PREPARATION AND EVALUATION OF MICROPARTICLES TO TARGET DRUGS TO DIFFERENT REGIONS OF THE GASTROINTESTINAL TRACT

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ABSTRACT

A novel method based on the emulsification solvent evaporation (ESE) technique has been used to prepare modified release microparticles. The aims of this study were to investigate whether the ESE method was suitable for the production of delayed release (pH sensitive and a combined pH sensitive and microflora responsive) microparticles and extended release (pH independent) microparticles. Further objectives included the characterisation of the microspheres and to identify the key parameters influencing the particle formation process and the distribution of drugs within the systems.

The ESE method used was shown to be a universal technique in the preparation of various types of modified release systems from a range of polymers (polymethacrylate (Eudragit L and S), hydroxypropylmethylcellulose phthalate, polyvinyl acetate phthalate, ethylcellulose and polyvinyl acetate) not only for targeting the upper and lower gastrointestinal tract but also for slow release applications. It was evident that the stability of the emulsion system could be used as a tool to screen for microparticle formulation since a stable emulsion provided particles of small size and with a spherical shape whereas an unstable emulsion led to the formation of irregular shaped structures. The physicochemical properties of the drug could also be used to predict the characteristics of the microparticles. Confocal laser scanning microscopy revealed that the pattern of drug distribution within the microparticles was greatly affected by the partition coefficient and the solubility of the drug in the internal phase.

Furthermore, it was found that the process parameters had great impact on the properties of the microparticles. The encapsulation efficiency and microparticle size were influenced by the concentration of the emulsifier, while the drug release characteristics were most affected by drug loading. Finally, the knowledge gained from the findings in this thesis can contribute to decision making in formulation design and development of microparticulate systems.
Dedicated To My Beloved

Father “Preecha” and Mother “Unchalee”
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PLAGIARISM STATEMENT

This thesis describes research conducted in The School of Pharmacy, University of London between 2004 and 2007 under the supervision of Dr. Abdul W. Basit. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

Signature

Date
LIST OF CONTENTS

ABSTRACT ..............................................................................................................................2
ACKNOWLEDGEMENTS ....................................................................................................4
PLAGIARISM STATEMENT ................................................................................................5
LIST OF CONTENTS .............................................................................................................6
LIST OF TABLES ..................................................................................................................10
LIST OF FIGURES ................................................................................................................12
LIST OF ABBREVIATIONS ...............................................................................................20

CHAPTER ONE

Introduction

1.1 Overview ..........................................................................................................................22
1.2 Oral drug delivery in relation to human gastrointestinal anatomy and physiology. 23
  1.2.1 The stomach .............................................................................................................24
  1.2.2 The small intestine ..................................................................................................26
  1.2.3 The large intestine ...................................................................................................26
  1.2.4 Physiological factors affecting the performance of oral modified release dosage forms ..................................................................................................................................27
    1.2.4.1 Gastrointestinal pH ...........................................................................................27
    1.2.4.2 Gastrointestinal transit ....................................................................................30
    1.2.4.3 Gastrointestinal fluid .......................................................................................35
    1.2.4.4 Gastrointestinal bacteria ..................................................................................38
1.3 Intestinal targeting via the oral route ............................................................................39
  1.3.1 Drug delivery to the small intestine .......................................................................39
  1.3.2 Drug delivery to the colon .....................................................................................40
1.4 The advantages of multiple unit dosage forms ............................................................45
1.5 Microencapsulation ........................................................................................................46
  1.5.1 Microencapsulation technique .............................................................................47
    1.5.1.1 Coacervation ....................................................................................................48
    1.5.1.2 Spray drying .....................................................................................................48
    1.5.1.3 Extrusion spheronisation .................................................................................50
    1.5.1.4 Emulsification solvent evaporation .................................................................50
1.6 Scope and Purpose of study ...........................................................................................56

CHAPTER TWO

Preparation and characterisation of modified release microparticles by emulsification solvent evaporation method

2.1 Introduction ....................................................................................................................59
SECTION ONE: PREPARATION AND CHARACTERISATION OF DELAYED RELEASE AND EXTENDED RELEASE MICROPARTICLES

2.2 Introduction ..................................................................................................................... 63
2.3 Materials ........................................................................................................................... 66
2.4 Methods ............................................................................................................................ 72
  2.4.1 Preparation of microparticles ................................................................................. 72
  2.4.2 Batch yield ............................................................................................................... 74
  2.4.3 Particle size analysis ............................................................................................... 74
  2.4.4 Scanning Electron Microscopy .............................................................................. 74
  2.4.5 Determination of prednisolone encapsulation efficiency .................................... 75
  2.4.6 In-vitro release studies ............................................................................................ 75
  2.4.7 Apparent miscibility/ immiscibility of organic solvent and liquid paraffin .... 79
2.5 Results and discussion ................................................................................................... 80
  2.5.1 pH sensitive microparticles .................................................................................... 80
    2.5.1.1 Eudragit L and S ............................................................................................... 80
    2.5.1.2 Hydroxypropylmethylcellulose phthalate ..................................................... 83
    2.5.1.3 Polyvinyl acetate phthalate ............................................................................. 91
    2.5.1.4 Cellulose acetate phthalate .............................................................................. 94
    2.5.1.5 Cellulose acetate trimellitate ........................................................................... 96
  2.5.2 pH independent microparticles .............................................................................. 97
    2.5.2.1 Ethylcellulose ................................................................................................... 97
    2.5.2.2 Polyvinyl acetate ............................................................................................ 101
2.6 Section one conclusions ............................................................................................... 103

SECTION TWO: PREPARATION AND CHARACTERISATION OF A COMBINED pH SENSITIVE AND MICROFLORA RESPONSIVE MICROPARTICLE SYSTEM

2.7 Introduction .................................................................................................................... 104
2.8 Materials ......................................................................................................................... 108
2.9 Methods .......................................................................................................................... 117
  2.9.1 Solubility Test ........................................................................................................ 117
  2.9.2 Method of preparation of microparticles ............................................................. 118
  2.9.3 In-vitro release study ............................................................................................ 119
2.10 Results and discussion ............................................................................................... 119
2.11 Section two conclusions ............................................................................................. 128
2.12 Overall conclusions ..................................................................................................... 128

CHAPTER THREE

An investigation into the process factors affecting the emulsion solvent evaporation process and the stability of the final product

3.1 Introduction .................................................................................................................... 131

SECTION ONE: STUDIES ON THE STABILITY OF THE EMULSION SYSTEM

3.2 Introduction .................................................................................................................... 132
  3.2.1 Stability of emulsion .............................................................................................. 133
3.3 Materials ......................................................................................................................... 137
CHAPTER FIVE

Conclusion and future perspectives

5.1 Conclusions ........................................................................................................ 216
5.2 Future perspectives ............................................................................................. 219

REFERENCES ............................................................................................................. 220
LIST OF TABLES

CHAPTER ONE

Table 1.1 Luminal pH measured along the small and large intestine ...........................................28
Table 1.2 Gastrointestinal free fluid volume of human subjects under fed and fasted condition .........................................................................................................................................36
Table 1.3 Location and contact of capsules with “free fluid” in the small and large intestine in fasted and fed volunteers .............................................................................................................37
Table 1.4 pH sensitive polymers commonly employed in the delayed release dosage form ..........................................................................................................................................................40
Table 1.5 Advantages of multiple units over single unit dosage forms ...........................................45
Table 1.6 Summary of the advantages and disadvantages of the microencapsulation techniques ................................................................................................................................................49
Table 1.7 Examples of pH sensitive and pH independent polymers that have been employed to produce microparticles ........................................................................................................51

CHAPTER TWO

Table 2.1 Summary of the parameters affecting the rate of microparticle solidification 61
Table 2.2 Examples of non-ionic surfactants ....................................................................................65
Table 2.3 Types and properties of HPMCP ....................................................................................68
Table 2.4 Grades and viscosities of EC .........................................................................................71
Table 2.5 Grades and viscosity of PVAc .......................................................................................71
Table 2.6 List of internal phase organic solvents and polymer concentrations used in the study ................................................................................................................................................73
Table 2.7 The properties of internal phase solvents .........................................................................85
Table 2.8 Characteristics of prednisolone loaded HP50 microparticles ........................................86
Table 2.9 Characteristics of HP55/ prednisolone microparticles ....................................................88
Table 2.10 Characteristics of PVAP microparticles ........................................................................93
Table 2.11 Characteristics of EC microparticles ..........................................................................98
Table 2.12 Characteristics of PVAc microparticles ......................................................................102
Table 2.13 Water insoluble polymer combined with polysaccharide or oligosaccharide for colon specific drug delivery .................................................................106
Table 2.14 Summary of aqueous solubility of polysaccharides ..................................116
Table 2.15 Terms of approximate solubility (British Pharmacopeia, 2007) ..............117
Table 2.16 Summary of solubility of various polysaccharides in organic solvents .......121

CHAPTER THREE
Table 3.1 Ranking of the effects of process parameters on microparticle properties ...162
Table 3.2 Standard storage conditions in accordance with ICH QIA (R2) and ICH QIF ..................................................................................................................163
Table 3.3 Percentage of prednisolone encapsulation efficiency of Eudragit L and S microparticles in screw cap bottles in various conditions .........................167

CHAPTER FOUR
Table 4.1 Summary of physicochemical properties of prednisolone, dipyridamole, acridine orange base, riboflavin and riboflavin sodium phosphate .......................191
Table 4.2 Summary of characteristics of the Eudragit L100 microparticles ............198
Table 4.3 Saturation solubility of fluorescent drugs in various pH .........................199
Table 4.4 Summary of characteristics of the riboflavin and dipyridamole loaded Eudragit L microparticles .................................................................205
Table 4.5 Summary of characteristics of the dipyridamole loaded Eudragit S microparticles .................................................................................................209
Table 4.6 Ranking of the effects of properties of drug on microparticle characteristics 212
LIST OF FIGURES

CHAPTER ONE

Figure 1.1 Diagram of the human gastrointestinal tract.................................................. 25
Figure 1.2 pH profiles in human gastrointestinal tract.................................................... 29
Figure 1.3 Distribution of selected bacteria in various sites of gastrointestinal tract... 38
Figure 1.4 Microparticle structures.................................................................................... 47

CHAPTER TWO

Figure 2.1 Schematic diagram of the emulsification solvent evaporation procedure.. ................................................................................................................................ 59
Figure 2.2 Chemical structure of prednisolone................................................................. 67
Figure 2.3 Chemical structure of Eudragit L and Eudragit S............................................ 67
Figure 2.4 Chemical structure of hydroxypropylmethylcellulose phthalate............... 68
Figure 2.5 Chemical structure of cellulose acetate phthalate......................................... 69
Figure 2.6 Chemical structure of cellulose acetate trimellitate..................................... 69
Figure 2.7 Chemical structure of polyvinyl acetate phthalate........................................ 70
Figure 2.8 Chemical structure of ethylcellulose............................................................... 71
Figure 2.9 Chemical structure of polyvinyl acetate........................................................ 72
Figure 2.10 Chemical structure of sorbitan sesquioleate............................................... 72
Figure 2.11 Chromatogram of blank dissolution medium.............................................. 78
Figure 2.12 Chromatogram of prednisolone dissolved in dissolution medium.......... 78
Figure 2.13 Chromatogram of dissolution sample......................................................... 79
Figure 2.14 SEM of blank Eudragit L microparticles...................................................... 80
Figure 2.15 SEM of prednisolone loaded Eudragit L microparticles............................. 81
Figure 2.16 SEM of blank Eudragit S microparticles..................................................... 81
Figure 2.17 SEM of prednisolone loaded Eudragit S microparticles............................. 81
Figure 2.18 In-vitro drug release profiles of Eudragit L and S microparticles.............. 82
Figure 2.19 SEM of prednisolone loaded HP50 microparticles formed from polymer 10
%w/v in ethyl acetate/ methanol (1:2)................................................................. 84
Figure 2.20 SEM of prednisolone loaded HP50 microparticles formed from polymer 5% w/v in ethyl acetate/methanol (1:2) .................................................. 84
Figure 2.21 SEM of prednisolone loaded HP50 microparticles formed from polymer 2.5% w/v in ethyl acetate/methanol (1:2) ........................................ 84
Figure 2.22 SEM of prednisolone loaded HP50 microparticles formed from polymer 10% w/v in ethyl acetate/ethanol (1:1) ............................................. 84
Figure 2.23 SEM of prednisolone loaded HP50 microparticles formed from polymer 5% w/v in ethyl acetate/ethanol (1:1) ............................................. 84
Figure 2.24 SEM of prednisolone loaded HP50 microparticles formed from polymer 2.5% w/v in ethyl acetate/ethanol (1:1) ............................................. 84
Figure 2.25 In-vitro release profile of prednisolone from HP50 microparticles ............. 87
Figure 2.26 SEM of prednisolone loaded HP55 microparticles formed from polymer 5% w/v in ethyl acetate/methanol (1:2) ............................................. 88
Figure 2.27 SEM of prednisolone loaded HP55 microparticles formed from polymer 2.5% w/v in ethyl acetate/methanol (1:2) ............................................. 88
Figure 2.28 In-vitro release of prednisolone from HP55 microparticles .................... 89
Figure 2.29 SEM of prednisolone loaded HP55S microparticles formed from polymer HP55S 2.5 %w/v in ethyl acetate/methanol (1:2) ................................. 90
Figure 2.30 SEM of prednisolone loaded HP55S microparticles formed from polymer 5% w/v in ethyl acetate/methanol (1:2) ................................. 90
Figure 2.31 SEM of prednisolone loaded PVAP microparticles formed from polymer concentration 5% w/v in methanol ................................................. 91
Figure 2.32 SEM of prednisolone loaded PVAP microparticles formed from polymer concentration 2.5% w/v in methanol ................................................. 91
Figure 2.33 SEM of prednisolone loaded PVAP microparticles formed from polymer concentration 5% w/v in dichloromethane/methanol (1:1) .................. 92
Figure 2.34 SEM of prednisolone loaded PVAP microparticles formed from polymer concentration 2.5% w/v in dichloromethane/methanol (1:1) .................. 92
Figure 2.35 In-vitro release of prednisolone from PVAP microparticles, produced by using the polymer concentration 5% w/v ........................................ 94
Figure 2.36 SEM of prednisolone loaded CAP microparticles, prepared by using acetone/ethanol (2:1) ................................................................. 95
Figure 2.37 SEM of prednisolone loaded CAP microparticles, prepared by using dichloromethane/ethanol (2:1) .................................................................................. 95

Figure 2.38 SEM of prednisolone loaded CAT microparticles, prepared by using acetone/ethanol (2:1) ................................................................................................. 96

Figure 2.39 SEM of prednisolone loaded CAT microparticles, prepared by using dichloromethane:ethanol (2:1) ................................................................. 96

Figure 2.40 SEM of prednisolone loaded N-7 microparticles ........................................ 98

Figure 2.41 SEM of prednisolone loaded N-100 microparticles .................................. 98

Figure 2.42 In-vitro prednisolone release from N-7 and N-100 microparticles .......... 100

Figure 2.43 SEM of prednisolone loaded B-500 microparticles .................................. 101

Figure 2.44 SEM of prednisolone loaded B-60 microparticles .................................. 101

Figure 2.45 In-vitro prednisolone release from PVAc microparticles ......................... 102

Figure 2.46 Chemical structure of chitosan .................................................................. 109

Figure 2.47 Chemical structure of pectin ..................................................................... 110

Figure 2.48 Chemical structure of guar gum .............................................................. 110

Figure 2.49 Chemical structure of chondroitin sulphate ............................................. 111

Figure 2.50 Chemical structure of dextran .................................................................... 112

Figure 2.51 Chemical structure of amylose ................................................................ 112

Figure 2.52 Chemical structure of inulin ...................................................................... 113

Figure 2.53 Chemical structure of Isomaltulose .......................................................... 113

Figure 2.54 Chemical structure of β-cyclodextrin ....................................................... 114

Figure 2.55 Eudragit S/prednisolone microparticles prepared by a mixture of water and ethanol .............................................................................................................. 120

Figure 2.56 SEM of Prednisolone loaded HP-β-CD microparticles, polymer to drug ratio (10:1) .............................................................................................................. 122

Figure 2.57 In-vitro drug release of HP-β-CD microparticles ........................................ 122

Figure 2.58 SEM of combined Eudragit S and HP-β-CD (8.8% w/w)/prednisolone microparticles .................................................................................................................. 123

Figure 2.59 In-vitro drug release of Eudragit S microparticles and combined Eudragit S and HP-β-CD (8.8% w/w) microparticles .............................................................. 124
CHAPTER THREE

Figure 3.1 A schematic diagram which represents the emulsion instability by various processes ................................................................. 136

Figure 3.2 Phase separation time of the various emulsion formulations ............... 139

Figure 3.3 SEM of Eudragit L microparticles .................................................. 140

Figure 3.4 SEM of Eudragit S microparticles .................................................. 140

Figure 3.5 SEM of HP55 microparticles ......................................................... 140

Figure 3.6 SEM of CAT microparticles ......................................................... 140

Figure 3.7 SEM of CAP microparticles ......................................................... 140

Figure 3.8 Particle size and size distribution of different emulsion formulation .... 142

Figure 3.9 Effect of stirring rate on the properties of microparticles ................. 146

Figure 3.10 SEM of microparticles formed from stirring at 300 rpm ................. 147

Figure 3.11 SEM of microparticles formed from stirring at 500 rpm ................. 147

Figure 3.12 SEM of microparticles formed from stirring at 1000 rpm ............... 147

Figure 3.13 SEM of microparticles formed from stirring at 1500 rpm ............... 147

Figure 3.14 In-vitro drug release of prednisolone loaded Eudragit S microparticles fabricated using various stirring speed ......................... 148
Figure 3.15 Effect of drug loading on particle size, batch yield and encapsulation efficiency of Eudragit S/prednisolone microparticles.............................149

Figure 3.16 SEM of drug free microparticles.............................................................150

Figure 3.17 Microparticles formed from drug content 9.1% w/w..................................151

Figure 3.18 Microparticles formed from drug content 16.7% w/w..................................151

Figure 3.19 Microparticles formed from drug content 50.0% w/w.................................151

Figure 3.20 Microparticles formed from drug content 66.7% w/w.................................152

Figure 3.21 In-vitro drug release of Eudragit S/prednisolone microparticles fabricated using various drug loading.................................................................153

Figure 3.22 Effect of polymer concentration on the properties of microparticles.............154

Figure 3.23 SEM of microparticles formed from polymer concentration 5% w/v............155

Figure 3.24 SEM of microparticles formed from polymer concentration 7.5% w/v.........155

Figure 3.25 SEM of microparticles formed from polymer concentration 10% w/v.........156

Figure 3.26 SEM of microparticles formed from polymer concentration 15% w/v.........156

Figure 3.27 In-vitro drug release of Eudragit S/prednisolone microparticles fabricated using various polymer concentrations.........................................................156

Figure 3.28 Effect of surfactant concentration on properties of microparticles...............157

Figure 3.29 SEM of Eudragit S microparticles formed from emulsifier 0.25% w/v..........159

Figure 3.30 SEM of Eudragit S microparticles formed from emulsifier 0.5% w/v...........159

Figure 3.31 SEM of Eudragit S microparticles formed from emulsifier 1% w/v...............159

Figure 3.32 SEM of Eudragit S microparticles formed from emulsifier 2% w/v...............159

Figure 3.33 Eudragit S microparticles formed from emulsifier 3% w/v........................160

Figure 3.34 In-vitro drug release of Eudragit S/prednisolone microparticle fabricated using various emulsifier concentrations....................................................161

Figure 3.35 A 14 mL clear glass screw cap bottle (left) and a 20 mL clear glass injection vials with aluminium cap seal (right) used in this experiment.................165
Figure 3.36 Dissolution profiles of Eudragit L microparticles stored at 40°C/0%RH....
........................................................................................................................................
168

Figure 3.37 Dissolution profiles of Eudragit L microparticles stored at 25°C/60%RH..
..........................................................................................................................................
168

Figure 3.38 Dissolution profiles of Eudragit L microparticles stored at 40°C/75%RH..
...........................................................................................................................................
169

Figure 3.39 Dissolution profiles of encapsulated Eudragit L microparticles stored in
screw cap bottle at various time points at 40°C/75%RH.................................169

Figure 3.40 Dissolution profiles of encapsulated Eudragit L microparticles stored in
crimped vial at various time points at 40°C/75%RH......................................170

Figure 3.41 Dissolution profiles of non-encapsulated Eudragit L microparticles stored in
screw cap bottle at various time points at 40°C/75%RH..............................170

Figure 3.42 Dissolution profiles of non-encapsulated Eudragit L microparticles stored in
crimped vial at various time points at 40°C/75%RH..............................171

Figure 3.43 Dissolution profiles of Eudragit S microparticles stored at 40°C/0%RH....
...........................................................................................................................................
171

Figure 3.44 Dissolution profiles of Eudragit S microparticles stored at 25°C/60%RH....
..........................................................................................................................................
172

Figure 3.45 Dissolution profiles of Eudragit S microparticles stored at 40°C/75%RH...
..........................................................................................................................................
172

Figure 3.46 Dissolution profiles of encapsulated Eudragit S microparticles stored in
screw cap bottle at various time points at 40°C/75%RH...............................173

Figure 3.47 Dissolution profiles of encapsulated Eudragit S microparticles stored in
crimped vial at various time points at 40°C/75%RH...............................173

Figure 3.48 Dissolution profiles of non-encapsulated Eudragit S microparticles, stored in
screw cap bottle at various time points at 40°C/75%RH...............................174

Figure 3.49 Dissolution profiles of non-encapsulated Eudragit S microparticles, stored in
crimped vial at various time points at 40°C/75%RH...............................174

Figure 3.50 SEM of cross section of prednisolone loaded Eudragit L microparticles,
initial............................................................................................................................175
Figure 3.51 SEM of cross section of prednisolone loaded Eudragit S microparticles, initial.................................................................175

Figure 3.52 SEM of encapsulated Eudragit L microparticles, stored in 25°C, 60%RH at 13 weeks...............................................................176

Figure 3.53 SEM of encapsulated Eudragit L microparticles, stored in 40°C, 75%RH at 13 weeks...............................................................176

Figure 3.54 SEM of Eudragit L microparticles, stored at ambient condition, 26 weeks.................................................................176

Figure 3.55 SEM of encapsulated Eudragit S microparticles, stored in 25°C, 60%RH at 13 weeks...............................................................177

Figure 3.56 SEM of encapsulated Eudragit S microparticles, stored in 40°C, 75%RH at 13 weeks...............................................................177

Figure 3.57 SEM of Eudragit S microparticles, stored at ambient condition 26 weeks.................................................................177

CHAPTER FOUR

Figure 4.1 Principal light pathways in confocal microscopy.................................183

Figure 4.2 Jablonski diagram illustrating the fluorescence process..................184

Figure 4.3 Structure of dipyridamole...............................................................185

Figure 4.4 Structure of riboflavin.....................................................................185

Figure 4.5 Structure of riboflavin sodium phosphate........................................186

Figure 4.6 Structure of acridine orange.............................................................186

Figure 4.7 CLSM and transmitted light images of blank Eudragit L microparticle ....192

Figure 4.8 Dipyridamole loaded microparticles ..............................................194

Figure 4.9 Riboflavin loaded microparticles ....................................................195

Figure 4.10 Riboflavin sodium phosphate loaded microparticles ......................196

Figure 4.11 Acridine orange loaded microparticles........................................197

Figure 4.12 In-vitro release of riboflavin sodium phosphate, riboflavin, dipyridamole and acridine orange from Eudragit L microparticles (polymer to drug ratio, 30:1).................................................................199
Figure 4.13 SEM images of cross section of riboflavin microparticles, polymer to drug ratio 5:1

Figure 4.14 SEM images of cross section of riboflavin microparticles, polymer to drug ratio 30:1

Figure 4.15 SEM images of cross section of riboflavin microparticles, polymer to drug ratio, 70:1

Figure 4.16 Eudragit L/ riboflavin (5:1) microparticles

Figure 4.17 Eudragit L/ riboflavin (30:1) microparticles

Figure 4.18 Eudragit L/ riboflavin (70:1) microparticles

Figure 4.19 Eudragit L/ dipyridamole (5:1) microparticles

Figure 4.20 Eudragit L/ dipyridamole (30:1) microparticles

Figure 4.21 Eudragit L/ dipyridamole (70:1) microparticles

Figure 4.22 In-vitro release of riboflavin loaded Eudragit L microparticles

Figure 4.23 In-vitro release of dipyridamole loaded Eudragit L microparticles

Figure 4.24 Eudragit L/ riboflavin (312:1) microparticles

Figure 4.25 In-vitro release of riboflavin loaded Eudragit L microparticles

Figure 4.26 Eudragit S/ dipyridamole (5:1) microparticles

Figure 4.27 Eudragit S/ dipyridamole (30:1) microparticles

Figure 4.28 Eudragit S/ dipyridamole (70:1) microparticles

Figure 4.29 In-vitro release of dipyridamole loaded Eudragit S microparticles
LIST OF ABBREVIATIONS

BP  British pharmacopeia
CAP  Cellulose acetate phthalate
CAT  Cellulose acetate trimellitate
CLSM  Confocal laser scanning microscopy
EC  Ethylcellulose
ESE  Emulsification solvent evaporation
GI  Gastrointestinal
HCl  Hydrochloric acid
HLB  Hydrophilic-lipophilic balance
HPLC  High performance liquid chromatography
HPMCP  Hydroxypropylmethylcellulose phthalate
ICJ  Ileocaecal junction
MMC  Migrating Myoelectric Complex
NaOH  Sodium hydroxide
Na\(_2\)PO\(_4\)  Tribasic sodium phosphate
O/O  Oil in oil emulsion
pKa  negative logarithm of ionisation constant
PVAc  Polyvinyl acetate
PVAP  Polyvinyl acetate phthalate
RH  Relative humidity
SCFA  Short chain fatty acid
SD  Standard deviation
SEM  Scanning electron microscopy
USP  United states pharmacopeia
UV  Ultraviolet
W/O  Water in oil emulsion
W/O/W  Water in oil in water emulsion
CHAPTER ONE

Introduction
1.1 Overview

Site specific drug delivery by the oral route offers great benefits such as being convenient for patients and improving drug therapy by maximising therapeutic activity and safety and minimizing systemic side effects. Delivering drugs to different regions in the gastrointestinal (GI) tract is challenging since the physiology of the GI tract is complicated and has a great impact on the effectiveness of modified release systems.

Multiple unit dosage forms such as granules, pellets or microparticles have advantages over conventional single unit systems (tablets or capsules) in modified release applications. Microparticles have further benefits over other multiple unit systems because of their small particle size. This should translate into rapid emptying from the stomach and more reproducible transit through the small intestine. Also the larger surface area of the microparticles should facilitate solubilisation and release of the drug resulting in rapid drug dissolution, more reproducible absorption and reproducible bioavailability.

This thesis addresses the potential of emulsion solvent evaporation (ESE) as a versatile technique in preparing small sized particles with modified release properties. Although ESE is a conceptually simple technique, it is normally difficult to achieve the desirable characteristics of modified release microparticles. This is because there are many variables influencing the manufacturing process and also the microparticle formation is a complicated process. The parameters affecting the process of microparticle formation and the key process parameters influencing the characteristics of microparticles are investigated and identified in this study. This study will provide a fundamental understanding of a means for achieving the production of a desirable quality of microparticles.
Chapter One

1.2 Oral drug delivery in relation to human gastrointestinal anatomy and physiology

The most popular and convenient route of drug administration is the oral route (Chien, 1992). There are various designs of dosage forms of pharmaceutical products for oral delivery such as semisolid, solid and liquid dosage form. The solid dosage forms are addressed in this thesis. Two types of oral drug delivery concepts, immediate release and modified release are discussed below.

The immediate release dosage form is designed for rapid release and absorption of the drug. Following administration, the dosage form disintegrates in the stomach, dissolves and then releases the drug rapidly. Most of the drug is absorbed in the upper regions of the small intestine. There are many limitations to this system due to its non site specific action and lack of ability to maintain therapeutic plasma concentrations.

The modified release dosage form provides an effective means to optimise the concentration of drug in the plasma and the drug bioavailability. Therefore, this type of dosage form improves the treatment of many chronic diseases, reduces side effects and also reduces the total amount of drug administered to a minimum. Modified release can be categorised into two types, delayed release and extended release. The delayed release dosage form is designed to protect the drug within the dosage form and release it at a specific location. An example of this system is the enteric coated dosage form, in which the enteric coating is resistant to dissolution in an acidic pH but dissolves rapidly at a pH greater than the pH threshold of the coating polymers; therefore, the drug is prevented from releasing in the stomach but is released at the site in the intestinal tract. The extended release dosage form refers to sustained release, controlled release and rate-controlled drug delivery systems. The aim of an extended release system is to maintain therapeutic levels of a drug in blood or tissue for an extended period. The extended release system releases drug slowly and the rate of drug release is controlled by the dosage form. This system is beneficial to drugs with short biological half-lives and a narrow therapeutic index by improving the therapeutic effect, patient compliance and also reducing systemic side effects.
The modified release dosage forms can be categorised into two types, the multiple units dose and single unit dose (Hunter et al., 1982). Multiple units are composed of many sub-units such as pellets, granules, microparticles and nanoparticles contained in a capsule or tablet, which disintegrates in the stomach into a large number of sub-units and then disperses throughout the GI tract. Each singular sub-unit behaves as an individual modified release. In contrast single unit dosage forms, which comprise one non-disintegrating unit, retain their structure through the digestive tract (Bechgaard and Christensen, 1982).

To achieve site specific drug delivery via the oral route, it is important to understand the human gastrointestinal physiology. There are three main anatomical areas of the gastrointestinal (GI) tract, namely the stomach, the small intestine and the large intestine (Figure 1.1) and the main functions of the GI tract are the digestion of food, absorption of nutrients and the elimination of waste materials.

1.2.1 The stomach

Anatomically, the stomach is divided into four parts, the fundus, body, antrum and pylorus. The stomach mucosa forms numerous longitudinal folds, called rugae and the mucosal surface is lined by a layer of simple columnar epithelium, containing a great number of narrow channels known as gastric pits, at the bottom of which are found gastric glands. There are three main types of secretary cells dominant in the gastric glands: mucous cells secrete mucus to protect the epithelium lining; chief cells secrete pepsinogen, the precursor for the enzyme pepsin and parietal cells secrete mainly hydrochloric acid. The stomach secretes about 1-1.5 litres of gastric juice per day. The stomach is not the main site for drug absorption due to its small surface area and physical barriers such as the mucus layer residing on the mucosal surface.
Chapter One

Figure 1.1 Diagram of the human gastrointestinal tract (adapted from Genesis Health System.com)

The main functions of the stomach are acting as a temporary reservoir for food, reducing and mixing the ingested materials with acid and enzymes to form chyme, which facilitates the absorption of nutrients from the small intestine and controls the delivery of food to the small intestine. Moreover, the stomach produces hydrochloric acid, a bacteriostatic, to maintain the pH of the stomach at the level at which pepsin, an enzyme secreted by the gastric pits of the stomach, which breaks down proteins (polypeptide chains) into smaller polypeptide chains, is active. Production of gastric acid is stimulated by the hormone gastrin, which is activated by the distension of the stomach, peptides and amino acid. In the fasted state, the pH of the stomach is 1 to 2.5 (Evans et al., 1988). In the presence of food the pH in the stomach rises to 5 or above, this increase in stomach pH is transient, lasting for only a short duration (Hardy et al., 1987a).
1.2.2 The small intestine

The length of the small intestine, the longest part of the GI tract, is about four to six metres and its diameter is approximately 6 cm. The small intestine can be divided into three parts which are the duodenum (30 cm), the jejunum (240 cm) and finally the ileum (360 cm) (Tortora and Grabowski, 1996). The major functions of the small intestine are digestion and absorption. Within the GI tract, the small intestine is the main site for absorption as it has a large surface area provided by the plicae circulares, villi and microvilli (brush border) with a total surface area of about 200 m² (in an adult) (Wynsberghe et al., 1995). Plicae circulares are deep, permanent circular folds of the mucous membrane and underlying submucosa. The surface of the mucous membrane of the small intestine consists of villi. The finger-like shaped fine structures projecting from the villi surface are called microvilli and these greatly increase the surface area. Most absorption takes place in the duodenum and jejunum since these sites contain more prominent plicae circulares and villi (Aiache and Aiache, 1985).

The mucosal epithelium in the intestine contains not only columnar absorptive cells but also the crypt of Lieberkühn, which consists of at least 4 different types of cell: the Paneth cells which secrete large amounts of protein-rich material, goblet cells which secrete mucus, undifferentiated cells responsible for the renewal process of epithelium and endocrine cells which produce peptides and hormones. The combination of the secretion from the cells in the crypt, the secretion of bicarbonate from Brunner's glands (found only in the duodenum) and the secretion of alkaline pancreatic juice help to neutralise the pH of the acidic chyme and also prevent mucosal damage by the rapid neutralisation of hydrochloric acid and the inactivation of pepsin. The pH rises gradually along the length of the small intestine from the duodenum to the ileum (Evans et al., 1988). The small intestine connects to the large intestine at the ileocaecal junction (ICJ).

1.2.3 The large intestine

The length of the large intestine, the last part of the GI tract, is about 150 cm. The large intestine also known as the colon is composed of the caecum (8.5 cm), the ascending (20
cm), the transverse (45 cm), the descending (30 cm), the sigmoid colon (40 cm) and the rectum (15 cm). The colonic structure is different from the structure of other GI tract sites as it possesses a larger diameter, thinner wall and very viscous luminal contents. The main functions of the large intestine are reabsorption of electrolytes and water, storage and the formation of faeces. Daily, approximately 1.5 litres of chyme enter the colon, of which only about 200 mL is discarded as faeces (Martini, 1995). The colon is a poor site for drug absorption as it has a low surface area with a lack of villi and the faeces within it is prone to impaction. The “tighter” junctions between the epithelial cells in the colonic mucosa also hinder the paracellular absorption of drugs (Hayton, 1980). However, the slow colonic transit increases the time available for drug absorption since the duration within which the drug is in contact with the absorptive surface is prolonged (Khosla and Davis, 1989).

Furthermore, the endogenous and exogenous substrates which are not digested in the upper GI tract are metabolised by colonic bacteria enzymes. The colon is colonized by a considerable number of various bacteria, up to 400 different species of both the aerobic and anaerobic type (Gorbach et al., 1967). However, it is mainly anaerobic bacteria that are found in the colon, particularly *Bacteroides*, *Bifidobacterium* and *Eubacterium*. The caecum is the main site of bacterial activity. Bacteria receive energy and nutrients from dietary fibre, undigested polysaccharide in the diet and the carbohydrate elements of secretions. The main end product from the fermentation of proteins and carbohydrates are short chain fatty acids (SCFA) which are readily absorbed in the colon and consequently stimulate water and sodium absorption (Cummings, 1981). The formation of SCFA results in a decrease in the luminal pH of the caecum (6-6.5). Thereafter the pH in the lumen increases toward the final part of the colon (Evans et al., 1988).

### 1.2.4 Physiological factors affecting the performance of oral modified release dosage forms

#### 1.2.4.1 Gastrointestinal pH

As mentioned earlier regarding the pH gradient of each region in the GI tract, there is a
variation in pH between different regions and the segments within those regions. The most comprehensive studies on pH measurement in different regions of the healthy human GI tract are the work of Evans et al (1988) and Fallingborg et al (1989). Both studies measured the gastrointestinal pH profiles of healthy adult humans conducting normal daily activities by using a radiotelemetry capsule. The summary of the intestinal pH profiles (performed in 66 volunteers) reported by Evans et al (1988) is shown in Table 1.1 and the pH profile of the gut measured in 39 subjects reported by Fallingborg et al (1989) is demonstrated in Figure 1.2.

