Physicochemical and Biopharmaceutical Investigations of Novel Drug Delivery Systems for Oral Administration of Poorly Water-Soluble Drugs: Silica-Lipid Hybrid Microcapsules

by *Angel Tan*

(B. Pharm. Hons)

A thesis submitted for the degree of Doctor of Philosophy

(Pharmacy)

May, 2010

School of Pharmacy and Medical Sciences,

University of South Australia

With love I dedicate this thesis to my parents:

K.T. Tan & L.H. Chow

TABLE OF CONTENTS

List of Figures ..... ***v***

List of Tables ..... ***ix***

Abbreviations ..... ***xi***

Abstract ..... ***xii***

Author's Statement of Originality ..... ***xiv***

Thesis Publications ..... ***xv***

Acknowledgements ..... ***xvi***

Chapter One: Introduction

1.1 Project scope and significance 1

1.2 Formulation of poorly water-soluble drugs 2

1.2.1 Biopharmaceutical classification of drug substances 2
1.2.2 Oral formulation strategies for poorly water-soluble drugs 4

1.3 Lipid-based carriers for oral delivery of poorly water-soluble drugs 12

1.3.1 Types of lipid excipients 12
1.3.2 Gastrointestinal processing of lipids and drug transport 17
1.3.2.1 Gastric processing of lipids 18
1.3.2.2 Intestinal processing of lipids 20
1.3.2.3 Intestinal uptake 22
1.3.2.4 Route of absorption 23
1.3.3 Types of lipid-based formulations and factors affecting drug absorption 26
1.3.3.1 Lipid solutions and suspensions 27
1.3.3.2 Lipid emulsions/ microemulsions 31
1.3.3.3 Dry emulsions 33
1.3.3.4 Self-emulsifying drug delivery systems 36
1.3.4 The role of silica-based materials in oral delivery of poorly water-soluble drugs 41
1.3.5 In vitro assessment tools for lipid-based formulations 43
1.3.5.1 Drug solubility assessment, dispersion test and formulation particle sizing 43
1.3.5.2 Lipid digestion test 46
1.3.6 Future directions and challenges for oral lipid-based formulations 48
1.3.6.1 Biopharmaceutical perspective 48
1.3.6.2 Regulatory perspective 50

1.4 Aims and significance of research project 52

Chapter Two: Development and Physicochemical Characterisation of Silica-Lipid Hybrid (SLH) Microcapsules

2.1 Introduction 55

2.1.1 Development considerations and formulation design 55
2.1.1.1 Lipid excipients 56
2.1.1.2 Emulsifiers 57

2.1.1.3 Stabilisers/ solid carriers *58*
2.1.2 Celecoxib as a model poorly water-soluble drug 59
2.1.2.1 Physicochemical properties *60*
2.1.2.2 Therapeutic applications *61*
2.1.2.3 Pharmacokinetic properties *62*

2.2 Specific aims and hypotheses 62

2.3 Materials 63

2.4 Methods 64

2.4.1 Analytical methodology and validation 64
2.4.2 Screening and optimisation of lipid solubility of CEL 65
2.4.3 Preparation of emulsions and SLH microcapsules 65
2.4.4 Physical characterisation of emulsions and SLH microcapsules 67
2.4.4.1 Solid state properties *67*
2.4.4.2 Colloidal properties *68*
2.4.5 Chemical characterisation of SLH microcapsules 69
2.4.5.1 Lipid load analysis *69*
2.4.5.2 Drug content analysis *70*

2.5 Results and Discussions 71

2.5.1 HPLC analysis 71
2.5.2 Celecoxib solubility in lipids 72
2.5.3 Solid state and colloidal properties of SLH microcapsules 74
2.5.3.1 Surface and internal morphology *75*
2.5.3.2 Chemical homogeneity *79*
2.5.3.3 Microcapsule redispersibility *81*
2.5.4 Chemical properties of SLH microcapsules 83
2.5.4.1 Lipid content *83*
2.5.4.2 Drug content *84*
2.5.5 Characterisation of control formulation systems 88

2.6 Summary 90

Chapter Three: *In Vitro* Assessment of SLH Microcapsules (I): Drug Dissolution/ Release Studies

3.1 Introduction 91

3.1.1 An overview of drug dissolution/ release studies 91
3.1.2 Specific aims and hypotheses 93

3.2 Materials 94

3.3 Methods 94

3.3.1 Solubility assessment and determination of sink conditions 94
3.3.2 Comparative dissolution studies 95

3.4 Results and Discussion 96

3.4.1 Aqueous solubility of celecoxib and selection of dissolution medium 96
3.4.2 Dissolution profiles of various celecoxib formulations 97

3.5 Summary 101

Chapter Four: *In Vitro* Assessment of SLH Microcapsules (II): Enzymatic Digestion of Various Lipid-based Formulations

4.1 Introduction 102

4.1.1 An overview of lipolysis study 102
4.1.2 Specific aims and hypotheses 104

4.2 Materials 105

4.3 Methods 106

4.3.1 Preparation of the lipolysis medium 106
4.3.2 Lipolysis studies 107
4.3.3 Morphological examination of the digested samples 110

4.4 Results and Discussion 110

4.4.1 Comparative lipolysis kinetics of various lipid-based vehicles 110
4.4.1.1 Lipolysis of Miglyol-based systems 111
4.4.1.2 Dose-dependent inhibitory effect of Aerosil silica on lipolysis 117
4.4.1.3 Lipolysis of Capmul-based systems 121
4.4.2 Mechanisms of digestion of SLH microcapsules 123
4.4.3 Phase partition of celecoxib during lipolysis 126

4.5 Summary 129

Chapter Five: *In Vivo* Assessment of SLH Microcapsules: Oral Absorption of Celecoxib from Non-lipid and Lipid-based Formulations

5.1 Introduction 131

5.1.1 Pharmacokinetic properties of celecoxib 131
5.1.2 An overview of formulation strategies for celecoxib 133
5.1.3 Specific aims and hypotheses 134

5.2 Materials 135

5.3 Methods 135

5.3.1 Animal study protocol 135
5.3.2 Preparation and oral administration of celecoxib formulations 136
5.3.3 Blood sample collection and analysis 137
5.3.4 Pharmacokinetic and statistical analysis 138

5.4 Results and Discussion 138

5.4.1 Comparative oral pharmacokinetics of celecoxib from non-lipid and lipid-based formulations 138
5.4.2 Dose-dependent oral pharmacokinetics of celecoxib from non-lipid and lipid-based formulations 143

5.5 Summary 149

Chapter Six: *In Vitro-in vivo* Correlations for Various Celecoxib Oral Dosage Forms

6.1 Introduction 151

6.1.1 An overview of in vitro-in vivo correlation studies 151
6.1.2 Specific aims and hypotheses 154

6.2 Theory for *in vitro-in vivo* correlations 154
6.2.1 Dissolution-based IVIVC 154
6.2.2 Lipolysis-based IVIVC 157
6.3 Results and Discussion 157
6.3.1 Dissolution-based IVIVC 157
6.3.2 Lipolysis-based IVIVC 160
6.3.3 Proposed mechanisms of drug release, solubilisation and absorption from the SLH microcapsules 169
6.4 Summary 171
Chapter Seven: Summary and Implications of Research Findings
7.1 Summary of Research Findings 173
7.1.1 Fabrication and physicochemical properties of SLH microcapsules 173
7.1.2 In vitro performance of SLH microcapsules 174
7.1.3 In vivo performance of SLH microcapsules 175
7.1.4 In vivo-in vivo correlations to probe the drug absorption mechanisms 175
7.2 Conclusions and future directions 176
References 179
Appendix I 197

LIST OF FIGURES

Figure 1.1 Biopharmaceutical classifications of drug substances: (A) the FDA Biopharmaceutics Classification Scheme (BCS), where the *x*-axis shows the volume required to dissolve the highest dose strength of the parent drug at the lowest solubility in the pH range of 1 to 7.5, and the *y*-axis shows the permeability of the drugs [17]; and (B) the Biopharmaceutics Drug Disposition Classification System (BDDCS), in which the major route of drug elimination (metabolised by hepatocytes vs. excreted unchanged) serves as the permeability criteria [1].....3

Figure 1.2 Schematic diagram (not to scale) illustrating the gastrointestinal digestion of triglyceride substrates and the possible fate of the encapsulated drug molecules: (A) Gastric lipolysis catalysed by gastric lipase, which is gradually inhibited by the accumulated digestion products (mainly DG and FA) at the lipid droplet surfaces (scanning electron micrographs adapted from [81]), and (B) duodenal lipolysis of lipid droplets emptied from the stomach catalysed by pancreatic lipase-colipase complexes, which results in the formation of a highly solubilising lipophilic microenvironment (comprised of liquid crystalline phase on the surface of the degrading oil droplet, vesicular and mixed micellar phases in the solutions) that subsequently facilitates drug trafficking/ transport across the unstirred water layer and enterocytes (photomicrographs adapted from [82, 88]). 19

Figure 1.3 Percentage dose of halofantrine absorbed via the lymphatic and portal route resulted from various structured triglycerides in conscious rats. In the schematic presentation of the structured triglycerides, M represents a medium-chain fatty acid group and L represents a long-chain fatty acid group [127].....25

Figure 1.4 Percentage photodegradation of amlodipine formulated in various pharmaceutical carrier systems [153, 158].....34

Figure 1.5 Solubility of progesterone in taurocholate micelles in the presence and absence of lipolytic products (n = 3). Compositions: simple bile salt micelles contained 15 mM ox bile salt, mixed micelles (MCPLs) contained 15 mM ox bile salt + 0.5% medium chain lipolytic products (MG:FA = 1:2), mixed micelles (LCLPs) contained 15 mM ox bile salt + long chain lipolytic products (MG:FA = 1:2). Taken from reference [93]. 44

Figure 1.6 The dose percentage of a lipophilic compound remained in water as a function of time after 1/100 dilutions of Type II, IIIA, IIIB and IV lipid-based formulations of the drug (mean ± standard deviation, n = 4). Prior to dispersion, the drug was dissolved at 80% saturated solubility in each formulation. Taken from reference [54].....45

Figure 1.7 Schematic representation of the typical lipolysis model consists of a thermostated lipolysis reaction vessel and a pH-stat meter controller connected to an autoburette for titration of the fatty acids liberated during lipolysis: (A) The volume of NaOH consumed in the titration is recorded by the computer software, which can be used to estimate the lipolysis kinetics based on a 1:1 stoichiometric reaction ratio between the OH^- ions and the liberated free fatty acids; (B) the typical phases formed after ultracentrifugation of the lipolysis samples, in which different proportions of digested lipid components (TG, DG, MG and FA) are recovered in each phase resulted from a 30-min digestion of 250 mg of LCT (peanut oil) and MCT (Captex 355) under both fasted (5 mM NaTDC:1.25 mM PL) and fed (20 mM NaTDC: 5 mM PL) conditions [8, 82, 189]. 47

Figure 1.8 Schematic flow of research on the silica-lipid hybrid (SLH) formulations of BCS Class II drugs. 54

Figure 2.1 The generic chemical structure of various formulation lipids: triglycerides, diglycerides, monoglycerides and phospholipids (e.g. lecithin) [13, 205]. 56

Figure 2.2 Surface chemistry and charge of hydrophilic fumed silica (Aerosil 380) as a function of pH: (A) schematic reaction of hydrated silica in acidic and basic solutions; (B) zeta potential of silica dispersion on the addition of 10 mM NaCl (drawing not to scale) [215]…… 58

Figure 2.3 Classification of celecoxib in the category of non-steroidal anti-inflammatory drugs (NSAIDs) approved by FDA and its molecular structure (M.W. = 381.38) and SEM micrographs [37, 221-223]. 61

Figure 2.4 Schematic representation of SLH microcapsule preparation from two different precursor emulsion systems containing (A) silica in the aqueous phase, and (B) silica in both aqueous and oil phases, which hypothetically leads to the formation of the core-shell structured SLH-M_1 microcapsules and the porous matrix-structured SLH-M_2 microcapsules, respectively (drawing not according to scale). 67

Figure 2.5 A typical calibration curve of celecoxib and chromatograms of rat plasma spiked with celecoxib (mean retention time = 11.5 min). 71

Figure 2.6 The effect of soybean lecithin on the equilibrium solubility of celecoxib at 22 °C in (A) Miglyol oil and (B) Capmul oil (mean ± S.E.M., n = 3). 74

Figure 2.7 The formation of highly porous SLH microcapsules from two different precursor emulsion systems: SLH-M_1 and SLH-C were prepared from emulsion-(A) which contained silica in the aqueous phase; and SLH-M_2 was prepared from emulsion-(B) which contained silica in both aqueous and oil phases. Note that the cross-section images of SLH-M_1 and SLH-M_2 are the ion beam induced secondary electron micrographs, whereas the SLH-C broken microcapsules were imaged by scanning electron microscopy. 77

Figure 2.8 Confocal microscopy images of (A) emulsion-A (stabilised by silica from the aqueous phase), (B) emulsion-B (stabilised by silica from both aqueous and lipid phases), (C) spray-dried Miglyol-based microcapsules and (D) spray-dried Capmul-based microcapsules (blue dye is Coumarin 102, red dye is Rhodamine B). 80

Figure 2.9 The z-average diameters (bar graphs) and zeta potentials (line graphs) of SLH microcapsules redispersed in phosphate buffer (pH 7.2), during storage at ambient temperature: (A) SLH-M_1, (B) SLH-M_2, (C) SLH-M_3 and (D) SLH-C (mean ± S.E.M., n = 3). The white bars correspond to the particle sizes of emulsions before spray-drying (PDI values <0.2), whereas grey bars the redispersed particle sizes after spray-drying (PDI values >0.5)…… 82

Figure 2.10 The celecoxib content of various SLH microcapsules stored at ambient temperature for up to 6 months: (A) screened microcapsule formulations and (B) optimised microcapsule formulations (mean ± S.E.M., n = 3). 86

Figure 2.11 DSC thermograms of (A) Celebrex® and pure celecoxib powder, and (B) celecoxib (1%)-silica physical mixture and celecoxib-loaded SLH microcapsules (drug loading levels are approximately 1, 2 and 4% for SLH-M_1/-M_2, SLH-M_3 and SLH-C, respectively) after 6 months of storage at ambient temperature. 87

Figure 2.12 XRD profiles of pure celecoxib powder and celecoxib-loaded SLH microcapsules (drug loading levels are approximately 1, 2 and 4% for SLH-M_1/-M_2, SLH-M_3 and SLH-C, respectively) after 6 months of storage at ambient temperature. 88

Figure 3.1 Mean dissolution profiles of CEL (2 mg) in phosphate buffer (0.05 M, pH 7.2) containing (A) 0% SLS, (B) 0.05% SLS and (C) 0.5% SLS at 37 °C (mean ± S.E.M., *n* = 3): Pure CEL (○), Celebrex® (◇), MaltoDE (□), SLH-M_1 (▲) and SLH-M_2 (∗). The inset shows the initial phase of dissolution at an enlarged scale for each formulation. 98

Figure 4.1 Time dependence of the enzyme-mediated lipolysis for various Miglyol oil-based systems under simulated fasted intestinal conditions at 37 °C (mean ± S.E.M., *n* = 3). The insets show the pseudo first-order descriptions of the lipolysis process for each formulation which can be described by monophasic, biphasic or triphasic kinetics..... 112

Figure 4.2 The lipolysis profiles of Miglyol-based emulsions stabilised by Aerosil silica from the aqueous phase at various concentrations (mean ± S.E.M., *n* = 3): (Inset A) pseudo first-order descriptions of the lipolysis process and (Inset B) the slow phase of lipolysis at an enlarged scale for each formulation. 118

Figure 4.3 The lipolysis behaviour of Miglyol-based emulsions stabilised by Aerosil silica from the oil phase at various concentrations (mean ± S.E.M., *n* = 3): (Inset A) pseudo first-order descriptions of the lipolysis process and (Inset B) the slow phase of lipolysis at an enlarged scale for each formulation. 119

Figure 4.4 Time dependence of the lipase-mediated lipolysis for various Capmul oil-based systems under simulated fasted intestinal conditions at 37 °C (mean ± S.E.M., *n* = 3). The inset shows the pseudo first-order descriptions of the lipolysis process for each formulation…. 121

Figure 4.5 Schematic representation of the possible fate of SLH microcapsules under simulated intestinal digesting conditions (drawing not according to scale). In the particle sizing results, D[4,3] is the volume-weighted mean diameter; D(*v*,0.1), D(*v*,0.5) and D(*v*,0.9) are the particle diameters at 10, 50 and 90% cumulative size, respectively, after 60 min of redispersion in the lipolysis medium (5 mM BS:1.25 mM PL). 124

Figure 4.6 Freeze-fracture SEM images for a Miglyol-based and a Capmul-based microcapsule system after 60 min of lipolysis under simulated fasted intestinal conditions (5 mM BS:1.25 mM PL). 124

Figure 4.7 Phase partition results for celecoxib in the simulated fasted intestinal medium (5 mM BS:1.25 mM PL) at 37 °C (mean ± S.E.M., *n* = 2): (A) percentage of celecoxib distributed in the aqueous and pellet phases, and the likely species of lipid products generated [262], and (B) concentration of celecoxib solubilised in the lipolysis aqueous phase. 127

Figure 5.1 Plasma concentration–time profiles of celecoxib in male S.D. rats following a single oral dose of various formulations equivalent to 5 mg/kg celecoxib (mean ± S.E.M., *n* = 5)……..... 139

Figure 5.2 Plasma concentration–time profiles of celecoxib in male S.D. rats following a single oral dose of various formulations equivalent to (A) 5 mg/kg (*n* = 5), (B) 20 mg/kg (*n* = 5), and (C) 50 mg/kg of celecoxib (*n* = 4) (mean ± S.E.M.)..... 144

Figure 5.3 Linearity in pharmacokinetics of celecoxib over the dose range of 5–50 mg/kg following a single oral administration of various celecoxib formulations to male S.D. rats: $AUC_{0\rightarrow\infty}$ (grey bars) and C_{max} (closed circles).....148

Figure 6.1 Single-point correlations between pure CEL (□), Celebrex® (△) and SLH-M_1 (○) or SLH-M_2 (●): (A) DE_{15} correlated to the bioavailability ($AUC_{0\rightarrow\infty}$) and (B) DE_{180} correlated to the maximum plasma concentration (C_{max}). The MaltoDE formulation (×) is an outlier which is not fitted into the least squares regression lines. The dashed lines correspond to the linear correlations between pure CEL, Celebrex® and SLH-M_1, whereas the solid lines correspond to the linear correlations between pure CEL, Celebrex® and SLH-M_2…… 158

Figure 6.2 Point-to-point correlations between the fraction of drug dissolved and the fraction of drug absorbed.....159

Figure 6.3 Single-point correlations between lipid solution (□), lipid emulsion (■) and SLH-M_1 (○) or SLH-M_2 (●): (A) percentage lipolysis (H) correlated to the bioavailability ($AUC_{0\rightarrow\infty}$) and (B) percentage lipolysis (H) correlated to the maximum plasma concentration (C_{max}). MaltoDE (×) is an outlier which is not fitted into the least squares regression lines. 161

Figure 6.4 Single-point correlations for various CEL formulations, including pure CEL (+), SLH-M_1 (○), SLH-M_3 (▲), and SLH-C (△). The final concentration of CEL solubilised in the post-lipolysis aqueous phase at 60 min ($[CEL]_{aq\ (60\ min)}$) was correlated to: (A) the bioavailability ($AUC_{0\rightarrow\infty}$) and (B) the maximum plasma concentration (C_{max}). The maximum concentration of CEL solubilised in the aqueous phase during lipolysis ($[CEL]_{aq\ (max)}$) was correlated to (C) $AUC_{0\rightarrow\infty}$ and (D) C_{max}. Note that in panel (A) and (B), SLH-C is an outlier which is not fitted into the least squares regression lines. 163

Figure 6.5 Multiple-point correlations between the percentage of *in vitro* lipid hydrolysis (H) obtained at t = 1, 5, 15, 30 and 60 min, and the *in vivo* plasma concentration of celecoxib (C_p) at t = 15, 30, 60, 120 and 180 min resulted from oral administration of the corresponding lipid dosage forms. 165

Figure 6.6 The time points selected from the *in vivo* absorption study ($t_{in\ vivo}$) against the time points chosen from the *in vitro* lipolysis study ($t_{in\ vitro}$) for the development of IVIVC (resembling the Levy plot). 167

Figure 6.7 Multiple-point correlations between the *AUC* of CEL solubilised in the *in vitro* lipolysis aqueous phase obtained at t = 5, 15, 30 and 60 min, and the *in vivo AUC* data of celecoxib obtained at t = 30, 60, 120 and 180 min resulted from oral administration of the corresponding lipid dosage forms. All *AUC* values are expressed in the unit of min.μg/ml…… 169

Figure 6.8 Schematic representation (not to scale) of the proposed mechanisms for the enhancement of CEL oral absorption resulting from the SLH microcapsule system: (*1*) CEL pre-dissolution in the lipid phase, (*2*) enhanced aqueous dispersion of the SLH microcapsules, (*3*) rapid CEL release from the high-surface-area porous microcapsule carrier, (*4*) enhanced digestion of the embedded, adsorbed or released lipid droplets, (*5*) formation of the amphiphilic colloidal species, and (*6*) supersaturated solubilisation of CEL in the colloidal phases leading to an enhanced absorption. 170

LIST OF TABLES

Table 1.1 Comparison of various formulation technologies for poorly water-soluble drugs [2, 23-25] 5

Table 1.2 Classes of currently marketed lipid excipients [4, 5, 64] 13

Table 1.3 Classification of lipids based on their interactions with water [5, 70] 16

Table 1.4 Comparison of the properties of gastric lipase and pancreatic lipase [76, 78, 79]..... 17

Table 1.5 Examples of studies examining the oral bioavailability of poorly water-soluble drugs from lipid solutions or suspensions..... 28

Table 1.6 Examples of studies examining the oral bioavailability of poorly water-soluble drugs from liquid and dry emulsions 32

Table 1.7 Lipid formulation classification system (LFCS) based on the types and compositions of lipid and surfactant excipients as proposed by Pouton [2] 38

Table 1.8 Examples of studies examining the oral bioavailability of poorly water-soluble compounds from SEDDS and SMEDDS..... 39

Table 1.9 Examples of *in vitro-in vivo* (rank order) correlations for lipid-based formulations using the lipid digestion model [189] 51

Table 2.1 Solubility of celecoxib in various solvents at 25 °C [220]..... 60

Table 2.2 List of materials used for formulation development 63

Table 2.3a Precision (%RSD) and accuracy (%Bias) of celecoxib assay method (mean ± S.E.M., n = 6).. 72

Table 2.3b Recovery of celecoxib from rat plasma (mean ± S.E.M., n = 4)..... 72

Table 2.4 Equilibrium solubility of celecoxib in various lipid excipients at 22 °C (mean ± S.E.M., n = 3) 73

Table 2.5 Solid state surface morphology, particle size distribution and zeta potential of various SLH microcapsules (mean ± S.E.M., n = 3)..... 76

Table 2.6 Lipid load and entrapment efficiency (mean ± S.E.M., n = 3)..... 84

Table 2.7 Drug loading level and entrapment efficiencies (mean ± S.E.M., n = 3)..... 84

Table 2.8 Physical and chemical stability of redispersed maltodextrin-stabilised dry emulsion (MaltoDE) stored at ambient conditions (mean ± S.E.M., n = 3)..... 89

Table 2.9 Comparison of the variables of interest in the studied celecoxib formulations…..... 90

Table 3.1 FDA recommended dissolution method for celecoxib [252] 93

Table 3.2 Equilibrium solubility (C_s) of pure celecoxib and the number of folds of saturation volume (FSV) in 900 ml of phosphate buffer (pH 7.2) containing different

amount of sodium lauryl sulphate (SLS) at 37 °C (mean ± S.E.M., *n* = 2). The dashed line indicates the position of the reported CMC level of SLS in water.....96

Table 3.3 Dissolution characteristics (with the number of fold of improvement compared to pure CEL) of various CEL formulations in phosphate buffer (pH 7.2) containing 0.5% SLS at 37 °C (mean ± S.E.M., *n* = 3).....99

Table 4.1 The experimental level of each lipolysis component in comparison with the likely concentration range encountered in the fasted state human duodenal contents.....106

Table 4.2 Lipolysis characteristics of various Miglyol oil-based systems: lag phase duration, initial hydrolysis rate constant (*k*), and the maximum percentage of hydrolysis (H at 60 min) (mean ± S.E.M., *n* = 3).....114

Table 4.3 Lipolysis characteristics of Miglyol oil-based emulsions stabilised by Aerosil silica from the aqueous phase: the percentages of hydrolysis (H) at 5 min and 45 min post-lipolysis (mean ± S.E.M., *n* = 3).....118

Table 4.4 Lipolysis characteristics of Miglyol oil-based emulsions stabilised by Aerosil silica from the oil phase: the percentages of hydrolysis (H) at 5 min and 45 min post-lipolysis (mean ± S.E.M., *n* = 3).....120

Table 4.5 Lipolysis characteristics of various Capmul oil-based systems: lag phase duration, initial hydrolysis rate constant (*k*), and the maximum percentage of hydrolysis (H at 60 min) (mean ± S.E.M., *n* = 3).....122

Table 5.1 Pharmacokinetic properties of celecoxib in various healthy subjects (reported mean values)….....132

Table 5.2 The composition of oral CEL formulations tested in rats 136

Table 5.3 Non-compartmental pharmacokinetic parameters of celecoxib following a single oral dose (5 mg/kg) of various celecoxib formulations to male S.D. rats (mean ± S.E.M., *n* = 5).. 140

Table 5.4 Dose-dependent pharmacokinetic parameters of celecoxib following a single oral dose of various celecoxib formulations to male S.D. rats based on non-compartmental calculation (mean ± S.E.M., *n* = 5 unless otherwise specified).....145

Table 6.1 Various parameters used in the IVIVC studies of poorly water-soluble drugs…… 152

Table 6.2 The values of area-under-the curve (with the number of fold of increment compared to blank micelles) derived from the CEL solubilisation plots for various CEL formulations during digestion under simulated fasted intestinal conditions at 37 °C (*n* = 2) …… 168

ABBREVIATIONS

ANOVA	Analysis of variance
AUC	Area-under-the curve
BCS	Biopharmaceutics Classification Scheme
BDDCS	Biopharmaceutics Drug Disposition Classification System
BET	Brunauer, Emmett and Teller
BS	Bile salts
CDs	Cyclodextrins
CEL	Celecoxib
C_{max}	Maximum plasma concentration
CMC	Critical micellar concentration
COX-2	Cyclooxygenase-2
C_p	Plasma concentration
%DE	dissolution efficiency
DG	Diglycerides
DLS	Dynamic light scattering
DSC	Differential scanning calorimetry
FA	Fatty acid
FFA	Free fatty acid
FDA	Food and Drug Administration
$F_{rel.}$	Relative bioavailability
FSV	Number of folds of saturation volume
GI	Gastrointestinal
GRAS	Generally recognised as safe
HLB	Hydrophile-lipophile balance
HPLC	High performance liquid chromatography
HPMC	Hydroxypropyl methylcellulose
IVIVCs	I*n vitro-in vivo* correlations
LCT	Long-chain triglycerides
LFCS	Lipid Formulation Classification System
LOQ	Limit of quantification
MaltoDE	Maltodextrin-stabilised dry emulsion
MCT	Medium-chain triglycerides
MG	Monoglycerides
M.W.	Molecular weight
NaTDC	Sodium taurodeoxycholate
NSAIDs	Non-steroidal anti-inflammatory drugs
o/w	oil-in-water
PCS	Photon correlation spectroscopy
PDI	Polydispersity index
PEG	Polyethylene glycol
P-gp	P-glycoprotein
PK	Pharmacokinetic
PL	Phospholipids
SCT	Short-chain triglycerides
SEDDS	Self-emulsifying drug delivery systems
SEM	Scanning electron microscopy
S.E.M.	Standard error of the mean
SLH	Silica-lipid hybrid
SLS	Sodium lauryl sulphate
SMEDDS	Self-microemulsifying drug delivery systems
SPSS	Statistical package for social sciences
$t_{50\%}$	Time to achieve 50% of drug dissolution
TBU	Tributyrin units
TG	Triglycerides
TGA	Thermogravimetric analysis
t_{max}	Time taken to reach the maximum plasma concentration
USP	United States Pharmacopoeia/ Pharmacopoeial
UWL	Unstirred water layer
XRD	X-ray diffraction

ABSTRACT

Formulation of poorly water-soluble and lipophilic drugs in lipid-based delivery system is recognised as a potential strategy for improving their oral absorption. With only 2–4% oral lipid-based formulations available in the pharmaceutical market worldwide, the development of lipid-based products is clearly behind the rate of discovery of new chemical entities exhibiting poor aqueous solubility.

A novel silica-lipid hybrid (SLH) microcapsule system for enhancing the oral absorption of low solubility and high permeability (BCS Class II) drugs has been developed and demonstrated for the model drug celecoxib (CEL). This thesis describes (*i*) the fabrication of the SLH microcapsule system, (*ii*) the systematic *in vitro* characterisation of its dissolution and lipolysis properties and (*iii*) the *in vivo* pharmacokinetic investigation of various CEL formulations in a rat model.

The formulation of SLH microcapsules takes advantage of the drug solubilising effect of dispersed lipids and the stabilising effect of hydrophilic silica nanoparticles to enhance drug dispersion/ solubilisation, which leads to an improved oral bioavailability. Such formulations originate from submicron emulsions composed of a dispersed oil phase (specifically medium-chain Miglyol and Capmul MCM oils) emulsified by lecithin. The emulsions are subsequently stabilised and encapsulated by Aerosil (hydrophilic) silica nanoparticles after being transformed into a powdery state by spray-drying. The readily redispersible SLH microcapsules have demonstrated appreciable physicochemical stability at ambient conditions for the study period of 6–12 months. A specific feature of the SLH microcapsules is the nano-structured internal porous matrix (BET surface area = 184 m^2/g) as evidenced by various microscopic analyses. This was shown to offer several physicochemical and biopharmaceutical advantages in comparison with unmodified drug, lipid solution, submicron emulsion and a maltodextrin-coated (core-shell) dry emulsion; moreover, the SLH formulations are at least comparable to the commercial product of CEL, Celebrex®.

An *in vitro* dissolution/ drug release study conducted under simulated intestinal sink conditions reveals a 2- to 5-fold increase in the dissolution efficiencies (%DE) for CEL formulated as SLH microcapsules, superior to that of pure CEL and Celebrex®. This highlights the significant roles of lipids and hydrophilic silica-based carrier in improving the dissolution (or dispersion) of CEL.

