AMYLOSE COATED PELLETS

FOR

COLON - SPECIFIC DRUG DELIVERY

by

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Drug targeting to the colon may be possible by using a coating comprising amylose in glassy form. Amylose is a natural polysaccharide, in this case extracted from pea starch, and possesses the ability to form films through gelation. Its gel microstructure has been found to be resistant to pancreatic $\alpha$-amylase but is digested by enzymes of colonic microflora origin. Amylose coatings swell in water, becoming porous and thus allowing drug release under simulated gastro-intestinal conditions. Incorporation of other polymers into the amylose film, to control the swelling of amylose has provided a solution to this problem. A range of coating materials (cellulose based polymers and acrylate based copolymers) were assessed of which a commercially available ethylcellulose (Ethocel®) was found to suppress amylose swelling. With additional thermal treatment of the coat, premature \textit{in vitro} drug release was prevented even after storage of the product for one year.

The \textit{in vitro} digestion of various coated pellets under simulated gastro-intestinal tract conditions (using commercially available pepsin and pancreatin powder) was determined and has demonstrated the resistance of the amylose/Ethocel® mixture to such conditions over a period of 12h. Coated pellets were further evaluated in a batch culture fermenter (simulated colon conditions) which contained mixed faecal bacteria. The \textit{in vitro} release of 5-aminosalicylic acid from coated pellets in the fermenter system was studied. The \textit{in vivo} performance of amylose/Ethocel coated pellets in 8 healthy human volunteers was evaluated using $^{13}$CO$_2$ excretion in breath after ingestion of $^{13}$C-labelled glucose (incorporated into the pellets) and measured using mass spectrometry. Inert lactose pellets containing amberlite resin and of the same diameter and density as the glucose were labelled with $^{99m}$Tc to provide a marker to follow gastro-intestinal transit. Gamma scan photographs showed the mean arrival time of $^{99m}$Tc labelled pellets at the caecum was 3.5h (range 2.50-4.75h). $^{13}$CO$_2$ in breath was not detected until 3.7h post dosing and did not become significant (1% recovery) until 6.4h. $^{13}$CO$_2$ was not found in breath before pellets had reached the caecum and there was a delay of 2.9h between arrival in the caecum and significant (1%) breakdown of the pellets. $^{13}$CO$_2$ enrichment in breath increased linearly for up to 15h and this was still evident 24h after administration of pellets, indicating a gradual release of glucose as the pellets passed through the colon.
To my
mother Breda, father Milan, brother Miško,
grandmother Rozalija and grandfather Anton
with love and grateful thanks
"Our desires presage the capacities within us; they are harbingers of what we shall be able to accomplish. What we can do and want to do is projected in our imagination, quite outside ourselves, and into the future. We are attracted to what is already ours in secret. Thus passionate anticipation transforms what is indeed possible into dream-for-reality."

"Weltanschauung"
J.W.Von Goethe (1749-1832)
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TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>List of Figures</td>
<td>10</td>
</tr>
<tr>
<td>List of Tables</td>
<td>15</td>
</tr>
<tr>
<td>List of Plates</td>
<td>16</td>
</tr>
</tbody>
</table>

CHAPTER 1: GENERAL INTRODUCTION

1.1 Oral sustained release dosage forms                                   18
   1.1.1 General principles, advantages and disadvantages                   18
   1.1.2 Multiunits versus single units                                     20

1.2 Oral drug delivery to the human large intestine                       20
   1.2.1 Anatomy, physiology, histology and microbiology of human gastro-intestinal tract  21
   1.2.2 Why to the colon?                                                 26

1.3 Inflammatory Bowel Diseases                                           30
   1.3.1 Definition                                                         30
   1.3.2 Epidemiology and etiology                                          30
   1.3.3 Pathology                                                         31
   1.3.4 Clinical features                                                 31

1.4 Treatment of Inflammatory Bowel Diseases                              31
   1.4.1 Routes of drug administration                                     31
   1.4.2 Principal drugs                                                   32

1.5 5-aminosalicylic acid as a model drug                                  33
   1.5.1 General pharmacology                                               33
   1.5.2 Sustained 5-aminosalicylic acid delivery systems:                 34
       A clinical and commercial success??                                  34
   1.5.3 A new perspective in drug targeting to the colon                  38

1.6 In vitro and In vivo methodologies for studying colon drug delivery    40
<table>
<thead>
<tr>
<th>Section</th>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.7</td>
<td>Rationale and scope of the present study</td>
<td>45</td>
</tr>
<tr>
<td>1.7.1</td>
<td>'Resistant' starches - general review and design rationale for use of Amylose</td>
<td>45</td>
</tr>
<tr>
<td>1.7.2</td>
<td>Scope of the present study</td>
<td>47</td>
</tr>
<tr>
<td>2.1</td>
<td>Introduction</td>
<td>50</td>
</tr>
<tr>
<td>2.2</td>
<td>Materials</td>
<td>52</td>
</tr>
<tr>
<td>2.3</td>
<td>Methods</td>
<td>52</td>
</tr>
<tr>
<td>2.3.1</td>
<td>Method of preparation</td>
<td>52</td>
</tr>
<tr>
<td>2.3.2</td>
<td>In vitro dissolution studies</td>
<td>56</td>
</tr>
<tr>
<td>2.4</td>
<td>Results and discussion</td>
<td>57</td>
</tr>
<tr>
<td>2.4.1</td>
<td>Extrusion and spheronisation</td>
<td>57</td>
</tr>
<tr>
<td>2.4.2</td>
<td>In vitro dissolution studies</td>
<td>61</td>
</tr>
<tr>
<td>2.5</td>
<td>Conclusion</td>
<td>66</td>
</tr>
<tr>
<td>3.1</td>
<td>Introduction</td>
<td>68</td>
</tr>
<tr>
<td>3.2</td>
<td>Choice of materials</td>
<td>74</td>
</tr>
<tr>
<td>3.2.1</td>
<td>Natural polysaccharides</td>
<td>74</td>
</tr>
<tr>
<td>3.2.2</td>
<td>Commercially available enteric coating materials</td>
<td>77</td>
</tr>
<tr>
<td>3.2.2.1</td>
<td>Ethylcellulose</td>
<td>77</td>
</tr>
<tr>
<td>3.2.2.2</td>
<td>Acrylate co-polymers</td>
<td>78</td>
</tr>
<tr>
<td>3.3</td>
<td>Methods</td>
<td>81</td>
</tr>
<tr>
<td>3.3.1</td>
<td>Fluid bed coating</td>
<td>81</td>
</tr>
<tr>
<td>3.3.1.1</td>
<td>Preparation of aqueous coating formulations</td>
<td>81</td>
</tr>
<tr>
<td>3.3.1.2</td>
<td>Preparation of organic solution formulations</td>
<td>82</td>
</tr>
<tr>
<td>3.3.1.3</td>
<td>Curing of film coated pellets</td>
<td>82</td>
</tr>
</tbody>
</table>
CHAPTER 4: EVALUATION OF GLUCOSE PELLETS FOR IN VIVO STUDIES

4.1 Introduction

4.2 Choice of materials

4.3 Methods
   4.3.1 Extrusion and spheronisation
   4.3.2 Fluid bed coating
   4.3.3 *In vitro* dissolution studies and photometric determination of glucose
   4.3.4 *In vitro* fermentation studies

4.4 Results and discussion
   4.4.1 Extrusion and spheronisation
   4.4.2 The effect of glucose load
   4.4.3 The effect of amylose concentration
   4.4.4 The effect of various binders
   4.4.5 The effect of glycerol monostearate concentration
   4.4.6 The effect of pH of a dissolution media
   4.4.7 *In vitro* fermentation results
   4.4.8 Scanning electron micrographs

4.5 Summary and Conclusions

CHAPTER 5: IN VIVO EVALUATION - $^{13}$CO$_2$ BREATH TEST AND GAMMA SCINTIGRAPHY STUDY

5.1 Introduction

5.2 Materials
5.3 Methods

5.3.1 *In vitro* methods

5.3.1.1 Preparation and coating of $^{13}$C glucose pellets 166
5.3.1.2 *In vitro* performance of coated $^{13}$C glucose pellets 166
5.3.1.3 Preparation and coating of 5% Amberlite pellets 167
5.3.1.4 Density determination 167
5.3.1.5 Radiolabelling of Amberlite pellets with $^{99m}$Tc 167
5.3.1.6 *In vitro* testing of $^{99m}$Tc binding to Amberlite pellets 168

5.3.2 *In vivo* protocol

5.3.2.1 Breath test 169
5.3.2.2 Gamma scintigraphy study 171

5.4 Results and discussion

5.4.1 *In vitro* results 172
5.4.2 *In vivo* results 174

5.5 Conclusions 207

*CHAPTER 6: SUGGESTIONS FOR FURTHER WORK* 208

*APPENDIX* Information sheet for volunteers 211

*REFERENCES* 216
# LIST OF FIGURES

<table>
<thead>
<tr>
<th>Chapter</th>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>CHAPTER 1</strong></td>
<td>Figure 1.1</td>
<td>The digestive system</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>Figure 1.2</td>
<td>Overall view of fermentation in the large intestine</td>
<td>27</td>
</tr>
<tr>
<td></td>
<td>Figure 1.3</td>
<td>Flow diagram showing the scope of the present study</td>
<td>48</td>
</tr>
<tr>
<td><strong>CHAPTER 2</strong></td>
<td>Figure 2.1</td>
<td>Diagram of the ram extruder</td>
<td>54</td>
</tr>
<tr>
<td></td>
<td>Figure 2.2</td>
<td>Force-displacement profiles of 50%, 60% and 70% 5-aminosalicylic acid formulae</td>
<td>59</td>
</tr>
<tr>
<td></td>
<td>Figure 2.3</td>
<td><em>In vitro</em> release of 5-aminosalicylic acid in distilled water from uncoated pellets containing varying quantities of 5-aminosalicylic acid</td>
<td>63</td>
</tr>
<tr>
<td></td>
<td>Figure 2.4</td>
<td><em>In vitro</em> release of 5-aminosalicylic acid from uncoated 60% 5-aminosalicylic acid pellets as a function of pH</td>
<td>64</td>
</tr>
<tr>
<td><strong>CHAPTER 3</strong></td>
<td>Figure 3.1</td>
<td>Schematic representation of the change of specific volume $v$ of a polymer with temperature $T$ for (i) a completely amorphous sample, (ii) a semi-crystalline sample and (iii) a perfectly crystalline material</td>
<td>71</td>
</tr>
<tr>
<td></td>
<td>Figure 3.2</td>
<td>Diagram of a fluid bed coater</td>
<td>73</td>
</tr>
<tr>
<td></td>
<td>Figure 3.3</td>
<td>Flow diagram of starch extraction from whole peas</td>
<td>76</td>
</tr>
<tr>
<td></td>
<td>Figure 3.4</td>
<td>Schematic diagram of (A) amylose, (B) ethylcellulose, (C) methacrylate ester co-polymers and (D) methacrylic acid co-polymers</td>
<td>80</td>
</tr>
<tr>
<td></td>
<td>Figure 3.5</td>
<td><em>In vitro</em> release of 5-aminosalicylic acid in various dissolution media from amylose coated pellets as a function of coat thickness (TWG)</td>
<td>92</td>
</tr>
<tr>
<td></td>
<td>Figure 3.6</td>
<td><em>In vitro</em> release of 5-aminosalicylic acid in various dissolution media from Ethocel® coated pellets as a function of coat thickness</td>
<td>93</td>
</tr>
<tr>
<td></td>
<td>Figure 3.7</td>
<td><em>In vitro</em> release of 5-aminosalicylic acid from amylose : Ethocel® mixture (1:4) coated pellets as a function of coat thickness (TWG), using pH change method</td>
<td>96</td>
</tr>
</tbody>
</table>
**Figure 3.8** *In vitro* release of 5-aminosalicylic acid from coated pellets as a function of amylose : Ethocel® ratio, using pH change method

**Figure 3.9** *In vitro* release of 5-aminosalicylic acid from coated pellets as a function of film coating composition (amylose to Ethocel® ratio)

**Figure 3.10** *In vitro* release of 5-aminosalicylic acid as a function of film coating composition, using pH change method (3h in pH 1.2, followed by 21h in pH 7.2)

**Figure 3.11** *In vitro* release of 5-aminosalicylic acid in water from pellets coated with amylose : Eudragit® RS/RL 30D mixture (1:4), as a function of coat thickness (TWG)

**Figure 3.12** Comparison of the release profiles of 5-aminosalicylic acid in various dissolution media, from pellets coated with amylose : Eudragit® RS/RL 30D mixture and amylose : Ethocel® mixture

**Figure 3.13** *In vitro* release of 5-aminosalicylic acid from pellets coated with amylose : Aquacoat® ECD-30 mixture (1:4), as a function of various plasticizers, using pH change method

**Figure 3.14** *In vitro* release of 5-aminosalicylic acid in water from pellets coated with separate amylose / ethylcellulose layers, as a function of coat thickness (TWG)

**Figure 3.15** Comparison of the release profiles of 5-aminosalicylic acid in various dissolution media, from pellets coated with separate amylose / Ethocel® layers and separate amylose / ethylcellulose layers (TWG = 0.9% / 0.6%)

**Figure 3.16** *In vitro* release of 5-aminosalicylic acid in water from pellets coated with separate amylose / Eudragit®L100 layers, as a function of coat thickness (TWG)

**Figure 3.17** *In vitro* release of 5-aminosalicylic acid from pellets coated with (1) multi layer system: (i) amylose/ethylcellulose, TWG=0.9%/0.6%; (ii) amylose / Eudragit®L100, TWG=0.9%/2.4%; and (2) single layer system: (iii) amylose : Ethocel®, TWG=4.8%; (iv) amylose : Eudragit®RS/RL 30D, TWG=14.4%

**Figure 3.18** (A) Square - root of time and (B) First - order release of 5-aminosalicylic acid from pellets coated with (1) multi layer system: (i) amylose / Eudragit® L100, (ii) amylose / ethylcellulose; (2) single layer system: amylose and Eudragit® RS/RL mixtures

**Figure 3.19** Effect of pepsin in 0.1N HCl (gastric fluid) and pancreatin
in 0.2M phosphate buffer (intestinal fluid) on the release of 5-aminosalicylic acid from pellets coated with the amylose : Ethocel® mixture (ratios 2:6 and 1:4)

**Figure 3.20** *In vitro* release of 5-aminosalicylic acid from pellets coated with amylose : Ethocel® mixture (1:4), as a function of storage time and curing conditions

**Figure 3.21** Release profile of 5-aminosalicylic acid in fermenter (simulated colon) system from pellets coated with amylose and Ethocel® mixture (ratio 1:4) and with amylose

**Figure 3.22** Schematic structure of portions of amylose showing bonds hydrolysed by various enzymes

**CHAPTER 4**

**Figure 4.1** Histogram showing distribution of pellet size versus pellet percentage weight

**Figure 4.2** *In vitro* release of glucose in water from amylose : Ethocel® mixture coated pellets, as a function of coat thickness (TWG) Coating conditions: fast spraying rate and high pressure

**Figure 4.3** *In vitro* release of glucose in water from amylose : Ethocel® mixture coated pellets, as a function of coat thickness (TWG) Coating conditions: slow spraying rate and low pressure

**Figure 4.4** *In vitro* release of glucose in water from amylose : Ethocel® mixture coated pellets, as a function of coat thickness (TWG)

**Figure 4.5** *In vitro* release of glucose in water from amylose : Ethocel® mixture coated pellets, as a function of amylose concentration and coat thickness (TWG)

**Figure 4.6** *In vitro* release of glucose in water from amylose : Ethocel® mixture coated pellets, as a function of various binders and coat thickness (TWG)

**Figure 4.7** Effect of thermal treatment (TT) on *in vitro* release of glucose in water from amylose (4.9%) : Ethocel® mixture coated pellets

**Figure 4.8** Effect of amylose concentration and coat thickness (TWG) on *in vitro* release of glucose in water from amylose : Ethocel® mixture coated pellets

**Figure 4.9** Effect of concentration of glycerol monostearate and amylose on *in vitro* release of glucose in water from amylose : Ethocel® mixture coated pellets

**Figure 4.10** Effect of concentration of glycerol monostearate on *in vitro* release of glucose in various dissolution media from uncoated pellets
**Figure 4.11** Effect of concentration of glycerol monostearate and amylose on *in vitro* release of glucose in water from amylose: Surelease® mixture coated pellets

**Figure 4.12** Effect of concentration of glycerol monostearate and amylose on *in vitro* release of glucose from amylose: Ethocel® mixture coated pellets using pH change method

**Figure 4.13** Effect of concentration of glycerol monostearate and amylose on *in vitro* release of glucose from amylose: Ethocel® mixture coated pellets using pepsin and pancreatin in the dissolution media

**CHAPTER 5**

**Figure 5.1** *In vitro* release of $^{13}$C glucose from amylose: Ethocel® mixture (1:4) coated pellets in simulated colon system and simulated gastro-intestinal system

**Figure 5.2** Volunteer 1: The appearance of $^{13}$CO$_2$ in breath as a function of time following administration of (300mg $^{13}$C-glucose) coated pellets

**Figure 5.3** Volunteer 2: The appearance of $^{13}$CO$_2$ in breath as a function of time following administration of $^{13}$C-glucose coated pellets

**Figure 5.4** Volunteer 3: The appearance of $^{13}$CO$_2$ in breath as a function of time following administration of $^{13}$C-glucose coated pellets

**Figure 5.5** Volunteer 4: The appearance of $^{13}$CO$_2$ in breath as a function of time following administration of $^{13}$C-glucose coated pellets

**Figure 5.6** Volunteer 5: The appearance of $^{13}$CO$_2$ in breath as a function of time following administration of $^{13}$C-glucose coated pellets

**Figure 5.7** Volunteer 6: The appearance of $^{13}$CO$_2$ in breath as a function of time following administration of $^{13}$C-glucose coated pellets

**Figure 5.8** Volunteer 7: The appearance of $^{13}$CO$_2$ in breath as a function of time following administration of $^{13}$C-glucose coated pellets

**Figure 5.9** Volunteer 8: The appearance of $^{13}$CO$_2$ in breath as a function of time following administration of $^{13}$C-glucose coated pellets

**Figure 5.10** Cumulative recoveries in volunteer of $^{13}$CO$_2$ in breath
after a single dose of $^{13}$C-glucose either as free glucose or in the pellets

**Figure 5.11** Cumulative percentage dose recovered over 25h period for eight volunteers after administration of 300mg $^{13}$C glucose dose
LIST OF TABLES

 Chapter 2

| Table 2.1 | Composition of uncoated 5-aminosalicylic acid pellets | 53 |
| Table 2.2 | Process conditions for the manufacture of 5-aminosalicylic acid pellets | 55 |
| Table 2.3 | Size distribution of 5-aminosalicylic acid pellets | 55 |

 Chapter 3

| Table 3.1 | Composition and coating thickness values for different formulations | 83 |
| Table 3.2 | Coating conditions for different coating formulation systems | 84 |
| Table 3.3 | In vitro fermentation results | 124 |

 Chapter 4

| Table 4.1 | Composition of uncoated glucose pellets and characteristics of the manufacturing process | 132 |
| Table 4.2 | Coating conditions for amylose : Ethocel® system | 131 |
| Table 4.3 | Composition of coated glucose pellets | 133 |
| Table 4.4 | In vitro fermentation results | 159 |

 Chapter 5

| Table 5.1 | Summary of gamma scintigraphy and $^{13}$CO$_2$ breath test results | 203 |
# List of Plates

## Chapter 3

**Plate 3.1** Scanning Electronmicrographs of 5-aminosalicylic acid pellets  
Page 125

## Chapter 4

**Plate 4.1** Scanning Electronmicrographs of glucose pellets  
Page 161

## Chapter 5

**Plate 5.1** Gamma scintigraphy photographs showing gastrointestinal transit of pellets in volunteer 1  
Page 176

**Plate 5.2** Gamma scintigraphy photographs showing gastrointestinal transit of pellets in volunteer 2  
Page 179

**Plate 5.3** Gamma scintigraphy photographs showing gastrointestinal transit of pellets in volunteer 3  
Page 182

**Plate 5.4** Gamma scintigraphy photographs showing gastrointestinal transit of pellets in volunteer 4  
Page 186

**Plate 5.5** Gamma scintigraphy photographs showing gastrointestinal transit of pellets in volunteer 5  
Page 189

**Plate 5.6** Gamma scintigraphy photographs showing gastrointestinal transit of pellets in volunteer 6  
Page 192

**Plate 5.7** Gamma scintigraphy photographs showing gastrointestinal transit of pellets in volunteer 7  
Page 196

**Plate 5.8** Gamma scintigraphy photographs showing gastrointestinal transit of pellets in volunteer 8  
Page 200
CHAPTER 1

General Introduction
1.1 ORAL SUSTAINED RELEASE DOSAGE FORMS

1.1.1 General principles, advantages and disadvantages

Sustained release dosage forms cover a wide range of prolonged and controlled release of their active ingredients at a predetermined rate and for a period of time (usually 8-12h). A plasma-concentration profile is produced to give optimal therapeutic activity.

One of the earliest attempts to control drug release in the gastro-intestinal tract was the use of enteric coating. The first acid resistant coating material comprising a mixture of lac gum, ammonia and alcohol was formulated by Wruble (1930). Among the earliest modern sustained release products was the 'Spansule' capsular dosage form marketed by Smith Kline & French Laboratories in the 1950’s (US Patent No 2738303). However, significant progress has been made in this area in the past two decades.

In general, two methods have been used in the formulation of sustained release products. The first is based on modification of those physical and/or chemical properties of the drug that affect bioavailability. These include the use of ion-exchange resins to form drug adsorbates, complex formation and prodrug based systems. A major advantage of this approach is that it operates independently from the dosage form and the mechanism of drug release is through either dissociation of the free drug into solution or through decreased rate of dissolution. The second set of methods are based on modification of the drug release characteristics of the dosage form. For those drugs with rapid dissolution, embedding them within a slowly dissolving or erodible matrix provides a means of retarding the dissolution rate. Another approach is to disperse the drug within an insoluble matrix, which is porous in nature, containing small channels, so that the drug release is delayed as the dissolved molecules diffuse through a network of capillaries. The kinetics of release from such matrices have been studied by Higuchi (1963). Another effective means of controlling drug release is by coating the drug particles or pellets with a barrier membrane, which can either be slowly soluble or insoluble in nature. There are two mechanisms whereby coated
pellets release their active principles: by diffusion through the intact coat (Peppas, 1985) and through erosion of the coat (Blythe, 1956). A typical product utilizing this second release mechanism may consist of a capsule containing numerous pellets coated with different coating materials to various thicknesses with some erodible material (Hermelin, 1957). Formulations in which osmotic pumping is a major release mechanism were described by Theeuwes (1975). The osmotically active solid (drug or dispersion) is surrounded by a rigid rate-controlling membrane which is semipermeable to water. Uptake of water via an orifice in the membrane is at a controlled rate and the rate of drug release is constant so long as excess solid remains within the device. Because the mechanism of this system is based on osmotic pressure, the system delivers the drug at a rate that is essentially independent of stirring rate and the environmental pH.

Several therapeutic advantages can be gained by using sustained release formulations. Prolonging the duration of action of the drug beyond that normally achieved with conventional dosage forms can be one such advantage. The frequency of drug administration can be reduced to once or twice daily leading to better patient compliance and convenience (Blackwell, 1973). Another benefit is the attainment of a relatively constant steady state blood level with reduced peak-trough fluctuations, thereby decreasing the toxicity and increasing the efficacy of the drug (Theeuwes, 1983). This is extremely beneficial for drugs with a low therapeutic index, such as theophylline, where blood levels must remain within a narrow range in order to protect asthma patients from attacks and to prevent toxic side effects.

Sustained release delivery systems are not suitable for all drugs. There are specific attributes that a drug must possess for it to be suitable for incorporation in a sustained release system (Notari, 1980). The drug must be effective in a relatively small dose or else the large dose required will make the preparation hazardous to swallow. Also the drug must not possess a very short (levodopa) or very long half-life (tricyclic antidepressants). Drug potency is another factor that must be considered. Very potent drugs generally have a small margin of safety and, therefore, present serious safety problems if the system fails. One future goal for sustained action products may be to bring about the retention of the drug at the site of absorption.
1.1.2 Multiunits versus single units

Oral sustained release preparations may be classified as single or multiunit dosage forms (Bechgaard and Nielsen, 1978). In single unit devices, the drug is often incorporated into a matrix tablet. A multi-unit system may consist of many small pellets contained in a hard gelatin capsule and these appear to have several advantages over the single unit systems. The pellets are easily coated with a rate controlling membrane which can be varied in nature and thickness due to the spherical nature of the pellets. This system permits easy dose adjustments, the risk of dose dumping is equally subdivided (Beckett, 1985) and ineffective plasma levels due to incomplete release of the drug is less likely to occur (Murthy et al., 1983).

Dispersion of the individual particles over a large area in the gastrointestinal tract would yield a more predictable drug release profile by reduction of the local differences in the gastrointestinal environment (Murthy et al., 1983). Local effects of an irritant drug can be reduced as a result of spreading particles over a wide area (Rowe, 1983). This is, however disputed by Wilson et al., (1983) and Devereux (1987) who suggested that particles agglomerate in the gastrointestinal tract.

Multi-unit dosage form empty gradually from the stomach, giving more predictability to the system than single units which empty randomly and would inherently result in a large inter-subject variation (Bechgaard, 1982).

1.2 ORAL DRUG DELIVERY TO THE HUMAN LARGE INTESTINE

Colon - selective drug delivery systems have been the focus of interest during the last decade. Colonic drug delivery has several attractive aspects including a prolonged residence time of luminal contents, reduced epithelial enzymatic activity compared to gastric and small intestine environment and increased tissue response to absorption enhancers. Successful approaches to drug targeting are based, first of all, on a sophisticated understanding of the basic biological and physiological functions of the stomach, the small intestine and the large intestine. A second aspect is to be
aware of the variety of delivery technologies which currently exist and be sensitive to their strengths and limitations.

1.2.1 Anatomy, physiology, histology and microbiology of human gastro-intestinal tract

The human gastro-intestinal tract is a very complex organ, which can be divided into three distinct sections, namely the stomach, the small intestine and the large intestine (Figure 1.1). An extensive description of their functions is given by Sanford (1982) and only functions which are directly related to the performance of a dosage form will be described here.

The stomach

The stomach can be divided into four anatomical regions: fundus, body, antrum and pylorus, which have different physiological functions. The major function is to temporarily store food. The antrum acts as a homogeniser and grinder of food, breaking down the large particles and propelling the contents toward the pylorus for ejection into the duodenum as a semi-solid chyme. A muscular pyloric sphincter regulates the flow between the stomach and small intestine. The entire mucosal surface of the stomach is lined with simple columnar epithelial cells involved in the secretory process. Three different cells can be recognised in the glands; mucous cells which secret mucus to form a protective barrier for the stomach lining, peptic cells which secret pepsinogen, an inactive precursor of pepsin and oxyntic cells which are responsible for the secretion of acid. The acid helps in the breakdown of connective tissue and muscle fibres, activates pepsin and provides a medium of low pH 1.0 - 2.5 (Evans et al., 1988) and has a bactericidal action. The hormone gastrin is the most potent stimulator of gastric acid production. Human pepsin is an endopeptidase and hydrolyses several peptide bonds within the interior of protein molecules. There is an evidence that some absorption can take place from the stomach. Several organic acids can be absorbed, depending on the pH of the luminal contents. Such substances are un-ionized, fat soluble and absorbable at one pH but ionized, water soluble and virtually unabsorbable at another (e.g. aspirin).
The small intestine

The small bowel is divided into three parts, duodenum, jejunum and the ileum. It is 5 - 6 metres in length and its main functions are to mix food with enzymes to facilitate digestion, absorption (from the lumen towards the bloodstream), secretion (from the bloodstream towards the lumen) and to circulate the intestinal contents. It has the high capacity for nutrient absorption due to the large surface area of 200 - 500m² (Davenport, 1977) provided by epithelial folding and the villous structures of the absorptive cells. The duodenum (pH 5.0-6.5) has a mucous membrane and contains the digestive glands and Brumer's glands. They produce a protective alkaline secretion (bicarbonate and mucus) which neutralizes gastric acid but does not contain any enzymes. The intestinal cells, which are present throughout the small intestine secrete mucus. The jejunum (pH 5.0-6.5) is more vascular. In the ileum (pH 7.0-9.0) the lymphatic follicles are larger and more numerous then elsewhere in the intestine. The lymphatic system has an important role in the absorption of nutrients, especially fat (pancreatic lipase) from the gastro-intestinal tract and provides a route by which electrolytes, protein and fluids can be returned in the blood. The blood vessels in the jejunum and ileum are derived from the superior mesenteric artery. The blood from the small intestine flows into the large hepatic portal vein which takes blood to the liver. The liver has the highest drug metabolising capacity and in a single pass (first-pass metabolism) can remove a large proportion of the absorbed drug before it reaches the systematic circulation. The human pancreas is a large gland secreting approximately 1 litre of pancreatic juice per day. The pancreatic juice has two major components: (i) alkaline fluid and (ii) enzymes. The secretion of the aqueous phase and the bicarbonate component is largely regulated by the pH of the chyme delivered into the small intestine from the stomach. All the pancreatic proteases (trypsin, chymotrypsin) are secreted as inactive enzyme precursors and are converted into the active forms in the lumen. Pancreatic amylase and lipase are secreted in active forms. Pancreatic amylase, like salivary amylase, is an α-amylase hydrolysing α-1,4 glucosidic bonds. The secretion of pancreatic enzymes is primarily regulated by the amount of fat and protein entering the duodenum. Bile is secreted by the liver, and is a complex mixture of water, organic (bile acids, phospholipids-lecithin, cholesterol and bilirubin) and inorganic solutes. Salivary and pancreatic amylases initiate the hydrolysis of starch and exhibit their optimal activity at near neutral pH.
Carbohydrates are absorbed in the proximal part of the small intestine. Glucose is absorbed rapidly and completely by both passive diffusion and active transport. The brush border possesses a sodium-dependent carrier which transports sugars across the membrane in either directions. Most drugs are absorbed by passive diffusion in a non-ionised state. The pH of the small intestine determines the degree of ionisation and hence controls the efficiency of absorption.