Table 1.1 Luminal pH measured along the small and large intestine (Evans et al., 1988)

<table>
<thead>
<tr>
<th>Intestinal site</th>
<th>Mean pH ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal small intestine</td>
<td>6.63±0.53</td>
</tr>
<tr>
<td>Mid small intestine</td>
<td>7.41±0.36</td>
</tr>
<tr>
<td>Distal small intestine</td>
<td>7.49±0.46</td>
</tr>
<tr>
<td>Ascending colon</td>
<td>6.37±0.58</td>
</tr>
<tr>
<td>Transverse colon</td>
<td>6.61±0.83</td>
</tr>
<tr>
<td>Descending colon</td>
<td>7.04±0.67</td>
</tr>
</tbody>
</table>

Both the studies of Evans et al (1988) and of Fallingborg et al (1989) showed that the pH in the stomach is highly acidic but on entry into the small intestine the pH rises rapidly and then slowly increases along the length of the small intestine. The pH drops when entering the caecum and then rises gradually on moving toward the distal colon (Table 1.1 and Figure 1.2). However, the Fallingborg study observed a more dramatic fall in luminal pH when the capsule emptied from the distal small intestine (medial pH = 7.3) into the large intestine (median pH = 5.7, 5.6, 5.7 and 6.6 in the caecum, ascending, transverse and descending colon, respectively) and unlike in the study by Evans, the median pH did not reach pH 7 again in the descending colon.

As seen from Figure 1.2, the human luminal pH is highly variable. The gastrointestinal pH is variable between individuals since there are many factors influencing the pH level in the GI tract such as age (Goldschmiedt et al., 1991; Cryer et al., 1992), SCFA (Pye et al., 1987) and diseases (Gilbert et al., 1988; Raimundo et al., 1992; Fallingborg et al., 1989).
1993). For instance, a considerable decrease in colonic pH from (6.4-7) to (2.3-4.7) was observed during acute attacks of inflammatory bowel disease (IBD) (Roediger et al., 1984; Fallingborg et al., 1993). Gastric pH is also influenced by gender, there being higher median gastric pH in females (Feldman and Barnett, 1991).

This variability in pH also has a considerable influence on the performance of pH based systems. Also, the higher the pH in the distal small intestine than that of the colon and the dramatic drop in the pH from the distal small intestine to caecum (Evans et al., 1988; Fallingborg et al., 1989) causes a potential problem for a pH based approach to colonic delivery. There have been many reports on the failure of the enteric coating single unit dosage form for colonic delivery due to irreproducible drug release and the unpredictability of the site where the drug dissolves and releases from the system (Schroeder et al., 1987; Ashford et al., 1993a; Ibekwe et al., 2006). It is hypothesised
that the *in-vivo* performance of a pH based microparticulate system might be better than that of single unit dosage form because the small sized microparticles should transit more slowly through the colon and their larger surface area to volume ratio would facilitate the dissolution of the enteric polymer.

### 1.2.4.2 Gastrointestinal transit

The postprandial (fed) and inter-digestive (fasted) motility patterns are two distinct patterns of contraction and transit occurring in the GI tract, especially in the stomach and small intestine. The transit of pharmaceutical dosage forms depends highly on these motility patterns. The GI transit time has a great impact on the effectiveness of modified release dosage forms since the site of the drug release depends on the transit time of the GI tract. Most of the data on the GI transit times of dosage forms discussed in this chapter was obtained using the technique of gamma scintigraphy. This technique provides detailed information on the *in-vivo* performance of dosage forms such as GI transit time and the site of disintegration (Davis, 1986; Wilding et al., 1992; Basit et al., 2004).

#### The Stomach

The contractile activity known as the migrating myoelectric complex (MMC) appears during the fasted state (Szurszewski, 1969). MMC is divided into four distinctive phases (Code and Marlett, 1975). Phase I is a period of no activity (which takes 40-60 minutes). Phase II is of the same duration as phase I but mixing contractions in the stomach and intestine occur. Phase III lasts for 10-15 minutes and is known as a housekeeper wave because there are powerful peristaltic contractions which remove and clear residual materials from the stomach. Phase IV is a short transition period between phase III and phase I. The cycle repeats itself approximately every two hours until it is interrupted when a meal is eaten.

In the fasted state, many studies reported that the gastric emptying of single units and multiple unit dosage forms occurs within 2 to 3 hours (Wilding et al., 1992; Tuleu et al.,
2002; Basit et al., 2004). However, the gastric emptying of such dosage forms is unpredictable since it is dependent on the time of the arrival of MMC phase III. Also, the interval of housekeeper waves between the subjects is greatly variable. Therefore, the actual gastric emptying time in inter-individual subjects is highly variable (Davis et al., 1990).

In the fed state, the grinding, mixing and emptying of food are present together. The peristaltic contractions of the antrum serve to mix and grind food and move it towards the pylorus. In this state, the motility responds to the calorific value of the food. For instance, a heavy, high calorie breakfast delayed gastric emptying time for a longer period than a light, low calorie breakfast (Davis et al., 1984a; Davis et al., 1988). In addition, the gastric emptying time of liquid relies on the calorific value of the fluid and its volume (Stubbs, 1977).

Food and the nature of the dosage form are the two main parameters governing the gastric emptying of a modified release dosage form. Liquid, digestible and indigestible solids are emptied from the stomach at different gastric emptying rates. Gastric emptying of the liquid components appears at an exponential rate and is controlled by gastro-duodenal pressure, produced by the contraction of the proximal stomach (Kelly, 1980). The emptying of digestible solid components occurs in a linear fashion after a variable lag time (Notivol et al., 1984). Large digestible solids are detained in the stomach until they are ground to sufficiently smaller particles and are then vacated with the chyme. Davis (1986) proposed that small indigestible particles can empty with food whereas large indigestible solids are retained in the stomach until the interdigestive motility pattern returns and phase III of the MMC will then empty them with the remaining contents. Nevertheless, the gastric emptying of MMC phase III is not always efficient, as the studies of Coupe et al (1991) showed that in 1 out of 8 subjects the non-disintegrated capsule was retained for over 12 h in the stomach, withstanding emptying by the housekeeper waves.

It has been reported that the mean resting pyloric diameter, 12.8±7 millimetres (Munk et al., 1978), may be an indicator of the cut-off site for gastric emptying. In a fed state, if
the size of non-disintegrating materials are larger than the mean diameter of the pylorus, they are retained in the stomach and will be emptied when it reverts to the fasted mode (Davis et al., 1984b). Khosla et al (1989) also suggested that the gastric emptying of tablets from the fed stomach was random and determined by the diameter of the resting pylorus and the pressure gradient between the antrum and duodenum. The variability of the gastric emptying time increased with the increase in tablet size and larger tablets showed an irregular pattern of emptying and on occasion a “fortuitous emptying” of the dosage form with a digestible meal. However, Khosla et al (1989) proposed that there was no cut off size per se that can empty from the fed stomach but a gradual change of size over which the stomach allows particles to empty.

Clarke et al (1993; 1995) reported that the size of the pellets (0.5, 1.2 and 4.8 mm) did not affect the gastric emptying of the dosage form in the fasted state and no evidence that the small pellets emptied from the stomach with the co-administered liquid was observed. The pellets were only emptied from the stomach by the interdigestive cycle, MMC phase III. Clarke et al (1993) also revealed that increasing the density (from 1.5 to 2.6 g/cm³) of the small pellets (0.5 mm diameter) resulted in the prolongation of gastric emptying. Further experimentation by Clarke et al (1995) demonstrated that no appreciable difference in the gastric emptying of the pellets of size 1.2-1.4 mm was observed at a density up to 2.4 g/cm³. The possible reason for the difference in the gastric emptying of these pellets was that the higher density pellets (2.6 g/cm³) were able to resist the normal contractions of the stomach whereas the lighter density pellets could not. They also suggested that there is likely to be a threshold density of the order of 2.4-2.6 g/cm³, above which the gastric residence is prolonged.

Sugito et al (1992) studied the effect of the size of microcapsules which have a mean diameter size of 100, 500, 1100 µm on the gastric emptying rate. In this study, the gastric emptying rate was determined by pharmacokinetic parameters. They found that under fasting conditions, the size of microcapsules had considerable influence on the gastric emptying rate. The smaller particle sizes were emptied from the stomach at a faster rate than those with larger particle sizes. On the other hand, under fed conditions the gastric emptying rate was almost entirely the same, regardless of the size of the
particles and the emptying rate from the stomach of all preparations was slower than when administered under fasting conditions. This would seem to indicate the benefit of a modified release system containing small sized microparticles (under 100 μm) over the pellets when administered in the fasting condition since the small sized particles were able to empty rapidly from the stomach, which lead to a rapid drug release, easing the delivery of the drug to the various regions of the GI tract.

Indeed, the gastric emptying time is influenced by many factors, not only food and types of dosage form but also the postural position (Moore et al., 1988), pathological conditions (Rosswick et al., 1967), stress (Kaus and Fell, 1984), exercise (Cammack et al., 1982) and the type of drug (Urbain et al., 1990).

**The Small intestine**

Similarly to the stomach, the motility of the small intestine during the fasted and fed state displays different patterns. In the fasted state, the MMC controls the activity of the small intestine whereas in the fed state, the segmental and peristaltic contractions govern the pattern of motility. Segmental contractions mix chyme and digestive juices together and bring the intestinal content in contact with absorptive surfaces while the peristaltic wave serves to move the chyme through the small intestine. In the fasted mode, the small intestinal transit of a non-digestible pharmaceutical dosage form is influenced by phase III of the MMC (Code and Marlett, 1975; Kaus et al., 1984).

Sugito et al (1990a) found that the small intestinal transit time was not effected by the size of the dosage form. This was in agreement with the finding of Davis et al (1986b), who reported that there was no significant difference in the small intestinal transit time for solutions, pellets and single units formulations and that the mean intestinal transit time was relatively constant, approximately 3±1 hours. The transit time of dosage forms in the small intestine is also unaffected by pathological conditions (Hardy et al., 1988), age (Davis et al., 1986c) and the absence or presence of food (Mundy et al., 1989; Davis et al., 1990)
Chapter One

The small intestinal transit time is a crucial factor in the bioavailability of the drugs because most drugs are absorbed in the small intestine. In addition, the small intestinal residence time of the dosage forms is a vital factor for the sustained release system, enteric coated dosage forms and drugs that dissolve gradually in the small intestine.

The Ileocaecal junction

The ileocaecal junction (ICJ) serves as a mechanical barrier to protect the small intestine from bacterial overgrowth and prevents the reflux of colonic contents. Devereux et al (1987) found that before pellets arrived at the caecum, they accumulated at the ICJ and were then emptied into the colon as boluses. Transit time in the ICJ relies on the rate of contents accumulating in the ileum and is unaffected by the interdigestive cycle, MMC (Quigley et al., 1984). The residence time at the ICJ of non-disintegrating dosage forms, either single unit or multiple units was variable (Sugito et al., 1990). The stagnation time of non-disintegrating matrix tablets varied from 2 to 20 hours (Marvola et al., 1987). Adkin et al. (1993) also reported that the size of the tablets had no influence on the transit time through the ICJ. However if the size of the units is too large, their transit time may be extended due to their prolonged residence time at the ICJ.

The colon

Segmental contractions are the predominant motility pattern in the colon whereas the propulsive contractions appear only 3-4 times daily in healthy human. The colonic transit times are highly variable (Hardy, 1989). Hardy et al. reported that the transit times for dosage forms ranged from 1 hour to more than 60 hours (Hardy et al., 1985; Hardy et al., 1987b). The factors that have an influence on colonic transit are diet, stress, disease and types of dosage form (Barrow et al., 1991).

The colonic transit time is an important parameter for successfully targeting drugs to the colon. Accelerated transit time within the colon would have a considerable impact on drug bioavailability because it would reduce the time for the absorption of the drug. Nevertheless, extended colonic residence may effect the accumulation of multiple doses (Haeberin and Friend, 1992).
Chapter One

Colonic streaming has been described as a process in which the solid phases transit more rapidly through the colon than the liquid phase (Eastwood, 1975). It is presumed this phenomenon occurs because small multiple dosage forms such as pellets are recognised by the colon as digestible substances or liquid phase whereas the large single non-digestible dosage forms such as tablets are identified as a solid phase substance (Goto et al., 2004). Thus, the colonic transit of large single non-digestible dosage forms is more rapid compared to that of small multiple dosage forms. Colonic streaming, a selective retention of small particles, can also be called “colonic sieving”. As a result of the streaming effect the dispersive system, such as pellets, become broadly distributed within the colon within a longer period of time (Hardy et al., 1985; Hardy and Perkins, 1985) whereas the large single units move rapidly through the colon.

Colonic transit scintigraphy studies confirm that the colonic transit time of large single unit formulations within the colon was more rapid than that of small particles (Davis et al., 1984b; Hardy et al., 1985; Adkin et al., 1993; Abrahamsson et al., 1996). In the studies of Hardy et al (1985), the pellets (0.5-1.8 mm) were widely dispersed and moved through the colon more slowly than the large radiotelemetry capsule (25x9 mm) even though both formulations entered the colon together.

1.2.4.3 Gastrointestinal fluid

The dissolution and absorption of drugs are greatly dependent on the amount of fluid in the gut lumen. Gotch (1957) investigated the intraluminal gastrointestinal water volume of 13 human subjects at post-mortem. The mean water content of three different regions in the GI tract was measured and found to be 118 ml (range of 11-233 ml) in the stomach, 206 ml (range of 60-352 ml) in the small intestine and 83 ml (range of 7-430 ml) in the caecum and proximal transverse colon. This data showed that the water content in the large intestine appeared to be the least volume and demonstrates the efficiency of the colon in the reabsorption of water. Furthermore, Cummings et al (1990) measured the amount of content in the entire large intestine of 46 adult subjects at post-mortem and found that the total amount of wet content in the whole colon was 221±21 g (42%w/w in the caecum and ascending colon, 30% w/w in the transverse
colon and 28% w/w in the descending colon, sigmoid and rectum) and after drying, the total dry matter was 35±4 g (14% w/w in the caecum and 23% w/w in the sigmoid and rectum). From this data, the calculated water content in the large intestine was found to be 187 g, which fell within the range of the water content in the proximal large bowel (7-430 ml) reported by Gotch (1957).

Recently, Schiller et al (2005) investigated the volume and distribution of free fluid along the gastrointestinal tract in 12 healthy humans by using a water sensitive imaging technique, magnetic resonance image (MRI). The MRI provided information on the fluid volume in the gut and the location of the capsules within the GI tract and also revealed the degree of contact of the capsules with gut fluid, whether the capsule was surrounded by liquid, partly surrounded by liquid or not in contact with liquid at all. The gastrointestinal free fluid volume under the fed and fasted condition is shown in Table 1.2.

Table 1.2 Gastrointestinal free fluid volume of human subjects under fed and fasted condition (adapted from Schiller et al., 2005)

<table>
<thead>
<tr>
<th>Gastrointestinal region</th>
<th>Fasted state free fluid volume (mL)</th>
<th>Fed state free fluid volume (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean Range</td>
<td>Mean Range</td>
</tr>
<tr>
<td>Stomach</td>
<td>45 ± 18 13 - 72</td>
<td>686 ± 93 534 - 859</td>
</tr>
<tr>
<td>Small intestine</td>
<td>105 ± 72 45 - 319</td>
<td>54 ± 41 20 - 156</td>
</tr>
<tr>
<td>Large intestine</td>
<td>13 ± 12 1 - 44</td>
<td>11 ± 26 2 - 97</td>
</tr>
</tbody>
</table>

\*The total volume of the stomach contents (not only free fluid)\*

Even though a total of 850 mL water was ingested within the 7 hours before imaging, a limited free fluid volume in the gastrointestinal tract was observed. It was also found that the free fluid volume in the gut was highly variable and was affected by the consumption of food. The intestinal fluid was located in pockets and was inhomogeneous in distribution along the small and large intestine. After the ingestion of food, the free fluid volume within the small intestine reduced dramatically. Schiller et al.
suggested that the decrease in the free fluid volume could be attributed to a meal-induced gastro-ileocaecal reflex; with this mechanism, therefore, solids and fluid were transported from the small intestine to the colon. During the gastro-ileocaecal reflex, the fluid which is transferred from the small intestine would be mostly absorbed in the colon. This is probably due to the high colonic absorption capacity of 2.7 mL water/min (Palma et al., 1981); thus, no increase in the free fluid volume was observed in the colon under the fed state.

In the stomach under fed and fasting conditions, all capsules were found to be fully covered with free fluid. On the other hand, the degree of contact of capsules with free fluid in the small and large intestine was variable and less predictable (Table 1.3).

Table 1.3 Location and contact of capsules with “free fluid” in the small and large intestine in fasted and fed volunteers (adapted from Schiller et al., 2005)

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

Unlike the in-vitro dissolution studies, the oral dosage forms may not be in permanent contact with the free fluid in the gut (Table 1.3) due to the random distribution of fluid pockets and dry segments along the intestinal tract. Therefore, the in-vivo release of the drug from the formulations is difficult to predict by using the in-vitro dissolution results.

For specific drug delivery to the intestinal regions, the degree of contact of dosage forms with fluid pockets and free fluid volume in the target areas are the important factors in determining the performance of drug delivery systems such as dissolution and the disintegration process. The main factors hindering the achievement of colonic drug
delivery are a very low fluid volume in the colon (Table 1.2) and the high possibility of
the lack of contact with fluid pockets (Table 1.3).

1.2.4.4 Gastrointestinal bacteria

The distribution of various bacteria throughout the GI tract is shown in Figure 1.3.

Figure 1.3 Distribution of selected bacteria in various sites of the gastrointestinal tract
(Reproduced from Abu Shamat, (1993)

In the stomach the microbial count is very low due to the acidic pH and the relatively
rapid transit of material through the small intestine. Moreover, in the small intestine, the
peristaltic waves, bile juice and enzymes reduce the bacterial growth. The microbial
population in the upper gastrointestinal regions is estimated to be up to $10^4$ CFU/mL
(Gorbach, 1971). In contrast, the proximal neutral pH of the colon and the slow passage
of the contents in the large intestine contribute to the increase in bacterial flora in this
region. The microflora population in the colon is approximately $10^{11}-10^{12}$ CFU/mL,
representing over 400 bacterial species and almost one third of the faecal dry weight
(Moore and Holdeman, 1975; Savage, 1977; Simon and Gorbach, 1984). In comparison
with the other regions of the GI tract, the colon is home to a vast amount of various
bacteria of which the majority are anaerobic. A bacterial sensitive system could
therefore exploit this fact in the utilisation of site specific colonic drug delivery.
1.3 Intestinal targeting via the oral route

1.3.1 Drug delivery to the small intestine

Targeting drugs to the small intestine is a benefit for drugs which have side effects such as nausea and gastric irritation and also for drugs which are easily destroyed by gastric enzymes or the acidity of gastric fluid. To target drugs to this region, the enteric coating approach is commonly used.

The enteric coating approach exploits the fact that the pH in the stomach is highly acidic but on entry into the small intestine the pH rises rapidly and then slowly increases along the length of the small intestine. Enteric coated dosage forms have been broadly used for modified release systems and pH sensitive polymers are the most commonly used enteric coating material. Many pH sensitive polymers are composed of ionisable carboxylic groups and a pKa between 4 and 6 which are unionized in the low pH condition of the stomach (pH 1.0-2.5) (Evans et al., 1988); thus these polymers are insoluble and resistant to dissolution in the stomach. On the other hand, in the intestinal fluid, carboxylic groups are ionized and the coating polymers therefore dissolve and release the drug (Agyilirah and Banker, 1991). The commonly used enteric polymers are shown in Table 1.4.

Nevertheless, the inter- and intra-individual variability of the pH in the small intestine (Fallingborg et al., 1993) is a limitation in this enteric coating approach due to the site of dosage form disintegration depending greatly on the intraluminal pH. Also the exposure time of the dosage form to the required pH, in which pH above the pH threshold of the polymer, may be limited. Therefore, the use of a pH based microparticulate system might help to overcome such limitations since the large surface area to volume ratio should allow rapid dissolution of the enteric polymer and fast drug release at the target site.
Table 1.4 pH sensitive polymers commonly employed in the delayed release dosage form

<table>
<thead>
<tr>
<th>Enteric polymer</th>
<th>Threshold pH</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Cellulosic polymer</strong></td>
<td></td>
</tr>
<tr>
<td>Cellulose acetate trimellitate (CAT)</td>
<td>4.8</td>
</tr>
<tr>
<td>Hydroxypropylmethylcellulose phthalate 50 (HP50)</td>
<td>5.0</td>
</tr>
<tr>
<td>Hydroxypropylmethylcellulose phthalate 55 (HP55)</td>
<td>5.5</td>
</tr>
<tr>
<td>Cellulose acetate phthalate (CAP)</td>
<td>6.0</td>
</tr>
<tr>
<td><strong>Acrylic polymer</strong></td>
<td></td>
</tr>
<tr>
<td>Poly(methacrylic acid, ethyl acrylate), 1:1, Eudragit L55</td>
<td>5.5</td>
</tr>
<tr>
<td>Poly(methacrylic acid, methyl methacrylate), 1:1, Eudragit L</td>
<td>6.0</td>
</tr>
<tr>
<td>Poly(methacrylic acid, methyl methacrylate), 1:2, Eudragit S</td>
<td>7.0</td>
</tr>
<tr>
<td><strong>Polyvinyl derivative</strong></td>
<td></td>
</tr>
<tr>
<td>Polyvinyl acetate phthalate (PVAP)</td>
<td>5.0</td>
</tr>
</tbody>
</table>

1.3.2 Drug delivery to the colon

Site specific drug delivery to the colon provides a major benefit for colonic disorders such as Crohn's disease, ulcerative colitis, irritable bowel syndrome (IBS) and colorectal cancer. Also drug delivery to the colon of peptides, proteins and vaccine would be of benefit as the colon has less adverse enzymatic activity than the other parts of the GI tract (Saffran et al., 1986; Rubinstein, 1995; Basit, 2000). In colonic delivery, the rectally delivered colonic formulations frequently failed to reach the transverse colon (Hay, 1982) and did not spread uniformly within the colon (Wood et al., 1985). Therefore, studies have been focused more on the oral modified release dosage forms due to the convenience of administration and effective delivery of the drug to the therapeutic site. Various approaches have been developed for targeting drugs to the
Chapter One

colon including pH based systems, prodrug, timed release systems, pressure dependent systems and microbial-enzyme triggered drug release systems.

**pH based system**

When using pH sensitive polymers as a film coating in enteric coated dosage forms for colonic drug delivery, the polymers control drug release in the colon by exploiting the differences in pH between the small and the large intestine. There are numerous reports on drug delivery to the colon using a pH sensitive delivery system (Dew et al., 1982; Thomas et al., 1985; Marvola et al., 1999). Copolymers of methacrylic acid and methyl methacrylate, Eudragit L and Eudragit S, are commonly used polymers in the colonic delivery system (Morishita et al., 1993). Marketed formulations derived from pH sensitive polymers such as Asacol® MR, Mersen® MR and Ipocol® which are Eudragit S coated mesalazine tablets, Salofolk® which is a Eudragit L coated mesalazine tablet and Budenofolk® which consists of Eudragit L/S coated granules of budesonide, are available for the treatment of inflammatory bowel disease.

As discussed earlier, the pH in the intestine is not stable and is variable in inter-individual subjects. pH sensitive systems suffer from unpredictable site-specific drug release, leading to poor reproducible drug release and to the drug leaking in the small intestine prior to delivery to the colon (Basit, 2005). Moreover, the low water flux and a high viscosity of the contents of the colon might also retard drug dissolution from a dosage form.

The *in-vivo* and *in-vitro* studies of Ashford et al showed the lack of reproducibility of drug release and poor site specificity when using Eudragit S-coated tablets for colonic targeting. The *in-vivo* studies in humans (n=7) demonstrated the variation of the disintegration site of the tablets from the terminal ileum to splenic flexure and none of the tablets disintegrated in the caecum or ascending colon. These results suggested that a single unit coated dosage form was not the best approach for colon specific drug delivery (Ashford et al., 1993a; Ashford et al., 1993b).
Schroeder et al (1987) also reported the variability in the performance of commercial products of Asacol® MR (Eudragit S coated tablet of mesalazine for treating ulcerative colitis) and that the site of tablet disintegration varied from the small intestine to the descending colon and in some cases the tablets have been seen passing through the GI tract intact and found in the stools of ulcerative colitis patients. The failure of this dosage form can be attributed to the low colonic pH of ulcerative colitis patients (Nugent et al., 2001).

Recent research by Ibekwe et al (2006) also observed the variability of the *in-vivo* performance of Eudragit S coated tablets in 8 healthy volunteers. A dosage form prepared by coating with Eudragit S organic solution was found to disintegrate at the ICJ (2 volunteers) and at the ascending colon (3 volunteers). However, in three volunteers the tablets remained intact in the ascending colon through to the end of the study.

Nevertheless, the release of drugs from coated dosage forms for terminal ileum and colon drug delivery not only depend on the gastrointestinal pH but also on the intestinal transit time. Increasing the thickness of the film coating and/or a combination of pH sensitive polymers and pH independent water insoluble polymers such as ethyl cellulose, cellulose acetate butyrate, Eudragit RL, RS or NE would help to control the time that the drug is released in the colon after the dissolution of the pH sensitive polymer coating in the terminal ileum (Rao and Ritschel, 1992; Rodriguez et al., 1998; Gupta et al., 2001). A marketed product for the treatment of Crohn’s disease that employs a combination of pH sensitive polymer and pH independent polymer is Entocort®, Eudragit L55 coated ethylcellulose granules of budesonide.

**Prodrug**

Prodrug systems for colonic delivery generally consist of a drug molecule linked to either a microbial enzyme degradable bond or linked using a microbial enzyme degradable carrier. This carrier molecule is able to transport the drug to the colon and release it at the target site. The concept of this approach is the enzymatic cleavage of the
Chapter One

linkage bond between the drug molecule and the carrier moiety via reduction and hydrolysis. Typical enzymes include azoreductase, glycosidase and glucuronidase.

For instance, sulfasalazine is composed of sulphapyridine conjugated with 5-aminosalicylic acid (5-ASA) by an azo-linkage, which is cleavaged by colonic bacteria enzymes. After administration, this prodrug is minimally absorbed in the stomach and small intestine but when it enters the colon the azo-bond is cleaved by the host bacteria and 5-ASA is released (Peppercorn and Goldman, 1972; Kopecek et al., 1992). The toxicity of sulphapyridine led to the development of a number of alternative azo prodrugs which are safer and less toxic, for example, olsalazine and balsalazide. Olsalazine is a dimer of 5-ASA linked via azo bond (Ryde et al., 1991) and balsalazide consists of 5-ASA azo linked to 4-aminobenzyl-β-alanine (Chan et al., 1983). Several prodrugs such as dextran prodrugs (Harboe et al., 1989), glycosidic prodrugs (Friend and Chang, 1985) and cyclodextrin conjugated prodrugs (Hirayama et al., 1996) have also been investigated for colonic drug delivery. Even though these prodrugs offer a great benefit for site specific delivery, they are considered as new chemical entities and are therefore require extensive toxicological studies before being allowed for use in humans. In addition, the availability of functional groups on the drug molecule for chemical cross linkage is a limitation of the prodrug approach. Therefore, this approach is not versatile.

Timed release system

The timed release system has been developed for a delayed release system for drug delivery to the colon (Gazzaniga et al., 1994). This approach relies on a perceived constant transit time through the small intestine. The idea for this system comes from the fact that the small intestinal transit time is relatively constant at three to four hours. The first device of this system was Pulsincap® (Stevens et al., 2002), composed of an impermeable capsule body comprising the drug and a hydrogel plug. In gastrointestinal fluid, the plug hydrates, swells and after a preset time is ejected from the body of the capsule; thereby allowing drug release. In addition, a “Time clock” tablet is a device in which the tablet core is coated with a mixture of hydrophobic surfactant and polymer. It was observed that drug released from the core tablet after a predetermined lag time and the release of the drug was independent from the pH in the GI tract but it did depend on
Chapter One

the thickness layer of the film coating (Pozzi et al., 1994; Wilding et al., 1994; Wilding, 2000). However, the limitations of a time based approach were the variability in gastric emptying and the small intestinal transit time (Basit, 2005).

Microbial-enzyme triggered drug release system

Polysaccharides such as amylose, chitosan, chondroitin, dextran, guar gum, inulin and pectin are extensively used as a carrier for drug delivery to the colon (Rubinstein et al., 1992b; Ashford et al., 1994; Sinha and Kumria, 2001) since they are only degraded by colonic bacteria. Polysaccharides are also natural materials and are safe, highly stable, and non-toxic. However, a major problem of polysaccharides is their high water solubility. To overcome this problem, polysaccharides are either chemically modified or mixed with insoluble polymers to improve the film forming and solubility properties. For instance, a combination of ethylcellulose (EC) and amylose was used as a pellet film coating in a formulation for targeting drugs to the colon (Milojevic et al., 1996b; Basit et al., 2004).

Pressure dependent system

Pressure within the GI tract is produced by the muscular contraction of the gut wall for grinding and propelling the luminal contents. The intraluminal pressure in the colon is high due to a combination of haustral contractions and a viscous environment. A pressure-controlled drug delivery system has been developed by exploiting the high intraluminal pressure in the colon produced by peristalsis (Takaya et al., 1995).

A pressure controlled colon delivery capsule (PCDC) system consists of a gelatine capsule with an inner ethylcellulose coating and a drug dispersed in a suppository base. Following the administration, the suppository base melts at body temperature resulting in the formation of a liquid, which is retained by the ethylcellulose wall. The system can resist the pressure of the upper GI tract but fractures in the colon. Furthermore, due to the small amount of fluid in the distal colon, this system, which has a solution filled capsule should be of benefit because it could enhance drug absorption and dissolution (Takaya et al., 1998). Nevertheless, with the influence of the administration of food in the fed state, the contractions in the stomach may be adequate to break down the
capsule. To overcome this problem, the system can be modified to resist and fracture at different pressures by manipulating the thickness of the ethylcellulose coating and changing the size of the capsule (Muraoka et al., 1998). However, the reproducibility of the luminal pressures and the duration of this high pressure phase still require intensive study due to the lack of information available leading to a difficulty in accurately predicting the exact time of drug release from the system (Leopold, 1999).

1.4 The advantages of multiple unit dosage forms

As mentioned earlier regarding the different in-vivo performance of multiple unit dosage forms and the single unit dosage forms, it is generally accepted that the multiple unit systems perform better in-vivo than single units (Bechgaard and Nielson, 1978; Abrahamsson et al., 1996; Asghar and Chandran, 2006). The advantages of multiple unit dosage forms are demonstrated in Table 1.5.

Table 1.5 Advantages of multiple units over single unit dosage forms

<table>
<thead>
<tr>
<th>Single unit</th>
<th>Multiple units</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transit relying on GE and strongly influence by food</td>
<td>Transit only moderately affected by food</td>
</tr>
<tr>
<td>The larger dosage form, the more delayed and unpredictable the GE</td>
<td>Reproducible GI transit time and less inter-individual subject variability</td>
</tr>
<tr>
<td>High variation of GI transit time in inter-individual subjects</td>
<td>Reduce risk of dose dumpling</td>
</tr>
<tr>
<td>Varying rate and extent of bio-availability</td>
<td>Reduce risk of systematic toxicity and risk of local irritation</td>
</tr>
<tr>
<td>Risk of accumulation of doses</td>
<td>More uniformly dispersed in GI tract, resulting in more uniform drug absorption and reproducible bioavailability</td>
</tr>
<tr>
<td>Risk of local irritation</td>
<td>Larger surface area facilitating the solubilisation and release of drug</td>
</tr>
</tbody>
</table>
To date, there is still no conclusion as to the ideal size for particles which can be emptied with co-administered water in both a fed and fasted state. As mentioned before there is no evidence of pellets with a particle size of 500 μm emptying from the stomach with co-administered water (Clarke et al., 1993). This would seem to indicate that if a cut-off size for particles emptying through the pylorus with liquid content exists, this size should be below 500 μm. In addition, Sugito et al (1992) reported that under a fasted state, the gastric emptying of microparticles with a size of 100 μm was faster than that of those with a size of 500 μm but that there was no difference between the two under fed state.

When compared to pellets, it would seem microparticles are more likely to empty rapidly from the stomach and provide a better in-vivo performance, which would offer greater benefits in a modified release system. It is hypothesized that modified release microparticles of a particle size less that 100 μm may be able to suspend in the stomach and empty rapidly through the pylorus with co-administered water in both a fed and fasted state since the stomach might not recognise such small particles as solid particles and therefore these particles were likely to empty rapidly with water. When the intraluminal pH reaches above the pH threshold of the polymer, the large surface area of the microparticles would lead to rapid polymer dissolution and fast drug release. Also, the reproducibility of drug release from a uniform size of microparticles would be expected. For drug delivery to the colon, a region with limited fluid volume, small sized microparticles would have more benefits than pellets. Since the microparticles possess a larger surface area to volume ratio, drug release and solubilisation would be more rapid.

In addition, due to the streaming effect in the colon, the microparticles are retained for a longer period of time, resulting in a diminishing of incidents of dosage forms evacuating from the colon in an intact form and an extension of the time for drug absorption.

1.5 Microencapsulation

Microencapsulation is a broad term used for describing terms such as microsphere, microcapsule, nanocapsule and liposome. The term microcapsule can be described as an encapsulated substance that is completely surrounded by a capsule wall whereas a microsphere is described as a monolithic or micromatrix structure with particles of
encapsulated substances dispersed throughout the microsphere matrix (Whateley, 1992). The microcapsule and microsphere structures are shown in Figure 1.4.

![Microcapsule, Homogeneous micromatrix, Heterogeneous micromatrix](image)

**Figure 1.4 Microcapsule and microsphere structures (adapted from Burgess and Hickey (2002)).**

Microspheres and microcapsules are employed in a wide variety of commercial products such as carbonless copy papers, pesticide and herbicide products and pharmaceutical products (Thies, 1996). The oral modified release microparticulate dosage form has been intensively studied for over 20 years and a large number of drugs with different physical and chemical properties have been encapsulated in microspheres, such as anti-cancer drugs (Eley et al., 1992), peptide (Mehta et al., 1996), protein (Jeffery et al., 1993) and amine based drugs (Maulding et al., 1986).

### 1.5.1 Microencapsulation technique

Microencapsulation is one of the techniques that has been employed for improving patient compliance, producing carriers for modified release systems, improving drug stability, avoiding substance degradation and for taste masking (Watts et al., 1990; Palomo et al., 1996). There are many methods which have been used for microencapsulation. Four methods, emulsification solvent evaporation (ESE), coacervation, extrusion spheronisation and spray drying are described below and the comparison of the advantages and disadvantages of these techniques are showed in Table 1.6.
Chapter One

1.5.1.1 Coacervation

Coacervation is the oldest method of the microencapsulation technique (Watts et al., 1990). Coacervation can be described as the macromolecular aggregation (or controlled phase separation) process (Arshady, 1990b). The three main steps of the coacervation process are phase separation of the coating polymer solution, adsorption of the coacervate around the drug molecules and in the last step, solidification of the microparticles (Nihant et al., 1995a). Simple coacervation is based on one macromolecule while complex coacervation is based on two or more macromolecules of opposite charges. A change in the conditions, the addition of a non-solvent, the addition of a chemical or a change in temperature induced a simple coacervation process, the consequence of which was the reduction in solubility of a dissolved polymer (macromolecule). The change in these conditions promotes polymer-polymer interaction over polymer-solvent interaction. The complex coacervation process, is induced by electrostatic interactive forces between two or more macromolecules (Burgess, 1990)

1.5.1.2 Spray drying

The spray drying process is described as follows: firstly, the polymer coating materials and the drug are dissolved in an aqueous or non-aqueous solvent or the drug is suspended in the polymer solution; secondly, a solution or suspension of the drug and polymer is then atomised by gas and dried quickly on contact with hot air and finally the product is recovered. The size of particles is controlled by various parameters such as the nozzle size, the rate of spray drying, the feed rate of the polymer drug solution and the temperature in the drying and collecting chambers. The spray drying technique is applicable to a wide variety of materials (Moretti et al., 2001; Lee, 2002; Gharsallaoui et al., 2007).
Table 1.6 Summary of the advantages and disadvantages of the microencapsulation techniques

<table>
<thead>
<tr>
<th></th>
<th>Coacervation</th>
<th>Spray drying</th>
<th>Extrusion spheronisation</th>
<th>Emulsification solvent evaporation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Advantages</strong></td>
<td>• Great range of polymers and drugs can be used</td>
<td>• Rapid process</td>
<td>• High yield output</td>
<td>• Simple procedure</td>
</tr>
<tr>
<td></td>
<td>• Ability to encapsulate liquid such as essential oil</td>
<td>• Practical in manufacturing</td>
<td>• Practical in manufacturing</td>
<td>• Providing the particle size in the range of nanometre to micrometer</td>
</tr>
<tr>
<td></td>
<td>• Providing the particle size in the range of nanometre to micrometer</td>
<td>• Producing the particle size in the range of nanometre to micrometer</td>
<td></td>
<td>• Wide range of polymers and drugs can be used</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>• Low cost process</td>
</tr>
<tr>
<td><strong>Disadvantages</strong></td>
<td>• The issue of residual solvents and coacervating agents remained in the final products</td>
<td>• High cost process</td>
<td>• Complex manufacturing process</td>
<td>• The issue of residual solvents left in final product</td>
</tr>
<tr>
<td></td>
<td>• Difficult to transfer to industrial scale</td>
<td>• Using a high temperature in a process, therefore, not suitable technique for temperature sensitive compounds</td>
<td>• High cost process</td>
<td>• Difficult to transfer to industrial scale</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Providing low yields (if batch size are small)</td>
<td>• Yielding the particle size in the range 0.5-5 mm. Not suitable for preparing microparticles in the low micrometer and nanometre size</td>
<td></td>
</tr>
</tbody>
</table>
1.5.1.3 Extrusion spheronisation

Extrusion spheronisation is one of the most popular methods of producing microspheres (Hellen et al., 1993). The process involves drug mixing, wet granulation, extrusion, spheronisation, drying, screening and film coating. At the beginning, the drug and excipient are mixed in suitable mixers until uniform, followed by the wet granulation process. The wet mass is passed through the extruder to form rods. The extruded strands are then processed in the spheronizer, this is followed by drying the pellets and finally the pellets are coated with the polymer. There are many critical factors that influence the properties of the pellets such as extruder type, spheronizer speed and formulation components (Ghebre-Sellassie and Knoch, 2002).

