be marked greater release in the enzymatic system. The greater release from the simulated small intestinal system could be due to digestion of DBS, a lipid, and / or amylose due to presence of lipases and amylases, respectively.

The in vitro testing time was very much longer than the actual residence time in
the small intestine in human which is reliably constant (Davis et al., 1986). Under normal human residence time in the small intestine (~ 3-6 hours), the amount of premature drug release was ~ 10 - 30%. This was considered acceptable for an oral colonic delivery system.

5.5.1.7 Glucose release

AG1 pellets coated with Surelease:amylose (1.5:1) + 4% DBS, TWG = 10% was also tested for premature release of glucose. These pellets contained equal amounts of glucose + 5-ASA. The test showed that while there was little detectable premature amount of 5-ASA released, all of the glucose had been released in the upper GIT conditions. This could be explained with the primary mechanism of release of each drug. 5-ASA, being very insoluble at 1 in 500, would be released primarily by diffusion through aqueous pores whereas glucose, which is very soluble at 1 in 1 could diffuse through the hydrated aqueous amylose pores as well as by osmosis (Dressman et al., 1994). Therefore, this coating, which was too weak to resist osmosis of glucose, would be unsuitable for oral colonic delivery of such a highly water-soluble drug. This served as a useful illustration of the limitation of this coating as a generic coating for all drugs. The coating developed so far would only be useful for 5-ASA and other drugs with similar or less soluble solubility profiles.

5.5.2 Reproducibility of coating material

During the course of spray-coating the pellets, it was noted that there was batch to batch variation in the performance of amylose as a coating material. It was not clear why this should be so. Various tests used to characterise the different batches of amylose suggested that this variation was due to possible accidental contamination of the amylose coating dispersion (Chapter 2). When the purity of the batch was tested using iodine-binding test, it was found that the batch was 200% pure! Amylose by definition, could only bind 19.5% of its own weight of iodine. The sample bound more than 40% its own weight of iodine. The complex was also shown by DSC to be in amorphous rather than crystalline form. However, the batch showed similar molecular weight distribution when
tested using gel exclusion chromatography. It also had very good free-film forming abilities. Due to lack of testing facilities, it was not possible to test further the samples to explain this phenomena.

This appeared to only affect experiments involving amylose complex dispersions made at The School of Pharmacy, University of London and not at The Institute of Food Research, Norwich. Experiments using these batches of amylose dispersions were not repeated. Instead these experiments were used as indicators of trends rather than definitive experiments. The study affected was the statistical analysis of variance of Aquacoat/amylose formulations. The whole study was done with one batch of amylose-butanol dispersion made at The School of Pharmacy. Therefore, the trends were believed to be similar despite batch to batch variation.

5.5.3 Aquacoat / amylose

5.5.3.1 The statistical analysis of variance

Aquacoat, another commercially available ethylcellulose coating dispersion, was also used in combination with amylose. Aquacoat required the addition of plasticizer to promote coalescence of polymeric particles. Therefore, the basic coating formulae contained Aquacoat:amylose and DBS. Statistical analysis of variance of effects of coat thickness, plasticizer content and ratio of polymers showed that all three factors studied i.e. the ratio of Aquacoat to amylose, plasticizer content and thickness of film, were related. The argument for increase ratio of Aquacoat and increase of thickness of film improving resistance to premature release was similar to that argued in the case of Surelease / amylose.

Increase in the plasticizer quantity also showed better resistance to premature release. This could be due to one or both of the following reasons. The increase of plasticizer improved the quality of the Aquacoat fraction of the film by making the fusion of polymers more complete, hence, it was stronger to withstand the swelling of amylose. In addition, the presence of plasticizer increased the overall hydrophobicity of
the film. This in turn could reduce the swelling of amylose as the film became more difficult to wet.

5.5.3.2 Stability studies

The films which contained the highest quantity of DBS, i.e. 36% w/w DBS, were found to be stable for at least 6 months' storage at ambient temperature and humidity. This was very encouraging as it had been suggested that it was not possible to form good Aquacoat films at low temperature (Goodhart et al, 1984, Lippold et al, 1990, Laicher et al, 1995, Aquacoat handbook). This confirmed the findings of Bodmeier and Paeratakul (1994a) that the use of higher plasticizer levels would eliminate the need for a curing step.