The amount of flora encountered in the small intestine in normal individuals is highly dependent on the location of sampling. The upper small intestine is generally sterile (0-10^4.5 colonies per millilitre) of both aerobic (streptococci, staphylococci, lactobacilli and yeasts) and anaerobic (streptococci and lactobacilli) bacteria. Of note is the almost complete absence of anaerobic Bacteroides. The lower small intestine contains a flora (10^3.5-10^6.3 colonies per millilitre) with high content of Bacteroides (Finegold et al., 1983) and more closely resembles colonic flora. Subjects with higher gastric pH have higher contents of both anaerobic and aerobic bacteria because gastric acid acts as a barrier to swallowed organisms.

The large intestine

The large intestine is responsible for the conservation of water and electrolytes, motor function, the formation of a solid stool and storage until time of defecation (Sanford, 1982). The colon extends from the ileo-caecal junction to the anus and is approximately 125cm long. It can be divided into the caecum, ascending (pH 5.5), transverse (pH 6.0), descending (pH 7.2), sigmoid colon (pH 7.5), rectum and anus. For the purposes of drug delivery, the colon has to be considered as two regions; the distal colon, which can be reached from the anus, and the proximal colon which is only accessible via the oral route. As it can be seen in Figure 1.1, the caecum, which is the widest part of the colon, is based at the ileo-caecal junction. The ileo-caecal sphincter has a regulatory function in controlling the movement of food from the small intestine into the large intestine. The ascending colon extends from the caecum to the hepatic flexure. The transverse colon hangs loosely between the hepatic and the splenic flexure, often following the greater curvature of the stomach. The descending colon extends downwards from the splenic flexure to the pelvic brim. The wall of the colon is divided into four layers: serosa, muscularis externa, submucosa and mucosa.
The epithelium of the serosa is covered with fat pouches of peritoneum known as appendices epiploical. The muscularis externa consists of an inner-circular muscle layer and an outer longitudinal layer composed of tripe known as taenia coli. The submucosa contains many blood and lymph vessels. The blood supply the to upper rectum derives from the superior and inferior mesenteric arteries. They join the splenic vein as part of the portal venous system to the liver. Drugs absorbed from the lower rectum and anal canal are transported via the haemorrhoidal plexuses to the vena cava, and have the advantage of avoiding first pass elimination. Colonic mucosa contains numerous T-lymphocytes, macrophages, plasma cells and these cells play an important role in the immune function of the colon. The colonic mucosa has a comparatively smooth surface without the villi or microvilli of the small intestine. Thus the surface area available for absorption in the colon is considerably less than that of the small intestine (Koch-Weser and Schechter, 1981). Mucus excreted by colonic mucosa cells lubricates the surface of the colon and facilitates the transit of dehydrated faecal material.

The bacterial counts in faeces average at least $10^{11}$ colonies per gram and anaerobic organisms predominate with 300-400 species usually present. The flora is of either luminal (represented by faeces) or mucosal origin (Finegold et al., 1983). The diverse bacterial populations of predominantly saccharolytic species (bacteria requiring carbohydrate for energy and growth) synthesise the many enzymes necessary for the fermentation of carbohydrates (Salyers and Leedle, 1983). Human intestinal bacteria primarily synthesise cell-associated (i) polysaccharidases which initiate polymer breakdown by hydrolysing the polysaccharide backbone. (ii) Glycosidases hydrolyse the polysaccharide side chain and, in addition, further break down the backbone oligosaccharides (Cummings et al., 1989). Human faeces contain high levels of amylase activity, most of which (92%) is extracellular. However, bacterial-cell-bound amylase are much more effective at breaking down starch than the soluble enzymes in cell-free supernatant from faeces. The predominantly amylolytic bacteria belong to the genera Bifidobacterium, Bacteroides, Fusobacterium and Butyrivibrio (Cummings and Englyst, 1987). Fermentation, has an important role in human metabolism as it is integral part of human digestion. An outline of human large gut fermentation is shown in Figure 1.2. Fermentation is described as a complex series of
anaerobic processes in which carbohydrates and proteins are broken down by the action of many bacterial species. Endogenous substrates mainly consist of the polysaccharides and proteins that are present in epithelial cells and in small intestinal secretions such as mucin and the pancreatic hydrolytic enzymes (amylase, lipases, proteases). The end products of these reactions are diverse but quantitatively ammonia, the short chain fatty acids (SCFA) acetate, propionate and butyrate, and the gases hydrogen, carbon dioxide and methane. The control of fermentation is of major importance in determining colonic function in both health and disease. The main site of the fermentation in the large gut is the caecum. SCFA absorption stimulates sodium and water absorption (Cummings et al., 1987). Butyrate has important regulatory effects on nucleic acid metabolism and so may be of benefit to the health of the large bowel epithelium. Once absorbed SCFA pass into the portal vein and hence to the liver where propionate and acetate are taken up. About 80% of fermentation gases are excreted as flatus and a proportion (10-20%) of all gas produced in the large gut is absorbed and excreted in breath. Intestinal bacteria is also involved in other physiological functions in the body besides the fermentation, such as synthesis of vitamins (e.g. K, B₁₂, thiamin and riboflavin).

### 1.2.2 Why to the colon?

Colon specific drug delivery needs to be designed to protect the active compound from the acidic environment of the stomach, and minimize systemic absorption from the small intestine (Dew et al., 1982). Side effects can be reduced and pharmacological response increased if the active drug can be delivered specifically to its site of action. Many diseases of the colon, for example ulcerative colitis, Crohn’s disease, irritable bowel syndrome, diarrhoea, constipation and also cancer of the colon, could be better treated if site specific delivery of the therapeutic agent could be effective. There are also situations (arthritis) other than the treatment of diseases of the colon where it is desirable to deliver a drug to the colon before it is released. The drug can then act locally in the colon, be available for possible absorption into the blood, doses can be lower and the efficiency therefore maintained. The large intestine is attracting interest as a site where poorly-absorbed drug molecules (heparin) may
Figure 1.2  Overall view of fermentation in the large intestine

(Taken from Cummings et al., 1989)
have an improved bioavailability.

Recent years have seen the development of systems associated with oral administration of peptide drugs to the colon (Saffran et al., 1986). They developed a copolymer of styrene and 2-hydroxyethyl methacrylate cross-linked with divinylazobenzene to coat oral dosage forms. These peptides include analgesic peptides (endorphin), contraceptive peptides (gonadotropin-releasing hormone), growth-promoting peptides, oral vaccines, plasminogen-activating peptides, insulin, vasopressin etc. These peptide molecules are of high molecular weight, polar in nature and are susceptible to degradation if exposed to the acidic environment of the stomach through conformational changes. Also other changes may occur due to the normal action of digestive enzymes breaking the polypeptide down into absorbable di- and tri-peptides. Thus material gets to the portal circulation and it could be metabolised on its first-pass through the liver or secreted into the bile. However, the colon is recognized as having a less hostile environment in terms of intensity of enzymatic activities than the stomach and small intestine. Pancreatic $\alpha$-amylase and total protease activities decrease distally from the small bowel to the sigmoid/rectum region of the large intestine, showing that considerable breakdown or inactivation of the enzymes occurs during gut transit.

The colon has a longer retention time (Macfarlane et al., 1989) which might improve total drug uptake. Colonic transit time varies considerably between individuals; ranging from about 23 to 168h in healthy subjects of the United Kingdom with a mean of approximately 70h. Although the colonic region has such drawbacks as impaction of faeces (which might act to entrap drug) and the presence of bacterial enzymes and toxins, many believe these concerns are easier to deal with than the degradation of drugs during their passage through the stomach and small intestine.

Successful oral colonic delivery first requires that the drug reaches the proximal colon at the precise time. Fluctuations in gastric residence time due to the presence of food (Davis et al., 1984, 1987) and the dosage form itself produce much of this variability. Gamma scintigraphy is a useful technique for the evaluation of the in vivo fate of pharmaceutical preparations (Fell and Digenis, 1984). They found that
following a light breakfast, pellets and osmotic pump systems are emptied quite rapidly and move through the small intestine in about 200min. After a heavy breakfast the pellets empty more slowly (3h) from the stomach. In contrast, the single unit (osmotic pump) are retained in stomach after a heavy breakfast for at least 9h. Clarke (1989) and Devereux et al., (1990) studied the influence of the density of the pellets on their gastric emptying. The 2.8gcm \(^3\) pellets had an extended residence time in both fed and fasted state.

Transit trough the small intestine is independent of the physical characteristics of dosage form (Davis et al., 1986). While gastro-intestinal transit time for females and males appear to differ slightly, age does not seem to be a factor (Metcalf et al., 1987). However, numerous other factors such as emotional stress are capable of increasing cecocolonic motility and altering gastro-intestinal transit time.

Factors affecting the transit of pharmaceutical preparations through the stomach and small intestine have been extensively investigated. If the colon is to be prime site of drug absorption then an understanding of ileo-caecal transit is necessary because the junction controls movement of intestinal contents between the ileum and the colon. Sugito et al., (1990) found that the entire transit time of solid dosage preparations (heavy and light tablets, pellets and micro-particles) from the stomach to the ascending colon is determined by the gastric emptying time and the residence time at the ileo-caecal junction. The residence time at the ileo-caecal junction (more than 2h) is found to be independent of pharmaceutical factors (size and density) (Sugito et al., 1990; Adkin et al., 1993). Metcalf et al., (1987) investigated the transit through the proximal colon (caecum to mid-transverse), right colon (mid-transverse to sigmoid colon) and sigmoid colon and found that it took 7-11h, 9-11h and 11.7-12.8h respectively. Solutions and small particles pass through the proximal colon more slowly than large units (Hardy et al., 1985), size and density of large units have little effect on transit time (Parker et al., 1988). Although residence time in the ascending colon is similar for solids and small volumes of liquids (Proano et al., 1991), meals and faeces can also affect local nerve function and therefore alter colonic residence time (Neri et al., 1991). In the colon, dispersive systems such as pellets, become widely distributed and have a longer residence time compared to single units. This phenomenon may explain
the mixing of pellets within the colon. If the drug was released from a dispersive preparation over 10h, drug levels would be difficult to control than if the active compound was released over a longer period, due to the variability in excretion patterns. One approach to overcome fluctuations in colonic residence times is to employ bioadhesives in colonic drug formulations (Kopecek, 1990) or prodrugs specific for colon delivery (Friend and Chang, 1985), whose approach will be discussed in Section 1.5.3.

1.3 INFLAMMATORY BOWEL DISEASES (IBD)

1.3.1 Definition

A comprehensive review of inflammatory bowel diseases and its significance is given by Harrison (1987). However, successful application of drug targeting requires understanding of disease processes involved. Inflammatory bowel diseases (IBD) is a general term for a group of chronic inflammatory disorders of unknown etiology involving the gastro-intestinal tract. Chronic IBD may be divided into:

(i) chronic nonspecific ulcerative colitis
(ii) Crohn’s disease

1.3.2 Epidemiology and Etiology

Epidemiologic consideration reveals that the diseases are more common in white populations than black, with a greater incidence amongst Jews and orientals. Age group between 15 and 35 years are mainly affected by the diseases, probably on a hereditary basis as well as environmental factors.

Several areas of possible etiological importance are:

(i) genetic
(ii) infections (bacterial, fungal, viral agents; there is evidence from isolation of cell wall variants from *Pseudomonas* which can cause many infections of
more acute than chronic nature)

(iii) immunologic (patients may have humeral antibodies to colon cells, bacterial antigens, such as *Escherichia coli*; lipopolysaccharide and foreign proteins

(iv) psychological factors (there is evidence of stress associated with IBD)

1.3.3 Pathology

Ulcerative colitis affects the distal colonic mucosa and extends a variable distance proximally. It is often limited to the rectum or recto-sigmoid colon. The primary inflammatory reaction involves the surface epithelium and no granulomas are present. Crohn’s disease is a segmental process that often affects the small bowel as well as the colon. It is complicated by localized perforations (granulomas) accompanied by abscess or fistula formation and nutritional deficiencies occur particularly if the small bowel is involved. Morphologic features distinguishing Crohn’s disease from ulcerative colitis is that chronic inflammation extends through all layers of the intestinal wall and also involves the mesentery and the regional lymph system.

1.3.4 Clinical features

Differentiation between the two diseases is not always possible and depends on clinical, endoscopic, pathological and radiologic assessment. The most common symptoms are bloody diarrhoea, abdominal pain with fever, weight loss, colonic neoplasms (carcinoma), rectal bleeding and constipation. The extracolonic manifestation which may accompany the IBD are arthritis, skin changes, anaemia, hypokalemia and hypotension.

1.4 TREATMENT OF INFLAMMATORY BOWEL DISEASES

1.4.1 Routes of drug administration

The aims of the therapy are to control the inflammatory process and replace
nutritional losses. For the purposes of drug delivery, the colon has to be considered as two regions; the distal colon, and the proximal colon which is only accessible via the oral route. The splenic flexure limits the area of exposure of drugs administered by the anal route to the descending and sigmoid colon, rectum and anus. Administration by the rectal route is preferable for drugs which are irritant when given orally, when administration by mouth is inappropriate, for example in the presence of nausea and vomiting, unconscious patients and when first-pass metabolism is to be avoided. Rectal dosing is achieved through suppositories, enema solutions and foam enemas. In general, the spreading of suppositories is confined to within the rectum and is complete within an hour of dosing. The spreading of suppositories is of importance when drugs are administered for their topical action (Hardy, 1989). Enema solutions usually spread sufficiently to provide effective topical treatment to the sigmoid and descending colon and spreading is usually complete within 2 hours. The structure of the foam enemas and the small amount instilled restrict the distribution to the rectum and sigmoid colon. However, drug administration to the proximal colon is best achieved with controlled-release, oral preparations which are discussed in Section 1.5.2. The objective of a peroral drug administration is to maintain a therapeutic concentration in all affected areas of the gut by the release of sufficient amounts of drug from the preparation. The release pattern of the drug preparation should, ideally, remain unaffected by variable the intestinal transit time.

1.4.2 Principal drugs

For many years the drug therapy for inflammatory bowel disease was limited to sulfasalazine and either topical or systemic anti-inflammatory corticosteroids. Recently, immunosuppressive agents (azathioprine and 6-mercaptopurine) and antibiotics (metronidazole) have assumed important roles in the treatment of Crohn’s disease and corticotropin has been established as a drug for treating patients with ulcerative colitis. Now the aminosalicylates are taking an important role in IBD pharmacotherapy (Van Rosendaal, 1989; Peppercorn, 1990)
1.5 5-AMINOSALICYLIC ACID AS A MODEL DRUG

1.5.1 General pharmacology

The principal therapeutic use of 5-aminosalicylic acid is an anti-inflammatory agent which acts locally and is used by patients with ulcerative colitis inducing remission in the acute phase (Dew et al., 1983). All dosage forms of 5-aminosalicylic acid in Europe carry the generic name mesalazine. A limited number of therapeutic trials suggest that orally administered mesalazine of 1.5 to 2.4g daily is effective in patients with mild to moderate ulcerative colitis. Schroeder et al., (1987) showed that oral 5-aminosalicylic acid administered in a dosage of 4.8g per day is effective therapy.

In vitro studies of fresh biopsies of colonic mucosa from patients with chronic IBD indicate elevated concentrations of prostglandin, leukotriene B₄ (LBT₄). Consequently many investigation have been concerned with potential modulation of chemical mediators. Mesalazine has been found to inhibit migration of intestinal macrophages to LBT₄ and thus may restrict migration of macrophages to inflamed areas (Brogden and Sorkin, 1989). It has also been suggested that mesalazine may act as a radical scavenger against reactive oxygen moieties (Williams and Hallett, 1989). They have shown that an inflammatory infiltrate in the mucosa contains macrophages and retains the ability to produce oxygen products such as hydrogen peroxide and hypochlorite. This latter metabolite may play an important role in mediating tissue damage. 5-aminosalicylic acid reacts with hypochlorite ions and provide protection against the potentially tissue damaging products of activated neutrophils in the inflamed bowel.

The pharmacokinetic properties of mesalazine administered as a single molecule of 5-aminosalicylic acid have been reviewed by Klotz et al., (1985) and are discussed in Section 1.5.2., using commercially available drugs as an example. When instilled in the proximal part of the small intestine, mesalazine is quickly absorbed (Yu et al., 1990). Presystemic metabolism of 5-aminosalicylic acid may involve bacterial (faecal) or mucosal acetylation, or both (Allgayer et al., 1989) when the drug is
targeting to the colon. The main metabolite of 5-aminosalicylic acid, N-acetyl-ASA however, seems to be therapeutically inactive with no potential toxicity (Ireland et al., 1992). To characterize this presystemic metabolism they used an indirect method by measuring systemic (plasma) and colonic (faecal) concentration of 5-aminosalicylic acid and N-acetyl-ASA in patients with IBD and found that acetylation was mainly mediated by a colonic mucosal enzyme and only to a small extent by bacterial enzymes. As the N-acetyltransferase enzyme is cytosolic, 5-aminosalicylic acid must be taken up by the epithelial cell before acetylation can take place. De Vos et al., (1992) measured mucosal concentration and used this to estimate the local concentration. Yu et al., (1990) conducted the study to describe oral 5-aminosalicylic acid pharmacokinetics after suspension dosing and found the elimination half life of 5-aminosalicylic acid to be 51.9 minutes, whereas the acetyl ASA half life has not been determined. This indicate that 5-aminosalicylic acid absorption is not the rate-limiting step in 5-aminosalicylic acid disposition.

The most commonly reported adverse effects of mesalazine are headache and nausea, indigestion, muscular ache and fever (Brogden and Sorkin, 1989).

1.5.2 Sustained 5-aminosalicylic acid delivery systems:
A clinical and commercial success??

Sulphasalazine (salazosulphapyridine), which consists of sulphapyridine and 5-aminosalicylic acid joined by a diazo bond, has been used in the acute treatment and maintenance therapy of IBD for over 40 years. When orally administered the drug reaches the colon where colonic bacteria, which possess intracellular azoreductase, cleave the azo bond releasing mesalazine and sulphapyridine. Azad Khan et al., (1977) demonstrated that mesalazine was the active moiety as it possesses the anti-inflammatory effect. Sulphapyridine has little therapeutic effect in ulcerative colitis but is absorbed from the colon and was found to exhibit adverse effects such as perimyocarditis, pancreatitis and watery diarrhoea (Das et al., 1973). Consequently, research has been focused on the development of new oral mesalazine formulations to enable mesalazine to reach the colon and to avoid the adverse reactions caused by
sulphapyridine (Riley et al., 1988). These new products use two methods to facilitate drug delivery to the colon:

(i) colonic flora (azo-bond cleavage enzymes)
(ii) local-luminal pH

Azo bonded systems composed of mesalazine linked through an azo bond to another mesalazine or to a carrier moiety are subject to bacterial azoreductases which cleave the bond and liberate the drug. The second method uses pH-dependent polymer coatings of specified thickness that dissolve during transit through the gut and supposedly release mesalazine in the colon.

Why the existing treatment is not satisfactory?

Examples of new azo-bonded drugs include: olsalazine which is a combination of two molecules of 5-aminosalicylic acid (van Hogezand, 1988); ipsalazide and belsalazide, which have mesalazine linked to 4-aminobenzoyl glycine and 4-aminobenzoyl β-alanine respectively (Chan et al., 1983; Fleig et al., 1988). First of all, azo bonded drugs have some advantages. Release of mesalazine from such drugs requires enzymatic cleavage by bacterial azo-reductase and is therefore restricted to the large bowel, however azo bond drug systems have several limitations. These prodrugs should be a pharmacologically inactive entity which are formed by chemical modification of the drug. It has been reported (Zinberg et al., 1987) that a significant proportion of patients with ulcerative colitis develop severe diarrhoea as an adverse effect to the azo bond drug system thus reducing colonic azo reduction and release of the active moiety. The azo bond itself is responsible for many of the side effects such as diarrhoea and nausea. In a study of besalazide (Chan et al., 1983) the recovery of 5-aminosalicylic acid in urine and faeces was similar to the findings with sulphasalazine. In contrast to sulphapyridine, the carrier molecule was poorly absorbed from the colon, as 72% was excreted in the faeces and only 18% in the urine. If the intestinal transit time is increased, the amount of active drug will be decreased and the efficacy of azo bond drug may be reduced in patients with more active or severe diarrhoea.
The most extensively studied new azo compound is olsalazine (Dipentum®, Pharmacia) which is poorly absorbed from the colon. After a single dose of olsalazine, at steady-state, which takes 6 to 19 days to achieve, the serum concentration of olsalazine was low and serum half-life was 6 to 10 days (van Hogezand, 1988), probably because of low clearance rate. This half-life is considerably longer than that of sulphasalazine (reported to be less than 2 days) and is caused by, strongly sulphated protein-bound metabolite which occurs in very low serum concentrations. There are several disadvantages of olsalazine. Olsalazine can accelerate gastric emptying and mouth to caecum transit time (Rao et al., 1987). It has been found that 30% of the daily dose was excreted in the urine (Willoughby et al., 1982). In contrast, a concentration of 5-aminosalicylic acid in faeces doubled when sulphasalazine is replaced by the same dosage of olsalazine. Thus, olsalazine gives the most reliable delivery of 5-aminosalicylic acid to the colon of all the new 5-aminosalicylic acid compounds, but is not suitable for treatment of small bowel disease.

To eliminate the need for bacterial cleavage of the azo bond mesalazine and to avoid chemical modification of the drug, controlled and slow release dosage forms which utilize the pH of colonic environment have been developed. These preparations do not require a carrier molecule or rely on bacterial action for the release of the active substance. These slow release preparations utilize two basic pharmaceutical technologies. One method is to formulate 5-aminosalicylic acid tablets that contain compressed microgranules of 5-aminosalicylic acid that are coated with ethylcellulose varying in thickness. The product is registered under the trade name Pentasa® (Ferring, Vanlose, Denmark). It disintegrates in the stomach to liberate microgranules at a certain rate. The rate is slow below pH 6 but increases at pH 7.5. When trying to evaluate how much of the administered drug reaches the colon, the urinary recovery as well as the early plasma 5-aminosalicylic acid peak give useful information about how much of drug is absorbed in the small intestine and thereby 'lost' for treatment of colonic lesions. Rasmussen et al., (1982) reported that at a steady-state, 40% of the 24h dose was recovered from the faeces and 53% from the urine, indicating that a large amount is absorbed in the small intestine. Of this excreted total, one third was still retained in granules and two-thirds of 5-aminosalicylic acid has been released. Christensen et al., (1987) studied the influence of intestinal transit time on the release
of 5-aminosalicylic acid from a peroral Pentasa® preparation and found 45% of 5-
aminosalicylic acid still retained in granules during normal conditions, with about 80%
released when the transit time is accelerated. Controlled trials of 46 patients receiving
either Pentasa® (1.5g/day) or a placebo indicated a trend in favour of Pentasa®
(Rasmussen et al., 1986). However, the difference was not statistically significant,
either because of the limited number of patients or because the patients were only
mildly to moderately ill and thus the effect of treatment was difficult to evaluate.

Another method is to use pH dependent polymer coatings of specific thickness
that dissolve during transit through the gut and release 5-aminosalicylic acid in the
intestine. The specific location of drug release is dependent on the type and thickness
of the polymer coating. These polymers have ionizable carboxyl groups and in the low
pH environment of the gastric fluid, are not ionised and therefore poorly soluble in
water.

Asacol® (Tillot, Smith Kline & French) is a tablet of mesalazine covered with
an 80 to 130µm thick coat of the acrylic polymer Eudragit®S, which is soluble at pH
7 and above (Riley et al., 1988). A proportion of tablet may be excreted unaltered in
the faeces (Schroeder et al., 1987), 5-aminosalicylic acid is released distally in the
ileum and in the colon (Dew et al., 1982) and urinary excretion is reported to be 20%
(Mardini et al., 1987). Time required to achieve maximum plasma concentration is
7.5h after administration of Asacol® tablet.

Salofalk® (Falk, Germany, Switzerland), Claversal® (Smith Kline Davelsberg,
Germany) and Mesasal® (Smith Kline & French) consisted originally of a tablet with
a compressed core of 5-aminosalicylic acid (250mg - 500mg) mixed with buffering
sodium carbonate and glycerine and coated with Eudragit® L100. This polymer is
acrylic based and formulated to resist gastric acid pH for 2h and dissolve in intestinal
fluids (pH 6) in 30min. When clinically considering this formulation, 44-66% of the
drug was excreted in the urine (Klotz et al., 1985). The site of tablet disintegration is
largely dependent on the transit rate through the intestine and the amount of polymer
coil present. When taken with a meal gastric acid-resistant tablet can remain in the
stomach for more than 11h (Davis et al., 1986), which the original Salofalk®
formulation probably could not stand without being dissolved. With healthy subjects it was found that 70% of the tablets disintegrated in the small intestine, on average 5h after emptying from the stomach (Hardy et al., 1987a) and in the case of patients after 3.2h (Hardy et al., 1987b; Healey, 1990). These findings indicate that this formulation might be a good choice for treatment of ileocecal disease but is not reliable for colonic treatment.

Use of such a system is based on the premise that there is a shift in pH from slightly acidic to slightly basic conditions at the ileocecal junction, but this may not be useful since studies with healthy humans suggest that the pH may actually decrease in the colon (Evans et al., 1988). In conclusion, high percentage of urinary excretion and maximum plasma concentration achieved in a very short period of time after administration of these formulations indicate that a great amount of 5-aminosalicylic acid is released in the small intestine and only a small portion of the dose reaches the colon. Therefore, utilization of pH difference between small intestine and large intestine can not be used to formulate colonic specific release formulations due to high inter-individual patient variation in pH through the gut. In addition, the release pattern of 5-aminosalicylic acid preparations should, ideally, remain unaffected by the intestinal transit time over a wide range.

1.5.3 A new perspective in drug targeting to the colon

An approach which has been extensively exploited lately includes targeting the colon via prodrug-based systems which rely on enzymes (glucosidases and polysaccharidases) produced by colonic microflora to release the active drug in the colon. Prodrugs are pharmacologically inactive and have different physico-chemical properties compared to the active molecule. There are two requirements for prodrug colonic drug delivery. First, the prodrug should not be absorbed in the small intestine. A high molecular weight or hydrophillic nature can be used to achieve this goal. Second the rate of conversion to the active drug should be faster in the colon than in the stomach and small intestine. These two factors ensure a slow rate of drug absorption in the upper GIT and a complete conversion of the drug in the colon. Prodrug-based colonic delivery systems which will be discussed here are: (i)
bioadhesive polymeric azobond (Kopeček et al., 1992), (ii) glucoside (Friend et al., 1991; Friend and Chang, 1985; Friend and Tozer, 1992), (iii) chondroitin sulphate matrix (Rubinstein et al., 1992) and (iv) hydrogels (Brøndsted and Kopeček, 1992; Kopeček et al., 1992).

Friend and Chang (1985) have developed an orally administered colon-specific prodrug by coupling a hydrophilizing promoiety, a glucose or galactose molecule to steroid (dexamethasone and prednisolone). As a result of the decreased permeability of the polar prodrugs, absorption in the small intestines is minimized resulting in an increased concentration of the prodrug reaching the site of action, the colon. At this target site, bacterial glucosidases cleave the promoiety which releases the therapeutically active steroid. The study was performed with the guinea pig as a model for humans.

The limitations in using the oral route for site specific delivery due to the rapid transit time of soluble macromolecules through the gastro-intestinal tract was discussed in Section 1.2.2. To modify the transit time of polymeric drugs the use of bioadhesives has been proposed by Kopeček et al., (1992). They synthesized N-(2-hydroxypropyl) methacrylamide (HPMA) copolymers containing 5-aminosalicylic acid bound via aromatic azo bonds and bioadhesive moieties (monosaccharides-fucose). The present bioadhesion formulation is based on the following concept. Some bacteria (e.g. Shigella flexneri), adhere to the colonic mucosa of guinea pigs and the binding is fucose and glucose specific. The higher the content of fucose in the co-polymer, the higher the amount associated with colonic tissue. The binding to the small intestine was lower and did not show a clear dependence on the content of fucose in the co-polymers. Microbial azoreductases predominantly present in the colon are able to cleave aromatic azobonds in water soluble polymers.

Another interesting approach explored by Brøndsted and Kopeček (1992) uses hydrogels for site specific peptide and protein drug delivery to the colon. The hydrogels contain acidic co-monomers and enzymatically degradable azoaromatic cross-links with a low degree of swelling which protects the drug against degradation by digestive enzymes. As the gel passes down the gastro-intestinal tract, the degree
of swelling increases as a result of increased ionisation. Upon arrival in the colon, the degree of swelling allows enzyme access to the cross-links which are degraded and the drug is released. Enzymatic degradation of cross-links in hydrogels strongly depends on their degree of swelling and the cross-linking density. By changing the structure of the polymer main chain and the crosslinking density, it is possible to control the degree of swelling and the pH-sensitivity of the gel. Gels containing a longer cross-linker were degraded faster than gels containing the shorter cross-linker.

Rubinstein et al., (1992) developed a matrix tablet system comprising cross-linked chondroitin sulphate, a soluble mucopolysaccharide consisting of D-glucoronic acid linked to N-acetyl-D-galactosamide. Cross-linked chondroitin matrix tablet is found to retain 70% of its indomethacin (drug model) content for over 28h at pH 7, which is close to the physiological pH of rat caecal content. In the presence of rat caecal content at the same pH enhanced release of indomethacin occurs. In the human colon, the natural sources of chondroitin are sloughed epithelial cells.

In summary, here were presented good examples of prodrugs which act selectively by a combination of targeting mechanisms and site specific activation. However, the feasibility of these approaches must be evaluated after more experimental in vivo data in humans is available.

Ashford et al., (1992) used gamma scintigraphy during in vivo studies in human subjects to deduce whether USP pectin coated tablets are capable of affording site specific delivery to the colon. They reported that all six of the tablets disintegrated in the colon and 5 out of 6 of them in the caecal/ascending colonic regions.

1.6 IN VITRO AND IN VIVO METHODOLOGIES FOR STUDYING COLON DRUG DELIVERY

A properly designed in vitro test is valuable in dosage form development, quality control of the final proven formulation and can also provide details of bioavailability. This project concerns specific drug delivery to the colon and therefore
general methods such as in vitro dissolution tests, in vitro fermentation tests and in vivo studies will be briefly discussed.