Due to the digestible nature of the lipids employed, an *in vitro* lipolysis study (inclusive of lipolysis kinetics and drug phase partition) is warranted to fully characterise the dynamic solubilisation state of the encapsulated drugs before, during, and after enzymatic digestion. Several important factors contributing to an enhanced or inhibited lipolysis of the medium-chain lipid carriers have been identified: the effect of emulsification and the influence of lecithin, maltodextrin and Aerosil silica nanoparticles. The findings emphasise the importance of the highly porous matrix structure of the SLH microcapsules in producing a more predictable and enhanced lipid digestibility (~100% lipolysis with up to 20-fold higher initial digestion rate constants) as compared to lipid solutions and submicron emulsions stabilised by silica nanoparticles.

Through an orally dosed *in vivo* study in a fasted rat model, we verify the hypothesis that enhanced drug dissolution (or dispersion) and lipid digestibility could improve the oral absorption of CEL. The SLH formulations were shown to produce a statistically higher relative bioavailability, $F_{rel.}$ (150 to 240%) in reference to the pure drug administered as an aqueous suspension at three CEL dose levels, ranging from 5 to 50 mg/kg ($p < 0.05$). The pharmacokinetic data (particularly $F_{rel.}$ and C_{max}) indicates a rank order of SLH microcapsules ≈ Celebrex® > maltodextrin-coated dry emulsion ≈ submicron emulsion ≈ lipid solution ≈ pure CEL (aqueous suspension).

The *in vitro-in vivo* correlations (IVIVCs) undertaken based on various dissolution and lipolysis characteristics are important for extracting the relevant parameters that could accurately predict the *in vivo* outcome of each studied formulation. Linear single-point and multiple-point IVIVCs ($R^2 > 0.8$) have been successfully developed based on %DE, the percentage of lipolysis and the concentration of CEL solubilised in the lipolysis aqueous phase. This provides a clear understanding of the mechanisms behind the improved oral absorption of CEL promoted by the SLH formulations.

Essentially, the present work provides the impetus for studies on a wide range of other poorly water-soluble compounds that require a higher and more predictable bioavailability that eventually leads to a desirable therapeutic outcome.

AUTHOR'S STATEMENT OF ORIGINALITY

I declare that this thesis presents work carried out by myself and does not incorporate without acknowledgement any material previously submitted for a degree or diploma in any university. To the best of my knowledge it does not contain any materials previously published or written by another person except where due reference is made in the text; and all substantive contributions by others to the work presented, including jointly authored publications, is clearly acknowledged.

_____

Angel Tan

(May, 2010)

THESIS PUBLICATIONS

Peer-reviewed Journal Articles:

Tan, A., Simovic, S., Davey, A.K., Rades, T., Boyd, B.J., Prestidge, C.A.. Silica nanoparticles to control the lipase-mediated digestion of lipid-based oral delivery systems. Molecular Pharmaceutics **2010**, 7 (2), 522–532.

Tan, A., Simovic, S., Davey, A.K., Rades, T., Prestidge, C.A., Silica-lipid hybrid (SLH) microcapsules: A novel oral delivery system for poorly soluble drugs. Journal of Controlled Release **2009**, 134 (1), 62–70.

Conference Proceedings:

Tan, A., Simovic, S., Davey, A.K., Rades, T., Boyd, B.J., Prestidge, C.A., Silica-lipid hybrid (SLH) microcapsules improving the oral absorption of pooly soluble drugs. 36th Annual Meeting and Exposition of the Controlled Release Society, 18–22 July **2009**, Copenhagen, Denmark. (Oral presentation)

Tan, A., Simovic, S., Davey, A.K., Rades, T., Boyd, B.J., Prestidge, C.A., Silica-lipid hybrid (SLH) microcapsules improving the oral absorption of pooly soluble drugs. Australasian Pharmaceutical Science Association Annual Conference, 6–9 Dec **2008**, Canberra, Australia. (Oral presentation)

Tan, A., Simovic, S., Davey, A.K., Rades, T., Boyd, B.J., Prestidge, C.A., Silica-lipid hybrid (SLH) microcapsules improving the oral absorption of pooly soluble drugs. Australasian Pharmaceutical Science Association Annual Conference, 18–22 Dec **2008**, Canberra, Australia. (Poster – awarded 'The Best Poster Prize')

Tan, A., Simovic, S., Davey, A.K., Rades, T., Prestidge, C.A., Nanoparticle encapsulated droplets: a novel delivery system for lipophilic drugs. Australasian Pharmaceutical Science Association Annual Conference, 8–11 Dec **2007**, Sydney, Australia. (Oral presentation)

Tan, A., Simovic, S., Davey, A.K., Rades, T., Prestidge, C.A., Nanoparticle encapsulated droplets: a novel delivery system for lipophilic drugs. 34th Annual Meeting and Exposition of the Controlled Release Society, 7–11 July **2007**, Long Beach, USA. (Poster)

ACKNOWLEDGEMENTS

Project supervisors

Prof. Clive Prestidge[a]

Dr. Andrew Davey[b]

Dr. Spomenka Simovic[a]

Scientific advisors

Dr. Ben Boyd[c]

Dr. Mihail Popescu[a]

Dr. May Song[b]

Program coordinators/ counselors

Assoc. Prof. Phillip Pendleton[b]

Assoc. Prof. Robert Milne[b]

Laboratory managers/ physical chemistry analysts

Ms. Nicole Sanders[b]

Dr. Ji-Ping Wang[b]

Mr. Andrew Robinson[a]

Mr. Eric Tavenner[a]

Ms. Maria Provatas[a]

Microscopy analysis contributors

Dr. Peter Heard[d]

Ms. Amanda Martin[a]

Ms. Li-Hui Lim (Christine)[a]

Animal surgery demonstrators

Ms. Hong-Ping Jin[b]

Ms. Siriluk Jaisue[b]

Technical work contributors

Ms. Siau-Lin Phang[b]

Mr. Thomas Jeh[b]

Ms. Kathy Lee[c]

Purchasing managers

Mr. Paul Wotley[a]

Ms. Jayne Skinner[b]

IT/ Facilities managers

Mr. Michael Bottroff[a]

Mr. Craig Hackney[a]

Laboratory- and office-mates

Ms. Lv Wang[b]

Ms. Sha-Sha Rao[b]

Ms. Shir-Ley Tan[b]

Ms. Jasmina Turkanovic[b]

Ms. Xiao-Xin Qi (Alexia)[b]

Dr. Nasrin Ghouchi Eskandar[a]

Dr. Feng Wang (Frank)[a]

Mr. Mohanraj Vellore Janarthanan[a]

Scholarships providers

Australian Government Department of Education, Science and Training (DEST)

University of South Australia (UniSA)

[a] Ian Wark Research Institute, UniSA

[b] Sansom Institute, UniSA

[c] Monash University

[d] University of Bristol

CHAPTER ONE

Introduction

1.1 Project scope and significance

The oral route is the most convenient and non-invasive way of drug administration that receives the highest degree of patient compliance. For a drug substance to be reasonably well absorbed following oral administration, it has to: (*i*) be sufficiently soluble in the gastrointestinal (GI) fluids and (*ii*) easily permeate across the GI membrane without undergoing significant degradation or elimination mediated by the GI enzymes and enterocyte transporters [1-4]. Drugs with poor aqueous solubility and dissolution properties are not suitable for oral delivery using conventional tablet formulations as this often produces low and variable bioavailability which leads to erratic biological effects [2, 4].

According to recent estimates, nearly 30% of the oral immediate-release drug products and 40–70% of the newly discovered chemical entities are poorly soluble in water [4-7]. In an attempt to improve the solubility-limited bioavailability of such compounds, lipid-based formulations have emerged as an effective and versatile solubilisation technology, as evidenced by the numerous proof-of-concept studies in more than four decades [2-5]. Yet, successful therapeutic applications of oral lipid-based formulations have accounted for only 2–4% of the pharmaceutical market worldwide [4, 5]. This underlines the need for more intensive and systematic studies on lipid-based systems to foster the growth of viable formulations in order to cope with the exponentially growing number of poorly water-soluble drug candidates.

Some potential barriers that impede further clinical studies and regulatory approval of many lipid-based dosage forms are the lack of complete understanding on the mechanisms of action of the formulations and the shortage of robust *in vitro* tests that are predictive of the *in vivo* performance [8, 9]. In light of this, various *in vitro* lipolysis models have been developed recently to allow more thorough investigations of the dynamic changes of various formulation lipids and the solubilisation state of drugs under simulated digesting conditions [10-14].

Guided by the current state of knowledge regarding the physiological and biopharmaceutical aspects of oral lipid-based systems (which is reviewed in this chapter), this research aims to develop a novel oral formulation (*i.e.* silica-lipid hybrid

microcapsules) for improving the absorption of poorly water-soluble drugs based on appropriately selected lipid excipients and the utilisation of nanoparticle technology. The prototype formulations have been systematically characterised for the physicochemical properties (**Chapter 2**), *in vitro* release (**Chapter 3**), and solubilisation of a model drug under digesting conditions (**Chapter 4**). The *in vivo* absorption performance of the prototype formulations was investigated through a dose-dependent oral pharmacokinetic study conducted in rats (**Chapter 5**). The *in vitro-in vivo* correlations undertaken (in **Chapter 6**) effectively provide an improved mechanistic understanding of the microcapsule formulations in enhancing the oral absorption of poorly water-soluble drugs.

1.2 Formulation of poorly water-soluble drugs

1.2.1 Biopharmaceutical classification of drug substances

As described in the Biopharmaceutics Classification Scheme (BCS), drug substances can be categorised into four classes based on their aqueous solubility and intestinal permeability properties, *i.e.* BCS Class I to IV as summarised in **Figure 1.1A**. This classification scheme was introduced by Amidon *et al.* in 1995 as a tool to predict the primary importance of solubility/ dissolution and permeability on drug absorption [15]. The BCS framework accounts for the following three factors which are important in governing the rate and extent of drug absorption [16]:

(i) *Solubility*: a drug substance is considered "highly soluble" when the highest dose strength is soluble in ≤ 250 ml of aqueous media over a pH range of 1 to 7.5;

(ii) *Permeability*: a drug substance is considered "highly permeable" when the extent of drug absorption in humans is ≥ 90% of an administered dose; and

(iii) *Dissolution rate*: a drug product is considered to be "rapidly dissolving" when > 85% of the labelled amount of drug substance dissolves within 30 min using the United States Pharmacopoeial (USP) Apparatus I or II in a volume of less than 900 ml of buffer solution.

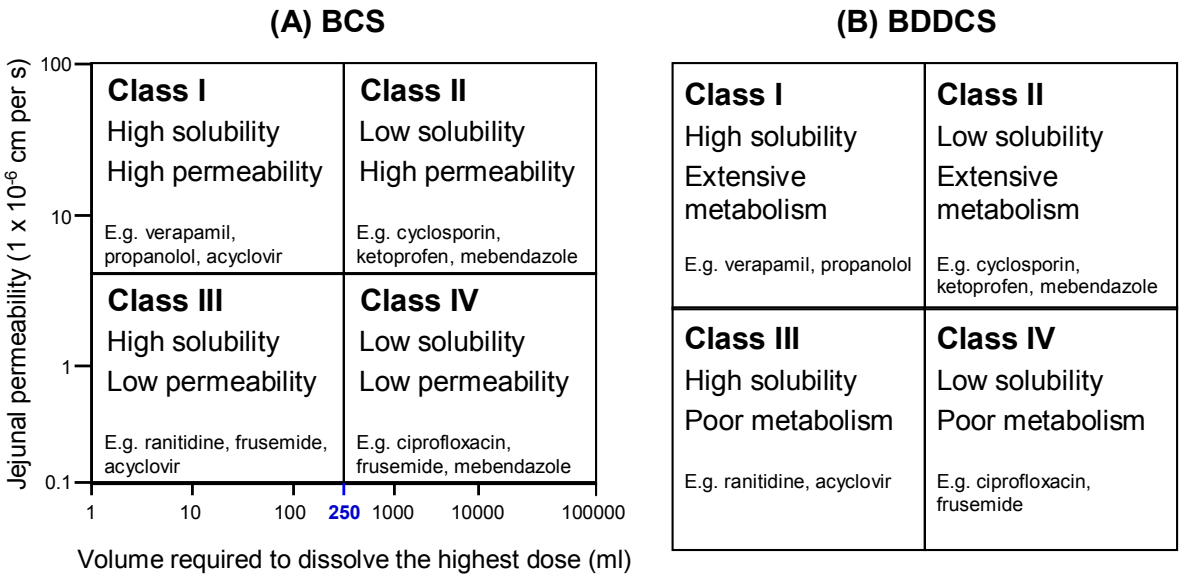


Figure 1.1 Biopharmaceutical classifications of drug substances: (A) the FDA Biopharmaceutics Classification Scheme (BCS), where the *x*-axis shows the volume required to dissolve the highest dose strength of the parent drug at the lowest solubility in the pH range of 1 to 7.5, and the *y*-axis shows the permeability of the drugs [17]; and (B) the Biopharmaceutics Drug Disposition Classification System (BDDCS), in which the major route of drug elimination (metabolised by hepatocytes vs. excreted unchanged) serves as the permeability criteria [1].

In simplified terms, poorly water-soluble drugs (BCS Class II and IV) are compounds with a dose/solubility ratio of >250 ml or with an aqueous solubility of <100 μg/ml [18]. Since year 2000, the BCS scheme has been implemented by the United States Food and Drug Administration (U.S. FDA) as an industrial guidance for justifying biowaiver through bioequivalence testings of immediate-release solid dosage forms for highly soluble–highly permeable (BCS Class I) drugs [19]. Recently, Wu and Benet have raised the concern of uncertainty in the human permeability data, which resulted in multiple categorisation of drugs in the BCS system [1]. For example, some highly soluble drugs such as acyclovir, amiloride and captopril are listed in both BCS Classes I and III; some drugs with low lipid passive diffusion permeability (as reflected in the log *P* term[e] [20]) such as frusemide, hydrochlorothiazide and methotrexate, are listed in Classes III and IV. Considering the BCS

[e] Log *P* is the logarithm of octanol-water partition coefficient, which is widely used as a quantitative descriptor for lipophilicity

definition that 'highly permeable' drugs are compounds that exhibit ≥90% absorption of an orally administered dose, the enterocyte transporter-enzyme functions (which produce either efflux or uptake effects) should be taken into consideration in addition to the drug lipophilicity properties. On this basis, Wu and Benet suggested a novel Biopharmaceutics Drug Disposition Classification System (BDDCS) (**Figure 1.1B**), in which the parameter 'permeability' is replaced with 'first-pass metabolism' to indicate the overall exposure of drugs following oral administration. An extensive study based on 168 compounds has highlighted some advantages of the BDDCS framework, including the expansion of the number of Class I drugs eligible for a biowaiver, reduced ambiguities (*i.e.* double/ triple listings) and improved predictability of drug disposition profiles for Classes II, III and IV compounds; this is essential in guiding the selection of drug models in pharmaceutical formulation design and development. While the BDDCS investigation has critically pointed out the limitation of the BCS framework, the BCS method has remained an official guide in drug classification and thus, it will be used to describe the characteristics of drug compounds throughout this thesis.

1.2.2 Oral formulation strategies for poorly water-soluble drugs

Among the four classes of drugs, BCS Class II (poorly soluble and highly permeable) drugs are more likely to benefit from formulation intervention especially by lipid dosage forms. This is partly ascribable to their reasonable solubility or miscibility in lipids, and in part due to the significance of food effects in enhancing their solubilisation and absorption *in vivo* [2, 21, 22]. While it is beyond the scope of this thesis to provide an in-depth account of all emerging formulation technologies designed for the oral delivery of BCS Class II drugs, a brief description of various solubility and dissolution-enhancing strategies is included in this section. Each formulation approach is endowed with specific pharmaceutical advantages as well as some limitations (mostly from the practical point of view), and this is outlined in **Table 1.1** [2, 23-25].

(a) Particle size reduction/ micronisation: According to the Noyes-Whitney principle, an increased wetted surface area (or reduced particle size) of a drug substance results in an increased rate of drug dissolution [16]. Therefore, particle size reduction is a relatively simple method of enhancing the dissolution rate (but not the saturation solubility) of drugs; this has been exemplified for poorly water-soluble drug models such as indomethacin [26],

Table 1.1 Comparison of various formulation technologies for poorly water-soluble drugs [2, 23-25]

Technology	References (examples)	Potential advantages	Potential disadvantages
Physical modifications			
Micronisation (conventional)	[26-28]	Known technology; freedom to operate; easily adaptable to pharmaceutical manufacturing set-ups; relatively inexpensive and reproducible production; solid dosage form	Insufficient improvement in dissolution rate; no effect on the saturation solubility of drugs (these limitations also apply to other micronisation techniques)
Fluid pearl- or ball-milling	[2, 23]	Established products on the market; experienced technology provider (Elan Corporation); versatility to produce solid or liquid dosage forms	Available only under license; secondary process required to avoid aggregation of nanocrystals
Dense gas technology	[29-31]	Alternative nanocrystal processing method; still room to develop new IP	Unproven technology for industrial use; secondary process required to avoid aggregation of nanocrystals
Rapid solvent exchange	[32]		
Cogrinding & spray-drying	[28]		
Surfactant and cosolvent system (also known as LFCS Type IV system)	[33, 34]	Relatively high solvent capacity for hydrophobic compounds	Possible poor tolerance to excipient-related side effects in chronic use; tendency of drug precipitation on excessive dilution
Crystal habit modification	[35-37]	Relatively inexpensive; versatility to produce solid or liquid dosage forms	Risk of polymorph instability; possible need of incorporating stabilisers to inhibit crystal growth/ change
Supersaturating drug delivery systems (solid solutions/ dispersions)	[38-44]	Freedom to operate; new melt-extrusion technology offers solvent-free continuous process operating at a lower temperature; direct filling of semi-solid materials into hard gelatin capsules	Unpredictable physical stability of drug/ polymer in the product; limited scale-up of the manufacturing processes; requirement of a surfactant- or amphiphilic polymer-based additive to assist complete dissolution or to maintain supersaturation state

Table 1.1 (continued)

Technology	References (examples)	Potential advantages	Potential disadvantages
Cyclodextrins (CDs)	[45, 46]	Versatility to produce solid or liquid dosage forms; may offer protection to acid-labile drugs in the GI tract	May reduce drug permeability depending on the drugs' affinity for carrier or membrane; potential toxicity associated with less hydrophilic CDs
Lipid-based formulations (LFCS Type I, II and III)	[8]	Versatility to produce solid or liquid dosage forms; potential in reducing food effect on bioavailability	Limited to highly lipophilic drugs; not feasible for formulating into conventional compressed tablets due to insufficient physical integrity and mechanical strength
Chemical modifications			
Salt formation	[47-49]	Better physical and chemical stability during storage compared to amorphous formulations	Not feasible for neutral and very weakly acidic/ basic compounds; potential reconversion into aggregates of the original poorly soluble acid/ base forms when dispersed in GI fluids
Prodrugs	[50-53]	Better physical and chemical stability during storage compared to amorphous formulations; a potential way of reducing GI toxicity and presystemic metabolism	Can be a time-consuming and costly process

fenofibrate [28], ibuprofen, itraconazole, ketoconazole and a multitude of other examples [31, 32]. The technique of particle micronisation have evolved from conventional air jet milling (which typically reduces particle sizes to 2–5 μm) to the more advanced wet pearl- or ball-milling (which reduces particle sizes to 100–250 nm) [2, 24]. The latter method, which is also known as the NanoCrystal® technology owned by Elan Corporation, has successfully brought to commercialisation of a few products: sirolimus tablets (Rapamune®), aprepitant capsules (Emend®), fenofibrate tablets (TriCor®) and a megestrol oral suspension (Megace® ES) [2, 23]. Other novel micronisation techniques yet to be industrialised include dense gas technology [29-31], rapid solvent exchange techniques [32], as well as cogrinding and spray-drying [28].

(b) Surfactant/cosolvent system: Surfactants and cosolvents are solubilising excipients which can be used alone or in combination to increase the solubilisation power of an aqueous medium. Surfactants are amphiphilic agents that improve drug solubility and dissolution by lowering the surface tension or by forming solubilising micelles at concentrations exceeding their critical micelle concentrations (normally in the range of 0.05–0.1% for most surfactants) [23, 34]. Some commonly used surfactant excipients are sodium lauryl sulphate (SLS, anionic), polysorbates (non-ionic) and polyoxyethylated castor oil (non-ionic) [23]. It has always been assumed that all surfactants can cause irritation as a result of their potential in penetrating and fluidising biological membranes [54]. Notwithstanding this problem, the commercialisation of products based particularly on non-ionic surfactants has supported their acceptability for oral administration. The risk-benefit ratio has always played a central role in determining the use of surfactants in chronically used products. Marked examples are the HIV anti-retroviral drugs, including amprenavir (Agenerase®, GSK), lopinavir/ritonavir tablet (Kaletra®, Soliqs), and ritonavir (Norvir®, Abbott Laboratories), in which case the patients consume up to ~3 g of surfactants daily (e.g. the polyoxyethylated castor oil, Cremophor) [54].

Most of the surfactant-based products have also included cosolvents as an additional solubilising excipient. Pharmaceutically employed cosolvents are water-miscible organic solvents which can confer high solubility to hydrophobic solutes via specific molecular interactions (such as hydrogen bonding) [23]. In general, the cosolvency effect (*i.e.* solute-water-cosolvent miscibility) is highly dependent on the solvent polarity: co-solvents of increasing polarity will produce a more ideal mixing effect with water; and those with a polarity near to that of a given hydrophobic solute will produce sufficient solute solubilising effects [55]. Essentially, these two have to be balanced to achieve an adequate cosolvency effect. Some commonly used cosolvents are ethanol, glycerin, polyethylene glycol (PEG) and propylene glycol [23, 34]. An example of a pharmaceutical product which employs the cosolvent approach is nifedipine (Procardia®, Pfizer), which contains glycerin, peppermint oil, PEG 400 and sodium saccharin encapsulated in soft gelatin capsules [56]. According to Pouton's Lipid Formulation Classification System (LFCS) (which is discussed in **Section 1.3.3.4**), a surfactant-cosolvent blend system is specifically classified as the Type IV formulation, which is regarded suitable for formulating hydrophobic but not lipophilic drugs [2].

(c) Crystal habit modification: A solid drug substance may exist in more than one crystalline form (*i.e.* polymorphs) which exhibits different physicochemical properties such as melting point and stability; this inherently has a key impact on the compound's solubility and dissolution rate [36, 56, 57]. Therefore, polymorphic modification represents an alternative way of changing the dissolution profile of a given drug. A metastable polymorph (with higher free energy) is often associated with a relatively higher solubility or dissolution rate than the thermodynamically stable form (with lower free energy) [56, 57]. The formation of metastable crytals can be achieved by controlled supersaturation and/or re-crystallisation of drugs from different solvents in the presence or absence of surfactants and polymers [37, 56, 57]. When selecting a polymorph for formulation, an important consideration is to balance between the manufacturability, stability and solubility of a solid compound, such that the drug's potency can be maintained over the shelf-life period [36, 56, 57]. The issue of polymorph instability is best illustrated by the 1998 post-marketing withdrawal of the ritonavir semi-solid capsules (Norvir®), when it was discovered that the originally identified crystal form is prone to conversion into a less soluble and therefore, a less absorbable polymorph within weeks in the marketed product [23, 36, 56]. Following this, Abbott Laboratories reformulated Norvir® in the lower energy polymorph form (still in solution-filled gel capsules), which requires refrigeration due to thermal instability of the crystals [56, 58].

(d) Solid solutions/ dispersions as supersaturating drug delivery systems: Supersaturating drug delivery systems are built on the basis that, presentation of drugs in the high energy, thermodynamically unstable forms (*i.e.* metastable or amorphous forms) can provide a higher apparent solubility and an accelerated dissolution in the GI tract; the supersaturated drug concentration can also enhance the intestinal uptake of drug molecules down the concentration gradient [42]. Solid solutions/ dispersions are formulations of this type of technology. Solid dispersions are produced by dispersion or immobilisation of drugs in a hydrophilic or water-soluble matrix in the solid state. It can be described as a *eutectic dispersion* when the drugs are presented in a microcrystalline state, or as a *solid solution* when a certain fraction of the drugs are molecularly dispersed in the matrix [23, 24, 42, 43, 59]. Conceptually, solid dispersions gain improvement in drug dissolution in two ways: intraluminal supersaturation of drugs as very fine, colloidal particles with greatly enhanced surface area (in which the risk of precipitation cannot be neglected), as well as the carrier-related increase in drug wettability [24, 42, 43]. Typically, a surface-active stabiliser (e.g.

SLS and PEG-polysorbate 80 mixture) is included in a solid dispersion system to prevent the formation of a drug-rich hydrophobic barrier on the surface of the dissolving matrix [2, 42, 43]. The inclusion of a polymer-based stabiliser (e.g. PEG and hydroxypropyl methylcellulose, HPMC) is an effective approach to maintain the supersaturation state or to delay precipitation (*i.e.* 'crystal growth') of drugs in the GI fluids [42]. The preparation method of solid dispersions has evolved from the conventional hot-melt and solvent-evaporation methods (which operates at high temperatures and employs organic solvents, respectively) to the more recent melt-extrusion technology (with shorter heating time/ lower operating temperatures, without using organic solvents) [2, 24, 44]. Currently, the commercial application of solid dispersion formulations is limited to a few products, this includes a griseofulvin-in-PEG solid dispersion (Gris-PEG®, Novartis), a nabilone-in-povidone solid dispersion (Cesamet®, Lilly), a lopinavir/ritonavir tablet using a proprietary melt extrusion (Kaletra®, Soliqs), and a itraconazole-in-HPMC solid dispersion coated on inert sugar spheres (Sporanox® capsules, Janssen Pharmaceutica) [27, 42, 43, 56].

(e) Cyclodextrins: Cyclodextrins (CDs) are starch-derived cyclic oligosaccharides with a hydrophilic exterior and hydrophobic interior, which may contain six (α-CD), seven (β-CD), eight (γ-CD) or more α-D-glucopyranose units [24, 46, 56]. The major mechanism by which CDs increase the apparent solubility and dissolution of drugs is via the formation of non-convalent inclusion complexes (which may also protect acid-labile drug molecules from degradation); CDs may also form non inclusion-based complexes and act as a surfactant, supersaturation stabiliser and precipitation inhibitor in the aqueous environment [42, 46]. Dissociation of drug molecules from the CD-drug complexes may be triggered by aqueous dilution, replacement by other suitably sized molecules such as dietary lipids (*i.e.* a 'competitive inclusion effect'), or by the affinity of drug molecules towards the biological membranes when located in close proximity with the mucosal membrane (such as the oral mucosa) [45, 46]. On the other hand, drug release from covalently bound CD-drug conjugates is induced by enzymatic degradation, and this prodrug-like formulation can be useful for colon-targeted drug delivery [45]. It has been recognised that the toxicity (*i.e.* haemolytic) effect of a CD system is highly dependent on its ability to bind or extract cholesterol from the biological membranes [46]. While many CD-based formulations are still under evaluation, a considerable number of CD-based oral products have already been commercialised: a α-CD-based cefotiam-hexetil HCl tablet (Pansporin T®, Takeda), a β-CD-based nicotine sublingual tablet (Nicorette®, Pfizer), a 2-hydroxypropyl-β-CD-based

itraconazole solution (Sporanox® oral solution, Janssen Pharmaceutica) and many more [46]. The company's product monograph of Sporanox® oral solution has stated that high oral doses of 2-hydroxypropyl-β-CD (*i.e.* ≥1500 mg/kg body weight) potentially result in osmotic diarrhea, because the CD excipient has a low systemic absorption and hence tend to produce a stimulant effect on the intestinal secretion and motility. Nevertheless, the versatility of CDs as a multifunctional drug carrier is anticipated to advance. Recently, the combined use of CDs and surfactants, polymers and liposomes has been investigated for increased complexation efficacy, targeted delivery and prolonged therapeutic effect of poorly water-soluble drugs [24, 46].

(f) Lipid-based formulations: Lipid-based formulations are developed to mimic the food (or post-prandial) effect based on the fact that co-administration of poorly-water soluble drugs with fatty food can increase the drug bioavailability [60]. Lipid-based dosage forms represent an important class of solubilised formulations particularly for lipophilic drugs when other physical modification methods do not provide sufficient improvement in drug dissolution and absorption. Based on the compositions of lipids and surfactants incorporated into the system, lipid-based formulations can be broadly divided into LFCS Type I (oil-based), Type II (oil and lipophilic surfactant-based), Type III (oil and hydrophilic surfactant-based) and Type IV (surfactant and cosolvent-based) formulations [2]. Principally, lipid-based formulations increase drug bioavailability by presenting the drug in a molecularly dispersed/ dissolved state during GI transit, as well as inducing a series of physiological responses (e.g. increased biliary secretions) and biochemical changes (e.g. increased intestinal permeability) in the GI tract which favours drug absorption [60-62]. As the key focus of the current literature review, the role of lipid-based formulations in oral delivery of poorly water-soluble drugs is discussed in detailed in **Section 1.3**.

(g) Salt formation: If a drug molecule is ionisable within the gastrointestinal pH range of 1–9 and exhibits a strong pH dependency of the distribution coefficient (expressed as log *D*), chemical conversion of the parent unionised drug into the crystalline salt form is the most fundamental approach for improving its solubility and dissolution [16]. Most of the pharmacopoeial monographs for drugs demonstrating a solubility of >10 mg/ml are hydrochloride, sulphate, maleate and citrate salts of basic drugs (e.g. chlorpromazine HCl and morphine sulphate); and potassium, calcium and sodium salts of acidic drugs (e.g. dicloxacillin sodium and losartan potassium) [63]. The major concern of this formulation type is the pH-mediated reconversion of the formulation salt into the parent free acid/ free

base, which may precipitate in the GI fluids [42, 56]. Nevertheless, it has been shown possible to maintain a supersaturation state for the recrystallised drugs in the presence of a precipitation inhibitor, which eventually leads to an improved bioavailability [42, 48]. Previous studies have evidenced that maximising the degree of supersaturation (even for a limited time period) is more important than maintaining the stability of supersaturation in the GI fluids for attaining a higher bioavailability of high permeability drugs (such as celecoxib and tacrolimus) [42].

(h) Prodrugs: Prodrugs are biologically inactive compounds that can undergo transformation into the active parent drug via chemical or enzymatic reactions [53]. Prodrug formulation is a suitable approach for drugs requiring high doses of administration and those displaying poor aqueous and lipid solubility [52]. It involves chemical modification of the pharmacophore by adding a polar (either ionisable or non-ionisable) functional group. As a general rule, the formulation design of a prodrug should always balance between the aqueous solubility and the membrane permeability to ensure adequate intraluminal solubilisation and intestinal uptake [52, 53]. The intestinal permeability is greatly influenced by the ionisation state and the molecular weight of a drug (which should not exceed 500 g/mol) [52, 53]. For example, prodrugs with ionised functionalities (hence with higher solubilities) will require reconversion into the unionised parent molecules (with higher lipophilicities) in the GI lumen prior to permeation across the intestinal membrane. In such cases, local accumulation of the less soluble parent drugs may generate a supersaturation state which create the driving force for drug absorption, although the risk of precipitation cannot be ruled out [42]. An example of ionised oral prodrugs is fosamprenavir calcium (Lexiva® or Telzir®, GlaxoSmithKline), which is a phosphate ester prodrug of the anti-retroviral drug, amprenavir [42, 52, 53]. This prodrug formulation is endowed with a significantly higher solubility than the parent drug (*i.e.* ~10 times increased solubility), which allows reformulation of the drug into a higher strength tablet (600 mg amprenavir) in comparison with the original capsules containing only 150 mg amprenavir (which requires higher frequency of administration). Besides solubility enhancement, the ability of a prodrug to mask local GI irritation is illustrated by the non-ionisable, sulfoxide prodrug of sulindac sulfide (Clinoril®, Merck), which is a non-steroidal anti-inflammatory drug (NSAID) [52].