1.5.1.4 Emulsification solvent evaporation

Emulsification solvent evaporation (ESE) is a conceptually simple, three step process (Watts et al., 1990). In the first step, the polymer and drug are dissolved in organic solvent (internal phase) and this solution is then emulsified into the continuous phase containing an emulsifying agent under agitation. In the following step, the solvent transports out from the emulsion droplets and evaporates through the emulsion/air interface resulting in polymer precipitation and particle hardening. In the last step, microparticles are separated from the emulsion by filtration or centrifugation and they are then cleaned up by an appropriate solvent. By using the ESE method, a wide range of polymers that have low or high aqueous solubilities can be used to encapsulate the drugs (Huang and Ghebre-Sellassie, 1989; Zinutti et al., 1996).

The selection of modified release microparticles produced by various types of polymers and methods are shown in Table 1.7. (Examples of modified release microparticles prepared by extrusion spheronisation were not included in the Table 1.7 as this method produces a large size of microparticles)
<table>
<thead>
<tr>
<th>Polymer</th>
<th>Encapsulated material</th>
<th>Method</th>
<th>Particle size (µm)</th>
<th>Morphology</th>
<th>In-vitro release studies</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPMCP</td>
<td>Insulin</td>
<td>ESE - O/O</td>
<td>500</td>
<td>Spherical particles with smooth surface</td>
<td>Able to control the release in acidic pH (no release observed at 2 hrs)</td>
<td>(Qi and Ping, 2004)</td>
</tr>
<tr>
<td></td>
<td>Ketoprofen</td>
<td>Spray drying</td>
<td>45±16</td>
<td>Clusters of irregular and sphere particles</td>
<td>Able to control the release in acidic pH (≈ 10% at 2hrs)</td>
<td>(Palmieri et al., 2002)</td>
</tr>
<tr>
<td></td>
<td>Paracetamol</td>
<td>Spray drying</td>
<td>Not reported</td>
<td>Clusters of small irregular particles</td>
<td>At 2 hrs in acidic pH, microparticles failed to control drug release but the tableted microparticles could control the release (≤ 10%)</td>
<td>(Palmieri et al., 2000)</td>
</tr>
<tr>
<td>HP 50</td>
<td>Ketoprofen</td>
<td>ESE - O/W</td>
<td>10.9±2.0</td>
<td>Spherical particles</td>
<td>Fail to control the release in acidic pH (≈ 60% at 2 hrs)</td>
<td>(Guzman et al., 1996)</td>
</tr>
<tr>
<td>HP 55</td>
<td>Ibuprofen</td>
<td>Coacervation phase separation</td>
<td>137-210</td>
<td>Irregular particles</td>
<td>Able to control the release in acidic pH (≈ 5% at 2hrs)</td>
<td>(Wei et al., 1995)</td>
</tr>
</tbody>
</table>

*Hydroxypropylmethylcellulose phthalate (HPMCP)

*Unspecified grade*
<table>
<thead>
<tr>
<th>Polymer</th>
<th>Encapsulated material</th>
<th>Method</th>
<th>Particle size (μm)</th>
<th>Morphology</th>
<th>In-vitro release studies</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CAT Tartarzine</td>
<td>Coacervation phase separation</td>
<td>488.4- 821.9</td>
<td>Spherical particles with rough and wrinkly surface</td>
<td>Only one formulation was able to control the release (≈ 10% at 2hrs)</td>
<td>(Sanghvi and Naim, 1991)</td>
<td></td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>Spray drying</td>
<td>70.0± 18</td>
<td>Irregular particles</td>
<td>Fail to control the release in acidic pH (release ≈ 15% at 2 hrs)</td>
<td>(Palmieri et al., 2002)</td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Spray drying</td>
<td>15-100</td>
<td>Cluster of small irregular particles with shrinkage surface</td>
<td>At 2 hrs in acidic pH, microparticles fail to control drug release but the tableted microparticles was able to restrict drug release (&lt; 3%)</td>
<td>(Palmieri et al., 2000)</td>
<td></td>
</tr>
<tr>
<td>Indomethacin</td>
<td>Spray drying</td>
<td>6- 10</td>
<td>Irregular particles with collapsed structure</td>
<td>Only one formulation was able to control the release (≈ 10% at 2hrs)</td>
<td>(Giunchedi et al., 1995)</td>
<td></td>
</tr>
<tr>
<td>CAP Propranolol hydrochloride</td>
<td>Coacervation phase separation</td>
<td>508.3- 754.5</td>
<td>Spherical particles</td>
<td>Fail to control the release in acidic pH (release ≥ 17% at 2 hrs)</td>
<td>(Manekar et al., 1992b)</td>
<td></td>
</tr>
<tr>
<td>Mycoplasma hyponeumonia vaccine</td>
<td>Coacervation phase separation</td>
<td>500-2300</td>
<td>Spherical particles</td>
<td>Not reported</td>
<td>(Lin et al., 1991)</td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Spray drying</td>
<td>30-80</td>
<td>Clusters of small irregular particles</td>
<td>At 2 hrs in acidic pH, microparticles fail to control drug release but the tableted microparticles released drug slowly (≈ 10%)</td>
<td>(Palmieri et al., 2000)</td>
<td></td>
</tr>
<tr>
<td>Phenacetin</td>
<td>ESE - O/O</td>
<td>600</td>
<td>Not mentioned</td>
<td>Fail to control the release in acidic pH (release ≈ 15% at 2 hrs)</td>
<td>(Silva and Ferreira, 1999)</td>
<td></td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>Spray drying</td>
<td>35± 11</td>
<td>Irregular particles</td>
<td>Fail to control the release in acidic pH (release ≈ 20% at 2 hrs)</td>
<td>(Palmieri et al., 2002)</td>
<td></td>
</tr>
</tbody>
</table>
Table 1.7 Continued

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Encapsulated material</th>
<th>Method</th>
<th>particle size (μm)</th>
<th>Morphology</th>
<th>In-vitro release studies</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit S</td>
<td>Ketoprofen</td>
<td>ESE (O/O)</td>
<td>250-1,000</td>
<td>Spherical with rough surface</td>
<td>Able to control the release in acidic pH</td>
<td>(Goto et al., 1986)</td>
</tr>
<tr>
<td></td>
<td>Hydroxycamptothecin</td>
<td>Spray drying</td>
<td>230</td>
<td>Spherical particles with smooth surface</td>
<td>Able to control the release in acidic pH</td>
<td>(Lu and Zhang, 2006)</td>
</tr>
<tr>
<td></td>
<td>Theophylline</td>
<td>ESE (O/O)</td>
<td>125-500</td>
<td>Spherical particles</td>
<td>Fail to control the release in acidic pH (release ≥ 25% at 2 hrs)</td>
<td>(Obeidat and Price, 2006)</td>
</tr>
<tr>
<td></td>
<td>5-fluorouracil loaded alginate microspheres</td>
<td>ESE (O/O)</td>
<td>103-185</td>
<td>Spherical particles with smooth surface</td>
<td>Able to control the release in acidic pH</td>
<td>(Rahman et al., 2006)</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>Spray drying</td>
<td></td>
<td>60±13</td>
<td>Clusters of small spherical particles</td>
<td>Able to control the release in acidic pH</td>
<td>(Palmieri et al., 2002)</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Spray drying</td>
<td></td>
<td>10-100</td>
<td>Clusters of small irregular particles</td>
<td>At 2 hrs in acidic pH, microparticles failed to control drug release (&gt; 50%) but a tablet form of microparticles released drug slowly (13%)</td>
<td>(Palmieri et al., 2000)</td>
</tr>
<tr>
<td>Eudragit L</td>
<td>Verapamil</td>
<td>ESE (O/O)</td>
<td>869.17</td>
<td>Spherical particles</td>
<td>Able to control the release in acidic pH</td>
<td>(Kilicsarslan and Baykara, 2004)</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>Spray drying</td>
<td></td>
<td>60±13</td>
<td>Clusters of spherical particles</td>
<td>Able to control the release in acidic pH</td>
<td>(Palmieri et al., 2002)</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>Spray drying</td>
<td></td>
<td>6.6-7.8</td>
<td>Spherical particles</td>
<td>The release of vitamin C in acid condition not report</td>
<td>(Esposito et al., 2002)</td>
</tr>
<tr>
<td>Ketoprofen</td>
<td>ESE (O/O)</td>
<td></td>
<td>250-800</td>
<td>Irregular particles with shrinking surface</td>
<td>Able to control the release in acidic pH</td>
<td>(Goto et al., 1986)</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>ESE (O/O)</td>
<td></td>
<td>180-500</td>
<td>Spherical with rough and porous surface</td>
<td>Fail to control the release of drug in acidic pH (&gt; 50% in 1 hour)</td>
<td>(Morishita et al., 1991)</td>
</tr>
<tr>
<td>Polymer Encapsulated material</td>
<td>Microencapsulation Method</td>
<td>Mean particle size</td>
<td>Morphology</td>
<td>Release studies</td>
<td>Reference</td>
<td></td>
</tr>
<tr>
<td>-------------------------------</td>
<td>---------------------------</td>
<td>--------------------</td>
<td>------------</td>
<td>----------------</td>
<td>----------</td>
<td></td>
</tr>
<tr>
<td>EC1 Terbutaline sulfate</td>
<td>Coacervation phase separation</td>
<td>407.1-790.2</td>
<td>Irregular particles with rough and porous surface</td>
<td>Able to prolong the release of drug up to 12 hrs</td>
<td>(Manekar et al., 1992a)</td>
<td></td>
</tr>
<tr>
<td>5-fluorouracil</td>
<td>ESE (O/O)</td>
<td>530-630</td>
<td>Spherical particles</td>
<td>Few formulations were able to extend the release of drug</td>
<td>(Zinutti et al., 1994)</td>
<td></td>
</tr>
<tr>
<td>Fenoterol hydrobromide</td>
<td>ESE (O/O)</td>
<td>49.8-77.0</td>
<td>Spherical particles</td>
<td>Initial burst release was observed in some formulations and a few formulations were able to sustain the release of the drug</td>
<td>(Lin and Wu, 1999)</td>
<td></td>
</tr>
<tr>
<td>Paracetamol</td>
<td>Spray drying</td>
<td>25-30</td>
<td>Clusters of irregular shape particles</td>
<td>Unable to control drug release</td>
<td>(Palmieri et al., 2001)</td>
<td></td>
</tr>
<tr>
<td>PVAc2 Theophylline</td>
<td>ESE (O/O)</td>
<td>550-1198</td>
<td>Not mentioned</td>
<td>Some formulations were able to extend the release of drug</td>
<td>(Sa, 1991)</td>
<td></td>
</tr>
<tr>
<td>Phenylpropanolamine HCl</td>
<td>Coacervation phase separation</td>
<td>Not mentioned</td>
<td>Porous surface</td>
<td>Release of drug was rapid and completed at 2 hours</td>
<td>(El-shattawy et al., 1992)</td>
<td></td>
</tr>
<tr>
<td>PVAc (aqueous dispersion, Kollicoat SR 30D)</td>
<td>Spray drying</td>
<td>13.5-25.8</td>
<td>Spherical particles with wrinkly surface</td>
<td>Able to extend drug release</td>
<td>(Sangkapat, 2004)</td>
<td></td>
</tr>
<tr>
<td>Furosemide</td>
<td>Spray drying</td>
<td>14.2-26.8</td>
<td>Shrunk particles with shrinkage of the surface wall</td>
<td>Few formulations were able to extend the release of drug</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1Ethylcellulose (EC), 2Polyvinyl acetate (PVAc)
The desired properties of modified microparticles, which it was the aim of this study to produce, are smooth and spherical with a small size (preferably less than 100 μm), non-aggregation, uniform particle size distribution and should also be possessed of a high drug encapsulation efficiency and modified release properties.

In comparison with the spray drying technique, the ESE method requires inexpensive instruments and is compatible with temperature sensitive drugs. Unlike the coacervation phase separation technique, the ESE method is far less complicated. The ESE method also provides microparticles in a small size range of nanometer to micrometer which could not be obtained by the extrusion spheronisation technique. Nevertheless, the issue of residual solvents remaining in the final product is a drawback to the ESE method. As the volatile organic solvents used in the method can not usually be completely removed by practical manufacturing processes and remain behind in the final product in low quantities. If organic solvents were to remain in the product at a high level or a very toxic solvent was used in the process, it would increase the risk of toxicity to human health. To reduce the side effects of the residual solvents left in the microparticles, the solvents used should be considered based on the International Committee for Harmonisation (ICH) guideline under the topic of impurities: residual solvent, ICH topic Q3C (R3) and the chosen solvent should be categorised in class II (solvent to be limited: non-genotoxic animal carcinogens or possible causative agents of neurotoxicity or teratogenicity) such as methanol, dichloromethane, hexane or class III (low toxic potential to human) such as ethanol, acetone, ethyl acetate.

As ESE is a versatile and inexpensive technique, provides microparticles with a uniformity of size and a low size range and is also compatible with a wide range of drugs and polymers, this method was selected to prepare modified release microparticles and the microparticle formation process of this technique has been investigated in this thesis.

When using the ESE technique it is important to select a suitable emulsion system and employ the appropriate manufacturing process parameters in order to obtain the desired microparticles. In general the type of emulsion system used in the ESE method is either
oil in water emulsion (O/W), water in oil in water (W/O/W) or oil in oil emulsion (O/O).

To select the suitable emulsion system, the solubility of the encapsulated drug in the continuous phase should be taken into consideration. For example, to encapsulate a hydrophilic drug, the use of the O/W system was found to be an inappropriate emulsion type due to the rapid partition of the drug from the dispersed droplets to the continuous phase (Bodmeier and McGinity, 1987b). In contrast, the use of the O/O emulsion system, where drug and polymer are dissolved in an organic solvent and emulsified into an immiscible lipophilic phase (such as mineral oil), was found to be a suitable system for encapsulating high water soluble drugs and can be a solution to the low encapsulation problem (Yang et al., 2005). However, due to the use of oil in the continuous phase, the disadvantages of the O/O emulsion system are the difficulties in the microparticle cleaning-up process to eliminate the oil and the removal of the organic solvent used in the cleaning up process. Nevertheless, the use of the W/O/W emulsion system can avoid the above problems of removing the oil and the cleaning-up of the organic solvent (Bodmeier et al., 1991).

1.6 Scope and Purpose of study

Even though ESE is a conceptually simple technique, the microparticle formation process is relatively complex and there are many factors governing the manufacturing process and final characteristics of the microparticles. The properties of microparticles are the outcome of a complex interplay between the compositions in the formulation which are the encapsulated drug, polymer, emulsifying agent, internal solvent phase and the continuous phase. To date some fundamental aspects of the microparticle formation process remain unclear. The findings in this work will help achieve a fundamental understanding of the relationship of key parameters in the ESE process and the characteristics and release properties of modified release microparticles, which will assist in the success of formulation development. The aims of this work are:

- To investigate whether the ESE method was applicable in preparing two types of modified release microparticles: delayed release (pH sensitive and a combined
Chapter One

pH sensitive and microflora responsive system) and extended release (pH independent) microparticles

- To study the effect of emulsion stability on the properties of microparticles and to understand the microparticle formation process.
- To identify the key process parameters effecting the particle formations and properties of microparticles.
- To investigate whether the final products of microparticles are stable under different types of storage and different accelerated conditions.
- To investigate the effect of physiochemical properties of drugs on their distribution patterns within microparticles and to correlate these observations with drug encapsulation efficiency and in-vitro drug release.
- To explore the potential of the confocal laser scanning microscopy technique in studying the distribution pattern of drugs within modified release microparticles.
CHAPTER TWO

Preparation and characterisation of modified release microparticles by emulsification solvent evaporation method
2.1 Introduction

In this thesis microencapsulation through the emulsion solvent evaporation (ESE) technique has been used to prepare modified release microparticles. The procedure of the ESE process is illustrated in figure 2.1.

In the first step, the drug dissolved in the polymer solution is added into the continuous phase, containing an emulsifier to form a dispersion of drug-polymer-solvent droplets. Stirring is continued until the solvent is removed from the dispersed droplets by evaporation at room temperature, resulting in polymer precipitation and particle hardening. Solidified microparticles can then be separated from the continuous phase by filtration. Finally, microspheres are washed by using an appropriate solvent and then dried under vacuum conditions or in an oven.
Chapter Two

It is important to select the suitable continuous phase and dispersed phase solvent to dissolve the drug and polymer. The following criteria for selection of these two liquid phase solvents were reported by Watts et al (1990). The internal phase solvent should have the ability to dissolve the polymer and ideally be able to dissolve the drug. Also, it should be immiscible with the continuous phase solvent and have a low toxicity and a lower boiling point than the continuous phase solvent. Moreover, the continuous phase should be unable to dissolve the polymer and have a low solubility toward the drug. Besides this, it should have low toxicity and be easy to wash away during the microparticle harvesting process.

There are many parameters influencing the process of microparticle formation. Among these variables, the polymer precipitation rate was of importance as it determined the properties of the microparticles. A summary of the factors affecting the rate of microparticle solidification and the outcome of the effect, influencing the encapsulation efficiency is demonstrated in Table 2.1.

From Table 2.1, it can be seen that the factors controlling the rate of solvent removal from the dispersed droplets are not only the boiling point of the solvent but also the solubility of the organic solvent in the external phase, phase volume ratio between the internal and external phase and the affinity between the polymer and the solvent (Bodmeier and McGinity, 1988; Arshady, 1990a; Sah, 1997). In addition, the rate of solvent diffusion into the continuous phase has a major impact on the rate of polymer precipitation and consequently the overall properties of the microparticles (Bodmeier and McGinity, 1988).
Furthermore, the partitioning of the drug into the continuous phase occurs during the early stage of the particle formation when the emulsion droplets have a non-solidification (semi-solid) status. After the polymer precipitates at the surface of the droplets, the partition of the drug into the external phase is impeded (Yeo and Park, 2004). If the drug is preferentially localised in the continuous phase rather than the internal phase, the longer the time the dispersed droplets will remain in a non-solidified state and the greater will be the amount of the drug that diffuses out from the droplets (Bodmeier and McGinity, 1987b).

Bodmeier and McGinity (1988) proposed that the successful entrapment of drug in the microparticles depends on three main parameters; low solubility of the drug in the external phase, a fast rate of polymer precipitation from the internal phase and a high viscosity of polymer solution in the organic solvent phase. If the drug has a high
favourability to solubilise in the continuous phase, then it will tend to partition out from
the dispersed droplets to the continuous phase; resulting in low drug encapsulation
efficiency within the particles. High concentration of polymer leads to fast polymer
precipitation on the surface of the emulsion droplets, thereby hindering the drug
diffusion through the continuous phase and a high polymer concentration also enhances
the viscosity of the dispersed phase; thus delaying the drug diffusion across the
continuous phase. The low solubility of the polymer in the internal phase also leads to
fast polymer precipitation; resulting in a decrease in drug diffusion through the external
phase and therefore, high drug encapsulation within the microparticles.

The aims of this chapter were to investigate whether the ESE method was appropriate in
the production of two types of microparticles; delayed release (pH sensitive and a
combination of pH sensitive and microflora responsive) and extended release (pH
independent). This chapter has therefore been divided into two sections.

- Section one: Preparation and characterisation of delayed release (pH sensitive)
  and extended release (pH independent) microparticles
- Section two: Preparation and characterisation of a combined pH sensitive and
  microflora responsive microparticle system
SECTION ONE: PREPARATION AND CHARACTERISATION OF DELAYED RELEASE AND EXTENDED RELEASE MICROPARTICLES

2.2 Introduction

The polymeric film coatings for modified release conventional solid dosage forms have also been used to produce modified release microparticles. As seen in Section 1.5.1 (Table 1.6), many techniques have been used to produce microparticles and some polymers produced microparticles with an undesirable morphology; large particle size, aggregation and unacceptable rough, wrinkly and porous surfaces. In addition, some modified release microparticles failed to retard release of drug. It is not surprising to observe many unsuccessful attempts at the production of microparticles since there are many variables influencing the properties of the microparticles such as the physicochemical properties of the drug and polymer, process parameters and the microencapsulation method.

Encapsulating drugs within various types of enteric polymers using the same method and same process conditions would seem to be the ideal way to produce microparticles on a manufacturing scale as it would reduce the cost and ease the production. Many studies have attempted to find a multi-application method to produce microparticles with various types of polymers. For example, Goto et al (1986) proposed an ESE method to encapsulate ketoprofen into various types of acrylic polymers such as Eudragit E, L and S. Ketoprofen loaded microparticles of Eudragit L and S were able to resist the release of drugs in acidic conditions. However, this method produced microparticles of a large size (250 to 1000 µm) with a rough surface.

Kilicarslan and Baykara (2004) also employed the ESE technique to load verapamil within various polymethacrylates such as Eudragit RS, RL, L and L55. The verapamil loaded microparticles of these polymers exhibited high drug encapsulation efficiency and some formulations were successful in controlling the release of the drug.
Nevertheless, the mean diameter size of the microparticles yielded from this study was quite large, ranging from 421-911 μm, and the surfaces of some of the microparticle formulations appeared to be rough and porous. In addition, the use of the spray drying technique for the encapsulation of ketoprofen into various types of pH sensitive polymers such as Eudragit S and L, cellulose acetate phthalate (CAP), cellulose acetate trimellitate (CAT) and hydroxypropylmethylcellulose phthalate (HPMCP) was reported by Palmieri (2002). This method provided small sized microparticles, ranging from 24-88 μm. Only microparticles of Eudragit L, S and HPMCP were able to retard the release of the drug in acidic conditions. However, the ketoprofen loaded microparticles exhibited aggregation, irregular shape and defects.

To date, very few reported methods could provide a multi-application system for producing microparticles with the required characteristics from various types of enteric polymer. A method developed by Kendall (2006), at The School of Pharmacy demonstrated the potential of the ESE method in encapsulating prednisolone within various types of acrylic polymers such as Eudragit L and S. The microparticles of these polymers exhibited high drug encapsulation efficiency (Eudragit L=86.4%, Eudragit S=90.0%), possessed a small size (Eudragit L=31 μm, Eudragit S=50 μm), were spherical, with non-aggregation and a smooth surface. Also, the yield of these microparticles was high (Eudragit L=96.4% and Eudragit S=97.1%). At acidic pH, Eudragit L and S microparticles restricted the release of prednisolone to a minimum.

The method invented by Kendall for producing modified release microparticles using the ESE technique was patented in 2005 (WO/2005/070391). The patent described how this method can overcome the problems of the production of microparticles of poor quality, i.e. with poorly morphology or poorly controlled drug release. Kendall proposed that sorbitan sesquioleate (Arlacel 83) was the most promising emulsifying agent in the oil in oil (O/O) emulsion system (using ethanol as an internal phase and liquid paraffin as a continuous phase) for producing Eudragit L and S microparticles by using the ESE technique since the microparticles produced by Arlacel 83 appeared to have an excellent morphology and were spherical, non-aggregated and non-porous. Compared with other non-ionic surfactants and surfactant mixtures with HLB of 3.7, Arlacel 83 has been
demonstrated to be superior to other emulsifying agents not only in producing an acceptable morphology of microparticles but also in stabilising the emulsion system. Examples of non-ionic surfactants and their HLB values are shown in Table 2.2.

Table 2.2 Examples of non-ionic surfactants

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Non-proprietary name</th>
<th>HLB value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Span 85</td>
<td>Sorbitan trioleate</td>
<td>1.8</td>
</tr>
<tr>
<td>Span 80</td>
<td>Sorbitan monooleate</td>
<td>4.3</td>
</tr>
<tr>
<td>Span 20</td>
<td>Sorbitan monolaurate</td>
<td>8.6</td>
</tr>
<tr>
<td>Oleic acid</td>
<td>Oleic acid</td>
<td>4.3</td>
</tr>
<tr>
<td>Brij 92</td>
<td>Polyoxyl 2 oleyl ether</td>
<td>4.9</td>
</tr>
<tr>
<td>Brij 52</td>
<td>Polyoxyl 2 cetyl ether</td>
<td>5.3</td>
</tr>
<tr>
<td>Arlacel 83</td>
<td>Sorbitan sesquioleate</td>
<td>3.7</td>
</tr>
</tbody>
</table>

Kendall also suggested that the stability of the O/O emulsion system was not only determined by the HLB value of the surfactant since an emulsifier mixture (Span 80 and Span 85) with the same HLB as Arlacel 83 was shown to be less effective in stabilising the emulsion system. The superiority in stabilising the emulsion system of Arlacel 83 is likely because this emulsifier consists of an equimolar mixture of sorbitan monooleate and sorbitan dioleate, therefore these two constituents help to form an effective and strong film around the emulsion droplets.

The novel method described by Kendall (for acrylic polymer) was believed to be superior to other methods in the literature and required only simple and inexpensive equipment, avoiding the use of harmful solvents and additive agents and also provided microparticles with a superior morphology together with a high yield, a high encapsulation efficiency and controlled release properties. It was therefore decided to use the Kendall method as a model for investigating the microparticle formation process using the ESE technique and studying the factors effecting the characteristics of microparticles. In addition, it was decided to attempt to investigate further whether this ESE method would be applicable in the production of various types of modified release microparticles in order to target drugs to the different regions in the gastrointestinal (GI)
tract. A wide range of polymers (polyvinyl derivative and cellulosic polymer) have been used in the studies. To target drugs to the upper part of the intestinal tract, the pH sensitive polymers: CAT (pH threshold 4.8), HPMCP (pH threshold 5) polyvinyl acetate phthalate (PVAP, pH threshold 5), CAP (pH threshold 6) were selected for use in this study. To formulate the extended release system, two types of polymers: ethyl cellulose (EC) and polyvinyl acetate (PVAc) were chosen for the study.

The aim of this study was to investigate whether the ESE method using a simple O/O emulsion was applicable for the production of delayed release (pH sensitive) microparticles and extended release (pH independent) microparticles from various types of polymers and to characterise the resultant microspheres.

2.3 Materials

Prednisolone

Prednisolone is a crystalline powder with a molecular weight of 360.4 Da and is slightly soluble in water (British Pharmacopeia 2003). The aqueous solubility of prednisolone was reported to be 0.24 mg/ml, at 25°C (Hayton et al., 1972). The chemical structure of prednisolone is shown in Figure 2.2. According to the Biopharmaceutics Classification Scheme (BCS), prednisolone is classified as BCS class 1 (Kasim et al., 2004; Lindenberg et al., 2004). Prednisolone is a corticosteroid and has a predominantly glucocorticoid activity. In clinical therapeutics, it is used for the treatment of inflammatory bowel disease, rheumatic disease, suppression of inflammatory and allergic disorder and asthma. In addition, prednisolone also has indications for immunosuppression. Gastro-intestinal side effects of prednisolone include dyspepsia, acute pancreatitis, peptic ulceration (with perforation), abdominal distension and esophageal ulceration.

Prednisolone has been chosen as a model drug as it is a neutral, non-ionisable drug and its solubility is independent of pH. Therefore, under the simulated gastrointestinal conditions in the in-vitro dissolution study, the release of prednisolone from the microparticles depends on the formulation of the microparticles. In addition, the
Chapter Two

modified release formulation of prednisolone loaded microparticles has potential for improving the treatment of diseases which require drugs to be targeted at the intestinal region and also reduces GI side effects. Prednisolone was purchased from Sanofi-Aventis (Romainville, France).

![Chemical structure of prednisolone](image)

*Figure 2.2 Chemical structure of prednisolone*

**Polymethacrylates**

Polymethacrylates are water insoluble polymers and are mainly utilized in oral tablet and capsule formulations as an enteric coating agent. Eudragit L and S are methacrylic acid, methyl methacrylate copolymers (Figure 2.3). Eudragit S is different from Eudragit L in the ratio of free carboxyl groups to ester groups, which is 1:1 in Eudragit L and 1:2 in Eudragit S and soluble in an aqueous buffer at pH > 7 while Eudragit L is soluble in an aqueous buffer at pH > 6. The Eudragit L and S used in this study had a molecular weight of 135000 Da and were obtained from Rohm Pharma (Darmstadt, Germany).

![Chemical structure of Eudragit L and Eudragit S](image)

*Figure 2.3 Chemical structure of Eudragit L and Eudragit S*

For Eudragit L and Eudragit S

\[ R', R^1 = CH_3 \]
\[ R^2 = H \]
\[ R^3 = CH_3 \]
Hydroxypropylmethylcellulose phthalate

Hydroxypropylmethylcellulose phthalate (HPMCP) is made by reacting hydroxypropylmethyl cellulose with phthalic anhydride. HPMCP is a pH sensitive cellulose derivative and insoluble in water and gastric fluid but swells and dissolves promptly in the upper small intestine. Hence, it is extensively used as an enteric coating material for granules and tablets. The chemical structure of HPMCP is shown in Figure 2.4. There are various grades of HPMCP which vary in physical properties and degrees of substitution (Table 2.3). HP50, HP55 and HP55S are studied in this work. The numerical designations that follow “HP” in each product refer to the pH value (x10) in an aqueous buffer solution in which the polymers dissolve. In addition, the symbol “S” in HP55S demonstrates a higher molecular weight grade. HP50, HP55 and HP55S were obtained from R.W.Unwin Limited (Hertfordshire, UK).

Table 2.3 Types and properties of HPMCP

<table>
<thead>
<tr>
<th>Grade of HPMCP</th>
<th>pH Threshold</th>
<th>Molecular weight</th>
<th>Solution viscosity (cSt)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP50</td>
<td>5.0</td>
<td>78000</td>
<td>55</td>
</tr>
<tr>
<td>HP55</td>
<td>5.5</td>
<td>84000</td>
<td>40</td>
</tr>
<tr>
<td>HP55S</td>
<td>5.5</td>
<td>132000</td>
<td>170</td>
</tr>
</tbody>
</table>

¹ Solution viscosity of HPMC (10% w/w) in a mixture of methanol and methylene chloride (1:1) at 20°C (Shinetsu product information sheet)

Figure 2.4 Chemical structure of hydroxypropylmethylcellulose phthalate
Cellulose acetate phthalate

Cellulose acetate phthalate (CAP) comes in the form of a white powder, granules or flakes. It is prepared by reacting a particle acetate ester of cellulose with phthalic anhydride. CAP is insoluble in water but dissolves and swells in an aqueous buffer at pH ≥ 6. The viscosity of CAP in an acetone solution at 25 °C is 80.5 cSt (Eastman product information sheet). CAP is used as a gastroresistant film coating or as a binding agent for tablets and capsules. CAP with a molecular weight of 60000 Da was obtained from Eastman Company UK Limited (Wales, UK). The chemical structure of CAP is demonstrated in Figure 2.5.

![Chemical structure of cellulose acetate phthalate](image)

Figure 2.5 Chemical structure of cellulose acetate phthalate

Cellulose acetate trimellitate

Cellulose acetate trimellitate (CAT) is a trimellitic acid homologue of CAP, which has a cellulose backbone; consisting, by weight, of 25-33% trimellityl content and 18-26% acetyl content. CAT is soluble in an aqueous buffer at a pH of over 4.8. Thus, it has an application for enteric coating pharmaceuticals. CAT was supplied by Acros Organics (Geel, Belgium). The chemical structure of CAT is shown in Figure 2.6.

![Chemical structure of cellulose acetate trimellitate](image)

Figure 2.6 Chemical structure of cellulose acetate trimellitate
Polyvinyl acetate phthalate

Polyvinyl acetate phthalate (PVAP) has a molecular weight in the range of 47000 – 60700 Da and its chemical structure is demonstrated in Figure 2.7. PVAP is prepared by the esterification of partially hydrolysed polyvinyl acetate with phthalic anhydride. The viscosity of 15% w/w PVAP in methanol at 25 °C is in a range of 70 to 110 mPas (Colorcon product information sheet). PVAP dissolves rapidly in an aqueous buffer at pH 5; therefore, it is commonly used as enteric coating for tablets, granules and hard/soft gelatin capsules. PVAP was provided by Colorcon Limited (Kent, UK).

Determining on the phthalyl content, \( a \) will vary with \( b \) in mole percent. The acetyl content \( c \) remains constant depending on the initial material.

Figure 2.7 Chemical structure of polyvinyl acetate phthalate

Ethylcellulose

Ethylcellulose (EC) is available in a wide range of molecular weights and its chemical structure is shown in Figure 2.8. EC is broadly utilised in microencapsulation, extended-release tablet coating, tablet coating and tablet granulation. EC is practically insoluble in water. Therefore, this hydrophobic property is of benefit for coating tablets and granules. There are various grades of EC which vary in solution viscosity. N-7 and N-100 were employed in this study (Table 2.4) and were supplied by Hercules Ltd (Salford, UK).
Chapter Two

Table 2.4 Grades and viscosities of EC

<table>
<thead>
<tr>
<th>Grades of EC</th>
<th>Molecular weight</th>
<th>Solution viscosity (mPas)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-7</td>
<td>58000</td>
<td>5.6-8.0</td>
</tr>
<tr>
<td>N-100</td>
<td>230000</td>
<td>80.0-105.0</td>
</tr>
</tbody>
</table>

¹ Solution of EC 5% w/v in a solvent mixture of toluene/ethanol (4:1) at 25°C (Pharmaceutical excipients)

Figure 2.8 Chemical structure of ethyl cellulose

Polyvinyl acetate

Polyvinyl acetate (PVAc) is a broadly used excipient in the development of oral delayed release products. PVAc is synthesized from vinyl acetate monomer via a free-radical polymerisation technique. The structure of PVAc is shown in Figure 2.9. There are various grades of PVAc, different in viscosity and product forms. In this experiment, PVAc B-60 and B-500, in a granular form, were used (Table 2.5) and supplied by Erkol (Tarragona, Spain).

Table 2.5 Grades and viscosity of PVAc

<table>
<thead>
<tr>
<th>PVAc grades</th>
<th>Solution viscosity (mPas)¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-60</td>
<td>40-50</td>
</tr>
<tr>
<td>B-500</td>
<td>80-100</td>
</tr>
</tbody>
</table>

¹ solution of PVAc (10%w/v) in ethyl acetate at 20 °C (Erkol product information sheet)
Sorbitan sesquioleate

Sorbitan sesquioleate is a mixture of sorbitan mono- and dioleate and its trade name is Arlacel 83. It is a non-ionic surfactant with a HLB of 3.7, a molecular weight of 561 Da and its chemical structure is shown in Figure 2.10.