**In vitro dissolution test**

The in vitro dissolution procedure and operating parameters must be optimized to be sensitive to critical manufacturing variables (excipient concentrations, compression pressure, coating thickness) and satisfy requirements necessary to simulate more closely the environment of gastrointestinal tract. The result obtained must be reproducible and correlated or associated with some characteristic of the in vivo data. The official in vitro dissolution methods described in current US Pharmacopoeia should be utilized unless shown to be unsatisfactory. In general, dissolution tests have evolved into two distinct types, a rotating basket assembly or a rotating paddle system and the flow through column systems (Abdou, 1989). The former is characterised by a relative large dissolution volume with minimal liquid exchange. The second type consists of a relatively small dissolution cell through which fresh solvent flows at a constant rate with no additional agitation. However, a suitable agitation intensity is important to enable discrimination of formulation parameters that are important in vivo. To mimic the variable pH of the gastrointestinal tract, composition of the dissolution medium should be aqueous in nature and be buffered at different pH values. Some formulations may contain retardants whose function depends on the presence of normal constituents of the gut (example pepsin, pancreatic secretions). For these constituents, the dissolution medium should be further refined to contain the appropriate materials. It is also important to maintain sink conditions by keeping the concentration of the dissolved drug in the bulk medium below 15 percent of saturation and to conduct the test at 37 ± 0.5°C, as both of these factors can affect the rate of drug release.

**Fermentation tests**

To evaluate the dosage form intended to deliver a drug to the colon, it is important to set up appropriate fermentation tests. There is not an established standard method to examine digestibility of the test substrate. Baring in mind that the human colon is a highly complex ecosystem with more than 400 different bacterial species, the method used depends on the test substrate and the testing stage. A number of IBD
animal models are available for the testing of colonic delivery systems. Previous workers have used rats or guinea pigs (Friend and Tozer, 1992; Kopecek et al., 1992) or rat caecal contents (Rubinstein et al., 1992) to study drug delivery to the colon. Friend and Tozer (1992) claimed the potential of dexamethasone-β-D-glucoside for the colon specific delivery of dexamethasone. The effect of a prodrug-based colonic delivery system was investigated on carrageenan-induced inflammatory bowel disease in guinea-pigs. It was found earlier by the same scientists that glucosidase is responsible for a liberation of drug. In humans, the bacterial β-glucosidase activity in the small intestine is well over 100-fold less compared with guinea pigs, while activity in the colon is comparable.

The experimental animal model is useful in the initial screening of new pharmacotherapeutic treatments (Mrsny 1992). These laboratory animals can differ considerably from the human with respect to transit times, pH of the GI tract, enzymology, and the location, concentration and metabolic activity of gut microflora. Because many colon-specific delivery systems are evaluated in animal model, physiologic and anatomic differences between the animal and human have to be considered. Macfarlane et al., (1992) provided evidence for regional variations in bacterial populations and their activities in the human colon by measuring the bacteria and their fermentation products in large bowel contents obtained soon after death in two people. They demonstrated that substrate availability and the structure of the large intestine strongly influence the activity of the microflora. Measurements of bacterial metabolites in colon contents showed that short-chain fatty acids and lactate concentration is highest in the caecum and ascending colon, while products of protein fermentation progressively increased from the right to the left colon, as did the pH of gut contents. These differences in pH and bacterial colonies prompted the use of a multi-vessel system to study the activities of gut microflora. Allison et al., (1989) showed that complex populations due to heterogeneity of physical conditions and nutrient availabilities of bacteria characteristic of those occurring in the large gut could be maintained when grown in a multistage continuous culture system.

The amylose (used in this study as drug carrier to the colon) is already proven to be non-toxic with wide use in food industry. Baring in mind this and that human
colon is a complex ecosystem, it has been decided to assess amylose coated pellets in a multistage continuous culture fermentation system (in vitro fermentation test) rather than in animal model. The system used has been explained by Macfarlane and Englyst (1986) and comprises a 5% faecal slurry prepared from fresh faeces obtained from healthy people homogenized in 0.1 mol/l phosphate buffer. Anaerobic conditions were maintained by sparing cultures with high purity nitrogen. A mixture of bacteria have been used to assess the degree of amylose fermentation. Anaerobic amylolytic bacteria have been identified previously (Macfarlane and Englyst, 1986) on the basis of Gram reaction. Gram negative rods which produced mainly acetate were identified as Bacteroides and those which formed butyrate as major VFA fermentation products were classified as Fusobacterium and Butyrivibrio. Gram positive bacteria which produce lactate and acetate were identified as Bifidobacterium.

**In vivo** methodologies (volunteers studies)

Several methods have been proposed to study drug delivery to the colon in vivo. Some of the early methods such as glass beads, knots of cotton, grape seeds (Hoelzel, 1930) of studying gastro-intestinal transit behaviour of dosage forms now appear crude, sophisticated imaging equipment was not available at that time. The discovery of X-rays at the turn of the century led to the first imaging technique for external monitoring of a dosage form in the GIT. X-rays were first used by Lozinski and Diver (1953) for studying the in vivo disintegration of enteric coated pellets. Due to the radiation hazard, the number of images that can be made is limited.

More recently, visualization of tablets in the gastrointestinal tract using sonography was reported by Maublant *et al.*, (1988), but the tablets could not be detected once they had entered the intestine.

Application of radionuclides in gastrointestinal studies was first used by Hansky and Connell (1962). Total transit times were measured by recording the activity of Chromium-51 labelled compounds excreted in the faeces after oral administration. Improvements in measuring the radioactivity was made when Harvey *et al.*, (1970) reported using a gamma camera in gastric emptying studies with the radionuclides Chromium-51. The gamma-camera enabled the whole region of interest
to be viewed continuously and produced frequent images of the distribution of radioactivity. This development together with the discovery of the most ideal radionuclides $^{99m}$Tc, $^{111}$In and $^{113m}$In have contributed to the subsequent growth in the use of this method (Davis, 1983). The subject is exposed to low radiation doses but the technique provides information on a continuous and quantitative basis. Unfortunately, such a technique does not provide direct anatomical detail. Neutron activation techniques can be used to overcome some of the problems encountered in labelling of the dosage forms (Christensen et al., 1984).

Bond and Levitt (1975) described a method of monitoring small bowel transit time by measuring the pulmonary hydrogen excretion following ingestion of a meal containing the non-absorbable disaccharide lactulose. Lactulose was utilized as the test substance because this disaccharide is not hydrolysed by the enzymes in the small intestine and therefore, small bowel absorption is negligible. However, this compound is fermented by colonic bacteria to yield hydrogen gas which can then be detected in breath. Therefore, a rise in hydrogen concentration in the breath signals the arrival of the meal at the caecum. Hydrogen concentrations were determined using gas chromatography. Since it was shown that there is a relation between malabsorption and hydrogen production, the hydrogen breath test has become very popular. This method has been used by several scientists to provide with a range of valuable information. Metz at al., (1976) studied carbohydrate malabsorption, Gilat et al., (1978) evaluated the effect of alterations in the colonic flora on the hydrogen appearing in the breath and Corbett et al., (1981) used hydrogen breath test to measure small bowel transit time of the unabsorbable carbohydrate lactulose in normal subjects and patients with irritable bowel syndrome. Technical improvement in breath hydrogen monitors has allowed accurate and sensitive measurements of the small bowel transit time of a meal containing unabsorbable carbohydrate (Read et al., 1985). However, this method did not reliably indicate the time when all of the meal had entered the colon. Furthermore, a lack of hydrogen producing intestinal flora or low colonic pH (Perman et al., 1981) may give rise to false results. High hydrogen levels make the interpretation difficult and extra intestinal influences such as smoking, may disturb test results in some patients.
More recently, a $^{13}$CO$_2$ breath test was used to study the effect of physicochemical characteristics of starch digestion (Hiele et al., 1990). They used this method to determine the effect of amylose/amyllopectin ratio and the degree of gelatinisation of starch on its rate of hydrolysis after intake of different corn starch preparations by healthy subjects.

In the present study the two more reliable techniques have been employed to study delivery of the model drug to the human colon; gamma scintigraphy and $^{13}$CO$_2$ breath test. Gamma scintigraphy is a crude technique, uses a radiomarker to follow transit through the GIT and $^{13}$CO$_2$ breath test is a very sensitive, easy to perform and non-invasive technique which uses a stable isotope ($^{13}$C). These methods are discussed in Chapter 5.

1.7 RATIONALE AND SCOPE OF THE PRESENT STUDY

1.7.1 'Resistant' starches - general review and design rationale for use of amylose

It has been considered for a long time that the non-starch polysaccharides (dietary fibre and lignin) provided the major source of fermentable carbohydrate for saccharolytic bacteria present in the colon. Dietary fibre is defined as the remnants of the plant cell walls (e.g. pectin, cellulose, hemicellulose) that are not digested in the small intestine. The delimitation between starch and non-starch is usually made on the basis of enzymatic degradation of the starch (Cummings and Englyst, 1987). However, recent work has shown that starch from potatoes, maize and rice can escape hydrolysis in the small intestine and consequently this starch is available for fermentation in the large gut. Such work as well as evaluation of the physiological effects of resistant starch have been the field of interest of several research scientists and its significance and possible application in pharmaceuticals will be discussed here.

Anderson et al., (1981) measured breath hydrogen in healthy subjects to assess the absorption of the carbohydrate in white, all-purpose wheat flour (bread and
macaroni). These studies demonstrate incomplete small intestine hydrolysis and absorption of wheat flour, in contrast to sucrose, low-gluten wheat flour and rice flour, which were almost completely hydrolysed and absorbed. They suggested that incomplete absorption was due to the interaction between the starch and protein (gluten) moieties of wheat flour.

Other general cause of resistance to pancreatic enzymes include the crystallinity investigated by Englyst et al., (1986) and Ring et al., (1987). Englyst et al., (1986) used a term resistant starch (RS) to designate a starch fraction that resisted pancreatic amylase degradation in vitro and undergoes degradation after incubation with (i) cell-free supernatant from faecal suspensions and (ii) washed faecal bacterial cell suspensions. Resistant starch is produced when foods are thermally processed and then cooled when dispersed starch undergoes reassociation called retrogradation. The RS (mainly retrograded amylose) resists redispersion in boiling water as well as hydrolysis with pancreatic amylase as a result of strong intermolecular hydrogen bonds. The amount of RS produced during the processing of starch foods is controlled by water content, pH, heating temperature and time, cooling temperature and time.

The retrogradation of amylose was identified as the main component of resistant starch by Ring et al., (1988). They measured the resistance to hydrolysis in vitro of raw and gelatinised starch from peas, maize, wheat and potatoes, and found that raw starch proved very resistant to amylolysis. In contrast, gelatinised starch hydrolyses quickly. Pea and potato starch were very resistant to hydrolyses while the cereal starches and wheat maize were slightly less so. One postulation given for resistance is particle size; potato starch granules are large (140μm) when compared to pea, wheat and maize starch granules (20-30μm). If the major site of enzyme action is at the granule surface, potato starch will be more slowly hydrolysed. They also investigated the onset of resistance to hydrolyses of dispersions of amylose and pancreatin. Concentrated amylose solutions rapidly gel on cooling to room temperature; the gel arises as a result of a phase separation which produces a polymer-rich network (Miles et al., 1985). The amylose gel was found (Ring et al., 1988) to be resistant to hydrolysis while the amylopectin gel was almost completely degraded by the enzyme.
Ring et al., (1988) proposed also crystallinity as a factor responsible for resistance. Percentage of crystallinity in the starch granules ranges from 30 to 50%. In general, starch granules showing X-ray diffraction patterns of the B or C type tend to be more resistant to digestion by pancreatic amylase. This resistance to hydrolysis with pancreatic amylase effects the digestibility of starchy foods normally eaten raw such as banana, and of processed foods, such as biscuits, where the starch has been incompletely gelatinised. The unhydrolysed amylose and in some cases amylopectin passes into the large intestine and is available for microbial fermentation.

In the present study amylose has been chosen as a candidate for the investigation as it has a number of attributes which make it applicable in pharmaceutical technology and they are listed below:

- resistance to pancreatic amylase present in the small intestine (discussed above)
- degraded and fermented by microbial enzymes present in the colon (Macfarlane and Englyst, 1986)
- safe for clinical application (widely used in food industry)
- natural gel forming material
- possesses good film forming properties (application in coating process)

1.7.2 Scope of the present study

As discussed in Section 1.1.2, a multiunit dosage form appears to offer a number of advantages over the single unit system in drug therapy with sustained release formulations. 5-aminosalicylic acid has been chosen, as a model drug for the development of the coat formulation because it is a drug intended for IBD. Amylose in the form of amylose - butan-1-ol complex has been exploited as a coating candidate. In vitro and in vivo studies have been performed to deduce if amylose is useful as a coating candidate for site specific drug delivery to the colon.

The study was conducted in various stages and the flow diagram of the present study is displayed in Figure 1.3.
Figure 1.3  Flow diagram showing the scope of the present study
CHAPTER 2

Preparation of 5-aminosalicylic Acid Pellets by Extrusion and Spheronisation
2.1 INTRODUCTION

Pellets for pharmaceutical uses are of interest for both conventional dosage forms and controlled drug delivery. An extrusion and spheronisation method was used to prepare spherical pellets, defined as multiparticulate oral dosage form. The process has been described by Conine and Hadley (1970) and Reynolds (1970).

The basic process consists of the following stages; mixing the active ingredient and excipient, incorporation of the fluid and binders, extrusion of the wet powder mass, spheronisation of the extrudate by breaking the cylindrical extrudate into shorter lengths and forming the spheres, and finally drying.

Various types of extruders are available and all have the common feature of forcing the extrudate through the restriction of the die. The force required and the characteristics of the extrudate are dependent on the rheological properties of the extrudate, the design of the die and the rate at which the material is forced through the die. The mass must possess inherent fluidity, allowing flow during the process and self-lubricating properties as it passes through the die. The resultant extrudate must remain non-adhesive to itself and retain a degree of rigidity so that the shape imposed by the die is retained. Precise formulation requirements depend upon subsequent processing.

Application of the theory, the basis of the process, depends on the ability to measure the extrusion force and rate. Most commercial extruders do not allow for these types of measurements as they work in bulk and continuously. The ram extruder used in this study is suitably designed for this type of experimental measurement. Harison et al., (1985) reported the use of a ram extruder in evaluation of the rheological characteristics of powder masses.

The second part of the process involves spheronisation. Rowe (1985) has reviewed the development of spheroniser design. The spheroniser consists of a grooved horizontal plate rotating at variable high speed. The design of spheronisers allows the use of plates with different configuration of grooves such as radial
geometry and cross-hatch pattern. In this process, the extrudate which should have sufficient plasticity is broken down by the motion of the plate into short cylindrical lengths which are transported centrifugally to the periphery of the plate. The extrudate then undergoes a series of shape changes from cylinders with rounded ends to dumbbells and ellipsoids, and finally spheres. Chapman (1985) has investigated the effect of different spheronisation variables and materials on resultant pellets. A poor formulation gives rise to pellets either size reduced if too dry or agglomerated if too wet or containing water with high mobility (Fielden, 1987). It is possible to obtain uniformly dense spheroids with a narrow size distribution when the extrudate is cylindrical and smooth. The optimum spheronisation speed, load of the extrudate, geometry and the diameter of the grooves of the plates are then carefully determined.

The aim of this part of the study was to manufacture pellets of suitable physical appearance with high 5-aminosalicylic acid loading. Formulations containing 50%, 60% and 70% of 5-aminosalicylic acid were prepared using extrusion and spheronisation processes. The following chapter describes their manufacture and performance of the pellets during in vitro dissolution tests.
2.2 MATERIALS

5-aminosalicylic acid

5-aminosalicylic acid was obtained from Sigma (94F3410) and from Nobel Chemicals Sweden (N° 27095/90). Both were certified 96-98% purity. The particle size distribution of the drug was determined using a Particle Sizer (Malvern Instruments Master M 4.4) and was found to be similar for 5-aminosalicylic acid powder obtained from both suppliers (50% smaller than 12.8μm). A crude comparison of particle shapes was carried out using a Scanning electron microscope (Phillips XL20, Eindhoven, Holland). 'Sigma' 5-aminosalicylic acid particles were more needle-like and that from 'Nobel' were irregular rod like, shaped.

Avicel® PH101, BH0747, FMC Corporation, USA

Microcrystalline cellulose (Avicel) is derived from native cellulose by the acid hydrolysis of wood pulp. It acts as a binding agent and has the ability to absorb large quantities of water and be readily deformable.

Bentonite, N° 26022, BDH Ltd., Poole, Dorset, UK

Bentonite is hydrated colloidal aluminium silicate clay. It exhibits properties which are highly desirable and is used as disintegrant, binder, filler or as a viscosity imparting agent. It has a high swelling capacity in water, forms gels at low concentrations and is chemically inert and stable over a wide range of temperature. Bentonite is supplied as a smooth, white to off-white powder.

2.3 METHODS

2.3.1 Method of preparation

The formulae used in the preparation of the pellets are shown in Table 2.1.

Avicel® PH101 powder was mixed for five minutes with 5-aminosalicylic acid and bentonite in a planetary mixer (Kenwood®, A707A, Havant Hants, UK). The total
amount of bentonite added in all batches was kept constant and represents 5% of the dry weight mixture. Deionised water was added to the mixing powder and mixing continued for 10 min to achieve a suitable consistency for extrusion.

**Table 2.1** Composition of uncoated 5-aminosalicylic acid pellets

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>% w/w* (ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-aminosalicylic acid</td>
<td>50 (5.0)</td>
</tr>
<tr>
<td></td>
<td>60 (6.0)</td>
</tr>
<tr>
<td></td>
<td>70 (7.0)</td>
</tr>
<tr>
<td>Avicel® PH101</td>
<td>45 (4.5)</td>
</tr>
<tr>
<td></td>
<td>35 (3.5)</td>
</tr>
<tr>
<td></td>
<td>25 (2.5)</td>
</tr>
<tr>
<td>Bentonite</td>
<td>5 (0.5)</td>
</tr>
<tr>
<td></td>
<td>5 (0.5)</td>
</tr>
<tr>
<td></td>
<td>5 (0.5)</td>
</tr>
<tr>
<td>Water</td>
<td>(4.9)</td>
</tr>
<tr>
<td></td>
<td>(4.5)</td>
</tr>
<tr>
<td></td>
<td>(3.9)</td>
</tr>
</tbody>
</table>

* Calculations are based on the total weight of dried pellets

The mixture was passed through the extruder driven by Instruments MX50 Lloyds (UK). Diagram of the ram extruder is shown in Figure 2.1.

The ram extruder, designed by Ovenston and Benbow (1968), consists of a stainless steel barrel (2.54 cm internal diameter, approximately 20 cm in length), which acts as the material reservoir. The extrusion is a noncontinuous operation; first the material (50-100 g) is packed into the barrel and partially consolidated to a plug by inserting the piston. The barrel-and-die assembly is mounted onto a rigid base and a load is applied to the piston sufficient to extrude the material through the die. The ram extruder rests on a load cell and the piston is attached to the cross-head. The cross-head was driven down at various constant rates with its displacement monitored by an attached displacement transducer. Output from the transducer and the load cell is fed into a computer enabling the force acting on the material during extrusion to be recorded as a function of the displacement of the piston. A force-displacement profile is then produced.

The extrudate obtained was then processed in the spheroniser (GB Caleva,
Ascot, Berks) using the 21.25cm plate. Details of the extrusion and spheronisation process are presented in the Table 2.2.

The pellets obtained were then dried in a fluidized bed dryer (P. R. L. Engineering Ltd., Flintshire, UK) for 30 min at 60°C. After drying the pellets were sieved through a nested set of sieves, arranged in a \( \sqrt{2} \) progression, for 10 min, using a mechanical sieve shaker (Endecott, London). The results are shown in Table 2.3. The sieve fraction 1.40-1.70 mm was used in further studies.

![Diagram of the ram extruder](image)

**Figure 2.1** Diagram of the ram extruder
Table 2.2  Process conditions for the manufacture of 5-aminosalicylic acid pellets

<table>
<thead>
<tr>
<th>5-aminosalicylic acid content (% w/w)</th>
<th>50</th>
<th>60</th>
<th>70</th>
</tr>
</thead>
</table>

**Extrusion process**

<p>| | | | |</p>
<table>
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<tr>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>extrusion speed (mm/min)</td>
<td>200</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>die diameter x length (mm)</td>
<td>1x4</td>
<td>1x4</td>
<td>1x4</td>
</tr>
<tr>
<td>steady state extrusion force (kN)</td>
<td>7.94</td>
<td>10.5</td>
<td>13.2</td>
</tr>
</tbody>
</table>

**Spheronisation process**

<p>| | | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>rotation plate speed (rpm)</td>
<td>1000</td>
<td>1000</td>
<td>1000</td>
</tr>
<tr>
<td>spheronising time (min)</td>
<td>25</td>
<td>30</td>
<td>35</td>
</tr>
</tbody>
</table>

Table 2.3  Size distribution of 5-aminosalicylic acid pellets

<table>
<thead>
<tr>
<th>5-aminosalicylic acid content (% w/w)</th>
<th>50</th>
<th>60</th>
<th>70</th>
</tr>
</thead>
</table>

<table>
<thead>
<tr>
<th>Size range (mm)</th>
<th>percent retained on sieve ± s. d.</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt; 1.70</td>
<td>3.3 ± 0.5</td>
</tr>
<tr>
<td>1.40 - 1.70</td>
<td>87.1 ± 7.0</td>
</tr>
<tr>
<td>1.00 - 1.40</td>
<td>9.0 ± 5.8</td>
</tr>
<tr>
<td>&lt; 1.00</td>
<td>0.6 ± 0.5</td>
</tr>
</tbody>
</table>

s.d. - standard deviation

* s.d. values have not been calculated; they are not valid due to the irregularity in pellet shape
2.3.2 *In vitro* dissolution studies

The *in vitro* release profiles of the three 5-aminosalicylic acid formulations were assessed. This study was carried out following the regulations for the assessment of a solid dosage form described in USP XXI Pharmacopoeia. The dissolution test apparatus used in this study was model PTWS, Pharma Test (Apparatebau, W. Germany). All the tests were conducted in 900ml of dissolution media maintained at 37°± 0.5°C with a paddle rotation speed of 100rpm. The dissolution media used in this study were deionised water, 0.1N hydrochloric acid and 0.2 M phosphate buffer of pH 7.2. 300mg of uncoated pellets were used in each case to ensure sink conditions. Three millilitre samples were withdrawn at pre-selected time intervals for a total of 6h by means of an automated sampler (Pharma Test, Apparatebau Type PTFC 1, W. Germany). The samples were diluted 1/10 and the absorbance measured using a UV-Vis spectrophotometer (Perkin-Elmer 554) at 296nm for water, 300nm for hydrochloride acid and 328nm for phosphate buffer. The percentage released over 6h was calculated using curves for 5-aminosalicylic acid in the different dissolution media according to Beer’s law.

The solubility of 5-aminosalicylic acid in water at 37°C was found to be 0.85mg/ml, in 0.1N hydrochloric acid (pH 1.2) 8.4mg/ml and in 0.2M phosphate buffer (pH 7.2) 3.4mg/ml.
2.4 RESULTS AND DISCUSSION

2.4.1 Extrusion and Spheronisation

In the present study, three basic formulas were developed as shown in Table 2.1. Microcrystalline cellulose (Avicel® PH101) was used in all the formulations because it has long been considered as an essential ingredient in the formulation of pellets. It exhibits rigidity and plasticity required for extrusion and spheronisation (Miyake et al., 1973, Avicel Handbook, 1985).

The basic starting point of the formulation was one in which the drug was used to replace lactose in the following formulation; microcrystalline cellulose 5 parts, lactose 5 parts and water 6 parts. The optimal processing conditions for a given mixture were described by Harrison et al. (1985) and Chapman (1985). These conditions were used as a starting point in the development of formulation containing 50% of 5-aminosalicylic acid supplied by Sigma at that stage of the study. Pellets with a narrow size distribution were obtained when the above given formulae was used. The next step was to develop a formulation with a higher drug load. Drug supplied by Nobel Chemicals behaved differently to that supplied by Sigma. The same formulae was used to manufacture pellets with Nobel 5-aminosalicylic acid but during the spheronisation process the pellets agglomerated. To improve the quality of the pellets, the proportion of water was altered, but a mixture of spheres, ellipsoids and dumb-bells was obtained, indicating that the rheological properties (such as plasticity) of the mixture had to be improved. Thus the extrusion properties of the mixture containing 5-aminosalicylic acid and microcrystalline cellulose had to be altered.

The approach taken to solve this problem involved changing the composition by adding a binding agent. Incorporation of 5% w/w bentonite in all formulations resulted in considerable improvement in the quality of the extrudate and in the pellets produced. Bentonite possibly suppresses the electrostatic forces of 5-aminosalicylic acid and modifies the rheological properties of the mixture, conferring a degree of plasticity which allows it to be readily extruded. Bentonite may act as a capillary binder (Carstensen and Su., 1971), where the inherent capillary attraction of the liquid
phase by the binder imparts strong cohesive forces. The interaction between the mixed powder and the water is a physical rather than a chemical reaction.

Little has been published on the extrusion of clay-like materials. The rheological factors governing transport of clay-like pastes along the extruder screw and flow through dies of various shapes have been investigated by Ovenston and Benbow (1968). However, an analysis of these results was made in terms of mathematical models.

The force-displacement profiles for the formulation containing different quantities of 5-aminosalicylic acid were compared for constant experimental conditions of die length (1×4 mm) and extrusion speed (200 mm/min). Three distinct regions are clearly visible from the profiles as shown in Figure 2.2, corresponding to a compression, steady state flow and forced flow stage as demonstrated by Harrison (1985). In the compression stage (A-B), the piston, descending into the barrel, consolidates the material into a plug prior to flow. The material is compressed to its maximum density and minimum volume. A large increase in load accompanied by a minimal change in displacement is shown in the profile. With an increasing amount of 5-aminosalicylic acid, a change in load was observed. The extent of consolidation depends on the moisture content of the mixture (Fielden, 1987). At the end of compression stage the pressure applied to the wet mass increases until it is high enough for the material to yield and commence flow. This is followed by a steady-state flow stage (B-C) in which the force required to maintain the extrusion remains relatively constant as the displacement increases. The duration of steady state flow for the three formulations, containing different percentages of 5-aminosalicylic acid and microcrystalline cellulose but a constant content of bentonite, was prolonged with increasing the velocity of throughput (from 100 mm/min to 200 mm/min). Bentonite acts as a thixotropic agent causing the viscosity to decrease with time during shear. Microcrystalline cellulose alone did not prove satisfactory for spheronisation when mixed with the drug. The analysis of the force-displacement profile indicates a jagged short steady state stage. However, when the mixture containing bentonite flowed through the orifice, the slippage at the die wall was significantly reduced and prolonged state flow stage was observed. As the content of drug was increased, the
**Figure 2.2** Force-displacement profiles of 50%, 60% and 70% 5-aminosalicylic acid formulae. Keys: A - B compression stage; B - C steady state flow stage; C - forced flow stage
extrusion of the mixture became more difficult to control and was critically dependent on the moisture content. The optimum water content was determined by systematically altering the proportion of water incorporated into the wet mass until a reproducible formulation was produced (Table 2.1). Forced flow (C-) occurs when steady-state flow could no longer be maintained leading to a gradual rise in extrusion force with displacement.

These preliminary investigations allowed a few points relevant to the extrusion/spheronisation process to be made. Examination of the force-displacement profile for the formulation containing 70% 5-aminosalicylic acid reveals poor flow characteristics of wet mass when compared with the 50% and 60% 5-aminosalicylic acid formulations. Insufficient moisture resulted in early onset of forced flow during extrusion of 70% formulation. Under these conditions, the extrudate produced was insufficiently plastic and could not be satisfactorily spheronised into spheres. A mixture of ellipsoids and spheres was produced. Satisfactory spherical products could not be obtained with a percentage of 5-aminosalicylic acid greater than 60%, as the further manipulation of the water content was of no avail. The increase in drug content possibly reduces the deformability of the microcrystalline cellulose and bentonite in the given formulation. This indicates that the amount of drug incorporated in the formulation is critical. Assessing the process variables (Table 2.2) it is noticed that at high drug content spheronisation had to be continued for up to 35 min. Spheronisation plate speed was kept constant at 1000 rpm as found optimal by Chapman (1985).

The size distribution of the pellets is shown in Table 2.3. The extrudate containing 50% and 60% drug could be spheronised in a controlled manner, producing round pellets of a narrow size distribution. The extrudate containing 70% drug spheronised uncontrollably. It was noticed that when increasing the drug content above 50% there was an increase in the size distribution of the pellets. The formation of pellets of a different size can be attributed to the low water solubility of the drug, the proportion of powder components and their water adsorption capacities. It was noticed (Table 2.3) that increase in 5-aminosalicylic acid amount resulted in increase of the fraction of larger size pellets (ie. from 1.40-1.70 mm to over 1.70 mm). At the
high drug concentration (70%), the bentonite concentration of 5% w/w is not sufficient to give an extrudate of desired plasticity. The resultant pellets were irregular and had a wide particle size distribution. The observation of a jagged forced-displacement profile (Figure 2.2) is related to these problems. Such a mixture exhibits poor flow characteristics which is thought to be due to a greater tendency of material to slip at the die wall. The 50% drug formulation flows through orifice with no slip at the die wall and shows a relatively smooth forced displacement profile (Figure 2.2).

The physico-chemical properties of the drug (i.e., solubility, particle shape and surface texture of particles) determines to a large extent the maximum quantity that can be incorporated in a particular formulation. Aqueous solubility of 5-aminosalicylic acid is very low and water distribution in the mass containing 70% of 5-aminosalicylic acid cannot be achieved homogeneously. The three formulations (50%, 60% and 70% of drug) were characterized in vitro despite less than 50% of the 70% formulation pellets being in the size range 1.40-1.70mm. Standard deviation values have not been calculated for the 70% drug formulation since they are not valid due to the irregularity in pellet shape.

2.4.2 In vitro dissolution studies

The release of 5-aminosalicylic acid in different dissolution media from uncoated pellets containing varying quantities of drug was investigated. The results are shown in Figure 2.3 and 2.4.

A number of factors contribute to the properties of the pellets and to the release of drug from them. It has been shown that particular attention must be paid to binder characteristics when the drug in the core is poorly water soluble (Laakso and Eerikäinen, 1991), as in this case is 5-aminosalicylic acid. The reason for incorporation of bentonite in the core has been discussed previously in Section 2.3.1. The literature contains numerous examples of clay performing different functions in tablets. Various grades of clay were studied for use as disintegrants, binders and lubricants (Wai et al., 1966). Another study found that the procedure used to
incorporate clay as the disintegrating agent affects the disintegration time of tablets (Nair and Bhatia, 1957).