1.3 Lipid-based carriers for oral delivery of poorly water-soluble drugs

The diversity of lipid excipients available commercially has opened up the options of formulation design for pharmaceutical scientists: solutions, suspensions, emulsions (liquid or dry form), micellar systems, solid lipid particles, liquid crystalline phase carriers, liposomes and various self-emulsifying systems. Successful development and application of these formulations greatly depends on the physicochemical properties of the lipid excipients, their compatibility with the drug molecules, as well as their nature of processing in the GI lumen. The following sub-sections provide an overview of the types of commonly used lipid excipients, followed by a review on the intraluminal processing of lipids and the impact of this process on drug transport. Next, the characteristics of various lipid dosage forms are covered in detail and some important factors that potentially affect drug absorption from these formulations are identified based on previous case examples. Finally, the opportunities and challenges for oral lipid-based formulations are discussed from both the biopharmaceutical and regulatory perspectives.

1.3.1 Types of lipid excipients

Based on differences in the chemical compositions and manufacturing methods, the commercially available lipid excipients can be categorised into the following classes (which is summarised in **Table 1.2**) [4, 5]:

(a) Fatty acids: Fatty acids (FA) are monocarboxylic acid derivatives of saturated or unsaturated aliphatic hydrocarbons. Saturated FA with ≤8 carbons in the chain length are flowable liquids (*i.e.* more water-miscible and have lower melting points) at room temperature, whereas those with ≥10 carbons are semi-solids (*i.e.* less water-miscible and have higher melting points) at room temperature. Unsaturated FA or lipids containing unsaturated FA are prone to oxidation catalysed by impurities such as peroxides, metal ions, and photochemical sensitisers (e.g. chlorophyll and riboflavin); this reaction can be identified from the unpleasant tastes and odours of the 'rancid' lipids. There are several ways of minimising instability of the lipids resulted from oxidative reactions, these include purification to remove the impurities, protection from light and excessive heat, addition of an antioxidant, maintaining a pH of ~6.5 during processing and in the final product, as well

Table 1.2 Classes of currently marketed lipid excipients [4, 5, 64]

Class of lipid excipients	Examples of excipients used in marketed lipid-based oral products	Pharmaceutical applications			
		Solubiliser	Surfactant	Emulsifier	Others
Fatty acids	Soy fatty acids, oleic acid	✓			
Natural oils and fats	***MCT*** Coconut oil, palm seed oil	✓			
	LCT Olive oil, soybean oil	✓			
Semi-synthetic MG, DG, TG	***medium-chain MG/DG*** Glyceryl mono- and dicaprylate (Capmul MCM)	✓		✓	Co-emulsifier
	MCT Glyceryl tricaprylate/caprate (Miglyol 810)	✓			Vehicle for capsule formulations
	long-chain MG Glyceryl monooleate (Capmul GMO)	✓		✓	Wetting agent, vehicle for capsule formulations
Semi-synthetic PEG derivatives of glycerides and fatty acids	***medium-chain glycerides*** PEG 400 glyceryl caprylate/caprate (Labrasol)	✓	✓		Vehicle
	long-chain glycerides PEG 300 glyceryl oleate (Labrafil M-1944CS)	✓			Vehicle for soft gels, co-emulsifier, lipid phase/ co-surfactant in microemulsions
	PEG 1500 glyceryl laurate (Gelucire 44/14)	✓		✓	Semi-solid matrix capsule vehicle
Polyglyceryl fatty acid esters	Polyglycerol-3 dioleate (Plurol Oleique CC497)	✓	✓	✓	Vehicle for capsules
	Polyglycerol-10 decaoleate (Caprol 10G 10O)	✓	✓	✓	Vehicle, lubricant, crystallisation inhibitor
Cholesterol and Phospholipids	Egg lecithin, soybean lecithin	✓	✓	✓	(in mixed micelles, emulsions, liposomes)

as hydrogenation to decrease the number of carbon-carbon double bonds. FA is primarily used as a solubiliser in pharmaceutical formulations.

(b) Natural oils and fats: Lipids of this class are mixtures of naturally occurring triacylglycerols or triglycerides (TG) which contains FA of different chain length and degree of unsaturation. Based on the hydrocarbon chain length of the component FA, oils and fats can be classified as short-chain triglycerides, SCT (<5 carbons), medium-chain triglycerides, MCT (6–12 carbons), or long-chain triglycerides, LCT (>12 carbons). They can be used as solubilisers in oral formulation products, but may require processing such as hydrogenation to improve resistance to oxidative degradation, or separation into the component glyceride fractions to obtain desirable physical and drug solubilisation properties (thus, these modified glycerides are semi-synthetic).

(c) Semi-synthetic mono-, di-, and triglycerides: Semi-synthetic mono-, di-, and triglycerides (MG, DG, and TG) are commercially available fractionated glycerides which have less variability in their compositions compared to naturally occurring oils and fats. In each excipient lot, the compositions of each glyceride components are strictly controlled within the specified range of acceptability. In general, fractionated glycerides offer greater applications in pharmaceutical formulation due to their specific compositions and improved uniformity. For example, coconut oil which is composed of saturated MCT and LCT appears as semi-solid at room temperature; whereas its fluid derivative, fractionated coconut oil (e.g. Miglyol 810 and Captex 300), contains ≥95% MCT and is more suitably used as an oily excipient in soft gelatin encapsulated products such as Advil®, Pfizer/Wyeth (ibuprofen) and Robitussin®, Pfizer/Wyeth (guiafenisin). This class of excipient has offered multiple pharmaceutical applications, including solubilising vehicles, emulsifiers, suspending and wetting agents, as well as in various controlled release dosage forms.

(d) Semi-synthetic PEG derivatives of glycerides and fatty acids: The chemically modified mixtures of MG, DG and TG with fatty acid esters of PEG are widely used as fluid or thermo-softening semi-solid solubilising vehicles, surfactants and wetting agents; they also serve as an emulsifier or co-emulsifier in self-emulsifying systems. The fluid excipients can be modified with the addition of colloidal silicon dioxide to form thixotropic formulations useful for preparing lipid suspensions of solid drugs. Thermo-softening semi-solids normally require melting (at 26–70 °C) before they can be used as a solubilising or suspending agent, which is then filled into hard gelatin capsules at the molten state; in this

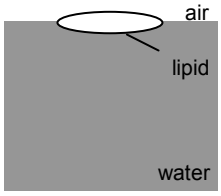
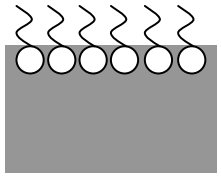
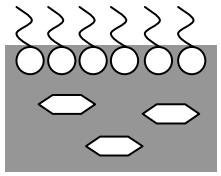
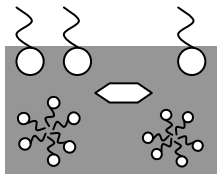
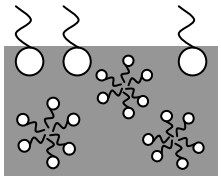
case soft gelatin capsules (with a melting point of 40 °C) are not suitable for encapsulation of the melted excipients.

(e) Polyglyceryl fatty acid esters: Polyglyceryl fatty acid esters are synthesised by linking a chain of glycerol molecules via ether linkages, followed by esterification with one or more FA molecules. Their hydrophilicity depends on the polyglycerol chain length and number of free hydroxyl groups present. Lipids of this class can act as surfactants, solubilisers, vehicles, emulsifiers and crystallisation inhibitors.

(f) Cholesterol and phospholipids: Cholesterol and phospholipids are most frequently used to form mixed micelles, emulsions and liposomes. Phospholipids can be subdivided into negatively charged lipids (e.g. phosphatidic acid and phosphatidylglycerol), Zwitterions (e.g. phosphatidylcholine and phosphatidylethanolamine) or a mixture of both (e.g. L-α-lecithin and egg lecithin). The use of charged phospholipids may confer stability to the emulsion and liposomal systems by preventing droplet aggregation and fusion. In addition, cholesterol and phospholipids have also found applications in the formulations of self-emulsifying solid dispersions by improving and minimising age-related changes in the dissolution characteristics of poorly water-soluble drugs [65-69].

Conceptually, the pharmaceutical functions (e.g. the ability to act as surfactants, emulsifiers and to form micelles) of each lipid excipient are greatly dependent on their polarity and interactions with water. Small [5, 70] has systematically classified lipids into two groups based on the specific phase behavior of various lipids when coming into contact with water: *polar lipids* (which interact with water) and *non-polar lipids* (which do not interact with water). The polar lipids are sub-divided into three categories (**Table 1.3**). *Class I lipids* are insoluble and non-swelling amphiphiles (e.g. TG, DG, cholesterol) which do not interact with water but spread at the air-water interface as a stable monolayer. *Class II lipids* refer to insoluble but swelling amphiphiles (e.g. MG and phospholipids) which interact with water via the exposed hydrophilic group (this is described as 'swelling'). As a result, these amphiphiles tend to form either a stable monolayer at the surface of water, or self-assemble into a laminated, lipid-water structure known as a liquid crystal in bulk water. *Class III lipids* are water-soluble and swelling amphiphiles which at the equilibrium state, spread as an unstable monolayer at the air-water interface and present more stably as micelles in relatively dilute solutions. Lipids of this class are capable of solubilising other classes of

Table 1.3 Classification of lipids based on their interactions with water [5, 70]

Class of lipids		Schematic physical appearances (drawing not to scale)		Examples
NON-POLAR LIPIDS		Surface: Will not spread to form a monolayer Bulk: Insoluble		Cholesteryl esters, paraffin oil, carotene, hydrocarbons
POLAR LIPIDS				
Insoluble	**I** (non-swelling)	Surface: Stable monolayer Bulk: Insoluble		TG, DG, cholesterol, fat-soluble vitamins (A,D,E,K), long-chain FA
Insoluble	**II** (swelling)	Surface: Stable monolayer Bulk: Liquid crystals		MG, phospholipids
Soluble	**IIIA** (Swelling amphiphiles with lyotropic mesomorphism)	Surface: Unstable monolayer Bulk: Liquid crystalline intermediates Micelles		Detergents, sodium and potassium salts of long-chain FA
Soluble	**IIIB** Swelling amphiphiles without lyotropic mesomorphism	Surface: Unstable monolayer Bulk: Micelles		Bile salts, saponins

lipids. They can be different in their propensity for lyotropic mesomorphism, *i.e.* aggregation of the lipid monomers to form an intermediate liquid crystalline phase prior to forming micelles when the critical micellar concentration is approached. Type IIIA lipids exhibit distinct polarity between the hydrophobic and hydrophilic regions of the molecule, therefore they possess the lyotropic mesomorphism property (e.g. detergents and sodium salts of long-chain FA). In contrast, Type IIIB lipids are generally aromatic compounds with fused rings that are not capable of forming liquid crystalline intermediates due to the

lack of distinct polarity between the ends of molecules. Consequently, Type IIIB lipids (e.g. bile salts) form micelles directly in bulk water.

1.3.2 Gastrointestinal processing of lipids and drug transport

When a lipophilic compound is formulated in digestible lipids, digestion of the lipid excipients mediated by the GI enzymes will have a crucial impact on the intraluminal release and solubilisation state of the compound. This will significantly affect the overall drug bioavailability. It is therefore essential to understand the fundamental process of lipid digestion and the possible drug transport pathways across the enterocytes. Knowledge in this area has been constantly in review over the past few decades [8, 71-75]. In humans, digestion of lipids is mainly catalysed by the water-soluble enzymes, lipases, producing the lipid monomers of FA, MG, and to a lesser extent, glycerols (**Table 1.4**). Other enzymes which are relatively minor in their lipolytic activities are also present in the GI tract, including pancreatic carboxyl esterase, a non-specific catalyst for non-emulsified substrates, and phospholipase A2, which is responsible mainly for the digestion of phospholipids (PL) [60, 76]. In this section, we review the GI processing of lipids catalysed by lipases based on the digestion of TG substrates, which is the major form of dietary fat in human beings [77].

Table 1.4 Comparison of the properties of gastric lipase and pancreatic lipase [76, 78, 79]

Properties	Gastric lipase	Pancreatic lipase
Origin	Gastric mucosa	Pancreas
Maximum catalytic pH	3.0–6.0	6.5–9.0
Measured medium pH	1.5–4.0	4.0–6.8
% TG hydrolysis	10–20% (in stomach) ~10% (in small intestines)	60–90 % (in small intestines)
Typical reactions	TG → DG + FA	TG → DG + FA DG → 2-MG + FA *Isomerisation at basic pH's:* 2-MG → 1-MG → Glycerol + FA
Cofactor	Nil	Colipase

1.3.2.1 Gastric processing of lipids

Physiological aspects: The digestion of lipids (up to 30%) is initiated in the stomach by gastric lipase and lingual lipase. The ingested lipids are prone to emulsification facilitated by the gastric shear forces, the presence of membrane-derived PL and the generated amphiphilic lipolysis products; consequently lipid droplets of 1–100 μm are formed prior to gastric emptying [8, 17, 80]. Gastric lipase has shown greater affinity to SCT and MCT in comparison with LCT, producing mainly FA and DG (with traces of MG) as the hydrolysis products [60, 79, 81]. Pafumi *et al.* [81] have studied the mechanisms of gastric lipolysis using LCT-based emulsions and identified a complete cessation of gastric lipase activity after 60 min. They proposed that the relatively limited extent of gastric lipolysis (in comparison with duodenal lipolysis) is resulted by the progressive accumulation of long-chain FAs (which are protonated at low pH) at the droplet surface. The FA products potentially re-organise into peripheral particles which entrap the gastric lipase that typically acts at the lipid-water interface (**Figure 1.2A**). However, the FA-entrapped gastric lipase has been found to retain its enzymatic activity following gastric emptying and continues to account for ~7.5% lipolysis in the small intestines [79].

Impact on drug delivery: In general, weakly acidic drugs have limited solubility and dissolution in the stomach; whereas for weak bases, the stomach is the principle site of dissolution prior to emptying into the intestines, where the risk of drug precipitation exists [17, 82]. Encapsulation of such poorly water-soluble drugs in lipids may present several advantages. Firstly, the drug compounds are molecularly dispersed or fully dissolved in the lipid phases. This inherently eliminate the need for pre-absorptive drug dissolution in the GI fluids, and the intraluminal processing of lipids which commences in the stomach may progressively increase the drug solubilisation degree (this is discussed in more detail in **Section 1.3.2.2**) [60, 82]. Secondly, the presence of lipids in the intestines has been shown to induce a dose-dependent feedback inhibition of gastric emptying. However, this is more prominent for long-chain lipids compared to short- or medium-chain lipids, probably due to the formation of chylomicron in the former case, which efficiently induce the feedback inhibition effect on the upper GI functions [83-85]. The slow down in gastric emptying has been suggested to be a means of controlling and prolonging the transit time of drugs at the absorption site, thereby increasing the drug bioavailability [82, 86, 87]. However, the likeliness of such effect has to be interpreted with care, given that a typical pharmaceutically relevant lipid dose is less than 2–3 g, while higher doses of lipids (at

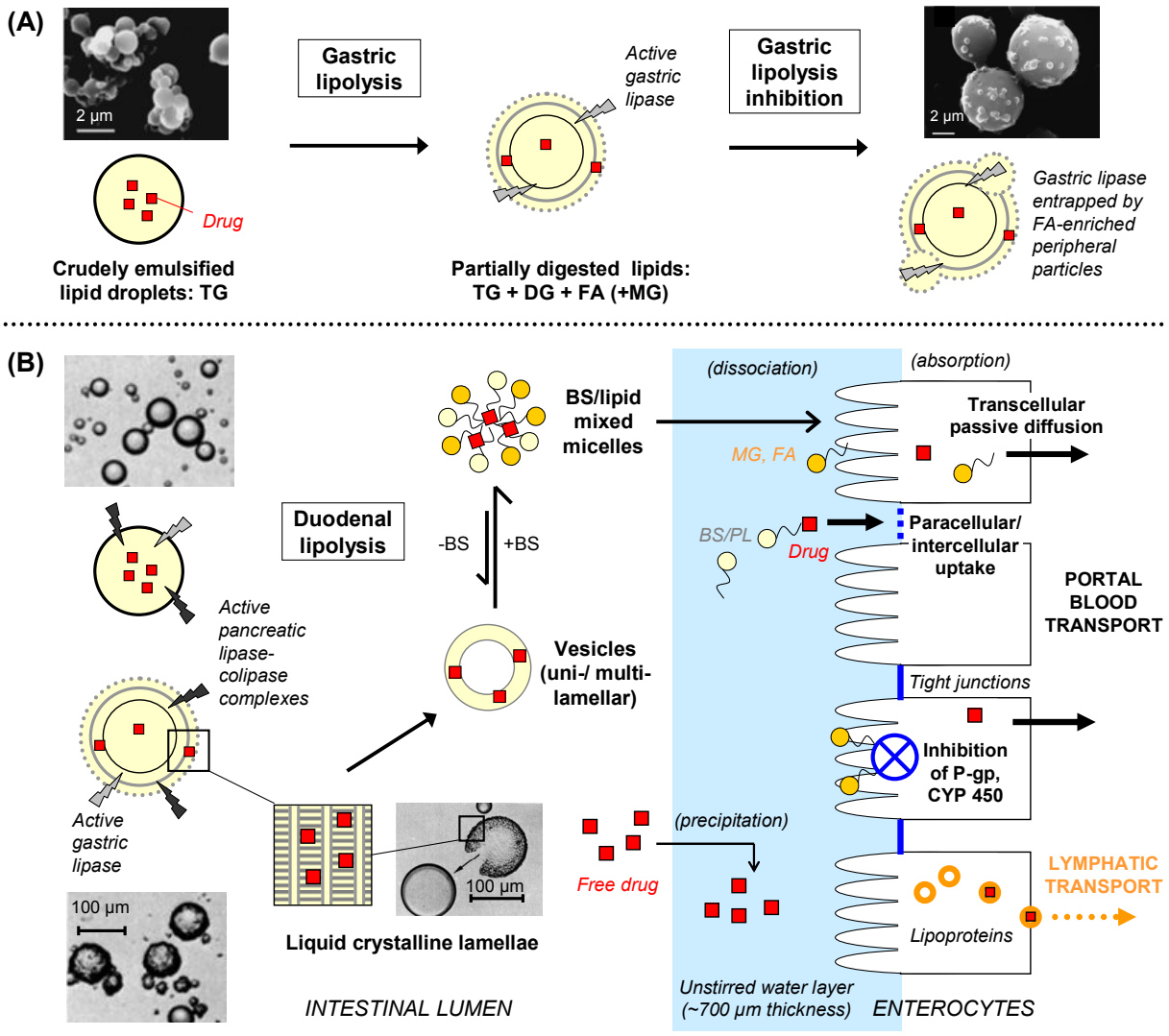


Figure 1.2 Schematic diagram (not to scale) illustrating the gastrointestinal digestion of triglyceride substrates and the possible fate of the encapsulated drug molecules: (A) Gastric lipolysis catalysed by gastric lipase, which is gradually inhibited by the accumulated digestion products (mainly DG and FA) at the lipid droplet surfaces (scanning electron micrographs adapted from [81]), and (B) duodenal lipolysis of lipid droplets emptied from the stomach catalysed by pancreatic lipase-colipase complexes, which results in the formation of a highly solubilising lipophilic microenvironment (comprised of liquid crystalline phase on the surface of the degrading oil droplet, vesicular and mixed micellar phases in the solutions) that subsequently facilitates drug trafficking/ transport across the unstirred water layer and enterocytes (photomicrographs adapted from [82, 88]).

least 7–10 g) are required to induce a significant post-prandial effect (including a change in the GI motility) [3, 85]. Although it has been reported that fat can produce a 30–60 min extension to the typical human intestinal transit time of 3.5–4.5 h, such effect may be considered modest and therefore this mechanism may not be easily exploitable by using lipid excipients that do not provide a specific GI retention effect [18, 89].

1.3.2.2 Intestinal processing of lipids

Physiological aspects: Upon entry into the duodenum, the partially digested lipid substrates (*i.e.* a mixture of TG, DG, MG and FA) stimulate the secretion of bile salts (BS) and biliary-derived lipids (PL and cholesterol) from the gall-bladder, as well as the release of lipase enzymes from the pancreas. The endogenous BS/PL components further reduce the droplet sizes and enhance the colloidal stability of the lipid droplets [60, 71, 90]. Pancreatic lipase, usually secreted in a 100-fold excess than necessary for lipid digestion, plays a major role in completing the hydrolysis of insoluble lipid substrates in a basic environment [76]. The full catalytic activity of pancreatic lipase is enhanced by the ion pairing of the cofactor, colipase, to its C-terminal domain in a 1:1 molar ratio [60, 76]. Colipase provides a strong linkage for pancreatic lipase to act at the substrate interface unaffected by the desorption effect of BS. For each TG molecule hydrolysed, one molecule of 2-MG and two molecules of FA (with traces of 1-MG and glycerols) are produced [76]. A complete intestinal absorption of fat typically occurs at 66% FA release from the glyceride substrates [79, 91].

The digestion of LCT is more efficient in the duodenum than in the stomach due to the presence of the endogenous BS/PL components which readily remove the lipolysis products from the droplet surfaces [71]. The process of surface removal of the lipolysis products and the subsequent formation of various colloidal species during duodenal lipolysis has been well described [82, 88, 92, 93]. As illustrated in **Figure 1.2B**, lipolysis of TG and DG produces the less hydrophobic lipid monomers (FA and MG) which accumulate at the droplet surface as the liquid crystalline structures (this is microscopically evidenced by the 'crusty' surface of the digesting droplets). In the presence of BS and PL, these relatively large crystalline structures (with hydrodynamic radii of >500 nm) subsequently dissociate from the droplet surface and form liquid crystalline vesicles (*i.e.* liposomes with hydrodynamic radii of 20–60 nm), which rapidly re-organise to form lipid-BS/PL mixed micelles in the presence of high BS/PL concentrations. The hydrodynamic radii of the

mixed micelles generated are highly dependent on the acyl chain length of the lipid monomers: the endogenous simple BS/PL micelles (typically 3–3.5 nm) can 'swell' to form relatively larger mixed micelles (reportedly >4 nm) in the presence of long-chain FA and MG molecules in comparison with the medium-chain products (<4 nm) [61].

Impact on drug delivery: As digestion of the parent lipid droplets progresses, the liquid crystalline lamellae and the colloidal species formed (*i.e.* vesicles and mixed micelles) serve as the key lipophilic reservoirs to keep the drug molecules solubilised in the bulk aqueous phase, until they are trafficked across the major hydrophilic barrier, *i.e.* the unstirred water layer (UWL), and ultimately reach the intestinal absorptive cells (**Figure 1.2B**). In healthy humans, the average BS level in the fasted state duodenal content is 4–6 mM; this can rise to 10–20 mM after lipid ingestion, and hence favouring the formation of highly solubilising mixed micelles [80, 92]. As the mixed micelles approach the pre-epithelial site, the relatively lipophilic FA and MG monomers (and presumably the drug molecules) are expected to dissociate from the micellar cores and diffuse into the intermicellar environment prior to absorption; such dissociation has been suggested to be triggered by a microclimate of lower pH associated with the intestinal brush border membrane [94].

Solubilisation in BS micelles has been found to increase the aqueous concentration of FA and MG 100- to 1000-fold; such saturation state in turn creates a concentration gradient that drives diffusion of the micellar structures across the UWL, and induces intestinal uptake of the lipolysis products [60]. Such solubilisation and intestinal uptake effect is anticipated to occur for drug molecules possessing properties similar to FA and MG monomers. Some previous work has reported that micellar solubilisation by endogenous BS and BS/PL is able to provide a 2- to 172-fold of solubilisation enhancement in aqueous media for drugs of intermediate lipophilicity (log P = 2 to 4) [95, 96]. Kossena *et al.* further expand the knowledge regarding solubilisation enhancement provided by the intermediate digestion phases in the presence of both endogenous and exogenous (formulation) lipids for drugs possessing low to high lipophilicity (log P = 1.4 to 5.2) [97]. They reported a solubility enhancement ranging from 10- to 2000-fold and 10- to 30,000-fold afforded by different liquid crystal phases (*i.e.* lamellar and cubic forms, respectively). Therefore, it is evident that the lipolysis products of exogenous lipids can further enhance the intrinsic solubilisation capacity provided by the endogenous BS/PL systems.

1.3.2.3 Intestinal uptake

Physiological aspects: The apical brush border membrane of the enterocytes is packed with microvilli (~100 nm), where the spaces between the microvilli range from 5–20 nm [5]. The absorption of lipolysis products has long been thought to be a concentration gradient-driven process (contributed by the saturation of BS micelles at the pre-epithelial phase), in which the FA and MG monomers are absorbed predominantly via passive diffusion across the enterocytes [60]. While a full mechanism has yet to be elucidated, there are proposals that the intestinal uptake of dietary lipids and lipophilic vitamins can possibly be facilitated and regulated by specific membrane bound carrier proteins (particularly the large family of FA binding proteins, FABP), which can be found in the tight junctions and crypt, as well as in the intestinal brush border membrane [98-101]. Such discovery of new transporter proteins is crucial in offering a new insight into the absorption pathway for lipophilic xenobiotics. Besides the uptake transporter systems, also embedded in the apical membrane are the multidrug efflux proteins (particularly P-glycoprotein, P-gp) and the metabolising enzymes (primarily the cytochrome P450 3A enzymes, CYP3A). Both P-gp and CYP3A act concomitantly to control the uptake and elimination of a wide range of lipophilic and amphiphatic xenobiotics [102, 103].

Impact on drug delivery: The transport and absorption of drug molecules across the intestinal wall often involve more complicated processes other than simple passive diffusion as in the case of dietary lipids, mainly due to the presence of various protective mechanisms in the enterocytes, such as small passage gaps, as well as the presence of P-gp efflux pump and the CYP3A metabolising enzymes (**Figure 1.2B**). In this regard, lipid-based carriers potentially act as 'permeability enhancers' to enhance the intestinal absorption of lipophilic drugs that are vulnerable to these protective barriers. The presence of BS, endogenous and exogenous lipolysis products has been shown to facilitate the intestinal uptake of lipophilic drug molecules via several mechanisms. Based on previous *in vitro* investigations using excised intestinal segments and Caco-2 cell lines [62, 104-106], it has been suggested that the digested lipids and BS components can enhance drug permeation by (*i*) increasing the intestinal membrane fluidity (thereby enhancing transcellular transport), or by (*ii*) disrupting the integrity of the tight junctions (thus facilitating paracellular or intercellular uptake) [107]. These events are mostly dose- and time-dependent, and such dietary-induced damage has known to be reversible in most cases [106]. In addition to this, there have been considerable reports suggesting the inhibitory effects of various lipid and surfactant

excipients on the intestinal P-gp efflux and CYP3A metabolising systems, which eventually leads to an enhanced drug bioavailability [22]. For example, vitamin E TPGS [108-110], Peocol (*i.e.* MG, DG FA) and Gelucire 44/14 (*i.e.* PEG, TG, DG) [111], sodium taurocholate (NaTC) [112], 1-monopalmitin (*i.e.* monoglycerides) [113] and the non-ionic surfactant Cremophor EL (*i.e.* polyoxyethylene-glycoltriricinoleate) [114], have been shown to down regulate the expression of P-gp. While there is an overlap in the substrate specificities and tissue distribution of CYP3A and P-gp [102], other lipid excipients that have been specifically identified as potent CYP3A inhibitors include micellar solutions and oleic acid [115].

1.3.2.4 Route of absorption

Physiological aspects: Following uptake into the enterocytes, the lipolysis products can either (*i*) diffuse directly across the cells and enter the systemic circulation via the portal blood (which drains through the hepatocytes), or (*ii*) undergo intracellular uptake in which the lipid monomers are re-synthesised into TG molecules and incorporated into the lipoprotein cores within the endoplasmic reticulum (ER), prior to secretion into the mesenteric lymph (**Figure 1.2B**) [3]. The mesenteric lymph drains into the systemic circulation at the junction of the left subclavian and internal jugular veins, therefore bypassing the liver and avoiding the first-pass metabolism [5]. Given that the flow rate of the portal blood is ~500 times higher than the mesenteric lymph flow, compounds of low molecular mass are anticipated to have greater access to the portal blood [75]. However, the vascular endothelium, featured by tight inter-endothelial junctions and a continuous basal lamina, has offered limited permeability to molecules with high molecular mass (>10,000 Da) and high hydrophilicity [116]. As such, transport of macromolecules or large colloidal structures is usually assisted by the lymph lipoproteins through the paracellular route [3, 75, 116]. Typically, lipids with <12-carbon acyl chains (*i.e.* SCT and MCT digestion products) are prone to portal blood transport, whereas lipids with >12-carbon acyl chains (*i.e.* LCT digestion products) are more likely to associate with the lipoproteins and undergo lymphatic transport [117, 118]. However, there is no absolute relationship between the lipid chain length and the transport pathway, and both portal blood and lymphatic transport may co-exist for a given lipid [117-119].

Impact on drug delivery: The intestinal lymphatic system is a high capacity drug transport pathway which potentially enhances the systemic exposure of lipophilic drugs by minimising the first-pass elimination effect. This delivery route is also beneficial for targeted lymphatic delivery of some anti-viral drugs, immuno-modulatory and anti-cancer drugs, since it is the primary route for the spread of some viruses and metastatic spread of some solid tumours [75]. Recently, research into the area of lymphatic drug transport has become increasingly important and a number of excellent reviews have been presented on this topic [5, 74, 75, 116, 120]. The precise mechanisms by which the lipophilic drugs gain access into the lymphatic system are still under investigation. Currently, it is believed that drug molecules are solubilised in the lipophilic core of lipoproteins (within the enterocytes) and subsequently secreted into the lymph. Fundamentally, there are two different types of lipoproteins generated within the enterocytes: the very-low-density lipoproteins which are generated under fasting conditions and thus, have very limited drug carrying capacity; in the fed state, the TG-enriched chylomicrons predominate and this lipoprotein type is the major drug transporter in the lymph [5].