2.4 Methods

2.4.1 Preparation of microparticles

A polymer to drug ratio of 30 to 1 was selected to be a model ratio for investigating the encapsulation of prednisolone within different types of polymers. The microparticle preparation described by Kendall was as follows, three grams of polymer and 0.1 grams of prednisolone were accurately weighed and dissolved in 30 ml of organic solvent. The resultant solution was added gradually into 200 ml of liquid paraffin containing 1% w/w of Arlacel 83. The emulsion was stirred, at an ambient temperature, using a Heidolph over head propeller stirrer, at a speed of 1000 rpm. Microspheres were collected by vacuum filtration through a glass filter (pore size 4) and then washed three times with 50
ml of n-hexane and dried for 24 hours in a vacuum oven. Each formulation was prepared in triplicate.

Many polymers employed in this study did not dissolve in ethanol. Therefore, the internal solvent phase and polymer concentration in this method was modified and optimised to suit the solubility of the polymers and to obtain good quality microparticles. A list of organic solvents used for dissolving the polymers is shown in Table 2.6.

Table 2.6 List of internal phase organic solvents and polymer concentrations used in the study

<table>
<thead>
<tr>
<th>Polymer</th>
<th>Concentration (%w/v)</th>
<th>Organic solvent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eudragit L</td>
<td>10</td>
<td>Ethanol</td>
</tr>
<tr>
<td>Eudragit S</td>
<td>10</td>
<td>Ethanol</td>
</tr>
<tr>
<td>HP50</td>
<td>10, 5, 2.5</td>
<td>Ethyl acetate/ methanol (1:2) and ethyl acetate/ ethanol (1:1)</td>
</tr>
<tr>
<td>HP55</td>
<td>5, 2.5</td>
<td>Ethyl acetate/ methanol (1:2)</td>
</tr>
<tr>
<td>HP55S</td>
<td>5, 2.5</td>
<td>Ethyl acetate/ methanol (1:2)</td>
</tr>
<tr>
<td>PVAP</td>
<td>5, 2.5</td>
<td>Dichloromethane/ methanol (1:1) and methanol</td>
</tr>
<tr>
<td>CAP</td>
<td>2.5</td>
<td>Dichloromethane/ethanol (2:1) and acetone/ ethanol (2:1)</td>
</tr>
<tr>
<td>CAT</td>
<td>2.5</td>
<td>Dichloromethane/ethanol (2:1) and acetone/ ethanol (2:1)</td>
</tr>
<tr>
<td>EC</td>
<td>2.5</td>
<td>Ethanol</td>
</tr>
<tr>
<td>PVAc</td>
<td>5</td>
<td>Dichloromethane/ methanol (1:1)</td>
</tr>
</tbody>
</table>
2.4.2 Batch yield

The yield of microparticles for each batch was calculated using the Equation 2.1.

\[
\% \ \text{Batch yield} = \frac{\text{Weight of the harvested microparticles}}{\text{Total amount of polymer and drug added}} \times 100 \quad \text{(Eqn.2.1)}
\]

2.4.3 Particle size analysis

After suspending the microparticles in 0.1M HCl, the particle size was measured by laser diffractometry using a Mastersizer S (Malvern Instruments Ltd, Worcestershire, UK). Triplicate measurements were conducted. The average size of microparticles was expressed as a median diameter (Dv50), which is a particle diameter at 50% cumulative volume. Particle size distribution was determined by a span value, which is a measure of the width of the volume distribution relative to the median diameter. The calculation of span is shown in the Equation 2.2.

\[
\text{Span} = \frac{(Dv90-Dv10)}{Dv50} \quad \text{(Eqn.2.2)}
\]

Dv90 and Dv10 have a particle diameter of 90% and 10% cumulative volume, respectively. The larger the value of the span the wider the range of particle sizes whereas the lower the value of the span, the narrower the distribution of particle sizes.

2.4.4 Scanning Electron Microscopy

The morphology and surface topography of microparticles were examined by Scanning Electron Microscopy (SEM). The samples were fastened to a holder using double sided carbon adhesive tape and then coated with gold using a gold sputter module in a high vacuum evaporator (Emitech K550, Ashford, Kent, England). The microspheres were observed and micrographs were taken using the scanning electron microscope (Phillips XL30, Eindhoven, Holland).
2.4.5 Determination of prednisolone encapsulation efficiency

For the pH sensitive microparticles, the encapsulation efficiency was determined by completely dissolving 40 mg of microparticles in 10 ml methanol; followed by the addition of 0.1M HCl to precipitate the pH sensitive polymer and made up to 100 ml. Samples were filtered through 0.22 micrometer disposable filters and assayed for prednisolone spectrophotometrically at 245 nm. The measured absorbance was converted to drug concentration using a standard curve for the known concentration of the drug in 10% methanol in 0.1M HCl. The amount of the encapsulated drug was determined in triplicate.

For the EC microspheres, forty milligrams of microparticles were weighed out accurately and dissolved in 100 ml of ethanol. The quantity of prednisolone was determined by ultraviolet spectrophotometry at a wavelength of 244 nm and calculated with reference to a calibration curve. In the case of PVAc, the assay method was almost the same as above but the solvent used to dissolve the sample was dichloromethane: methanol (1:1) and the wavelength used for detection was 243 nm.

The experiment was carried out in triplicate for each formulation. Prednisolone encapsulation efficiency is calculated from the Equation 2.3.

\[
\text{Encapsulation efficiency} = \frac{\text{Calculated amount of prednisolone in microparticles} \times 100}{\text{Theoretical amount of prednisolone in microparticles}} \quad (\text{Eqn.2.3})
\]

2.4.6 In-vitro release studies

To ensure the sink conditions of prednisolone in the in-vitro dissolution studies, the saturation solubility of prednisolone in the dissolution medium was calculated. As prednisolone is a non-ionisable drug, the saturation solubility study was performed only in 0.1 M HCl and pH 6.8 phosphate buffer. The excess amounts of prednisolone were added to glass vials containing 10 ml of 0.1M HCl and 10 ml of pH 6.8 phosphate buffer. The vials were agitated at 37 °C in a horizontal shaker for 24 hours. The solution
was filtered with a 0.22 micrometer disposable filter and 0.5 ml of solution made up to 10 ml in volumetric flasks using the appropriate medium. The filtrate was assayed for prednisolone spectrophotometrically at 247 nm and the saturation concentration was calculated with reference to a standard curve of prednisolone in 0.1M HCl or pH 6.8 phosphate buffer.

The saturation solubility at 37°C was calculated to be 218.67±1.96 (n=3) and 222.65±1.22 (n=3) mg/L in 0.1M HCl and pH 6.8 phosphate buffer, respectively. From this result, it was shown that the solubility of prednisolone does not depend on the pH and these saturation solubility values are close to an aqueous solubility value of prednisolone at 25°C, 240 mg/L, reported by Hayton et al (1972). The amount of microparticles used in in-vitro dissolution studies was calculated based on the final concentration of the prednisolone in the dissolution medium, which was less than 10% of the saturation solubility of the prednisolone in the dissolution medium.

Release studies were carried out under sink conditions. Three hundred milligrams of microparticles were accurately weighed and filled into a size 0 gelatin capsule. The dissolution test method followed USP27 (United State Pharmacopeia) for the testing of extended release and delayed release dosage forms. The experiments were carried out in triplicate for each formulation.

For pH sensitive microparticles, to evaluate the in-vitro drug release at gastric and intestinal pH, the dissolution test was carried out using a pH change method and USP II dissolution apparatus. The microparticle filled capsule, secured inside a stainless steel sinker was introduced into 750 ml of 0.1M HCl. After 120 minutes, 250 ml of 0.2M trisodium phosphate, which had been equilibrated to 37±0.5 °C, was added to each vessel and the pH was adjusted to 6.8 or 7.4 ±0.05 with 2M NaOH. The experiment was then run for a further 180 minutes. Throughout the experiment, the speed of the paddle was 100 rpm and the temperature of the medium was maintained at 37±0.5. For pH independent microparticles, the dissolution test was conducted for 24 hours in 900 ml of 0.1M HCL using USP II dissolution apparatus. The speed of the paddle was 100 rpm and the temperature of the medium was maintained at 37±0.5 °C.
Chapter Two

During the dissolution test, the samples were taken and filtered through 0.2 μm filters and prednisolone content was measured spectrophotometrically at 247 nm. The amount of prednisolone in the dissolution media was calculated with reference to a standard curve of prednisolone, which was prepared in triplicate for prednisolone in 0.1M HCl solution and in pH 6.8 and 7.4 phosphate buffer. The drug release profiles were calculated and plotted based on the encapsulation efficiency of the drug in the microparticles.

To ensure there was no interference by the polymer on the UV absorbance reading of prednisolone, the pH sensitive polymers and water-insoluble polymers in the dissolution media were spectrophotometrically scanned from 190 to 500 nm. Most of the polymers were shown to have no UV absorbance in the range of testing. However, the scanning results of HPMCP and PVAP polymers found that they had absorbed UV at the same wavelength as prednisolone (247 nm). The content of prednisolone in HPMC and PVAP microparticles was therefore quantified by high performance liquid chromatography (HPLC), described below.

**HPLC-UV for assaying prednisolone**

A Hewlett-Packard 1050 Series HPLC system (Agilent Technologies, UK) was used to analyse the dissolution samples. Ten microlitres of dissolution samples were injected into a Water Symmetry C8 (5 μm particle size) column (Waters, Massachusetts, USA). The chromatographic conditions were as follows: a column temperature of 35 °C, a pressure of 1800 psi, a mobile phase recommended by USP27 consisting of water/ tetrahydrofuran/ methanol (68.8:25:6.2 v/v), and a flow rate of 1.0 mL/min. Prednisolone was detected at 254 nm. The standard curve was constructed for prednisolone in the concentration range of 3 to15 μg/mL. A good linear relationship was observed between the concentrations of prednisolone and peak area (r² = 0.9998). The retention time for prednisolone was found to be 9 minutes. The HPLC assay method was validated in terms of accuracy and precision. The HPLC method was found to be accurate and precise, as indicated by a high recovery of 97.5 to 99.7% of prednisolone and a coefficient of variation of 1.4 %. Chromatograms of blank dissolution medium,
Chapter Two

Prednisolone dissolved in dissolution medium and dissolution sample are demonstrated in Figure 2.11-2.13.

Figure 2.11 Chromatogram of blank dissolution medium

Figure 2.12 Chromatogram of prednisolone dissolved in dissolution medium

78
2.4.7 Apparent miscibility/immiscibility of organic solvent and liquid paraffin

Organic solvent (200 μL) was added to 5 mL of liquid paraffin, contained in a glass vial. The solution was then vortex mixed for 2 minutes (Whirlimixer, Fisons Scientific Apparatus Ltd, Leicestershire, UK). The miscibility was observed visually after mixing for 30 minutes. If the organic solvent was miscible with liquid paraffin, another 200 μL of organic solvent was added into the solution and then vortex mixed for 2 minutes. This step was repeated and carried out until immiscibility of these 2 phases was observed. The last volume of organic solvent, miscible with liquid was recorded. The experiment was performed at room temperature and in triplicate for each sample.
Chapter Two

2.5 Results and discussion

2.5.1 pH sensitive microparticles

2.5.1.1 Eudragit L and S

The aims of this preliminary experiment were to standardise the Kendall method of microparticle preparation and to characterise the potential of resultant microparticles to control the release of drug. The results found that the batch yield for both Eudragit L and S microparticles was high (being 94.3% and 96.8% respectively) and the median diameters were 26.61± 0.3 and 35.49± 0.5 μm for Eudragit L and S microparticles respectively and the size distribution of these microparticles was narrow (span value = 0.77 and 0.91 for Eudragit L and S respectively). The prednisolone encapsulation efficiency of Eudragit L and S was calculated to be 86.72± 1.9 and 87.43± 2.1, respectively. SEM images of blank Eudragit L and S microparticles and prednisolone loaded microparticles are shown in Figures 2.14 to 2.17.

![Figure 2.14 SEM of blank Eudragit L microparticles](image1)

![Figure 2.15a SEM of prednisolone loaded Eudragit L microparticles (high magnification)](image2)
SEM images revealed that both Eudragit L and S microparticles possessed a spherical shape with smooth surfaces, non aggregated and non porous microparticles. The *in-vitro* prednisolone release profiles from Eudragit L and S microparticles in 0.1M HCl for 2 hours and pH 6.8 (Eudragit L microparticles) or pH 7.4 (Eudragit S microparticles) phosphate buffer are shown in Figure 2.18.
Within two hours in 0.1M HCl, prednisolone release from Eudragit L and Eudragit S microspheres was minimal (being 5% and 0.6%, respectively). The difference in the rate of drug release between Eudragit L and S in 0.1M HCl could be due to the smaller size of Eudragit L microparticles and the resultant larger surface to volume ratio, consequently causing the faster release of the drug. Also some molecules of prednisolone might localise on the surface of the Eudragit L microparticles resulting in the leaking of prednisolone at an early stage of dissolution. The study of the pattern of drug distribution within microparticles is described in Chapter Four.

After two hours in an acidic medium, the pH of the dissolution medium was rapidly adjusted to a pH of 6.8 and 7.4 for Eudragit L and S microspheres, respectively. It appeared that both Eudragit L and S microspheres dissolved rapidly and released most of the prednisolone immediately, the release being 96 and 97% within 6 minutes of the change in pH, for Eudragit L and S, respectively.

According to the USP acceptance for delayed release in the acid stage, drug release from enteric coated dosage forms should be less than 10% at 2 hours. Therefore, both Eudragit L and S microparticles met the USP specification for enteric coated dosage forms.
forms. The data from this preliminary experiment (particle size, batch yield, drug encapsulation efficiency, release characteristics) was in accordance with the result reported by Kendall (2006) and proved that this method was reproducible and valid. Therefore, the next studies were to investigate whether the ESE method could be applied universally in the production of various types of modified release microparticles. However, since many polymers used in this experiment did not dissolve in ethanol (internal phase solvent for Eudragit L and S), the organic solvent and polymer concentrations in the Kendall’s method were modified to suit the solubility of the polymers.

2.5.1.2 Hydroxypropylmethylcellulose phthalate

**HP50**

Two types of solvent mixtures, ethyl acetate/ethanol (1:1) and ethyl acetate/methanol (1:2) were used as internal phase solvents to prepare HP 50 microparticles. The SEM images of HP50 microparticles are shown in Figures 2.19 to 2.24.

SEM images (Figure 2.19 to 2.24) demonstrate the difference in morphology of prednisolone loaded HP50 microparticles prepared by using different types of solvents. Microparticles produced using ethyl acetate/ethanol (1:1) showed poor morphology and an unacceptable irregular shape whereas the morphology of microparticles prepared by ethyl acetate/methanol (1:2) appeared to be spherical. This showed clearly that the internal phase solvent had a considerable impact on the morphology of the microparticles. To understand this phenomenon, it is important to know the properties of the internal phase solvents and these are listed in Table 2.7.
Figure 2.19 SEM of prednisolone loaded HP50 microparticles formed from polymer 10% w/v in ethyl acetate/methanol (1:2)

Figure 2.20 SEM of prednisolone loaded HP50 microparticles formed from polymer 5% w/v in ethyl acetate/methanol (1:2)

Figure 2.21 SEM of prednisolone loaded HP50 microparticles formed from polymer 2.5% w/v in ethyl acetate/methanol (1:2)

Figure 2.22 SEM of prednisolone loaded HP50 microparticles formed from polymer 10% w/v in ethyl acetate/ethanol (1:1)

Figure 2.23 SEM of prednisolone loaded HP50 microparticles formed from polymer 5% w/v in ethyl acetate/ethanol (1:1)

Figure 2.24 SEM of prednisolone loaded HP50 microparticles formed from polymer 2.5% w/v in ethyl acetate/ethanol (1:1)
Chapter Two

Table 2.7 The properties of internal phase solvents

<table>
<thead>
<tr>
<th>Organic solvent</th>
<th>Boiling point (°C)</th>
<th>Dielectric constant</th>
<th>Amount of solvent (ml), able to solubilise in liquid paraffin (5 ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate</td>
<td>77.1</td>
<td>6.02</td>
<td>&gt; 10</td>
</tr>
<tr>
<td>Ethanol</td>
<td>78.3</td>
<td>24.3</td>
<td>immiscible</td>
</tr>
<tr>
<td>Methanol</td>
<td>64.7</td>
<td>33</td>
<td>immiscible</td>
</tr>
</tbody>
</table>

*at room temperature*

Since ethyl acetate was miscible with liquid paraffin (Table 2.7), a solvent mixture containing a high ratio of ethyl acetate would have a higher rate of solvent diffusion into liquid paraffin compared to solvent mixtures with a low ratio of ethyl acetate. Although the overall boiling point of ethyl acetate/ethanol (1:1) was higher than that of ethyl acetate/methanol (1:2), it was hypothesised that the higher ratio of ethyl acetate in ethyl acetate/ethanol (1:1) should lead to a faster rate of solvent diffusion from the emulsion droplets into liquid paraffin and consequently a more rapid rate of polymer precipitation, in which the particles would probably solidify rapidly before the microparticles were formed. Consequently, aggregated structures with an irregular shape were formed (Figures 2.22 to 2.24). On the other hand, the use of ethyl acetate/methanol (1:2) as an internal solvent phase should slow the rate of solvent removal from the emulsion droplets, consequently resulting in the slowing of the polymer precipitation rate and increasing the time for the microparticles to be formed.

These effects of solvent removal on the morphology of particles are in agreement with many reports (Tice and Gilley, 1985; Bodmeier et al., 1994; Sah, 1997; Meng et al., 2003). Tice and Gilley (1985) reported that while the wall of the microparticles is being formed, too rapid partition of the internal phase solvent into the external phase and too fast solvent evaporation will disrupt the particle formation process and consequently, affect the morphology of the final microparticles. However, if the rate of solvent removal is slowed down, it will provide a higher quality of microparticle. Bodmeier et al (1988) also observed that irregular structures were formed when using water miscible
solvent as a dispersed phase in the O/W emulsion system. This was due to the rapid diffusion of the solvent into the aqueous phase.

As the HP50 microparticles produced using acetate/ethanol (1:1) exhibited an unacceptable morphology no further analysis of these products was carried out. The median diameter, span value and % batch yield of HP50 microspheres, produced by ethyl acetate/methanol (1:2) are shown in Table 2.8.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Polymer concentration (%w/v)</th>
<th>Dv50 (µm) ±1SD</th>
<th>Span value</th>
<th>Batch yield %</th>
<th>Encapsulation efficiency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethyl acetate/methanol (1:2)</td>
<td>10</td>
<td>186.68 ±15.7</td>
<td>1.6</td>
<td>92.7±1.1</td>
<td>93.7±1.8</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>77.74 ±0.7</td>
<td>1.2</td>
<td>91.5±0.9</td>
<td>92.4±1.1</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>65.91 ±1.1</td>
<td>0.7</td>
<td>93.6±1.3</td>
<td>90.2±0.8</td>
</tr>
</tbody>
</table>

As shown in Table 2.8 and the SEM images (Figures 2.19-2.21), the size and morphology of microparticles are influenced by the polymer concentration. When the polymer concentration increased, the size of the HP50 microparticles increased considerably. A similar trend of the increased size of microparticles with an increase in polymer concentration was reported in many literature reports including the findings of Jeffery et al (1991). They suggested that this was because the increase in the concentration of the polymer led to an increase in the viscosity of the internal phase solution; thereby contributing to an increase in the frequency of collisions and fusions of semi-formed particles, which would result in enlarging the emulsion droplet size and eventually lead to a greater size of microparticles and higher degree of aggregation.

The drug encapsulation efficiency of HP50 microparticles was also found to be slightly increased when increasing the polymer concentrations. Rafati et al (1997) and Shukla and Price (1991) also observed a similar phenomenon in which a higher viscosity of the
polymer solution tended to restrict the diffusion of the drug to the continuous phase, resulting in a lower amount of drug loss.

Due to possessing a spherical morphology with no aggregates, the microparticles, prepared by using HP50 2.5%w/w in ethyl acetate/methanol were selected to study the in-vitro release of prednisolone. The drug release profile of these microparticles in a 0.1M HCl and pH 6.8 phosphate buffer is shown in Figure 2.25.

![Figure 2.25 In-vitro release profile of prednisolone from HP50 microparticles at pH 1.2 for 2 hours followed by an adjustment to pH 6.8](image)

After 2 hours incubation at acidic pH, the microparticles released 19% prednisolone and following a change in pH to 6.8, the drug was released rapidly, the release being complete within 10 minutes after the pH change. As more than 10% of the drug was released from the microparticles in acidic conditions, these HP50 microparticles did not meet the USP specification for enteric coated products.

**HP55**

In the previous study it was seen that the solvent mixture of ethyl acetate/methanol (1:2) and polymer concentrations of 2.5% and 5%w/v were able to produce HP50 microparticles with a good morphology. Therefore, this solvent mixture and
concentrations of polymer were used for preparing the HP55 microparticles. SEM images revealed that prednisolone loaded microparticles of HP55 concentration 5 and 2.5%w/v possessed a spherical shape with no aggregation (Figures 2.26 and 2.27). The median diameter, span value and % batch yield of HP55 microspheres are shown in Table 2.9.

![Figure 2.26 SEM of prednisolone loaded HP55 microparticles formed from polymer 5%w/v in ethyl acetate/methanol (1:2)](image1)

![Figure 2.27 SEM of prednisolone loaded HP55 microparticles formed from polymer 2.5%w/v in ethyl acetate/methanol (1:2)](image2)

**Table 2.9 Characteristics of HP55/prednisolone microparticles**

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Polymer concentration (%w/v)</th>
<th>Dv50 (µm)</th>
<th>Span value</th>
<th>Batch yield (%)</th>
<th>Encapsulation efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ethyl acetate/</td>
<td>5</td>
<td>155.81±6.4</td>
<td>1.1</td>
<td>94.5±1.7</td>
<td>92.2±1.5</td>
</tr>
<tr>
<td>methanol (1:2)</td>
<td>2.5</td>
<td>69.27±2.6</td>
<td>1.2</td>
<td>93.8±2.1</td>
<td>88.79±2.1</td>
</tr>
</tbody>
</table>

The polymer concentration has a great impact on microparticle size and a slight effect on the encapsulation efficiency. Particle size was decreased dramatically by decreasing the polymer concentration (Table 2.9). The encapsulation efficiency was increased when the polymer concentration increased. This can probably be explained by the fact that
increasing the polymer concentration resulted in the increase in viscosity of the dispersed droplets and consequently lowered the diffusion rate of the drug towards the continuous phase.

The release profiles of HP55 microparticles in 0.1 M HCl and pH 6.8 phosphate buffer are shown in Figure 2.28.

![Figure 2.28 In-vitro release of prednisolone from HP55 microparticles at pH 1.2 for 2 hours followed by an adjustment to pH 6.8](image)

After 2 hours in 0.1M HCl, the release of prednisolone from HP55 microparticles, prepared by polymer concentrations of 2.5% and 5% w/v was restricted to less than 5%. Following the increase in pH from 1.2 to 6.8, the release of prednisolone from both sets of HP55 microparticles was rapid and complete within 5 minutes.

The HP55 microparticles appeared to prevent the release of entrapped prednisolone in an acidic condition better than HP50 microparticles (Figure 2.25 and 2.28). The leakage of a large amount of the drug from the HP50 microparticles during incubation in an acidic condition was attributed to the low molecular weight and high water permeability of the HP50 polymer. It has been reported that the amount of water permeating through free films of HP50 and HP55 in 24 hours was 246 and 168 g/m², respectively (Shin-Etsu Chemical Company, 1984). Therefore, when the HP50 microparticles were immersed in
the dissolution medium, the water penetrated into the polymer matrix rapidly and dissolved the encapsulated drug; consequently the drug diffused throughout the fluid filled channels. As a result, a high amount of the drug was released in the early stages of dissolution. In contrast, the drug release from HP55 microparticles was slower due to the higher chain entanglement capability of this high molecular weight polymer and a tighter matrix structure, resulting in a low water penetration rate. In addition, as HP55 contained a higher degree of phthalyl substitution, the pKa of HP55 (4.49) was higher than that of HP50 (4.20); therefore the resistance to the gastric dissolution media of HP55 was observed to be more than HP50 (Davis et al., 1986a).

**HP55S**

HP55S microparticles were prepared by using the same internal solvent and polymer concentration of HP55. The SEM images (Figures 2.29 and 2.30) revealed a large irregular shape of HP55S structures, prepared by polymer concentrations of 2.5 and 5% w/v and the particle size of these microparticles was found to be more than 300 µm. Comparing the morphology of HP55 and HP55S microparticles it was interesting to observe that with the same formulation HP55S could not produce spherical microparticles whereas HP55 provided small spherical microparticles (Figures 2.26 and 2.27).
The formation of HP55S structures with an unacceptable morphology may be explained by the fact that the use of a high molecular weight of HP55S (Mw of HP55 and HP55S is 84000 and 132000, respectively) resulted in a high viscosity in the internal phase solution; thereby contributing to the rapid precipitation of the polymer at an earlier stage before the formation of the spherical microparticles. As a result, irregular shaped HP55S microparticles were formed. Due to the unacceptable morphology of these microparticles, the experiment was not continued further.

2.5.1.3 Polyvinyl acetate phthalate

The SEM micrographs reveal the distinct difference in the morphology of PVAP microparticles prepared by using methanol and dichloromethane/ methanol (1:1) (Figures 2.31 to 2.34). As seen in Figures 2.31 to 2.34, the internal solvent phase had a dramatic effect on the morphology of the PVAP microparticles. The PVAP microparticles produced by a solvent mixture of dichloromethane/ methanol (1:1) appeared to have a better morphology than those microparticles prepared using methanol. It was observed that at room temperature dichloromethane is miscible in liquid paraffin, more than 10 mL of dichloromethane was soluble in 5 ml of liquid paraffin whereas the methanol is immiscible in liquid paraffin. Furthermore, the boiling point of dichloromethane (39.8°C) is much lower than that of methanol (64.7 °C).
As a result of the difference in the boiling point and solubility of dichloromethane and methanol in liquid paraffin, the rate at which these two solvents diffused out from the emulsion droplets into the liquid paraffin phase would be different, the diffusion rate of dichloromethane/ methanol (1:1) being faster than that of methanol. Therefore, the difference in the morphology of the microparticles produced by these solvents may have resulted from the difference in the solvent removal rate between dichloromethane/ methanol (1:1) and methanol.

As the methanol diffused slowly from the emulsion droplets into the liquid paraffin, the time for the polymer precipitation and the duration of the emulsion droplets in a semisolid state is likely to be prolonged, resulting in the emulsion droplets being more susceptible to coalescence and to forming agglomerate structures before the solidification of the microparticles. On the other hand, the faster rate of dichloromethane/ methanol (1:1) removal from the droplets is likely to contribute to the production of spherical particles.

Since the PVAP microparticles, prepared by methanol possessed an unacceptable morphology no further analysis of the product was carried out. The summary of characteristic properties of PVAP microparticles, produced by using dichloromethane/ methanol (1:1) is shown in Table 2.10.
PVAP microparticles produced using polymer concentrations of 5% and 2.5% w/v differed considerably in morphology, as shown in the SEM images (Figures 2.33 and 2.34). At a polymer concentration of 5% w/v the microparticles were unaggregated, spherical, free flowing and exhibited high prednisolone encapsulation efficiency whereas at a polymer concentration of 2.5% w/v they presented large clusters of aggregated small particles and obtained lower drug encapsulation efficiency. The agglomeration of small particles and the low encapsulation efficiency of microparticles at a polymer concentration of 2.5% w/v was probably because the low concentration of polymer led to its slow solidification rate; consequently, the emulsion droplets were in a semi-solid state longer and were therefore susceptible to coalescence and the formation of agglomerates before the solidification of the microparticles. In addition, the slow polymer solidification is also likely to contribute to low drug encapsulation within the microparticles since there was more drug diffusion into the continuous phase.

Due to the large degree of aggregation of PVAP microparticles produced using a polymer concentration of 2.5% w/v, it was decided not to continue further experiments with these particles. The in-vitro release profile of prednisolone loaded PVAP microparticles, prepared using PVAP 5% w/w in dichloromethane/ methanol (1:1) is shown in figure 2.35.
Figure 2.35 In-vitro release of prednisolone from PVAP microparticles, produced using the polymer concentration 5% w/v at pH 1.2 for 2 hours followed by an adjustment to pH 6.8

After 2 hours exposure to 0.1M HCl, the release of prednisolone from PVAP microparticles was restricted to less than 3%. Following the increase in pH of the dissolution media from 1.2 to 6.8, the release rate of the drug was increased, taking approximately 60 minutes for the complete release of prednisolone. At pH 6.8, the slow release of prednisolone is probably due to the large size of PVAP microparticles (99 μm); therefore, the low surface to volume ratio of microparticles results in the slow dissolution of the matrix polymer and the slow release of the drug.

To date, there have been no other published studies on the use of the ESE method to prepare PVAP microparticles. This study shows the potential application of the ESE method in producing PVAP modified release microparticles.

2.5.1.4 Cellulose acetate phthalate

When using acetone/ethanol (2:1) and dichloromethane/ethanol (2:1) and a polymer concentration of 2.5% w/v, it was found that this condition could not provide a desirable morphology of CAP microparticles. The CAP structures were revealed to be of an
unacceptable irregular shape and larger than 600 μm in diameter (Figures 2.36 and 2.37); therefore, no further analysis of the product was carried out.

Figure 2.36 SEM of prednisolone loaded CAP microparticles, prepared using acetone/ ethanol (2:1)

Figure 2.37 SEM of prednisolone loaded CAP microparticles, prepared using dichloromethane/ ethanol (2:1)

There are many reports on the use of CAP for microencapsulation and several methods have been employed to produce CAP microparticles. However, it seemed that the CAP microparticles reported in several studies appeared to be large in size (> 500 μm). For example, Silva and Ferreira (1999) employed CAP for encapsulating various drugs such as thiamine hydrochloride (highly water soluble) and phenacetin (practically water insoluble). By using the ESE method (O/O emulsion system), the mean particle size of the CAP microparticles was found to be approximately 600 μm. However, the morphology of these particles was not reported. The in-vitro drug release profiles in 0.1M HCl showed thiamine hydrochloride released from CAP microparticles rapidly (release > 90% in 1 hour) whereas the release of phenacetin from these microparticles was slow but failed the USP acceptance (release > 10% in 2 hours). Amorim and Ferreira (2001) also used the same method reported above to produce protein loaded CAP microparticles. These microparticles exhibited a large size (1-1.2 mm) and regular shape with a rough surface. Drug release from the CAP microparticles was found to be minimal in acidic conditions (<5% in 2 hours).
Palmieri et al (2002) reported the use of spray drying for preparing ketoprofen loaded CAP microparticles. These microparticles possessed irregular shape and were of small size (24-46 μm). In acidic conditions, CAP microparticles failed to control the release of the drug (>10% in 1 hour).

Moreover, Lin et al (1991) used the coacervation phase separation technique to encapsulate *mycoplasma hyponemonia* vaccine within CAP microparticles. The morphology of these microparticles appeared to be spherical with a large particle size (ranging from 0.5-2.3 mm). Mankar (1992b) also reported the use of coacervation phase separation to produce propranolol hydrochloride (water soluble drug) loaded CAP microparticles. The size of CAP microparticles was in the range of 508-754 μm. The burst release of the drug from these microparticles was found to occur at an early stage of the dissolution study (>10% in 1 hour).

### 2.5.1.5 Cellulose acetate trimellitate

It was found that the use of acetone/ethanol (2:1) and dichloromethane /ethanol (2:1) with a polymer concentration of 2.5% w/v did not provide satisfactory microparticles from CAT. SEM images revealed an unacceptable morphology, large sized and irregular shaped CAT particles (Figures 2.38 and 2.39), therefore no further analysis was carried out.

![Figure 2.38 SEM of prednisolone loaded CAT microparticles, prepared by using acetone/ethanol (2:1) (96)](image1)

![Figure 2.39 SEM of prednisolone loaded CAT microparticles, prepared by using dichloromethane/ethanol (2:1) (96)](image2)
Many studies have been reported on CAT microparticles, produced by various techniques. Sanghvi and Nairn (1991) prepared tartarazine loaded CAT microparticles by using the coacervation phase separation method. The microparticles exhibited a spherical shape with a wrinkly surface and a size in the range of 488-822 μm. Only one formulation could control the release of the drug in acidic conditions (10% at 2 hours).

The use of the spray drying technique to produce paracetamol loaded CAT microparticles was reported by Palmieri et al. (2000). The size of the paracetamol loaded microparticles was in the range of 15-100 μm and the morphology of these microparticles was irregular. At 2 hours in an acidic pH, the microparticles failed to control drug release but the tableted microparticles were able to restrict drug release (<3%). Giunchedi et al. (1995) also prepared CAT microparticles by spray drying. They found that indomethacin loaded CAT microparticles exhibited a small size (6-10 μm) and had an irregular shape with a collapsed structure. However, drug release from CAT microparticles was found to be approximately 10% at 2 hours in an acidic condition.

To date very few studies on the use of the ESE method to produce CAT microparticles have been reported whereas the spray drying and coacervation phase separation techniques have been widely employed to prepare these microparticles. However, the morphology of CAT microparticles, produced by the spray drying method appeared to be small in size and of irregular shape (Giunchedi et al., 1995; Palmieri et al., 2000; Palmieri et al., 2002) while that of microparticles prepared by the coacervation phase separation method exhibited a spherical shape with a large size and wrinkly surface (Sanghvi and Nairn, 1991; Sanghvi and Nairn, 1992).

2.5.2 pH independent microparticles

2.5.2.1 Ethylcellulose

Ethylcellulose (EC) completely dissolves in ethanol and therefore ethanol was used as an internal phase solvent. The distinct differences in the properties of EC microparticles
from different grades of EC can be seen in SEM images Figures 2.40, 2.41 and Table 2.11.

![Figure 2.40 SEM of prednisolone loaded N-7 microparticles: (a) low magnification, (b) high magnification](image)

![Figure 2.41 SEM of prednisolone loaded N-100 microparticles: (a) low magnification, (b) high magnification](image)

**Table 2.11 Characteristics of EC microparticles**

<table>
<thead>
<tr>
<th>Grades of EC</th>
<th>Polymer Concentration % (w/v)</th>
<th>Dv50 (μm) ± SD</th>
<th>Span value</th>
<th>Batch yield %</th>
<th>Encapsulation efficiency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>N-7</td>
<td>2.5</td>
<td>50.18±1.0</td>
<td>1.1</td>
<td>89.41±2.1</td>
<td>81.35±0.9</td>
</tr>
<tr>
<td>N-100</td>
<td>2.5</td>
<td>81.31±7.4</td>
<td>1.2</td>
<td>85.38±3.1</td>
<td>76.31±1.6</td>
</tr>
</tbody>
</table>
Chapter Two

It was observed that large sized microparticles were produced from N-100 (the high viscosity grade of EC) whereas smaller sized microparticles were produced by N-7 (EC of lower viscosity grade) (Figures 2.40, 2.41 and Table 2.11). This result could be explained by the increase in the viscosity of the dispersed phase when a high viscosity grade of EC was used resulting in the emulsion droplets being more susceptible to coalescence and fusion and consequently leading to an increase in microparticle size. Many studies also reported a similar trend in which the particle size increased with the use of a higher viscosity grade of EC (Arabi et al., 1996; Guyot and Fawaz, 1998; Cheu et al., 2001).

It was not expected that the prednisolone encapsulation efficiency of the microparticles produced by N-100 would be lower than that of the microparticles yielded by N-7 (Table 2.10), since the high viscosity of the emulsion droplets of the N-100 formulation would be expected to act as a barrier and impede drug diffusion into the continuous phase; consequently resulting in a high drug encapsulation efficiency within the microparticles.

The low drug encapsulation efficiency in N-100 microparticles could be because of pinholes, which were observed to be scattered on the surface (Figure 2.41b). Therefore, the drug could diffuse out from the particles into the continuous phase through the pores. Qi and Ping (2004) described how the formation of pores on the surface of microparticles was attributed to the use of solvent with a high boiling point as an internal phase. After the solvent on the particles surface completely evaporated, the remaining solvent in the core of the particles vaporised leading to the formation of pores on the surface of the particles. This is related to the explanation of Tice and Gilley (1985) that pinholes were a result of a fast solvent evaporation out of the microparticles due to the vaporization of the remaining solvent within them leading to the disruption of the polymer wall. Tice and Gilley also suggested that if the rate of solvent removal was slowed down, the solvent would have time to diffuse into the continuous phase and then evaporate at the interface of the continuous phase/air resulting in a better morphology of microparticles being obtained.
Chapter Two

As the N-100 produced a very high viscosity in the internal phase, the rate of microparticle solidification could occur very rapidly. It was hypothesised that after the solvent on the surface of N-100 particles had evaporated, the walls of the microparticles solidified. Due to the high boiling point of ethanol (78 °C), an amount of ethanol would have remained inside the particles. As a result, the vaporisation of the remaining solvent led to the formation of pores on the surface of the particles.