Figure 2.3 shows the profiles of 5-aminosalicylic acid release from uncoated pellets of varying drug content. A comparison of the dissolution profiles reveals an increase in the percentage of drug released with increase in drug loading. 5-aminosalicylic acid binds weakly and was readily released from drug - excipient system in the 60% and 70% formulations. Release rates of drug from 60% and 70% formulation pellets were similar, however, only 60% of the drug was released after 6h when the concentration of drug in the core pellet was 50% w/w. All three formulation pellets did not remain intact, but swelled and disintegrated during the dissolution test. In all formulations bentonite content was kept the same (5% w/w). Gros and Becker, (1952) found that neither bentonite nor magnesium aluminium silicate in concentrations up to 17% produced any disintegrating affect in tablets. The swelling of clay depends upon two main mechanisms, absorption and osmosis (Wai and Banker 1966). The disintegrating activity of the clay - microcrystalline cellulose system in pellets may be hypothesized as primarily due to absorption swelling rather than osmotic swelling of the clay. Bentonite can absorb water both via a capillary system between the particles and into the particles (Carstensen and Su, 1971). Microcrystalline cellulose can only absorb water into a capillary system (Nogami et al., 1969). This explains the disintegration phenomenon as pellets with a higher drug to excipient ratio will yield a formulation with a lower retardation of the drug and dissolution is hence faster.

The formulation containing 60% 5-aminosalicylic acid had an adequate (the highest) rate of release in water and was further characterized in vitro using different pH media. Results are shown in Figure 2.4. The percentage drug released in hydrochloric acid (pH 1.2) and phosphate buffer (pH 7.2) are similar. In aqueous solution 5-aminosalicylic acid has amphoionic properties. The pKa values are the following; -COOH group 3.0, -NH₃⁺ group 6.0, -OH group 13.9 (Allgayer et al., 1985). Therefore, the release of drug from the pellets should, in principle, take place both at acid and alkaline pH. However, the percentage of drug released in water was lower. The difference in release patterns could also be explained on the basis of lower
Figure 2.3  *In vitro* release of 5-aminosalicylic acid in distilled water from uncoated pellets containing varying quantities of 5-aminosalicylic acid (5-ASA).

Keys: ■ 50% 5-ASA; ▲ 60% 5-ASA; ● 70% 5-ASA
Figure 2.4 *In vitro* release of 5-aminosalicylic acid from uncoated 60% 5-aminosalicylic acid pellets as a function of pH.

Keys: ■ pH 7.2; ▲ pH 1.2; ▼ distilled water
drug solubility in water than at pH 1.2 and pH 7.2 (refer to the solubility values given in Section 2.3.2). The dissolution profiles, particularly the high initial concentration of the drug in the acid (pH 1.2) media, are indicative of drug-excipient interaction involving physical adsorption with weak van der Waals bonds. McGinity and Lach (1977) have investigated products obtained by interaction of montmorillonite clays (e.g. bentonite) with cationic drugs or amine salts and certain nonionic drugs and found that both ion-exchange and strong chemisorption contribute to the interaction.
2.5 CONCLUSION

The results obtained demonstrate that pellets prepared with specific quantities of 5-aminosalicylic acid can be processed via extruder/spheroniser technology. Incorporation of bentonite improves the rheological properties of a given mixture, conferring a degree of plasticity which allows it to be readily extruded. Three formulations (50%, 60% and 70% 5-aminosalicylic acid) were assessed in vitro. Research was continued with the pellets containing 60% 5-aminosalicylic acid as they showed rapid rate of dissolution and are the highest drug loaded pellets which possess the appropriate spherical shape for coating.
CHAPTER 3

Development of Film Coat and Preparation of Formulation with Gastric and Small Intestine Resistance
3.1 INTRODUCTION

The present study relates to the development of a delayed release formulations with gastric and small intestine resistant properties, in which the delayed release characteristic is due to the coating. 5-aminosalicylic acid was used as a model drug. The rationale behind the use of pellets as a basis for this study has been described previously (Chapter 1). Being spherical, the pellets possess the ideal shape for application of the coat in view of their low surface area to volume ratio and lack of edges.

The task of this study is to provide a coated medicament for drug targeting to the human large intestine with the following properties:

1) be resistant to gastric acid (pH 1.2) and enzyme (pepsin) action at a temperature of 37°C
2) be resistant to neutral pH and enzymatic activities (pancreatic α-amylase) and bile salts of the small intestine (pH 7.2)
3) undergo enzymatic degradation in the presence of amylose - cleaving enzymes provided by the microbial flora normally present in the large intestine

The design rationale for use of amylose as a coating was discussed in Chapter 1. Some physical forms of the starch polysaccharide are resistant to digestion by pancreatic amylase, but are digested by enzymes of colonic microflora origin (Englyst and Macfarlane, 1986; Ring et al., 1988; Szczodrak and Pomeranz, 1991; Asp and Björck, 1992). It has been suggested that especially advantageous results in drug targeting to the colon may be obtained using a particular form of coating, comprising glassy amylose. The amylose is a natural polysaccharide, in this case extracted from pea starch, and possesses the ability to form a film through gelation and its characteristic gel microstructure has potential to be resistant to pancreatic enzymes (Ring et al., 1987). However, there was a need to enhance the release characteristics of the amylose coating composition as its swells in water and allows drug release. Commercially available polymers that have had a long history of safe use in the food
industry with a wide regulatory acceptance were considered. The following polymers are among those commonly used for the purposes of controlled release coatings: cellulose polymers and acrylate copolymers. The degradation of cellulose polymers in vivo is in general not pH dependent (Porter, 1989a; Kohri et al., 1992). There is now an extensive range of different forms of acrylate (Eudragit®) available, which are classified into two groups: (a) methacrylic acid co-polymers, which are used for enteric coating because they contain carboxylic groups that are transformed to carboxylate above pH 5.5. Their dissolution pH depends primarily on their content of carboxylate groups. (b) methacrylate ester co-polymers, the products in this group are neutral or weakly cationic polymers and are insoluble in pure water, dilute acids, buffer solutions or digestive fluids over the entire physiological pH range. The designation of type was originally technologically oriented e.g. 'E' for final coating, 'R' for retard dosage forms and 'L/S' for gastric juice resistant preparations. The chemical structure and the properties of permeability and solubility in pH range were therefore used as criteria for rational application (Lehmann and Dreher, 1981).

After the early development of film coating in the 1950's the polymers used for the film coating were invariably dissolved in an organic solvent. Modern techniques have now tended to rely on water as a solvent because of the following disadvantages which are associated with the use of organic solvents: (a) environmental - the venting of untreated organic solvent vapour into the atmosphere is ecologically unacceptable whilst efficient solvent vapour removal from gaseous effluent is expensive, (b) safety - organic solvents provide explosion, fire and toxic hazard to plant operators, (c) financial - the use of organic solvents necessitates the building of flame- and explosion-proof facilities and ingredient cost is also comparatively high and the associated cost of storage and quality control must also be taken into consideration (Hogan, 1983). If one eliminates organic mixtures, what is left? Hot melts presently have application in the food field (Kester and Fennema, 1986), and they are still in their infancy in the pharmaceutical field. The role of solvent in the film formation process and the importance of choosing the right solvent has been reported by Spitael and Kinget (1980). However, there are several drawbacks associated with the use of water as a solvent. Due to its high latent heat of vaporization, energy costs and drying times are increased and water penetration into
the tablet core may degrade the drug and make it susceptible to hydrolysis (Pondell, 1984).

Aqueous colloidal dispersions are referred to as latices and pseudolatices. Physically, pseudolatices are indistinguishable from true polymer emulsions or latices. A true latex is formed from a synthetic polymer with a liquid monomer and is prepared by emulsion polymerization (Vanderhoff and El-Asser, 1978; Chang et al., 1987). In contrast, pseudolatices may be prepared from virtually any existing water-insoluble polymer. Both are fluid even at polymer concentration of 20 - 40% w/w, and both systems form films by the same mechanism (Miller and Vadas, 1984). In order to understand how drug release is mediated and controlled over time through a film, it is helpful to understand how that film has formed - the mechanism of film formation and the effect of plasticizer inclusion in lowering the minimum film forming temperatures (MFT). In the literature, it has been widely reported that the mechanism of film formation from solution (organic solvent based) and aqueous dispersion are different (Onions, 1986; Bindschaelder et al., 1983).

Films prepared from pure polymers are frequently brittle and crack on drying. The plasticizer alters the polymer - polymer interactions to improve the flexibility of the film by relieving molecular rigidity which leads to a decrease in the glass transition temperature \( T_g \), the temperature at which a polymer undergoes a marked change in material properties (Banker, 1966). At sufficiently low temperatures all polymers are hard rigid solids. As the temperature rises, each polymer eventually obtains sufficient thermal energy to enable its chains to move freely enough for it to behave like a viscous liquid. There are two ways in which a polymer can pass from the solid to the liquid phase, depending on the internal organisation of the chains in the sample. The different types of thermal response, illustrated by following the change in specific volume, are shown schematically in Figure 3.1. A polymer may be completely amorphous in the solid state, which means that the molecules are arranged in a totally random fashion. The volume change in amorphous polymers follows the curve A-D. In the region C-D the polymer is a glass, but as the sample is heated it passes through a temperature \( T_g \), beyond which it softens and becomes rubberlike. A continuing increase in temperature along C-B-A leads to change of the
rubbery polymer to a viscous liquid. In a perfectly crystalline polymer, the molecules display three-dimensional order, called crystallites, and no glass transition would be observed. The crystalline polymer, on heating, would follow curve H-B-A and at \( T_m \), melting would be observed and the polymer would become a viscous liquid. Perfectly crystalline polymers are not encountered in practice and instead polymers may contain varying proportions of ordered and disordered regions. These semi-crystalline polymers usually exhibit both \( T_g \) and \( T_m \), and follow curve similar to F-E-G-A. As a general rule, the film increases resistance to mechanical stress when a plasticizer is added to a coating composition. The plasticizers are generally low molecular weight compounds, or even the aqueous solvent. There is an optimal concentration of plasticizer to use for any film composition and the compatibility and efficiency of selected plasticizer in a polymer system can be evaluated by thermogram analysis (Lippold et al., 1990).

**Figure 3.1** Schematic representation of the change of specific volume \( v \) of a polymer with temperature \( T \) for (i) a completely amorphous sample (A-C-D), (ii) a semi-crystalline sample (A-G-F), and (iii) a perfectly crystalline material (A-B-H). *(Taken from Cowie, 1973).*
Application of coating

The fluidized bed technique is being used increasingly in the development and production of solid dosage forms. Liquids can be applied to fluidized particles in a variety of ways, including top, bottom and tangential spraying. For a given product, each method can offer markedly different finished - product characteristics, which is revealed from scanning electron micrographs (Mehta and Jones, 1985; Mehta et al., 1986). Imperfections, seen when using top spraying, were attributed to the manner in which the liquid is applied. Because the coating solution is sprayed against the heated air stream, the evaporation of the solvent is rapid and causes a formation of rough surface. The bottom coating provides a smooth, continuous film of polymer. When tangential coating was used, the morphological characteristics of the applied film appear very similar to those of the coating applied using bottom spray method.

In the present study coating was performed using bottom fluidised bed equipment (Figure 3.2). In this method, air is introduced from the bottom of the coating chamber, flowing upwards to fluidize the pellets. The coating is then sprayed onto the pellets in a steady stream of air from an atomizer, which is located centrally at the bottom of the chamber. An upward movement of the fluidized pellets in the centre of the bed is promoted by the atomizing air. Since the top part of the coating chamber is widened, air pressure is reduced in this area, and the pellets upon reaching the top will flow back down along the side to the bottom to be resprayed. This movement of the pellets together with a proper spray rate of the coating dispersion will ensure that the pellets are uniformly coated. Historically, the application of the coatings to pharmaceutical tablets has involved skill, experience and educated guesswork. During the coating process special attention should be paid to a process variables such as temperature, volume and humidity of fluidized air, spray rate and atomization pressure (Mehta, 1989).

When dealing especially with aqueous latex coating systems, drying conditions might greatly influence the coalescence process and determine whether curing processes need to be employed (Ghebre-Sellassie et al., 1988; Porter, 1989a).
Figure 3.2  Diagram of a fluid bed coater
In this section, preliminary experiments necessary to develop a suitable film coat are described. The development of the pellet coating with gastric and small intestine resistant properties entailed a number of stages. Amylose as a film former was first examined. This was followed by evaluation of several coating candidates: (a) the water based, ethylcellulose (Ethocel®, Aquacoat®) and acrylic (Eudragit® RS/RL 30D); (b) the organic solvent based, ethylcellulose (in methanol and dichlormethane) and acrylic (Eudragit® L100 in isopropylalcohol). For this purpose, two methods were employed: making a coat from mixed dispersions (amylose with acrylic polymers or with ethylcellulose in different ratios), or combining single films to make a multilayer coat. A few multilayer compositions were examined, comprising of an inner amylose layer and an outer layer of either ethylcellulose or acrylic polymer material. The in vitro digestion of the various coating pellets under simulated gastrointestinal tract conditions (using commercially available pepsin and pancreatin powder) has been determined to demonstrate the resistance of the amylose to such conditions. Because enzymes are present in the gastrointestinal fluids, their inclusion in the dissolution medium would simulate the physiological conditions that would be encountered by an ingested dosage form (containing amylose) as it travels through the gastrointestinal tract. The 5-aminosalicylic acid pellets which had been coated with varying mixtures of amylose and Ethocel® were further evaluated by being introduced to a batch culture fermenter which contained mixed faecal bacteria. In an attempt to assess the degree of coat fermentability, gases (H$_2$ and CO$_2$) and short chain fatty acids (acetate, propionate, butyrate) were measured. In vitro release of 5-aminosalicylic acid in fermenter system from pellets coated with amylose and Ethocel mixture has been studied.

3.2 CHOICE OF MATERIALS

3.2.1 Natural polysaccharides

Humans have used products extracted from natural materials as food and medicine since the dawn of history. A variety of polysaccharides and their derivates have been tested for potential use as edible films or coatings. These include alginate,
pectin, carrageenin, starch and cellulose derivatives. The most important functional characteristic of an edible film or food coating is resistance to migration of moisture (Kester and Fennema, 1986). Many of the polysaccharides are widely used in pharmacy because they have acid groups which can aid gelation by binding to oppositely charged molecules or by phase separation (Glicksman, 1982).

In the present work, the emphasis has been on starch. Starch is encountered throughout the plant kingdom. It is laid down inside small cells, the plastids, in the form of particles that are insoluble in cold water, the starch granules. The insoluble granule contains densely packed polysaccharides and is therefore an efficient means of storing reserve energy in plants. A very high proportion of the world’s food energy intake is starch: over 80 percent of all food crops are composed of cereals and starch root-crops. The size, shape, levels of structural organization (Gidley and Cooke, 1991) and properties of starch granule are infinite in variety and being controlled by plant genes are characteristic of the botanical source. Starch consists of two polymers: amylose and amyllopectin both comprising of $\alpha$-(1→4) linked $D$ glucosyl units. Whereas amylose is essentially linear, amyllopectin is a highly branched molecule made of short chains (10-50 residues) linked together through $\beta$-(1→6) glucosidic branch points (5-6% of total linkages) as shown in Figure 3.4. At room temperature, most forms of starch are insoluble in water. Starch may be isolated from various botanical sources such as pea, maize and potato.

For the purpose of this study pea starch (Nutrio P-starr 33 supplied from Grinstead Products Ltd., UK) obtained from the extraction and purification of smooth-seeded leafless peas by an aqueous extraction procedure was used (Adkins and Greenwood, 1966). The isolation of amylose has been undertaken by Dr. R. L. Botham at the AFRC Institute of Food Research, Norwich. The flow diagram of starch extraction from pea is shown in Figure 3.3. Amylose content in P-starr 33 is 38% (Nutrio brochures). Amylose fractions are prepared by sequential aqueous leaching at $70^\circ C$ of the starch powder, with nitrogen passing through the slurry to minimize the probability of oxidative degradation. Swollen gelatinized granules (largely amyllopectin) are removed by mild centrifugation (2000G) and filtration through a glass sinter (porosity 2). Amylose is precipitated as its butan-1-ol complex by the
Figure 3.3  Flow diagram of starch extraction from whole peas
addition of butan-1-ol to the filtrate. After 24h at +1°C, butan-1-ol complex was collected by centrifugation (2000G), giving 4% w/w dispersion. The advantage of using the butan-1-ol complexes that it is relatively stable, whereas aqueous amylose is unstable and the amylose will irreversibly precipitate. It is preferred that the amylose contains no more than 5% w/w of amylopectin and also any material containing glucoside linkages of the type found in amylopectin. The usual method of demonstrating that a given sample of amylose is pure is based on the fact that the linear polymer binds 19.5 % w/w of iodine at 20°C (Banks and Greenwood, 1975), under conditions in which amylopectin does not absorb iodine to any appreciable amount.

3.2.2 Commercially available enteric coating materials

Due to the large numbers of materials used or proposed for enteric coating, it was not possible to explore all of them in this study. This study therefore, is limited to enteric materials that have been widely used (Porter, 1989a).

3.2.2.1 Ethylcellulose

Ethylcellulose is the ethyl ether of cellulose and can contain between 44.0 and 51.0 % of ethoxy (-OC₂H₅) groups (USNF XVI). The structures of ethylcellulose and that of the parent cellulose are shown in Figure 3.4, where it can be seen that each anhydroglucose unit has three reactive hydroxyl groups that can be ethoxylated. The structure as shown for ethylcellulose has all three hydroxyl groups ethoxylated and consequently is said to have a degree of substitution (D.S.) of 3.0. In the practice, D.S. may vary, depending on the end use of the polymer. As with most film coating polymers, ethylcellulose is available as various grades of different molecular weights, each grade being represented by a viscosity number as determined by measuring the viscosity of a 5% solution of the polymer in an 80:20 toluene : ethanol solvent mixture. Ethylcellulose is insoluble in water but it is soluble in a variety of organic solvents and is non-toxic, tasteless and odourless. Ethylcellulose exhibits a glass transition temperature at 120°C and therefore needs to be adequately plasticized. The two commercially available forms of aqueous ethylcellulose dispersions (Ethocel® and Aquacoat®) and ethylcellulose in a powder form have been studied.
Ethylcellulose N-100, N° BG 9708, Glaxo Group Research

The number 100 represents a viscosity of 80 - 105 cps and 'N' refers to the number of ethoxyl groups which is 48.0 - 49.5.

Aquacoat® ECD-30, N° J911, FMC, USA

The ethylcellulose pseudolatices is made by first dissolving the polymer and cetylalcohol in an organic solvent. The polymer solution is then emulsified in water with the aid of the anionic surfactant sodium lauryl sulphate. The emulsion is homogenized to reduce the particle size of the polymer droplets, and then the organic solvent is removed by steam distillation, leaving a dispersion of 30% w/w solid content. The process is called emulsion polymerization. The dispersion is low in viscosity and is fluid even at the high solid content (Aquacoat® Handbook). Aquacoat dispersion has to be plasticized before coating. Ethylcellulose is present in the dispersion as spherical particles in the size range of 0.1 - 0.3 μm. The weight average molecular mass quoted by FMC is 100,000.

Ethocel®, N° MA880708, Colorcon, UK

In Ethocel, the plasticizer is already incorporated in the dispersion during the manufacturing process. A hot melt of the polymer (ethylcellulose 20 cps), the plasticizer (dibutyl sebacate) and the stabilizer (oleic acid) is created to form a homogenous mixture. The mixture is then diluted with an alkali solution (ammonium hydroxide) to obtain a dispersion of polymer in water (20% w/w ethylcellulose, 26% w/w solid content).

3.2.2.2 Acrylate co-polymers

Acrylate co-polymers are sold as dry powdered solids, and aqueous suspensions under the trade names of Eudragit®. There are several structural variations. These polymethacrylates are copolymers of methacrylic acid, amino-ethyl methacrylates and neutral esters of acrylic and methacrylic acid (Lehmann and Dreher, 1981). They possess characteristic properties of solubility and permeability in the digestive juices of the gastrointestinal tract, depending upon the content of acidic, basic and hydrophillic groups in the polymers. From the entire range of combinations, the following have been chosen for this particular study.
Eudragit® RS 30D (N° 18-80118) and Eudragit® RL 30D (N° 80026),
Röhm Pharma, Germany

Eudragit® RS 30D and Eudragit® RL 30D are 30% aqueous dispersions of
copolymers of acrylic and methacrylic esters (Figure 3.4), with a low content of
quaternary ammonium groups. The mean molecular weight is about 150,000. They are
neutral polymers and are insoluble in the entire physiological pH-range. However, they
possess a defined swelling capacity and permeability in the presence of water. The
release of active ingredient is controlled by the permeability of the polymer by either
using one of the two types, RL (high permeability) or RS (low permeability), or by
mixing the two materials in a desired ratio. Addition of plasticizer is recommended.

Eudragit® L100, N° 0390031, Röhm Pharma, Germany

Eudragit® L100 is an anionic copolymer based on methacrylic acid and
ethylacrylate. It is a solid substance, insoluble in pure water, in buffer solutions of pH
less than 5.5 and in gastric fluid (Eudragit® Handbook).

The other materials used in this study are as follows:

Methanol, technical grade, BDH Chemicals Ltd., England
Dichlormethane, technical grade, BDH Chemicals Ltd., England
PEG 300, Koch-Light Laboratories, England
Isopropylalcohol, BDH Chemicals Ltd., England
Glycerol triacetate (triacetin), BDH Chemicals Ltd., England
Triethyl citrate, Aldrich Chemicals Co. Ltd, England
Pepsin, from porcine stomach mucosa, Sigma, England
Pancreatin, from porcine pancreas, Sigma, England
Figure 3.4  Schematic diagram of: (A) amylose (α-1,4) glucose polymer
(B) ethylcellulose, (R = C₅H₁₁)  (C) methacrylate ester co-polymers (Eudragit RS/RL),
R = CH₂-CH₂-N'(CH₃)Cl, n₁:n₂:n₃ for RS is 1:2:0.1 and for RL is 1:2:0.2
(D) methacrylic acid co-polymers (Eudragit L100), R₁=R₂=CH₃ and n₁:n₂ is 1:1
3.3 METHODS

3.3.1 Fluid bed coating

Two coating systems were employed in this study: aqueous and organic. The composition of the formulations and film thicknesses are given in Table 3.1. Aqueous formulations (including amylose alone) were heated to 70°C prior to spraying and maintained at that temperature throughout the coating process. Coating was performed using an Aeromatic AG Strea-1 fluidized bed coater (ACM Machinery, Tadley) and operated under optimized conditions listed in the Table 3.2. Sample batches of 40g were coated at each time. Preliminary studies were performed to optimize operating conditions shown in Table 3.2. A series of products with different film thicknesses were prepared by varying the amount of coating dispersion sprayed. The film thickness is expressed as the theoretical weight gain (TWG) of the polymer(s) used relative to the weight of the coated pellets.

3.3.1.1 Preparation of aqueous coating formulations

Amylose : Ethocel®

The coating formulation was prepared by the simple mixing of the two dispersions in the following ratios: 1:4, 2:6, 2:5. The resulting mixtures were then heated to 70°C and sprayed hot on to the pellets.

Amylose : Eudragit® RS / RL 30D

A 1:1 mixture of the two Eudragit (RS and RL) aqueous dispersions was stirred for at least 30 min before coating. The resulting mixture had 10% w/w level of Eudragit solids. The plasticizer polyethylene glycol 300 (PEG) was added to 10% w/w of the amount of polymers. PEG is readily soluble in water. The resulting mixture was mixed with the amylose and heated up to 70°C.

Amylose : Aquacoat® ECD-30

After intensively shaking the Aquacoat dispersion for five minutes, appropriate quantities of dispersion and plasticizer were combined and mixed for 30 min. Two
different formulations were obtained by incorporating the plasticizers triacetin or triethylcitrate. The amount of plasticizer, glycerol triacetate (triacetin), in the modified coating formulation is 20% w/w Aquacoat solids. Triethyl citrate, a partially water soluble plasticizer, was added to 24% w/w Aquacoat solids. The aquacoat plasticized formulations were mixed with amylose and heated prior to coating.

3.3.1.2 Preparation of organic solution formulations

The following organic solution formulations were prepared to be investigated in this study. Different multilayer coated pellets were obtained in the following way. Amylose was heated up and applied as a first layer followed by the application of one of the organic solvent formulations listed below.

Ethylcellulose N-100 in dichlormethane and methanol

Ethylcellulose N-100 was dissolved in a 50:50 mixture of dichlormethane and methanol to obtain a 5% w/w solution. Higher concentrations of ethylcellulose were more difficult to atomize due to higher viscosities (see section 3.4). No plasticizer was added.

Eudragit® L100 in isopropylalcohol

A solution was prepared by dissolving Eudragit L100 (12.5% of dry polymer substance) in a mixture of 82.75 g of isopropylalcohol, 1.25 g of plasticizer PEG 300 (10% w/w calculated on the amount of polymer) and 3.0 g of water under constant stirring at room temperature.

3.3.1.3 Curing of film coated pellets

The need for a curing step during the development of a dispersion based controlled release coated system is essentially well established (Aquacoat Handbook; Ghebre-Sellassie et al., 1988). A film sprayed from an aqueous ethylcellulose dispersion normally has to be cured at an elevated temperature in order to coalesce into a continuous film (Steuernagel, 1989), since the temperature of minimum film formation, MFT, is often higher than the processing temperature. Also, the structure
Table 3.1 Composition and coating thickness values for different formulations

<table>
<thead>
<tr>
<th></th>
<th>Aqueous system</th>
<th>Organic systems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>single layer</td>
<td>separate layers</td>
</tr>
<tr>
<td></td>
<td>mixture (1:4)</td>
<td>Amylose / Ethocel®</td>
</tr>
<tr>
<td>Ethocel®</td>
<td>4.8% 9.6%</td>
<td>1.2% 2.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.8% 9.6%</td>
</tr>
<tr>
<td>Amylose</td>
<td>2.0% 4.8%</td>
<td>Amylose : Eudragit® RS/RL 30D</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ PEG 300</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4.8% 9.6% 14.4%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amylose : Aquacoat® ECD-30</td>
<td>4.8% 9.6%</td>
<td>Amylose / Eudragit® L-100</td>
</tr>
<tr>
<td></td>
<td></td>
<td>+ TEC or triacetin</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>mixture (2:6 and 2:5)</td>
<td>Amylose : Ethocel®</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.8% 9.6%</td>
<td></td>
</tr>
</tbody>
</table>

*Percentage (%) refers to TWG (coating thickness)
Table 3.2  Coating conditions for different coating formulation systems

<table>
<thead>
<tr>
<th></th>
<th>Aqueous mixtures systems</th>
<th>Organic solvent systems</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td><em>Amylose : Ethocel</em></td>
<td>Amylose : Eudragit*</td>
</tr>
<tr>
<td>Batch size (g)</td>
<td>40</td>
<td>40</td>
</tr>
<tr>
<td>Inlet temperature (°C)</td>
<td>60</td>
<td>40</td>
</tr>
<tr>
<td>Outlet temperature (°C)</td>
<td>42</td>
<td>32</td>
</tr>
<tr>
<td>Atomizing air pressure (bar)</td>
<td>0.2 - 0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Spray rate (ml/min)</td>
<td>0.5 - 0.6</td>
<td>0.5</td>
</tr>
<tr>
<td>Fluidization air** (units)</td>
<td>9</td>
<td>9</td>
</tr>
<tr>
<td>Drying time (min)</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Curing time (min)</td>
<td>60</td>
<td>120</td>
</tr>
<tr>
<td>Curing temperature (°C)</td>
<td>60</td>
<td>40</td>
</tr>
</tbody>
</table>

* amylose and Ethocel films were applied under the same conditions as their mixtures

** fan capacity
of the amylose may be varied by drying, which has the effect of reducing pore size and hence permeability. Depending upon the nature of polymers used, curing of the coated pellets was performed under different conditions (Table 3.2). However, half of the amount of the pellets coated with the Amylose : Ethocel mixture in ratio 1:4 were cured for 1h and for 2h at 60°C. Another half of the coated products were stored in a screw capped container at room temperature (10 - 25°C) without curing. Drug release studies were performed on all the products shortly after preparation and repeated 2 months and 1 year later.

3.3.2 In vitro dissolution studies

Drug release studies were performed using the method described in Section 2.4, using 900ml of dissolution media. The in vitro evaluation of the coated pellets was conducted in different dissolution media as follows: distilled water; 0.1 N HCl; phosphate buffer (KH$_2$PO$_4$) pH 7.2; pH change method which involves treatment with 0.1N HCl for 3h followed by 21h treatment in phosphate buffer at pH 7.2. The in vitro release of 5-aminosalicylic acid from the most acceptable coating formulation (amylose : Ethocel in the ratios 1:4 and 2:6), has been further evaluated under the simulated gastrointestinal conditions as described in USP XXI. The studies were conducted with stirring rate of 100 rpm in 900ml fluid media at 37°C. Freshly prepared simulated gastric fluid (0.1N HCl containing 0.32% w/v pepsin) was used as the dissolution media for the first 3h and then replaced with 900ml of freshly prepared simulated intestinal fluid (0.2M phosphate buffer containing 1% w/v pancreatin) for an additional 21h in order to maintain sink conditions throughout the study (Gibaldi and Feldman, 1967). A commercial mixture of pancreatin extracted from pig pancreas, contains lipases, amylases and proteases. The human digestive juice is very similar to that of the pig in enzyme composition (Peschke, 1989). Two ml samples were withdrawn for determination of drug concentration, after predetermined time intervals. Samples were first centrifuged for 15 min at 9600G to remove turbidity due to the pancreatin and other insoluble particles. The supernatant was diluted by 1/10. Drug concentrations were determined using a UV spectrophotometer (model PTWS, Pharma Test, Apparatebau, W. Germany).
3.3.3 *In vitro* fermentation studies

*In vitro* fermentation studies were performed at the Dunn Clinical Nutrition Centre in Cambridge by Dr. Glenn Gibson.