The propensity for a drug compound to undergo lymphatic transport can be predicted partly from its partition coefficient (*i.e.* the log *P* values), and partly from the solubility of the drug in LCT, because this mostly reflects the drug solubility in the lipid core of chylomicrons [5, 74, 75, 116, 120]. As a general guide, lipophilic molecules with a log *P* of ≥5, a solubility in LCT of ≥50 mg/g, and are metabolically stable (in the intestinal lumen and within the enterocytes) can be considered a good candidate for lymphatic transport [75, 121]. Some successful examples of using lipid-based delivery system to increase the bioavailability of lipophilic drugs via lymphatic transport include DDT or 1,1-bis(*p*-chlorophenyl)-2,2,2-trichlorethane [122], halofantrine [123], and retinoids [124]. According to previous reviews [75, 116], a lipid-based carrier system can be engineered to achieve effective lymphatic transport by considering the following formulation criteria: (*i*) lipids with acyl chain length of 14 or greater have considerably high extent of lymphatic transport (*i.e.* approximately 40–60% of the lipid dose) compared to lipids with shorter acyl chain lengths; (*ii*) unsaturated lipids are better triggers of lymphatic transport and readily form relatively larger-sized chylomicrons favoring drug solubilisation in comparison with saturated lipids (but it is noted that unsaturated FA-containing lipids are prone to oxidation [4, 5]); and (*iii*) phospholipids (particularly phosphatidylcholine and its digestion product, lyso-phosphatidylcholine) are potential enhancers of lymphatic drug transport.

In addition to exploiting the lymphotropic potential of the commercially available LCT excipients, there has been considerable research focusing on the synthesis of structured triglycerides (STG) [75, 125, 126] and lipid-based prodrugs [127, 128], which take advantage of the different metabolic fate of the incorporated FA chains to quantitatively control portal and lymphatic drug transport. For example, formulation of halofantrine in STG combining medium (M) and long (L) chain FAs at different proportions (**Figure 1.3**) has resulted in an increased portal absorption (7–12%) and total bioavailability (>23%) of halofantrine in comparison with the long-chain sunflower oil formulation (*i.e.* 5.5% portal absorption and 19% total bioavailability) [127]. The level of lymphatic transport was equivalent between the STG and sunflower oil formulations (*i.e.* 12–15% of the administered dose). In the lipid-based prodrug approach, the drug molecules are covalently linked to selected lipid moieties (such as phospholipids) and this has been shown to enhance the lymphatic uptake of an anti-tumour drug, fluorouridine [125].

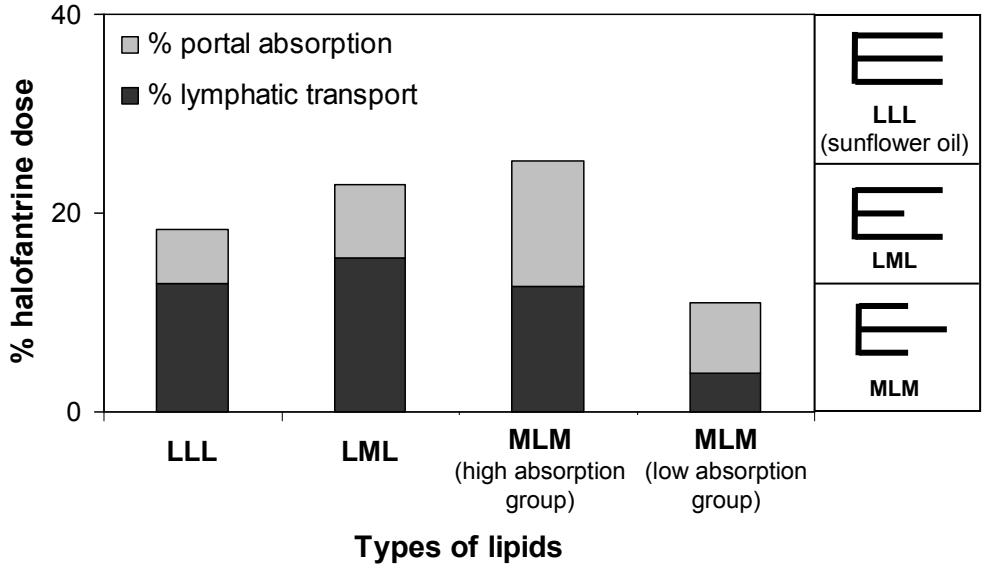


Figure 1.3 Percentage dose of halofantrine absorbed via the lymphatic and portal route resulted from various structured triglycerides in conscious rats. In the schematic presentation of the structured triglycerides, M represents a medium-chain fatty acid group and L represents a long-chain fatty acid group [127].

1.3.3 Types of lipid-based formulations and factors affecting drug absorption

The bioavailability-enhancing effect promoted by co-administration with a fat-rich meal has been demonstrated for a substantial number of lipophilic drugs, such as isotretinoin [129], halofantrine [130] and danazol [89]. Some potential mechanisms by which lipid-based vehicles enhance the oral absorption of poorly water-soluble drugs have been described in the previous sections: (*i*) stable presentation of drugs in the molecularly dispersed/ dissolved state, (*ii*) slowing down of gastric emptying/ increased GI residence time, (*iii*) improved intestinal solubilising capacity due to the formation of lipophilic microenvironments resulting from lipolysis, (*iv*) down regulation of the intestinal efflux and metabolism functions, and (*v*) recruitment of the intestinal lymphatic transport which minimises the first-pass metabolism effect.

Lipid-based formulations is a broad class encompassing a wide range of delivery systems consisting not only of lipids, but also including the use of other formulation technologies such as surfactants (which can be hydrophilic or lipophilic) and cosolvents, drug micronisation, salt formation and prodrugs. The current reviews on oral lipid-based formulations identify two main periods. From 1960's up to 1980's, lipid-based formulations have been limited to single-component lipid solutions/ suspensions or oil-in-water (o/w) macroemulsions; these are summarised in the review by Armstrong and James [131]. The more advanced lipid dosage forms developed in the subsequent 30 years include microemulsions, dry emulsions, solid lipid particles/ dispersions, micellar systems, liquid crystalline phase carriers, liposomes and various types of self-emulsifying systems. In the last decade, a substantial number of reviews [2, 3, 8, 17, 54, 74, 77, 132-136], including some textbooks [5, 137], have been devoted to these formulations, while only few [60, 138] have been published in the 1990's. Recently, Mullertz's research group has also conducted an excellent review on published clinical (pharmacokinetic) studies of orally administered lipid-based formulations in humans [139].

In this section, the bioavailability assessment of the various types of lipid-based formulations is discussed. Case examples have been selected from the literature to specifically illustrate the formulation factors that potentially influence the absorption of drugs delivered using lipid-based vehicles. An important concept behind the development of each type of lipid-based carrier is to mimic the food effect or to imitate the intermediate phases of dietary lipid digestion for creating a sufficient lipophilic microenvironment for

drug trafficking and absorption along the GI tract, where the rate and extent of drug release can be modified by using various coating and polymer technologies. Interested readers are encouraged to consult the original review articles for more comprehensive knowledge regarding each type of lipid-based formulation [2, 3, 5, 8, 17, 54, 74, 77, 132-137].

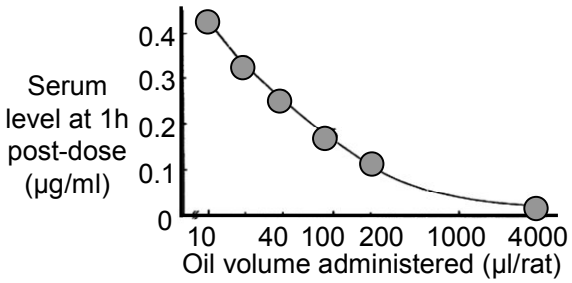
1.3.3.1 Lipid solutions and suspensions

Lipid solutions are oily vehicles which contain the drug substance in a fully solubilised form, whereas lipid suspensions are two-phase systems in which the finely divided drug substance is dispersed in the lipid matrix [4, 5]. Both lipid solutions and suspensions are relatively simple approaches that have been shown to enhance the oral bioavailability of many lipophilic drugs prior to the development of other lipid-based systems (**Table 1.5**). For example, **cinnarizine** when administered as a lipid (oleic acid) solution, has been reported to produce approximately 3- and 4-fold higher maximum plasma concentration (C_{max}) and bioavailability (BA), respectively, in comparison with the conventional tablet formulation [140].

While in some cases, simple lipid solutions/ suspensions do not seem to produce significant bioavailability-enhancing effects, the concomitant use of other formulation technology may improve the performance of the lipid vehicles. One compelling example is the absorption study of **progesterone** conducted by Hargrove *et al.* [141], in which they demonstrated that for micronised progesterone administered as a lipid suspension, the C_{max} produced (30.3 ± 7.0 ng/ml) is significantly higher than that of other formulations, including a lipid suspension containing plain-milled progesterone (11.3 ± 3.0 ng/ml), micronised progesterone powder (13.2 ± 2.4 ng/ml), followed by plain-milled powder (9.6 ± 2.5 ng/ml). Apparently, the particle size of the dispersed drug is an important factor in governing the extent of drug absorption in addition to the lipid solubilising effect.

The application of lipid-based vehicles is somewhat limited for poorly water-soluble drugs that require high therapeutic doses and do not possess sufficiently high solubility in lipids. In such case, the prodrug approach (*i.e.* chemical modification) can be used to modify the solubility profile of the drug substance. As an example, **phenytoin** is a high-melting, weakly acidic drug that is poorly soluble in the GI fluids (especially in the gastric fluid) as well as in lipids (e.g. 3.9 mg/ml in tributyrin, a SCT). In an attempt to efficiently deliver phenytoin by using

Table 1.5 Examples of studies examining the oral bioavailability of poorly water-soluble drugs from lipid solutions or suspensions

Compound	Study subject, route of administration	Rank order of bioavailability (BA) or peak plasma level (C_{max}) ($p < 0.05$)	Ref.
Factor: Lipid (post-prandial) effect			
Cinnarizine	Beagle dogs, oral	BA: oleic acid solution (hard capsule) (4-fold) > standard tablet C_{max}: oleic acid solution (3-fold) > standard tablet	[140]
Progesterone	Humans, oral	C_{max}: micronized powder suspended in long-chain FA (2- to 3-fold) > plain-milled powder suspended in long-chain FA ≈ micronized powder > plain-milled powder	[141]
Phenytoin lipophilic prodrug	Rats, intragastric	BA: 3-pentanoyloxymethyl prodrug in SCT solution (4-fold) > phenytoin sodium aqueous solution	[142]
Factor: Lipid digestibility (digestible vs. non-digestible lipids)			
SL-512	Rats, oral	C_{max}: digestible MCT solution (4-fold) > non-digestible MBLA solution	[143]
Factor: Lipid dose			
SL-512	Rats, intraduodenal		[143]
Factor: Lipid acyl chain length (i.e. lipid digestibility vs. lymphotropic potential)			
Probucol	Rats, oral	BA: LCT solution > MCT solution > liquid paraffin (non-digestible)	[144]
Vitamin E	Rats, oral	BA: MCT solution > LCT solution	[145]
Griseofulvin	Rats, intragastric	BA: MCT suspension > LCT suspension > SCT suspension	[104]

lipid solutions, Stella's research group [142] has synthesised a series of low-melting, lipophilic phenytoin prodrugs, *i.e.* the 3-acyloxymethyl derivatives, with significantly improved lipid solubility (*i.e.* >100 mg/ml in tributyrin); the lipid solubility of the prodrugs

increases with lower melting points. In comparison with the salt form (*i.e.* phenytoin sodium) administered as an aqueous solution, the lipid solutions of the prodrugs produced a superior bioavailability in rats. The authors proposed that the SCT digestion products have effectively created a highly solubilising micellar phase for the prodrugs until they were trafficked across the GI membrane, where the promoeity was rapidly cleaved by the tissue and plasma esterases prior to releasing the active parent drug. Phenytoin sodium was likely to reconvert into the poorly soluble acid form in the acidic gastric fluid and therefore was liable to precipitation before reaching the intestinal absorptive site.

As summarised in **Table 1.5**, the absorption performance of a lipid-based vehicle is a function of its digestibility, acyl chain length, dose and state of emulsification (which is discussed in the next section). It is well recognised that the release and absorption of drugs is not simply governed by the dispersion/ miscibility of the lipid phase with the GI aqueous medium, but also by endogenous processing of the lipid vehicles that changes the intraluminal solubilisation capacity [60]. The effect of digestible (*i.e.* polar) versus non-digestible (*i.e.* non-polar) lipids on drug absorption has been extensively studied. For the lipophilic compound **SL-512** (*i.e.* 1-cyclopropyl-4-phenyl-6-chlor-2(1H)-quinazolmone), the bioavailability in intact rats was greatly enhanced (4-fold higher) when formulated with the digestible MCT solution, in comparison with the synthetic, non-digestible N-α-methylbenzyllinoleamide (MBLA) solution, although SL-512 has shown similar partition coefficients in both lipids [143]. Further bioavailability assessment in bile-duct ligated rats (*i.e.* with inhibited bile and pancreatic secretions) has revealed a decreased absorption from the MCT solution but an unchanged absorption from the MBLA solution; this confirms the importance of lipid digestion (*i.e.* micellar solubilisation) in enhancing the absorption of SL-512. The rate of SL-512 absorption from the MCT solution was found to decrease with increasing lipid doses (as shown by the plot in **Table 1.5**). The authors suggested that the exponential decrease in the serum SL-512 levels was mainly related to a slower digestion as the MCT dose was increased; it is important to note that as the oil formulations were administered intraduodenally, the effect of lipid dose on gastric emptying rate was irrelevant.

The lipid acyl chain length is an important factor governing the absorption of drugs from a lipid-based vehicle because this is closely related to the digestibility and lymphotropic potential of the formulation. In general, the overall rate of digestion is the highest for short-chain lipids, followed by medium-chain and long-chain lipids; long-chain lipids are likely to

trigger the lymphatic transport of lipophilic drugs (**Section 1.3.2**). In the case of highly lipophilic drugs such as **probucol**, administration as a LCT solution (arachis oil) has produced better bioavailability over a MCT solution (Miglyol oil), and the absorption was found negligible from the non-digestible liquid paraffin oil [144]. The authors have found significantly higher recoveries of probucol in the fistulated lymphs for the LCT solution treated rats as compared to the groups treated with MCT and liquid paraffin oil, thereby confirming the trigger of lymphatic drug transport when delivered using LCT, which is the main reason for an improved bioavailability.

Medium-chain lipids may play a better role than long-chain lipids particularly for drugs exhibiting lower lipophilicity (e.g. griseofulvin), as well as for compounds that are known to be absorbed exclusively via the lymphatic route even without a trigger by exogenous lipids, such as vitamin E. A study on the absorption of **vitamin E** has reported a greater bioavailability from a MCT (trioctanoin) solution than a LCT (triolein) solution [145]. This finding essentially shows that the absorption of vitamin E is greatly promoted by an enhanced dispersion and solubilisation in the GI lumen due to a faster digestion rate and more efficient micelle formation in the case of MCT; whereas the presence of LCT did not further enhance the drug transport through the lymphatic route. In the case of **griseofulvin**, the bioavailability was greatly enhanced following an oral administration as a MCT (Captex 355) suspension in comparison with a LCT (peanut oil) and a SCT (triacetin) suspension [104]. The improved absorption from the MCT vehicle is presumably attributed to the enhanced micelle formation resulted from a relatively faster digestion of MCT, as well as a higher rate of gastric emptying as compared to LCT. The lowest bioavailability produced by the SCT vehicle was most probably resulted from the rapid dissipation of SCT, which subsequently caused massive drug precipitation upon exposure to the aqueous surrounding due to the lack of solubilising capacity (*i.e.* less 'swelling' effect) produced by the SCT digestion products.

It is noted that in addition to the case examples given here, there are also other studies showing insignificant differences in the drug bioavailability resulting from lipids of various acyl chain length (such as seocalcitol and dexamethasone) [8]. Therefore, the effect of lipid acyl chain length on the oral bioavailability is believed to be drug-specific, depending on the physicochemical properties of the drug compounds.

1.3.3.2 Lipid emulsions/ microemulsions

The presence of lipid vehicles in the emulsified form has proved to be effective in increasing the rate of drug release and/or the rate of lipid digestion via an increased total surface area [60]. Microemulsions used for oral delivery of lipophilic drugs are thermodynamically stable, isotropically clear dispersions of oil droplets stabilised by surfactants/ emulsifiers or co-surfactants in an aqueous continuous phase [5, 146, 147]. While ingested lipid solutions/ suspensions are capable of forming crude emulsions contributed by the gastric movements and the endogenous amphiphiles, microemulsions are less affected by these mechanisms and their droplet sizes can range from a few nanometers up to a few hundred nanometers [5, 146, 147].

Table 1.6 shows some examples of studies examining the effect of lipid dispersion on the absorption of drugs. It was clearly demonstrated for **phenytoin** that emulsification of the LCT (corn oil) vehicle led to a higher bioavailability when compared to the non-emulsified LCT suspension; and both LCT emulsions and suspensions produced a ~50% and ~90% higher C_{max} as compared to the aqueous suspension [87].

In another study on the cytotoxic agent **penclomedine**, Myers and Stella [148] have systematically examined the effect of lipid acyl chain length, lipid digestibility and lipid emulsification on drug absorption in rats. It was evident that the MCT (trioctanoin) emulsion produced the highest systemic exposure of penclomedine in comparison with two LCT (soybean and triolein) emulsions, followed by a non-digestible mineral oil emulsion and a SCT (tributyrin) emulsion; all of which were superior to the aqueous suspension. The rationale behind this finding was that the release of penclomedine from the lipid vehicles was governed by both lipid vehicle digestion and drug diffusion. Whilst digestion of the MCT emulsion rapidly generated a highly solubilising micellar phase for penclomedine, both LCT emulsions were not completely digested, hence leading to an incomplete absorption; the low bioavailability resulting from the SCT emulsion was plausibly a result of drug precipitation where the drug molecules have to be redissolved in the GI fluids prior to absorption. Another insight given by this study was that lipid emulsification plays an important role in enhancing drug absorption from both digestible and non-digestible oils, via an increased surface area for vehicle lipolysis and drug diffusional release, respectively.

Table 1.6 Examples of studies examining the oral bioavailability of poorly water-soluble drugs from liquid and dry emulsions

Compound	Study subject, route of administration	Rank order of bioavailability (BA) or peak plasma level (C_{max}) ($p < 0.05$)	Ref.
Liquid emulsions			
Phenytoin	Rats, intragastric	BA and C_{max}: LCT emulsion > LCT suspension > aqueous suspension	[87]
Penclomedine	Rats, intraduodenal	BA: MCT emulsion > LCT emulsion > mineral oil emulsion > SCT emulsion > aqueous suspension	[148]
		BA: MCT emulsion > mineral oil emulsion ≈ MCT-lecithin mixture ≈ mineral oil-lecithin mixture	
		C_{max}: MCT emulsion > mineral oil emulsion > MCT-lecithin mixture ≈ mineral oil-lecithin mixture	
REV 5901	Rats, oral	BA: PEG 400 solution ≈ polysorbate 80 solution > LCT solution > aqueous suspension	[149]
Ibuprofen	Rats, intragastric	BA: MCT microemulsion ≈ PEG200/ethanol/water solution > aqueous suspension	[150]
Dry emulsions			
Vitamin E acetate	Beagle dogs, oral	BA and C_{max}: Dry emulsion ≈ liquid emulsion ≈ drug-oil mixture (all in hard gelatin capsules)	[151]
5-PDTT	Rabbits, intragastric	BA: Dry emulsion (redispersed) > β-cyclodextrin solution	[152]
Amlodipine	Rats, intragastric	BA: Dry emulsion (redispersed) ≈ amlodipine besylate (Norvasc® tablet) > aqueous suspension	[153]

Furthermore, bioavailability enhancement afforded by improved lipid dispersion was also demonstrated for the lipoxygenase inhibitor **REV 5901**, which has an appreciably high solubility in TG lipids (*i.e.* >100 mg/g), and water-miscible solvents and surfactants (*i.e.* 250 mg/g in both PEG 400 and polysorbate 80) [149]. Administration of REV 5901 as a PEG 400 and a polysorbate 80 solution has produced approximately 2-fold higher bioavailability than an aqueous suspension, and 30–50% higher than a LCT (peanut oil) solution. It was reported that both PEG 400 and polysorbate 80 are readily redispersed in

the aqueous medium to form fine oily globules, hence facilitating the dispersion of drug molecules. As for the LCT solution, the drug absorption was plausibly limited by the incomplete digestion or insufficient dispersion of the administered volume of oil. It is noteworthy that there are also studies reporting insignificant differences between lipid emulsions and organic solvent-based solutions in enhancing the bioavailability of some lipophilic drugs, such as that demonstrated for ibuprofen [150].

1.3.3.3 Dry emulsions

The transformation of liquid emulsions into the solid state by spray-drying has been described in the early 1960's; this was followed by the technique of lyophilisation and more recently, by solvent evaporation [152, 154-156]. The use of dry emulsions as a solid dosage form has gained much popularity due to the elimination of some shortcomings associated with liquid emulsions, such as physical instability, relatively large volume of administration as compared to solid dosage forms, strict storage conditions (e.g. refrigeration required), dosage precision problem, possible patient incompliance, and difficulty in sterilisation [60, 152, 153, 157]. Additionally, dry emulsions have also found great applications in improving the storage stability of lipophilic substances that are photosensitive, volatile and susceptible to oxidation [153, 155, 156]. The light-protective effect of dry emulsions has been demonstrated for the photosensitive drug amlodipine; Jang *et al.* [153] have specifically assessed the photostability of amlodipine in various systems for 24 h. They identified a four to ten-fold lower rate and extent of drug degradation for a dextrin-based dry emulsion as compared to that formulated as an ethanol solution and the pure powder. When combined with the photostability results obtained by Ragno *et al.* for other systems, such as liposomes, tablets, cyclodextrins and polymer microspheres [158], the potential of dry emulsions in prolonging the shelf-life of an emulsion product is clearly exalted (**Figure 1.4**).

The formation of dry emulsion can be achieved by incorporating a solid carrier into an emulsion system to encapsulate the lipids when the water phase is removed by one of the aforementioned methods. Water-soluble solid carriers were more extensively used in previous work; some examples are gelatin, glycine, sugars (lactose, sucrose, trehalose, maltodextrin and mannitol), povidone and HPMC [152-154, 156, 159, 160]. On the other

hand, water-insoluble carriers such as magnesium alumino metasilicate, Neusilin (US2 or UFL2) and colloidal silica have also been employed [151, 154, 161].

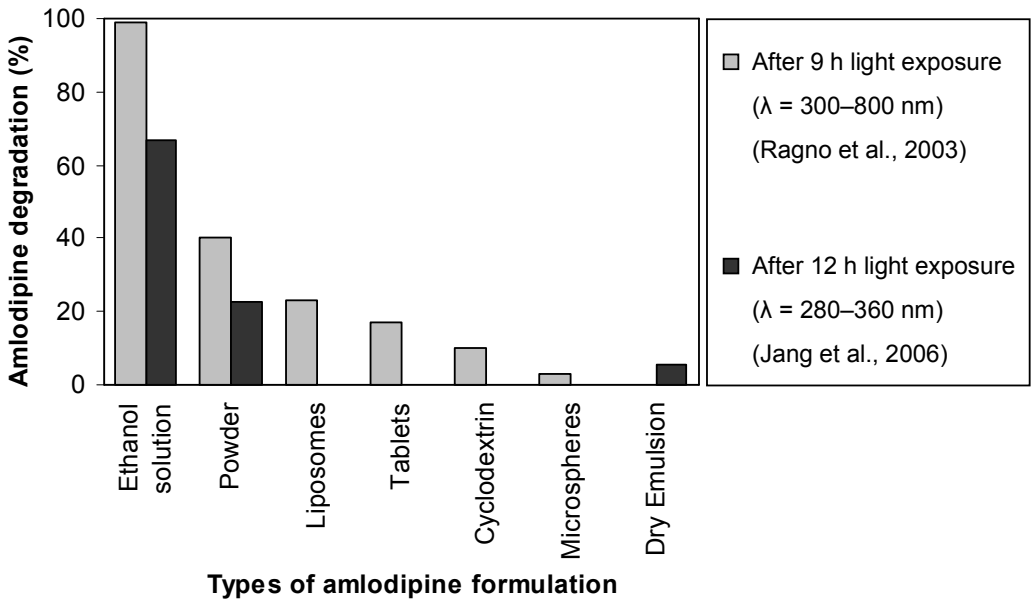


Figure 1.4 Percentage photodegradation of amlodipine formulated in various pharmaceutical carrier systems [153, 158]

Smaller-sized insoluble carriers are always preferred over the larger-sized carriers in order to achieve reasonably high powder yield and lipid encapsulation efficiency. Hansen *et al.* [154] demonstrated that Neusilin US2 with larger particle sizes, *i.e.* D(*v*,0.5)[f] of 112 μm, yielded significantly lower powder mass and encapsulation efficiency (*i.e.* >20% lower) than the small-sized carrier Neusilin UFL2, which has a D(*v*,0.5) value of 3 μm. The substantial loss of lipids is ascribable to two reasons: firstly, the large solid carrier was not associated with the lipid droplets effectively, thus causing adherence of the lipid components to the surfaces of the spray-drying chamber; and secondly, Neusilin US2 tends to promote deposition of lipids in the drying chamber due to its high moisture content and high diffusion path during the spray-drying process. However, larger particles may be favourable in producing powders with better flowability (*i.e.* less cohesive) and increased mechanical strength when formulated into tablet forms.

Ideally, a dry emulsion formulation is expected to readily redisperse in an aqueous medium to reform the original emulsions. However, most studies have reported an increment of

[f] D(*v*,0.5) is the median volumetric diameters, *i.e.* the volume (*v*) diameters at 50% cumulative volume

17–40% in the median droplet sizes for the reconstituted dry emulsions in comparison with the original liquid emulsions. This is possibly due to coalescence of droplets during the process of water removal or reconstitution in water [152, 153, 159], or precipitation of the insoluble carrier particles [159]. Even so, other important properties including the droplet surface structure (*i.e.* well separated spherical particles), homogeneity, *in vitro* drug release kinetics and *in vivo* bioavailability of the encapsulated drugs have been successfully retained before and after the liquid-solid state transformation. Many studies have also evidenced excellent preservation of the reconstituted droplet sizes for at least 6 months when stored at room temperature and elevated temperature (*i.e.* 40 °C) [153, 156, 157]. It is believed that recovery of the original droplet sizes is not necessary for orally ingested emulsions because the human fat digestive system is capable of enhancing lipid dispersion in the GI fluids [154, 159]. Previously, Armand *et al.* [90] have assessed the effect of droplet sizes on the digestion and absorption of fat emulsions in healthy humans. They analysed the content of the gastric and duodenal aspirates over a 4 h period and detected a higher total percentage of lipolysis for the fine emulsion (0.7 μm) compared to a coarse emulsion (10 μm). However, the overall fat absorption over a 7 h period was insignificantly different between the two emulsions. The authors concluded that lipolysis mediated by the pancreatic lipase is unlikely to be affected by droplet sizes in the range of 0.7–10 μm because of the high efficiency of the digesting enzymes. Therefore, it is possible that a difference in the extent of drug absorption is not detectable between lipid solutions and emulsions of this size range at a fixed dose and same type of lipids. More importantly, most of the lipid-based systems have proved to be effective in increasing the drug bioavailability when compared to the pure drugs or conventional tablet formulations.

Some examples of lipophilic drugs that have been formulated as dry emulsions are listed in **Table 1.6**. In the study of Takeuchi *et al.* [151], the absorption characteristics of **vitamin E acetate** was found to be equivalent for a silica-adsorbed dry emulsion, an o/w emulsion, and a drug-oil mixture, although there was a slight trend showing the bioavailability and C_{max} in the order of liquid emulsion > dry emulsion > oily mixture. The lack of significant differences observed across the different treatments was probably due to the low number of dog subject per treatment group (n = 2), and the effect of feeding each dog with 250 g of solid food prior to drug administration; this makes it difficult to draw a conclusive remark regarding the performance of the test formulations.

There are other studies reporting at least comparable or better oral bioavailability for the dry emulsion formulations as compared to the pure drugs, cyclodextrin formulations and the marketed products. As an example, for the potential anticancer agent **5-phenyl-1,2-Dithiole-3-thione (5-PDTT)**, its administration as a maltodextrin-coated dry emulsion produced approximately 3-fold higher bioavailability than a β-cyclodextrin solution [152]. For the photosensitive anti-hypertensive drug **amlodipine**, Jang *et al.* [153] reported an equivalent bioavailability between the dextrin-coated dry emulsion and the marketed salt form product, amlodipine besylate (Norvasc® tablet), both of which were significantly better than the pure drug.

1.3.3.4 Self-emulsifying drug delivery systems

Self-emulsifying drug delivery systems (SEDDS) are physically stable, isotropic mixtures of oil, surfactant/ emulsifier (and co-solvent) and drug, which spontaneously emulsify to form fine o/w emulsions on exposure to an aqueous medium (such as the GI fluids) under mild agitation [4, 54, 60, 133]. Depending on the types and concentrations of each excipient incorporated, SEDDS may redisperse to form turbid and white emulsions with the lipid droplet sizes ranging from 100–250 nm, or form transparent microemulsions containing lipid droplets of <50 nm in the average size; the latter are classified as self-microemulsifying drug delivery systems (SMEDDS) [4, 5, 54, 82]. The formulation of SEDDS/ SMEDDS has offered several advantages in comparison with lipid solutions, such as increased drug loading capacity (via the inclusion of surfactants/ cosolvents), increased rate and extent of absorption (due to rapid dispersion of the lipids to form fine droplets for drug release and enzymatic degradation), as well as improved reproducibility in the bioavailability (due to more controllable and uniform droplet sizes) [8]. Additionally, the formation of small droplets may promote rapid stomach emptying and wide dispersion throughout the GI lumen, thereby minimising local exposure of the GI mucosa to surfactants at high concentrations and reducing their irritation potential (at a specified frequency and duration of dosing) [5]. Recently, there has also been considerable work reporting successful conversion of liquid SEDDS/ SMEDDS into the solid form by incorporating a solid carrier as in the case of dry emulsions, where the *in vitro* and *in vivo* performance was well preserved as in the liquid state [162-164].

The LFCS framework proposed by Pouton [2, 8] has served as a useful guide in predicting the likely performance characteristics of various lipid-based systems: dispersion particle sizes, susceptibility to drug precipitation upon dilution, the significance of digestibility in promoting drug absorption, and the likely effect on gastric emptying (**Table 1.7**).

LFCS *Type I systems* are not capable of self-emulsifying and are the least hydrophilic lipid vehicles among the four LFCS categories. This formulation class encompasses simple oil-based solutions and suspensions, as well as o/w emulsions stabilised by a low quantity of emulsifiers (e.g. 1% polysorbate 60 and 1.2% lecithin) [8]. As shown by some case examples in the previous sections (**Section 1.3.3.1** and **Section 1.3.3.2**), these systems are highly dependent on the enzyme-mediated digestion to create a sufficiently solubilising environment in the GI lumen for facilitating drug trafficking and absorption. Some formulation factors that have been known to influence the solubilisation degree and absorption of drugs are the lipid digestibility nature, lipid acyl chain length, lipid dose, and the state of emulsification of the systems.