However, a porous surface was not observed in the N-7 microparticles (Figure 2.40b). This was probably because when N-7 was used, a lower viscosity in the internal solvent phase was obtained; resulting in a slower rate of microparticle solidification and a slower rate of solvent removal; therefore, the time in which ethanol diffused from the droplets and evaporated at the surface in the continuous phase was extended. As a result the microparticles formed a smooth surface. The in-vitro prednisolone release profiles of EC microparticles at pH 1.2 are shown in Figure 2.42.

![In-vitro prednisolone release profiles](image)

Figure 2.42 In-vitro prednisolone release from prednisolone loaded N-7 and N-100 microparticles at pH 1.2

The rate of prednisolone release from microparticles produced by N-7 was faster than that produced by N-100 (Figure 2.42). The rapid release of prednisolone from N-7 microparticles could be attributed to the larger surface area of the smaller size
microparticles and the low viscosity of N-7. This allowed water to penetrate rapidly into the microparticles and dissolve the entrapped drug which resulted in fast drug release throughout the water filled pores at the early stage.

Even though N-100 microparticles exhibited pores on the surface, these microparticles showed a slow drug release. This might be explained by the fact that N-100 has a high molecular weight, a long molecular chain and a large molecule; therefore, by using the N100 polymer a tighter structure of matrix was formed. As a result it was more difficult for water to penetrate into the N-100 microparticles and consequently a slow drug release was observed. This effect of viscosity of EC on the rate of drug release agrees with many findings (Arabi et al., 1996; Dashevsky and Zessin, 1997; Guyot and Fawaz, 1998; Song et al., 2005).

2.5.2.2 Polyvinyl acetate

Due to the previous study, the dichloromethane/ methanol (1:1) and a polymer concentration of 5 %w/v were able to produce PVAP microparticles with a good morphology. Therefore, this solvent and concentrations of polymer were used for preparing PVAc microparticles. The SEM images of these microparticles and the summary of properties of PVAc microparticles are shown in Figures 2.43, 2.44 and Table 2.12.
SEM micrographs show that both B-60 and B-500 microparticles appeared to be spherical and the size of B-60 microspheres was smaller than that of B-500 microspheres.

Table 2.12 Characteristics of PVAc microparticles

<table>
<thead>
<tr>
<th>Grades</th>
<th>Dv50 (μm)</th>
<th>Span value</th>
<th>Batch yield %</th>
<th>Encapsulation efficiency %</th>
</tr>
</thead>
<tbody>
<tr>
<td>B-60</td>
<td>119.55±8.7</td>
<td>1.2</td>
<td>90.13±2.1</td>
<td>76.51±1.4</td>
</tr>
<tr>
<td>B-500</td>
<td>133.68±3.7</td>
<td>0.9</td>
<td>92.4±1.7</td>
<td>78.82±0.7</td>
</tr>
</tbody>
</table>

The difference in the size of the microparticles can probably be attributed to the difference in the viscosity of the polymer in the dispersed solution. The less viscous grade of polymer produced a smaller particle size. This observation agrees well with the previous study of EC microparticles. When a higher viscosity of PVAc was used, the viscosity of the dispersed droplets was increased, leading to an increase of the emulsion droplet size and finally, to an increase in microparticle size. In addition, the encapsulation efficiency also slightly increased when a high viscosity grade of PVAc was used. This is likely to be because the increase in the viscosity of the dispersed droplets restricted the diffusion of the drug to the continuous phase. As a result, less drug loss was observed. The *in-vitro* release profiles of PVAc microparticles at pH 1.2 are

![Figure 2.45 In-vitro prednisolone release from prednisolone loaded PVAc microparticles at pH 1.2](image-url)
B-500 microparticles controlled the release more effectively than B-60 microparticles as the rate of prednisolone release from B-500 microparticles was slower than that from B-60 microparticles (Figure 2.45). The difference in the release rate was attributed to the difference in the size of microparticles and the structure of their matrix. The B-500, high viscosity grade of PVAc provided a denser and less porous matrix; therefore, holding the drug more firmly and the porosity of the microparticles decreased, consequently decreasing the amount of water penetration into the microparticles during the dissolution. Also the size of B500 microparticles was larger than that of B-60; therefore the low surface area to volume ratio of B500 microparticles resulted in a slow drug release.

2.6 Section one conclusions

By modification of the ESE method reported by Kendall (2006), it was found that a wide variety of modified release microparticles, produced from various types of pH sensitive polymers such as HPMCP, PVAP and water insoluble polymers such as EC and PVAc could be prepared using the ESE technique. In addition, the morphology and modified release properties of the microparticles produced in this study were superior to that of the microparticles reported in much of the literature (Table 2.1).

However, some polymers such as CAT and CAP could not form microspheres under these experimental conditions. The emulsion stability of CAT and CAP formulations could be one of the possible parameters that contributed to the unsuccessful formation of CAT and CAP microparticles. It was, therefore, decided to investigate the possible effect of the stability of the emulsion on the formation of these microparticles. This study is described in Chapter Three: section one.

As the ESE method proved to be applicable to the preparation of various types of pH sensitive microparticles and pH independent microparticles, it was attempted to produce a combination of pH sensitive and microflora responsive microparticles. This investigation is represented in section two.
Chapter Two

SECTION TWO: PREPARATION AND CHARACTERISATION OF A COMBINED pH SENSITIVE AND MICROFLORA RESPONSIVE MICROPARTICLE SYSTEM

2.7 Introduction

Several systems have been developed for colonic drug delivery such as pH sensitive systems, time dependent systems, pressure dependent systems and microbial-enzyme triggered release systems. However, the physiological properties of the gastrointestinal (GI) tract have been an obstruction for many systems to achieve colon specific drug delivery.

Microbial-enzyme triggered drug delivery to the colon appears to be the most promising approach as it exploits a specific property of the colon (Basit, 2005). A great diversity of gram negative bacteria, which are accommodated in the colon, produce a large variety of enzymes that can in turn digest a wide variety of oligosaccharides, non-absorbable disaccharides and polysaccharides. Moreover, regarding safety and cost, naturally occurring polysaccharides are potential polymers for drug delivery to the colon since these polymers are categorised as GRAS (generally regarded as safe) and are inexpensive.

Naturally occurring polysaccharides are derived from many sources such as animal (chondroitin sulphate, chitosan), algal (alginites), plant (guar gum, inulin) and microbial origin (dextran). These polysaccharides are degraded in the colon by the colonic bacterial enzymes to simple saccharides. When polysaccharide based delivery systems reach the colon, the glycosidic linkages within polysaccharides are hydrolysed by the colonic bacterial enzymes; resulting in the release of the drug from a system. Both Bacteroides and Bifidobacterium are the main saccharolytic species. For colonic drug delivery, polysaccharides have been commonly used in matrix systems and film coating agents. Non-digestible oligosaccharides (NDOs) are low molecular weight carbohydrates of an intermediate nature between simple sugars and polysaccharide.
NDOs recognised as prebiotic, resist hydrolysis and absorption in the stomach or small intestine and stimulate the growth of bacteria in the colon (mainly Bifidobacteria species) and also enhance the bacteria fermentation. The most studied prebiotics are inulin and fructooligosaccharide (Howard et al., 1995; Cummings et al., 2001).

The common problems of the polysaccharides and oligosaccharides are their high water solubility, swelling behaviour and poor filming properties which lead to premature drug release in the upper GI tract. To overcome these problems, these polymers are commonly chemically modified to increase their hydrophobicity and/or mixed with other hydrophobic film forming polymers (Rubinstein et al., 1993; Vervoort and Kinget, 1996; Damian et al., 1999; Gliko-Kabir et al., 2000). Polysaccharide in combination with pH dependent or pH sensitive polymers as a film coating using conventional coating methods is commonly used to control the swelling of polysaccharide and its drug release profile. For example, Lorenzo-Lamosa (1998) studied the copolymer system by using the pH sensitive polymer and polysaccharides. Chitosan microspheres containing diclofenac sodium were produced by the spray drying technique and then microencapsulated into Eudragit L and S. The results of in-vitro released studies showed that in an acidic medium, there was no drug release from Eudragit-chitosan microspheres but when the pH of the dissolution medium reached Eudragit pH solubility, the drug was released continuously.

Furthermore, amylose in combination with ethyl cellulose as a film coating for tablets or pellets has been used for colonic drug delivery (COLAL™). A number of gamma scintigraphic and pharmacokinetic studies in humans have revealed the potential of the COLAL™ system for colonic targeting (Cummings et al., 1996; Basit et al., 2004). To date, the COLAL™ drug delivery system is the only polysaccharide-based formulation to progress to clinical trial. The clinical data for the targeting performance of COLAL™-PRED, containing prednisolone metasulpho-benzoate has been assessed in phase II clinical trials (Thompson et al., 2002). Phase III clinical evaluation of this product is being currently undertaken. Table 2.13 shows examples of polysaccharide in combination with pH dependent or pH sensitive polymers as a film coating for colon specific drug delivery.
<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>Water insoluble polymer</th>
<th>Drug</th>
<th>Dosage form prepared</th>
<th>System performance</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amylose</td>
<td>Ethylcellulose</td>
<td>5-aminosalicylic acid</td>
<td>Film coating for pellets</td>
<td>The release of the drug was restricted in the simulated upper GIT pH. Drug release was rapid under simulated colonic conditions.</td>
<td>(Siew et al., 2000)</td>
</tr>
<tr>
<td>Ethylcellulose</td>
<td>Glucose</td>
<td>Film coating for pellets</td>
<td>Glucose resisted release in-vitro gastric and small intestinal conditions. In fermentation studies, the formulation was susceptible to enzyme degradation.</td>
<td>(Milojevic et al., 1996a)</td>
<td></td>
</tr>
<tr>
<td>Ethylcellulose</td>
<td>Ranitidine</td>
<td>Film coating for pellets</td>
<td>In-vivo studies, drug resisted release until the pellets had reached the colon.</td>
<td>(Basit et al., 2004)</td>
<td></td>
</tr>
<tr>
<td>Pectin</td>
<td>Ethylcellulose</td>
<td>5-fluorouracil (5-FU)</td>
<td>Film coating for pellets</td>
<td>Small amount of 5-FU released in the simulated upper GI tract pH. 5-FU mainly released in the caecum and colon.</td>
<td>(He et al., 2007)</td>
</tr>
<tr>
<td>Ethylcellulose</td>
<td>Paracetamol</td>
<td>Film coating for tablet</td>
<td>Pectin in the mixed film was susceptible to degradation by pectinolytic enzyme.</td>
<td>(Wakerly et al., 1997)</td>
<td></td>
</tr>
<tr>
<td>Hydroxypropylmethy1cellulose</td>
<td>5-aminosalicylic acid</td>
<td>Compression coated tablet</td>
<td>Drug resisted release in the simulated gastric and intestinal pH. In simulated colonic conditions, pectin was degraded by the enzyme, resulting in rapid drug release.</td>
<td>(Turkoglu and Ugurlu, 2002)</td>
<td></td>
</tr>
<tr>
<td>Polysaccharide</td>
<td>Water insoluble polymer</td>
<td>Drug</td>
<td>Dosage form prepared</td>
<td>System performance</td>
<td>Reference</td>
</tr>
<tr>
<td>------------------------</td>
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<td>------------</td>
<td>----------------------</td>
<td>-------------------------------------------------------------------------------------------------------------</td>
<td>----------------------------------</td>
</tr>
<tr>
<td>Pectin</td>
<td>Eudragit S</td>
<td>5-fluorouracil</td>
<td>Film coating beads</td>
<td>Drug resisted release in the simulated gastric and intestinal pH. Drug mainly released in colon due to degradation of pectin by colonic bacteria.</td>
<td>(Jain et al., 2007)</td>
</tr>
<tr>
<td>Inulin with high degree of polymerization</td>
<td>Eudragit RS</td>
<td>-</td>
<td>Film</td>
<td>Films resisted gastric and intestinal fluid. During In-vitro degradation, films were degraded by fecal microflora</td>
<td>(Vervoort and Kinget, 1996)</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Eudragit L or S</td>
<td>Sodium diclofenac (SD)</td>
<td>SD loaded chitosan microparticles was encapsulated in Eudragit L or S microparticles</td>
<td>No release was observed in acidic pH for either formulations. In simulated intestinal pH, rapid drug release was observed from Eudragit L formulation whereas slow drug release was found in the Eudragit S formulation. Enzyme degradation was not studied.</td>
<td>(Lorenzo-Lamosa et al., 1998)</td>
</tr>
<tr>
<td>Chitosan-pectin</td>
<td>Hydroxypropylmethylcellulose</td>
<td>Radiolabelled (¹⁹⁹⁰Tc)</td>
<td>Film coating tablet</td>
<td>Gamma scintigraphy revealed that the tablet passed through the stomach and small intestine intact and disintegrated in the colon due to degradation by bacteria</td>
<td>(Macleod et al., 1999)</td>
</tr>
</tbody>
</table>
The limitations of using a pH sensitive polymer in a formulation as a film coating, employing a conventional coating method, are the inter- and intra-individual variability of pH in the gastrointestinal tract and the low colonic pH of patients with inflammatory bowel disease. As a result, the mechanism of release is unreliable and dosage forms could remain intact through the entire colon.

However, these limitations could be overcome by using a mixed polysaccharide and pH sensitive microparticle system. In this study combined polysaccharide and Eudragit S microparticles were investigated. Eudragit S will prevent excessive rapid swelling and solubilisation of polysaccharide and also restrict drug release during the microparticles transit within the upper GI tract. It is expected that the incorporation of polysaccharide into the Eudragit S microparticles would provide a dual mechanism of release (a fail-safe mechanism); hence these microparticles guarantee the specific release of drugs in the colon. Even though the pH in the lower GI tract (e.g. low colonic pH of ulcerative colitis patients) is not above the pH threshold of Eudragit S, when the microparticles reach the colon, the polysaccharide is degraded by bacteria and subsequently the drug is released and its absorption occurs solely within this region. The use of different polysaccharides in this system was also attempted during this study.

The aims of this study were to investigate whether the ESE method was applicable to the preparation of a combined polysaccharide and Eudragit S microparticles and the characterization of these microparticles.

2.8 Materials

Chitosan

Chitosan is sparingly soluble in water and practically insoluble in ethanol (95%). It is a weak base, cationic polyamine and soluble in dilute and aqueous acidic solution (pH<6.5). Chitosan is obtained by the alkaline deacetylation of chitin and its chemical structure is shown in Figure 2.46. Due to the biodegradability, good biocompatibility and low toxicity, chitosan has been extensively used in a number of pharmaceutical
formulations for drug delivery applications such as colonic drug delivery formulations (Tozaki et al., 1997; Lorenzo-Lamosa et al., 1998; Tozaki et al., 1999) and modified release systems (Aiedeh et al., 1997). Chitosan also exhibits good bioadhesive properties and is therefore used as a component in mucoadhesive dosage forms (Genta et al., 1998; Martinac et al., 2005). Chitosan has been produced in various pharmaceutical forms such as microparticles, films, tablets, gels and beads. There are several types and grades of chitosan, varying in molecular weight, viscosity and degree of deacetylation. In this study, a medium molecular weight chitosan (95 kDa.) was used and purchased from Sigma-Aldrich (Poole, UK).

![Chemical structure of chitosan](image)

**Figure 2.46 Chemical structure of chitosan**

**Pectin**

Pectin is a carbohydrate-like plant constituent consisting primarily of chains of galacturonic acid units linked as 1, 4-α-glucosides. The chemical structure of pectin is demonstrated in Figure 2.47. Pectin can be degraded by *Bacteroides* (Rubinstein et al., 1993). The molecular weight of pectin ranges from 30- 100 kDa. Pectin is soluble in water and insoluble in ethanol (95%) and other organic solvents. Due to its gelling properties, pectin has been widely used in oral sustained delivery, transdermal delivery and colonic delivery (Macleod et al., 1997; Semde et al., 1998). Pectin (from apples) was purchased from Sigma-Aldrich (Poole, UK).
Guar gum

Guar gum is a galactomannan and its structure is shown in Figure 2.48. It is practically insoluble in organic solvent but swells and disperses almost instantly in hot or cold water. Guar gum can be degraded by Bacteroides in the colon (Bayliss and Houston, 1986). The molecular weight of guar gum ranges from 220-250 kDa. Guar gum has been generally used in pharmaceuticals as a stabilizing, thickening, suspending and viscosity increasing agent, tablet disintegrant, tablet binder and also as a controlled release carrier (Feinstein and Bartilucci, 1966; Elsabbagh et al., 1978). Guar gum has also been utilised in colonic drug delivery (Prasad et al., 1998; Krishnaiah et al., 1999). Guar gum was purchased from Sigma-Aldrich (Poole, UK).

Xanthan gum

Xanthan gum \((\text{C}_{35}\text{H}_{49}\text{O}_{29})_n\) is an anionic polysaccharide and is soluble in water but practically insoluble in ethanol and ether. The molecular weight of xanthan gum ranges from 2000-50000 KDa. Xanthan gum is a non-toxic material and its aqueous solution is
Chapter Two

stable over a wide pH range (pH 3-12). It has therefore been broadly employed in topical and oral liquid formulations as a thickening, suspending, stabilizing, emulsifying and also viscosity enhancing agent. It has also been used as drug carrier for controlled release formulations (Dhopeshwarkar and Zatz, 1993; Talukdar et al., 1998). Xanthan gum was purchased from Sigma-Aldrich (Poole, UK).

Chondroitin sulfate

Chondroitin sulphate is an important structural element in cartilage and connective tissues and is very highly soluble in water. The molecular weight of chondroitin sulphate ranges from 5-50 kDa and its structure is shown in Figure 2.49. Chondroitin sulphate is degraded by the anaerobic bacteria residing in the large intestine, mainly by Bacteroides (Rubinstein et al., 1992a). Chondroitin sulphate was purchased from Sigma-Aldrich (Poole, UK).

![Figure 2.49 Chemical structure of chondroitin sulphate](image)

Dextran

Dextran is a water soluble polysaccharide with a linear polymer backbone with mainly 1,6-α-D-glucopyranosidic linkages and is degraded mainly by Bacteroides in the colon (Hehre and Sery, 1952). The chemical structure of dextran is shown in Figure 2.50. Dextran has been commonly used for the preparation of hydrogels due to its low tissue toxicity and high enzymatic degradability at the desired site. A molecular weight of Dextran from Leuconostoc mesenteroides ranges from 9000-11000 Da. was used and was purchased from Sigma-Aldrich (Poole, UK).
Amylose

Amylose, a linear polymer of glucose linked with \( \alpha-(1-4) \) bonds, can be comprised of several thousands of glucose units and is one of the two elements of starch, the other being amylopectin. The chemical structure of amylose is demonstrated in Figure 2.51. The molecular weight of amylose ranges from 40-340 kDa. Amylose in a glassy amorphous form is specifically resistant to pancreatic enzymes but is sensitive to digestion by amylase produced by colonic bacteria (Macfarlane and Englyst, 1986). Thus, this form of amylose has been used as a film coating agent for colonic drug delivery (Milojevic et al., 1996a; Siew et al., 2000; Basit et al., 2004). Amylose (Hylon® VII) was supplied by National Starch & Chemical Limited (Manchester, UK).

Inulin

Inulin is a naturally occurring polysaccharide and is mainly composed of fructose units and typically has a terminal glucose. Inulin is soluble in hot water and in a solution of
dilute acid and alkalis. The chemical structure of inulin is demonstrated in Figure 2.52 and its average molecular weight is 1700 Da. Colonic bacteria, especially \textit{Bifidobacteria}, are responsible for the degradation of inulin (Wang and Gibson, 1993). In pharmaceutical applications, inulin has been used as a tablet filler-binder. It is also used in colon specific drug delivery systems (Vervoort and Kinget, 1996). Inulin was purchased from Sigma-Aldrich (Poole, UK).

\begin{figure}[h]
\centering
\includegraphics[width=0.3\textwidth]{inulin.png}
\caption{Chemical structure of inulin}
\end{figure}

\textbf{Isomaltulose}

The solubility of isomaltulose in water is increased when the temperature increases. Isomaltulose has an average molecular weight of 342.2 Da and its chemical structure is demonstrated in Figure 2.53. Isomaltulose is susceptible to degradation by \textit{Bifidobacteria} and \textit{Lactobacilli} (Cummings et al., 2001; Mussatto and Mancilha, 2007). In pharmaceuticals, isomaltulose has been employed as a tablet and capsule diluents, film coating agent, granulating agent and sweetening agent. Isomaltulose was purchased from Sigma-Aldrich (Poole, UK).

\begin{figure}[h]
\centering
\includegraphics[width=0.3\textwidth]{isomaltulose.png}
\caption{Chemical structure of isomaltulose}
\end{figure}
Cyclodextrin

Cyclodextrins (CD) are cyclic oligosaccharides produced from starch. Three natural cyclodextrins, α, β and γ, have different solubilities and contain different numbers of glucose monomers, being six, seven and eight glucose units for α, β and γ cyclodextrin, respectively. The structure of cyclodextrins, cone like toroid molecules, is rigid and contains a central cavity (Figure 2.54).

\[ R', R'' = \text{CH}_3 \text{ for methyl cyclodextrins} \]
\[ R', R'' = \text{CHOHCH}_3 \text{ for 2-hydroxyethyl cyclodextrins} \]
\[ R', R'' = \text{CH}_2\text{CHOHCH}_3 \text{ for 2-hydroxypropyl cyclodextrins} \]

*Figure 2.54 Chemical structure of β cyclodextrin*

Due to the arrangement of hydroxyl groups within the molecule, the interior surface of the cavity is hydrophobic whereas outside of the torus is hydrophilic. This arrangement allows cyclodextrin to form a non-covalent complex with the drug molecule within the internal cavity. The cavity of cyclodextrin can take up a whole molecule of a drug or some part of it. In pharmaceutical applications, cyclodextrins have been employed to improve the solubility, dissolution and enhancement of the bioavailability of various drugs by forming inclusion complexes (Szejtli, 1994; Loftsson and Brewster, 1996). The cyclodextrins inclusion complexes have been also used to mask the unpleasant taste of some drugs. The complexation between drug and cyclodextrin can be illustrated by the Equation 2.4.
Chapter Two

\[
[\text{Drug}] + [\text{CD}] \xleftrightarrow{K_{\text{assoc}}} \frac{[\text{Drug} - \text{CD}]}{[\text{Drug}][\text{CD}]}
\] (Eqn.2.4)

Where \([\text{Drug}]\) and \([\text{CD}]\) represent the free concentration of drug and cyclodextrin, 
\(K_{\text{assoc}}\) is an association constant and \([\text{Drug} - \text{CD}]\) is the concentration of complex. Drug within the complex is dissociated by dilution and/or displaced by a competing agent, such as an endogeneous or exogeneous substance. At the absorption site, the displacement of the drug from the cyclodextrin cavity by competing agents results in the acceleration of drug absorption (Tokumura et al., 1986).

As cyclodextrins are susceptible to digestion by colonic bacteria especially \textit{Bacteroides} (Sinha and Kumria, 2001), they have been exploited as a drug carrier for colonic drug delivery. In an oral specific colonic delivery system, cyclodextrins were commonly used in a prodrug approach (Uekama et al., 1997; Yano et al., 2002; Zou et al., 2005). \(\beta\)-CD is the most broadly used natural cyclodextrin (Bibby et al., 2000) and is sparingly soluble in water (1.85 g/100 ml at 25 °C (Szejtli, 1988). To improve the solubility, toxicity and enhanced drug absorption, natural cyclodextrins have been chemically modified. Hydroxypropyl-\(\beta\)-cyclodextrin (HP-\(\beta\)-CD) is more water soluble and of less toxicity than the native \(\beta\)-CD (Irie and Uekama, 1997). In this experiment, 2- hydroxypropyl-\(\beta\)-cyclodextrin, (HP-\(\beta\)-CD) has been used due to it being more soluble in organic solvent in comparison with \(\beta\) cyclodextrin (Filipovic-Grcic et al., 1996). The average molecular weight of HP-\(\beta\)-CD is 1380 Da. It was provided by Cargil (Surrey, UK)

**Fructooligosaccharide**

Fructooligosaccharides are water soluble oligosaccharides. Fructooligosaccharides are found in a variety of common plants, fruits and vegetables. They are resistant to digestion in gastric acid and pancreatic enzymes but susceptible to being degraded by \textit{Bifidobacteria} and \textit{Lactobacilli}, resident microflora in the large intestine (Cummings et al., 2001). Fructooligosaccharide was purchased from Shangdong Baolingbao Biotechnology Co.Ltd (Shangdong, China).
Chapter Two

Xylooligosaccharide

Xylooligosaccharide is obtained by the enzymatic hydrolysis of xylan. Xylooligosaccharides exhibit prebiotic activity, resist hydrolysis and absorption in the upper GIT and are susceptible to digestion by colonic bacteria especially *Bifidobacteria* in the colon (Howard et al., 1995). They can be used as ingredients in food, cosmetics, pharmaceuticals or agricultural products (Vasquez et al., 2000). Xylooligosaccharide was purchased from Shangdong Baolingbao Biotechnology Co.Ltd (Shangdong, China).

The aqueous solubility of polysaccharides used in this study is shown in Table 2.14.

Table 2.14 Summary of aqueous solubility of polysaccharides (Pharmaceutical excipients, Shangdong product information sheet 2006)

<table>
<thead>
<tr>
<th>Aqueous solubility</th>
<th>Polysaccharide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sparingly soluble</td>
<td>Chitosan</td>
</tr>
<tr>
<td></td>
<td>β-Cyclodextrin</td>
</tr>
<tr>
<td>Soluble</td>
<td>Pectin</td>
</tr>
<tr>
<td></td>
<td>Xanthan gum</td>
</tr>
<tr>
<td></td>
<td>Dextran</td>
</tr>
<tr>
<td></td>
<td>Amylose</td>
</tr>
<tr>
<td></td>
<td>Innulin(^{2})</td>
</tr>
<tr>
<td></td>
<td>Isomaltose(^{2})</td>
</tr>
<tr>
<td></td>
<td>Guargum(^{2})</td>
</tr>
<tr>
<td></td>
<td>Fluctooligosaccharide(^{1})</td>
</tr>
<tr>
<td></td>
<td>Xylooligosaccharide(^{1})</td>
</tr>
<tr>
<td>Very soluble</td>
<td>Chondroitin sulphate</td>
</tr>
</tbody>
</table>

\(^{2}\) *Soluble in hot water*

Other reagents

\(\alpha\)-Amylase (source *Aspergillus Oryzae*), containing enzyme activity 887 FAU/g and a density 1.26 g/ml was purchased from Sigma-Aldrich (Poole, UK). All other reagents were of analytical grade and were used as received.
2.9 Methods

2.9.1 Solubility Test

Polysaccharides have a reasonable solubility in water (Table 2.13) but limited information is available on their solubility in organic solvents. Therefore, this study was conducted to gain data on their solubility in organic solvents. The solubility test in this experiment was based on the solubility terms described in British Pharmacopeia, 2007. The meaning of the terms used in statements of approximate solubility is shown in Table 2.15.

Table 2.15 Terms of approximate solubility (British Pharmacopeia, 2007)

<table>
<thead>
<tr>
<th>Descriptive terms</th>
<th>Approximate volume of solvent in millilitres per gram of solute</th>
</tr>
</thead>
<tbody>
<tr>
<td>Very soluble</td>
<td>Less than 1</td>
</tr>
<tr>
<td>Freely soluble</td>
<td>1-10</td>
</tr>
<tr>
<td>Soluble</td>
<td>10-30</td>
</tr>
<tr>
<td>Sparingly soluble</td>
<td>30-100</td>
</tr>
<tr>
<td>Slightly soluble</td>
<td>100-1,000</td>
</tr>
<tr>
<td>Very slightly soluble</td>
<td>1,000-10,000</td>
</tr>
<tr>
<td>Practically insoluble</td>
<td>More than 10,000</td>
</tr>
</tbody>
</table>

The solubility test was conducted at room temperature and during the experiment the solution in a beaker was covered with aluminium foil to prevent the evaporation of the organic solvent.

1. Ten milligrams of polysaccharide were slowly added to 100 ml of organic solvent under magnetic stirring (Jenway 1002 Magnetic Stirrer, Dunmow, Essex). Stirring was continued for 24 hours. If the polysaccharide did not dissolve in the organic solvent within 24 hours, it was assumed that this polysaccharide was practically insoluble. However, if it dissolved completely, the experiment continued following step 2.
2. Ten milligrams of polysaccharide were slowly added to 10 ml of organic solvent under magnetic stirring and the solution was stirred for 24 hours. After 24 hours, if the polysaccharide did not dissolve in the organic solvent, it suggested that this polysaccharide was very slightly soluble in organic solvent. If it dissolved completely, the experiment continued following step 3.

3. Ten milligrams of polysaccharide were slowly added to 1 ml of organic solvent under magnetic stirring and the solution was stirred for 24 hours. If the polysaccharide did not dissolve in the organic solvent within 24 hours, it implied that this polysaccharide was slightly soluble in organic solvent. However, if it dissolved completely, the experiment continued following step 4.

4. One gram of polysaccharide was gradually added to 30 ml of organic solvent under magnetic stirring and the solution was stirred for 24 hours. If the polysaccharide did not dissolve in the organic solvent within 24 hours, it was assumed that this polysaccharide was sparingly soluble in organic solvent. If it dissolved completely, the experiment continued following step 5.

5. One gram of polysaccharide was slowly added to 10 ml of organic solvent under magnetic stirring and the solution was stirred for 24 hours. If the polysaccharide did not dissolve within 24 hours, it suggested that this polysaccharide was soluble in organic solvent. If it dissolved completely, the experiment continued following step 6.

6. One gram of polysaccharide was gradually added to 0.99 ml of organic solvent under magnetic stirring and the solution was stirred for 24 hours. If the polysaccharide dissolved completely in organic solvent, it was assumed that this polysaccharide was very soluble in organic solvent. If it did not dissolve within 24 hours, this implied that this polysaccharide was freely soluble in organic solvent.

### 2.9.2 Method of preparation of microparticles

*Preparation of HP-β-CD microparticles:*
Prednisolone loaded HP-β-CD microparticles (polymer to drug ratio 10 to 1) were used in this experiment. The method of preparation is described in Section 2.4.1.
Preparation of mixed Eudragit S and HP-β-CD microparticles:

A polymer to drug ratio of 30 to 1 (containing 88%, 8.8% and 3.2% w/w of Eudragit S, HP-β-CD and drug, respectively) was used in this experiment. A combination of Eudragit S and polysaccharide microparticles were prepared in an identical procedure to Section 2.4.1, except HP-β-CD (8.8% w/w) was added to the drug solution prior to addition of the polymer.

2.9.3 In-vitro release study

Drug release from the Eudragit combined HP-β-CD microparticles was assessed for 2 hours under a simulated gastric condition (0.1M HCl). This was followed by adjustment of the dissolution medium to small intestinal conditions in the absence and presence of amylase. Three hundred milligrams of microparticles were accurately weighed and filled into a size 0 gelatin capsule. The dissolution test method described in Section 2.4.6 was used, except under the presence of enzyme conditions, α-amylase from Aspergillus Oryzae (50 unit) 0.045 ml was added after the pH value in the dissolution medium had been adjusted to pH 6.5. The rationale for using the α-amylase from Aspergillus Oryzae was because it has been reported that this enzyme was a suitable glycolytic enzyme for the in-vitro assay of colonic degradation of β-CD (Fetzner et al., 2004).

2.10 Results and discussion

Most polysaccharides are soluble in water (Table 2.13). The use of a mixture of water and organic solvent as an internal phase solvent to dissolve the polysaccharides was attempted. It was decided to conduct a preliminary experiment using a mixture of water and ethanol to prepare Eudragit S loaded prednisolone microparticles. The SEM images of Eudragit S/ prednisolone microparticles prepared by employing a 9:1 ratio of absolute ethanol to water as an internal phase are shown in Figure 2.55.
Figure 2.55 Eudragit S/prednisolone microparticles prepared by a mixture of water and ethanol

Figure 2.55 illustrates the irregular shape and large size of the structures. This can be explained by the fact that the addition of water in the dispersed phase reduced the solubility of Eudragit S, a water insoluble polymer, in the internal phase; resulting in the fast precipitation of the polymer. It was therefore decided not to use water and organic solvent mixtures as the internal phase. Thus, polysaccharides used in this experiment need to be able to dissolve in an organic solvent as the microparticle preparation process is an O/O emulsion system.

To select the appropriate polysaccharide, various types of polysaccharides were examined to discern their solubility in organic solvents. Table 2.16 shows the solubility of different types of polysaccharide in organic solvents and it was found that most of the polysaccharides were insoluble in an organic solvent. However, HP-β-CD was freely soluble in ethanol and methanol. Therefore, in this experiment HP-β-CD and ethanol were selected to use as a model for polysaccharide and internal phase solvent, respectively.
### Table 2.16 Summary of solubility of various polysaccharides in organic solvents

<table>
<thead>
<tr>
<th>Polysaccharide</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Acetone</th>
<th>Ethyl acetate</th>
<th>Dichloromethane</th>
<th>Isopropanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>HP-β-CD</td>
<td>Freely soluble</td>
<td>Freely soluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Very slightly soluble</td>
</tr>
<tr>
<td>Isomaltulose</td>
<td>Very slightly soluble</td>
<td>Sparingly soluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>Fluctooligosaccharide</td>
<td>Very slightly soluble</td>
<td>Sparingly soluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>Xylooligosaccharide</td>
<td>Practically insoluble</td>
<td>Very slightly soluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>Chitosan</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>Pectin</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>Amylose</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>Chondroitin sulfate</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>Xanthan gum</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>Guar gum</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>Dextran</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
</tr>
<tr>
<td>Inulin</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
<td>Practically insoluble</td>
</tr>
</tbody>
</table>
To investigate whether HP-β-CD could be formulated into microparticles, prednisolone loaded HP-β-CD microparticles, with a polymer to drug ratio of 10:1 were prepared. The SEM images of these microparticles are shown in Figure 2.56.

![SEM images of microparticles](image)

*Figure 2.56 Prednisolone loaded HP-β-CD microparticles, polymer to drug ratio (10:1)*

Most of the HP-β-CD microparticles appeared to be spherical. The median diameter, encapsulation efficiency and batch yield of HP-β-CD microparticles were found to be 137.41 ± 5.1μm, 78.3 ± 1.7 % and 86.8 ± 1.3%, respectively. The prednisolone release profile of these microparticles in 0.1M HCl is shown in Figure 2.57.

![Prednisolone release profile](image)

*Figure 2.57 In-vitro drug release of HP-β-CD microparticles in pH 1.2*
As expected, the drug release from HP-β-CD microparticles was rapid. This was because HP-β-CD is soluble in water and HP-β-CD enhanced the aqueous solubility of prednisolone by forming an inclusion complex. As a result, a rapid dissolution of prednisolone was observed. This result was in agreement with the findings of Kamada et al. (2002) who reported that the cyclodextrin complex is not suitable for oral drug delivery to the colon due to the premature release of the drug as a result of dilution and/or competitive inclusion effect. At this point, it was clear that using polysaccharide alone in the system would lead to the failure of system due to the rapid drug release.

To investigate whether Eudragit S could improve the dissolution behaviour of HP-β-CD microparticles, combined HP-β-CD and Eudragit S/prednisolone microparticles, polymer to drug ratio 30 to 1 (containing 88%, 8.8% and 3.2% w/w of Eudragit, HP-β-CD and the drug, respectively) were prepared. The SEM image of prednisolone loaded mixed Eudragit S and HP-β-CD microparticles is shown in Figure 2.58.