5-aminosalicylic acid pellets, which had been coated with amylose and with amylose : Ethocel mixtures, were introduced (0.7% w/v) into a batch culture fermenter inoculated with mixed faecal bacteria (5% w/v). Fermenters were prepared by homogenizing the human faeces in anaerobic 0.1 mol/l sodium phosphate buffer pH 7.0 ([Macfarlane and Englyst, 1986](#)). Gas head space and liquid samples were then removed at varying time intervals during a 48h time course. Gases (H$_2$ and CO$_2$) and volatile fatty acids (acetate, propionate, butyrate) were measured to assess the degree of coat fermentation. Liquid samples were spun to remove bacteria and were immediately frozen for subsequent analysis of 5-aminosalicylic acid using HPLC.
3.4 RESULTS AND DISCUSSION

For the purpose of this study, the starch was extracted from smooth-seeded peas. Pea starch has a high amylose content (30-40%), with a gelatinisation temperature of about 70°C. The effect of botanical source on the gelation of starch was investigated by Orford et al., (1987). All the properties of the starch granules are related to a combination of two factors, namely: (a) its chemical composition, depending on the ratio of the two major components, amylose and amylopectin, and (b) its physical constitution, involving the organisation of these polymers to form a unique structural entity. Starch granules contain densely packed polysaccharide and include regions of long range ordering (crystallinity), thought to involve amylopectin branches. Amylose is considered to be present predominantly in the amorphous phase of starch granules (Banks and Greenwood, 1975). Therefore, starch is classified as a semicrystalline material. The crystalline fraction is formed by the packing of double helices into one or other of the well known A, B or C polymorphs as distinguished by X-ray diffraction. Starches of different botanical source give different diffraction patterns (e.g. cereals A-type, tuber and root starches B-type). The difference between A and B polymorphs comes not from the conformation of the double helices, which appears to be identical in both, but from the packing of the helices within the crystal (Imberty and Perez, 1988).

Based on the selective precipitant action of butan-1-ol towards amylose, the technique described in Section 3.2.1, has been used. The advantage of the technique was first described by Schoch (1942). Selective leaching of the amylose occurs from the starch granule by the action of hot water (i.e. heating aqueous dispersions of starches causes the granule to expand and amylose to be leached). As the temperature is raised appreciably above that of gelatinisation, amylopectin is also removed from the granule. The addition of butan-1-ol precipitates only the amylose to form an insoluble helical complex (Banks and Greenwood, 1975). The other reason for the use of butan-1-ol is that amylose in dilute, neutral, aqueous solution is a flexible coil and at room temperature the solutions are inherently unstable (Kitamura and Kuge, 1989). After a limited period of time, dependent on polymer concentration and molecular weight, the amylose irreversibly precipitates from solution. Amylose is stable if it is
in complex with butan-1-ol, where adopts a helical conformation (of approx. 200 glucose units) as a result of inter-residue bonding (e.g. hydrogen bonding). The interior of the helix is composed of hydrogen atoms attached to carbon atoms and a hydrophobic surface results (Zobel, 1988). Complex formation is associated with a large decrease in the hydrodynamic volume of the macromolecules. Banks and Greenwood (1975) studied the reaction of amylose in alkaline and acid solution with butan-1-ol by measuring the changes in the viscosity number of the polysaccharide as the complexing agent was added. The viscosity number of amylose decreases smoothly and progressively with the addition of butan-1-ol. The important conclusion to be drawn is that a conformational transition does take place on adding a complexing agent (butan-1-ol) to an aqueous solution of amylose, at either neutral or alkaline pH and furthermore, on heating the amylose - butan-1-ol complex (described below) and cooling, the amylose adopts a random coil conformation leading to gelation and network formation, which is thought to be responsible for pancreatic amylase resistance.

Prior to the coating process the amylose - butan-1-ol complex was melted (to prevent nuclei formation) by heating to 70°C. During the coating process, the temperature was kept constant and butan-1-ol was removed from the aqueous amylose - butan-1-ol complex. When heating, the amylose inclusion complex (butan-1-ol) undergoes a thermal dissociation (Whittam et al., 1989). The thermal dissociation behaviour of amylose inclusion complexes was found to be dependent on chain length of the guest molecule and whether the complex was crystalline or amorphous. They have also found that thermal dissociation of the complexes requires disruption of the various forces. For the crystalline material, energy is required both to melt the crystal and to overcome the attractive intermolecular forces between host and guest molecule. In the case of the amorphous complexes the energy requirement is lower since there is no regular lattice to melt. When an aqueous suspension of starch is heated above its gelatinisation temperature, the suspension behaves as a viscoelastic paste. On cooling, the paste is transformed into an opaque elastic gel. The gel arises as a result of a phase separation which produces a polymer-rich network (Miles et al., 1985) and crystals are formed by the processes of nucleation and crystal growth. The changes observed after cooling the viscoelastic paste are called retrogradation. The mechanical
and rheological properties of amylose gels have been studied by Ellis and Ring (1985). Biopolymer gels are usually formed by the physical association of parts of polymer chains at regions of local order or 'junction zones' to produce a continuous three dimensional polymer network. The gelation of amylose has been regarded as a partial crystallisation process and it has been suggested that the ordered 'junction zones' of the gel consist of microcrystalline regions. Crystalline amylose is not readily attacked by α-amylase (Ring et al., 1988) and therefore can be available as substrate for fermentation by enzymes derived from the microflora present in the colon. Whilst many studies have been made on crystalline amylose, little attention has been paid to the amorphous form of amylose complexes. In this study, glassy amylose was used. Amylose can be prepared in glassy form, with possibly no crystalline order by melting the amylose - butan-1-ol complex and cooling rapidly. This amorphous material may be either brittle or rubbery, depending on whether it is above or below the glass transition temperature ($T_g$) of the amylose mixture. There is a strong dependence of $T_g$ on degree of plasticization and Orford et al., (1989) has estimated the $T_g$ of pure amylose to be 227°C. As water is such a strong plasticizing agent for carbohydrate, small variations in water content can have a large effect on the observed $T_g$. Both butan-1-ol and water act as plasticizer, by depressing the $T_g$ of amylose (Whittam et al., 1989). The effect can be interpreted on the basis of increased free volume and a weakening of the inter chain interaction and it is assumed that interactions in such plasticized polymers are predominantly intramolecular, or conformational, although the intermolecular interaction between the molecular segment and solvent molecules may be appreciable (Chan et al., 1986).

The coating was carried out with hot aqueous amylose, then the product was cooled to produce a glassy system, below the $T_g$. Formation of glass is a result of cooling the melt at a rate (see fan capacity data in Table 3.2), which is too fast for the molecules to relax into their lowest energy state, which below $T_m$ is ideally that of a crystal. At slow rates of cooling the orientation of the polymer chains occurs more readily enabling them to pack into a regular formation (ie. arrangement of the polymer chains into crystalline array). Supercooling, a liquid leads to an increase in viscosity, until at a temperature $T_g$, the liquid-like structure is frozen in to form an amorphous solid. The glass transition is affected by kinetic factors, $T_g$ being influenced strongly
by the thermal history of the sample. Rising through this temperature, there is a sharp increase in the heat capacity of the amylose. This heat capacity increment allows the $T_g$ to be identified and can be measured by differential scanning calorimetry. The rate of cooling has been determined by optimizing the inlet temperature and fan capacity. In order to produce glass, the rate of drying has to be sufficiently rapid and is dependent on the rate of diffusion of water to the drying surface (Banker, 1966).

Zachariasen (1932) showed that if the atomic coordination number is sufficiently small, atoms can be assembled together in a continuous random network which can properly model a glass. Glassy state is characterized by a somewhat ordered structure in which there is minimum polymer chain movement. Rubbery state is characterized by increased polymer chain movement and polymer elasticity. There is an excellent review by Turnbull (1969), which provides information on the conditions a glass can be formed and what are the conditions for bypassing crystallization. It is preferred that the glassy amylose does not contain hydroxy groups in derivate form, and any derivatization is present to an extent 1-2% or less. This is because if they are regularly distributed along the chain, it will produce a distinct tendency for the formation of laterally ordered chains. The closer the polar groups are to one another along the chain, the better their lateral fit and the more pronounced will be their effect to promote crystallinity, which will not be preferable in this study. Molecular weight values depend on the methods of isolation and fractionation, and it is preferred that amylose has a high molecular weight.

Another important parameter was amylose concentration. Amylose was applied from amylose - butan-1-ol complex and the amylose concentration was 4% w/w. Miles et al., (1985) examined the influence of different amylose concentrations on the physical properties of associated amylose chains. For polymer concentration above $C^*$ (entanglement concentration determined as 1.5% w/w), gelation occurred on cooling whereas, for concentration less than $C^*$, precipitation of amylose tended to occur on cooling. Molecular mechanisms underlying amylose aggregation and gelation have been examined by Gidley (1989), who found that critical concentration for gelation was 1.0% w/v. Therefore, 4% w/w has been chosen as starting point and it is used in this part of the study. The influence of different amylose concentration on release of
glucose from coated pellets is examined in Chapter 4. When applied to 5-
aminosalicylic acid pellets, two amylose coating thicknesses were examined (TWG 2% and 4.8%) and the release profiles in various dissolution media are shown in Figure 3.5. Comparison of dissolution profiles of TWG 2% and TWG 4.8% formulation in water shows that TWG 4.8% can delay 100% release of drug for 2h, whereas TWG 2% formulation shows 100% release after 4h. The dominant factor controlling permeability is water uptake of the film. Amylose films swell in water, became porous and allowed the release of 5-aminosalicylic acid through the swollen pores. Amylose still retains its glassy properties at physiological temperature (37°), because the T_g is above 37°. There are not a lot of reports in the literature regarding drug release from glassy polymers. Lee and Lum, (1992); Peppas and Franson, (1983) showed that when coated pellets come into contact with water, the resultant transport into the glassy polymer follows non-Fickian kinetics. This drug delivery systems are based on glassy polymers which are known to follow the so-called glassy-rubbery transition and the resulting drug release is affected by chain macromolecular relaxation and is governed by a Fickian diffusion mechanism with a square-root of time dependence. There is a report in the literature where starch matrix was used as a controlled release device (Wing et al., 1988), in contrast to this study, release is influenced by amylose retrogradation which is formed of aggregates, resulting from hydrogen bonding between hydroxyl groups of amylose chains.

Amylose coated pellets are brittle and do not stay intact during dissolution studies. Therefore, amylose is unsatisfactory as a sole coating material and it was necessary to incorporate some of the commercially available impermeable coatings in order to control the swelling of amylose films and to improve the general mechanical properties of the film coat.

The in vitro 5-aminosalicylic acid release profiles of ethylcellulose (Ethocel®) coated pellets are shown in Figure 3.6. Two film thicknesses (TWG) were examined. The ethylcellulose films at both TWG examined, appeared to be poorly permeable to the drug. In the case of 4.8% w/w less than 5% of the dose was released in 6h, and in the case of the 9.6% w/w less than 2% was released in 6h in water. The rate of drug release is related inversely to the thickness of the coat, suggesting that the film
Figure 3.5  *In vitro* release of 5-aminosalicylic acid in various dissolution media from amylose coated pellets as a function of coat thickness (TWG).

Keys: ■ distilled water (TWG=2.0%); ▲ distilled water (TWG=4.8%);

▼ pH 7.2 (TWG=4.8%); ♦ pH 1.2 (TWG=4.8%)
Figure 3.6  In vitro release of 5-aminosalicylic acid in various dissolution media from Ethocel® coated pellets as a function of coat thickness (TWG).

Keys: □ pH 7.2; ▲ pH 1.2; ▼ distilled water; (open symbols, TWG = 4.8%; solid symbols, TWG = 9.6%)
is controlling the release process. The profile shows the pH independence of the Ethocel® coating. The release profiles in water were essentially linear. Porter (1989b) has shown that the thickness of the retarding Ethocel coat has a major effect on the ultimate rate of drug release. Similar results regarding the importance of film thickness have been described by Mehta (1986). There is no evidence that Ethocel is fermented by microbial enzymes (Gibson, 1991).

To improve release profile characteristics of the amylose film, the amylose was mixed with Ethocel in the various ratios (i.e. 1:4; 2:5 and 2:6). The situation becomes complex for a binary mixture. As a rule, components of lower molecular weight depress the $T_g$ of higher molecular weight compounds (amylose Mwt = 500,000). The $T_g$ of the amylose : Ethocel mixture was found to be 41-43°C using Dielectric Mechanical Thermal Analyzer (Botham, 1991). The components of Ethocel (e.g. ammonium oleate) possibly act as plasticizer to amylose. The glass transition temperature of Ethocel is about 41°C. The appearance of $T_g$ for the mixed film at the same temperature as Ethocel film confirms the film is phase separated. The glass transition temperature of the amylose occurs at a much higher temperature and a homogeneous film would show a glass transition temperature in between the two. Some spray coating variables have shown to affect the properties of ethylcellulose films prepared from aqueous dispersions, and therefore have an impact on drug release profiles (Yang and Ghebre-Sellasie, 1990; Arwidsson et al., 1991b). Film formation depends on entanglement and packing of polymer molecules as the solvent evaporates. In the first step of film formation, the dispersed particles become closely packed upon water evaporation and an irreversible coalescence towards a continuous film may start. Evaporation of the final amount of water, filling the voids between the particles, may promote further coalescence due to surface tension effects. Dibutyl sebacate, incorporated in the Ethocel acts as a plasticizer and causes the latex particles to soften. The temperature of the inlet air serves the dual function of evaporating the water, softening and coalescing the latex particles in the Ethocel film. The coating was carried out when the actual bed temperature was above $T_g$ of the final film (Table 3.2). In order to avoid amylose crystallization and produce glassy amylose, evaporation rate has been optimized. Problems were encountered during the optimization of the coating parameters. When the temperature was too high, it caused
electrostatic interactions and agglomeration because of excessive drying and softening the latex film. A balance was achieved between product temperature and the rate of evaporation to give glassy amylose. The most desirable inlet air temperature setting was one that allowed equilibrium between the application of liquid and subsequent evaporation so that film formation occurred. The rate of drying during the coating process depends on the quantity of air flowing through the pellet bed, the temperature of the air and the quantity of water that the outlet air contains. Evaporation of the water causes the outlet air temperature (40°C) to be cooler than the inlet (60°C). In order to avoid vigorous fluidization, which can cause problems such as bonding to the side wall and attrition of the core substrates, air volume has been optimized. Another important parameter is the spraying rate. Increasing the liquid spraying rate at a given atomization air pressure of 0.2 bar will result in larger droplets and a higher possibility of overwetting the pellets. Slower spray rates could cause electrostatic problems because of low humidity in the bed, especially at high temperature settings. A spraying rate of 0.5 ml/min has been found optimal. However, it has been demonstrated (Arwidsson, 1991) that the dissolution rate of drug can be affected by spray rate.

In conclusion, all coating parameters are interrelated. Optimization and control of the coating variables (Table 3.2) resulted in desired and reproducible properties of the coat which consists of a mixture of amylose and Ethocel. Figure 3.7 shows the effect of various coating levels applied to pellets when amylose : Ethocel ratio in the coating composition is 1:4. An ideal in vitro procedure should simulate conditions in vivo as far as possible. Gastric residence time for multiunit dosage forms is within the range of 90 - 180 min depending upon fasting or non-fasting state (Christensen et al., 1985). In normal subjects, transit time through the small intestine is 180 - 240 min (Davis et al., 1984). Gastrointestinal transit can be greatly influenced by factors such as diet, presence of food, administration of medicine and emotional state which may account for the inter- and intra- subject variations in transit time. Therefore, the dissolution test was performed for 3h at pH 1.2 without enzymes, followed by 21h at pH 7.2. It was observed from the dissolution rate profiles that there was an increase in the rate of drug release with change in film thickness when the amount of coating polymer mixture used was low. A small reduction in the amount of polymer was
Figure 3.7  *In vitro* release of 5-aminosalicylic acid from amylose : Ethocel® mixture (1 : 4) coated pellets as a function of coat thickness (TWG), using pH change method. Keys: ■ TWG=1.2%; ▲ TWG=2.4%; ▼ TWG=4.8%; ◆ TWG=9.6%
observe to cause a large increase in the release rate. For TWG 1.2% and 2.4% drug release was rapid at pH 1.2, while slower rates of release were observed with increasing the pH to 7.2. This may be attributed to the higher 5-aminosalicylic acid solubility at pH 1.2 than at pH 7.2. At pH 7.2 both (1.2% and 2.4%) TWG formulations sustain drug release in an apparent zero-order function, as opposed to initial activity (Figure 3.7). On the basis of all these findings, it was concluded that film thicknesses of the preparations which correspond to 4.8% and 9.6% produced the most satisfactory drug release profiles as they showed a continued impermeability to 5-aminosalicylic acid for up to 18h and 24h respectively.

Different types of 5-aminosalicylic acid coated products were obtained, by varying the amylose to Ethocel ratios (2:6, 2:5) in the coating formulation. Figure 3.8-3.10 show the 5-aminosalicylic acid release profiles from such coated pellets, when the pH change method was employed. Referring to Figure 3.8 it can be seen that when the ratio of amylose to Ethocel is 2:5, the release rate is decreased by increasing the coating loading (from 4.8% to 9.6%), indicating that film thickness is controlling the release process. A slight deviation from linearity was observed when the coat thickness was decreased. The release was faster and was accompanied by cracking of the pellets. Observation of the release profiles of the formulation where amylose to Ethocel ratio is 2:6 show that altering the film thickness of the coat does not influence the dissolution profiles to any significant degree. 5-aminosalicylic acid release was retarded for up to 3h at pH 1.2, independent of the ratio in the coating formulations. However, there was a significant improvement in the overall release profile when amylose was mixed with Ethocel in different ratio compared to pure amylose film (Figure 3.9).

The results obtained so far are summarized in the Figures 3.9 and 3.10. The figures show the effect of film composition on percentage release of 5-aminosalicylic acid over 24h. A correlation exists between the amylose : Ethocel ratios and corresponding release rates (degree of resistance), of the coated pellets using pH change of media. A small increase in the amount of amylose was observed to cause a large increase in the release of 5-aminosalicylic acid. This can be attributed to the hydration and swelling properties of amylose as it probably forms a 'swelling
Figure 3.8  *In vitro* release of 5-aminosalicylic acid from coated pellets as a function of amylose : Ethocel® ratio, using pH change method.

Keys: ▼ 2:5; ■ 2:6; (open symbols, TWG = 4.8%; solid symbols, TWG = 9.6%)
**Figure 3.9** *In vitro* release of 5-aminosalicylic acid from coated pellets as a function of film coating composition (amylose to Ethocel® ratio). TWG = 4.8%

Keys: ■ 10:0; ▲ 2:5; ▼ 2:6; ♦ 1:4
Figure 3.10  *In vitro* release of 5-aminosalicylic acid as a function of film coating composition, using pH change method (3h in pH 1.2, followed by 21h in pH 7.2). TWG = 4.8%
controlled system' upon water penetration through the swollen pores. In the formulation where the ratio is 2:5 and the thickness is 4.8%, the amount of Ethocel used is not sufficient to inhibit the swelling of amylose and therefore drug release occurred. Pellet coating formulations where amylose to Ethocel ratio is 1:4 and 2:6 do not exhibit any significant difference in consistency before and after treatment with acid and neutral media, as observed visually. Recently, Lenaerts et al., (1991) has introduced a new matrix system in which different reticulation degrees were observed by varying the ratio of epichlorhydrine to amylose. In contrast to this study they used retrograded amylose to control the release of a drug. Retardation in the release of theophylline from the matrix in their system is related to the high amylose interchain associations. Linear drug release is correlated to an increase in amylose amount.

As promising release profiles were obtained with amylose and Ethocel mixture in ratio 1:4 as shown in Figure 3.10, further studies were performed by introducing Eudragit® RS/RL aqueous dispersions. The effect of film thickness on the drug release from pellets coated with amylose : Eudragit mixtures (in the ratio 1:4) are shown in Figure 3.11. The release profiles of 5-aminosalicylic acid loaded pellets require a coating level of the final product to be 4.8% or 9.6%. In contrast, almost equal rates of drug release were obtained for coat thicknesses 9.6% and 14.4% w/w. Figure 3.11 also shows that 5-aminosalicylic acid release in water, is linear. All coated pellets appeared swollen, after removal from water. When dealing with Eudragit dispersions it has been shown by Goodhart et al., (1984), that release rates are a function of coating thickness, plasticizer concentration and film coalescence (the effect of drying temperature and duration). From the literature search (Lehmann 1989; Jenquin et al., 1990) and the Eudragit® Handbook, the form of Eudragit (discussed previously in the section 3.2.2.2), nature of plasticizer and its concentration, to be miscible and compatible with amylose under the required coating conditions was selected. The amount of quaternary ammonium groups in Eudragit, despite their low content in an excess of neutral ester in the polymer chain, is of high influence to the permeability and swelling of the films. The permeability is independent of the pH. However, the formation of the latex-like aqueous dispersion is also caused by the hydrophilic ammonium groups (Lehmann, 1989). The RS has a lower content of these charged groups and is considered only slightly permeable to water vapour in comparison with
the more readily permeable RL. Husson et al., (1991) has shown that RS and RL aqueous dispersions can be mixed in any proportion to adopt the desired permeability. Another important characteristic of Eudragit polymers is that they are noncrystalline polymers, as they are copolymers of two or more dissimilar monomer constituents in which an irregularity of structure occurs (Banker, 1966). Because the latex film forming process involves the fusion of individual polymer particles into a homogeneous film, particle coalescence is dependent upon the presence of an appropriate concentration of plasticizer to soften the latex particles. PEG 300 was used as a plasticizer in this study. Muhammad et al., (1991) has shown that, for a given concentration of PEG, the extent of changes in the release properties of Eudragit films is dependent upon the molecular weight of PEG incorporated with the polymer. Lehmann and Dreher (1981) have attributed film resistance to mechanical shock to the incorporation of PEG in a coating formulation. Films from latex systems are practically free of pores when film forming conditions are optimal. Mixtures of RS and RL in equal parts give minimum film forming temperature (MFT) in the range of 30-40°C, which was further reduced to 16-20°C by adding 10% PEG (Lehmann, 1989). The application temperature should be 10-20°C above the MFT. As previously mentioned the T_g of glassy amylose is above 40°C. Based upon this information, and in order to balance optimal conditions for film coalescence of the mixed film, subsequent coating trials were conducted with PEG-plasticized Eudragit with inlet air temperature maintained at 40°C, and some retardation effect in drug release was obtained.

The amylose : Eudragit RS/RL formulation was further evaluated under different pH conditions using preparations of film thickness 4.8% w/w. A comparison was made between two aqueous system mixtures; amylose : Eudragit RS/RL 30D and amylose : Ethocel and the results are displayed in Figure 3.12. The previous results have shown that when using amylose : Ethocel formulation the drug release is not affected by pH to any significant degree. In contrast, 5-aminosalicylic acid release from amylose : Eudragit coated pellets was greater in all the pH media examined, more than 80% of the dose has been released in pH 1.2 media within 6h. Higher drug release at pH 1.2 compared to 7.2 and water may be attributed to pH solubility of the drug (being three times higher at pH 1.2 than at pH 7.2). The deviation from linearity
Figure 3.11  In vitro release of 5-aminosalicylic acid in water from pellets coated with amylose : Eudragit® RS/RL 30D mixture (1 : 4), as a function of coat thickness (TWG). Keys: ■ TWG=4.8%; ▲ TWG=9.6%; ▼ TWG=14.4%
in the initial part of the curve (pH 1.2 and pH 7.2) may be associated with swelling of amylose or Eudragit, resulting in an increase in the diffusional path length and decrease in the effective area of diffusion. Eudragit films possess a defined swelling capacity, being unable to control the swelling of amylose. Drug release could also be attributed to diffusion through the pores formed as result of PEG dissolution. Greater amounts of amylose : Eudragit material are needed to get a sufficient resistance to acid and neutral media. In conclusion, Eudragit formulation coated pellets released the drug at faster rates than Ethocel formulation coated pellets.

Another commercially available ethylcellulose aqueous dispersion Aquacoat® was tested in the present study. It was applied as a mixture with amylose to form a single layer (Figure 3.13). To facilitate film formation, the ethylcellulose dispersion required plasticization. A primary goal was to clarify whether minimal release in the desired period of time could be obtained for pellets coated with amylose : Aquacoat mixture in 1 : 4 ratio. Also it was required to establish if desired release profile can be achieved by optimizing influential parameters, such as plasticizer content for the Aquacoat and process variables. Two plasticizers (triacetin and triethylcitrate-TEC) were employed in this study because of their different solubility properties. Pellets were prepared under the coating conditions listed in Table 3.2. Yang and Ghebre-Sellassie (1990) evaluated the effect of coating temperature on the performance of the deposited Aquacoat film on diphenhydramine hydrochloride pellets. Optimum coalescence of an Aquacoat formulation plasticized with triethylcitrate was achieved when the bed temperature was kept between 34-40°C. Coating applied outside this temperature range resulted in the formation of poorly coalesced films and faster release rates. At high bed temperature, the rate of evaporation of water is so fast and may overcome the diffusion of water from between the polymeric spheres to the surface, resulting in the formation of porous films. The fast release rates (more than 90% of the drug released after only 2h) at low bed temperature (22°C) were attributed primarily to drug migration into the film layer during the coating process and to incomplete film formation due to hardening of the uncoalesced polymer particles, which have a relatively higher MFT. Arwidsson et al., (1991a, 1991b) found 22% TEC close to optimal amount of plasticizer needed to obtain complete coalescence of Aquacoat ECD-30 film at 35-40°C. In addition, a low release rate is favoured by a
Figure 3.12  Comparison of the release profiles of 5-amino salicylic acid in various dissolution media, from pellets coated with amylose : Eudragit® RS/RL 30D mixture, ratio 1:4 (solid symbols) and amylose : Ethocel® mixture, ratio 1:4 (open symbols). TWG = 4.8%. Keys:  ■ distilled water;  ▲ pH 7.2;  ▼ pH 1.2;
Figure 3.13  In vitro release of 5-aminosalicylic acid from pellets coated with amylose : Aquacoat® ECD-30 mixture (1 : 4), as a function of various plasticizers, using pH change method. Keys: □ 24% triethylcitrate; ▲ 20% triacetin; (open symbols TWG = 4.8%, solid symbols TWG = 9.6%)
short spraying distance, a high atomising air pressure, a low concentration of solid, a high TEC content and high drying temperature (Arwidsson et al., 1991b). Minimum film formation temperature for film plasticized with 20% triacetin was found to be 20°C (Aquacoat Handbook). Optimal film formation and linear plots are obtained if the microcapsules are heat treated 10°C above the MFT of Aquacoat (Lippold et al., 1989). The most logical criterion to assess the nature of coating systems was to monitor the release behaviour of the coated product employing pH change method. Two coating thicknesses (4.8% and 9.6%) were examined in this study and the results are displayed in Figure 3.13. It is evident that neither the nature of the plasticizer, nor the coating thickness act in such a manner to retard drug release to any significant degree. Rapid drug release at pH 1.2 is accompanied with cracking of the pellets. The release rate in the present study fitted first-order kinetics. The effect of pH on release rate was also found by Goodhart et al., (1984). They suggested that the sodium lauryl sulphate's (surfactant) state of ionisation (pKₐ 1.9) under acidic or basic conditions affects partition of the drug into the gastrointestinal fluid. A similar effect was observed by Chang et al., (1987), who found that the ionic surfactant (sodium lauryl sulphate) increases the effect of pH dissolution on the drug release from Aquacoat coated pellets. Arwidsson et al., (1991a) suggested that the release through Aquacoat ECD-30D films plasticized with TEC is likely to take place by diffusion through pores. The pores may either be built in as imperfections in the film or created by leaching of a water soluble TEC. Ozturk et al., (1990) studied PPA.HCl release from Aquacoat film plasticized with dibutylsebacate and suggested that osmotically driven release is a major mechanism of release. Observations in this study suggest that drug release may be attributed to an incompletely formed polymer film coating due to incompatibility of amylose with Aquacoat aqueous dispersion.

Comparison of drug release from Aquacoat formulation (Figure 3.13) and from Ethocel formulation (Figure 3.6) the difference may be noticed, and attributed to the fact that Aquacoat is plasticized by mechanical means. To interact with the polymer, the plasticizer has to partition from the aqueous to the polymer phase and subsequently diffuse throughout the polymeric particles. Although the first partitioning step takes approximately 30min (mixing time of Aquacoat and plasticizer), a relatively long period of time may be required for the actual diffusion of the plasticizer, from
the periphery of the core, followed by the complete solvation of the polymeric spheres. Consequently, the degree of plasticization during the preparation of Aquacoat may be less than the optimum in comparison with the Ethocel formulation, and this property is bound to have adverse effects on the final film. Arwidsson et al., (1991b) studied the mechanical properties of Aquacoat film and found that it is not affected by any of the coating process factors. Since the film formation from Aquacoat mainly takes place by coalescence after the coating procedure (curing step), the spraying conditions give the starting point for the film formation and do not directly affect the final properties. An important factor found to induce coalescence was a curing temperature of 60°C and the time of 1h (Aquacoat Handbook). It was concluded that the amylose : Aquacoat coating system, plasticized with triacetin or triethylcitrate does not possess the capacity to retard 5-aminosalicylic acid in acidic and neutral media. Aquacoat can not control a swelling of amylose. Moreover, the films were found (Lippold et al., 1990) to squeeze out cetylalcohol and sodium lauryl sulphate during storage, which may further change the permeability of the films.

Donbrow and Samuelov, (1980); Wouessidjewe et al., (1991) and Wan and Lai (1992) have shown that in producing sustained release pellets, the multiple film coverage (film laid upon film) has a significant effect on the dissolution performance of coated pellets. On the other hand, it was thought that the release characteristics of the multilayer composition may be controlled by variations in the nature of the two coatings. The degree of permeability of the outer coating of film forming cellulose or acrylic polymer material, can be varied to achieve the desired mode of release of the drug particularly through the thickness of the coat. The rate of break down of the amylose by microflora in the colon may be varied through selection of different thicknesses of amylose for the inner coating. The present study was undertaken to develop a new delayed release model using multilayer coated pellets prepared by consecutively spraying aqueous amylose solution as inner layer followed by spraying ethylcellulose dissolved in methanol and dichlormethane, onto 5-aminosalicylic pellets. The release profiles in water, of the multilayer coated pellets are shown in Figure 3.14. Four coating formulations were examined; in three formulations a thickness of inner coat was kept constant (0.9%), while increasing the thickness of outer coat, and the fourth formulation was obtained by increasing the thickness of both layers. It can
be seen that drug release decreases with increasing the thickness of the ethylcellulose layer. There are several reasons behind the rationale for use of high molecular weight ethylcellulose dissolved in dichloromethane and methanol, without incorporation of a plasticizer. The mechanical properties (tensile strength, elongation) are important in governing the incidence of film cracking. The tensile strength of film has been found to increase with increasing molecular weight of ethylcellulose (Rowe 1982, 1986). Moreover, drug release was found to decrease with increasing molecular weight and on the addition of plasticizer, diethylphthalate, to the films prepared from the low molecular weight grades. At molecular weight in excess of 35,000 the addition of a plasticizer had no effect on drug release. Films prepared from low molecular weight polymers with short chains are relatively weak and the release is a consequence of the presence of flaws and cracks, but as the chain length and hence, molecular weight is increased, the mechanical properties of the films improve until at some critical molecular weight (35,000) there is no further improvement. In this case, drug release is primarily due to transport through intact film.