On the contrary, the surfactant and cosolvent-based *Type IV systems* are the most hydrophilic formulations among the four LFCS classes, and details on such formulations have already been presented in **Section 1.2.2** under the category of surfactant/ cosolvent system. In general, digestion of the formulation carrier is not required for drug release or absorption to occur. However, surfactants containing the ester functional groups (such as the commonly used ethoxylated esters) are liable to hydrolysis in the presence of the hydrolytic enzymes, which may inherently induce a profound change in the total solvent capacity in the GI lumen, in addition to the aqueous dilution effect [2].

LFCS *Type II systems* are composed of oil and lipophilic (water-insoluble) surfactants, and are typically referred to as SEDDS. According to Pouton and Porter [54], in order to achieve efficient lipid dispersion/ emulsification, the concentration of lipophilic surfactants should lie in the optimum range of 30–40%, whilst a concentration exceeding 50% will possibly cause slow emulsification due to the likely formation of viscous liquid crystalline phases at the oil-water interface. The potential of SEDDS in improving the oral bioavailability has been demonstrated for many poorly water-soluble compounds. For example, the SEDDS solution comprising of peanut oil and polyglycolysed glycerides has been shown to increase the bioavailability and C_{max} of **Ro-15-0778** (a lipophilic arotinoid)

at least 3-fold in comparison with a PEG 400 solution, a capsule formulation of wet-milled spray-dried powder, and a tablet of micronised drug [165] (**Table 1.8**).

Table 1.7 Lipid formulation classification system (LFCS) based on the types and compositions of lipid and surfactant excipients as proposed by Pouton [2]

Formulation type / Properties	Increasing hydrophilicity Increasing rate of gastric emptying →				
	Type I	**Type II**	**Type IIIA**	**Type IIIB**	**Type IV**
Description	Simple lipid solution or suspension	SEDDS	SEDDS/ SMEDDS	SMEDDS	Surfactant/ cosolvent blend
Typical compositions					
TG, mixed glycerides	100%	40–80%	40–80%	<20%	—
Surfactants	—	20–60 % (HLB[a] < 12)	20–40% (HLB[a] > 12)	20–50% (HLB[a] > 12)	0–20% (HLB[a] < 12) 30–80% (HLB[a] > 12)
Hydrophilic cosolvents	—	—	0–40 %	50–100%	0–50%
Particle size of dispersion	Coarse	100–250 nm	100–250 nm	50–100 nm	<100 nm
Significance of aqueous dispersion	Limited importance	Solvent capacity unaffected	Some loss of solvent capacity[b]	Significant phase changes and potential loss of solvent capacity[b]	Loss of solvent capacity[b]
Significance of digestibility	Crucial requirement	Not crucial but likely to occur	Not crucial but may be inhibited	Not required and not likely to occur	Not required and not likely to occur

[a] The hydrophile-lipophile balance (HLB) system is a measure of the overall hydrophilicity/ hydrophobicity of a surfactant or emulsifier; compounds with HLB < 12 is considered lipophilic, whereas those with HLB > 12 is regarded hydrophilic

[b] The loss of solvent capacity is likely to increase the risk of drug precipitation during dispersion in an aqueous medium

Table 1.8 Examples of studies examining the oral bioavailability of poorly water-soluble compounds from SEDDS and SMEDDS

Compound	Study subject, route of administration	Rank order of bioavailability (BA) or peak plasma level (C_{max}) ($p < 0.05$)	Ref.
Factor: Lipid emulsification (i.e. droplet sizes)			
Ro-15-0778	Dogs, oral	BA and C_{max}: SEDDS solution (≥3-fold) > PEG 400 solution > wet-milled spray-dried powder ≈ micronized drug	[165]
Vitamin E	Humans, oral	BA: LC-SEDDS (3-fold) > LCT solution	[166]
Cyclosporine	Humans, oral	BA and C_{max}: SMEDDS (Neoral®) > SEDDS (Sandimmune®) t_{max}: SMEDDS (Neoral®) < SEDDS (Sandimmune®)	[167, 168]
Solvent green 3	Rats, oral	BA: semi-solid SMEDDS (1.7-fold) > LCT emulsion	[169]
Factor: Lipid acyl chain length (i.e. lipid digestibility vs. lymphotropic potential)			
Danazol	Beagle dogs, oral	BA: LCT solution ≈ LC-SMEDDS > MC-SMEDDS ≈ micronized powder (all under fasting conditions) LCT solution ≈ LC-SMEDDS ≈ micronized powder > MC-SMEDDS (all under fasting conditions except for micronized powder)	[170]
Seocalcitol	Rats, oral	BA: LC-SMEDDS ≈ MC-SMEDDS ≈ LCT solution ≈ MCT solution	[171]

Whilst Takeuchi *et al.* previously reported a similar bioavailability of vitamin E between a dry emulsion, a liquid emulsion and a drug-oil mixture in a dog absorption study under non-fasting conditions [151], Julianto *et al.* have obtained a significantly higher bioavailability of **vitamin E** when administered as a SEDDS formulation (containing palm oil, Tween 80 and Span 80) compared to the marketed soft gelatin capsule (Natopherol® containing a soybean oil solution) in a human fasted state absorption study [166]. However, the higher lipid dose contained in the SEDDS formulation than that in the oil solution product has limited the validity of the conclusion regarding the pharmacokinetic benefits of the SEDDS formulations.

LFCS *Type III systems* are excellently well dispersing lipid vehicles which are capable of forming either SEDDS or SMEDDS, due to the incorporation of hydrophilic (water-soluble) surfactants and hydrophilic cosolvents to the oil systems [2, 8]. This class of formulation is susceptible to the loss of solvent capacity upon aqueous dilution; therefore the overall hydrophilicity of the system has to be balanced between the ease of lipid dispersion and the solvent capacity for the poorly water-soluble compounds [54]. The most well known example of a Type III lipid system is the marketed SMEDDS formulation of the immunosuppressant, **cyclosporine A** (Neoral®, Novartis), which is a microemulsion preconcentrate containing corn oil-derived glycerides as lipids, polyoxyl 40 hydrogenated castor oil (Cremophor RH40) as a water-soluble surfactant, propylene glycol and ethanol as cosolvents, as well as DL-α-tocopherol as an antioxidant [8]. Neoral® has outperformed the earlier marketed SEDDS formulation of cyclosporine (Sandimmun®, Novartis), which contains corn oil, a semi-synthetic polyoxyethylated glycerides (Labrafil M-2125-CS) and ethanol, in several ways: (*i*) Neoral® produces faster absorption and better bioavailability and C_{max} (independent of the bile flow) than Sandimmun® [167, 168]; (*ii*) Neoral® improves the dose linearity of cyclosporine and reduces the effect of food intake on its absorption [167, 172]; and (*iii*) Neoral® demonstrates a reduced intra- and inter-subject variability in the absorption of cyclosporine [173]. It is apparent that enhanced lipid dispersion and the rapid formation of smaller lipid droplets in the GI tract is advantageous for improving drug absorption via an increased interfacial area for more efficient lipid digestion and drug release. In another study, the lymphatic transport of a model lipophilic dye, **solvent green 3**, was enhanced by the SMEDDS formulation based on Gelucire 44/14 (*i.e.* PEG, TG, DG) in comparison with a LCT (soybean oil) emulsion, mainly via an enhanced lipid digestion as suggested by the authors [169]; it is worth noting that Gelucire 44/14 is also known to be a down regulator of the intestinal P-gp efflux system (**Section 1.3.2.3**).

The findings of Porter *et al.* [170] emphasised the influence of lipid acyl chain length on the absorption of lipophilic drugs, in which the bioavailability of **danazol** was enhanced to an equal extent by the LCT solution and LC-SMEDDS formulations, superior to that of a MC-SMEDDS. The poor absorption of the micronised powder was significantly improved by the co-administration of a fatty meal (~30 g lipid content), giving similar results as the LCT-based systems. These results clearly point out the prime importance of the post-digestion solubilisation capacity in the GI lumen in determining the extent of drug

absorption, whereas the dispersion quality of the lipid vehicles did not seem to be a key factor in the absorption of danazol.

Another study conducted by Grove *et al.* [171] provided a further insight into the physiological requirement to accomplish the self-emulsifying effect of SMEDDS formulations. For the vitamin D analogue, **seocalcitol**, the authors reported a similar bioavailability data across the treatment groups receiving the formulations of LC-SMEDDS, MC-SMEDDS, LCT solution and MCT solution. An explanation given for this observation was that a sufficient amount of GI fluids is required for an efficient self-emulsification of the SMEDDS systems, and this criterion was probably not fulfilled in the rat model at the administered formulation dose. Furthermore, the authors have also found limited stability of seocalcitol in the SMEDDS formulations (*i.e.* >4-fold higher percentage of degradation) as compared to the lipid solutions when stored at various conditions (including low, ambient and elevated temperatures and humidity levels). Such findings highlight the important consideration of possible drug-excipient interactions in an attempt of developing a viable lipid-based formulation particularly when multiple excipients are in use.

1.3.4 The role of silica-based materials in oral delivery of poorly water-soluble drugs

Nanoparticulate vehicles have received considerable interests as a high-surface-area carrier for controlled and/or targeted drug delivery via various administration routes (e.g. intravenous, oral and transdermal) [174, 175]. Inorganic silica-based materials have emerged as a biocompatible, stable and 'stealthy' option for drug delivery. This is superior to organic materials (e.g. micelles, liposomes and polymeric nanoparticles) that are typically thermo-labile and susceptible to chemical degradation on storage as well as by the body's immune system [174, 176]. Silica-based materials have been demonstrated as an excellent particulate carrier for a variety of poorly water-soluble drugs. A few mechanisms have been proposed through which silica-based materials improve the oral bioavailability of poorly water-soluble drugs:

(*i*) preservation of drug molecules in the amorphous or molecularly dispersed form and increased drug wettability in aqueous media, which lead to enhanced dissolution or release kinetics [41, 177]; and

(*ii*) solubilisation of drugs at a supersaturation state in the GI fluids, which creates a driving force favouring drug absorption across the intestinal membrane [42].

Silica-based drug delivery systems can be categorised into three types: mesoporous silica xerogels [176, 178], ordered mesoporous silica-based materials [179], and mesoporous hollow silica spheres [180]. Silica-based xerogels are amorphous, porous materials in which the drug molecules are encapsulated inside the silica matrix using sol-gel technology. This has been investigated for some poorly water-soluble drugs such as cisplatin [181], nifedipine [182] and ibuprofen [183], in which controlled drug release profiles could be achieved via modulation of the gel structure and porosity, as well as via the chemical interactions between the gel network and the embedded drug. Ordered mesoporous silica-based materials typically have more well-defined surface properties, and their porous interiors can be tuned to be suitable for encapsulating different molecules of interest. This has been demonstrated for drugs such as itraconazole [177], fenofibrate [41] and carbamazepine [184], in which the encapsulated drug molecules are released mainly via a diffusion-controlled mechanism. Mesoporous hollow silica spheres are somewhat similar to mesoporous silica with respect to the mechanism of drug release, but they can facilitate a higher drug loading capacity through the hollow core structure. This has been demonstrated specifically for the model drug ibuprofen [180, 185].

Many of the silica-based materials have thus far been investigated using *in vitro* dissolution models for controlled, sustained, and enhanced drug release, whilst very few reports on their *in vivo* evaluation are available. One of the few examples is the absorption study of itraconazole conducted in rabbits and dogs [177], in which an ordered mesoporous silica formulation has been shown to provide an enhanced *in vitro* drug dissolution or release, and a bioavailability of at least 2-fold higher than the crystalline drug. Interestingly, the mesoporous silica-based formulation was found to be comparable to the marketed capsule product Sporanox®, which contains HPMC-based solid dispersions of itraconazole [see **Section 1.2.2 (d)**]. This study represents one of the important reports supporting the use of solid solutions or dispersions as supersaturating drug delivery systems for enhancing the oral bioavailability of poorly water-soluble drugs.

A review of the types of solid carrier commonly used for transforming liquid emulsions into dry emulsions has been presented in **Section 1.3.3.3**, in which colloidal silica has been identified as one of the potential carrier materials for lipid-based systems. However,

systematic investigations on the *in vitro* and *in vivo* performance of such silica-lipid hybrid drug delivery systems are still lacking. Some clinical studies conducted by Llabres *et al*. [186-188] in year 1982 have demonstrated an enhanced bioavailability effect for a poorly water-soluble antibiotic, amoxicillin trihydrate, resulting from a tablet formulation containing fat-silica matrix; the type of synthetic fat and silica excipient employed was not specified in their reports. Importantly, such enhancement effect was found to increase with higher silica content in the tablet formulation. Later, the study of Takeuchi *et al.* [151] has not been successful in demonstrating the superiority of a silica-containing dry emulsion over a conventional oily mixture and a liquid emulsion in enhancing the bioavailability of vitamin E acetate in dogs. The synergistic role of lipid and silica excipients in enhancing the oral absorption of poorly water-soluble drugs thus remains largely unexplored.

1.3.5 In vitro assessment tools for lipid-based formulations

While the *in vitro* assessment method for lipid-based formulations has yet to be standardised for regulatory use, more well defined formulation tools have been progressively developed for screening, characterisation and optimisation of various lipid-based vehicles. Based on fact that lipid vehicle dispersion and digestion are the key determinants of the subsequent state of drug solubilisation and absorption in the GI tract, a number of useful diagnostic tests have been implemented for lipid-based formulations: (*i*) assessment of drug solubility in simulated BS mixed micelles (plus digestion products), (*ii*) drug dispersion or release test, (*iii*) formulation particle size analysis, and more importantly (*iv*) the enzyme-mediated digestion test for digestible lipid-based formulations [2, 54, 93].

1.3.5.1 Drug solubility assessment, dispersion test and formulation particle sizing

The assessment of drug solubility in simulated BS/PL mixed micelles has been recommended as a simple screening method to determine if the intestinal micellar solubilisation is of any exploitable value to the absorption enhancement of a chosen lipophilic drug. In humans, the average BS concentration is approximately ~5 mM in the fasted state, and ~10 to 20 mM in the fed state; the measured ratio of BS:PL in human bile is 4:1 [82]. Macgregor *et al.* [93] illustrated the simplicity of such solubility tests in predicting the likely solubilisation capacity produced by different simulated GI media for

progesterone. As illustrated in **Figure 1.5**, simple BS micelles are capable of providing a 4-fold higher solubility for progesterone than the blank aqueous medium; the addition of medium- and long-chain lipolytic products (containing MG:FA in the molar ratio of 1:2) further increased the solubility of progesterone up to >14-fold. These data clearly indicate that progesterone is likely to benefit from the post-prandial solubilisation effect, and both medium- and long-chain digestible lipids are potential formulation lipids for the development of a viable progesterone formulation.

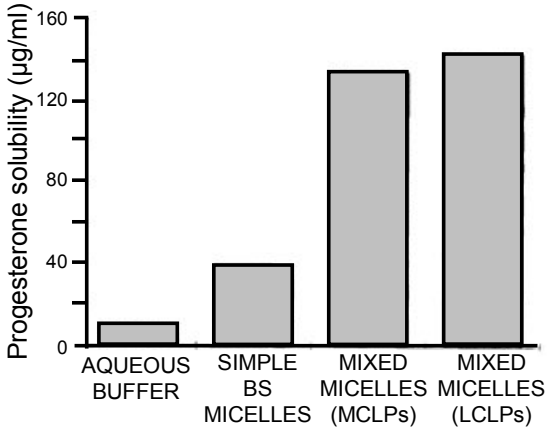


Figure 1.5 Solubility of progesterone in taurocholate micelles in the presence and absence of lipolytic products (*n* = 3). Compositions: simple bile salt micelles contained 15 mM ox bile salt, mixed micelles (MCPLs) contained 15 mM ox bile salt + 0.5% medium chain lipolytic products (MG:FA = 1:2), mixed micelles (LCLPs) contained 15 mM ox bile salt + long chain lipolytic products (MG:FA = 1:2). Taken from reference [93].

The drug dispersion (or release) test can be easily developed employing the standard pharmacopoeial dissolution apparatus [2]. The experimental method is somewhat similar to the general procedure of dissolution testing, which involves collection of the sample aliquots from the dispersion vessel at specified time followed by immediate filtration or centrifugation for removing undissolved materials; thereafter a spectrophotometry or chromatography assay can be used to analyse the drug content in the samples. In contrast to the dissolution tests in which the solid-liquid dissolution transitions are emphasised, the main purpose of dynamic dispersion tests is to determine the likelihood of drug precipitation from the lipid vehicles upon exposure to an aqueous medium [2, 54]. This is because the drugs are already solubilised in the lipid phase and the tendency of precipitation in the aqueous continuous phase will limit the absorption process. Pouton and Porter [2, 54] have stressed the importance of conducting a dispersion test for the relatively hydrophilic

LFCS Type III(B) and IV systems due to the higher risk of drug precipitation upon dilution for these systems (**Figure 1.6**). Although the potential loss of solvent capacity can be predicted from a dispersion test, the beneficial solubilisation effect resulting from the digested lipid components cannot be inferred from such analysis. Therefore, the dynamic digestion tests represent a more useful predictor of the *in vivo* fate of both lipid vehicles and drugs.

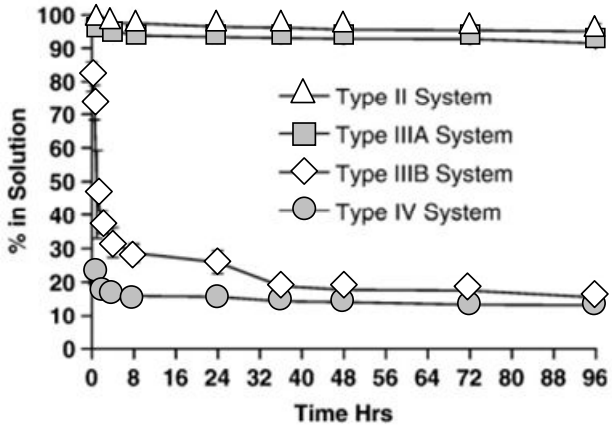


Figure 1.6 The dose percentage of a lipophilic compound remained in water as a function of time after 1/100 dilutions of Type II, IIIA, IIIB and IV lipid-based formulations of the drug (mean ± standard deviation, *n* = 4). Prior to dispersion, the drug was dissolved at 80% saturated solubility in each formulation. Taken from reference [54].

Particle sizing of lipid formulations can be conducted in conjunction with a dispersion test, or as a separate test to verify the quality of dispersion. This is particularly useful for self-emulsifying systems where adequate and reproducible dispersion is essential to ensure a consistent performance [2, 54]. Visual observation is a simple way of discerning an acceptable or poor self-emulsifying formulation in the early stage of formulation development. For many solid lipid dosage forms (e.g. dry emulsions and solid SMEDDS), particle sizes of the redispersed vehicles have generally been used as an indicator of the product storage stability and performance consistency [152-156, 162-164]. More precise particle sizing is usually conducted by using the photon correlation sizer or the Fraunhofer diffraction instrument, which has a size detection limit of 0.6 nm to 6 μm, and 0.1 to 100 μm, respectively [2, 54].

1.3.5.2 Lipid digestion test

Lipid digestion (or lipolysis) testing is the major assessment tool for LFCS Type I, II and III formulations due to the presence of digestible lipid components, and probably for Type IV formulations if the surfactant component is susceptible to hydrolytic degradation [54]. While separate information regarding the dispersion of lipid vehicles or drugs can be obtained from the aforementioned solubility assessment, dispersion test and particle size analysis, the *in vitro* digestion test offers the advantage of producing more comprehensive data regarding the lipolysis kinetics of the vehicles, as well as the fate of drugs throughout the process of digestion [2, 54, 189].

As depicted in **Figure 1.7**, a typical lipolysis model is composed of (*i*) a thermostated reaction vessel which contains a buffered digestion medium consisting of BS/PL micelles under continuous stirring condition, (*ii*) a pH-stat system equipped with an autoburette for titrating any pH change to the initial set point (normally lies in the pH range of 6.5 to 7.5, well representing the intestinal conditions), and (*iii*) a computer with specific software for recording the lipolysis kinetic parameters [82, 189]. The specific experimental procedure of a lipolysis test is outlined in the method section in **Chapter 4**. Briefly, the digestion of a known amount of lipid formulation is initiated by adding excess pancreatic enzymes (containing lipase/ co-lipase) into the reaction vessel, and the lipolysis is allowed to run for a fixed time period (normally 30 min) [82]. As lipolysis proceeds, the liberated FA molecules are automatically titrated with NaOH to maintain the initial pH, and the consumption of NaOH can then be used to infer the extent of lipolysis based on a 1:1 stoichiometric reaction ratio between OH^- ions and the liberated FA.

In order to characterise the chemical changes of the lipid vehicles and the state of solubilisation of the encapsulated drug molecules during lipolysis, multiple samplings can be carried out in which the lipase activity in the collected samples is immediately inhibited by the addition of a lipase inhibitor, *i.e.* 4-bromobenzeneboronic acid solution [189]. The reaction samples are then subjected to ultracentrifugation to separate into the undigested oil phase, the micellar and vesicular solubilising aqueous phase, and the pellet phase which is rich in the insoluble Ca^{2+}-FA soaps. The drug content in each phase is usually determined with a high performance liquid chromatography (HPLC) assay, whereas the distribution of various lipid components (TG, DG, MG and FA) across the separated phases can be

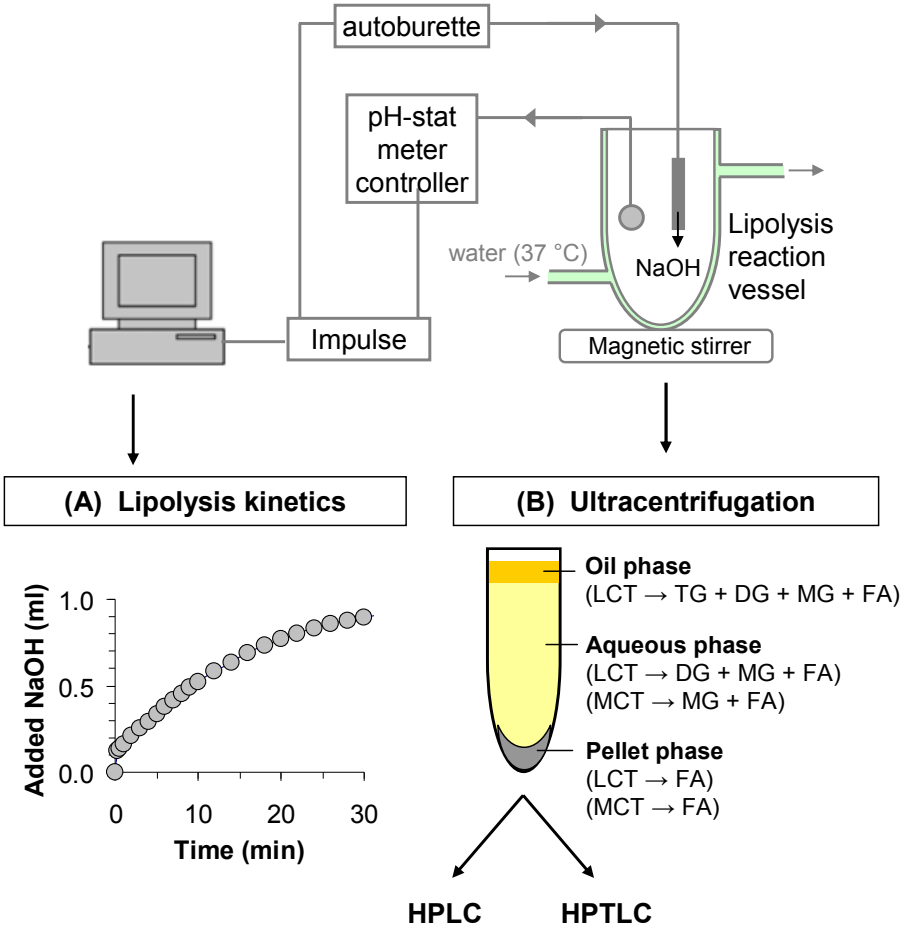


Figure 1.7 Schematic representation of the typical lipolysis model consists of a thermostated lipolysis reaction vessel and a pH-stat meter controller connected to an autoburette for titration of the fatty acids liberated during lipolysis: (A) The volume of NaOH consumed in the titration is recorded by the computer software, which can be used to estimate the lipolysis kinetics based on a 1:1 stoichiometric reaction ratio between the OH^- ions and the liberated free fatty acids; (B) the typical phases formed after ultracentrifugation of the lipolysis samples, in which different proportions of digested lipid components (TG, DG, MG and FA) are recovered in each phase resulted from a 30-min digestion of 250 mg of LCT (peanut oil) and MCT (Captex 355) under both fasted (5 mM NaTDC:1.25 mM PL) and fed (20 mM NaTDC: 5 mM PL) conditions [8, 82, 189].

quantified by using either a HPLC [79] or a high performance thin layer chromatography (HPTLC) method [12, 82, 190, 191].

In parallel to the digestion test, the sequential morphological changes of the digesting lipids can also be visualised by using various microscopy techniques: light microscopy [88],

freeze fracture electron microscopy [192], and cryogenic transmission electron microscopy (Cryo-TEM) [193]. Among these methods, only Cryo-TEM has been used by Fatouros *et al.* [193] to follow the lipolysis process of a pharmaceutical lipid-based formulation (*i.e.* a SMEDDS), while other methods have been used mainly for characterising the physicochemical changes of dietary lipids. Some example images of the digesting droplets have been presented previously in **Figure 1.2**. Other biophysical methods that have been in use for characterising the structures of the intermediate digestion phases are small-angle neutron scattering (SANS), small-angle X-ray scattering (SAXS), as well as nuclear magnetic resonance (NMR) and Raman spectroscopy; electron paramagnetic resonance (EPR) spectroscopy has recently been introduced as a novel approach to determine the partitioning of drugs into the intermediate colloidal phases during lipolysis [189].

1.3.6 Future directions and challenges for oral lipid-based formulations

1.3.6.1 Biopharmaceutical perspective

The oral lipid-based products have first gained entrance into the pharmaceutical market in 1981, mainly in the form of simple lipid solutions [5]. The subsequent commercialisation of the lipid-surfactant based SEDDS and SMEDDS formulations after 1990's, such as cyclosporine A (Sandimmun® followed by Neoral®, Novartis) , ritonavir (Norvir®, Abbott Laboratories) and saquinavir (Fortovase®, Roche) have highlighted the importance of the physicochemical aspect of a lipid-based vehicle in effectively delivering poorly water-soluble (and lipophilic) drugs via the oral route [139]. While a standard pharmacopoeial protocol is yet to be established for the evaluation of lipid-based formulations, there is noticeable progress in the development of *in vitro* assessment tools for lipid-based formulations. Interestingly, the *in vitro* digestion models have long been utilised by biochemists, but have only recently been used by pharmaceutical formulation scientists in conjunction with an *in vivo* absorption study to effectively explore the performance and viability of various lipid-based vehicles [2]. As a positive outcome, there has been increasing literature elucidating the roles of various formulation lipids in affecting the absorption of a wide range of lipophlic drugs. This enabled Porter *et al.* to conclude the following seven guiding principles for effective biopharmaceutical design of a lipid-based formulation [8]:

(*i*) the *in vivo* formulation performance is greatly reflected by the drug solubility in the formulation, as well as by the maintenance/ enhancement of such solubilisation state upon dispersion in the stomach followed by digestion in the intestines (although gastric lipolysis may play a role);

(*ii*) the maintenance/ enhancement of the drug solubilisation state at the absorptive site is highly dependent on the properties of the colloidal species formed during digestion, rather than on the properties of the formulation itself – this can be easily elucidated by the *in vitro* digestion models (particularly the drug phase partition study);

(*iii*) for self-emulsifying formulations, a relatively high proportion (w/w) of long-chain lipids (~60%) and a lower amount of surfactants (~30%) and cosolvents (<10%) can produce reasonably effective drug solubilisation properties upon both dispersion and digestion, but the drug solubility in the formulation may be compromised;

(*iv*) there is a general trend that long-chain digestion products are superior to medium-chain lipolysis products in supporting the solubilisation of highly lipophilic drugs (*i.e.* log $P > 5$) due to slower drug transfer into the aqueous environment (because of a slower digestion), greater swelling capacity (which in turn creates micelles with higher solubilising capacity), and the potential stimulation of lymphatic drug transport (which minimizes the first-pass metabolism);

(*v*) self-emulsifying systems containing high levels of surfactants (*i.e.* Type IIIB formulations) are often endowed with excellent dispersion properties (*i.e.* forming smaller particle sizes) where the risk of drug precipitation can be reduced by employing less readily digestible surfactants rather than those liable to digestion;

(*vi*) the inclusion of a secondary cosolvent to a surfactant system (*i.e.* Type IV formulations) can enhance the overall dispersion rate of the vehicle; and

(*vii*) when comparing a Type IV formulation with a glyceride-based system, the former typically provides a higher solubility for poorly water-soluble drugs than the latter; however, it is important to consider the risk of drug precipitation associated with the use of highly water-miscible components.

1.3.6.2 Regulatory perspective

A successful formulation is built on a careful selection of excipients based on their safety, as well as drug-excipient miscibility and compatibility, which can ultimately produce the desirable pharmacological availability of a drug substance without causing unwanted adverse effects [59]. The FDA refers this process as quality by design. From the regulatory point of view, the issues of irritancy, toxicity and history of use related to the formulation excipients have always been prioritised over scientific understandings of a drug formulation [54, 194]. Generally, toxicity testing will not be required for a newly developed formulation provided that: (*i)* the formulation excipient is listed as "generally recognised as safe" (GRAS) by the FDA [195], and (*ii*) the formulation excipient is one of the inactive ingredients that have been used in the marketed products [196].