Figure 2.58 SEM of combined HP-β-CD (8.8% w/w) and Eudragit S/prednisolone microparticles

Prednisolone loaded mixed Eudragit S and HP-β-CD microparticles exhibited spherical shape with a smooth surface. The batch yield of combined Eudragit S and HP-β-CD microparticles was 89.5% ±1.2 and the median diameter and span value were 53.81±0.9, 9.3 respectively. Prednisolone encapsulation efficiency was calculated to be 85.32 ±1.8. Compared to the encapsulation efficiency of Eudragit S microparticles in the absence of
Chapter Two

HP-β-CD, the value (86.27±1.9%) was very similar to the value of those composing HP-β-CD. The addition of HP-β-CD, therefore, did not affect the ability of Eudragit S in encapsulating prednisolone.

For in-vitro release studies, drug release from the mixed Eudragit S and HP-β-CD microparticles was assessed under simulated gastric and colonic conditions in the presence and absence of α-amylase (source *Aspergillus Oryzae*), suitable enzyme degrading β-CD (Fetzner et al., 2004). The prednisolone release profile of Eudragit S microparticles and combined HP-β-CD (8.8%w/w) and Eudragit S microparticles in 0.1M HCl for 2 hours followed by a pH change to 6, 6.5 and 6.8 are shown in Figure 2.59.

![Figure 2.59 In-vitro drug release of Eudragit S microparticles and mixed Eudragit S and HP-β-CD (8.8%w/w) microparticles in 0.1M HCl for 2 hours followed by a pH change to 6, 6.5 and 6.8. Error bars have been omitted for clarity.](image)

After 2 hours in acid, the release of prednisolone was found to be less than 7% from all formulations. It is thought that the restricted release of prednisolone at pH 1.2 can be attributed to the insolubility of Eudragit S which acted as a matrix polymer and effectively protected the release of prednisolone from the microparticles.
Following the pH change to 6, the release of prednisolone from Eudragit S microparticles and combined Eudragit S and HP-\(\beta\)-CD microparticles was slow, as a result of slow polymer dissolution and the swelling of Eudragit S. Increasing the pH values to greater than 6 resulted in an increased prednisolone release rate with pH. When the pH was increased from 1.2 to a range of between pH 6 to 6.8, the release rate of prednisolone from combined Eudragit S and HP-\(\beta\)-CD microparticles was faster than that of Eudragit S microparticles. This could be attributed to the effect of HP-\(\beta\)-CD, the water soluble polysaccharide and the inclusion complex of HP-\(\beta\)-CD with prednisolone. Following the pH increase, the dissolved and swollen Eudragit S led to the creation of pores in the microparticles, resulting in the water diffusing into the channel and dissolving the HP-\(\beta\)-CD and the drug. The rapid dissolution of HP-\(\beta\)-CD led to the increase in fluid filled channels, which consequently facilitated drug release and also the dissociation of the free drug from the inclusion complex.

The effect of cyclodextrin enhancing drug release has been reported by many researchers (Gursoy et al., 1995; Utsuki et al., 1996). Villar-Lopez reported that drug release was enhanced by increasing the porosity of the pellet and the dissociation of the free drug from the inclusion complex after cyclodextrin dissolved upon contact with water (Villar-Lopez et al., 1999). Giunchidi et al. also found that the addition of cyclodextrin increased drug release by promoting the erosion of the matrix polymer and enhancing the aqueous solubility of the drug (Giungkini et al., 1994). Incorporation of cyclodextrins in the formulation can modify the drug release of the system by effecting drug solubility, diffusivity of the drug and promoting hydration of the polymer system.

From the results above, pH 6.5 was selected to investigate the release of prednisolone from combined Eudragit S and HP-\(\beta\)-CD microparticles in the presence of \(\alpha\)-amylase. After incubating the microparticles for 2 hours, the pH in the dissolution medium was adjusted to pH 6.5 and then \(\alpha\)-amylase was added. The release profiles of prednisolone from combined Eudragit S and HP-\(\beta\)-CD (8.8%w/w) microparticles are shown in Figure 2.60.
Chapter Two

Figure 2.60 In-vitro drug release of Eudragit S microparticles and mixed Eudragit S and HP-β-CD (8.8% w/w) microparticles in pH 1.2 for 2 hours followed by pH change to 6.5 in the presence and absence of α-amylase

No obvious differences in the dissolution performance of combined Eudragit S and HP-β-CD (8.8% w/w) microparticles were seen in the absence and presence of glycolytic enzyme, α-amylase. This was because the HP-β-CD dissolves rapidly after the increase in pH; therefore, there is an insufficient amount of HP-β-CD left in the microparticles for the enzyme to degrade.

To prove the conclusion above, the release study and characteristics of combined Eudragit S and HP-β-CD microparticles (polymer to drug ratio 30:1), containing a higher amount of CD than in the previous study, were investigated. Figure 2.61 shows the morphology of combined Eudragit S and HP-β-CD microparticles, containing 3.2%, 16.1% and 80.7% w/w of the drug, HP-β-CD and Eudragit S, respectively.

An SEM image of combined Eudragit S and HP-β-CD (16.1% w/w) microparticles revealed aggregated particles with a wide size distribution. The increase in the amount of HP-β-CD might lead to the increase in the viscosity of the internal phase solution, resulting in the increase in the frequency of collision and the fusion of partially formed particles and consequently to the aggregation of the final microparticles. The median size of the microparticles was 136.95±26.7 μm and the span value was 1.85. The prednisolone encapsulation efficiency was calculated to be 84.65±1.1%.
Chapter Two

Figure 2.61 SEM of combined Eudragit S and HP-β-CD (16.1%w/w) prednisolone microparticles

The in-vitro prednisolone release from combined HP-β-CD (16.1%w/w) and Eudragit S microparticles under simulated gastrointestinal tract conditions (2 hours in acid and then adjusted to pH 6.5) in both the presence and absence of α-amylase is shown in Figure 2.62.

Figure 2.62 In-vitro prednisolone release from combined Eudragit S and HP-β-CD (16.1%w/w) microparticles in pH 1.2 for 2 hours followed by pH change to 6.5 in the presence and absence of α-amylase

In the absence of amylase, the rate of prednisolone release from the microparticles prepared by using HP-β-CD 16.1%w/w was faster than that of HP-β-CD 8.8%w/w
(Figures 2.60 and 2.62). This is due to the increase in the concentration of HP-β-CD, leading to the increase in fluid filled pores and aqueous diffusion into the microparticles, resulting in an increase in the rate of drug release. However, no difference in the drug release rate from mixed Eudragit S and HP-β-CD (16.1% w/w) microparticles was observed under either the presence or absence of α-amylase.

To conclude; the amount of HP-β-CD was not the key factor in the unsuccessful formulation but the rapid release of HP-β-CD was the reason for the lack of success in the formulation of combined Eudragit S and HP-β-CD microparticles, since the rapid dissolution of HP-β-CD in the simulated intestinal pH resulted in an insufficient amount of HP-β-CD left for the enzyme to degrade.

2.11 Section two conclusions

Limitation in the study was in the choice of polysaccharide that could be used in the experiment, as this had to be soluble in an organic solvent. HP-β-CD appeared to be the polysaccharide of choice as it was highly soluble in an organic solvent. Nevertheless, incorporating HP-β-CD into the system was a challenge due to it being water soluble. Even though the combined HP-β-CD and Eudragit S microparticles could retard drug release in acidic conditions, in simulated colonic conditions these microparticles failed to show a difference in the drug release in the presence or absence of an enzyme. The lack of success of a mixed Eudragit S and HP-β-CD formulation could be due to the rapid release of the HP-β-CD from the particles, resulting in an insufficient amount of HP-β-CD left for the enzyme to degrade.

2.12 Overall conclusions

The ESE method used in this study was shown to be a potential technique in the production of pH sensitive and pH independent modified release microparticles, since microparticles with a good morphology and the ability to modify the release of drugs in simulated gastrointestinal tract conditions were obtained. This technique also provided the mixed HP-β-CD and Eudragit S microparticles with a spherical shape and a smooth
surface. However, unsuccessful release characteristics were observed in these microparticles. This was due to the rapid release of the HP-β-CD from the microparticles, resulting in an insufficient amount of HP-β-CD remaining for the enzyme to degrade.

To help in understanding the process of particle formation in the ESE method, it was therefore decided to investigate the effect of the stability of the emulsion and the influence of process parameters such as stirring rates, concentration of polymer and emulsifier and drug loading. These studies are described in Chapter Three. Studies on the release characteristics of drugs in correlation to their distribution within microparticles have been conducted and are described in Chapter Four.
CHAPTER THREE

An investigation into the process factors affecting the emulsion solvent evaporation process and the stability of the final product
3.1 Introduction

As described in Chapter two: section one, the emulsification solvent evaporation (ESE) method was applicable in producing a variety of modified release microparticles. However, it could not be used to prepare microparticles from the enteric polymers such as cellulose acetate phthalate (CAP) and cellulose acetate trimellitate (CAT). It is clear that although the emulsification solvent evaporation (ESE) method is a conceptually simple technique, the microparticle formation process is relatively complex and there are many factors governing the characteristics of the microparticles. Studies of the stability of the emulsion system and investigation of the effect of process parameters on the properties of microparticles would help in understanding the microparticle formation process and aid in the improvement of the quality of the microparticles.

This chapter is concerned with the processing and stability of the product. The aims are to study the effect of emulsion stability on the morphology and size of microparticles and to investigate and identify the key process parameters influencing the properties of modified release microparticles and also to study the stability of the final product in different conditions. This chapter has been divided into three sections.

- Section One: Studies on the stability of the emulsion system
- Section Two: Effect of process parameters on the properties of microparticles
- Section Three: Stability of the final product
SECTION ONE: STUDIES ON THE STABILITY OF THE EMULSION SYSTEM

3.2 Introduction

Emulsification of the dispersed phase and continuous phase containing the emulsifier is the first step in the ESE method. This step is a crucial process for the formation of microparticles and it is also determines the properties of the microparticles such as morphology and size distribution (Nihant et al., 1994). In the ESE process, the stability of the emulsion is required for short periods of time until the particles have hardened or microparticles are formed. The particle formation is a complex mechanism as it involves emulsion stabilisation, solvent removal and the polymer precipitation process. Also the various factors such as polymer, drug, organic solvent, external phase and emulsifying agent influence the particle formation process.

Nevertheless, studies of the stability of the emulsion system would help in understanding the microparticle formation process and aid in the improvement of the quality of the microparticles. To date, there are few reports on the effect of emulsion stability on the properties of microparticles, prepared by the ESE method. For example, the effect of the emulsion stability on the morphology of polylactide microparticles, prepared by double water-oil-water (W/O/W) emulsion has been reported by Nihant et al (1994; 1995b) and Schugens et al (1994). These studies found that the stability of the primary emulsion had a dramatic influence on the microparticle formation process and greatly determined the structure of the microparticles. In addition, Blanco-Prieto et al (1996) reported that the stability of the primary emulsion effected the protein encapsulation efficiency within poly(lactide-co-glycolide) microparticles, prepared by W/O/W emulsion. They observed that formulations in which the emulsion was stable provided high protein encapsulation efficiency.

To understand more about the emulsification process, the emulsion background and the stability of emulsion are described in detail below.
3.2.1 Stability of emulsion

An emulsion is a heterogeneous system composed of two immiscible liquid phases, one of which is dispersed as small droplets distributed through the other. In a stable emulsion, the dispersed droplets retain their initial character and maintain uniformity distributed throughout the continuous phase within the experiment timescale (Binks, 1998). In the emulsification process, the emulsifying agent plays a crucial role in reducing the interfacial tension, stabilising emulsion droplets from flocculation and coalescence and maintaining the dispersed state of small disperse droplets. To stabilize the emulsion droplets, emulsifying molecules arrange themselves in a particular way to lower the interfacial energy between the two phases and to form an interfacial film around the dispersed droplets.

The emulsion is a thermodynamically unstable system as it has high surface energy, therefore, the emulsion tends to reduce the surface area of the dispersed droplets through coalescence and finally returns to its original state of two separate phases. The instability of the emulsion may be classified as follows.

Creaming

Creaming (or sedimentation), a layer of concentrated emulsion may be observed in a stable emulsion due to the difference in density of the two liquid phases. The creaming position, rising to the top or sinking to the bottom, depends on the relative density of the disperse phase and continuous phase. Creaming is a reversible process as the droplets of the creamed layer may redisperse throughout the continuous phase and be restored to their original state by gentle shaking. However, since the droplets are in close proximity in the cream, it is likely that coalescence will then occur.

According to Stoke's law (Equation 3.1), the creaming rate is determined by the distinction of density between the dispersed droplets and the continuous phase, the viscosity of the continuous phase and the droplet size. The rate of creaming is therefore
reduced by decreasing the difference in density between the two phases, increasing the viscosity of the continuous phase and reducing the size of the droplets.

\[ v = \frac{2ga^2(\sigma-\rho)}{9\eta} \]  
(Eqn. 3.1)

Where \( v \) is the velocity of creaming, \( g \) is the acceleration due to gravity, \( a \) is the droplet radius, \( \sigma \) is the density of the sphere (i.e. droplet of the dispersed phase), \( \rho \) is the density of the continuous phase and \( \eta \) is the velocity of the continuous phase.

**Flocculation**

Flocculation is described as the aggregation of the emulsion droplets without a rupture of the interfacial film at the interface. This is because of the attractive forces between the droplets, and may result from the change in the properties of the adsorbed emulsifier layer, such as changes in the electrostatic or steric repulsion or a change in the solvent quality due to the addition of other substances (Becher, 2001).

Flocculation induces a rapid creaming as flocs, clusters of droplets with a large radius, rise more rapidly than individual droplets. Compared to the monodisperse system, the polydisperse system further increases the degree of flocculation due to the difference in the creaming velocity of large and small droplets, resulting in the droplets being in close proximity which may result in aggregation. Flocculation is a reversible process, the emulsion droplets may be redispersed by shaking. However, following flocculation, coalescence may occur due to the weakness of the interfacial films of the droplets which are in contact with each other in the flocculation state.

**Coalescence and breaking**

Coalescence is an irreversible process whereby the droplets collide and contiguously fuse together to form a large single droplet. Eventually, this process leads to the separation of two liquid phases. Cracking or breaking are the terms used to describe the emulsion system in which the constituent phases of the emulsion separate into individual
Chapter Three

phases. Due to the fact that the interfacial film surrounding the droplets has been ruptured when a breaking occurs; simple shaking of the emulsion fails to redisperse the droplets.

Ostwald ripening

Oswald ripening is a process in which the emulsion droplet size increases by the diffusion of material from smaller droplets to larger droplets due to the solubility differences of materials within droplets of different size. This process is also irreversible. According to the Kelvin equation (Equation 3.2), the solubility of the material within the droplets increases with a decrease in droplet size. As a result the material from smaller droplets tends to dissolve and diffuse through the bulk phase, recondensing into larger droplets; consequently, there is an overall increase in the average droplet size of the emulsion.

\[
c(r) = c(\infty) \exp \left( \frac{2\gamma V_m}{rRT} \right) \quad \text{(Eqn. 3.2)}
\]

Where \( c(r) \) is the solubility of the material in the droplets of radius \( r \), \( c(\infty) \) is the solubility in the system with only a planar interface, \( \gamma \) is the interfacial tension, \( V_m \) is the molar volume of the material within the droplets.
The processes described are summarised in Figure 3.1.

Assessment of emulsion stability

There are many ways to investigate the instability of an emulsion. The simplest and most direct way to characterise the stability is to monitor the macroscopic phase separation of the emulsion since the gross instability in the emulsion is indicated by the separation of the dispersed phase into a distinct layer (i.e. cracking). However, a more precise
technique for evaluating the emulsion stability is to measure the particle size and size
distribution with time, due to the fact that an unstable emulsion is generally
characterized by a progressive increase in the particle size and the widening of particle
size distribution with time. As the wide range of size distribution brings the droplets
packing closer, it increases the chance for droplets to coalesce (Nihant et al., 1994;
Indiran et al., 1995). In this study, both particle size measurement and monitoring of the
phase separation time were used to assess the stability of the emulsions.

As CAT and CAP could not form microspheres under the previous study conditions, the
lack of emulsion stability of the respective formulations could be one of the possible
reasons that contributed to the unsuccessful formation of CAT and CAP microparticles.
It was, therefore, decided to investigate the relationship between the stability of the
emulsion and the morphology of the final microparticles. The emulsion stability of the
formulations providing a good morphology of microparticles was compared with that of
formulations producing bad morphology. Since the microparticles of polymethacrylate
(Eudragit L and S) and hydroxypropylmethylcellulose phthalate (HP55) exhibited a
spherical shape with a smooth surface, the formulations of these microparticles were
selected as good models. Also the formulation of CAP and CAT were selected to
represent bad formulations.

The aims of this study were to investigate whether emulsion stability in the ESE method
is an important parameter for the formulation of polymers into modified release
microparticles and to examine whether it is possible to use a simple assessment of
emulsion stability as a pre-formulation scanning tool to select the formulation for
producing the microparticles.

3.3 Materials

Full details of prednisolone, polymethacrylate (Eudragit L and S), hydroxypropyl-
methylcellulose phthalate (HP55), cellulose acetate phthalate (CAP) and cellulose
acetate trimellitate (CAT) are shown in Section 2.3.
3.4 Methods

3.4.1 Emulsion stability study

The emulsion formulations (polymer to drug ratio 30:1) were prepared by using different types of pH sensitive polymers: Eudragit L and S, CAP, CAT and HP55 and experiments were carried out in triplicate for each formulation. The detail of each of the formulations was as follows:

(a) Preparation of Eudragit L and S emulsion: polymers (3 g) and drug (0.1 g) were dissolved in 30 ml of ethanol and emulsified into 200 ml of liquid paraffin containing 1% Arlacel 83.

(b) Preparation of CAP and CAT emulsion: polymers (0.75 g) and drug (0.025 g) were dissolved in 30 ml of acetone/ethanol (2:1) and emulsified into 200 ml of liquid paraffin containing 1%w/w of Arlacel 83.

(c) Preparation of HP55 emulsion: HP55 (0.75 g) and drug (0.025 g) were dissolved in 30 ml of ethyl acetate/methanol (1:2) and emulsified into 200 ml of liquid paraffin comprising 1%w/w of Arlacel 83.

The emulsion was stirred for 10 minutes using a Heidolph overhead propeller stirrer. After that the emulsion was poured into a 250 ml measuring cylinder and then immediately covered with parafilm to prevent evaporation of the organic solvent. At room temperature, the stability of the emulsion was regularly observed and the time required for macroscopic phase separation, with emulsion completely separated into two phases, was recorded.

3.4.2 Determination of droplet size and size distribution

The emulsion formulations were prepared as described in the emulsion stability study. However, the emulsion was stirred continuously until the end of the experiment and the
emulsion beaker was covered with aluminium foil and paraffin to prevent evaporation of the organic solvent. The size of the emulsion droplets was determined at 30, 60, 180 and 360 minutes. The sample was pipetted onto a slide and immediately observed under an optical microscope (Nikon Microphot FXA) at x4 and x10 objective magnification. Random pictures of the globules from different locations on the slide were taken by a digital camera and used for measuring the particle size. From each system, a population of 500 globules was sized.

3.5 Results and discussion

Figure 3.2 Phase separation time of the various emulsion formulations

Figure 3.2 illustrates the stability of the various emulsion formulations. Within a few minutes of the studies, the emulsion formulations of CAT and CAP showed a distinct layer separation of the disperse phase and continuous phase and the emulsion systems of these formulations could not be restored to their original state by gentle shaking. It could therefore be concluded that these emulsion formulations were unstable with cracking. Moreover, the emulsion of the Eudragit S formulation showed more stability than that of the HP 55 formulation due to the slower phase separation rate. However, the emulsion of the Eudragit L formulation appeared to be the most stable. It was found that the emulsion stability data correlated well with the morphology of the final microparticles.
The SEM images of these microparticles are shown in Figures 3.3 to 3.7 (Images are the same as shown in a previous chapter).

Figure 3.3 SEM of Eudragit L microparticles
Figure 3.4 SEM of Eudragit S microparticles
Figure 3.5 SEM of HP55 microparticles
Figure 3.6 SEM of CAT microparticles
Figure 3.7 SEM of CAP microparticles

It was observed that large particles with an irregular shape were produced from the unstable emulsion formulation of CAP and CAT (Figures 3.6 and 3.7). Since the phase separation time of these emulsions was short, it indicated that at the beginning of the
study the interfacial film surrounding the droplets had been ruptured rapidly, resulting in the break down of the emulsion. These results also show that Arlacel 83 could not stabilise CAT and CAP emulsion droplets. This might be because either the amount of Arlacel 83 used was insufficient to stabilise the droplets and to protect them from coalescence or because Arlacel 83 was an unsuitable stabiliser for these emulsions.

It is hypothesised that at the beginning of the emulsification process in the ESE method, the weak stabilizing film covering the emulsion droplets of CAP and CAT allowed the droplets to collide and fuse rapidly and consequently breakdown the emulsion. After the solvent had completely evaporated from the unformed particles, large particles with an irregular shape were obtained.

In contrast to the morphology of the CAP and CAT emulsion systems, the microparticles produced from a more stable emulsion system (Eudragit L, S and HP55) exhibited a smooth surface and were spherical (Figures 3.3 to 3.5). These data suggest that Arlacel 83 could stabilise the emulsion of these polymers better than the emulsion of CAP and CAT by forming a more rigid stabilizing film around the droplets. Even though the phase separation of Eudragit L, S and HP55 occurred in less than a day, in the ESE method, microparticles with a good morphology could be obtained from these emulsion formulations. This suggested that in the ESE method, the stability of emulsion required only a short period of time before droplet solidification occurred. During the emulsification process, Arlacel 83 could protect the emulsion droplets of Eudragit L, S and HP55 from rapid coalescence; therefore, after solvent removal and polymer precipitation, spherical particles were formed. Nihant et al (1994) also characterised the emulsion stability of primary emulsion by monitoring phase separation time. They found that an undesirable structure of polylactide microparticles resulted from an unstable emulsion formulation.

The classic method of droplet size measurement by direct visual observation of the emulsion using an optical microscope was used to investigate the change in size of droplets over time. Since formulations of Eudragit L, S and HP55 formed a more stable emulsion than formulations of CAT and CAP, therefore only the formulations of
Eudragit L, S and HP55 were evaluated further. Figure 3.8 shows mean globules size of each emulsion formulation at various sampling times.

![Graph showing mean globules size over time for Eudragit L, S, and HP55. The graph indicates that Eudragit L has a slower rate of globule size increase compared to Eudragit S and HP55. The size of the final microparticles for Eudragit L, S, and HP55 are 26.6, 35.5, and 69.3 µm, respectively.](image)

Figure 3.8 Particle size and size distribution of different emulsion formulations: (a) mean globule size at different sampling time (b) particle size distribution measured at 3 hours.

The slow rate of globule size increase of the Eudragit L formulation indicated that it was more stable than Eudragit S and HP55. These results correlate to the size of the final microparticles (26.6, 35.5, 69.3 µm for Eudragit L, S and HP55 respectively). In effect, the more stable the emulsion, the smaller the size of the resultant particles.
Chapter Three

As seen in SEM images (Figures 3.3-3.5) and Figure 3.8 both the globule size and particle size distribution for HP55 was broader than the Eudragit L and S formulations, showing that the globule size distribution is related to the microparticle size distribution (span value = 0.77, 0.91 and 1.2 for the microparticles of Eudragit L and S and HP55, respectively). Therefore, the size distribution of the final microparticles could be predetermined by the globule size distribution of the emulsion droplets.

3.6 Section one conclusions

In conclusion, the stability of the emulsion was one of the key factors controlling the properties of microparticles. To obtain a spherical and narrow size distribution of microparticles, a stable emulsion formulation was required. It is possible to use the simple assessment of emulsion stability as a pre-formulation screening tool for microparticle formulation, as the stability of the emulsion is important and predetermines the morphology of the microparticles.
SECTION TWO: EFFECT OF PROCESS PARAMETERS ON THE PROPERTIES OF MICROPARTICLES

3.7 Introduction

The characteristics of modified release microparticles prepared by the ESE method are substantially affected by formulation and manufacturing variables (Watts et al., 1990; Arshady, 1991; Shukla and Price, 1991; Perumal, 2001) such as drug loading, type of polymer and drug, polymer and emulsifier concentration, volume ratio of the internal and external phase, stirring speed and solvent evaporating temperature. For example, an increase in the molecular weight of the biodegradable polymer, poly (d, l-lactide) resulted in a slower release of the drug from the microspheres (Suzuki and Price, 1985). In addition, Fu and colleagues reported that the decrease in encapsulation efficiency and a porous microparticle surface resulted from an increase in the evaporation temperature (Fu et al., 2005). It also has been reported that the type of equipment used for producing microparticles also affected the characteristics of microparticles. For instance, addition of baffles to the wall of the mixing vessel could decrease the average particle size and prevent aggregation and lumping formation and increase the yield of microparticles (Bodmeier and McGinity, 1987a).

Eudragit S has been selected to be a model polymer in this study since in the previous study it was able to form microparticles with good properties. The effect of the parameters in the preparation procedure such as stirring speed, amount of drug content, concentration of polymer and emulsifier on the characteristics of microparticles has been investigated in order to understand the relationship between the process parameters and the properties of Eudragit S microparticles such as morphology, particle size, in-vitro release profile and encapsulation efficiency.

The objective of this study was to investigate the relationship between process parameters and the characteristics of microparticles and to identify the key process variables affecting the properties of microparticles prepared by the ESE method.
Chapter Three

3.8 Methods

The ESE method described in section 2.4.1 was used to prepare prednisolone loaded Eudragit S microparticles. All the batches of microparticles were prepared in triplicate. The parameters varied in each experiment are detailed below:

Variation of stirring rate
Microparticles with a polymer to drug ratio (10:1) were prepared and four different stirring rates were employed; 1500, 1000, 500 and 300 rpm.

Variation of drug loading
Prednisolone loaded Eudragit S microparticles with a drug to polymer ratio of 1:10, 1:5, 1:1 and 1:0.5 ratio were prepared (9.1, 16.7, 50 and 66.7% w/w drug, respectively). For all ranges of drug loading, the amount of prednisolone used for preparing microparticles was under the level of its saturation solubility in ethanol (30 ml).

Variation of concentration of polymer
Microparticles (polymer to drug ratio (10:1)) were prepared and four different polymer concentrations of 30 ml ethanolic Eudragit S solution (5%, 7.5%, 10% and 15% w/v) were employed.

Variation of emulsifier
Prednisolone loaded Eudragit S microparticles, polymer to drug ratio (10:1) were produced by using five different concentrations of Arlacel 83; 0.25, 0.5, 1, 2 and 3% w/w.
3.9 Results and discussion

3.9.1 Effect of the stirring speed on microparticle properties

The influence of the stirring rate on the properties of prednisolone loaded Eudragit S microparticles is shown in Figure 3.9.

![Graph showing the effect of stirring speed on microparticle properties](image)

*Figure 3.9 Effect of stirring rate on the particle size, batch yield and encapsulation efficiency of Eudragit S/prednisolone microparticles (polymer to drug ratio, 10:1)*

The stirring rate is one of the important process parameters because it provides the energy to disperse the organic phase in the liquid paraffin phase. As expected, the particle size decreased with the increase in stirring speed (Figure 3.9). When the stirring rate was increased from 300 to 1000 rpm, the median diameter of the particles decreased considerably (from 111.45 to 36.16 μm) and the size distribution was narrower (span value= 1.92, 1.43 and 0.95 for 300, 500 and 1000 rpm respectively). As described by O’Donnell and McGinity (1997) the increase in the stirring rate leading to a decrease in size was a result of the high shear generated by the propellers. Therefore, when the stirring speed was increased, the dispersed droplets decreased; as a result, a smaller particle size and narrower particle size distribution were obtained. The impact of the stirring rate on the particle size was also in accordance with many reports (Benita et al., 1984; Huang and Ghebre-Sellassie, 1989; Jeffery et al., 1991; Gabor et al., 1999)
However, increasing the stirring speed from 1000 rpm to 1500 rpm did not further reduce the particle size and size distribution (span value = 0.91 for 1500 rpm).

As seen in figure 3.9, the encapsulation efficiency was increased slightly with the increase in the stirring rate. This could be due to the increase in the stirring rate leading to the reduction in size of the emulsion droplets resulting in a larger surface area for rapid solvent removal and rapid polymer precipitation; consequently hindering drug diffusion to the continuous phase, resulting in a low amount of drug loss. The variation of stirring rate had no effect on the batch yield. This was in contrast to the findings of Yang and colleagues who reported that increasing the stirring speed resulted in a decrease of the batch yield due to the fragmentation of microspheres at a high stirring rate (Yang et al., 2001). The morphological features of microparticles prepared by varying the stirring rate are shown in Figures 3.10 to 3.13.
The morphology of microparticles produced by the range of stirring rates showed spherical particles with a smooth surface. The in-vitro drug release profiles of the Eudragit S particles at pH 1.2 for 2 hours following by pH 7.4 are shown in Figure 3.14.

![Figure 3.14 In-vitro drug release of prednisolone loaded Eudragit S microparticles fabricated using various stirring rates at pH 1.2 for 2 hours followed by an adjustment to pH 7.4. (Error bars have been omitted for clarity).](image)

After 2 hours incubation in acid, the release of prednisolone from microparticles produced at the entire range of stirring rates was restricted to less than 4%. Following the increase in pH from 1.2 to 7.4, the rate of drug release from the microparticles was increased with the increase in the stirring rates. The rapid release of prednisolone from microparticles fabricated at stirring rate of 1500 and 1000 rpm was seen after adjusting the pH to 7.4. In contrast, a slow release of prednisolone was observed from microparticles prepared using a stirring speed of 300 and 500 rpm. The rapid dissolution of prednisolone observed in the particles produced by the stirring rates of 1000 and 1500 rpm was due to the smaller size of these particles and therefore a larger surface area to volume ratio, resulting in faster drug release.
3.9.2 Effect of drug loading on the microparticle properties

The impact of drug loading on the characteristic properties of microparticles is demonstrated in Figure 3.15.

![Graph showing the effect of drug loading on particle size, batch yield, and encapsulation efficiency.](image)

**Figure 3.15** Effect of drug loading on particle size, batch yield and encapsulation efficiency of Eudragit S/prednisolone microparticles

As seen in Figure 3.15, it was observed that the median diameter of the microparticles increased considerably with the increase in drug content from 9.1 % (polymer to drug ratio 10:1) to 66.7 % w/w (ratio 0.5:1). A similar trend of particle size being influenced by drug loading was observed in many studies (Shukla and Price, 1991; Jeffery et al., 1993; Dinarvand et al., 2002). This may be because the increase in drug concentration led to an increase in drug quantity in the emulsion droplets resulting in the increase in droplet size and subsequently a greater particle size (Krishnamachari et al., 2007). However, there was no difference in the particle size of drug content at 16.67% (polymer to drug ratio 5:1) and 9.1% w/w (ratio 10:1). As drug loading increased, the size distribution tended to be wider (span value= 0.95, 0.99, 2.4 and 2.9 for drug content 9.1, 16.7, 50 and 66.7 %w/w, respectively) and the encapsulation efficiency was slightly decreased.

The decrease in drug encapsulation efficiency with an increase in drug concentration was observed in much of the literature (Benoit et al., 1999; Yang et al., 2001; Yang et
al., 2005). Yang et al (2001) reported that the increase in drug loading led to an enhancement of the drug loading in the emulsion droplets and consequently increased the concentration gradient between the emulsion droplets and the continuous phase; as a result increasing the amount of drug partitioning into the continuous phase. Another possible explanation would be that at high drug loading (drug content of 50% and 66.7% w/w), the amounts of the polymer present were insufficient to encapsulate the entire drug inside the microparticles; therefore the encapsulation efficiency was decreased. Furthermore, Figure 3.15 shows that as drug loading increased the batch yield slightly decreased. This was because microparticle batches with a drug content of 50% and 66.7% w/w contained some irregular and broken particles. The morphology of the Eudragit S microparticles produced from varied drug loading is shown in Figures 3.16 and 3.20.

As seen in the SEM images (Figures 3.16 and 3.17), there was no distinct difference in the particle size of drug-free microparticles and prednisolone loaded microparticles (drug content of 9.1% w/w); these being 33.2±0.8 μm and 36.16±1.2 μm for drug free and prednisolone loaded microparticles, respectively. SEM images also reveal that the microparticles produced by using high drug loading (66.7 and 50% w/w) exhibit a totally distinct morphology to microparticles of lower drug loading (9.1 and 16.7% w/w). Microparticles of high drug loading appeared to be hollow and had an extensive amount of crystalline prednisolone on the surface of the microparticles (Figures 3.19 and 3.20) whereas the morphology of lower prednisolone loading microparticles showed a smooth surface and dense particles (Figures 3.17 and 3.18).
Figure 3.17 Microparticles formed from drug content 9.1% w/w (polymer to drug ratio 10:1): (a) SEM image (b) SEM cross section image

Figure 3.18 Microparticles formed from drug content 16.7% w/w (polymer to drug ratio 5:1): (a) SEM image (b) SEM cross section image

Figure 3.19 Microparticles formed from drug content 50.0% w/w (polymer to drug ratio 1:1): (a) SEM image (b) SEM cross section image
Figure 3.20 Microparticles formed from drug content 66.7 %w/w (polymer to drug ratio 0.5:1): (a) SEM image (b) SEM cross section image

At high drug loading (drug content of 50% and 66.7% w/w), the high amount of prednisolone and low amount of Eudragit S polymer may contribute to the particles being hollow and the presence of the drug in a crystalline form on the surface of the microparticles. This was likely to be because the low quantity of polymer in the dispersed droplets led to the formation of a thin polymer wall. The presence of the drug in a crystalline form on the surface of the microparticles could probably be explained by the increase in drug loading leading to an increase in the drug quantity in the emulsion droplets; therefore, during the microparticle formation process, when the solvent was diffusing out from the emulsion droplets, the solubility of the drug in the droplets decreased. Therefore crystallisation of the drug occurred after solvent removal, resulting in the drug being crystallised and precipitated on the wall of the microparticles.

The effect of drug loading on the release profiles of the Eudragit S particles in 0.1M HCl and pH 7.4 phosphate buffer is shown in Figure 3.21. It was observed that the degree of drug loading affected the release rate and initial burst of the drug. The initial burst release was observed from the microparticles of high drug content (50% and 66.7 % w/w), being 59% and 29%, respectively after 2 hours in 0.1M HCL; consequently, these high drug loading microparticles fail the USP test for enteric coat products. The considerable amount of prednisolone found on the surface of these high loading microparticles accounts for the burst release of the drug.
Chapter Three

![Graph showing in-vitro drug release of Eudragit S' prednisolone microparticles fabricated using various drug loading at pH 1.2 for 2 hours followed by an adjustment to pH 7.4 (Error bars have been omitted for clarity)](image)

Figure 3.21 In-vitro drug release of Eudragit S' prednisolone microparticles fabricated using various drug loading at pH 1.2 for 2 hours followed by an adjustment to pH 7.4 (Error bars have been omitted for clarity)

In contrast to the release patterns of high drug loadings, prednisolone release from microparticles of drug contents of 9.1% and 16.6% w/w was restricted to less than 5% after 2 hours in acid. Following the increase in pH from 1.2 to 7.4, a rapid release was observed in these microparticles due to the large surface area of small microparticles. In contrast, the release rate of prednisolone from microparticles of high drug loading (drug content of 50% and 66.7% w/w) was found to be slower, taking approximately 20 and 70 minutes, respectively for the complete release of prednisolone. The slow dissolution of prednisolone at pH 7.4 could be due to the large size of the microparticles (being 169.9 μm, 228.4 μm for drug content of 50% and 66.7% w/w, respectively) and therefore a low surface area to volume ratio. Since the high drug loading had a great impact on the release profile, the degree of drug loading should be taken into consideration when formulating the modified release microparticles.
3.9.3 Effect of the concentration of polymer on the microparticle properties

The influence of polymer concentration on the characteristic properties of microparticles is shown in Figure 3.22.