Mixtures of dichloromethane (strongly hydrogen bonding solvent) and methanol (poorly hydrogen bonding solvent) have been successfully used for the film coating of dosage forms with ethylcellulose (80-105 cps) when an organic system is required (Rowe, 1986; Clarke, 1989; Course, 1992) and were therefore used in this study. However, a few studies have been published reporting the influence of different solvents on the mechanical properties of ethylcellulose films. Arwidsson and Nicklasson (1989, 1990) claimed that dilute solution properties, intrinsic viscosity and interaction constant of ethylcellulose 10cps can be of great theoretical and practical importance when selecting a solvent system, because they indirectly reflect the ability of a solvent system to dissolve a polymer. The binary mixtures of dichloromethane and ethanol generated the interaction constant and indicate that this solvent system dissolve each ethylcellulose more effectively than dichloromethane or ethanol per se. In contrast, Iyer et al., (1990) found that dichloromethane provides more transparent and flexible films than the mixed solvent system. They claim that when a mixed solvent is used, the composition of the solvent system is likely to change as a function of time, because of the faster evaporation of the lower boiling solvent - dichloromethane (40°C). The polymer is less soluble in ethanol than in dichloromethane, thus premature
Figure 3.14  *In vitro* release of 5-aminosalicylic acid in water from pellets coated with separate amylose / ethylcellulose layers, as a function of coat thickness (TWG).

Keys: ■ TWG= 0.9% / 0.6%; ♦ TWG= 0.9% / 1.2%; ▲ TWG= 0.9% / 2.4%; ▼ TWG= 2.0% / 4.8%;
precipitation of the polymer might occur as the amount of dichlormethane in solution is depleted. As a result, internal stress may be created which may cause the faster release rate. Different observations regarding the efficiency of the solvents for ethylcellulose could possibly be due to the impact of using different grades (molecular weight and ethoxy content) of ethylcellulose. However, maximum interaction between the polymer and solvent typically results in maximum chain extension of the polymer such that interaction between the polymer chains in the resultant dried coating will also be high, (yielding a film without good mechanical properties). However, the release profiles obtained (Figure 3.14) give an indication of the relative efficiency of ethylcellulose-methanol-dichlormethane system applied as a second layer. The thickness of inner amylose layer was also varied, hoping that the rate of breakdown of the amylose by microflora enzymes of the colon may be varied through selection of different thickness of amylose. The degree of permeability of the outer ethylcellulose coating is varied attempting to achieve the desired mode of release of 5-aminosalicylic acid, particularly through variation of the thickness of the coat. When the TWG is greater than 1.2%, ethylcellulose allows viscous forces to exhibit their adhesive action and thereby limit the swelling phenomenon of amylose. In contrast, the thinner ethylcellulose film allowed water penetration, hydration and subsequent swelling of amylose in the underlying layer. Drug release in water, which follows linear regression is accelerated with the cracking of the pellets, probably due to amylose swelling, resulting in the breaking of the ethylcellulose film.

Another double layer system was investigated, which comprised an inner amylose layer and an outer ethylcellulose aqueous dispersion - Ethocel® layer. Comparison of the release rates of 5-aminosalicylic acid in various dissolution media from the pellets coated with amylose/Ethocel and amylose/ethylcellulose-methanol-dichlormethane systems, coated at the same thickness (0.9%/0.6%) is shown in Figure 3.15. Difference in release rates is thought to be due to different mechanisms of the film formation. The steady-state release from pellets coated with the organic solvent formulation is considered to be the end point, because film formation from a polymer solution is dependent only upon the polymer chain entanglement with solvent evaporation, which results in the formation of dense polymeric network. Onions (1986) and Porter (1989) stated that formation of coating from polymer solutions involves a
few stages, conversion of viscous liquid into a visco-elastic solid. In contrast, film formation from aqueous polymeric dispersion is based upon the plasticizer/particle softening/surface tension/temperature mechanism. Therefore, end point/coalescence release profiles are dependent solely upon determination of the proper drying conditions at fixed plasticizer levels. A close examination of Figure 3.15 indicates that pH of the dissolution media affects the behaviour of the coating materials to a different degree. Release rates of both formulations demonstrated pH dependency and were slower at pH 7.2 than at pH 1.2. The pH dependent release rates can be attributed to pH solubility of a drug as the amount of applied coating is not sufficient to retard drug release. It is obvious that the profile of drug release from amyllose/ethylcellulose organic system follows linear release, whilst the amyllose/Ethocel system exhibits deviation from linearity. While no attempts were made to study the effect of coating parameters on the mechanical and hence release properties of the two formulations, the difference in coating parameters for organic and aqueous system can be seen in Table 3.2. Difference in spray rate may be due to difference in polymer concentration in different solvents, properties of the carrier liquid being sprayed and the zone (in a fluid bed coater), in which the application of coating takes place (Jones, 1985). Arwidsson et al., (1991b) found that droplet size from the aqueous dispersion is not significantly affected by any of the spraying variables studied (see Table 3.2). In a similarly designed study the release of the drug from ethylcellulose - organic solvent system coated pellets, the spraying factors were found to have an impact on the droplet size (Arwidsson, 1991). This difference may be attributed to the fact there is a viscosity increase, due to higher concentration of solids in the aqueous dispersion, compared to the corresponding change in the organic formulation. The experiment performed in this study confirms that ethylcellulose films from organic formulation form less permeable films than aqueous dispersion when it is applied as a second layer.

The influence of Eudragit® L-100 organic system was also examined. Release rates of drug from pellets coated with amyllose as an inner layer and Eudragit L-100 isopropylalcohol as an outer layer are shown in Figure 3.16. Two coating formulations were examined; the thickness of amyllose coat was kept the same (0.9%) and the
Figure 3.15  Comparison of the release profiles of 5-aminosalicylic acid in various dissolution media from pellets coated with separate amylose / Ethocel® layers (solid symbols) and separate amylose / ethylcellulose layers (open symbols). Coat thickness TWG = 0.9% / 0.6%. Keys: □ ■ distilled water; △ ▲ pH 1.2; ▼ ▼ pH 7.2
Figure 3.16  *In vitro* release of 5-aminosalicylic acid in water from pellets coated with separate amylose / Eudragit® L100 layers, as a function of coat thickness (TWG).

Keys: ■ TWG= 0.9% / 1.2%; ▲ TWG= 0.9% / 2.4%
thickness of Eudragit L100 was varied. As shown by Lehmann (1989) the most effective additives for lowering the MFT of anionic methacrylate latex are hydrophilic plasticizers (e.g. PEG). MFT is in the range of 20 - 30°C, which could be further reduced to 3 - 16°C by adding 10% PEG. Thus, Eudragit - organic solution system was plasticized with PEG 300. The release rates depend on level of outer coating and presence of plasticizer. The plasticizer possibly forms a gel-like mass with the polymer when the solvent evaporates and the plasticizer diffuses onto the outer surface of the polymer particles. Greater amounts of Eudragit (more than 2.4%) were needed to get sufficient resistance to water for 6h. This may be attributed to pore formation between polymer particles and the hydrophilic PEG 300. Furthermore, some of the carboxylic groups of Eudragit may begin to ionize and cause softening and swelling of the film. It was observed that the coating level and the presence of water soluble plasticizer played a major role in drug release, which is in agreement with Ghebre-Sellassie et al., (1987) observations for Eudragit L100 film. The water penetration through the outer coat influenced the swelling of the inner amylose layer.

A comparison of the dissolution profiles (Figure 3.17) of 5-aminosalicylic acid coated pellets with the single or with multilayer systems showed that the slower (almost zero) release was obtained when amylose : Ethocel mixture was used as a single coating layer, and the coat thickness was 4.8%. It was noticed that in multilayer systems, where ethylcellulose - organic system comprised the outer layer, even at very low thickness, the coat have capacity to slow drug release to a certain degree. Furthermore, a comparison of Eudragit L100 to ethylcellulose systems, show that higher drug release is obtained with Eudragit system and this may be attributed to leaching of the plasticizer from the polymer with formation of pores through which water penetrates into the inner amylose layer.

To analyze the mechanism of release from the multilayer coated pellets, the experimental data obtained are fitted to two commonly used models, namely, the first order and square - root of time equations (Higuchi's model), for diffusion controlled release (Higuchi, 1963). The results are shown in Figure 3.18. 5-aminosalicylic acid release from multilayer coated pellets, where ethylcellulose organic system comprise outer layer, is likely to be due to the simultaneous operation of more than one release
Figure 3.17  In vitro release of 5-aminosalicylic acid from pellets coated with (1) multi layer systems: ▼ amylose / ethycellulose, TWG = 0.9% / 0.6%; ■ amylose / Eudragit® L100, TWG = 0.9% / 2.4%; and (2) single layer systems: ♦ amylose : Ethocel®, TWG = 4.8%; ▲ amylose : Eudragit® RS/RL 30D, TWG = 14.4%
Figure 3.18  (A) Square-root of time and (B) First-order release of 5-aminosalicylic acid from pellets coated with (1) multi layer system: ■ amylose / Eudragit® L100, ▼ amylose / ethylcellulose; (2) single layer system: ▲ amylose and Eudragit® RS/RL mixtures.
mechanism because release profiles fit both models. In contrast, when analysing multilayer coated pellets where Eudragit organic system is the second layer the situation is slightly different. In the first 2h of the drug dissolution test, the two mechanisms simultaneously operate from 2h to 6h, release following first order kinetics. From 6h to 15h release is square - root of time dependent. The results obtained suggest that drug release is affected by other rate - determining factors than drug diffusion. This may include penetration of aqueous solvent, swelling, dissolution of drug and plasticizer and the formation of pores in the amylose layer. The relative contribution of each of these factors to the overall release process is difficult to assess and apparently varies with the composition of the multilayer polymeric coat.

From the results obtained so far, the amylose and Ethocel mixture appears to be the best coating candidate. The formulation was further evaluated under the addition of pepsin and pancreatin to the dissolution fluid using the two formulations of varying amylose : Ethocel ratio of 1:4 and 2:6. The release rate profiles are shown in Figure 3.19. The proteolytic enzymes pepsin and pancreatin were added to the dissolution media (section 3.3.2) in order to simulate more closely the physiological conditions in the gastro-intestinal tract. It was noticed that 5-aminosalicylic acid release is associated with an increase of amylose content in the coating formulation. Pancreatic α-amylase also known as α-1,4-glucan-glucanohydrolase, hydrolyses α-1,4-glucan links in saccharides containing three or more α-1,4-linked D-glucose units. Generally, there are three mechanisms by which amylolytic activity may take place (Ruyssen and Lauwers, 1978); (a) a single-chain mechanism in which the enzyme, having formed a complex with a substrate molecule, hydrolyses that molecule completely before attacking a second molecule of substrate; (b) a multichain mechanism in which the enzyme acts randomly on all the substrate molecules; and (c) a multiple-attack mechanism in which the enzyme splits off several maltose molecules per encounter with the substrate molecule, and then diffuses away to combine with another molecule of substrate. However, the relationship amylase-amylose in this study is far from being understood. Mammalian enzymes are large, and thus the amylose network may be unaccessible to them.
Figure 3.19  Effect of pepsin in 0.1N HCl (gastric fluid) and pancreatin in 0.2M phosphate buffer (intestinal fluid) on the release of 5-aminosalicylic acid from pellets coated with the amylose : Ethocel® mixture. Keys: □, ■ TWG = 4.8%; △ TWG = 9.6%; ratio 2 : 6 (open symbols); ratio 1 : 4 (solid symbol).
The drug release profiles, as a function of storage and curing time for the pellets that were and that were not subjected for 1h or 2h to additional curing of the film coat are shown in Figure 3.20. It can be seen that the release rates of the preparations that were not subjected to curing increased slightly after storage of 2 months and 1 year. In contrast, the release profiles of the fractions that were cured for 1h and 2h remained stable even after 1 year of storage. Those results clearly indicate that thermal treatment is crucial for complete curing as ethylcellulose undergoes coalescence upon curing of the film coat so that the release will remain stable during storage. Moreover, the physical nature of the amylose may be varied by additional drying which has the effect of reducing the pore size and hence permeability.

Results presented in Figure 3.21 show that the gut microflora could degrade sole amylose and amylose : Ethocel (1:4) coating in the colon. In the presence of faecal flora, 5-aminosalicylic acid release is initially slow but appears to accelerate rapidly between 2h and 5h after commencing fermentation. Release is largely complete after 8 hr. Inclusion of Ethocel in the coating mixture was necessary to control the swelling of the amylose in the gastro-intestinal tract. Once in the colonic region the ethylcellulose is weakened enough to allow the amylose to swell and then be fermented. Further studies are clearly required to determine the interactions and mechanism by which gut bacterial enzymes hydrolyse amylose in the colon. Gibson (1991) found that Bacteroides and Clostridia bacterial groups utilise amylose at a higher rate that the other tested genera. The data of Finegold et al., (1983) indicate that these bacteria are of a high incidence in man and therefore suggest that amylose will be utilized in all persons tested. High levels of amylase activity were observed in human faeces and starch or its degradation products are required as an inducer for amylase synthesis. In the absence of the inducer the amylolytic enzymes are not produced (Macfarlane and Englyst, 1986). Williamson et al., (1992) examined the hydrolysis of polycrystalline amylose by glucoamylase, β-amylase and α-amylase (all produced by bacteria) and found that each enzyme has a characteristic degradation pattern and that the type and arrangement of polymer structure, the average molecular weight and the existence of other materials (lipid, protein) will also play a role in determining the rate of hydrolysis by a given enzyme. Possibly the size of bacterial enzymes plays a role in degradation of amylose. Being smaller than mammalian
Figure 3.20 In vitro release of 5-aminosalicylic acid from pellets coated with amylose: Ethocel® mixture (1:4), as a function of storage time and curing conditions. TWG = 4.8% Keys: Curing conditions: □ without curing; △ cured for 1h; ○ cured for 2h; Storage time: — initial release; - - - after 2 months; --- after 12 months
Figure 3.21  Release profile of 5-aminosalicylic acid in fermenter (simulated colon), system from pellets coated with (●) amylose and Ethocel® mixtures (ratio 1 : 4). TWG 4.8% and (■) amylose TWG 4.8%
enzymes, they possess the ability to access the amyllose microstructure. The enzymes differ from each other by the site of enzymatic attack on the macromolecular chain, and by number of bonds cleaved as a result of each enzymic attack (Figure 3.22).

\[
\begin{align*}
\alpha\text{-amylase} & \quad \beta\text{-amylase} & \quad \text{glucoamylase} \\
\phi & -O-O-O-O-O-O-O-O
\end{align*}
\]

Figure 3.22  Schematic structure of portions of amyllose showing bonds hydrolysed by various enzymes. Keys: O glucose residue; \(\phi\) glucose with an exposed reducing group; 1,4-\(\alpha\)-linkage

Colonic transit time (23-168h, mean of approximately 70h) may be an important influence on carbohydrate fermentation by intestinal bacteria (Macfarlane et al., 1989). It should be added that enzymic reactions involving insoluble substrate consist of several steps: diffusion, adsorption and catalysis which is difficult to generate in small intestine, considering the short transit time in that region.

Studies were performed to estimate the degree of amyllose and Ethocel mixture, fermentability and the results are shown in Table 3.3. A slight delay in volatile fatty acids (VFA) production was observed when comparing to the amyllose film, where a rapid increase in fermentation VFA occurred. High levels of volatile fatty acids confirmed that the amyllose film and amyllose: Ethocel mixture film have been fermented. In contrast, VFA, \(H_2\) and \(CO_2\) values indicate that Ethocel film has not been fermented. However, the effect of adding Ethocel to the coating mixture delayed the time in which active fermentation occurs, possibly due to limited amyllose content.

Plate 1 shows the scanning electromicrographs of the pellet construction. The micrographs (A) and (B) were taken of the pellets before and after coating with amyllose and Ethocel mixture (1:4). It can be seen that the pellet surface is relatively rough before application of the coat (micrograph C). In contrast, the film surface deposited appears smooth (micrograph D), indicating that the polymeric particles were
adequately deformed and fused, to yield a film where amylose and Ethocel are phase separated. In addition, the film thickness also appears very uniform as seen with the 4.8% coatings in micrograph (E) and (F). A very thin film of approximately 13 μm was obtained.

Table 3.3 In vitro fermentation results

<table>
<thead>
<tr>
<th>Film Formulation</th>
<th>Volatile Fatty Acids (mM)</th>
<th>H₂ (nmol/ml/h)</th>
<th>CO₂ (μmol/ml/h)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0h</td>
<td>5h</td>
<td>8h</td>
</tr>
<tr>
<td>Ethocel</td>
<td>14.2</td>
<td>14.6</td>
<td>14.9</td>
</tr>
<tr>
<td>Amylose:Ethocel*</td>
<td>15.2</td>
<td>16.2</td>
<td>17.0</td>
</tr>
<tr>
<td>Amylose</td>
<td>-</td>
<td>17.1</td>
<td>17.1</td>
</tr>
</tbody>
</table>

* mixture (1:4)
Plate 3.1 Scanning Electronmicrographs of 5-aminosalicylic acid pellets

(A) uncoated pellet (x50)  (B) coated pellet (x50)

(C) uncoated pellet surface (x500)  (D) coated pellet surface (x500)

(E) cross-section of coated pellet (x45)  (F) cross-section of coated pellet (x500) TWG 4.8%
3.5. CONCLUSION

In conclusion, a coating formulation comprising the mixture of glassy amylose and Ethocel® in the ratio 1 : 4, appears to allow the controlled delivery of 5-aminosalicylic acid to the colonic region. The coating formulation exhibits gastric and small intestine resistance (less than 15% of drug dose was released over 24h), and it is fermented by enzymes of colonic bacteria origin. The mechanism of release is one of degradation of the amylose fraction by bacterial enzymes and not a mechanical breakage of the coating. Stability of release during storage can be achieved with additional curing of the coat. The manufacturing process shows good reproducibility as assessed by in vitro drug release.
CHAPTER 4

Evaluation of Glucose Pellets for *In Vivo* Studies
4.1 INTRODUCTION

The previous chapters have described a coating formulation with gastric and small intestine resistant properties. On the basis of these studies the amylose : Ethocel® mixture in the ratio 1 : 4 appears to be the best coating candidate for drug delivery to the human large intestine. A delayed release formulation of 5-aminosalicylic acid with satisfactory in vitro dissolution characteristics was successfully developed.

The validation of a delayed release system is best achieved by in vivo testing on human volunteers. It was decided to employ a non-invasive $^{13}$CO$_2$ breath test (Hiele et al., 1990) and gamma scintigraphy (Christensen et al., 1985) to deduce whether the coating is capable of affording site specific drug delivery to the colon. For that purpose $^{13}$C glucose was proposed as the diagnostic agent. The test is based on the fact that digestion of amylose coating would allow release of the glucose which could then be absorbed and further metabolised to $^{13}$CO$_2$. $^{13}$CO$_2$ is excreted in the breath, where it can be detected.

Preliminary studies with plain ($^{12}$C) glucose were performed on a small scale as a feasibility study, due to the limited availability (10g) and cost of $^{13}$C glucose. Glucose pellets were prepared employing the extrusion and spheronisation process. The basic principle of the technique has been described in Chapter 2. The aim of this part of the study is to replace 5-aminosalicylic acid with glucose and to evaluate the formulation in vitro, testing release characteristics into acid and alkaline dissolution fluids over 12h and digestion by fermentation in the simulated colonic environment. The nature of glucose (high water solubility, high osmotic potential) has a significant effect on the behaviour of the final coated product.

The general impression is that incorporation of an insoluble binder into the core, will slow the dissolution of soluble drug by decreasing the effective solid - solvent interface or by making the mass hydrophobic by physically coating the drug particles. The effect of the various binding agents incorporated in the pellet core on the dissolution of glucose was assessed. Binding agents were added in order to impart
cohesive qualities to the powder material which contained glucose as the active ingredient. In all systems studied two constituents, glucose and microcrystalline cellulose (Avicel® PH101) were the same, while the third, the binder, was changed. The binding agents selected in this study have been chosen because they represent a range of different physical and chemical properties. They are soluble PEG 6000 and PEG 35000, a water insoluble wax GMS and swellable sodium carboxymethylcellulose (NaCMC). The ability of these binders to maintain the spherionisation process was assessed. Furthermore, the influence of various concentrations (4.9%, 6.8% and 8.5% w/w) of amylose in the amylose : Ethocel® (1 : 4) coat on glucose release from the pellets was examined. The performance of various formulations was evaluated by means of in vitro dissolution and in vitro fermentation studies.

4.2 CHOICE OF MATERIALS

Glucose, N° 51119, BP grade, BDH, UK

Dextrose anhydrous, is a monosaccharide sugar and is manufactured on a large scale by the acid hydrolysis of starch. It is freely soluble in water (1g in about 1ml) which increases with increasing temperature.

Polyethylene Glycol, (PEG 6000, N° P1537 and PEG 35000, N° G18992), technical grade, Hoechst, Germany

Polyethylene glycol is water soluble and at high molecular weight can form gels and enhance the effectiveness of pellet or tablet binders. They are also known to impart plasticity to granules (Handbook of Pharmaceutical Excipients).

Glycerol monostearate (GMS), N° 16/879, BP grade, Croda surfactants Ltd., UK

Glycerol monostearate is a mixture of mono-, di- and triglycerides of stearic and palmitic acids which is practically insoluble in water. The addition of an emulsifying agent in the form of a soluble soap enhances surface active properties of GMS. The melting point of GMS is 58 - 60°C.
Avicel® RC591, FMC, USA

Avicel RC591 is a mixture of microcrystalline cellulose and a colloid, 11% sodium carboxymethylcellulose. It is not a water soluble cellulose derivate, but a water dispersible organic hydrocolloid, which at concentration less than 1% solids content forms colloidal dispersion and at concentration greater than 1% forms thixotropic gel (Avicel® , FMC Handbook).

The other materials used in this study are described previously in Section 3.2.2, and are as follows:

Avicel® PH101, N° BN6511, FMC, USA
Surelease® E-7-7050, N° 600064, Colorcon, UK
Ethocel®, N° MA880708, Colorcon, UK
Amylose (aqueous dispersion containing 8% butan-1-ol), AFRC, Norwich

4.3 METHODS

4.3.1 Extrusion and Spheronisation

Extrusion and spheronisation of the glucose, excipient and binder was performed on a small scale. Table 4.1 shows the formulae and characteristics of the extrusion process. A powder was mixed with water using a mortar and pestle and the wet powder mass was allowed to equilibrate for a few minutes before extrusion was performed. Extrusion was carried out with the 12.5mm barrel to allow for the small quantity used, giving only 0.6g of waste material. The cylindrical extrudate was then processed in a 10cm spheroniser (Caleva) plate rotating at 2250rpm. The extrudate is broken into short lengths by the friction plate and then rounded into pellets. The aim was to prepare spherical pellets with a narrow size distribution with a minimum of waste material. The pellets were dried in an oven at 40°C overnight, and a specified sieve fraction (see Table 4.1) was used in further studies. Pellets composed of glucose homogeneously distributed throughout a wax matrix (GMS) were prepared by both melt and traditional methods as explained below:
(i) Glucose was suspended in melted (60°C) wax (GMS) in an evaporating dish. Following the removal of heat, the melt was continually stirred until solidification (big lumps) had occurred, thus preventing any separation of glucose and wax. Finally, Avicel® PH101 and water were added and the solid was granulated by means of a mesh and spatula. The resulting mix was subjected to extrusion.

(ii) This method involves the simple incorporation of wax (GMS) into the powder mix as above but without the thermal treatment.

4.3.2 Fluid bed coating

Glucose pellets (12 - 16g) were coated using a laboratory - scale fluid bed machine (STREA-1, AEROMATIC). The following coating formulations were used: (i) amylose : Ethocel® mixture (1 : 4) and (ii) amylose : Surelease® mixture (1 : 4). The mixtures were heated up to 70°C and then sprayed. Coating conditions were varied during the coating process and are shown in Table 4.2. The pellets were cured in a forced - air oven at 60°C for 1h. Composition of coated glucose pellets is shown in Table 4.3.

<table>
<thead>
<tr>
<th>Table 4.2</th>
<th>Coating conditions for amylose : Ethocel® system</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coating parameters</td>
<td></td>
</tr>
<tr>
<td>spraying rate (ml/min)</td>
<td></td>
</tr>
<tr>
<td>slow</td>
<td>0.05</td>
</tr>
<tr>
<td>fast</td>
<td>0.5 - 0.6</td>
</tr>
<tr>
<td>inlet temperature (°C)</td>
<td>60</td>
</tr>
<tr>
<td>outlet temperature (°C)</td>
<td>40 - 41</td>
</tr>
<tr>
<td>fan capacity (units)</td>
<td>10, 11</td>
</tr>
<tr>
<td>atomizing air pressure (bar)</td>
<td></td>
</tr>
<tr>
<td>low</td>
<td>0.02</td>
</tr>
<tr>
<td>high</td>
<td>0.2</td>
</tr>
</tbody>
</table>
Table 4.1 Composition of uncoated glucose pellets and characteristics of the manufacturing process

<table>
<thead>
<tr>
<th>Core composition (% w/w) g</th>
<th>Extrusion process die/diameter length extrusion speed (mm/min)</th>
<th>Sieve analysis model pellet size (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Glucose (50) 10 Avicel® PH101 (50) 10 Water 7.5</td>
<td>1/2 75</td>
</tr>
<tr>
<td>B</td>
<td>Glucose (25) 5 Avicel® PH101 (75) 15 Water 11</td>
<td>1/4 90</td>
</tr>
<tr>
<td>C</td>
<td>Glucose (50) 10 Avicel® PH101 (25) 5 Avicel® RC591 (25) 5 Water 7.5</td>
<td>1/2 75</td>
</tr>
<tr>
<td>D</td>
<td>Glucose (50) 10 Avicel® PH101 (40) 8 PEG 6000 (10) 2 Water 6.5</td>
<td>1/2 100</td>
</tr>
<tr>
<td>E</td>
<td>Glucose (50) 10 Avicel® PH101 (40) 8 PEG 35000 (10) 2 Water 5.5</td>
<td>1/2 100</td>
</tr>
<tr>
<td>F</td>
<td>Glucose (50) 10 Avicel® PH101 (40) 8 GMS (10) 2 Water 5.5</td>
<td>1/2 100</td>
</tr>
<tr>
<td>G</td>
<td>Glucose (50) 10 Avicel® PH101 (30) 6 GMS (20) 4 Water 5</td>
<td>1/2 100</td>
</tr>
<tr>
<td>H</td>
<td>Glucose (50) 10 Avicel® PH101 (20) 4 GMS (30) 6 Water 4.5</td>
<td>1/2 100</td>
</tr>
</tbody>
</table>

* based on dry weight
## Table 4.3 Composition of coated glucose pellets

<table>
<thead>
<tr>
<th>Core composition (%)</th>
<th>Coating composition (Amylose: Ethocel® mixture, ratio 1:4)</th>
<th>Formulation Code</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose 50</td>
<td>Amylose (4.0%)</td>
<td>A1a</td>
</tr>
<tr>
<td>Avicel® PH101 50</td>
<td>TWG=28%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TWG=53%</td>
<td>A1b</td>
</tr>
<tr>
<td></td>
<td>Amylose (8.5%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TWG=28%</td>
<td>A2a</td>
</tr>
<tr>
<td>Glucose 25</td>
<td>Amylose (4.0%)</td>
<td>B1a</td>
</tr>
<tr>
<td>Avicel® PH101 75</td>
<td>TWG=14%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TWG=28%</td>
<td>B1b</td>
</tr>
<tr>
<td>Glucose 50</td>
<td>Amylose (4.9%)</td>
<td>C1a</td>
</tr>
<tr>
<td>Avicel® PH101 25</td>
<td>TWG=24%, 90μm</td>
<td></td>
</tr>
<tr>
<td>PEG 6000 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TWG=29%, 90μm</td>
<td></td>
</tr>
<tr>
<td>Glucose 50</td>
<td>Amylose (4.9%)</td>
<td>D1a</td>
</tr>
<tr>
<td>Avicel® PH101 40</td>
<td>TWG=14%, 60μm</td>
<td></td>
</tr>
<tr>
<td>PEG 35000 10</td>
<td>TWG=24%, 80μm</td>
<td>D1b</td>
</tr>
<tr>
<td>Glucose 50</td>
<td>Amylose (4.9%)</td>
<td>E1a</td>
</tr>
<tr>
<td>Avicel® PH101 40</td>
<td>TWG=12%, 60μm</td>
<td></td>
</tr>
<tr>
<td>PEG 35000 10</td>
<td>TWG=24%, 80μm</td>
<td>E1b</td>
</tr>
<tr>
<td>Glucose 50</td>
<td>Amylose (4.9%)</td>
<td>F1a</td>
</tr>
<tr>
<td>Avicel® PH101 40</td>
<td>TWG=14%, 53μm</td>
<td></td>
</tr>
<tr>
<td>GMS 10</td>
<td>TWG=29%, 87μm</td>
<td>F1b</td>
</tr>
<tr>
<td></td>
<td>Amylose (6.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TWG=12%</td>
<td>F2a</td>
</tr>
<tr>
<td></td>
<td>TWG=25%</td>
<td>F2b</td>
</tr>
<tr>
<td>Glucose 50</td>
<td>Amylose (4.9%)</td>
<td>G1a</td>
</tr>
<tr>
<td>Avicel® PH101 30</td>
<td>TWG=15%</td>
<td></td>
</tr>
<tr>
<td>GMS 20</td>
<td>TWG=30%</td>
<td>G1b</td>
</tr>
<tr>
<td></td>
<td>Amylose (6.8%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TWG=15%</td>
<td>G2a</td>
</tr>
<tr>
<td></td>
<td>TWG=30%</td>
<td>G2b</td>
</tr>
<tr>
<td>Glucose 50</td>
<td>Amylose (4.9%)</td>
<td>H1a</td>
</tr>
<tr>
<td>Avicel® PH101 20</td>
<td>TWG=15%</td>
<td></td>
</tr>
<tr>
<td>GMS 30</td>
<td>TWG=30%</td>
<td>H1b</td>
</tr>
</tbody>
</table>

* based on dry weight
4.3.3 *In vitro* dissolution studies

Dissolution studies were performed using the method described in Section 2.2.3, with 400ml distilled water as the dissolution medium. In addition, glucose pellets containing 20% and 30% w/w glycerol monostearate and coated with amylose : Ethocel® mixture (1:4), where the amylose concentration was 4.9% and 6.8% w/w were also evaluated. The dissolution media used were water, hydrochloric acid (pH 1.2 for 3h) and phosphate buffer (pH 7.2 for 9h) and simulated gastric (pepsin) and intestinal (pancreatin) conditions. The glucose concentration was determined using a UV spectrophotometer. For carbohydrates, the absorption maximum lies between 187 and 188nm (*Binder, 1980*). Below 200nm there is a significant absorption by most solvents and gaseous substances, such as oxygen and therefore measurements made at these wavelength can be unreliable. Thus, chemical modification of sugars is a useful way of shifting their absorption maxima into a range which facilitates a more reliable UV detection. The Glucose - GOD PERID® diagnostic kit (Boehringer Mannheim UK, Lenes, East Sussex) takes into account this factor and thus was used for the determination of glucose concentration.