While the lack of predictive *in vitro* tools has been recognised as a major barrier to the regulatory approval of many lipid-based formulations, Pouton and Porter who have constantly reviewed the area of lipid-based formulations within the last decade believed that standard test methods will emerge over the next few years [54]. Thus far, the use of *in vitro* digestion models to predict the *in vivo* performance of lipid-based formulations has been attempted with some degree of success (**Table 1.9**). However, there is still little evidence that the currently established digestion models can be used to establish correlations of a higher level (*i.e.* multiple points) rather than the rank order correlations established based on a selected end-point. In some cases, the *in vitro* drug solubilisation or precipitation levels have failed to accurately reflect the *in vivo* absorption outcome. The major concern regarding the current digestion models is the simulation of the pre-enterocyte processes without considering the effect of the intracellular events on the overall systemic exposure of drugs [189, 197]. It has generally been assumed that the intraluminal solubilisation state is of prime importance while the membrane permeability is not a limiting factor for the absorption of lipophilic drugs. Dahan and Hoffman [197] have clearly demonstrated that this assumption should not be generalised to all lipophilic drugs. For the highly lipophilic compound **vitamin D_3** (log P = 9.1), the *in vitro* solubilisation state in the post-digestion aqueous phase was the highest for a MCT (Captex 355) solution, followed by a LCT (peanut oil) solution, which was equivalent to that of a SCT (triacetin) solution [197]. However, the fasted state rat absorption study has given a different bioavailability trend of LCT > MCT > SCT. The reason behind such discrepancy is that administration of the LCT

Table 1.9 Examples of *in vitro-in vivo* (rank order) correlations for lipid-based formulations using the lipid digestion model [189]

Compound	Study subject, fasted/ fed state	*In vitro* ranking of drug solubilisation (in aqueous phase)	*In vivo* ranking of drug bioavailability (BA)	Ref.
Acceptable correlations				
Danazol	Beagle dogs, fasted	(30-min digestion) LC-SMEDDS > MC-SMEDDS	($AUC_{0\rightarrow10}$) LC-SMEDDS > MC-SMEDDS	[170]
Dexa-methasone	Rats, fasted	(30-min digestion) MCT ≈ LCT ≈ SCT	(Absolute BA) MCT ≈ LCT ≈ SCT	[104]
Griseofulvin	Rats, fasted	(30-min digestion) MCT > LCT > SCT > water	(Absolute BA) MCT > LCT > SCT > water	[104]
Progesterone	Rats, fasted	(30-min digestion) MCT > LCT > SCT	(Absolute BA) MCT > LCT > SCT	[197]
Halofantrine	Beagle dogs, fasted	(Low lipid mass: 5mg/ml) LCT solution > MCT solution (High lipid mass: 25mg/ml) MCT > LCT	($AUC_{0\rightarrow10}$) LCT solution > LCT/MCT blend > MCT solution ($AUC_{0\rightarrow\infty}$) LCT solution ≈ LCT/MCT blend ≈ MCT solution	[198]
Poor correlations				
Cyclosporine	Rats (bile duct-cannulated), fed	(12-min digestion) MCT > LCT	(cumulative radioactivity excretion over 72 h) *(Pre-digested lipids)* LCT ≈ MCT *(Non pre-digested lipids)* LCT > MCT	[191, 199]
Vitamin D_3	Rats, fasted	(30-min digestion) MCT > LCT ≈ SCT	(Absolute BA) LCT > MCT > SCT	[197]

formulation has the potential to enhance lymphatic transport of vitamin D_3, thus contributing to a higher overall bioavailability than MCT and SCT. Regretfully, this mechanism could not be deduced from the dynamic digestion model employed. This suggests that a complementary *ex vivo* permeability model may be valuable to better simulate the overall pre-systemic events that a lipid-based formulation may face. At present,

there is some ongoing work in the development and optimisation of digestion-permeation models [5, 104, 200]. Such novel models have yet to be utilised more extensively by the formulation scientists to justify their usefulness in improving the establishment of *in vitro-in vivo* correlations, and ultimately increasing the approval opportunity for various lipid-based formulations containing lipophlic drugs.

1.4 Aims and significance of research project

Lipid-based drug delivery systems are potential carrier systems for encapsulating BCS Class II drugs, mainly attributed to their appreciable solubility or miscibility in lipid excipients, and the significance of food effect in enhancing their state of solubilisation and absorption *in vivo* [21, 22]. There is a general opinion that the clinical application of many lipid-based formulations is limited by the lack of clear guidelines on their formulation design, incomplete understanding of the absorption mechanisms of the formulations, and the lack of standard *in vitro* tools for predicting the *in vivo* performance of the formulations. Notwithstanding these problems, the availability of the 3–4% oral lipid-based products in the pharmaceutical market worldwide has supported the use of lipid-based technology in addressing the low bioavailability issue of BCS Class II drugs. Among the existing marketed oral lipid-based formulations (of which a full list is summarised in the textbook [5]), most of the products are in either capsule (*i.e.* liquid, semi-solid, or solid formulation filled into soft/ hard gelatin capsules) or solution forms. An interesting exception is the antibiotic ciprofloxacin (Cipro®, Bayer), which is a microcapsule formulation for constitution to a suspension with the lipid-based diluent composed of MCT, sucrose, lecithin, water and strawberry flavour [5].

Silica-based materials have been demonstrated as an excellent particulate carrier for a variety of poorly water-soluble drugs. Some potential mechanisms by which silica-based materials enhance the oral absorption of drugs are mainly via the preservation of the drug amorphous or molecularly dispersed form, as well as increased wettability in the aqueous medium, which lead to enhanced dissolution or release kinetics [41, 177]. In addition, the maintenance of a supersaturated state in the GI fluids may also present a driving force for enhanced drug absorption [42, 48].

The current research therefore aims to develop a novel lipid-based dry microcapsule carrier as a potential alternative for effective oral delivery of BCS Class II drugs. The current formulation design is rationalised as follows (**Figure 1.8**):

(*i*) the use of lipid (o/w) emulsions offers an increased drug solubilisation capacity upon aqueous dispersion and digestion in the GI lumen;

(*ii*) stabilisation of lipid emulsions with the biocompatible silica nanoaparticles is preferred over the use of synthetic surfactants or polymers from the safety point of view;

(*iii*) silica nanoparticles can act as a solid carrier facilitating the transformation of a liquid emulsion system into a dry powdery form for improved shelf-life (as previously reviewed in **Section 1.3.3.3**); and

(*iv*) the dry silica-lipid hybrid (SLH) system is endowed with adequate reconstitution properties in the GI fluids, where the presence of silica nanoparticles offer several biopharmaceutical advantages to the hybrid system over the conventional dosage forms. For examples, the hydrophilic and 'stealthy' characters of silica nanoparticles offer adequate lipid/ drug dispersion and stability in the aqueous medium; the small particle size of silica provides a large surface area for desirable interactions between the carrier system and the GI environment, such as drug release and lipid digestion in a controllable fashion.

Overall, this formulation research has focused on a series of model BCS (or BDDCS) Class II compounds exhibiting different lipophilicities (*i.e.* log P = 2 to 6) and ionisation constants (*i.e.* pK_a = 3 to 11). Such extensive evaluation is anticipated to provide a thorough understanding of how the physicochemical properties of drugs (*i.e.* different lipid solubility, pH-dependent aqueous solubility and drug-excipient interactions) can affect the viability of the SLH formulations. This thesis particularly focuses on the investigation of a weakly basic anti-inflammatory drug, celecoxib, as a model compound with an intermediate lipophilicity (log P = 3.5). Through a series of physicochemical and performance assessments using various *in vitro* and *in vivo* models, we verify the novelty of the SLH microcapsule system for its unique porous morphology and homogenous hybrid compositions which produce several attractive biopharmaceutical advantages. This essentially opens up the formulation option for many poorly water-soluble drug candidates.

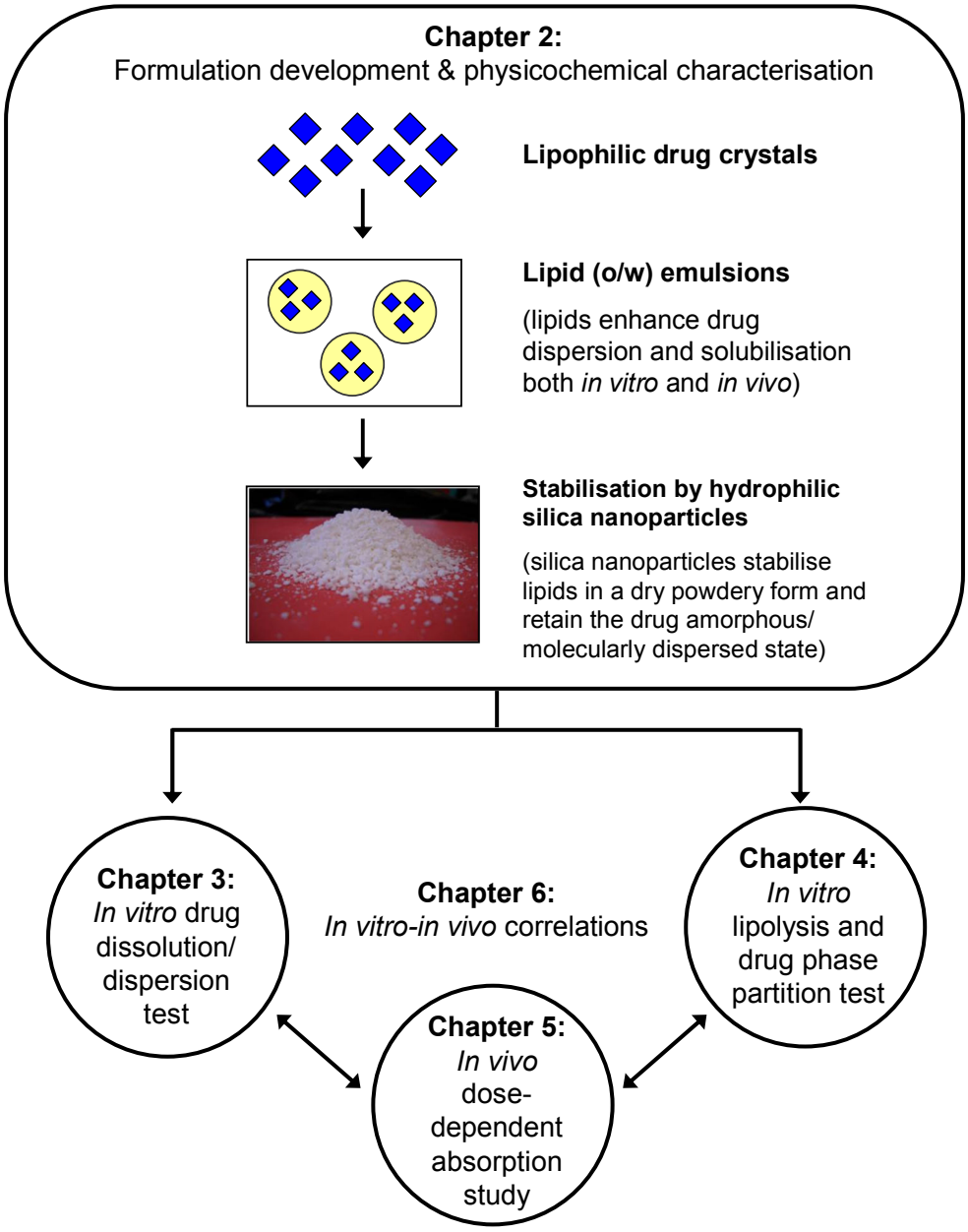


Figure 1.8 Schematic flow of research on the silica-lipid hybrid (SLH) formulations of BCS Class II drugs.

CHAPTER TWO

Development and Physicochemical Characterisation of Silica-Lipid Hybrid (SLH) Microcapsules

2.1 Introduction

This chapter describes the formation and physicochemical characterisation of the silica-lipid hybrid (SLH) microcapsules. The formulation excipients were selected based on their pharmaceutical functionality and established administration safety, which is reviewed in **Section 2.1.1**. The properties of the chosen model lipophilic drug, celecoxib, are presented in **Section 2.1.2**. Physicochemical characterisation of the prototype formulations was conducted using the commonly used chromatography, microscopy, particle sizing, X-ray diffraction and thermal analysis techniques. These are textbook methods for which a large number of literature is available [16, 201-204].

2.1.1 Development considerations and formulation design

Lipid-based formulation strategies have captured extensive pharmaceutical interest for optimising the oral absorption of poorly water-soluble and lipophilic drugs. Whilst many successful examples have been reported (refer to **Section 1.3.3**), there are several challenges that potentially compromise the therapeutic use of many lipid-based formulations, such as the need to employ synthetic surfactants to facilitate dispersibility of some oily vehicles, the use of organic solvents in the manufacturing process, as well as the lack of solid dosage forms that normally gains better patient compliance and have reasonably long shelf-life [2]. Ideally, a lipid-based formulation should take advantage of the solubilisation and bioavailability enhancement from dispersed lipids without requiring the addition of potentially toxic surfactants. With this in mind, the current formulation strategy has been designed to couple the solubilising effect of lipids and the stabilising effect of silica nanoparticulate material to produce stable emulsified carriers in the solid dosage form which can lead to enhanced drug dissolution and oral absorption profiles. By using the GRAS-listed excipients, such formulations originate from a simple oil-in-water emulsion (consisting of a dispersed oil phase in the presence of an emulsifier) which is subsequently stabilised and encapsulated by a solid carrier prior to transformation into the dry state.

2.1.1.1 Lipid excipients

The types of formulation lipids that have been proven proficient in enhancing the oral bioavailability of a wide range of poorly water-soluble drugs have been comprehensively reviewed by previous researchers [4, 5, 8]. The commercially available lipid excipients are tabulated in **Table 1.2** (Chapter 1). The generic chemical structure of various formulation lipids, including triglycerides (TG), diglycerides (DG), monoglycerides (MG) and phospholipids (PL) is depicted in **Figure 2.1**.

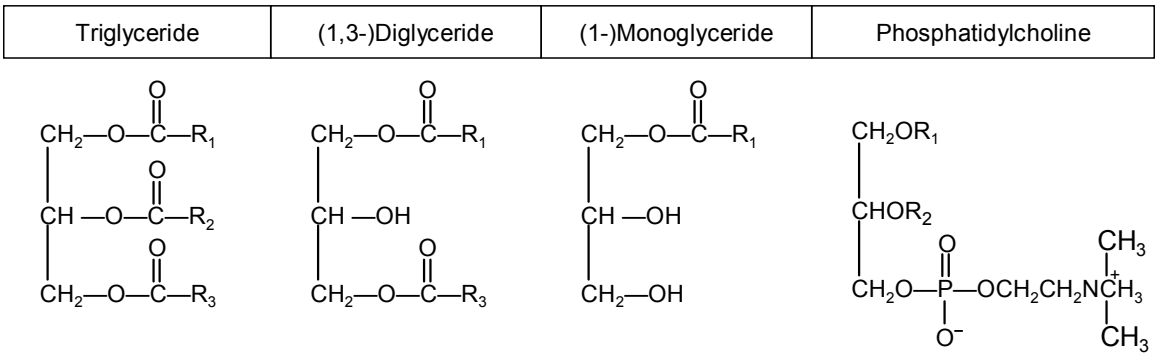


Figure 2.1 The generic chemical structure of various formulation lipids: triglycerides, diglycerides, monoglycerides and phospholipids (e.g. lecithin) [13, 205].

Natural MCT oils or the semi-synthetic medium-chain glycerides (e.g. Miglyol and Capmul oils) have gained much popularity in formulating drugs of intermediate lipophilicity (log $P < 5$), mainly because of their adequate solvent capacity, high fluidity and appreciable self-emulsifying efficiency in aqueous media in comparison with LCT [133]. The primary TG oil used in the current formulation development is the commercially available fractionated coconut oil (Miglyol 812), which is a mixture of saturated caprylic/capric (C8/C12) triglycerides. Miglyol 812 has been a widely preferred choice for developing a stable formulation due to its resistance against oxidative decomposition [133, 156].

In contrast to TG oils, C8/C12 mono-diglyceride blend (Capmul MCM) has been shown to provide a relatively higher solubility for some lipophilic drugs with low to intermediate log *P* characters (e.g. griseofulvin, diazepam and danazol [206]), thus made possible the formation of isotropic oil-water solutions without the use of co-surfactants [207-210]. Owing to its unique amphiphilic character, Capmul MCM with a hydrophile-lipophile

balance (HLB)[g] of 5–6 is more often used as an emulsifier, solubiliser and nonionic surfactant rather than as the oil phase in lipid-based formulations. Therefore, it is of particular interest in the current work to explore the effect of Capmul MCM when used as an oil phase to form an emulsified formulation, as well as to made comparison between Miglyol and Capmul oils in the microcapsule formulations.

In addition to the aforementioned saturated digestible oils, oleic acid is another lipid of interest due to its completely different compositions and properties, *i.e.* indigestible and unsaturated long-chain (C18) fatty acids. Oleic acid has been shown to potentiate the lymphatic uptake of lipophilic drugs through the formation of chylomicrons (80–1000 nm) in the enterocytes and thereby increasing the oral bioavailability with insignificant histological changes in the intestinal tissues [75, 211]. The toxicological data for each lipid excipient, expressed as the oral median lethal dose (LD_{50}), are available from the corresponding material safety data sheet. The LD_{50} in rats has been taken into consideration during formulation development prior to *in vivo* testing: Miglyol 812 (>5 g/kg body weight), Capmul MCM (>5 g/kg body weight) and oleic acid (≥74 g/kg body weight).

2.1.1.2 Emulsifiers

Lecithins are natural heterogeneous materials extracted from biological sources (e.g. soybean, eggs, sunflower seeds) that have been extensively used in stabilising oil-water systems because of their high surface active properties and biodegradability [60, 91, 212]. The presence of acidic lipids (typically 1–2 %) in lecithins significantly contributes to a zeta potential of -40 to -60 mV for lecithin-stabilised oil droplets [212]. However, a previous study evaluating the safety of various lecithins used in intravenous emulsion formulations has revealed a higher risk of haemolysis for hydrogenated (*i.e.* saturated) phospholipids in comparison with unsaturated lecithins; the relative haemolysis percentages decreased in the following trend: hydrogenated soybean lecithin > hydrogenated egg yolk lecithin > egg yolk lecithin > soybean lecithin [213]. The authors explained that hydrogenated phospholipids form relatively less stable monolayers at the oil-water interface, therefore are more freely dispersed in the circulation potentially causing haemolytic effects. Soybean lecithin (not

[g] The hydrophile-lipophile balance (HLB) is a measure of the relative hydrophilicity and lipophilicity of amphiphilic molecules; compounds with HLB values <12 are considered to be hydrophobic, whereas those with values >12 are considered hydrophilic

hydrogenated), which showed the least haemolytic risk among the emulsifying agents investigated, is selected for the current formulation development. Considering that the current formulations are intended for oral administration in which the lecithin component would undergo enzymatic digestion prior to intestinal absorption, the potential for haemolytic effects is expected to be negligible.

2.1.1.3 Stabilisers/ solid carriers

A solid carrier is an excipient incorporated into an emulsion system for encapsulating the lipids prior to conversion into the dry state. In previous work [152-154, 156, 159], water-soluble carriers (e.g. gelatine, sugars, glycine, povidone, and HPMC) have been more widely studied for lipid encapsulation than water-insoluble carriers (e.g. magnesium alumino metasilicate, Neusilin and colloidal silica). The current work further explores the use of hydrophilic fumed silica (Aerosil 380) as an insoluble nanoparticulate stabiliser and solid carrier for lipid encapsulation. It is noted that colloidal silica is a commonly used pharmaceutical/ food excipient approved by the Therapeutic Goods Administration without specific restriction on the daily oral dose [214].

Aerosil silica has been well characterised in respect to the dissociation behaviour of its surface silanol groups (-Si-OH) as a function of medium pH (**Figure 2.2**) [215].

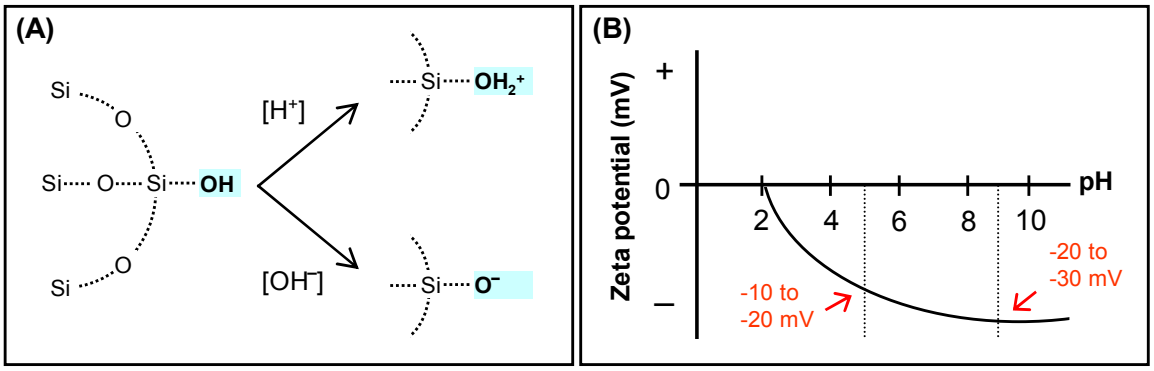


Figure 2.2 Surface chemistry and charge of hydrophilic fumed silica (Aerosil 380) as a function of pH: (A) schematic reaction of hydrated silica in acidic and basic solutions; (B) zeta potential of silica dispersion on the addition of 10 mM NaCl (drawing not to scale) [215].

In aqueous media, the weakly acidic silanol groups (pK_a = 6.25) are predominantly protonated (-Si-OH_2^+) in highly acidic conditions and ionised (-Si-O^-) at basic pH's. At pH > 2, the electrostatic repulsion between negatively charged silica and anionic lecithin droplets may be overcome by the attractive forces (namely hydrogen bonding and dispersion forces) in the system, therefore rendering an efficient interfacial adsorption of silica (free energy of adsorption, ΔG_{ads} = -15 to -23 kJ/mol) [216]. Assuming triglyceride droplets with an average diameter of 0.5 μm and the existence of Aerosil silica as hard spherical particles of either 20, 50 or 80 nm, previous investigations have predicted oil:silica ratios of 1:0.2, 1:0.4 and 1:0.6 (w/w), respectively, for the nanoparticles to form hexagonally packed monolayers at the interfaces [217]. The adsorption behaviour of Aerosil silica nanoparticles at the oil-water interface has been well described and visualised in previous works [217, 218]. For a Miglyol oil-based emulsion system emulsified by lecithin, Aerosil silica when added from the water phase, are randomly dispersed in the aqueous phase and only weakly adsorbed at the droplet interface; this is mainly ascribable to the electrostatic repulsion existing between the anionic nanoparticles and droplets. When silica nanoparticles are incorporated from the oil phase, residence of the nanoparticles in the oil bulk phase/ interface is enhanced by the presence of lecithin due to reduced droplet interfacial tension, as well as via the so-called 'hydrophobisation' mechanism (*i.e.* the particles acquire a more hydrophobic character when initially added to the lipid phase [219]). As a result, Ghouchi Eskandar *et al.* [218] have observed a synergistic stabilisation effect of lecithin and silica nanoparticles in the emulsion system when silica was incorporated from the oil phase.

Based on this information, an oil:silica ratio of 1:0.5 is regarded as optimum for droplet stabilisation in the liquid state, and this composition ratio is further investigated for lipid/ drug encapsulation efficiency in the dry state. The formation of the microcapsules is also examined using different precursor lipid emulsions stabilised by silica nanoparticles either from the lipid phase or from the aqueous bulk phase.

2.1.2 Celecoxib as a model poorly water-soluble drug

Celecoxib (CEL), or 4-[5-(4-Methylphenyl)-3-trifluoromethyl)-1H-pyrazol-1-yl] benzene-sulfonamide, has been chosen as a model BCS Class II drug based on several selection criteria, *i.e.* well characterised drug physicochemical properties, increasing therapeutic

values and readily available animal and human pharmacokinetic data. An adequate pre-formulation knowledge regarding the model drug is essential to rationalise the formulation design and to facilitate the selection of research methodology.

2.1.2.1 Physicochemical properties

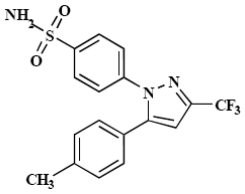
CEL is relatively well characterised for its solubility [208-210, 220] and solid state bulk properties [37, 221]. CEL is a weakly acidic compound (pK_a ~11) that is practically insoluble at gastrointestinal pH due to its non-ionised character over the pH range of 1–9. Its solubility in water is 3–7 μg/ml (at pH 7.0), which can be increased in the presence of various organic solvents (**Table 2.1**).

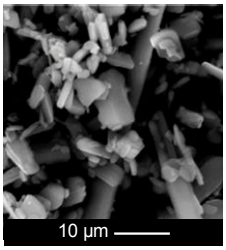
Table 2.1 Solubility of celecoxib in various solvents at 25 °C [220]

Solvent	Solubility of celecoxib (mg/ml)
Water	0.007
Methanol	114
Ethanol	63
Propylene Glycol	30
Polyethylene Glycol (PEG) 400	415
Ethanol–PEG 400 mix. (ratio 8:2–1:9)	171–392
Water-ethanol mix. (ratio 9:1–1:9)	0.01–49

CEL appears as a white to off-white crystalline powder (**Figure 2.3**) with an intermediate lipophilicity (log P = 3.5). CEL molecules exist in three polymorphic crystalline forms that do not undergo solid state interconversion: Form I, Form II, and Form III (which are thermodynamically stable at ambient conditions) with reported melting points of 163, 161.5 and 160.9 °C, respectively; a novel Form IV exhibiting a melt onset at 145–148 °C has also been identified recently [37]. CEL is commercialised in an oral capsule dosage form (*i.e.* Celebrex®) at 100 mg and 200 mg doses. Advanced formulation technology is warranted to ease tableting of CEL powder which is complicated by its high cohesiveness, low bulk density and compressibility, and poor flow properties [208].

Selective COX-2 inhibitors: COX-2 selectivity not diminished at higher doses	**Relatively selective NSAIDs:** COX-2 selectivity diminished at higher doses	**Non-selective NSAIDs:** Non-selectively inhibits both COX-1 and COX-2	
Rofecoxib (1999 – 2004)	Etodolac	Diclofenac	Nabumetone
Valdecoxib (2001– 2005)	Meloxicam	Diflunisal	Naproxen
Celecoxib (1999 – present):		Fenoprofen	Oxaprozin
		Flurbiprofen	Piroxicam
		Ibuprofen	Salsalate
		Indomethacin	Sulindac
		Ketoprofen	Tolmetin
		Mefenamic acid	





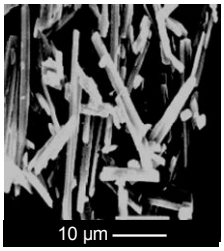


Figure 2.3 Classification of celecoxib in the category of non-steroidal anti-inflammatory drugs (NSAIDs) approved by FDA and its molecular structure (M.W. = 381.38) and SEM micrographs [37, 221-223].

2.1.2.2 Therapeutic applications

CEL (Celebrex®, Pfizer/Pharmacia) is the first FDA-approved selective cyclooxygenase-2 (COX-2) inhibitor in the non-steroidal anti-inflammatory drugs (NSAIDs) family used primarily for the management of rheumatism, osteoarthritis and dysmenorrhea, as well as an adjuvant for treating familial adenomatous polyposis [222]. CEL has been regarded to be relatively safer than other traditional NSAIDs because of its selective inhibition of the prostaglandin synthesis (via the inflammation-related COX-2 pathway) while negligibly affecting platelet aggregation (which is associated with the COX-1 pathway), hence producing minimal gastrointestinal side effects. The superiority of CEL in its efficacy-safety profile is undisputed after the other two COX-2 inhibitors, namely rofecoxib (Vioxx®, Merck) and valdecoxib (Bextra®, Pfizer), have been withdrawn by the drug companies due to increased cardiovascular risks (**Figure 2.3)**. Although newer COX-2 inhibitors such as etoricoxib (Arcoxia®, Merck Sharp & Dohme) and lumiracoxib (Prexige®, Novartis) have been approved for use by the Therapeutic Goods Administration, their prescriptions have been limited by less favorable efficacy-safety profiles.

Furthermore, recent preclinical/ clinical studies have supported the use of COX-2 inhibitors as chemoprevention or anticancer agents with the evidence that suppression of COX-2 derived prostaglandin E_2 (PGE_2) can inhibit tumour growth [224, 225]. Furthermore, therapeutic and preventive opportunities for neurodegenerative diseases can also be foreseen with the use of some NSAIDs that have been found to exhibit significant anti-amyloidogenic properties [226]. The increasing therapeutic value of CEL therefore urges the need to develop more diversified oral formulations with improved bioavailability to meet various therapeutic requirements.

2.1.2.3 Pharmacokinetic properties

The basic human pharmacokinetic data for CEL, with the exception of absolute bioavailability, is outlined in the product information [222]. In addition, extensive information on its pharmacokinetics, tissue distribution, metabolism, and excretion in animals (*i.e.* dogs and rats) and humans have been presented by Paulson *et al.* [227-229]. These *in vivo* data are detailed in **Section 5.1** (Chapter 5), which provide an essential basis for the selection of appropriate pharmacokinetic parameters for evaluation and comparisons between the test CEL formulations.

2.2 Specific aims and hypotheses

The major aims of this chapter were: (*i*) to develop several prototype formulations of SLH microcapsules based on different types of oil excipients (*i.e.* digestible medium-chain Miglyol and Capmul oils, and the indigestible long-chain oleic acid); (*ii*) to evaluate the effect of the initial location of silica nanoparticles in the precursor o/w emulsions on the subsequent formation of the SLH microcapsules (*i.e.* core-shell or porous matrix); and (*iii*) to determine the physicochemical properties of the SLH microcapsule formulations containing CEL. It was hypothesized that the SLH microcapsule formulations offer several physicochemical benefits as follows: excellent formulation storage stability, good formulation redispersibility in aqueous media, and preservation of CEL in the non-crystalline state in the solid dosage form.

2.3 Materials

Materials used for formulation development are specified in **Table 2.2**, including CEL powder, lipid excipients such as Miglyol 812, Capmul MCM, oleic acid and soybean lecithin, as well as solid carriers including hydrophilic fumed silica and maltodextrin.

Table 2.2 List of materials used for formulation development

Material	**Manufacturer**	**Properties** (based on material data sheet)
Celecoxib	ChemPacific (USA)	≥99.0% purity
Caprylic/capric triglyceride (Miglyol 812)	Hamilton Laboratories (Australia)	Caprylic acid ($C_{8:0}$) 59.5% Capric acid ($C_{10:0}$) 40.0% Lauric acid ($C_{12:0}$)/ other fatty acids 0.5% (Average M.W. 520 g/mol)
Glyceryl mono-dicaprylate (Capmul MCM)	Abitech Corporation (USA)	Caprylic acid ($C_{8:0}$) 81.5% Capric acid ($C_{10:0}$) 18.3% Lauric acid ($C_{12:0}$)/ other fatty acids 0.2% (58% MG, 36% DG, 5% TG) (Average M.W. 281 g/mol)
Oleic acid	Chem-Supply (Australia)	Oleic acid ($C_{18:1}$) 72% min. Linoleic acid ($C_{18:2}$) <13% Saturated C_{18} & below <13% (Average M.W. 282.47 g/mol)
Soybean lecithin	BDH Merck (Australia)	>94% phosphatidylcholine <2% triglycerides
Hydrophilic fumed silica (Aerosil 380)	Evonik Degussa (Australia)	Primary average diameter 7 nm Specific surface area 380 ± 30 m^2/g 2.5 silanol groups per nm^2 Hydrophobicity index 0.60 ± 0.05
Maltodextrin	Sigma-Aldrich (Australia)	Dextrose equivalent 13.0–17.0

Chemicals used for the preparation of buffer solutions include potassium dihydrogen phosphate (BDH Merck, Australia), sodium hydroxide pellets (Chem-Supply, Australia) and 1 M hydrochloric acid solution (Ajax Chemicals, Australia). Other reagents used for the preparation of analytical solutions, including acetonitrile, methanol and glacial acetic acid (Merck KGaA, Germany), were of analytical grade. Two types of dyes were used for confocal imaging, namely Rhodamine B and Coumarin 102 (95% and 99% purity, respectively), which were purchased from Sigma-Aldrich, Australia. All resources were

used as received without further processing. High purity (Milli-Q) water was used throughout the experimental work.