![Figure 3.22](image)

**Figure 3.22 Effect of polymer concentration on particle size, batch yield and encapsulation efficiency of Eudragit S/prednisolone microparticles (polymer to drug ratio, 10:1)**

The median diameter as well as drug encapsulation efficiency tended to increase when polymer concentrations were increased from 5% to 15%/v (Figure 3.22). The size distribution of microparticles was slightly narrower when polymer concentration decreased (Span value= 0.89, 0.93, 0.95 and 1.3 for polymer concentration 5, 7.5, 10 and 15%, respectively). A similar trend of the polymer concentration influencing size and encapsulation efficiency was shown in the studies of Dinarvand et al (2002), Zhu et al (2003) and Yang et al (2005). There are two possible explanations for the effect of the polymer concentration on the size of microparticles. Jeffery et al (1991) proposed that the increase in the polymer concentration in a fixed volume of internal solvent led to an increase in the viscosity of the internal phase and therefore increased the frequency of collision, resulting in the fusion of emulsion droplets and increasing the size of the droplets which would subsequently solidify as large sized microparticles. In addition, at the same stirring rate, when the viscosity of the internal phase increased, the efficiency
of the stirring in breaking the emulsion into small droplets was reduced and as a result large sized microparticles were formed.

Furthermore, during particle formation in the emulsion solvent evaporation process, the solvent gradually diffused from the internal droplets into the external phase and the drug migrated to the preferential phase. The increase of polymer concentration could lead to the increase in the viscosity within the internal emulsion droplets, which would impede drug mobility and thus decrease the diffusion rate of the drug from the inner droplets to the external phase; as a consequence the encapsulation efficiency was increased. In addition, the increase in the polymer concentration might lead to rapid polymer precipitation on the emulsion droplet surface and rapid microparticle solidification resulting in the hindrance of the diffusion of the drug to the continuous phase and consequently the increase in drug encapsulation efficiency (Bodmeier and McGinity, 1988). Moreover, there was no noticeable difference in the batch yield produced using the different range of polymer concentrations.

Figures 3.23 to 3.26 illustrate the morphology of Eudragit S/prednisolone microparticles (10:1), prepared from different concentrations of Eudragit S solution.
Figure 3.25 SEM of microparticles formed from polymer concentration 10%w/v

Figure 3.26 SEM of microparticles formed from polymer concentration 15%w/v

The SEM images reveal that the morphology of microparticles prepared at all ranges of polymer concentration appear spherical, non-aggregated and non-porous (Figures 3.23-3.26). The SEM image of microparticles of polymer concentration 15%w/w confirmed that a high polymer concentration produced large sized microparticles (Figure 3.26). The effect of polymer concentrations on the release profiles of the Eudragit S particles in 0.1M HCl and pH 7.4 phosphate buffer are shown in Figure 3.27.

Figure 3.27 In-vitro drug release of Eudragit S prednisolone microparticles (10:1) fabricated using various polymer concentrations at pH 1.2 for 2 hours followed by an adjustment to pH 7.4 (Error bars have been omitted for clarity)
Chapter Three

After 2 hours in 0.1M HCL, the release of prednisolone was restricted to less than 4% for all formulations. Following the increase in pH from 1.2 to 7.4, a rapid prednisolone release was observed from the microparticles prepared at the entire range of polymer concentrations. It is likely that the polymer concentrations used in this range of study have less impact on the release profile of prednisolone.

3.9.4 Effect of the concentration of emulsifier on the microparticle properties

The effect of the surfactant concentration on characteristic of microparticles is shown in Figure 3.28.

![Figure 3.28 Effect of surfactant concentration on particle size, batch yield and encapsulation efficiency of prednisolone loaded Eudragit S microparticles (polymer to drug ratio, 10:1)](image)

The median diameter of microparticles and size distribution were decreased when the surfactant concentration was increased from 0.25% to 1%w/w (span value = 1.2, 0.99 and 0.95, respectively). A similar trend of surfactant concentration influencing particle size was observed in many studies (Jeffery et al., 1993; Sansdrap and Moes, 1993; Dinarvand et al., 2002).

Since the function of the surfactant is to decrease the interfacial tension between the dispersed droplets and the continuous phase and to protect the droplets from collision...
and coalescence, the concentration of surfactant had an influence on the droplet stabilisation (Arshady, 1990a). At a low emulsifier concentration, the amount of emulsifier might be insufficient to shield the entire droplet surface and to reduce the interfacial tension; consequently, this resulted in the droplets being susceptible to collision and fusion which in turn resulted in a reduction of the surface area of the droplets. As a result larger droplets were formed, which subsequently became larger microparticles. In contrast, high emulsifier concentrations (1% and 2% w/w) formed a more stable emulsion, resulting in a smaller size of emulsion droplets being formed. Due to the high surface area of the small emulsion droplets, the solvent evaporation and particle hardening processes occurred rapidly resulting in a small size of microparticles. There was little change in the size of the microparticles prepared by using emulsifier 1% and 2% w/w. This was probably due to the optimum concentration of emulsifier, required to stabilize the stable emulsion, having already been achieved by using 1% w/w emulsifier.

However, increasing the emulsifier concentration from 2% to 3% w/w resulted in a dramatic increase in the particle size and an increase in the size distribution (span value=0.98 and 2.7 for emulsifier concentration 2% and 3% w/w, respectively). A possible explanation of this phenomenon was that a 3% emulsifier concentration might lead to a dramatic increase in the viscosity of the emulsion, resulting in it being more difficult to break up the emulsion into smaller droplets by using the same stirring rate and as a result large sized microparticles were formed.

As the emulsifier concentration increased to 3% w/w, the encapsulation efficiency was dramatically decreased. This can be explained by the increase in the emulsifier concentration leading to an increase in the solubilisation and the solubility of the drug in the oil phase resulting in an increase in drug partitioning into the oil phase; as a consequence there was a loss of drug in the continuous phase and then a decrease in the encapsulation efficiency (Kristmunsdottir and Ingvarsdottir, 1994). An emulsifier concentration of 1% was considered to be an optimum concentration as it provided small particle size with high drug encapsulation efficiency. Furthermore, the batch yield
of microparticles, prepared by 3%w/w of surfactant decreased as some irregular structures were found to adhere to the stirring blades and the wall of the beaker.

The SEM images of Eudragit S/prednisolone microparticles, prepared by varying the concentrations of emulsifier are shown in Figures 3.29 to 3.33.
Chapter Three

The SEM images (Figures 3.29 to 3.33) revealed that the morphology of microparticles, prepared by using emulsifier 3% w/w was unacceptable as they had a non-smooth surface and large irregular particles whereas the appearance of microparticles produced by using the lower emulsifier concentrations (2%, 1%, 0.5% and 0.25% w/w) was smooth and spherical. Yang et al (2005) also found that at very high concentration of emulsifier (span 80 or span 85) a good morphology of microparticle could not be produced when using O/O emulsion. As both Span 80 and Span 85 were highly soluble in ethanol, employing a very high concentration of these emulsifiers led to a considerable decrease in the ethanolic solubility of the polymer. As a result, the polymer precipitated rapidly at the beginning of the emulsification. According to this explanation, since Arlacel 83 was soluble in ethanol (http://www.surfactant.co.kr/surfactants/sorbitan.html), using a 3% w/w concentration of Arlacel 83 could probably decrease the solubility of Eudragit S in ethanol, resulting in the fast precipitation of the polymer and consequently to irregular structures being formed.

The effect of emulsifier concentration on the release profiles of the Eudragit S microparticles in 0.1 M HCl and pH 7.4 phosphate buffer are shown in Figure 3.34.
Chapter Three

Figure 3.34 In-vitro drug release of Eudragit S' prednisolone microparticle (10:1) fabricated using various emulsifier concentrations at pH 1.2 for 2 hours followed by an adjustment to pH 7.4 (Error bars have been omitted for clarity)

After 2 hours of exposure to 0.1M HCL, the release of prednisolone from all formulations was found to be less than 6%. Following the increase in pH from 1.2 to 7.4, rapid prednisolone release was observed from the microparticles prepared by using emulsifier concentration 0.5%, 1% and 2% w/w. The fast release of these microparticles might be due to the fact that the small sized microparticles exhibited a large surface area for drug release. In contrast, the rate of drug release from microparticles with an emulsifier concentration of 0.25 and 3% w/w was slower due to the larger size of these microparticles. Compared to microparticles with an emulsifier concentration of 0.25%w/w, the faster drug release of 3%w/w may have contributed to the surfactant remaining on the surface, resulting in the better wetting of the microparticles which enhanced the penetration of the dissolution medium into the matrix.

The effects of process parameters on microparticle properties are ranked in Table 3.1. At this point, it could be seen that the effects of some process parameters on the properties of microparticles did not follow the trend reported in the literature. This was because of the difference in the microparticle preparation conditions used in this study and in the published studies. This confirms that ESE is a complicated process and that the effect of
process parameters on the properties of the microparticles under one preparation condition could not be assumed to occur under other preparation conditions.

Table 3.1 Ranking of the effects of process parameters on microparticle properties

<table>
<thead>
<tr>
<th>Microparticle properties</th>
<th>Effects of process parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Size</td>
<td>Emulsifier concentration &gt; drug loading &gt; stirring rate &gt; polymer concentration</td>
</tr>
<tr>
<td>Yield</td>
<td>Drug loading ≥ emulsifier concentration &gt; stirring rate = polymer concentration</td>
</tr>
<tr>
<td>Encapsulation efficiency</td>
<td>Emulsifier concentration &gt; polymer concentration &gt; stirring rate &gt; drug loading</td>
</tr>
<tr>
<td>Drug release</td>
<td>Drug loading &gt; emulsifier concentration &gt; stirring rate &gt; polymer concentration</td>
</tr>
</tbody>
</table>

3.10 Section Two Conclusions

It is clear that process parameters had a great impact on the characteristic properties of microparticles. In this study, the particle size of microparticles was affected to increasing degrees by polymer concentration then stirring speed then drug loading with the greatest effect being produced by the emulsifier concentration. The encapsulation efficiency was greatly influenced by the concentration of emulsifier and polymer and was slightly effected by the stirring rate and drug loading. The release of prednisolone from the microparticles was shown to depend strongly on drug loading and the size of the microparticles. However, it was found that the variation of the process parameters present in this study had less impact on batch yields of microparticles. By varying the key process parameters and selecting the appropriate preparation conditions, the properties of microparticles could be successfully modified for a modified delivery system.
Chapter Three

SECTION THREE: THE STABILITY OF THE FINAL PRODUCT

3.11 Introduction

Stability studies are a crucial part of the development and marketing phase of a pharmaceutical product, since the knowledge provided by stability tests helps to determine the shelf life of the product, the retest period for the drug substance, recommended storage conditions (humidity, light and temperature), the packaging best suited to protect the product and the necessary transport conditions.

ICH (International Conference on Harmonization of Technical Requirements of Registration of Pharmaceuticals for human use) established the guidelines for the harmonization of the regulatory requirement for stability testing procedures in the regions with ICH representation (USA, Europe and Japan). The standard storage conditions defined in ICH guidelines for stability testing are shown in Table 3.2.

Table 3.2 Standard storage conditions in accordance with ICH Q1A (R2) and ICH Q1F

<table>
<thead>
<tr>
<th>Types of study</th>
<th>Temperature</th>
<th>Relative humidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>For climate zone I and II</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long term studies</td>
<td>25°C±2°C</td>
<td>60%±5%</td>
</tr>
<tr>
<td>Intermediate conditions</td>
<td>30°C±2°C</td>
<td>65%±5%</td>
</tr>
<tr>
<td>Accelerated conditions</td>
<td>40°C±2°C</td>
<td>75%±5%</td>
</tr>
<tr>
<td>For climate zone III and IV</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Long term studies</td>
<td>30°C±2°C</td>
<td>65%±5%</td>
</tr>
<tr>
<td>Accelerated conditions</td>
<td>40°C±2°C</td>
<td>75%±5%</td>
</tr>
</tbody>
</table>

Great Britain has been classified in climate zone I.

During storage, the changes of the drug in physical, chemical or microbiological terms might effect the appearance, pH value, dissolution rate, characteristics, degradation rate and drug content of the product, which could consequently result in changes to the
Chapter Three

therapeutic effect and/or safety of the product. There are many methods to investigate the changes on the physical stability of the active ingredient in products such as Differential Scanning Calorimetry (DSC) and X-ray powder diffraction (XRPD).

An important function of packaging is to protect the active ingredient in a product. The tightness and light protection of packaging is considered to be the most important aspect. In this study, clear screw cap bottles and clear glass injection vials with aluminium cap seals were selected to investigate the effect of light on the product and the moisture protection ability of packaging since the vials crimped with aluminium seals were thought to be able to protect the product from moisture better than screw cap bottles.

Microparticles of Eudragit L and S were chosen to be models in this study because these microparticles possessed ideal properties: small particle size with controlled release properties and high encapsulation efficiency. The stability of prednisolone loaded Eudragit L and S microparticles was studied in two dosage forms; non-encapsulated and encapsulated (within a hard gelatin capsule) microparticles which were stored in two types of packaging; clear glass screw cap bottles and clear glass injection vials with aluminium cap seals, under three different storage conditions; 25±2°C/ 60±5% RH (ambient condition), 40±2°C/ 75±5% RH (accelerated condition) and 40±2°C/ 0 ±5% RH (for comparing the data with the accelerated condition, but with no effect of humidity) for a period of 13 weeks.

The aim of this study is to investigate the stability of entrapped prednisolone on drug dissolution and release from Eudragit L and S microparticles during storage in different conditions for 13 weeks.

3.12 Materials

Clear glass screw cap bottles (14 mL) were purchased from Scientific Laboratory Supplies (Nottingham, UK). Clear glass injection vials (20 mL) with aluminium cap seals were purchased from Münnerstädtner Glaswarenfabrik GmbH (Bayern, Germany).
Chapter Three

An image of a clear glass screw cap bottle and a clear glass injection vial with an aluminium cap seal, which were used in this experiment, is shown in Figure 3.35.

![Figure 3.35 A 14 mL clear glass screw cap bottle (left) and a 20 mL clear glass injection vial with aluminium cap seal (right) used in this experiment.](image)

Prednisolone was purchased from Sanofi-Aventis (Romainville, France). Eudragit L and S were obtained from Rohm Pharma (Darmstadt, Germany). Size 1 gelatine capsules were provided by Capsugel (Colmar, France). All other reagents were of analytical grade and were used as received.

3.13 Methods

3.13.1 Preparation of controlled samples and capsules containing prednisolone loaded Eudragit L and S microparticles

Nine batches of prednisolone loaded Eudragit L and S microparticles (polymer to drug ratio 5:1) were prepared by the method described in Section 2.4.1. For each formulation, microparticles from nine batches were combined and blended for 15 minutes in a Turbula mixer. Sixty milligrams of microparticles were accurately weighed and filled into a size 1 gelatin capsule. Six capsules of each formulation were placed in a 14 mL clear glass screw cap bottles and 20 mL clear glass injection vials. Non-encapsulated microparticles (360 mg) were weighed in 14 mL clear glass screw cap bottles and 20 mL
clear glass injection vials. The injection vials containing product were crimped with aluminium covers. In this study, the non-encapsulated microparticles were also stored in a light protected bottle, prepared by wrapping clear glass screw cap bottles with aluminium foil.

3.13.2 Preparation of desiccators for stability study

In the stability study, the specific relative humidities in air-tight glass desiccators were maintained by saturated salt solutions. For the ambient (60±5% RH, 25±2°C) and accelerated condition (70±5% RH, 40±2°C), a saturated solution of sodium bromide was used to maintain a relative humidity of 57.5±0.2% at 25 °C and a saturated solution of sodium chloride was employed to maintain a relative humidity of 74.7±0.2% at 40 °C (Nyqvist, 1983). The saturated salt solutions were prepared by dissolving an excess amount of salt in 400 ml distilled water at 60 °C and then cooling to room temperature before transferring to air-tight glass desiccators. For the stability study condition 0% RH at 40±2°C, phosphorous pentoxide, a moisture-absorbing agent, was used to maintain the relative humidity at zero. The temperature in the desiccators was maintained by a temperature controlled oven, 25 °C for the desiccators containing a saturated sodium bromide solution and 40 °C for the desiccators containing a saturated sodium chloride solution and phosphorous pentoxide.

3.13.3 In-vitro dissolution studies

Samples were analysed at 4, 8 and 13 weeks after microparticle preparation. The In-vitro dissolution studies conducted are described in Section 2.4.6.

3.14 Results and discussion

Since the stability of the final product of encapsulated microparticles stored in a screw cap bottle was thought to be vulnerable to the accelerated conditions, the effect of gelatine capsules on the prednisolone encapsulation efficiency of the final product stored
in a screw cap bottle was investigated under various conditions. The results are shown in Table 3.3.

Table 3.3 Percentage of prednisolone encapsulation efficiency of Eudragit L and S microparticles in screw cap bottles in various conditions

<table>
<thead>
<tr>
<th>Storing condition</th>
<th>Eudragit L microparticles</th>
<th>Eudragit S microparticles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial</td>
<td>83.5±1.2</td>
<td>84.7±0.9</td>
</tr>
<tr>
<td>13 weeks at 25°C, 60% RH encapsulated microparticles,</td>
<td>82.0±0.7</td>
<td>84.1±1.3</td>
</tr>
<tr>
<td>13 weeks at 40°C, 75% RH encapsulated microparticles,</td>
<td>81.3±1.1</td>
<td>83.8±0.7</td>
</tr>
<tr>
<td>13 weeks at 40°C, 0% RH, encapsulated microparticles,</td>
<td>83.2±0.8</td>
<td>82.9±1.1</td>
</tr>
<tr>
<td>26 weeks, ambient condition, non-encapsulated microparticles</td>
<td>81.7±1.5</td>
<td>83.5±1.4</td>
</tr>
</tbody>
</table>

When comparing the freshly prepared microparticles, both encapsulated (within a hard gelatin capsule) Eudragit L and S microparticles stored at stressed conditions for a period of 13 weeks and non-encapsulated Eudragit L and S microparticles in ambient condition for 26 weeks, there appeared to be no change in their appearance and prednisolone encapsulation efficiency (Table 3.3). This showed that the microparticle final product of gelatin capsules being stored in a screw cap bottle had no effect on the prednisolone encapsulation efficiency.

In-vitro dissolution studies were performed for 2 hours in 0.1M HCL followed by a pH change to pH 6.8 (for Eudragit L microparticles) and pH 7.4 (for Eudragit S microparticles). The in-vitro dissolution profiles of Eudragit L and S microparticles in the form of either encapsulated microparticles or non-encapsulated microparticles at 40°C/0%RH, 25°C/60%RH and 40°C/75%RH for 13 weeks and at 40°C/75%RH for storage time points of 4, 8, 13 and 26 weeks are shown in Figures 3.36 to 3.49.
Chapter Three

Dissolution profiles of Eudragit L microparticles in various storage conditions

![Graph showing dissolution profiles of Eudragit L microparticles in various storage conditions.](image)

**Figure 3.36** Dissolution profiles of Eudragit L microparticles stored at 40°C/0%RH for 13 weeks

![Graph showing dissolution profiles of Eudragit L microparticles stored at 25°C/60%RH for 13 weeks.](image)

**Figure 3.37** Dissolution profiles of Eudragit L microparticles stored at 25°C/60%RH for 13 weeks
Figure 3.38 Dissolution profiles of Eudragit L microparticles stored at 40°C/75%RH for 13 weeks

Figure 3.39 Dissolution profiles of encapsulated Eudragit L microparticles stored in screw cap bottles at various time points at 40°C/75%RH
Figure 3.40 Dissolution profiles of encapsulated Eudragit L microparticles stored in crimped vials at various time points at 40°C/75%RH

Figure 3.41 Dissolution profiles of non-encapsulated Eudragit L microparticles stored in screw cap bottles at various time points at 40°C/75%RH
Chapter Three

Figure 3.42 Dissolution profiles of non-encapsulated Eudragit L microparticles stored in crimped vials at various time points at 40°C/75%RH

Dissolution profiles of Eudragit S microparticles in various storage conditions

Figure 3.43 Dissolution profiles of Eudragit S microparticles stored at 40°C 0%RH
Chapter Three

Figure 3.44 Dissolution profiles of Eudragit S microparticles stored at 25°C/60%RH

Figure 3.45 Dissolution profiles of Eudragit S microparticles stored at 40°C/75%RH
Chapter Three

Figure 3.46 Dissolution profiles of encapsulated Eudragit S microparticles stored in screw cap bottles at various time points at 40°C/75%RH

Figure 3.47 Dissolution profiles of encapsulated Eudragit S microparticles stored in crimped vials at various time points at 40°C/75%RH
Chapter Three

Figure 3.48 Dissolution profiles of non-encapsulated Eudragit S microparticles stored in screw cap bottles at various time points at 40°C/75%RH

Figure 3.49 Dissolution profiles of non-encapsulated Eudragit S microparticles stored in crimped vials at various time points at 40°C/75%RH
As seen in Figures 3.36 to 3.49, no noticeable difference in the rate of prednisolone released from encapsulated and non-encapsulated Eudragit L or S microparticles, stored at different time points and conditions, was observed. The release rate of prednisolone from both encapsulated and non-encapsulated microparticles stored in clear glass injection vials and clear glass screw cap bottles at 4, 8 and 13 weeks and at 40°C/0%RH, 25°C/60%RH and 40°C/75%RH were similar to the release rates of freshly prepared microparticles and non-encapsulated microparticles stored in an ambient condition for a period of 26 weeks (data shown only for 40°C/75%RH). It was shown that during 13 weeks storage, light, temperature & humidity, types of packaging and dosage form had no effect on the release rate of prednisolone from Eudragit L and S microparticles.

For both Eudragit L and S microparticles, after a pH change to pH 6.8 (for Eudragit L100 microparticles) or 7.4 (for Eudragit S microparticles), the rapid prednisolone release from both forms (encapsulated and non-encapsulated microparticles) at a range of storage conditions can probably be attributed to the amorphous nature of the drug encapsulated within the microparticle matrix, revealed by the absence of crystalline prednisolone on the surface and in cross section images of microparticles (Figures 3.50 to 3.57).
Figure 3.52 SEM of Eudragit L encapsulated microparticles, stored in 25°C, 60%RH at 13 weeks: (a) low magnification, (b) high magnification

Figure 3.53 SEM of Eudragit L encapsulated microparticles, stored in 40°C, 75%RH at 13 weeks: (a) low magnification, (b) high magnification

Figure 3.54 SEM of Eudragit L non-encapsulated microparticles, stored at ambient condition, 26 weeks: (a) low magnification, (b) high magnification
Figure 3.55 SEM of Eudragit S encapsulated microparticles, stored in 25°C, 60%RH at 13 weeks: (a) low magnification, (b) high magnification

Figure 3.56 SEM of Eudragit S encapsulated microparticles, stored in 40°C, 75%RH at 13 weeks: (a) low magnification, (b) high magnification

Figure 3.57 SEM of Eudragit S non-encapsulated microparticles stored at ambient condition 26 weeks: (a) low magnification, (b) high magnification
3.15 Section three Conclusions

No appreciable changes in physical appearance, drug content and dissolution performance were seen for either Eudragit L and S microparticles in either encapsulated or non-encapsulated microparticles after a storage period of 13 weeks in clear glass screw cap bottles and clear glass injection vials with an aluminium cap seal under accelerated conditions, thereby confirming the stability of both products in storage.

3.16 Over all conclusions

It was evident that both process parameters and the stability of the emulsion were crucial in the preparation of modified release microparticles using the ESE method. The properties of modified release microparticles could be modified to achieve a desirable quality using this knowledge. This chapter also showed that the final products of prednisolone loaded Eudragit L and S microparticles were stable in accelerated conditions for 13 weeks.

To help in comprehending more about the particle formation process, it was decided to investigate the effect of the physicochemical properties of an encapsulated substance on the distribution of the drug within the microparticles using confocal laser scanning microscopy. These experiments are described in the next chapter.
CHAPTER FOUR

Use of confocal laser scanning microscopy to investigate the distribution of drugs in microparticle systems
Chapter Four

4.1 Introduction

The effects of the physiochemical properties of drugs, such as solubility and partition behaviour, on the distribution of drugs within microparticles is of interest as it helps in understanding the particle formation process and can be used to tailor a modified release system. This is because the localisation of the drug whether in the inner core or on the surface of the particle affects the diffusion path length; therefore it has an influence on the release profile (Yang et al., 2001; Kim and Park, 2004). There are many studies in which it is reported that the initial burst release was mainly attributed to the drug being located on the surface of microparticles. For example, Sun et al (2003) reported that the initial rapid release of protein from poly(lactide-glycolide) microparticles is caused by the drug being located on the surface of microparticles. The finding of Yang et al (2001) also showed a burst release of protein in the earlier stage of the dissolution studies and attributed this to the distribution of protein on the surface of biodegradable polymeric microspheres.

The solubility and partition behaviour of the encapsulated drug are crucial factors influencing the quality of microparticles (Arshady, 1991) and the undesirable characteristics of microparticles such as low encapsulation efficiency and the initial burst release, depend on these parameters (Matsumoto et al., 1997). The effect of partition coefficient depends on the property of the drug molecule (in a given condition), lipophilicity and molecular weight. Many papers have reported that the drug encapsulation efficiency within microparticles is strongly dependent on the partition coefficient and solubility of the drug (Bodmeier and McGinity, 1987a; Alex and Bodmeier, 1990; Hombreiro-Perez et al., 2003; Weidenauer et al., 2003). In an oil in water (O/W) emulsion system, for example, the successful encapsulation of the drug in the microparticles was influenced by the solubility of the drug in the aqueous phase. The use of poorly water soluble drugs provided high drug encapsulation efficiency whereas the more water soluble drugs failed to encapsulate within the microparticles due to the fact that during microparticle formation the drug was entirely partitioned into the aqueous continuous phase (Bodmeier and McGinity, 1987b).
To date, little information has been given on the effect of the physicochemical properties of drugs on their distribution patterns within microparticles. Therefore, the influence of the partition behaviour and solubility of drugs on their distribution behaviour within modified release microparticles is investigated in this chapter. The techniques used to visualise the patterns of drug distribution are detailed below.

The most commonly used techniques in visualising the morphology of microparticles are light microscopy (LM) and scanning electron microscopy (SEM) (Benita et al., 1984; Tice and Gilley, 1985). However, these LM and SEM conventional techniques, non-fluorescent systems, have certain limitations when analysing the internal structure of such particles. For example, the resolution and quality of the images produced by LM are reduced due to the scattered or emitted light from structures outside the focal plane. To visualize the internal structures of samples, the SEM requires a relatively intensive sample pre-treatment and mechanical sectioning. Furthermore, SEM techniques cannot identify and localise the different compounds in the same sample (Lamprecht et al., 2000a). Even though the identification and localisation of the different fluorescent compounds can be performed with a non-confocal fluorescent microscope, the resolution and quality of the images provided by this microscope is poor and three dimensional images can not be obtained.

Confocal laser scanning microscopy (CLSM) has several advantages over conventional LM, SEM and non-confocal fluorescent microscopy such as eliminating out of focus fluorescent light from the focal plane, producing non-destructive three dimensional optical sections without prior sample preparation and improving the axial (z; along the optical axis) and lateral (x and y; in the specimen plane) optical resolution and imaging contrast (Yan et al., 1994). Moreover, using the CLSM technique, it is possible to obtain thin (0.5-1.5 μm) or thick (up to 100μm) optical sections of a specimen along the z axis and reconstruct its three dimensional (3D) structure, produced by compiling multiple optical sections at different depths of the specimen (Prasad et al., 2007). The advantage of optical over physical sectioning is that it eliminates artefacts which appear during manual sectioning.
By using sufficient fluorescent markers, CLSM also allows one to visualize and identify different compounds and structures within the same specimen. The CLSM technique therefore provides a useful approach for investigating the drug distribution and internal structure of microspheres. However, CLSM can only visualize fluorescent materials. To visualize non-fluorescent materials, fluorescent labelling is required and this may alter considerably the physicochemical properties of the compound, particularly in the case of drugs with a low molecular weight (Lamprecht et al., 2000a).

CLSM has been extensively used in biological and medical studies (Shotton, 1989; Zemanova et al., 2003) and is being increasingly employed in pharmaceutics for evaluation and characterisation of solid dosage forms. For example, it has been used to evaluate the film coating properties of tablets and pellets (Guo et al., 2002; Ruotsalainen et al., 2003; Missaghi and Fassihi, 2004), to investigate drug release mechanisms from controlled release dosage forms (Cutts et al., 1996; Lamprecht et al., 2000a; Guo et al., 2002; Kim and Park, 2004) and to analyze the polymer distribution in microcapsules (Lamprecht et al., 2000b). However, there were a few studies that employed CLSM to investigate the factors influencing the patterns of drug distribution within microparticles (Yang et al., 2001; Berkland et al., 2003). Therefore, this work emphasizes the applicability and usefulness of CLSM in the characterization of drug distribution within microparticles.

Principle of confocal microscopy

In the mid-1950s the basic principle of confocal microscopy was developed by Marvin Minsky. CLSM has sub-micrometer spatial resolution. The laser has been selected as a light source in the confocal microscope because it has advantages over other light sources. For example, laser supplies light at a discrete band of wavelength and has a high intensity which is also beneficial for fluorescence excitation. The light pathways in confocal microscopy are shown in Figure 4.1.
Chapter Four

The excitation light (green line) from the laser source passes through a pinhole aperture and is reflected by a dichromatic mirror, before passing through the microscope objective and scanning across the specimen in a defined focal plane. The fluorescence light (red line) from the specimen is collected by the objective and then passes back through the dichromatic mirror and is focused as a confocal point at the detector pinhole aperture, which locates in the conjugate focal plane of the specimen. Therefore, only light from the focal plane that emerges from the pinhole is detected by the photomultiplier tube. The out of focus fluorescent emission from the sample, which appears at points below and above the objective focal plane, is rejected by the detector pinhole aperture. Finally, the resulting image is reconstructed and displayed by a computer. The transmitted light image and confocal image are combined by using image processing software. The benefit of using this combined image mode is that the precise locations of fluorescent and non-fluorescent regions within the sample are distinguishable. Therefore, the fluorescent materials can be mapped within the non-fluorescent parts.
Fluorescence excitation and emission fundamentals

A fluorophore is a molecule that is able to fluoresce and it has a relatively low energy in its ground state. There are three steps in the fluorescence process, shown in the Jablonski diagram (Figure 4.2). Firstly, the fluorophore molecule is excited via the absorption of light energy at a particular wavelength; the molecule then reaches a higher energy state, an excited state. This process is known as excitation. Secondly, the fluorophore adopts the lowest energy excited state, semi-stable because the fluorophore is unstable at high energy configurations. Finally, the fluorophore reorganises from the semi-stable excited state back to the ground state, consequently excess energy is released and emitted as light, which is of a longer wavelength than the absorbed light.

Model fluorescent compounds

Since prednisolone does not have a fluorescent property, it could not be used in the studies for this chapter. Hence, auto-fluorescent materials with different physical and chemical properties have been employed as model compounds.

Dipyridamole

Dipyridamole is a yellow crystalline powder with a molecular weight of 504.6 Da and its chemical structure is shown in Figure 4.3. It is a weakly basic drug, practically insoluble.
in water and freely soluble in ethanol. Dipyridamole inhibits platelet aggregation and causes vasodilatation and is used with other drugs, such as aspirin, for prophylaxis of thromboembolism associated with prosthetic heart valves.

Figure 4.3 Structure of dipyridamole

Riboflavin

Riboflavin or vitamin B₂, is an orange-yellow crystalline powder with a molecular weight of 376.4 Da and its chemical structure is demonstrated in Figure 4.4. It is very slightly soluble in water, very soluble in dilute solutions of alkali hydroxides and practically soluble in alcohol and in ether. However, riboflavin exhibits variation in solubility, due to a variation in its internal crystalline structure. Riboflavin is an essential nutrient for humans and is a component of two coenzymes and is therefore requirement of all flavoproteins.

Figure 4.4 Structure of riboflavin

Riboflavin sodium phosphate

Riboflavin sodium phosphate, which has molecular weight of 473.8 Da, is an orange-yellow crystalline powder, soluble in water, very slightly soluble in alcohol and
practically insoluble in ether. The chemical structure of riboflavin sodium phosphate is shown in Figure 4.5. It is used in foods as a yellow food dye.

![Figure 4.5 Structure of riboflavin sodium phosphate](image)

**Acridine orange**

Acridine orange is a brown to orange-brown powder with a molecular weight of 265.4 Da. and is soluble in water. It is a fluorescent dye. The chemical structure of acridine orange is demonstrated in Figure 4.6.

![Figure 4.6 Structure of acridine orange](image)

The aims of this chapter are to investigate the effect of the physiochemical properties of drugs on their distribution patterns within microparticles by using the CLSM technique and to correlate these observations with drug encapsulation efficiency and *in-vitro* drug release.

The investigations in this chapter cover the following:

- The effect of the physicochemical properties of riboflavin, riboflavin sodium phosphate, dipyridamole and acridine orange on the properties of Eudragit L microparticles.
• The effect of drug loading on the distribution of dipyridamole and riboflavin within Eudragit L microparticles.
• The effect of different pH sensitive polymers, Eudragit S and Eudragit L on the distribution of dipyridamole within microparticles.

4.2 Materials

Eudragit L and S were obtained from Rohm Pharma (Darmstadt, Germany). Dipyridamole, riboflavin, riboflavin sodium phosphate, acridine orange, Arlacel 83 were purchased from Sigma Aldrich, (Poole, UK). All other reagents were of analytical grade and were used as received.

4.3 Methods

4.3.1 Determination of saturation solubility of the drugs in ethanol

Excess amounts of fluorescent drugs were added to 10 ml quantities of ethanol in a vial and saturated solutions were prepared by the agitation of the vials in a horizontal shaker at room temperature for 24 hours. The contents of the vials were filtered through a 0.22 micrometre disposable filter and then the filtrate was diluted with the ethanol. The UV absorbance of the dipyridamole, riboflavin, riboflavin sodium phosphate and acridine orange filtrate was read at wavelengths of 229, 260, 270 and 270 nm, respectively and the saturation concentration was calculated with reference to a standard curve.

4.3.2 Determination of the apparent partition coefficient of drugs in ethanol/liquid paraffin

The apparent partition coefficient (K) of the ethanol/liquid paraffin system was studied by the shake-flask method. Prior to the start of the experiment, both phases (ethanol and liquid paraffin) have to be presaturated with each other. Ethanol was equilibrated with an excess of liquid paraffin and left overnight at room temperature and vice versa. Stock
solutions of drugs were used; dipyridamole, riboflavin, riboflavin sodium phosphate and acridine orange base, prepared in an ethanol phase, which provided better solubility. The drug stock solution (3 ml) was added to 33 ml of liquid paraffin in the centrifuge tube and left shaking at room temperature for 18 hours, simulating the duration that the drug would be in the emulsification process when making microparticles. To be assured of complete phase separation, the tube was then centrifuged at 2500 rpm for 30 min to separate the two phases. The ethanol phase was then analyzed by UV spectrophotometry for drug concentration. The ethanol/liquid paraffin apparent partition coefficient ($K_{\text{ethanol/liquid paraffin}}$) was calculated using Equation 4.1.

$$K_{\text{ethanol/liquid paraffin}} = \frac{\text{Concentration of solute in ethanol}}{\text{Concentration of solute in liquid paraffin}}$$

$$K_{\text{ethanol/liquid paraffin}} = \frac{W_{\text{ethanol}}}{V_{\text{ethanol}}} / \left( W - W_{\text{ethanol}} \right) / V_{\text{liquid paraffin}}$$  \hspace{1cm} (Eqn.4.1)

Where $W$ is the weight of the initial solute in stock solution (3 ml), $W_{\text{ethanol}}$ is the weight of the solute remaining in ethanol at equilibrium, $V_{\text{ethanol}}$ and $V_{\text{liquid paraffin}}$ are volumes of ethanol and liquid paraffin, respectively. To obtain reliable values, the experiments were carried out in triplicate and at least three different concentration levels of drug stock solution covering at least 10 fold ranges (Hansch and Leo, 1995).

4.3.3. Microparticle preparation

The rationale for selecting Eudragit L as the main model polymer for the study was because as noted in Chapter Two section 1, prednisolone was found to leak from Eudragit L microparticles in 0.1M HCl (5% within 2 hours). It was therefore decided to investigate whether the localisation of the drug on the surface of Eudragit L microparticles was the cause of the drug leakage. However, prednisolone does not have fluorescent properties; therefore, auto-fluorescent drugs were used in the study. Eudragit L microparticles were prepared by the emulsification solvent evaporation (ESE) method as described in Section 2.4.1. As the encapsulated materials used in this study are photosensitive molecules, all the preparation steps were carried out in a dark room.
4.3.4 Determination of encapsulation efficiency

The encapsulation efficiency is defined as the ratio of actual over theoretical content of the drug in the microparticles and it was determined by completely dissolving 40 mg of microparticles in 10 ml methanol; followed by the addition of 0.1M HCl to precipitate the pH-sensitive polymer and made up to 100 ml. Samples were filtered through 0.22 micrometer disposable filters and the drug content was analysed by UV spectrophotometry at a wavelength of 283 for dipyridamole and 267 for acridine orange, riboflavin and riboflavin sodium phosphate. The measured absorbance was converted to drug concentration using a standard curve for the known concentration of the drug in 10% methanol in 0.1M HCl. The amount of encapsulated drug was determined in triplicate.