The method of determination was originally described by Werner *et al.*, (1970). The sensitivity of the method is very high. 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 are mixed with the GOD PERID reagent and incubated at 37°C for 10min in which time the following reaction occurs:

\[
glucose + O_2 + H_2O \xrightarrow{GOD} \text{gluconate} + H_2O_2
\]

\[
H_2O_2 + ABTS \xrightarrow{POD} \text{coloured complex} + H_2O
\]

where: GOD is glucose oxidase

POD is peroxidase (catalyses oxidation in which molecular oxygen serves as an electron acceptor)
During the dissolution studies, media was removed at certain time periods and diluted by 1/5. The resulting coloured complex absorbs UV light at 420nm and the absorbance is directly proportional to the amount of glucose present.

4.3.4 *In vitro* fermentation studies

Four coating formulations which were found to delay the release of glucose under simulated gastric and small intestine conditions, were further evaluated in a batch fermenter containing 5% faecal slurry. The faecal slurry in the fermenter was continually stirred and the pH controlled. CO$_2$ and volatile fatty acids (VFA) concentrations were measured over a 48h period. These fermentation studies were performed at the Dunn Clinical Nutrition Centre, Cambridge by Dr. G. Gibson.
4.4 RESULTS AND DISCUSSION

4.4.1 Extrusion and Spheronisation

In this study eight glucose formulations were developed as listed in Table 4.1. Observation of Table 4.1 and Figure 4.1 reveals two interesting points. The differences in the amount of water used in extrusion are clearly seen when different batches were investigated. The incorporation of the binding agents into the core (comprising 50% glucose and microcrystalline cellulose) resulted in changes to the most frequently occurring size of the pellets (from 1.00 - 1.40mm to 1.40 - 1.70mm). Pellet size distribution relates to a difference in the core composition and was determined by sieve analysis. A large number of factors contribute to the properties of pellets produced from mixtures of powders, and direct and quantitative measurements of the properties of such mixtures are difficult. The binders used in this study differ from each other in terms of their solubility in water and water uptake capacity. It was found that extrudate containing 50% glucose and microcrystalline cellulose were difficult to spheronize because of the difficulty of maintaining the preferred wet consistency throughout the process. Glucose, because of its crystalline nature, turned out to be insufficiently plastic at 50% w/w to allow perfect pellet formation. When the amount of glucose is 25%, fairly rounded pellets were obtained.

Jaiyeoba and Spring (1981) claimed that the physical properties of powders, the proportion of components in the powder mixture and the type and quantity of binder are the variables which mostly influence the mean sizes of the resulting pellets. The water soluble binders PEG 6000 and 35000 will dissolve in water and increase the liquid volume available for extrusion and thus give mainly large pellets. Dissolved binders form solid bridges between particles. During the spheronisation of the extrudate containing PEG binder, water drops were identified on the surface of the pellets. The appearance of moisture is a result of centrifugal force pulling the liquid from the interstitial spaces to the outer surface and causing densification of the pellets. Similar findings were observed by Chien and Nuessle (1985), who claimed that this phenomenon is almost always accompanied by the migration of water soluble chemicals in the formulation and that physical appearance of the final product is critically dependent on the amount of liquid used.
Figure 4.1 Histogram showing distribution of pellet size versus pellet percentage weight. Keys: (A) Formulation A; (B) Formulation B; (C) Formulation C; (D) Formulation D; (E) Formulation E; (F) Formulation F; (G) Formulation G; (H) Formulation H; (see Table 4.1)
The effect of hydration and swelling on the amount of water needed for extrusion and spheronisation, were clearly observed when microcrystalline cellulose and Avicel® RC591 (sodium carboxymethylcellulose) were used. Their ability to incorporate water both into and between the particles led to the increase in amount of water needed for extrusion as compared with other binders, specially to insoluble GMS (Table 4.1).

An increase in the mean pellet size was also obtained with pellets containing glycerol monostearate (GMS) as binder. Generally, in the mixture the liquid is in a pendular state of wetting, which has the greatest binding force because of the existence of liquid bridges. Commercially available GMS contains a non-ionic surfactant which lowers the surface tension at the liquid - solid interface and this may change the liquid surrounding the particles from pendular to funicular state. Bode-Tunji and Jaiyeoba (1984) explained that the mechanism by which a surfactant influence the increase in pellet size may be due to the effect of the surfactant on the interfacial tension of the water.

4.4.2 The effect of glucose load

On the basis of previous studies (Chapter 3), the amylose : Ethocel mixture (1:4) appears to be the best coating candidate as its exhibits in vitro gastric and small intestine resistance. Pellets containing 5-aminosalicylic acid as a model drug showed minimal release over a 24h period with no evidence of mechanical failure of the coating when the coat thicknesses (expressed as theoretical weight gain TWG) are 4.8% and 9.6%. The in vitro glucose release profile from amylose : Ethocel (1:4) coated pellets of different thickness are shown in Figures 4.2 and 4.3. The glucose content is 50%. Figure 4.2 shows the release profile from coated pellets where the coating was applied under identical conditions as for the 5-aminosalicylic acid pellets, with a fast spraying rate and a high atomising pressure. Figure 4.2 reveals that pellet formulations of average film thickness 28% and 53% exhibit fast release rates. The rate of glucose release is inversely related to the thickness of the coat. Although the release profile of the formulation of film thickness 53% was essentially linear, it was excessively high for a desirable profile. Coated glucose pellets in water have shown a mechanical breakdown of the coating at short time intervals. Glucose is a highly
Figure 4.2  *In vitro* release of glucose in water from amylose : Ethocel® mixture coated pellets, as a function of coat thickness (TWG). Coating conditions: fast spraying rate and high pressure. Keys: ■ Formulation A1a; ▲ Formulation A1b; (see Table 4.3)
soluble substance and in the presence of water exerts a considerable osmotic pressure against any barrier. During the coating process when aqueous conditions prevail there is the possibility that some glucose has dissolved and migrated/diffused into the coating and subsequently becomes distributed throughout the coating. When the coated pellet comes into contact with dissolution media the glucose in the coat dissolves leaving holes through which the dissolution media can reach the core glucose which then causes the rupture of the coat.

Several approaches have been tested in a bid to limit the release of glucose over a 12 to 24h period. It was thought that premature release of glucose could be prevented by the application of amylose : Ethocel® (1:4) mixture in multilayers (Figure 4.3). The thickness of a the layers are in the range of 5% - 22%. In between each application of the layers, the pellets were dried for 10min in a fluid bed dryer under the same conditions as during the coating procedure. Slow spraying rate and low atomization pressure was determined as the optimal conditions for the amount of glucose pellets (12 - 16g), to be coated such that no coating residue was deposited in the chamber after the coating process.

From a comparison of Figures 4.2 and 4.3 the glucose release profiles are very similar, for the formulations with coat thickness of 22% (♦) (Figure 4.3) and 28% (■) (Figure 4.2), irrespective of the coating conditions used.

It was thought that reduction in the amount of glucose to 25% in the pellet core may possibly slow down glucose release. In vitro release profiles from pellets containing 25% glucose in the core and coated with amylose : Ethocel® (1:4) mixture under fast spraying rate and high atomization pressure are shown in Figure 4.4. The formulation of the two film thicknesses (14% and 28%) were analyzed in terms of dissolution profiles. Drug release from the formulation with the coat thickness of 14% is associated with the burst effect of coated pellets. No significant difference in dissolution rates of 25% (Figure 4.4) and 50% (Figure 4.2) glucose formulations of the same coat thickness is observed.
Figure 4.3  *In vitro* release of glucose in water from amylose (4.0%) : Ethocel®
mixture coated pellets, as a function of coat thickness (TWG). Coating conditions:
slow spraying rate and low pressure. Formulation A (see Table 4.1). Keys: ■ one
layer (TWG= 5%); ▲ three layers (TWG= 12%); ▼ four layers (TWG= 15%); ♦ five
layers (TWG= 22%).
Figure 4.4  *In vitro* release of glucose in water from amylose : Ethocel*®* mixture coated pellets, as a function of coat thickness (TWG). Keys: ■ Formulation B1a; ▲ Formulation B1b; (see Table 4.3)
On the basis of all these results it can be concluded that the cause of the premature glucose release lies in the nature of the core. Two approaches have been taken in order to overcome the problem. Firstly, the concentration of the amylose fraction in the coating mixture was increased, expected to form a denser coat and therefore to slow glucose release. Secondly, glucose has been mixed with various additives of differing concentration to try and increase binding and therefore slow the release.

4.4.3 The effect of amylose concentration

Effect of amylose concentration on the release of glucose from the coated pellets is shown in Figure 4.5. The pellet core consists of glucose and microcrystalline cellulose with no binders added. Figure 4.5 shows that with the increase in the concentration of amylose fraction (from 4% to 8.5%), there is a decrease in glucose release. This indicates that the amylose network at higher concentration is more dense. This finding is in agreement with the earlier work of Leloup et al., (1990, 1992), who studied the influence of amylose concentration on pore size of amylose gels and the diffusion of a macromolecular probe (bovine serum albumin) into the gels. They found that the increase in amylose concentration reduced the porosity to probe molecule.

4.4.4 The effect of various binders

The effect of the various binders on the release of glucose from coated pellets is shown in Figure 4.6. Two coat thicknesses were examined for a range of formulations containing different binders. For the formulation containing sodium carboxymethylcellulose as a binder only one film thickness (24%) was examined. The size of the uncoated pellets differed (see Table 4.1). Therefore, a comparison of the release profiles of the final coated formulations by coat thickness expressed as percentage is not valid. The coat thickness was expressed as (μm) and was determined from scanning electron micrographs. The binders effect glucose release in the following order:

GMS > No binders > PEG 35000 > PEG 6000 > NaCMC

The results indicate that the formulation containing glycerol monostearate was
**Figure 4.5**  *In vitro* release of glucose in water from amylose : Ethocel<sup>®</sup> mixture coated pellets, as a function of amylose concentration and coat thickness (TWG).

Keys: ■ Formulation A2a; ▲ Formulation A1a; (see Table 4.3)
the most efficient at retaining glucose within the core over a 12h period. In Figure 4.6 comparison of the dissolution profiles for the two formulations of similar thicknesses, incorporating PEG 6000 (14%) (■) or PEG 35000 (12%) (▼) show no significant difference. Within two hours 80% of the glucose dose has been released. An increase in coating thickness, to 29% (□) and 24% (▼) respectively, is accompanied with a decrease in the release rate of glucose. The formulation containing PEG 35000 at coat thickness 24% shows slower release, which can partially be attributed to the different rates of migration of PEG 35000 - glucose and PEG 6000 - glucose system during the coating process, being slower for PEG 35000 - glucose system. During film deposition, there may be partial surface dissolution of glucose and binder in the sprayed droplets which remains embedded in the film after the evaporation of water. The lower molecular weight polyethylene glycols exhibit higher water solubility and therefore higher migration rate. It is thought that among the process variables, spheronisation time (35 min) might increase the migration of solvent - soluble materials to the surface. Both centrifugal force and interparticulate friction and collisions contribute to this migration as discussed previously in Section 4.4.1. As more and more layers of film are deposited to constitute the coat, the migration of glucose is hindered mechanically by thickness. Since the presence of glucose in the inner parts of the coating leads to a porous structure during dissolution, a thicker coat is required to generate the desired release profile. On the other hand the dissolution rate of PEG 6000 - glucose is greater than for pure glucose. PEG 6000, possibly enhances dissolution by increasing the wetting of glucose core, and allows better solvent penetration into the mass (Levy and Gumbow, 1963). The release rates of PEG 35000 - glucose and glucose alone are similar. In contrast to this observation, Solvang and Finholt (1970) showed that phenobarbitone tablets granulated with polyethylene glycol 6000 dissolved in dissolution media much slower than with other binders. They proposed that PEG 6000 forms a poorly soluble complex with the drug, probably during dissolution test. Probably, the concentration of PEG and chemical properties of the drug are critical in controlling the dissolution of the drug. However, with the PEG 35000 - glucose pellets, after 12h dissolution test, there were no ruptures in the coat of the pellets. The situation was clearly different in the case of pellets containing Avicel RC591 (sodium carboxymethylcellulose). The capacity of NaCMC to swell was
Figure 4.6  *In vitro* release of glucose in water from amylase : Ethocel® mixture coated pellets, as a function of coat thickness (TWG). Keys: ■, □  Formulations D1a, D1b; ▼, ▽  Formulations E1a, E1b; ♦, ◇  Formulations F1a, F1b; ★  Formulation C1a; △  Formulation B1b; (see Table 4.3)
powerful enough to break the coat and enhance the dissolution of glucose. Previous studies (O'Connor and Schwartz, 1985) have shown that there may be some gel formation beneath the coat during core dissolution, when NaCMC concentration was low. On swelling NaCMC may have not exerted enough force to break the matrix and drug may therefore have diffused rather slowly from the pellets, the gel retarding the release of drug. They also suggested that NaCMC does not form a gel in acid and in buffer and that it is converted in the low pH of gastric juice into a less soluble acid form. The impression is that decrease in concentration of NaCMC to a certain level will slow the dissolution of the drug. However, above a certain concentration, disintegration of the pellets occurred. Thus the amount of the colloid is critical in controlling drug release from the pellets.

On the other hand, GMS behaved differently. As shown in Figure 4.6, the rate of glucose release is decreased by the incorporation of 10% GMS in the pellet core and by application of the coating mixture to a thickness of 29%. Decreasing the coating thickness to 14% increases the rate of release. It is assumed that GMS as a wax is extremely soft (plastic) (Iranloye and Parrott, 1978) and will coat the drug particles during application of pressure (e.g. extrusion) and therefore slow the penetration of dissolution media in the pellets. Schwartz et al., (1968a) presented the schematic model where drug particles are isolated in a matrix of wax, emphasizing that model was a reasonable approximation at low drug concentration and that in these cases diffusion is more difficult in the wax regions connecting the pores (left behind after the drug is leached) than in the pores themselves.

4.4.5 The effect of GMS concentration

Attempts were made to determine whether melting of the wax would aid in matrix formation and slow drug release from the coated pellets. Schwartz, (1989) showed that the melting and resolidification of GMS wax, castor wax and beeswax resulted in a redistribution of the wax throughout the pellets with a possible change in the nature of the pores within pellets. Figure 4.7 shows the release profiles of 10% GMS/glucose pellets (with and without treatment). The wax is not effective in reducing the dissolution rate when subjected to thermal treatment at 60°C, because more drug is released from that formulation. This is in agreement with Ghali et al.,
Figure 4.7 Effect of thermal treatment (TT) on in vitro release of glucose in water from amylose (4.9%) : Ethocel® mixture coated pellets. Keys: ▼, ▽ Formulation F1 (see Table 4.1) TWG= 15%, 31% TT; ♦, ◊ Formulation F1a, F1b; (see Table 4.3)
(1989) observations of dissolution rates from pellets which had been thermally treated and untreated. The results of that study showed that the drug release from pellets is dependent on the wax selected, the wax level and the degree of thermal treatment. On the basis of the results in this study it has been decided to carry on studies with the pellets which were prepared without melting of GMS.

Analysing all the results available it was noticed that two parameters are critical in lowering the release of glucose; presence of GMS in the core and increase in the concentration of the amylose fraction in the coat. Subsequently formulations were developed incorporating these two features. The results of dissolution studies from these formulations are shown in Figure 4.8. Figure 4.8 presents the dissolution profiles of pellets containing 10% GMS in the core with amylose concentration of either 4.8% or 6.8% in the coat. It can be noticed that increase in the coat thickness decrease a dissolution rate.

Another approach has been to increase the GMS content in the core (from 10% to 20 and 30%) at constant drug concentration and increase the thickness of the coat with a varying concentration of the amylose fraction. The results obtained are displayed in Figure 4.9. Figure 4.9 shows that the increase in the GMS content in the formulation (TWG 30%), increase a glucose release rate. It is noticed that increase in GMS content above 20% (TWG 15%) does not further retard glucose release. By increasing the concentration of the amylose and thus the density of the network, the porosity is reduced. Similar release profiles of 20% and 30% GMS formulations with the coat thickness of 15%, raised the question of factors involved in glucose release from a given formulations. To reveal such a mechanism, uncoated pellets containing different amounts of GMS have been tested in water, pH 1.2 and pH 7.2, the results are shown in Figure 4.10. Figure 4.10 shows that glucose was released within 60min from all three formulations in all dissolution media tested. In water within 15min almost 100% of glucose dose is released. In 0.1N HCl and in buffer solution (pH 7.2) GMS matrices slightly slow release rates, as the pellets tend to agglomerate, thus presenting less surface area to the media and thus decreasing the dissolution rate of glucose. These results suggest that the GMS matrix itself is not the factor which retards glucose release. This is consistent with some other processes thought to occur.
Figure 4.8  Effect of amylose concentration and coat thickness (TWG) on \textit{in vitro} release of glucose in water from amylose : Ethocel\textsuperscript{®} mixture coated pellets.

Keys: ▼, ▽ Formulation F2a, F2b; ■, □ Formulation F1a, F1b; (see Table 4.3)
Figure 4.9  Effect of concentration of GMS and amylose on *in vitro* release of glucose in water from amylose : Ethocel® mixture coated pellets. Keys: ▼, ▼ Formulation G1a, G1b; ■, □ Formulation G2a, G2b; ♦, ♦ Formulation H1a, H1b. (see Table 4.3)
Figure 4.10  Effect of concentration of glycerol monostearate on in vitro release of glucose in various dissolution media from uncoated pellets. (A) distilled water (B) pH 1.2. Keys: □ Formulation F; △ Formulation G; ○ Formulation H (see Table 4.1)
Figure 4.10 Effect of concentration of glycerol monostearate on \textit{in vitro} release of glucose in various dissolution media from uncoated pellets. (C) pH 7.2

Keys: □ Formulation F; △ Formulation G; ○ Formulation H (see Table 4.1)
during the preparation of the final glucose - GMS coated formulation. During the coating process GMS probably interacts with the coating mixture which contains butan-1-ol, recrystallizes and remains deposited on the pellet surface. Another possible explanation is that GMS interacts with amylose, leading to formation of an insoluble complex (Schoch and Williams, 1944).

Those formulations which exhibited minimal release of glucose were chosen for further study. During the testing of the formulations, Ethocel® was resupplied under the trade name of Surelease®. Although supposedly the same, differences to the original Colorcon preparation were noticed. The Surelease® suspension is more viscous than Ethocel® and has a slightly darker colour. Free films that were cast also differed in appearance, Ethocel® form clear films whereas the Surelease® films were opaque with the presence of particulate matters. The amylose - butan-1-ol complex was mixed with Surelease® in the 1 : 4 ratio and applied to the pellets as previously described. The results of dissolution studies are shown in Figure 4.11. Comparison of release profiles for pellets coated with amylose : Ethocel® (Figure 4.9) with those of amylose : Surelease® (Figure 4.11), under the same dissolution conditions, show a much higher glucose release from amylose : Surelease® system. The release was accompanied with disintegration of the pellets, suggesting poor film formation from the amylose : Surelease® system. Thus, the two preparations were tested for physical compatibility. Films were prepared and the tensile modulus as a function of temperature was determined (Botham, 1992) using a dynamic mechanical thermal analyzer (Polymer Labs, Loughborough). The tensile modulus showed a slight difference and the onset of the modulus change representing the glass transition for Surelease® occurred after that of the Ethocel®, indicating a different chemical composition. It was disclosed that Surelease® contained a higher percentage of silicon dioxide. However, being aware of a finding that use of Surelease® may cause problems with the coating formulated with Ethocel® in mind (Chapter 3), research has been carried on with the Ethocel® suspension.

4.4.6 The effect of pH of dissolution media

Figures 4.12 and 4.13 show the effect of various dissolution media on the release of glucose from the selected formulations: (i) 20% GMS in the core with
Figure 4.11  Effect of concentration of GMS and amylose (Am) on *in vitro* release of glucose in water from amylose : Surelease® mixture coated pellets. Keys: ▼, ▽ Formulation 20% GMS & Am 4.9% & TWG= 16%, 31%; ■, □ Formulation 30% GMS & Am 8.5% & TWG= 14%, 22%; ♦, ♦ Formulation 20% GMS & Am 4.9% & TWG= 15%, 30%.
The proteolytic enzymes pepsin and pancreatin (α-amylase, protease and lipase) were added to the dissolution media in order to simulate more closely the physiological conditions of the gastrointestinal tract. Comparison of dissolution rate profiles in alkaline (Figure 4.12) and alkaline - pancreatin solution (Figure 4.13) of the two formulations (amylose concentration 4.9%) which differ from each other in terms of GMS content (Formulation G1b and H1b), shows that an increase in GMS content (Formulation H1b), increases the amount of glucose released after 12h in alkaline - pancreatin solution. This is probably due to partial solubilization of the wax particles deposited on the pellet surface by the surfactant action of pancreatin enzymes. This is in agreement with the findings of John and Becker (1968) that surfactant consistently promotes faster release in alkaline pancreatin solution. However, the increase of amylose concentration in the coating does not affect the glucose release in simulated intestinal fluid, this highlights amylose resistance to α-amylase in pancreatin. The results indicate that the formulation containing 20% GMS in the core and the higher amylose concentration in the coat (Formulation G2b) is the most effective formulation for retaining glucose over a 12h period. The average release over that time is 7%.

4.4.7 In vitro fermentation results

In vitro fermentation studies were carried out with four formulations listed in Table 4.3 and the results are shown in Table 4.4. The formulations differ in the concentration of amylose used in the coat, the coating thickness and the content of GMS used as binder in the core. The most efficient coating is the one that gave the highest volatile fatty acid (VFA) production from the glucose coated pellets during fermentation compared to an equivalent glucose pellet and that releases CO₂ continually. Although the formulation (G2a) containing 20% GMS and amylose concentration of 6.8% and coat thickness of 15% gave high VFA production, in vitro dissolution studies (Figure 4.13) showed an early release of glucose. Two formulations which differ only in concentration of amylose fraction in the coat (4.9% and 6.8%) were chosen as suitable candidates and since the formulation with 6.8% amylose concentration produced more CO₂ and more VFA, this formulation was chosen to
Figure 4.12 Effect of concentration of GMS and amylose on in vitro release of glucose from amylose : Ethocel® mixture coated pellets using pH change method.

Keys: ▼, ▽ Formulation G1a, G1b; ■, □ Formulation H1a, H1b; ◆, ◆ Formulation G2a, G2b; (see Table 4.3)
Figure 4.13 Effect of concentration of GMS and amylose on in vitro release of glucose from amylose: Ethocel® mixture coated pellets using pepsin and pancreatin in the dissolution media. Keys: ▼ Formulation H1b; ♦, ◊ Formulation G2a, G2b; △ Formulation G1b; (see Table 4.3)
Table 4.4  In vitro fermentation results

<table>
<thead>
<tr>
<th>Formulation</th>
<th>CO₂ concentration (µmol/ml)</th>
<th>VFA concentration after 48h (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>6h</td>
<td>24h</td>
</tr>
<tr>
<td>No additions</td>
<td>0.69</td>
<td>2.26</td>
</tr>
<tr>
<td>Glucose</td>
<td>3.09</td>
<td>3.43</td>
</tr>
<tr>
<td>H1b*</td>
<td>1.07</td>
<td>5.13</td>
</tr>
<tr>
<td>G2a*</td>
<td>0.81</td>
<td>3.90</td>
</tr>
<tr>
<td>G2b*</td>
<td>0.83</td>
<td>5.24</td>
</tr>
<tr>
<td>G1b*</td>
<td>0.89</td>
<td>1.93</td>
</tr>
</tbody>
</table>

* see Table 4.3
proceed to *in vivo* studies.

### 4.4.8 Scanning Electronmicrographs

Scanning Electronmicrographs of the uncoated and amylose : Ethocel (1:4) coated glucose pellets are shown in Plate 4.1. The pellet core comprises glucose, glycerol monostearate and Avicel PH101. These pellets have been chosen for further evaluation *in vivo*.

The surface of the uncoated pellets (Plate 4.1 A, B, C) was observed to contain numerous crystals. The surface of the coated pellets (D, E, F, G, H) appeared similar to the uncoated pellet surface. In contrast, a surface of the amylose : Ethocel film, which was applied onto the 5-aminosalicylic acid pellets appeared to be smooth (Plate 3.1) and it was not possible to see the presence of crystals on the film surface. A greater concentration of the structures at the surface of glucose pellets supports the postulation that these are actually glycerol monostearate crystals, dissolved in butan-1-ol during the coating process and recrystallized after drying (Section 4.4.5). The cross-section shown in Plate 4.1 (D and H) reveals, however, that the film coating is distinguishable from the core. The measured coat thickness was 170 μm.
Plate 4.1 Scanning Electronmicrographs of glucose pellets

(A) uncoated glucose pellet (x50)
(B) uncoated glucose pellet surface (x2000)
(C) uncoated glucose pellet surface (x2000)
(D) coated glucose pellet cross-section (x35)

(E) coated glucose pellet (x35)
(F) coated glucose pellet surface (x500)
(G) coated glucose pellet surface (x2000)
(H) coated glucose pellet cross-section (x250)
4.5. SUMMARY AND CONCLUSIONS

The results of the investigation of this part of the study show:

- The ability of different binders (PEG 6000, PEG 35000 and NaCMC) to spheronise and form pellets which contain 50% glucose.

- Pellets prepared with specific quantities (10%, 20%, 30%) of waxy material (GMS) can be processed via extrusion and spheronisation technology.

- Binders incorporated in the pellet core affect glucose release to a different degrees. Release from pellets containing PEG and NaCMC was found to be faster and therefore not suitable.

- Glycerol monostearate, when present in concentration of 20% was found to repress the dissolution rate of glucose in simulated gastric and intestinal conditions and to augment the rate in alkaline - pancreatin solution when the concentration is increased above 20%.

- Varying the amylose concentration in the coating mixture can alter glucose release rate.

- The formulation containing 20% GMS, 50% glucose and 30% Avicel® PH101 in the core and coated with amylose : Ethocel® (1 : 4), where the concentration of the amylose fraction is 6.8%, was chosen to proceed to in vivo studies. It is resistant to in vitro simulated gastric and intestinal conditions but fermented by colonic microflora.
CHAPTER 5

In Vivo Evaluation: $^{13}$CO$_2$ Breath Test and Gamma Scintigraphy Study
5.1 INTRODUCTION

The formulation selected for the in vivo study had been evaluated by in vitro studies which estimated the dissolution and fermentation profile of the coated pellets. The results showed simulated in vitro gastric and small intestine resistant properties and high SCFA and CO$_2$ release rate when the formulation was introduced to the in vitro fermentation system. However, in vitro tests alone cannot be used to predict the in vivo performance of the sustained release dosage form and validation is best achieved by in vivo testing in human volunteers. This usually entails a comparison of the blood level profile achieved with the dosage form and then the rate and extent of absorption of the drug can then be determined by relevant pharmacokinetic analysis of the data. In vivo evaluations based on blood level data alone may not be sufficient to optimise dosage form designed to deliver a drug to the colon as these studies are not applicable to drugs that have local actions or which are absorbed at specific sites in the gut. The integrity, distribution, time and site of in vivo dissolution and disintegration of the dosage forms can not be detected (Digenis, 1982). Thus, direct visualization of the in vivo behaviour of dosage forms within the gastrointestinal tract is important.

The aim of this part of the study, is to evaluate the in vivo performance of the formulation in healthy human volunteers employing two methods: gamma scintigraphy and $^{13}$CO$_2$ breath test and to assess the correlation between the methods of evaluation. Transit of the dosage form in the gastro-intestinal tract was determined using gamma scintigraphy with Technetium-99m ($^{99m}$Tc) as the radiomarker. An equal amount of inert lactose pellets of similar size and density containing 5% Amberlite (labelled with $^{99m}$Tc) was administered together with the $^{13}$C glucose pellets. This was necessary as the radiolabelling procedure might affect glucose release if both were incorporated in the same pellet. Furthermore, pellets of similar size and density have been shown to have the same gastric emptying, intestinal transit and caecum arrival time (Devereux et al., 1990). Incorporation of a small amount (5%) of the anionic ion exchange resin (Amberlite CG 400) has been shown to produce stable and satisfactory labelling with the radionuclide $^{99m}$Tc (Clarke, 1989; Yuen, 1991). $^{13}$CO$_2$ excretion in breath after ingestion and oxidation of the amylose coat by the anaerobic bacteria in the colon
followed by absorption and metabolism of the $^{13}$C-labelled glucose to $^{13}$CO$_2$ was measured using mass spectrometry (Lacroix et al., 1973).

5.2 MATERIALS

**Stable isotope** ($^{13}$C glucose, Cambridge Isotope Laboratories, Woburn, USA, BJ - 1177)

$^{13}$C glucose used in this study is single labelled glucose ($^{13}$1-C). The natural abundance of $^{13}$C represents 1.1 percent of the earth’s carbon atoms.