2.4 Methods

2.4.1 Analytical methodology and validation

A simple and rapid high performance liquid chromatography (HPLC) method employing UV detection developed by Zarghi *et al.* [230] was adopted with some modifications for quantification of CEL-containing samples. The HPLC procedure, as outlined below, was validated for method specificity, linearity, precision, accuracy, and limit of quantification (LOQ), *i.e.* the minimum detectable concentration with a signal-to-noise ratio of 8:1 that produces a precision and accuracy of ≥ 90%. This section serves as the core reference for all the analytical investigations for CEL throughout this thesis.

(a) Chromatographic conditions. The HPLC apparatus (Hewlett Packard/ Agilent 1100) consists of a G1310A isopump, a G1313A auto-sampler, and a G1314A UV detector connected to a Shimadzu C-R6A Chromatopac Integrator. Chromatographic separation was performed on a Waters XTerra RP C_{18} column (5 µm, 3.0 mm ID x 150 mm) at a detection wavelength of 254 nm. The mobile phase was a mixture of acetonitrile, methanol and water (50:10:40 v/v) containing 0.2% (v/v) glacial acid (pH 3.5), eluted at a flow rate of 1.0 ml/min. The mobile phase was degassed by ultrasonication before use and was not allowed to recirculate during the analysis. The samples were injected at a volume of 50 µl at ambient temperature.

(b) Calibration curve generation and method validation. Stock solutions (1.0 mg/ml) of CEL were prepared in methanol and serially diluted with the mobile phase solution to obtain ten CEL working solutions in the range of 0.05–20 µg/ml for HPLC analysis. Calibration curves were constructed by plotting the peak area against CEL concentrations and the linearity was assessed by linear regression analysis, *i.e.* correlation coefficient (R^2) as close as possible to 1.000.

For examining the intra- and inter-day precision and accuracy, quality control (QC) samples of CEL at low, medium and high concentrations (0.08, 1 and 10 µg/ml) were prepared as described above from independently made stock solutions (1.0 mg/ml). Each QC sample was assayed for 6 replicates on the day of preparation, and on three different days. The

precision and accuracy of the assay method was expressed as the relative standard deviation (%RSD) and the relative error (%Bias), respectively:

$$\%\text{RSD} = \frac{\text{standard deviation}}{\text{mean}} \times 100 \qquad (1)$$

$$\%\text{Bias} = \frac{\text{Concentration(detected)} - \text{Concentration(added)}}{\text{Concentration(added)}} \times 100 \qquad (2)$$

The reproducibility of the analytical method for biological samples was assessed by analysing the recovery of CEL (50 µl) spiked into rat plasma (250 µl) at three concentration levels: 0.4, 1.3 and 3.3 µg/ml. An aliquot of spiked plasma was vortex-mixed with an equal volume of acetonitrile and centrifuged at 3500 *g* for 10 min to remove proteins, prior to HPLC analysis as described above.

2.4.2 Screening and optimisation of lipid solubility of CEL

The equilibrium solubility of CEL in various oils with or without added solubilisers (soybean lecithin) was determined by using the conventional 'shake-flask' or batch agitation method [16]. Based on previously reported lipid solubility of CEL [208-210], excess CEL powder (50–100 mg/g) was added into each selected oil excipient (*i.e.* Miglyol 812, Capmul MCM and oleic acid) and ultrasonicated (Bransonic® Model 2510, USA) for 30 min prior to continuous shaking in a mixer chamber thermostated at 22 °C for 48 h. The ultrasonication step is essential in reducing the time required for reaching equilibrium state by reducing the particle sizes of the CEL crystals, thereby increasing the wettable surface area of the drug compound. Equilibrated mixtures were centrifuged at 9400 *g* for 15 min to precipitate the undissolved drugs. The supernatant was subjected to three cycles of methanol extraction and centrifugation (9400 *g* x 15 min) prior to dilution with the mobile phase solution for HPLC analysis.

2.4.3 Preparation of emulsions and SLH microcapsules

The SLH microcapsules were prepared in two elementary steps: (*i*) the preparation of submicron o/w emulsions by high pressure homogenisation (Avestin® EmulsiFlex-C5,

Canada), followed by (*ii*) the formation of dry powdery SLH microcapsules by spray-drying (BÜCHI Mini Spray Dryer B-290, Switzerland). **Figure 2.4** depicts these processes without specifying the exact ratio of the ingredients used, and visualises two possible types of microcapsules generated from different precursor emulsions. One precursor system was stabilised by silica from the aqueous phase, which is anticipated to produce core-shell structured microcapsules (designated as SLH-M_1); whereas the other precursor emulsion contains silica in both aqueous and oil phases, which is expected to form porous matrix structures (tagged SLH-M_2). These assumptions stem from the basis that silica nanoparticles are not freely diffusible across the oil-water interface [218], therefore the resultant microcapsule morphology is dependent on the initial location of the nanoparticles. The preparation steps are described as below:

(Step 1) Excipient mixing – lecithin and CEL (plus silica in the case of SLH-M_2) were sequentially dissolved or dispersed in the selected oil via ultrasonication (for 10, 20, and 30 min, respectively), followed by the addition of water as the continuous phase to form coarse o/w emulsions by hand held-shaking;

(Step 2) High pressure homogenisation – the coarse emulsions were homogenised under a pressure of 1000 bar for 5–6 volume cycles;

(Step 3) Stabilisation by silica nanoparticles – an aqueous dispersion of 5% silica (which was pre-dispersed in water for at least 6 h by ultrasonication to achieve an average hydrodynamic diameter of 50 ± 5 nm) was added to the homogenised emulsions and rotary mixed for at least 12 h; and

(Step 4) Spray-drying – the silica-stabilised emulsions were spray-dried to form powdery SLH microcapsules under the following conditions: emulsion flow rate 5 ml/min, aspirator setting 10, air flow rate 0.6 m^3/min, inlet and outlet temperature 160 °C and 65 °C.

Four optimised microcapsule systems containing either Miglyol oil (designated as SLH-M_1, SLH-M_2 and SLH-M_3) or Capmul oil (designated as SLH-C) were investigated for the physicochemical properties as described in the following sections.

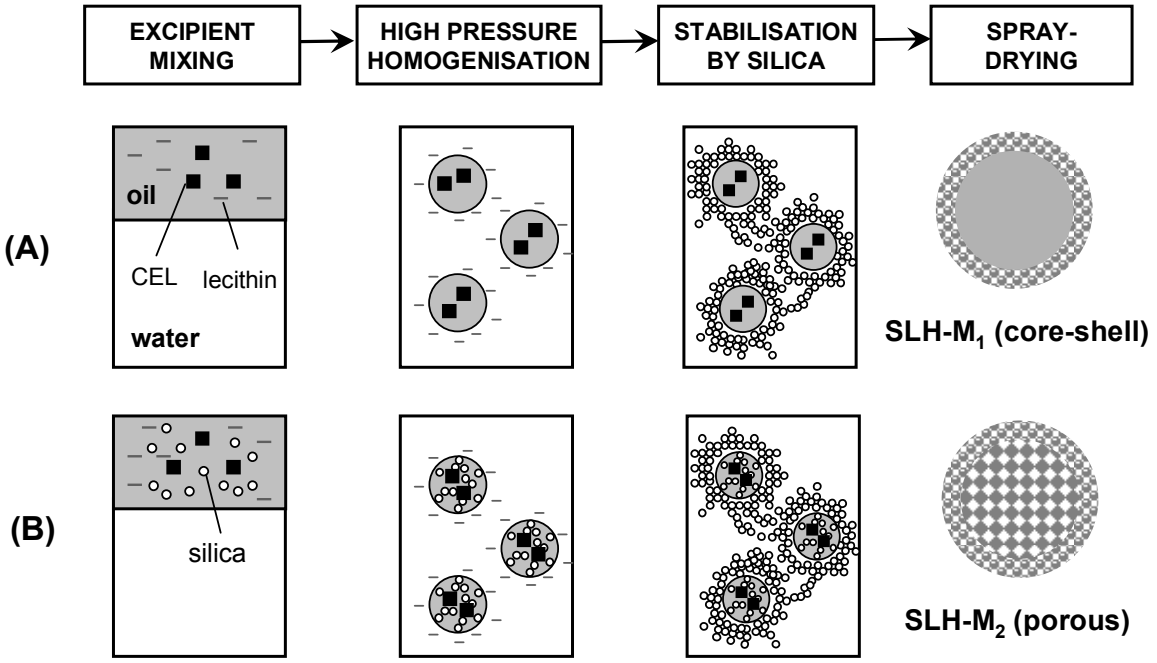


Figure 2.4 Schematic representation of SLH microcapsule preparation from two different precursor emulsion systems containing (A) silica in the aqueous phase, and (B) silica in both aqueous and oil phases, which hypothetically leads to the formation of the core-shell structured SLH-M_1 microcapsules and the porous matrix-structured SLH-M_2 microcapsules, respectively (drawing not according to scale).

2.4.4 Physical characterisation of emulsions and SLH microcapsules

The physicochemical characterisation was carried out for up to 12 months for two initially formulated SLH microcapsule systems (SLH-M_1 and SLH-M_2), and up to 6 months for another two formulations that were optimised for CEL loading level at a later stage of the research (SLH-M_3 and SLH-C).

2.4.4.1 Solid state properties

(a) Morphology. The surface morphology of the SLH microcapsules was examined by high resolution analytical scanning electron microscopy, SEM (CamScan CS44FE). Each sample was mounted on double-faced adhesive tape and sputter-coated with gold/palladium (60%:40%) prior to imaging at an accelerating voltage of 10 kV, spot size 10 μm, and an aperture of 50 μm.

In addition to this, the internal cross-sections of the microcapsules were visualised by a Focused Ion Beam (FIB) imaging tool (FEI Strata FIB201 system instrument located at the University of Bristol, U.K.). Briefly, a conducting deposit was produced on the insulated microcapsule sample by delivering a platinum-bearing organometallic gas to the ion beam focused sample region. The sample was cut and polished by the gallium ion beam at 30 keV and a channeltron detector was used to collect secondary electrons to produce high resolution images of the sample.

(b) Homogeneity. To specify the relative location of silica particles and the structure homogeneity of the studied systems, confocal fluorescence microscopy (Leica TCS-SP5 spectral scanning confocal microscope located at the University of Adelaide, Australia) was used to visualise the fluorescently labelled samples of precursor emulsions and redispersed SLH microcapsules. The silica particles were labelled with a hydrophilic dye (Rhodamine B), and the oil phase with a lipophilic dye (Coumarin 102) prior to formulation preparations, which were detected as red and blue images at the emission wavelengths of 588 and 488 nm, respectively (with the corresponding excitation wavelengths of 514 and 405 nm). Z-stacks were performed to obtain images of planes at various depths within the sample for determining the co-localisation of the dyes.

(c) Surface area analysis. The BET (Brunauer, Emmett and Teller) measurement of powder surface area can be valuable in correlating the exposed surface area of a solid sample to its pharmaceutical properties such as dissolution [16]. Nitrogen gas adsorption measurement was performed for spray-dried silica and oil-extracted SLH microcapsules (in which the oil content was extracted with hexane prior to analysis) by using a Micrometrics ASAP 2010 porosimeter. The total surface area and pore volume/ size of the SLH microcapsules were compared to that of the original silica material and spray-dried silica, and described based on the International Union of Pure and Applied Chemistry (IUPAC) nomenclature of micro-pores (≤2 nm), meso-pores (2–50 nm) and macro-pores (≥50 nm).

2.4.4.2 Colloidal properties

(a) Particle size. Particle sizes of the precursor emulsions and the redispersed SLH microcapsules were characterised using dynamic light scattering (DLS) [or photon correlation spectroscopy (PCS)] technique which has a size detection range of 0.6 nm to 6 μm (Malvern Zetasizer Nano ZS, UK). Size distribution results are expressed as the

z-average diameter (*i.e.* the intensity-weighted mean hydrodynamic diameter) together with the polydispersity index (PDI) indicating the width of the size distribution.

To confirm the absence or presence of larger particles, additional particle sizing by laser diffraction analysis (Malvern Mastersizer 2000, UK) was also undertaken, which is capable of detecting particles in the size range of 0.02 to 2000 μm. The size analysis results are expressed as the volume (*v*) diameters at 10, 50 and 90% cumulative volume, designated as D(*v*,0.1), D(*v*,0.5) and D(*v*,0.9), respectively. The polydispersity of the sample is measured by the span, in which span = [D(*v*,0.9)-D(*v*,0.1)]/D(*v*,0.9)].

Water (refractive index = 1.33) was used as the dispersant in both DLS and laser diffraction analyses. The particle refractive index was pre-set as 1.45 in the laser diffraction measurements; it is noted that the known refractive indices for medium-chain oils and silica particles are 1.45 and 1.46, respectively, according to the Mastersizer database information. Each dry microcapsule sample (5 mg/ml) was firstly redispersed in a phosphate buffer solution (0.05 M, pH 7.2) by brief hand held-shaking (10 s), followed by continuous mixing for 2 h using a rotator mixer, similar to the method of Jang *et al.* [153]. Both liquid emulsion samples and redispersed microcapsules were diluted 100-fold with water prior to measurement at 25 °C.

(b) Zeta potential. The zeta potentials of the precursor emulsions and redispersed SLH microcapsules were determined by using phase analysis light scattering (PALS) technique (Malvern Zetasizer Nano ZS, UK) at 25 °C, with the detection limit of 5 nm to 10 μm particles. The mean zeta potential was computed based on the electrophoretic mobility (*i.e.* the ratio of the velocity of particles to the field strength) by applying the Smoluchowski or Huckel theories (the Malvern Instrument website is recommended for detailed description of the measuring technique [231]).

2.4.5 Chemical characterisation of SLH microcapsules

2.4.5.1 Lipid load analysis

The lipid content of the SLH microcapsules was determined by thermogravimetric analysis (TGA) (TA Instruments TGA). Each microcapsule sample (approximately 10 mg) was heated at a scanning rate of 10 °C/min from 20–600 °C under a nitrogen gas purge. The lipid content (both oils and lecithin) was evaporated in the range of 240–350 °C and the

silica component remained thermally stable. After correction for the water content of spray-dried silica (previously determined to be 1.5 ± 0.0 %), the subtracted weight loss was computed by using the associated TA Universal Analysis software and this corresponds to the lipid content of the microcapsules.

2.4.5.2 Drug content analysis

(a) CEL payload and entrapment efficiency. The amount of CEL loaded into the SLH microcapsules was determined by a solvent extraction method. The encapsulated CEL was extracted by dissolving 10 mg of the formulation powder in 4 ml methanol (which has been tested to give a 100 ± 1 % extraction efficiency). The supernatant (0.5 ml) was taken and the solvent was completely evaporated under a dry nitrogen stream at 30 °C (Pierce Reacti-Therm™ Heating Module, US). The resulting residue was re-dissolved in 10 ml of the HPLC mobile phase solution by vortex-mixing for 1 min, and centrifuged at 9400 *g* for 15 min prior to HPLC analysis for CEL content. The mean drug payload and entrapment efficiency was calculated as below:

$$\text{CEL loading}\,(\%) = \frac{\text{Mass of CEL detected in SLH microcapsules}}{\text{Mass of SLH microcapsules}} \times 100 \qquad (3)$$

$$\text{CEL entrapment efficiency}\,(\%) = \frac{\text{Experimental CEL loading}}{\text{Initial CEL loading}} \times 100 \qquad (4)$$

(b) Crystallinity of CEL. The degree of crystallinity of encapsulated CEL was monitored by differential scanning calorimetry (DSC) and X-ray diffraction (XRD) techniques [16] for the formulation powder samples stored in airtight glass containers at ambient conditions.

Calibration of the DSC instrument (TA Instruments Q100 DSC) was undertaken using an indium standard. Approximately 5 mg of the sample powder was heated in a hermetically sealed aluminium pan at a rate of 5 °C/min over a temperature range of 25–200 °C, under a flow of dry nitrogen gas (80 ml/min). The endothermic event was recorded by the TA Instrument Control software as the heat flow required for maintaining the equality in temperature between the sample and reference (blank).

XRD patterns of the CEL formulation samples were obtained using Philips (PW 1050/25) X-ray diffractometer. The samples were scanned between 10 and 50° (2θ) at 0.02° intervals

at a rate of 0.05°/min. Co-K-α radiation (60 kV, 30 mA, 1.7902 Å) were used with a 1/12° collimation slit. Peak analysis was performed using the SIROQUANT V3 software.

2.5 Results and Discussions

2.5.1 HPLC analysis

(a) Specificity and linearity. Evaluation of the HPLC method showed that the calibration curves (n = 8) were linear over the range of 0.05–20 µg/ml ($R^2 > 0.9998$), with a LOQ value of 0.08 µg/ml. The method is highly specific for CEL, in which the CEL peaks (average retention time 11.5 min) were well resolved with negligible interference for both working solution samples and spiked plasma samples (**Figure 2.5**).

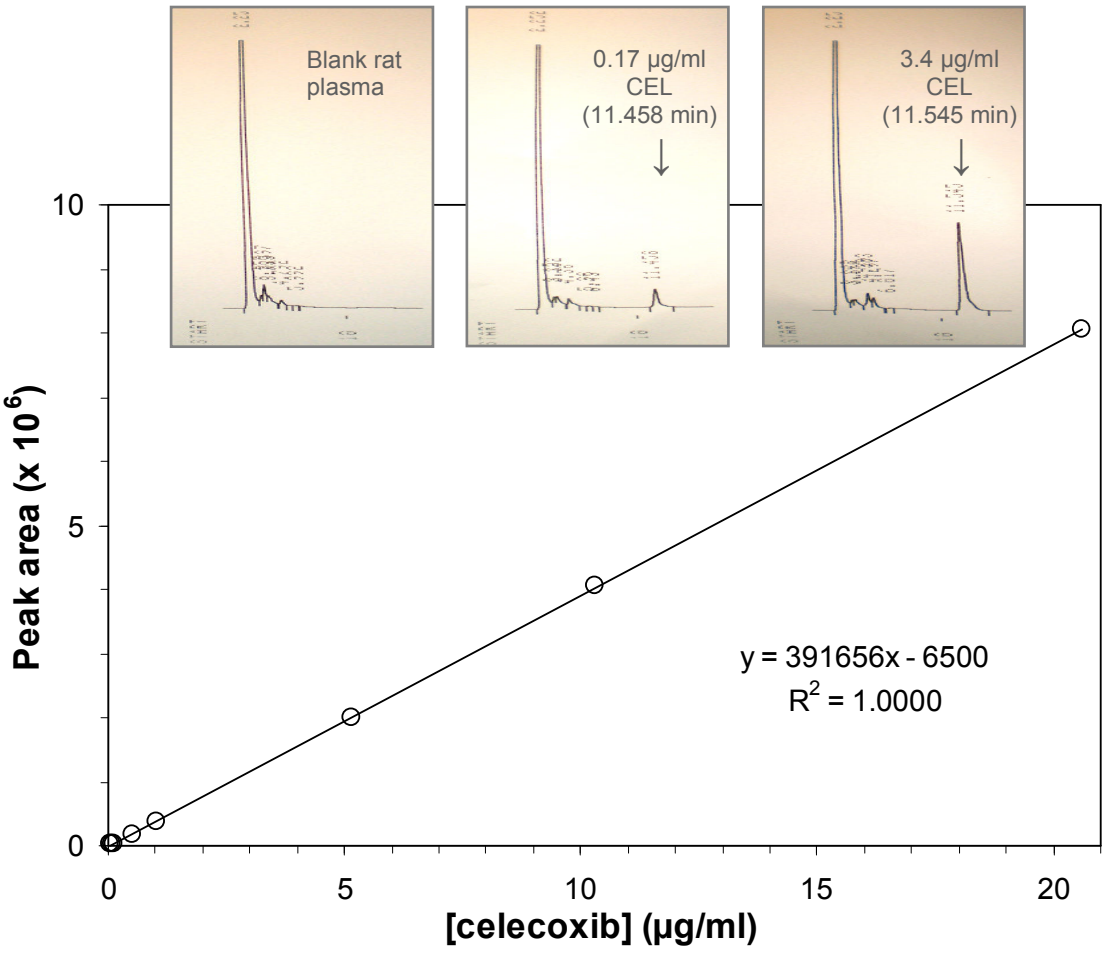


Figure 2.5 A typical calibration curve of celecoxib and chromatograms of rat plasma spiked with celecoxib (mean retention time = 11.5 min).

(b) Precision and accuracy. The precision and accuracy for both intra- and inter-day analyses were within the acceptable analytical limits (*i.e.* <10%) (**Table 2.3a**), even without the use of an internal standard. The high specificity and reproducibility of the assay was further supported by the high extraction recovery (102–108%) of CEL from rat plasma samples (**Table 2.3b**). Repeated analysis of the plasma specimens has shown excellent precision (<3%) in the peak area results. Therefore, the selected HPLC method is highly applicable for CEL quantification in both *in vitro* and *in vivo* studies discussed throughout the thesis.

Table 2.3a Precision (%RSD) and accuracy (%Bias) of celecoxib assay method (mean ± S.E.M., *n* = 6)

[CEL] known (µg/ml)	Intra-day			Inter-day		
	[CEL] found (µg/ml)	%RSD	%Bias	[CEL] found (µg/ml)	%RSD	%Bias
0.082	0.089 ± 0.002	3.2	+8.2	0.090 ± 0.005	6.1	+9.2
1.03	0.991 ± 0.004	0.4	-3.7	1.004 ± 0.015	1.6	-2.48
10.3	10.39 ± 0.051	0.5	+0.9	10.40 ± 0.222	2.1	+0.97

Table 2.3b Recovery of celecoxib from rat plasma (mean ± S.E.M., *n* = 4)

Spiked [CEL] (µg/ml)	Detected [CEL] (µg/ml)	%Recovery	%RSD
0.17	0.174 ± 0.002	102.7 ± 1.3	1.2
1.7	1.808 ± 0.044	106.4 ± 2.6	2.5
3.4	3.677 ± 0.097	108.1 ± 2.9	2.6

2.5.2 Celecoxib solubility in lipids

Assessment of drug solubility in lipids is a key component in pre-formulation screening, considering that the lipid phase is the major reservoir accommodating the poorly water-soluble drugs and therefore determining the final drug payload in a formulation. The screening of CEL solubility in lipids involved two parts: (*i*) equilibrium solubility of CEL in pure oils (*i.e.* Miglyol 812, Capmul MCM and oleic acid), and (*ii*) the effect of solubilisers (*i.e.* lecithin) on lipid solubility.

Among the three types of non-ionic but polar lipids examined, CEL showed the highest solubility in Capmul MCM (a medium-chain MG/DG blend), followed by Miglyol 812 (a medium-chain TG) and oleic acid (a long-chain FA solution) (**Table 2.4**). This solubility trend is comparable to previous literature [208-210]. Based on the solubility data obtained, Capmul MCM and Miglyol 812 are anticipated to confer sensible dose sizes for oral administration in rats (<1 g of powder mass at a minimum CEL dose of 5 mg/kg body weight), therefore these oils were further investigated for the effect of solubilisers on drug solubility.

Table 2.4 Equilibrium solubility of celecoxib in various lipid excipients at 22 °C (mean ± S.E.M., n = 3)

Types of lipids	CEL solubility (mg/g)
Oleic acid (C18 fatty acids)	2.1 ± 0.04
Miglyol 812 (C8/C10 triglycerides)	11.2 ± 0.3
Capmul MCM (C8/C10 mono-diglycerides)	93.4 ± 1.2

Lecithin was tested as a non-synthetic lipidic solubiliser (or termed 'biosurfactant' [232]) for improving the solubility of CEL in various oils. The anionic lecithin has a HLB value of 8 (*i.e.* lipophilic) and is highly miscible with oils. As shown in **Figure 2.6A**, increasing the concentration of lecithin from 0 to 80% enhanced approximately 3-fold the solubility of CEL in Milgyol oil (*i.e.* from 11 to 32 mg/g), in conjunction with the observation that higher lecithin content resulted in higher viscosity of the oil mixture. Mixtures containing up to 50% lecithin appeared to be highly viscous but pourable, yellowish liquid, whereas the 80% lecithin mixture exhibited a non-pourable gel-like texture. Although the underlying mechanism is not fully understood, the increased solubility of CEL in the presence of lecithin is accredited to its surface active and amphiphilic characters. As described by Shchipunov [233], lecithin tends to self-assemble into reversed spherical micellar structures in non-aqueous media; this potentially changes the polarity of the oil medium that favours the accommodation of higher amount of CEL whose solubility in pure triglycerides could be limited by its intermediate lipophilicity (log P = 3.5). Seedher and Bhatia [220] have previously reported that the solubility of CEL and other poorly water-soluble NSAIDs are significantly affected by the physicochemical properties of a non-aqueous solvent, which include polarity, intermolecular interactions, and the ability of the solvent to form a hydrogen bond with the drug molecules.

Following the results in **Figure 2.6B,** the addition of lecithin did not improve the solubility of CEL in Capmul oil. Capmul MCM is an amphiphilic lipid that has been widely used as a surfactant/ emulsifier for oil-water systems [207, 234, 235]; therefore its surface active and self-emulsifying properties is expected to resemble that of lecithin. This plausibly elucidates the subtle effect of lecithin on the solubility of CEL in Capmul oil due to insignificant changes in the medium polarity. Importantly, this solubility data streamlined the selection of oil and emulsifier at desirable ratios for the development of SLH microcapsules: Miglyol oil with lecithin at low and high levels (for achieving feasibly low and high drug loading levels to compare the effect of lipid load on drug absorption as detailed in **Chapter 5**), and Capmul oil with minimum lecithin levels.

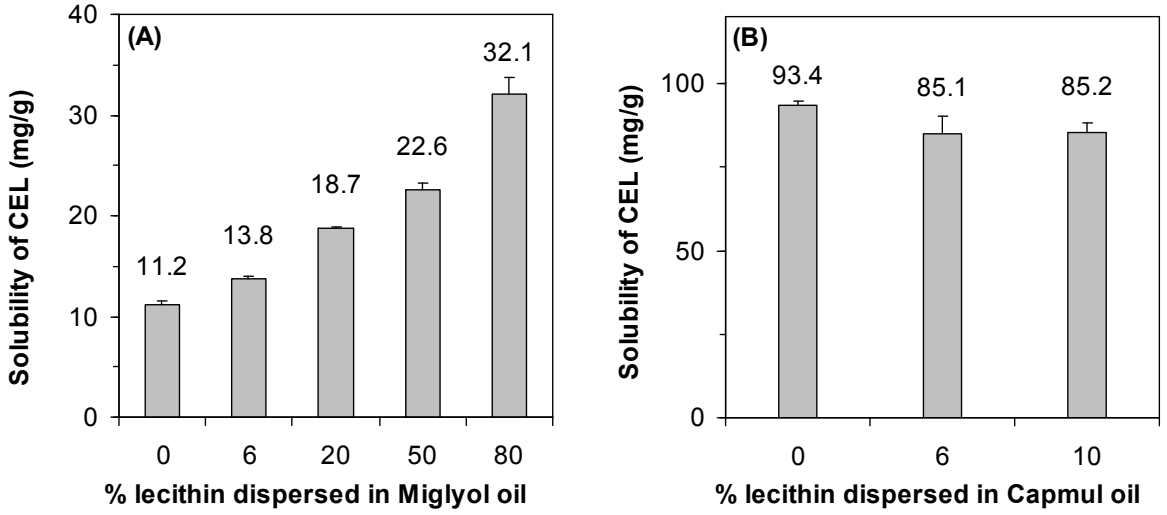


Figure 2.6 The effect of soybean lecithin on the equilibrium solubility of celecoxib at 22 °C in (A) Miglyol oil and (B) Capmul oil (mean ± S.E.M., *n* = 3).

2.5.3 Solid state and colloidal properties of SLH microcapsules

The current formulation technology employs a simple two-step procedure involving emulsion homogenisation followed by spray-drying to generate dry powdery SLH microcapsules encapsulating CEL. Two important formulation parameters that were hypothesized to impart different physicochemical properties to the resultant microcapsules have been systematically examined:

(*i*) the initial phase of silica nanoparticles (*i.e.* in aqueous or in aqueous-and-oil phases), which gives different degree of stabilisation to the precursor emulsions and potentially affects the morphology of the microcapsules; and

(*ii*) the types of lipids (*i.e.* TG versus MG/DG mixtures), which possess varying hydrophilic/ lipophilic character resulting in different interaction with silica particles in the liquid, dry and redispersed states.

Consideration of these compositional factors has led to the fabrication of four types of SLH microcapsules: three Miglyol-based microcapsules designated as SLH-M_1, SLH-M_2 and SLH-M_3; and another Capmul-based microcapsule system named as SLH-C. These SLH microcapsules were compared in terms of solid state morphology and redispersed state colloidal properties as a key indicator of the formulation stability.

2.5.3.1 Surface and internal morphology

The four types of SLH microcapsules developed were prepared from different precursor submicron emulsions as specified in **Table 2.5**. Based on visual observation, all SLH microcapsules appeared as white (in the presence of <1% lecithin) to pale yellowish (>1% lecithin) agglomerated powders. Microscopic observation by SEM showed that the SLH microcapsules prepared from either Miglyol or Capmul oils consist of well separated spherical structures with heterogeneous size distributions ranging from 1–15 μm in the solid state. All four types of SLH microcapsules showed external morphologies that resembled one another. Some microcapsules presented smooth round surfaces while some exhibited concave or indented exteriors, probably resulted from partial collapse of the aggregated silica nanoparticles during spray-drying. In all cases, efficient lipid encapsulation in a closed shell form was successfully achieved at the oil:silica ratio of 1:0.5 (w/w). It is apparent that the initial location of silica nanoaparticles, varying lecithin levels and different types of oils do not exert significant influence on the external morphology of the spray-dried microcapsules.