4.3.5 In-vitro release studies

To assure the sink conditions of drugs in the dissolution studies, the saturation solubility of drugs in different dissolution media was examined and calculated. Excess amounts of fluorescent drugs were added to each glass vial containing 10 ml of 0.1M HCL, pH 6.8 and pH 7.4 phosphate buffer and the vials then agitated at 37°C in a horizontal shaker for 24 hours to achieve saturation. After that the solution was filtered with a 0.22 micrometer disposable filter and then the filtrate was diluted with the appropriate solvent (pre-warmed to 37°C). The UV absorbance of dipyridamole, riboflavin, riboflavin sodium phosphate and acridine orange filtrate was read at different wavelengths, 282, 266, 266 and 268 nm respectively and the saturation concentrations were determined by calculating with reference to an appropriate standard curve. The dissolution studies were performed as described in Section 2.4.6, except the amount of the microparticles used was calculated based on the saturation solubility of the drug in the dissolution media and the drug content was measured spectrophotometrically at 282, 266, 266 and 268 nm for dipyridamole, riboflavin, riboflavin sodium phosphate and acridine orange, respectively.
4.3.6 Confocal laser scanning microscopy

The drug distribution and internal structure within the microparticles was investigated using a Zeiss LSM 510 Meta laser scanning confocal microscope, equipped with an argon laser. An exciting wavelength of 488 nm was employed and confocal images were taken with an x63 objective. The iris, amplifier offset, detector gain control and all other settings were kept constant during all experiments. To determine drug distribution, optical cross sections were taken at various depths of the microparticles and the Z section images were recorded at intervals of 1.05 μm in the Z direction. The microparticles were placed onto a glass slide, and the images were captured. Combining the green fluorescence channel with the transmitted light channel allows observation of the internal structure of the microparticles.

4.4 Results and discussion

4.4.1 Determination of partition coefficient and solubility

During particle formation in the ESE process, the drug and polymer solution was emulsified in the continuous phase to form discrete droplets and the solvent later diffused throughout the droplets into the oil phase and evaporated at the oil/air interface; subsequently, the microspheres were hardened. While the solvent evaporation was occurring, the drug was partitioning to the preferential phase. If the drug preferentially diffuses out of the emulsion droplet, the high drug loss would be an undesired result (Bodmeier and McGinity, 1987b). Therefore, it is important to investigate the partition of the drug molecule across the border of the two immiscible interfaces. Since the drug has to partition across two interfaces, ethanol and liquid paraffin, the apparent partition coefficient of the model drugs was evaluated (Table 4.1). The physicochemical properties of prednisolone are also shown in Table 4.1 in order to compare the characteristic behaviour with other drugs.
Table 4.1 Summary of physicochemical properties of prednisolone, dipyridamole, acridine orange base, riboflavin and riboflavin sodium phosphate

<table>
<thead>
<tr>
<th>Drug</th>
<th>Molecular weight</th>
<th>Log Pow</th>
<th>pKa</th>
<th>Solubility in ethanol (mg/ml)</th>
<th>$K_{\text{ethanol/liquid paraffin}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisolone</td>
<td>360.4</td>
<td>1.6</td>
<td>-</td>
<td>25.37±1.9</td>
<td>882.3±6.1</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>504.6</td>
<td>2.7</td>
<td>6.4</td>
<td>26.22±1.3</td>
<td>79.5±5.3</td>
</tr>
<tr>
<td>Acridine orange</td>
<td>265.4</td>
<td>3.4</td>
<td>9.8</td>
<td>154.13±2.1</td>
<td>13.6±4.2</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>376.4</td>
<td>-1.5</td>
<td>10.2</td>
<td>0.107±0.01</td>
<td>292.5±5.8</td>
</tr>
<tr>
<td>Riboflavin sodium phosphate</td>
<td>478.3</td>
<td>-</td>
<td>-</td>
<td>0.049±0.01</td>
<td>364.9±4.7</td>
</tr>
</tbody>
</table>

1 Clarke's Analysis of Drugs and Poisons 2004, British Pharmacopoeia, 2006
2 (Lober and Achtert, 1969)

Table 4.1 shows that the partition of all the drugs used in this study was in favour of ethanol rather than liquid paraffin. However, a difference in the degree of preferential localisation in ethanol was observed in the different drugs. Compared with other drugs, prednisolone, which had a highest $K$ value (882.3), was the most preferentially localised in the ethanol phase whereas the acridine orange partition was the least in favour in ethanol ($K = 13.6$). The apparent partition coefficient of model drugs in ethanol and liquid paraffin did not correlate with the partition coefficient of drugs in water and n-octanol, usually expressed as its logarithm ($\log P_{\text{ow}}$) (Table 4.1). This was because the system used in this study was fundamentally different from the water and n-octanol system. Therefore, $\log P_{\text{ow}}$ could not be used to predict the localisation of the drug between the ethanol and liquid paraffin phase.

4.4.2 Effect of physicochemical properties of drug on its distribution within Eudragit L microparticles and confocal microscopy studies

Blank Eudragit L microparticles were investigated to confirm that the polymer, surfactant and residual oil and solvents are not fluorescent (Figure 4.7).
Chapter Four

Figure 4.7 CLSM and transmitted light images of blank Eudragit L microparticle. Top left, top right and bottom left show CLSM, transmitted light and combined CLSM and transmitted light image, respectively.

The CLSM image was taken at the plane section through the approximate centre of this microparticle. It was found that no fluorescence emission was detected from the blank microparticle, indicating that the Eudragit L, Arlacel 83 and residual oil and solvent are not fluorescent. The results of CLSM and transmitted light images and CLSM cross section images of Eudragit L microparticles with various encapsulated fluorescent drugs, polymer to drug ratio 30 to 1, are shown in Figures 4.8 to 4.11.

Dipyridamole, which had an apparent partition coefficient of 79.5, was distributed throughout the microparticle matrix (Figure 4.8a); however, the CLSM cross section images revealed the high intensity of the drug towards the surface of the particle (Figure 4.8c); that might be because some of the drug molecules partitioned towards the external phase during the evaporation process. SEM cross section image of dipyridamole microspheres showed no drug crystals inside (Figure 4.8b).

Riboflavin, behaved in an opposite manner to dipyridamole, with the drug preferentially located in the inner core of the particles and a tendency to form large crystals visible under CLSM and transmitted light microscopy (Figure 4.9a). The large drug crystals inside the particles could also be seen in the SEM cross section image (Figure 4.9b) and beside this, it was observed that the polymer oriented around the outer surface of the microparticles. The optical cross section image of the riboflavin microparticles (Figure
4.9c) also confirmed that the drug largely distributed in their interior. This indicates that the particles were not homogeneous, matrix structures. As the solubility of riboflavin in ethanol was very low (0.107 mg/ml), during the preparation only a small amount of riboflavin was dissolved in ethanol and a large amount of non-dissolved riboflavin was suspended in the ethanol. As a result, the non-dissolved riboflavin, in a crystalline form was observed encapsulated within the microparticles.

Riboflavin sodium phosphate also had a low solubility in ethanol (0.049 mg/ml). Due to it being practically insoluble in ethanol, during the preparation process the majority of the drug was suspended in the ethanolic solution. As a result, riboflavin sodium phosphate was found mainly aggregated in the core of the microparticles (Figure 4.10a). This was also because the lack of solubility prevents drug partition into the liquid paraffin. Although the aggregation of the drug was found to a considerable degree in the interior of the microparticles, the CLSM cross section images revealed that some of the drug molecules distributed evenly throughout the particles (Figure 4.10c). The SEM cross section image (Figure 4.10b) revealed no drug crystals found in the riboflavin sodium phosphate microspheres (as seen with the riboflavin microspheres), which was probably because the raw material morphology of riboflavin sodium phosphate is a fine powder while riboflavin is a crystalline powder.

The distribution of acridine orange, which had the lowest apparent partition coefficient value (13.6), was distinct from the other drugs. It was evident in the CLSM images that acridine orange was found to be preferentially distributed on the surface (Figure 4.11a) which was likely to be due to the migration of the drug towards the liquid paraffin during the solvent evaporation process. In Figure 4.11c, the images of CLSM optical section from the centre towards the further end of the microparticles were highly attenuated because the laser could not penetrate the optically dense material. However the images confirmed that the distribution of acridine orange was located mainly near the microsphere surface. The SEM image (Figure 4.11b) showed no drug crystals in the acridine orange microparticles.
Figure 4.8 Dipyridamole loaded microparticles: (a) CLSM and transmitted light images, (b) SEM cross section image (c) CLSM cross section images
Figure 4.9 Riboflavin loaded microparticles: (a) CLSM and transmitted light images, (b) SEM cross section image, (c) CLSM cross section images
Figure 4.10 Riboflavin sodium phosphate loaded microparticles: (a) CLSM and transmitted light images, (b) SEM cross section image, (c) CLSM cross section images
Figure 4.11 Acridine orange loaded microparticles: (a) CLSM and transmitted light images, (b) SEM cross section image, (c) CLSM cross section images
4.4.3 Effect of physicochemical properties of the drug on its encapsulation efficiency in Eudragit L microparticles and its release properties

The properties of Eudragit L microparticles are summarised in Table 4.2.

Table 4.2 Summary of characteristics of the Eudragit L100 microparticles

<table>
<thead>
<tr>
<th>Drug</th>
<th>$K_{\text{ethanol/liquid paraffin}}$</th>
<th>Encapsulation Efficiency %</th>
<th>Size</th>
<th>Batch yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prednisolone</td>
<td>882.3±6.1</td>
<td>86.72±1.9</td>
<td>26.61±0.3</td>
<td>0.77</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>79.5±5.3</td>
<td>75.46±1.2</td>
<td>47.75±1.8</td>
<td>0.72</td>
</tr>
<tr>
<td>Acridine</td>
<td>13.6±4.2</td>
<td>50.21±1.5</td>
<td>82.82±3.6</td>
<td>1.12</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>292.5±5.8</td>
<td>79.56±1.6</td>
<td>31.82±2.2</td>
<td>1.10</td>
</tr>
<tr>
<td>Riboflavin sodium phosphate</td>
<td>364.9±4.7</td>
<td>86.86±1.1</td>
<td>45.50±1.9</td>
<td>1.97</td>
</tr>
</tbody>
</table>

Prednisolone, which was soluble in ethanol and preferentially localised in the ethanol phase rather than the liquid paraffin phase showed a high percentage of encapsulation efficiency. On the other hand, as the apparent partition coefficient of acridine orange was the lowest ($K=13.6$), acridine orange microparticles demonstrated the lowest percentage of encapsulation efficiency due to significant drug loss in the external phase. The considerable drug loss could also be explained by the possibility that during the particle formation process the large droplet particles of acridine orange hardened slowly due to the slow rate of solvent removal. This resulted from the low surface to volume ratio of the large droplets. As the duration of the particle hardening process increased, it extended the time for the drug to partition out to the continuous phase; hence resulting in the large extent of drug loss (Berkland et al., 2003).

According to the partition behaviour of dipyridamole, which is partially partitioned into the oil phase, drug loss was also found as a consequence of drug diffusion out of the internal phase. Consequently, dipyridamole loaded microparticles exhibited low encapsulation efficiency. In contrast, most of the riboflavin and riboflavin phosphate are efficiently encapsulated inside the microparticles during the emulsification and the
subsequent solvent evaporation process, resulting in high encapsulation efficiency. This is because these drugs had low partition in the continuous phase and had poor solubility in ethanol which meant they could not diffuse across into the continuous phase. To ensure sink conditions in the in-vitro dissolution studies, the saturation solubility of fluorescent drugs in the pH range (1-7.4) was calculated and is presented in Table 4.3.

Table 4.3 Saturation solubility of fluorescent drugs in various pH

<table>
<thead>
<tr>
<th>Drug</th>
<th>Saturation solubility (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.1N HCL</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>20.41±1.5</td>
</tr>
<tr>
<td>Acridine orange</td>
<td>22.99±1.1</td>
</tr>
<tr>
<td>Riboflavin</td>
<td>0.11±0.0</td>
</tr>
<tr>
<td>Riboflavin sodium phosphate</td>
<td>45.19±2.5</td>
</tr>
</tbody>
</table>

To perform dissolution under sink conditions, the amount of microparticles used was calculated based on the final concentration of the drug in the dissolution medium, which was less than 10% of the saturation solubility of the drug in the dissolution medium. The in-vitro dissolution profiles of the dipyridamole, riboflavin, riboflavin phosphate sodium and acridine orange loaded Eudragit L microparticle are shown in Figure 4.12.

![Figure 4.12 In-vitro release of riboflavin sodium phosphate, riboflavin, dipyridamole and acridine orange from Eudragit L microparticles (polymer to drug ratio, 30:1) microparticles at pH 1.2 for 2 hours followed by an adjustment to pH 6.8](image-url)
It was found that the release profile of the drug was affected by the size of the microparticles, the distribution of the drug and the solubility of the drug in the dissolution medium. After 2 hours in 0.1M HCl, the release of riboflavin and acridine orange was restricted to 3% and 10% respectively, whereas the release of dipyridamole and riboflavin sodium phosphate were observed at over 10%, being 18 and 15%, respectively. The burst release of dipyridamole occurred at the beginning of incubation in 0.1M HCl, which could have been caused by the nature of the drug itself, a weak basic drug and the release of the surface accumulated drug. In the case of acridine orange, a weakly basic dye, although the CLSM image showed that the drug mainly located towards the surface of the particles, the release was less than that of dipyridamole in 0.1M HCl. This could be explained by the larger particle size of the acridine orange loaded microparticles, 82.8 μm (Table 4.2) having a lower surface area to volume ratio which resulted in slower drug release.

The resistance to the release of riboflavin in 0.1M HCl could be explained by the low solubility of riboflavin in the dissolution medium and its distribution pattern within the microparticles. CLSM images of riboflavin loaded microparticles showed that large drug crystals were encapsulated inside the microspheres and the polymer was oriented around the outer surface of the microparticles (Figure 4.9). Therefore, the release of the drug was totally controlled by the surrounding Eudragit L polymer. On the other hand, the leaking of riboflavin sodium phosphate in 0.1M HCL was observed; this was due to the hydrophilic nature of riboflavin sodium phosphate, its high solubility in an acidic medium and its distribution pattern within the microparticles. The CLSM images revealed that although the distribution of the drug was mainly aggregated inside, some molecules of the drug were evenly distributed in the microparticles. Therefore, after being exposed to the acidic medium, the riboflavin sodium phosphate that was located close to the surface started dissolving and caused burst release.

After an increase in pH from 1.2 to 6.8, a rapid release of dipyridamole, acridine orange and riboflavin sodium phosphate was observed. This was particularly surprising because acridine orange and dipyridamole are weak basic substances and as such were expected to dissolve very slowly in the simulated environment of the small intestine lumen as a
result of being poorly soluble in basic conditions. After changing the pH, the rate of riboflavin release from the microparticles was slow. This was because the drug was in a crystalline form and therefore it dissolved slowly in the dissolution medium.

4.4.4 Effect of drug loadings on the distribution of dipyridamole and riboflavin within the Eudragit L microparticles

Dipyridamole and riboflavin were selected to be the model drugs in this part of the study. The SEM and CLSM images of various loadings of riboflavin loaded Eudragit L microparticles are shown in Figures 4.13 to 4.15 and 4.16 to 4.18, respectively. No difference in drug distribution was observed between high and low drug loading (drug to polymer ratio 1:5, 1:30 and 1:70). Riboflavin was found to preferentially locate in the inner core of the microparticles and it was observed that the polymer oriented around the outer surface in each set of microparticles. This indicates that riboflavin loaded microparticles were not homogeneous matrix structures. It was observed that the CLSM optical section images of high drug loading (1:5, drug to polymer ratio) were significantly attenuated from the centre towards the further end of microparticles as the laser could not penetrate the optically dense material.

No noticeable difference in the distribution of dipyridamole at various drug loadings was observed. For all drug loadings, dipyridamole exhibited an even distribution within the microparticles and the CLSM cross section images demonstrated a high intensity of the drug accumulated on the surface of the particle (Figures 4.19-4.21).

With both dipyridamole and riboflavin loaded microparticles, it was observed that an increase in drug loading resulted in a larger size of microparticles and higher encapsulation efficiency (Table 4.4).
Chapter Four

Figure 4.13 SEM images of cross section of riboflavin microparticles, polymer to drug ratio 5:1

Figure 4.14 SEM images of cross section of riboflavin microparticles, polymer to drug ratio 30:1

Figure 4.15 SEM images of cross section of riboflavin microparticles, polymer to drug ratio, 70:1
Chapter Four

Figure 4.16 Eudragit L/riboflavin (5:1) microparticles: (a) CLSM and transmitted light images, (b) CLSM cross section images

Figure 4.17 Eudragit L/riboflavin (30:1) microparticles: (a) CLSM and transmitted light images, (b) CLSM cross section images

Figure 4.18 Eudragit L/riboflavin (70:1) microparticles: (a) CLSM and transmitted light images, (b) CLSM cross section images
Chapter Four

Figure 4.19 Eudragit L' dipyridamole (5:1) microparticles: (a) CLSM and transmitted light images, (b) CLSM cross section images

Figure 4.20 Eudragit L' dipyridamole (30:1) microparticles: (a) CLSM and transmitted light images, (b) CLSM cross section images

Figure 4.21 Eudragit L' dipyridamole (70:1) microparticles: (a) CLSM and transmitted light images, (b) CLSM cross section images
Table 4.4 Summary of characteristics of the riboflavin and dipyridamole loaded Eudragit L microparticles

<table>
<thead>
<tr>
<th>Encapsulated drug</th>
<th>Polymer to drug ratio</th>
<th>Encapsulation efficiency %</th>
<th>Size Dv50(µm)</th>
<th>Span</th>
<th>Batch yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riboflavin</td>
<td>5:1</td>
<td>86.90±3.1</td>
<td>64.88±0.7</td>
<td>0.81</td>
<td>91.5±1.4</td>
</tr>
<tr>
<td></td>
<td>30:1</td>
<td>79.56±0.5</td>
<td>31.82±0.3</td>
<td>1.10</td>
<td>90.3±0.9</td>
</tr>
<tr>
<td></td>
<td>70:1</td>
<td>78.87±1.0</td>
<td>35.61±2.6</td>
<td>1.35</td>
<td>91.1±2.7</td>
</tr>
<tr>
<td>Dipyridamole</td>
<td>5:1</td>
<td>85.59±0.1</td>
<td>58.43±1.5</td>
<td>1.12</td>
<td>91.8±1.3</td>
</tr>
<tr>
<td></td>
<td>30:1</td>
<td>77.46±0.3</td>
<td>47.75±0.8</td>
<td>0.62</td>
<td>89.5±1.1</td>
</tr>
<tr>
<td></td>
<td>70:1</td>
<td>72.01±1.3</td>
<td>39.99±1.0</td>
<td>0.80</td>
<td>88.8±0.7</td>
</tr>
</tbody>
</table>

The release profiles of riboflavin loaded Eudragit L microparticles are shown in Figure 4.22.

Figure 4.22 In-vitro release of riboflavin loaded Eudragit L microparticles at pH 1.2 for 2 hours followed by an adjustment to pH 6.8

After 2 hours in 0.1M HCl the riboflavin release from Eudragit L microspheres was less than 5% and this observation was found for all drug loadings. Following the pH change, drug release from microparticles at all ranges of loading was slow. This resulted from the crystalline form of the riboflavin being encapsulated within the microparticles.
The *in-vitro* release profiles of dipyridamole loaded Eudragit L microparticles are presented in Figure 4.23.

![Graph showing in-vitro release of dipyridamole loaded Eudragit L microparticles at pH 1.2 for 2 hours followed by an adjustment to pH 6.8](image)

*Figure 4.23 In-vitro release of dipyridamole loaded Eudragit L microparticles at pH 1.2 for 2 hours followed by an adjustment to pH 6.8*

At the beginning of incubation in 0.1M HCl, a burst release of dipyridamole was observed for all ranges of drug loading of Eudragit L microparticles and dipyridamole release was found to be more than 15% after 2 hours in acid conditions. This could have been caused by the nature of the drug itself, a weakly basic drug and the release of the surface accumulated drug. Following the increase in pH, the release of dipyridamole from all ranges of drug loading of Eudragit L microparticles was rapid.

### 4.4.5 Effect of the solubility of drugs in the internal phase on the pattern of drug distribution within microparticles

As shown in Table 4.1, the solubility of riboflavin is very poor in ethanol. In the previous study, riboflavin microparticles which have polymer to drug ratios 5:1 to 70:1 were prepared from the partially dissolved riboflavin in ethanol. To investigate the effect of the solubility of drugs in the internal phase on the distribution of microparticles, the concentration of riboflavin used in this study was less than the saturation concentration of the drug in ethanol and microparticles of polymer to drug ratio (312:1) were prepared.
The CLSM and SEM images of these riboflavin loaded Eudragit L microparticles are shown in Figure 4.24.

Figure 4.24 Eudragit L riboflavin (312:1) microparticles: (a) CLSM and transmitted light images, (b) SEM cross section image, (c) CLSM cross section images

The CLSM images (Figures 4.24a and 4.24c) show the even distribution of riboflavin within Eudragit L microparticles, which contained a polymer to drug ratio (312:1) and were prepared by using the completely dissolved riboflavin solution. The SEM image (Figure 4.24b) confirms the absence of large drug crystals located within the
microparticles. This observation was found to be opposite to the distribution pattern of riboflavin in the previous study, where the distribution of riboflavin in microparticles prepared using partially dissolved riboflavin solution showed aggregation of the crystalline drug inside the particles and the polymer oriented around the outer surface of the particles. Therefore, it could be concluded that the solubility of the drug in the internal phase is a crucial factor that determines the pattern of the distribution of the drug. The dissolution profiles of riboflavin at various loadings are shown in Figure 4.25.

Figure 4.25 In-vitro release of riboflavin loaded Eudragit L microparticles at pH 1.2 for 2 hours following by an adjustment to pH 6.8

The drug release was found to be less than 5% for all formulations after incubation for 2 hours in 0.1M HCl. When comparing the release rate of riboflavin from the microparticles containing the polymer to drug ratio 5:1, 30:1, 70:1 and 312:1 in a pH 6.8 condition, the release rate of the drug from the microparticles (312:1) was found to be faster than that of the microparticles 70:1, 30:1 and 5:1. This was probably because the microparticles with a ratio of 312:1 had an absence of an outer coating polymer and the crystalline form of riboflavin.
4.4.6 Effect of polymers on the distribution of drugs within the microparticles

Eudragit L and S were used to investigate the effect of the matrix polymer on the distribution of drugs within the microparticles. Due to the slight difference in the ratio of free carboxyl groups to ester groups, which is 1:1 in Eudragit L and 1:2 in Eudragit S, it would be of interest to investigate whether the polymer structure has an influence on the distribution of the drug within the microparticles. The CLSM images of dipyridamole loaded Eudragit S microparticles are shown in Figures 4.26 to 4.28.

Both dipyridamole loaded Eudragit L and S microparticles showed similar distribution patterns (Figures 4.19-4.21 and 4.26-4.28). However, the prominent localisation of dipyridamole toward the surface was observed in Eudragit S microparticles. Various drug loadings of such particles showed slight differences in the degree of intensity of the drug towards the surface. A polymer to drug ratio of 70:1, showed a significant migration of the drug towards the surface (Figure 4.28). The characteristics of the dipyridamole loaded Eudragit S microparticles are summarised in Table 4.5.

<table>
<thead>
<tr>
<th>Polymer to drug ratio</th>
<th>Encapsulation efficiency %</th>
<th>Size</th>
<th>Batch yield %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5:1</td>
<td>86.24±0.1</td>
<td>68.05±0.5</td>
<td>0.71</td>
</tr>
<tr>
<td>30:1</td>
<td>81.46±0.5</td>
<td>58.46±0.3</td>
<td>0.65</td>
</tr>
<tr>
<td>70:1</td>
<td>76.85±1.6</td>
<td>55.55±0.3</td>
<td>0.54</td>
</tr>
</tbody>
</table>
Figure 4.26 Eudragit S / dipyridamole (5:1) microparticles: (a) CLSM and transmitted light images, (b) CLSM cross section images

Figure 4.27 Eudragit S / dipyridamole (30:1) microparticles: (a) CLSM and transmitted light images, (b) CLSM cross section images

Figure 4.28 Eudragit S / dipyridamole (70:1) microparticles: (a) CLSM and transmitted light images, (b) CLSM cross section images
In the previous studies, the CLSM cross section images showed that dipyridamole loaded Eudragit S microparticles exhibited a distinct migration of the drug towards the surface when compared with Eudragit L microparticles; it was therefore expected that the encapsulation efficiency of dipyridamole in Eudragit L microparticles would be considerably higher than that of dipyridamole in Eudragit S microparticles. Unexpectedly, no difference in the percentage of encapsulation efficiency was found (Table 4.5). As seen in Tables 4.4 and 4.5 the encapsulation efficiency of both Eudragit L and S microparticles, polymer to drug ratio 70:1 was much lower than that of ratio 5:1 and 30:1, which can be attributed to the higher amount of drug partition from the emulsion droplets to the continuous phase. Since the microparticle size of polymer to drug ratio, 70:1 was smaller than other ratios, it was postulated that the emulsion droplet size of these microparticles was also smaller. During the microparticle formation process, the high surface area of the small emulsion droplets led to more partition of the drug to the continuous phase. As a result low drug encapsulation efficiency was observed in microparticles with a polymer to drug ratio of 70:1. This explanation is supported by confocal images (Figure 4.28). Comparing the confocal images of the microparticles of 5:1 and 30:1, the confocal image of 70:1 exhibited less drug distribution in the inner core due to more diffusion of the drug to the continuous phase. The release profiles of dipyridamole loaded Eudragit S microparticles are shown in Figure 4.29.

![Figure 4.29 In-vitro release of dipyridamole loaded Eudragit S microparticles at pH 1.2 for 2 hours followed by an adjustment to pH 7.4](image-url)
After incubating in 0.1M HCl for 2 hours, the release of dipyridamole from all drug loadings of Eudragit S microparticles was found to be more than 15%. The rate of dipyridamole release from the Eudragit S microparticles was observed as being slightly slower than that from the Eudragit L microparticles (Figures 4.23 and 4.29). Following the increase in pH, the rate of dipyridamole release from the Eudragit S microparticles at all ranges of drug loading was rapid. Even though the CLSM optical section images of Eudragit S microparticles revealed more distribution of dipyridamole towards the surface, the rate of drug release from the Eudragit S microparticles in acidic and neutral conditions was slightly slower than that of the Eudragit L microparticles. This can probably be attributed to the effect of the size of the microparticles over the pattern of distribution of the drug; the larger size and the lower area to volume ratio therefore resulted in the slower release.

### 4.5 Overall conclusions

The physiochemical properties of drugs had a great influence on the release, encapsulation efficiency and distribution of drug within the microparticles. The effects of properties of drug on microparticle characteristics are ranked in Table 4.9.

*Table 4.6 Ranking of the effects of properties of drug on microparticle characteristics*

<table>
<thead>
<tr>
<th>Microparticle characteristics</th>
<th>Properties of drug</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drug distribution</td>
<td>Apparent partition coefficient of drug in internal phase and external phase &gt; form of drug used in internal phase &gt; molecular weight</td>
</tr>
<tr>
<td>Encapsulation efficiency</td>
<td>Apparent partition coefficient of drug in internal phase and external phase &gt; molecular weight</td>
</tr>
<tr>
<td>Release behaviour in acidic condition</td>
<td>pKa &gt; aqueous solubility of drug &gt; molecular weight</td>
</tr>
</tbody>
</table>
Chapter Four

It was found that the apparent partition coefficient of the drug in the internal and external phases was the most influential parameter affecting the pattern of drug distribution within microparticles and encapsulation efficiency. Drugs that preferentially localised in the ethanol phase rather than liquid paraffin phase tended to distribute evenly throughout the microparticles and were efficiently encapsulated inside them; as a consequence, the encapsulation efficiency of such drugs was high. In contrast, drugs which had low partition in favour of the ethanol phase were likely to localise towards the surface of the microparticles and exhibited low encapsulation efficiency. The burst release of such drugs, as a result, was undesirable. Nevertheless, in the case of acridine orange the burst release effect from the surface accumulated drug was muted due to the large size of the microparticles. The use of the partially dissolved drug in the internal phase resulted in the aggregation of drug located within the microparticles. On the other hand, the drugs which were soluble in internal phase exhibited fine distribution within the microparticles. The effect of molecular weight on the characteristics of microparticles was not pronounced as there was little difference in the molecular weight of the encapsulated drugs used.

Moreover, there was no apparent difference in the drug distribution pattern of various loadings, polymer to drug ratio, 5:1, 30:1 and 70:1 in Eudragit L/ riboflavin microparticles and Eudragit L and S/dipyridamole microparticles. The overall distribution pattern of dipyridamole within microparticles of Eudragit L and S also showed little difference.

From the knowledge gained in this study it could be predicted that prednisolone would homogeneously distribute within Eudragit L (polymer to drug ratio 30:1). This was because prednisolone completely dissolved in ethanol (during the microparticle preparation) and it had high $K_{\text{ethanol/liquid paraffin}}$ value, which enabled the prednisolone to preferentially localise in the ethanol phase rather than the liquid paraffin phase. In the dissolution study, Section 2.5.1 the leaking of prednisolone from Eudragit L microparticles was observed at the initial state of dissolution. It is hypothesized that this leakage cannot necessarily be attributed to the drug that had accumulated on the surface.
of the microparticles but could actually have been caused by the small size of the Eudragit L microparticles.

In conclusion, this study has shown the potential of CLSM as a tool for investigating the internal structure of the microparticles and drug distribution. The distribution of drugs within microparticles appeared to correlate well with the drug encapsulation efficiency and the release of the drugs from the microparticles.
CHAPTER FIVE

Conclusions and future perspectives
5.1 Conclusions

The potential problems which occur with conventional modified release dosage forms (capsules or tablets) arise from variabilities in the gastrointestinal tract, including; pH, transit times, fluid volumes and scarceness of the latter in the colon. All of these problems can be overcome to a great extent by the use of microparticulate systems. This thesis describes the use of a novel method based on the emulsion solvent evaporation (ESE) technique in producing various types of modified release microparticles to slow the release of drugs for an extended period and to target drug delivery to different regions of the GI tract (upper and low gastrointestinal tract). This method has been used as a universal technique for producing microparticles from a wide range of modified release polymers such as Eudragit L and S, HPMCP (HP50, HP55), PVAP, EC (N-7, N-100) and PVAc (B60, B500). The microparticles prepared using these polymers possessed a good morphology, high drug encapsulation efficiency and modified release properties. However, the polymers CAP and CAT could not produce microparticles when using this multi-application technique. This could be due to the instability of CAP and CAT emulsion formulations. Changing the type and/or concentration of emulsifiers or the emulsion system might help to improve the characteristics of CAP and CAT structures.

When preparing mixed pH sensitive and microflora responsive microparticle systems for colonic delivery, a restriction to the study was in the choice of polysaccharide as this had to be soluble in an organic solvent. It was found that HP-β-CD was the only suitable polysaccharide. Even though the combined HP-β-CD and Eudragit S microparticles could retard drug release in acidic conditions, in simulated colonic conditions these microparticles failed to show a difference in the drug release in the presence or absence of an amylase enzyme. This was due to the release of the HP-β-CD from the particles, resulting in an insufficient amount of HP-β-CD remaining to be degraded by the enzyme.

This thesis also focused on factors affecting the microparticle formation process and the properties of microparticles. The knowledge gained from these findings can contribute
Chapter Five

to formulation design and development. The characteristics of microparticles are the outcome of a complex interplay between the components in the formulation: drug, polymer, emulsifier, internal solvent phase and continuous phase. It was found that it is possible to use emulsion stability as a screening tool for the formulation of microparticles since the stability of emulsions at early stages of the ESE process is crucial in the particle formation process and consequently predetermines morphology of the microparticles.

Investigating the pattern of drug distribution and the internal structure of microparticles by using CLSM provided an extensive understanding of the effect of the physicochemical properties of drugs on the characteristics of microparticles. The properties of the microparticles, such as encapsulation efficiency, drug distribution and drug release characteristics can be predicted by using knowledge of the drugs’ physicochemical properties such as partition coefficients of drug in the internal and external phases, solubility, pKa and molecular weight. Drugs which preferentially localise in the internal phase rather than the continuous phase were likely to distribute evenly throughout the particles and were efficiently encapsulated inside the particles. In contrast, drugs which had low partition in favour of the internal phase tended to localise toward the surface of the microparticles and exhibited low encapsulation efficiency. Due to the distribution of the drug on the surface, a burst release of this drug was likely to be observed. The solubility of drugs in the internal phase is also of importance in determining the pattern of drug distribution. For instance, the use of the partially dissolved drug in the internal phase resulted in the drug being mainly aggregated and localised in the core of the microparticles. Furthermore, drug pKa, aqueous solubility and molecular weight have a considerable effect on the drug release characteristics. Drugs which tended to exhibit a burst release at the early stage of the dissolution in the simulated gastric pH were shown to be weakly basic, with high aqueous solubility and/or low molecular weight.

The next focus of the study was on the effect of process parameters on the particle formation process and the outcome characteristics of the microparticles. It was found that the emulsifier is one of the crucial factors in the process of microparticle formation.
as it governs droplet stabilization and stability of the emulsion. The microparticle size and drug encapsulation efficiency seemed to be most affected by the concentration of emulsifier. Furthermore, the boiling point and solubility in the continuous phase of the solvent mixture had a dramatic influence on the morphology of the microparticles as the polymer precipitation rate was found to depend greatly on the rate of solvent removal from the emulsion droplets by diffusion into the continuous phase and evaporation at the air/continuous phase interface. Irregular shaped microparticles were observed to be produced from the use of a solvent mixture which was removed too rapidly from the dispersed droplets by either diffusion or evaporation through the dispersed droplet into the continuous phase. This can be explained by the fact that solvent removal occurring at too rapid a rate will disrupt the particle formation process and lead to very rapid polymer precipitation before spherical microparticles can be formed. However, if the solvent is removed from the emulsion droplets too slowly, it will slow down the process of particle solidification, resulting in the particles remaining in a semi-solid form for a prolonged period and increasing the likelihood of aggregated particles being formed.

Moreover, it was found that the properties of the polymer (viscosity grade and molecular weight) and its concentration affected the particle size, drug encapsulation efficiency and drug release characteristics depending on the viscosity of the internal phase in which the polymer is distributed. The viscosity of the internal phase is a factor that affects the speed of the polymer precipitation and the extent of drug partition into the continuous phase. A highly viscous internal phase led to fast particle solidification and also hindered the partitioning of drug into the external phase. As a result large particles with high drug encapsulation efficiency were formed. The microparticles prepared with a high molecular weight or high viscosity grade polymer tended to release drugs slowly due to their tighter structure.

By varying the key process parameters and selecting the appropriate preparation conditions, the properties of the microparticles could be successfully modified for a modified delivery system. In conclusion, the purposes of the study were achieved and its findings aid in understanding the fundamental nature of the ESE process and will contribute to successful formulation development.
Chapter Five

5.2 Future perspectives

- Investigation of the influence of internal phase solvents on drug distribution within microparticles would be of interest as it will aid the understanding of the pattern of drug migration during the particle formation process and the relationship between the solvent removal process and the pattern of drug distribution within microparticles.

- Investigation of the solubility of polymers in the internal phase solvent would help to understand the particle formation process as the affinity of polymer to organic solvent is an important factor in determining how fast the solvent will be removed from the emulsion droplets and the polymer precipitation.

- Due to the superiority of modified release particles produced by this novel method, investigation of the in-vivo performance of these microparticles would be the next step in providing proof of efficacy of this drug delivery system.
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