5.5.3.3 In vitro fermentation studies

The ratio of Aquacoat to amylose within the film needed to be lower than (3:1) to show a faster release in simulated colonic condition than the test condition. However, at such high ratio of Aquacoat to amylose of (3:2), the predominant mechanism of release appeared to be by mechanical failure rather than by digestion as the drug release profile in both test and simulated colonic conditions were comparable, see Fig 5.67.

5.5.3.4 The enzymatic dissolution test mimicking the enzymic environment in the upper GIT

The best formulation, AG1 pellets coated with Aquacoat:amylose (2:1) + 36% DBS, TWG of 10%, was tested for resistance in upper GIT using in vitro simulated gastric and small intestinal conditions with enzymes. The drug release profile in the enzymatic environment was similar to drug release profile in non-enzymatic conditions, see Fig 5.71. This would suggest that the film was not affected by digestion of enzymes found in the upper GIT. The combination of Aquacoat/amylose appeared to be more resistant to digestion in the upper GIT compared to that of Surelease/amylose, see 5.5.1.6.
5.5.3.5 Glucose release

When AG1 pellets coated with Aquacoat:amylose (2:1) + 36% DBS, TWG = 10% was tested for premature release of glucose, nearly all of the glucose content was released within the first 3 hours, see Fig 5.72. The coat was clearly not suitable for oral colonic delivery of drugs with a greater solubility profile than 5-ASA. These drugs tend to be released prior to digestion of the amylose fraction through the hydrated film.

5.6 Conclusion

At coating temperatures of (32 to 36)°C, coating formulations consisting of a mixtures of glassy amylose and Aquacoat or Surelease which could control premature 5-ASA release in the upper GIT could be formed. The control of premature 5-ASA release was achieved by changing such formulation parameters as the ratio of ethylcellulose to amylose, the thickness of coatings and the quantity of plasticizer. Premature release of 5-ASA was reduced by increasing the ratio of ethylcellulose to amylose, increasing the thickness of coating and the quantity of plasticizer present.

The batch culture fermentation system was successfully developed as an in vitro testing model to test the drug release profile of the coated pellets in simulated colonic condition. The system was found to be active against resistant glassy amylose films.

AG1 pellets coated with Surelease:amylose (1.5:1) + 4% DBS, TWG of 10% and Aquacoat:amylose (2:1) + 36% DBS, TWG of 10% had been shown to successfully resist 5-ASA release in the upper GIT yet give a rapid onset of release in the simulated colon. However, both these coating formulations are not suitable for use with a drug that is more soluble than 5-ASA eg. glucose.
Chapter 6: Suggestions for future work

Future work in a number of other areas regarding the use of glassy amylose at low temperature would be beneficial to gain a better understanding of the system.

1. Investigation on the limitations of the system with drugs of different solubilities

   The suitability of the coating formulation for use with drugs of different solubilities should be investigated. To achieve a sufficiently quick onset of drug release, the coating formulation should possess a reasonably high content of amylose. This may limit the use of the coating formulation to drugs of low solubilities due to the possible diffusion of highly soluble drugs through the hydrated fraction of amylose within the film.

2. *In vivo* study with volunteers using the 5-ASA coated pellets in the fed and fasted states

   The *in vivo* performance of these low-temperature coated pellets had yet to be investigated in man. It would be useful to know if there is a good correlation between *in vitro* and *in vivo* 5-ASA release. *In vivo* study would also give an indication of the performance of the coated formulation under the variable conditions found in man.

3. Application of the coating system to thermolabile drugs

   The application of the coating system to thermolabile model drugs with potential therapeutic benefits when delivered by oral colonic delivery should be tested. This is to ensure that the physicochemical properties of the coating formulations, such as pH, would not adversely affect the efficacy of the model drugs. These model drugs include peptides, proteins and pro-pre- and symbiotic microorganisms.
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