**Radioisotope** (Technetium-99m) and **ion-exchange resin** Amberlite CG 400

The lack of any particulate emissions of $^{99m}$Tc, together with its half life of 6 hours, result in a relatively low radiation dose to the subject per unit of radioactivity administered. The radionuclide is readily available. Amberlite CG400 (Aldrich Chemicals Co Ltd., No 29793) ion exchange resin has been chosen because it has a high specificity for $^{99m}$TcO$_4^-$.

Radiolabelling was achieved by the radionuclide permeating the film coat and binding to the ion exchange resin displacing the appropriate ion, as shown below:

$$RN^+ (CH_3)_3Cl^- + TcO_4^- \rightleftharpoons RN^+ (CH_3)_3TcO_4^- + Cl^-$$

Other materials are listed in Section 4.3.
5.3 METHODS

5.3.1 In vitro methods

5.3.1.1 Preparation and coating of $^{13}$C glucose pellets

The formulae comprises $^{13}$C glucose, glycerol monostearate and Avicel PH101; the same ingredients used for the preparation of $^{12}$C glucose pellets. The extrusion and spheronisation process was performed on a small scale (pellet yield in one fraction size was 13g which is 70% of the original mixture). The formulae and the characteristics of extrusion and spheronisation process are described in Section 4.3.1. The pellets obtained were dried in an oven at 40°C overnight. After drying the pellets were sieved and the specific sieve fraction, 1.40 - 1.70mm was used for coating. The pellets were coated using amylose (6.8%) : Ethocel (20%) mixture in ratio 1:4, to obtain a film thickness of theoretical weight gain (TWG) 30 - 35%. The coating conditions were as described in Section 4.3.2.

5.3.1.2 In vitro performance of coated $^{13}$C glucose pellets

The performance of coated $^{13}$C glucose pellets was evaluated by means of in vitro dissolution and fermentation tests. In vitro dissolution test was performed as described in Section 4.3.3, using simulated gastric fluid for 3h, followed by simulated small intestine fluid for 9h. Glucose concentration was determined using Glucose - GOD PERID® (Boehringer Mannheim) diagnostic kit.

In vitro fermentation test was performed at the Dunn Clinical Nutrition Centre, in Cambridge as described in Section 4.3.4. $^{13}$C glucose fermentation was followed over 48h and $^{13}$C recoveries were measured using mass spectrometry. $^{13}$C recoveries are expressed relative to PDB. PDB value represents $^{13}$C enrichment relative to Peedee Belemnite limestone (Schoelller et al., 1977). A belemnite limestone was found long ago in the Pee Dee range of North Carolina. Most natural levels contain less $^{13}$C than the standard PDB, so the values usually are negative with respect to this standard. The CO$_2$ evolved from it has an agreed $^{13}$C/$^{12}$C ratio ($^{13}$R) of 0.0112372 and laboratory
standard gases are generally calibrated relative to this.

\[
\text{rel PDB} = \frac{^{13}\text{C}}{^{12}\text{C}} = 0.0112372
\]

Units are mmol excess $^{13}\text{C}$ per mol CO$_2$.

5.3.1.3 Preparation and coating of 5% Amberlite pellets

The 5% amberlite pellets comprised Avicel PH101 (45%), Lactose (50%), Amberlite (5%). They were prepared by extrusion and spheronisation and only pellets of the size fraction similar to the $^{13}\text{C}$ glucose pellets (1.40 - 1.70mm) was used. They were coated using the aqueous colloidal dispersion of ethyl cellulose (Ethocel®) with a film thickness of TWG 5.8%. To increase the permeability of the film, for easier radiolabelling, methylcellulose (viscosity grade 15cp) was incorporated into the ethylcellulose coat. The ratio of methylcellulose to ethylcellulose used was 0.4. After coating, the pellets were cured for 24h at 60°C in an oven.

5.3.1.4 Density determination

The density of the coated Amberlite and coated $^{13}\text{C}$ glucose pellets was determined by measuring the volume, by air displacement, of a known weight of the substance in a Beckmann Air Comparison Pycnometer (Model 930). Values were obtained as follows; Amberlite pellets 1.46gcm$^{-3}$ and for $^{13}\text{C}$ glucose pellets 1.23gcm$^{-3}$. Because the two types of pellets have similar density, it was assumed that they also have the same gastrointestinal transit properties.

5.3.1.5 Radiolabelling of Amberlite pellets with $^{99m}\text{Tc}$

The radionuclide $^{99m}\text{Tc}$ was eluted daily from a commercial generator (Elumatic III, CIS France) and obtained as a sterile pyrogen free solution of sodium pertechnetate ($\text{Na}^+\text{Na}^{99m}\text{TcO}_4$) in normal saline. All assays of the $^{99m}\text{Tc}$ activity were performed using an isotope assay ionisation chamber (Vinten Instruments Isocal II). The film coated Amberlite pellets (400mg) were soaked in 3ml of the$^{99m}\text{Tc}$ solution
containing approximately 290MBq of activity. After half an hour of soaking, the solution was removed and the pellets washed twice with 5ml of normal saline whilst rotating on a vertical gantry at 10rpm. This was to ensure that the unbound radiolabel was rinsed off from the surface of the pellets. The solution was removed and the pellets dried, in an oven at 50°. The pellets were weighed and assayed for $^{99m}$Tc activity.

The radiolabel uptake efficiency of the Amberlite pellets with respect to the amount of activity added was 44.1% ± 2.1 (number of determinations was 8).

5.3.1.6 In vitro testing of $^{99m}$Tc binding to Amberlite pellets

After labelling, the batch of pellets was placed in separate glass tubes containing 20ml of hydrochloride acid at pH 1.2. The tubes were sealed and placed in a vertical gantry rotating at 10rpm. After 4h the pellets were removed and the remaining hydrochloride acid was assayed for $^{99m}$Tc activity. The radioactivity released into the supernatant was calculated as a percentage of the initial activity after correcting the decay. The experiment was repeated with phosphate buffer at pH 7.4. The integrity of the pellets was also visually inspected during the course of the studies and no change in pellets integrity was observed.

In all the media used, less than 4% of the radiolabel was released (pH 1.2, 2.0% and at pH 7.4, 3.7%).

5.3.2 In vivo protocol

A group of 8 healthy male volunteers, 27-57 years, height 1.71-1.89m, weight 68.1-84.0kg, participated in this study. Although subjects were healthy at the time of recruitment two had flu-like illnesses one week prior to the study, one of whom had taken antibiotics during the episode. Each volunteer was provided with a written and oral explanation of the study and signed an informed consent agreement. In vivo protocol is given in Appendix. The protocol was approved by the Dunn Clinical
Nutrition Ethical Committee. Volunteers were supplied with a basal diet (see Appendix) which contained minimal naturally occurring $^{13}$C enriched substrate (e.g. maize products, sugar, pineapple) to reduce background $^{13}$CO$_2$ excretion in breath. Studies were performed in the Department of Nuclear Medicine, Adenbrooke’s Hospital, Cambridge and supervised by Dr. J. H. Cummings.

5.3.2.1 Breath test

Subjects commenced the diet on day 1 and in evening breath sampling (by direct exhalation) commenced using an end-expiratory breath sampler. The device (Patent No 9124312.1) is fitted to a plastic syringe with a needle to allow transfer of expiratory air to glass tubes for storage prior to analysis for CO$_2$. This particular device is simple and does not require particular training on behalf of the operator. All samples were taken in duplicate. On day 2 the subject took 300mg (range 293-310mg) of $^{13}$C glucose pellets divided into two hard gelatin capsules size 0 (Elanco Qualicaps, Lilly Industries, UK). These capsules were administered together with the 5% Amberlite pellets labelled with 7.4MBq $^{99m}$Tc in a separate capsule. The three capsules were taken with 200ml of water. A further two hours was then allowed before solid food was eaten although the subject was allowed liquids during this interval. Breath sampling commenced a few minutes prior to dosing and then every half hour for the first 4h and then hourly until 8pm on day 2. Five further breath samples were collected between 8pm and 9am on the following day. The appearance of $^{13}$CO$_2$ in breath as a function of time for each volunteer is shown in Figures 5.2-5.9.

Analysis of breath samples

The expired air is injected directly into the mass spectrometry. Smallest excesses of exogenous $^{13}$CO$_2$ arising from the oxidation of glucose are calculated from the increase in the ratio of $^{13}$CO$_2$ to $^{12}$CO$_2$. The ratios are obtained on a dual-inlet, dual-collector, isotope ratio mass spectrometer. The ratio is then compared to standard CO$_2$ (PDB calcium carbonate) sample of known isotopic abundance. $^{13}$C isotopic abundance is expressed as delta per mil ($\delta$%) relative difference to PeeDee Belemnite.
Limestone (PDB), (i.e. \( ^{13}\text{CO}_2/^{12}\text{CO}_2 \) ratio of the sample (\( R_{\text{smpl}} \)) and the standard (\( R_{\text{PDB}} \)) as defined by Schoeller et al., (1977).

\[
\delta^{13}\text{C} = \frac{R_{\text{smpl}}}{R_{\text{PDB}}} - 1 \times 1000, \%
\]

On this scale most biological material has a natural enrichment in the range -10 to -30\%, or 1 to 3\% less enriched than PDB.

The breath \(^{13}\text{CO}_2\) recoveries are expressed as a percent of the administered \(^{13}\text{C}\) glucose dose and were calculated from the increase in \(^{13}\text{C}\) abundance after administration of the substrate (Watkins et al., 1982).

\[
\% \text{ administered dose} = \frac{\text{mmol excess } ^{13}\text{C}/\text{mmol } \text{CO}_2}{\text{mmol } ^{13}\text{C} \text{ administered}} \times (\text{mmol } \text{CO}_2 \text{ excreted} \times 100)
\]

where:

\[
\text{mmol excess } ^{13}\text{C}/\text{mmol } \text{CO}_2 = (\delta^{13}\text{C}_{\text{PDB}} - \delta^{13}\text{C}_{\text{PDB}=0}) \times R_{\text{PDB}} \times 10^{-3}
\]

\[
\text{mmol } ^{13}\text{C} \text{ administered} = \text{mg substrate/}M_w \times P\times n/100
\]

where:

- \( P \) is atom \% excess = 99
- Atom \% = number of \(^{13}\text{C}\) atoms/ number of \(^{13}\text{C}\) atoms + number of \(^{12}\text{C}\) atoms \times 100
- \( n \) is number of labelled C atoms = 1
- \( M_w \) is molecular weight = 181

Many measurable process, particularly those involving interchanges of matter and energy with the environment (e.g. pulmonary gas exchange), take place across surfaces which might be expected to vary as a function of the square of body length. Body surface area was estimated by the equation of Haycock et al., (1978) and is
given below. The CO₂ production rate was assumed to be 5 mmol/m² of body surface area per minute.

\[
\text{mmol CO}_2 \text{ excreted} = 5 \text{mmol/m}^2/\text{min}
\]

where: \( m^2 = (\text{wt in kg}^{0.5378} \times \text{height in cm}^{0.3964}) \times 0.024265 \)

5.3.2.2 Gamma Scintigraphy study

Imaging of the pellets in the GIT was performed using a single head IGE400 ATMAXI Gamma Camera. The detector was fitted with a low energy parallel hole collimator (max. energy 170KeV) suitable for \(^{99m}\text{Tc}\) imaging and an on line digital computer was connected to the camera for data processing. During this part of the study, the volunteer was laying down on the bed and the head of the camera was positioned above the volunteer. After administration of the dose, an image of 100s per frame was immediately acquired from the anterior detectors. Gamma scans were then taken at the same time as breath samples every half an hour for the first 4h and then hourly until 8pm, in total for 12h. External anatomical reference markers comprising an adhesive patch labelled with a small quantity of \(^{57}\text{Co}\) (200kBq) were positioned anteriorly to mark the ileal crests.

Scintigraphic data were analyzed qualitatively and quantitatively as the main goal of this study was to determine caecal arrival of the pellets. The location of the pellets was determined by viewing the full sequence of images, from gamma scan pictures taken from computer images and also from direct photographs of the scans. On the latter, markers for the ileal crests were visible and this allowed the position of gamma-emitting substances relative to the pelvis to be assessed. On the basis of these pictures, the location of the radiomarker reaching caecum has been determined. Gamma scintigraphy photographs for each volunteer are shown in Plates 5.1 - 5.8.
5.4 RESULTS AND DISCUSSION

5.4.1 In vitro results

Figure 5.1 shows a graph of the in vitro release (%) of $^{13}$C glucose from amylose : Ethocel® (1:4) coated pellets in simulated gastro-intestinal conditions and the in vitro $^{13}$C enrichment ($^{13}$C-rel PDB) in simulated colon (fermenter) system. The percentage release values (□) refer to the left 'y' axis and $^{13}$C-rel PDB values (●) refer to the right 'y' axis. The graph shows that under simulated gastric and small intestine conditions, the coat is able to retard glucose release for between 6 to 9h. Only 6.2% of the total glucose dose had been released after 12h. However, average mouth to colon transit time is accepted to be 4-7h. The resistance can be attributed to the formulation of the coat as amylose by itself allows glucose release within a few minutes. The graph shows that bacteria present in the simulated colon system are able to ferment the coat accelerating the release of glucose from the coated pellets. For the first 6h of the study there was no release, but after 24h substantial release had occurred with a further increase to 48h.
Figure 5.1  *In vitro* release of $^{13}$C glucose from amylose : Ethocel® (1:4) coated pellets. Keys: • simulated colon system; □ simulated gastro-intestinal system
5.4.2 *In vivo* results

The preliminary experiments to optimize the amount of $^{13}$C glucose to be administered to the volunteers were performed by Dr. J.H. Cummings. The dose of $^{13}$C glucose was adjusted so that the detected increase in $^{13}$CO$_2$ in breath is substantial enough not to be affected by slight changes in basal $^{13}$CO$_2$ excretion. On the basis of these studies it was decided to carry out experiments with a dose of 300mg $^{13}$C glucose.

A typical breath $^{13}$CO$_2$ profile obtained in a volunteer after ingestion of the amylose : Ethocel mixture coated $^{12}$C glucose pellets is shown in Figure 5.2. The breath profile shows the change in $^{13}$CO$_2$ enrichment ($\delta$ rel PDB) over a 25h period. The initial points of the curves fall within the limits of standard deviation for the baseline value, so can not be considered as a significant increase. A detectable rise above background levels of $^{13}$CO$_2$ in breath began at about 5h but it was not until about 7h that $^{13}$CO$_2$ began to rise significantly. The detection of $^{13}$CO$_2$ in breath by mass spectrometry is a highly sensitive technique able to peak up as little as 0.01% of the administered dose. By contrast, gamma scintigraphy is able to identify only 5% or more of the dose arriving in the colon. $^{13}$CO$_2$ peaks for the volunteer reached maximum at 15h following administration of coated glucose pellets. Plate 5.1 shows the gastro-intestinal transit of the labelled pellets in volunteer 1 using gamma scintigraphy. The correlation with $^{13}$CO$_2$ appearance in breath (Figure 5.2) was assessed. At 4h Plate 5.1 shows material tracking up from the ileum towards the ileocecal junction and at 5h there is clearly material in the caecum. The arrival in the caecum was between the 4h and 5h scan. The time of arrival therefore was designated 4.5h. Analysis of Figure 5.2 shows that $^{13}$CO$_2$ appeared in the breath at 7h. However, there is a delay of 2.5h between 5% of the dose reaching the caecum and 1% being recovered. This is probably the time it takes for the bacteria to break down a significant part of the pellets and release the glucose.

Analysis of gamma scan photographs (Plate 5.1-5.8) and a corresponding $^{13}$CO$_2$ appearance in breath profile (Figure 5.2-5.9) show that $^{13}$CO$_2$ was not found in breath before the pellets had reached the caecum. A summary of the range of the arrival of
Figure 5.2  Volunteer 1: The appearance of $^{13}$CO$_2$ (solid line) in breath as a function of time (hours) following administration of (300mg $^{13}$C-glucose) coated pellets. (dotted line represents background)
Plate 5.1  Gamma scintigraphy photographs showing gastro-intestinal transit of pellets in volunteer 1
Figure 5.3  Volunteer 2: The appearance of $^{13}$CO$_2$ in breath as a function of time (hours) following administration of (300mg $^{13}$C-glucose) coated pellets.
Plate 5.2  Gamma scintigraphy photographs showing gastro-intestinal transit of pellets in volunteer 2
Figure 5.4  Volunteer 3: The appearance of $^{13}$CO$_2$ in breath as a function of time (hours) following administration of (300mg $^{13}$C-glucose) coated pellets.
Plate 5.3  Gamma scintigraphy photographs showing gastro-intestinal transit of pellets in volunteer 3
Figure 5.5  Volunteer 4: The appearance of $^{13}$CO$_2$ in breath as a function of time (hours) following administration of (300mg $^{13}$C-glucose) coated pellets.
Plate 5.4  Gamma scintigraphy photographs showing gastro-intestinal transit of pellets in volunteer 4
Figure 5.6   Volunteer 5: The appearance of $^{13}$CO$_2$ in breath as a function of time (hours) following administration of (300mg $^{13}$C-glucose) coated pellets.
Plate 5.5  Gamma scintigraphy photographs showing gastro-intestinal transit of pellets in volunteer 5
Figure 5.7  Volunteer 6: The appearance of \(^{13}\text{CO}_2\) in breath as a function of time (hours) following administration of (300mg \(^{13}\text{C}\)-glucose) coated pellets.
Plate 5.6  Gamma scintigraphy photographs showing gastro-intestinal transit of pellets in volunteer 6
Figure 5.8  Volunteer 7: The appearance of $^{13}\text{CO}_2$ in breath as a function of time (hours) following administration of (300mg $^{13}$C-glucose) coated pellets.
Plate 5.7 Gamma scintigraphy photographs showing gastro-intestinal transit of pellets in volunteer 7
**Figure 5.9** Volunteer 8: The appearance of $^{13}$CO$_2$ in breath as a function of time (hours) following administration of (300mg $^{13}$C-glucose) coated pellets.
Plate 5.8  Gamma scintigraphy photographs showing gastro-intestinal transit of pellets in volunteer S
radiolabelled pellets in the colon and the appearance of $^{13}$CO$_2$ in the breath for all volunteers is given in Table 5.1. The table shows that >5%, the minimum amount that could be detected, of $^{99m}$Tc reached the caecum an average at 3.5h with the whole of the small bowel cleared an average by 7.1h. Various methods, visual and statistical, were used to estimate the first significant rise in $^{13}$CO$_2$ in breath and % recoveries with time. The $^{13}$CO$_2$ recoveries are expressed as percent of administered $^{13}$C glucose dose and were calculated according to the equation given in Section 5.3.2.1. The start of the rise in $^{13}$CO$_2$ in the breath (usually less than 0.1% of the dose) was seen an average at 3.8h (visual) or 3.7h (calculated); 1% recovery of the dose was observed at 6.4h, with maximum recovery, 38.3%, at 25h. These data indicate that $^{13}$CO$_2$ appearance in breath was after capsules appear in the colon.

Table 5.1 Summary of gamma scintigraphy and $^{13}$CO$_2$ breath test results

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gamma scanning</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;5% activity in caecum</td>
<td>3.5h</td>
<td>2.5-4.75h</td>
</tr>
<tr>
<td>&gt;25% in caecum/colon</td>
<td>4.2h</td>
<td>3.0-6.0h</td>
</tr>
<tr>
<td>Maximum in colon</td>
<td>7.1h</td>
<td>4.5h-10.0h</td>
</tr>
<tr>
<td>Maximum amount in colon</td>
<td>88.8%</td>
<td>85-93%</td>
</tr>
<tr>
<td><strong>$^{13}$CO$_2$ in breath</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First rise (visual method)</td>
<td>3.8h</td>
<td>3.0-5.5h</td>
</tr>
<tr>
<td>First rise (calculated)</td>
<td>3.7h</td>
<td>2.7h-6.0h</td>
</tr>
<tr>
<td>1% cumulative recovery</td>
<td>6.4h</td>
<td>3.9-8.9h</td>
</tr>
<tr>
<td>Cumulative recovery at 15h</td>
<td>14.6%</td>
<td>7.8-20.3%</td>
</tr>
<tr>
<td>Total recovery (25h)</td>
<td>38.3%</td>
<td>22.1-48.8%</td>
</tr>
</tbody>
</table>

Figure 5.10 shows cumulative recoveries of $^{13}$CO$_2$ in breath after single dose of $^{13}$C glucose either as free glucose or in the pellets. After free glucose
Figure 5.10  Cumulative recoveries in volunteer, of $^{13}$CO$_2$ in breath after a single dose of $^{13}$C-glucose either as free glucose or in the pellets.
administration, $^{13}\text{CO}_2$ appears in breath within 30 minutes and peaks at 1.5h with 28% recovery at 6h. With the pellets the first traces of $^{13}\text{CO}_2$ appear in 5.5h but there is no significant recovery (1% of dose) until 9h. Recovery at 25h is 34%.

Figure 5.11 shows the cumulative % recovery of $^{13}\text{C}$ in breath for each volunteer. At 5h less than 1% on average had been recovered but by 10h recovery is 4.8% and by 15h it is 14.6%. One percent cumulative % recovery occurs at 6.4h (range 3.9-8.9h). Since total recovery of $^{13}\text{CO}_2$ in breath rarely exceeds about 35% of the dose due to sequestration in biomass, lipid, bicarbonate, bone etc. a recovery of 14.6% at 15h is equivalent to 42% of the amount administered, being released and metabolised in the colon. However, when the pellets reached the colonic region, $^{13}\text{CO}_2$ enrichment was detected for a period of approximately 10h. This slow release profile could possibly be of potential value and have advantage over other commercial systems, which are designed to deliver drugs to the lower colon.
Figure 5.11 Cumulative percentage dose recovered over 25h period for eight volunteers after administration of 300mg (range 293-310mg) $^{13}$C-glucose dose.
5.5 CONCLUSIONS

These results suggest that the non-invasive $^{13}$CO$_2$ breath test and gamma scintigraphy are valuable procedures to study drug delivery to the colon. Assessment of the correlation between these methods indicated that the amylose : Ethocel mixture (1:4 ratio) appears to allow the delivery of $^{13}$C glucose to the colonic region. $^{13}$CO$_2$ was not detected in breath until 3.8h post dosing and did not become significant (1% recovery) until 6.4h. Gamma scintigraphy of $^{99m}$Tc labelled pellets indicated >5% activity in caecum at 3.5h (range 2.5-4.75) with a delay of 2.9h between arrival in caecum and significant (1%) cumulative recovery. $^{13}$CO$_2$ was not found in breath before the pellets had reached the caecum. The increasing appearance of $^{13}$CO$_2$ in breath indicated that there was a gradual release of $^{13}$C glucose as the pellets passed through colon over a 12-24h period.
CHAPTER 6

Suggestions for Further Work
From this work it is evident that further work in a number of areas would lead to a better understanding of the in vivo performance of the amylose : Ethocel® mixture coated system.

1. single dosage form studies

Pellets were used as a model dosage form in this study. As being spherical, the pellets possess the ideal shape for application of the coat in view of the low surface area to volume ratio and the absence of edges. However, tablets still remain the most commercially used dosage form. Therefore, the amylose : Ethocel coating system might be investigated for suitability as a tablet coating.

2. application of coating system to other drugs

The results presented in this study indicated that the amylose : Ethocel mixture system, developed for 5-aminosalicylic acid, produced satisfactory control of the drug release in vitro (Chapter 2) and for glucose in both in vitro and in vivo (Chapter 4). In Section 1.2.2, the importance of delivering the following group of drugs to the colon was discussed: peptide drugs (systemic drug delivery), blood pressure controlling drugs, steroids for colonic diseases, antibiotics and drugs with short half life (anti-angina drugs). Drugs can be incorporated into the capsule, and thus avoid exposure to the pressures expended during extrusion. The gelatin capsule could subsequently be coated with the amylose : Ethocel mixture, with minor adjustments to the coat thickness. Furthermore, the coated capsule system might provide an opportunity for the delivery of probiotics for treating bowel disease due to imbalance of the local microflora.

3. targeting to the different parts of the colon

This system provides sustained drug delivery to the caecum where the thickness of the coat has been shown to be an important variable influencing the release of the drug. Factors such as thickness, ratio of amylose to Ethocel and different amylose concentrations may be explored further and their manipulation might provide a system for the delivery of drug to the ascending colon and the other regions of the colon.
4. *in vivo* study with volunteers in the fed state

Another interesting area which could be further investigated is the *in vivo* performance of the preparation in volunteers in the fed state. The systems designed to deliver drugs to the colon that are available on the market suffer from some limitations due to inter- and intra-individual differences in gastro-intestinal transit time (Section 1.5.2). Therefore, it would be of great interest to examine the effect of gastric residence and small intestine transit time on the release of the drug from the amylose : Ethocel (1:4) coated system for fed and fasted subjects.

5. Glassy amylose properties

Further investigation may be undertaken into what is special about the glassy amylose that makes it resistant to gastric and small intestine environment.

6. *in vitro* colon system

The previous experiments (*Gibson*, 1991) identified that *Bacteroides* and *Clostridia* bacterial groups utilize amylose at a higher rate than the other tested genera. The enzymes responsible for amylose fermentation were identified. On the basis of this information the *in vitro* colon system (without human colonic bacteria) could be developed. The test would be of benefit in terms of finance and time scale to the future evaluation of amylose delivery systems which contain different drugs.
Information sheet for volunteers

Project: Use of coated microspheres to deliver drugs to the large intestine

Most drugs when given by mouth are absorbed from the stomach. For someone with bowel disease, such as colitis, it would be much better to have drugs which passed through the stomach and were released directly into the lower bowel where they are needed.

We have developed a new coating for drugs which should allow this to happen. The present study is designed to test that coating for the first time.

No drug will be used in this study; instead some glucose will form the basis of the microspheres. The glucose will be broken down in the large bowel. In order to detect this process, the microspheres will contain some $^{13}$C glucose. This particular type of glucose is normally present in the diet in minute quantities but we will enrich the glucose for the purposes of this study. Once the glucose is broken down, the $^{13}$C will be excreted in breath as carbon dioxide.

We will need to take breath samples at the start of the study and every half-hour throughout. This is achieved very simply by blowing through a tube for a minute or two.

The microspheres are in the form of small spheres onto which we have attached a minute amount of radio-active material to allow us to track their progress through the bowel. The tracking will be done using a gamma-camera in the Nuclear Medicine Department of Addenbrooke’s Hospital. None of the radio-isotope will be absorbed into the body and the total dose will be equivalent to only 1/20 that which you experience normally from natural radiation each year.

What you have to do

1. Arrive at the main concourse of Addenbrook’s Hospital at 7.45a.m. on the day of the study having had nothing to eat for 12 hours. Proceed to Department of Nuclear Medicine with Dr. Cummings.

2. Breath samples will be collected.
3. At 8 a.m., swallow the capsule with a drink of water.

4. 8.05 a.m. Gamma-camera scan. This simply means lying still on a table for 2-3 minutes. You may have a drink of tea or coffee.

5. The scan will then be repeated at hourly intervals until 8 p.m. with some extra scans at half past the hour where needed.

6. 10.00 a.m. Breakfast. We will provide lunch at 12, tea at 3 p.m. and an evening meal at 6 p.m. You may drink tea or coffee freely during the day.

7. Further breath samples will be taken at half-hourly intervals during the study.

8. You may return home at 8.15 p.m.

Please feel free to ask as many questions as you like about this project.
Detailed Protocol

Friday

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>19.00</td>
<td>Breath samples x2 (b.s.)</td>
</tr>
<tr>
<td>21.00</td>
<td>b.s.</td>
</tr>
</tbody>
</table>

Remember to label all tubes clearly with name, date, time
Do not eat anything after 9 p.m.

Saturday

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>07.45</td>
<td>Arrive at Addenbrooke's Hospital</td>
</tr>
<tr>
<td>07.45</td>
<td>b.s.</td>
</tr>
<tr>
<td>08.00</td>
<td>Take 2 x $^{13}$C glucose capsules and $^{99m}$Tc capsules</td>
</tr>
<tr>
<td>08.05</td>
<td>Initial scan..... Tea/coffee</td>
</tr>
<tr>
<td>08.30</td>
<td>scan</td>
</tr>
<tr>
<td>09.00</td>
<td>b.s.</td>
</tr>
<tr>
<td>09.30</td>
<td>scan</td>
</tr>
<tr>
<td>10.00</td>
<td>b.s.</td>
</tr>
<tr>
<td>11.00</td>
<td>scan breakfast - provided capsules have left stomach</td>
</tr>
<tr>
<td>12.00</td>
<td>scan</td>
</tr>
<tr>
<td>13.00</td>
<td>scan......lunch more scans/breaths</td>
</tr>
<tr>
<td>14.00</td>
<td>scan may be needed</td>
</tr>
<tr>
<td>15.00</td>
<td>scan to identify exact</td>
</tr>
<tr>
<td>16.00</td>
<td>scan......tea/coffee time of arrival of</td>
</tr>
<tr>
<td>17.00</td>
<td>scan capsules in caecum</td>
</tr>
<tr>
<td>18.00</td>
<td>scan</td>
</tr>
<tr>
<td>19.00</td>
<td>scan</td>
</tr>
<tr>
<td>20.00</td>
<td>scan......dinner</td>
</tr>
</tbody>
</table>

Leave Department of Nuclear medicine

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>21.00</td>
<td>b.s.</td>
</tr>
<tr>
<td>22.00</td>
<td>b.s.</td>
</tr>
<tr>
<td>23.00</td>
<td>b.s.</td>
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</table>

Sunday

<table>
<thead>
<tr>
<th>Time</th>
<th>Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>08.00</td>
<td>b.s.</td>
</tr>
<tr>
<td>09.00</td>
<td>b.s.</td>
</tr>
</tbody>
</table>

Fill in expenses form
Return tubes/breath samples etc. to Dr. Cummings
Diet

2 or 3 days as needed

**Breakfast**
- Rice Krispies
- Trifyba (1 packet)
- Milk
- Brown bread, butter, honey

**Lunch**
- Beef sandwich (Days 1 and 3)
- Chicken sandwich (Day 2)

**Evening**
- Lamb (Day 1); Pork (Day 2);
- Fish/Seafood (Day 3); with
- Potato (+ milk/butter)
- Salad - lettuce
tomato
cucumber
radish
mushroom
- French dressing (olive oil, vinegar, mustard)
- Fresh fruit salad and cream

Tea or coffee + milk - freely
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