Further examination of the cross-sectional view of the SLH-M_1 and SLH-M_2 microcapsules reveals that the initial location of silica nanoparticles is not a major factor controlling the internal structures of the resultant microcapsules. As illustrated by the ion beam induced SEM images in **Figure 2.7**, both SLH-M_1 and SLH-M_2 similarly displayed a highly porous

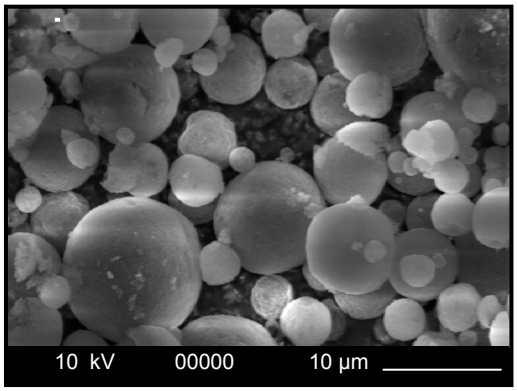
Table 2.5 Solid state surface morphology, particle size distribution and zeta potential of various SLH microcapsules (mean ± S.E.M., *n* = 3)

Formulation	**Solid state SEM images**	**Colloidal properties** (in water)

SLH-M$_1$

Precursor:

Oil	5.00%
Lec	0.30%
Si(aq)	2.50%
CEL	0.05%
Water	92.15%

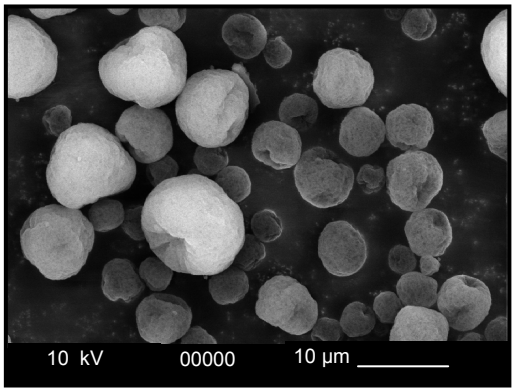


Size (µm)	1 month	12 months
D(v,0.1)	0.136 ± 0.0	0.138 ± 0.0
D(v,0.5)	0.842 ± 0.0	0.802 ± 0.1
D(v,0.9)	2.896 ± 0.0	3.536 ± 0.3
Span	3.28 ± 0.0	4.32 ± 0.6
ζ (mV)	1 month	12 months
Average	-67.5 ± 12.1	-80.1 ± 6.0

SLH-M$_2$

Precursor:

Oil	5.00%
Lec	0.30%
Si(aq)	2.50%
Si(oil)	0.30%
CEL	0.05%
Water	91.85%

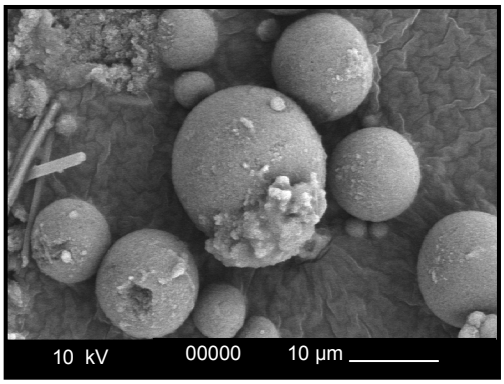


Size (µm)	1 month	12 months
D(v,0.1)	0.125 ± 0.0	0.131 ± 0.0
D(v,0.5)	0.745 ± 0.0	0.766 ± 0.0
D(v,0.9)	3.010 ± 0.0	3.478 ± 0.2
Span	3.87 ± 0.0	4.39 ± 0.3
ζ (mV)	1 month	12 months
Average	-62.3 ± 12.8	-72.6 ± 4.4

SLH-M$_3$

Precursor:

Oil	5.00%
Lec	2.50%
Si(aq)	2.50%
CEL	0.30%
Water	89.70%

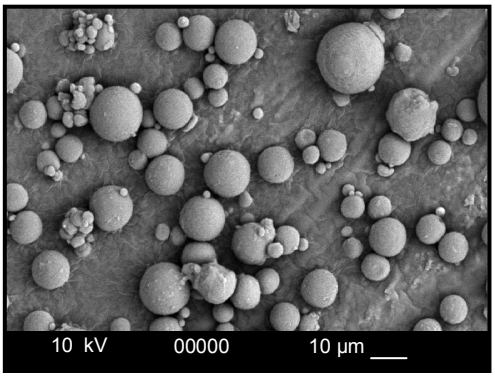


Size (µm)	1 month	6 months
D(v,0.1)	0.107 ± 0.0	0.106 ± 0.0
D(v,0.5)	0.616 ± 0.0	0.485 ± 0.0
D(v,0.9)	3.989 ± 0.0	2.584 ± 0.1
Span	6.30 ± 0.1	5.26 ± 0.1
ζ (mV)	1 month	6 months
Average	-68.6 ± 3.8	-73.1 ± 1.0

SLH-C

Precursor:

Oil	5.00%
Lec	0.30%
Si(aq)	2.50%
CEL	0.45%
Water	91.75%



Size (µm)	1 month	6 months
D(v,0.1)	0.133 ± 0.0	0.131 ± 0.0
D(v,0.5)	0.696 ± 0.0	0.575 ± 0.0
D(v,0.9)	5.665 ± 0.2	4.072 ± 0.4
Span	7.94 ± 0.2	6.82 ± 0.4
ζ (mV)	1 month	6 months
Average	-83.1 ± 13.7	-77.7 ± 3.4

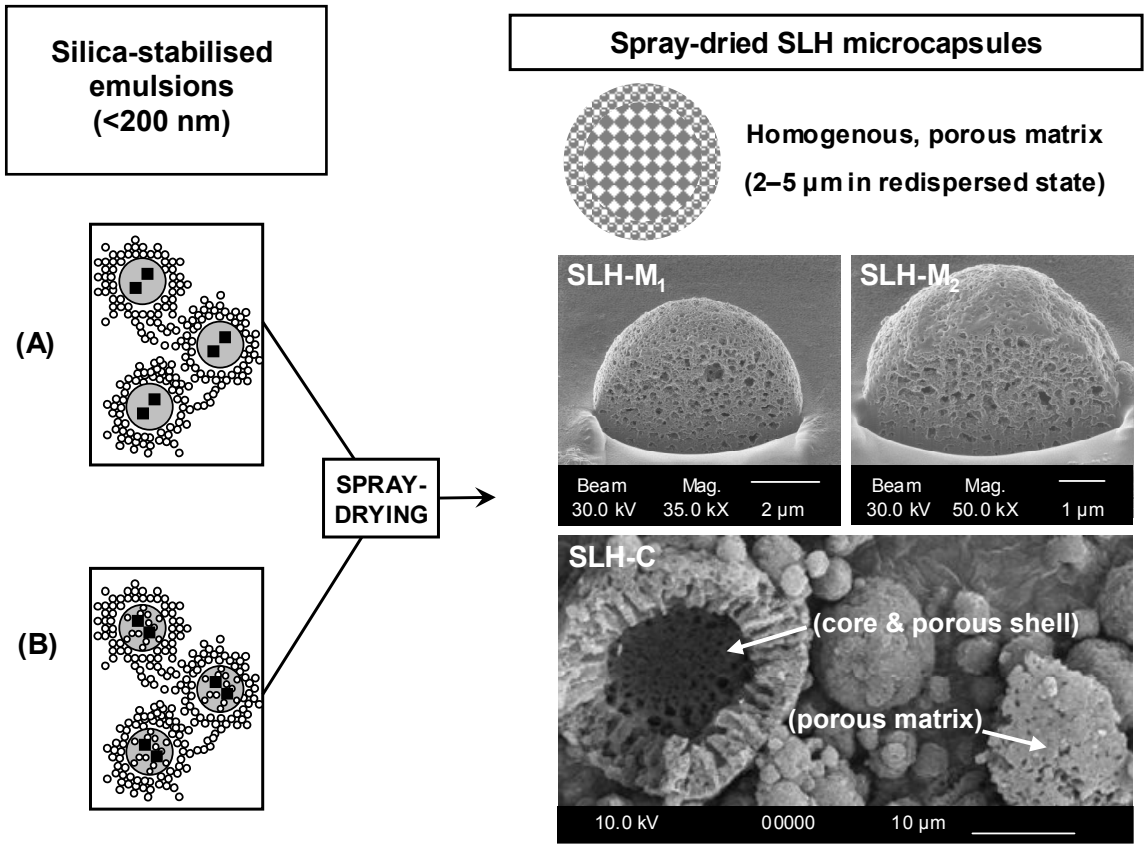


Figure 2.7 The formation of highly porous SLH microcapsules from two different precursor emulsion systems: SLH-M_1 and SLH-C were prepared from emulsion-(A) which contained silica in the aqueous phase; and SLH-M_2 was prepared from emulsion-(B) which contained silica in both aqueous and oil phases. Note that the cross-section images of SLH-M_1 and SLH-M_2 are the ion beam induced secondary electron micrographs, whereas the SLH-C broken microcapsules were imaged by scanning electron microscopy.

internal matrix with pore sizes ranging from 50–200 nm, even though the precursor emulsions were differently stabilised by silica from the aqueous or aqueous-and-oil phases, respectively. Therefore, the hypothesis illustrated in **Figure 2.4** is not evident. The production of core-shell structured SLH microcapsules was not feasible with the Miglyol oil-based emulsions by using the specified spray-drying technology. The process of water removal by spray-drying has seemingly induced redistribution of silica nanoparticles from the continuous phase onto or into the dispersed oil phase. By virtue of its ability to form hydrogen bonds via the silanol functional groups, silica nanoparticles tend to agglomerate into larger units, hence forming the "sponge-like" porous microcapsule structures capable of adsorbing various organic molecules, which are the lipid components in this context. The

formation of such silica-lipid hybrid network is evidenced by a reduction in the BET surface area from 311 m^2/g (for the spray-dried silica) to 184 m^2/g (for the spray-dried microcapsules), compared to the original silica material reportedly having a surface area of 380 m^2/g. The SLH microcapsules generated can be described as macro-porous (≥50 nm in pore sizes), originating from micro-/ meso-porous fumed silica (7 nm in average primary diameters).

Based on the finding that the initial location of silica nanoparticles did not significantly influence the resultant microcapsule structure, the SLH-C microcapsules were prepared from Capmul-based emulsions stabilised by silica nanoparticles from the aqueous phase. Ion beam induced secondary electron images of the SLH-C microcapsules are not available because the samples have yet to be sent and further analysed at the University of Bristol, U.K. The internal morphology of the SLH-C microcapsules was inferred from the high resolution SEM analysis which imaged some broken microcapsules. As seen in **Figure 2.7**, the SEM image shows the co-existence of porous matrix structures (as in the case of Miglyol-based microcapsules) and core-shell structures for the SLH-C system. The reason behind the formation of different structures for Miglyol- and Capmul-based systems is yet to be elucidated; however, it is assumed that the polarity and surface activity of the lipid components does play a role in affecting the resultant internal morphologies. It is noted that such morphological differences between the Miglyol- and Capmul-based microcapsule systems are lack of significance in influencing the *in vitro* lipid digestion and *in vivo* absorption of CEL, which are presented in **Chapter 4** and **Chapter 5**, respectively.

Collectively, the morphological data emphasised that the SLH microcapsules formed are fundamentally different from the existing liquid colloidosomes and polymer-coated dry emulsions, which have been described as core-and-shell systems coated with either nanoparticles or polymers [155, 160, 236-238]. It is interesting to note that the structural design of the SLH microcapsules has showed some degree of similarity to the mixed silk-drug microspheres developed by Wang *et al.* for protein macromolecules delivery [239], as well as the solid polymer-lipid hybrid nanoparticles (PLN) investigated by Li *et al.* for the controlled release of water-soluble drugs, in which the ionic drug-polymer complexes (resembling the drug containing oil-silica composite in the present case) were uniformly distributed throughout the formulation matrices [240]. All these formulations are regarded as novel technologies endowed with the potential of encapsulating different types of therapeutic compounds to meet specific objectives of modified release and absorption.

2.5.3.2 Chemical homogeneity

Specific knowledge on the chemical homogeneity of a formulation is essential to enable predictions of the pattern and consistency of drug release from a carrier system. In this regard, confocal fluorescence microscopy presents a powerful tool for discerning the structural differences of fluorescently tagged compounds by revealing co-localisation of two or more chemicals of interest when appropriate excitation/emission wavelengths are applied. According to previous reports detailing the adsorption behaviours of colloidal silica in equivalent oil-water systems [217, 218, 241-243], one would expect to observe varying structures for the precursor emulsions and spray-dried microcapsules as schematically described in **Figure 2.8**: (*i*) emulsion-A as 'particle-surrounded' or sparsely coated droplets due to weak interfacial adsorption of silica from the aqueous phase; (*ii*) emulsion-B as partially coated droplets resulted from (slow) self-assembly of silica at the interface from bulk oil in conjunction with weak silica adsorption from water; (*iii*) a uniform silica-lipid distribution in the matrix of Miglyol-based microcapsules and (*iv*) a combination of core-shell and uniform silica-lipid matrix for the Capmul-based microcapsules, as informed by the cross-sectioned SEM images described previously. These hypothesized remarks are adequately supported by the confocal images shown in **Figure 2.8**.

The chemical homogeneity is highly dependent on the mobility and interaction of silica nanoparticles with the lipid droplets. In both silica-stabilised liquid emulsion systems A and B, silica nanoparticles tend to equilibrate in their initial locations [218] without absolute partitioning into either phase according to their hydrophilicity. For emulsion-A in which silica nanoparticles were initially dispersed in water, the adsorption of silica at the oil-water interface is restricted mainly by the electrostatic repulsion that exists between the negatively charged fumed/ fused silica nanoparticles and the anionic lecithin-based droplets (at ~pH 7). Therefore silica nanoparticles favourably remained in the continuous phase as a massive flocculated network [241, 242]. In the case of emulsion-B, considerable distribution of silica in both aqueous and lipid phases could be visualised, in which case the lipids and silica particles are heterogeneously mixed. Solid particles (nano- or micrometer sized) are known to possess surfactant-like characters [244]. Therefore, there is a likelihood for the silica nanoparticles to either adsorb to the droplet interface, or reside in the oil bulk phase as enhanced by the hydrophobic emulsifier, lecithin, mainly via reduced droplet interfacial tension and increased nanoparticle wettability in oil (or 'enhanced particle hydrophobicity' as described by Ghouchi Eskandar *et al.* [218]).

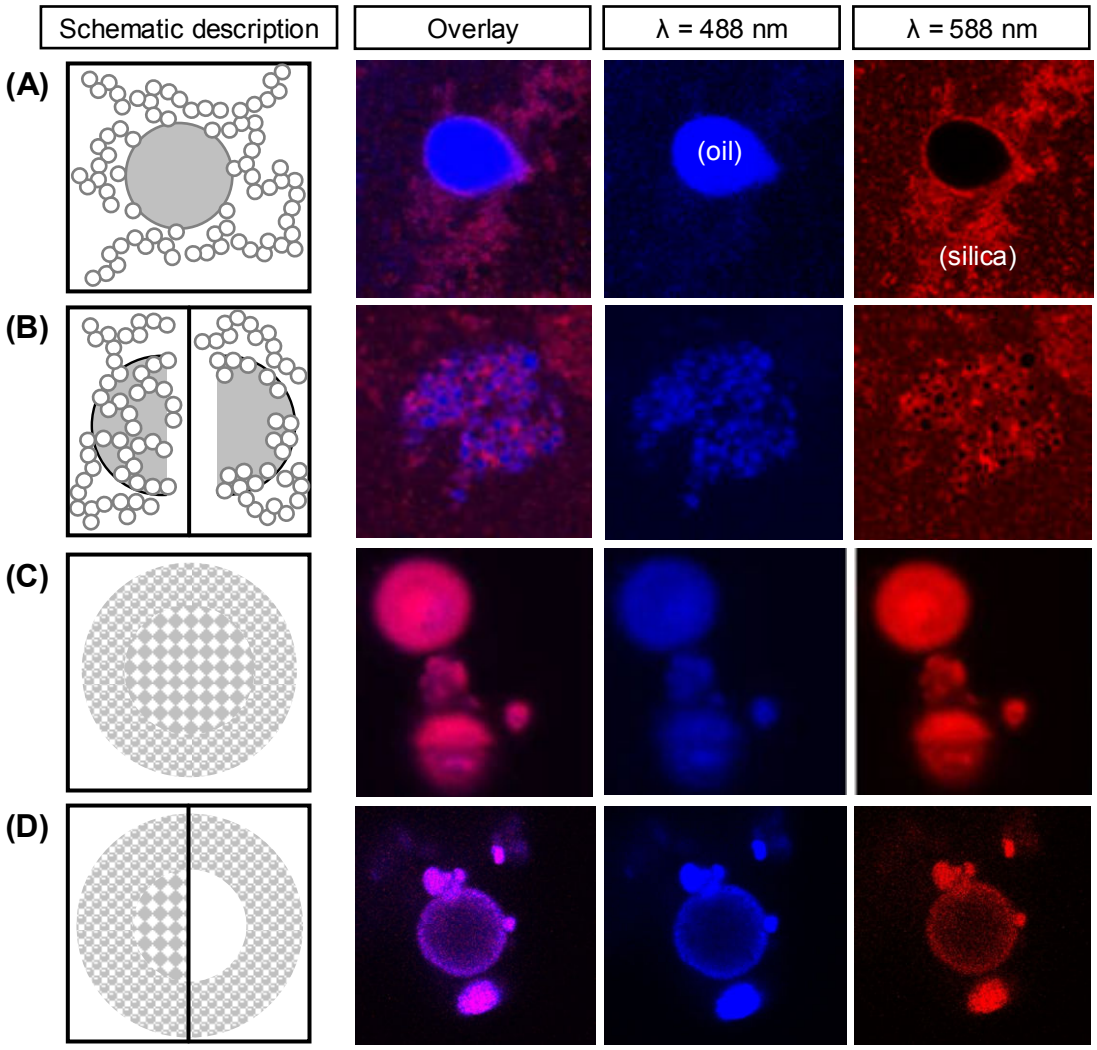


Figure 2.8 Confocal microscopy images of (A) emulsion-A (stabilised by silica from the aqueous phase), (B) emulsion-B (stabilised by silica from both aqueous and lipid phases), (C) spray-dried Miglyol-based microcapsules and (D) spray-dried Capmul-based microcapsules (blue dye is Coumarin 102, red dye is Rhodamine B).

In contrast to the heterogenous structures observed for the homogenised emulsions-A and -B, the SLH microcapsules exhibited homogenous distribution of lipids and silica without a clear separation between these two components. Such structural differences emphasise the importance of the spray-drying process in enhancing excipient interactions (mainly agglomeration) which resulted in the formation of the homogenous hybrids that could not be achieved by high pressure homogenisation alone in the liquid state. It should be noted that the current confocal images do not provide specific quantitative details of the full structure. Nevertheless, the relative localisation of silica nanoparticles and lipids can be

inferred from the fluorescence intensity at specified image depths, which reveals the differences in homogeneity for the systems investigated.

2.5.3.3 Microcapsule redispersibility

Similar to the spray-dried food products such as instant coffee or milk powder, it is desirable that the SLH microcapsule powders can be easily redispersed in water under mild agitation to ensure efficient drug release/ absorption upon oral administration. The ease of powder redispersion was assessed based on the time (10–30 s) and strength of mechanical mixing (*i.e.* whether or not vortex-mixing is required in addition to hand-held shaking) needed to reform suspension in water without lumpy appearances. Careful visual assessments revealed that SLH-C is the most easily redispersible powder (without the need of vortex-mixing) ascribable to the surface-active property of Capmul oil; this is followed by SLH-M_1 and SLH-M_2, which required vortex-mixing for 10–20 s in addition to hand-held shaking; and lastly SLH-M_3 which is relatively more 'sticky' probably because of the higher concentration of lecithin, thus requiring additional ultrasonication (for 10 s) to break the lumps before a homogenous suspension could be formed.

The long-term redispersibility (or shelf-life stability) of the SLH microcapsules was further evaluated based on changes in droplet size and zeta potential after formulation preparation and during storage at ambient conditions. The DLS (or PCS) measured hydrodynamic diameters and PALS determined zeta potentials for the emulsion systems before and after spray-drying are summarised in **Figure 2.9**. In contrast to the precursor submicron emulsions (<0.2 μm, PDI < 0.2), all four SLH microcapsules redispersed to produce polymodal micron-sized particles with an average diameter of 2–3 μm (PDI > 0.5). In all cases, the zeta potentials (ζ) of the anionic emulsion systems remained at >40 mV in magnitude before and after spray-drying. This indicates the existence of sufficiently high electrostatic repulsion between the particles, which resulted in acceptable colloidal stability at pH 7 (*i.e.* a simple simulation of the intestinal condition).

Additional particle sizing by laser diffraction (previously summarised in **Table 2.5**) indicated a relatively larger D(v,0.9) value for Capmul-based microcapsules (4–5 μm) compared to that of Miglyol-based microcapsules (2–4 μm); yet the D(v,0.1) and D(v,0.5) diameters are comparable for all formulations (<1 μm). More importantly, the presence of particles larger than 10 μm was confirmed to be negligible in all four types of microcapsule

systems. Both DLS and laser diffraction analyses consistently revealed a well preservation of the redispersed particle sizes with no significant changes over the study period of 6 to 12 months, thus highlighting considerable redispersibility and physical stability of the dry formulation powders stored in airtight containers at ambient conditions.

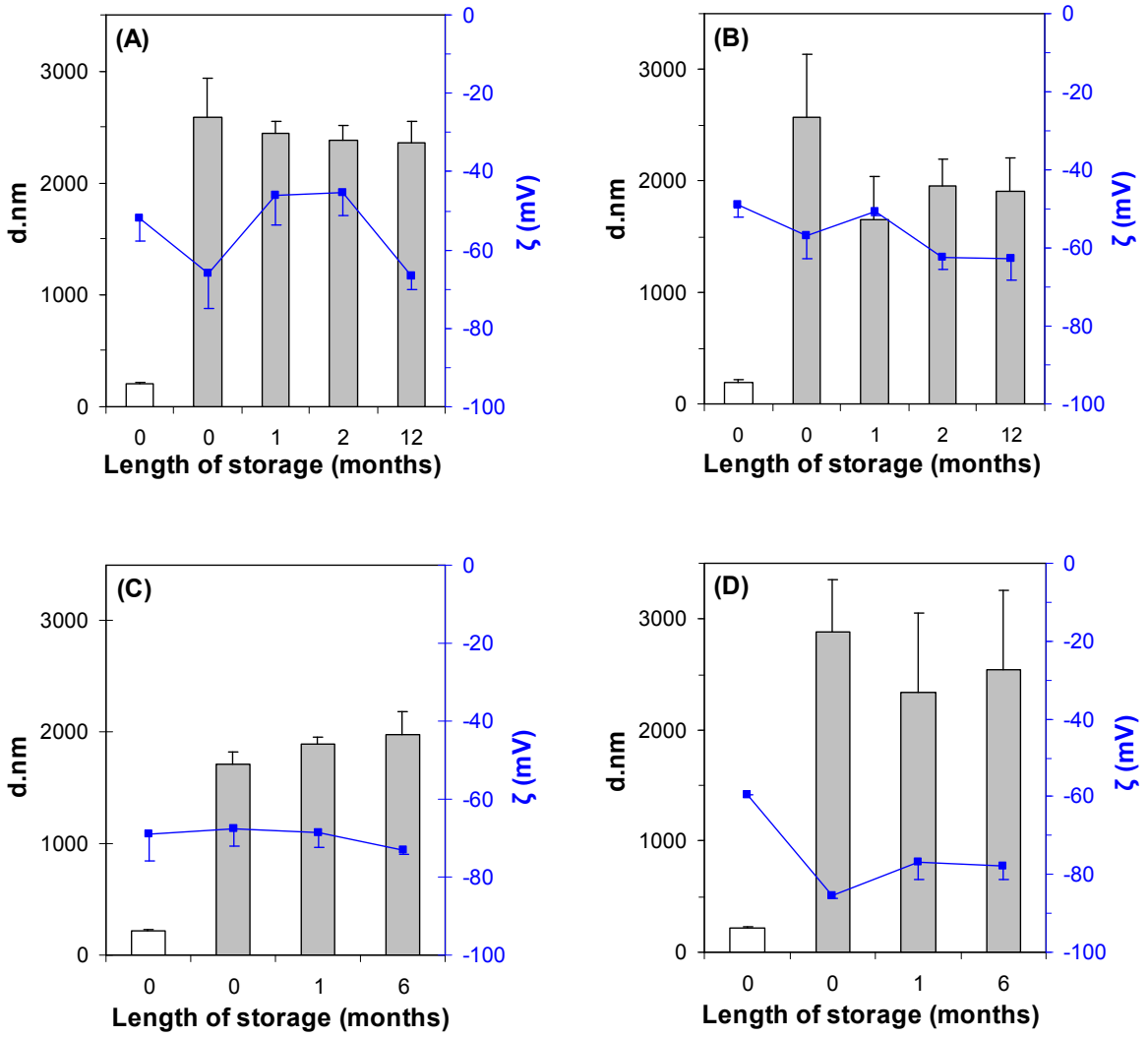


Figure 2.9 The z-average diameters (bar graphs) and zeta potentials (line graphs) of SLH microcapsules redispersed in phosphate buffer (pH 7.2), during storage at ambient temperature: (A) SLH-M_1, (B) SLH-M_2, (C) SLH-M_3 and (D) SLH-C (mean ± S.E.M., *n* = 3). The white bars correspond to the particle sizes of emulsions before spray-drying (PDI values <0.2), whereas grey bars the redispersed particle sizes after spray-drying (PDI values >0.5).

2.5.4 Chemical properties of SLH microcapsules

2.5.4.1 Lipid content

Bearing in mind that the drug of interest is principally encapsulated or solubilised in the lipid core, the loading and recovery level of lipids (mainly oils) in the resultant carrier system is extremely important in ensuring efficient drug delivery and absorption. TG analysis has proved to be valuable in quantifying the total volatile content of a solid formulation [16]. When a known amount of SLH microcapsule powder is heated up to 600 °C, the lipid content (inclusive of oil and lecithin) can be easily estimated from the mass change resulting from liquid evaporation. These values were compared to the theoretical lipid load based on the liquid state emulsion compositions to determine the lipid entrapment efficiency or recovery. It is noted that the theoretical lipid load refers to the maximum achievable lipid content based on the initial mass of non-aqueous ingredients added into the precursor emulsions.

As presented in **Table 2.6**, the lipid content determined for the SLH microcapsules was in the vicinity of 42–62% (w/w), along with an acceptable lipid entrapment efficiency ranging from 65–93%. Among the four types of microcapsules investigated, the lipid entrapment efficiency (*i.e.* lipid recovery) was the highest for the SLH-C system, presumably due to more effective encapsulation of the amphiphilic Capmul oil by the hydrophilic silica nanoparticles. For the Miglyol-based systems, the lipid entrapment efficiency was found to be relatively higher for SLH-M_3 than SLH-M_1, and the lowest for SLH-M_2. Such trend indicates that a higher concentration of the surface active lecithin in the oil phase may enhance the adsorption interactions between Miglyol oil and hydrophilic silica nanoparticles, thereby reducing the risk of lipid loss during the homogenisation and/or spray-drying processes. It is also deduced that the incorporation of silica nanoparticles in the oil phase without increasing the level of lecithin (as in the case of SLH-M_2) does not favour the encapsulation of lipids by the silica nanoparticles; this consequently leads to a higher extent of lipid loss during the preparation procedures.

By combining the lipid load data and the known solubility of CEL in the various oil-lecithin media as detailed in **Section 2.5.2**, it is predicted that the CEL content of the SLH formulations follows the decreasing rank order of SLH-C > SLH-M_3 > SLH-M_1 > SLH-M_2, a trend similar to the lipid entrapment efficiency. If this trend is supported by the drug loading results which are to be discussed in the next section, this would indicate a strong correlation between the lipid recovery and final drug payload in the powder mass produced.

Table 2.6 Lipid load and entrapment efficiency (mean ± S.E.M., *n* = 3)

Formulation	Lipid load (% w/w)		Lipid entrapment efficiency (%)
	(experimental)	(theoretical)	
SLH-M_1	51.95 ± 0.03	67.5	77
SLH-M_2	42.72 ± 0.20	65.4	65
SLH-M_3	62.34 ± 0.24	73.6	85
SLH-C	61.05 ± 0.03	65.4	93

2.5.4.2 Drug content

(a) Drug load and real-time stability. The CEL payload and encapsulation efficiency was evaluated as a function of initial drug fed mass. For the initially screened formulations of SLH-M_1 and SLH-M_2, CEL was loaded according to the equilibrium solubility in Miglyol oil, *i.e.* approximately 1% or CEL:lipid ratio of 1:100 (**Table 2.7**). The HPLC analysis indicated a final CEL load of 0.5% and 0.6% for SLH-M_1 and SLH-M_2, respectively, corresponding to an entrapment efficiency of ≥85%.

Table 2.7 Drug loading level and entrapment efficiencies (mean ± S.E.M., *n* = 3)

Formulation	Initial ratio of CEL:lipid	CEL load (% w/w)		CEL entrapment efficiency (%)
		(experimental)	(theoretical)	
Screened microcapsule formulations (drug dissolved at the maximum lipid solubility)				
SLH-M_1	1:100	0.54 ± 0.01	0.64	85 ± 1
SLH-M_2	1:100	0.62 ± 0.02	0.62	100 ± 3
Optimised microcapsule formulations (drug dissolved above the maximum lipid solubility)				
SLH-M_1	1:10	0.89 ± 0.01	6.02	15 ± 0
SLH-M_2	1:10	0.98 ± 0.05	5.85	16 ± 1
SLH-M_3	1:16	1.81 ± 0.07	2.96	61 ± 3
SLH-C	1:11	3.84 ± 0.03	5.47	70 ± 1

Further investigation was conducted by incorporating CEL in Miglyol oil at a supersaturated state (*i.e.* at concentrations 10 times higher than the equilibrium solubilities determined) via continuous ultrasonication, in which some drugs remained undissolved. With this approach, significantly higher CEL loading levels were obtained for both SLH-M_1 and SLH-M_2 (0.9% and 1.0%, respectively). However, the entrapment efficiency was

dramatically reduced in both cases. To minimise CEL dose dumping in the preparation procedures, CEL was dissolved in Miglyol and Capmul oils by gradual addition above the equilibrium solubilities until the drugs could not be dissolved any further via ultrasonication. This second approach was found to produce sufficiently high entrapment efficiencies (60–70%), in which case SLH-M_3 and SLH-C contained two- and four-times higher CEL loading levels than the earlier formulations. This is partly due to higher CEL intrinsic solubility in the different oil-lecithin mixtures, as well as appropriate saturation of CEL in the mixtures through a serial addition approach. The ranking of the four optimised microcapsule formulations in terms of drug payload is therefore SLH-C > SLH-M_3 > SLH-M_2 > SLH-M_1, where the latter two formulations are in a reverse trend compared to the previous proposal. SLH-M_2 though containing a relatively lower lipid mass, has presented significantly higher CEL load than SLH-M_1. This finding is suggestive of possible CEL adsorption onto silica nanoparticles that were pre-dispersed in the oil phase for SLH-M_2, in addition to drug encapsulation in the lipid cores. Deposition of poorly water-soluble compounds on silica has proved to be efficient by Sanganwar and Gupta [41] who successfully achieved fenofibrate loading of up to 27.5% (w/w) via an air depressurization technique. Taken together, these data imply that both the recovery of lipids and silica component are highly influential in the determining the final drug payload for the SLH microcapsules.

Following optimisation of drug payload, the real-time stability of the encapsulated drug was examined for up to 6 months. **Figure 2.10** shows insignificant reduction in the CEL content for all SLH microcapsules encapsulating both low (<1%) and high (≥1%) drug levels. However, it is noticeable that the CEL content for the optimised SLH-M_2 system (as shown in panel B) has followed a declining trend, with a 16% decrease in the CEL content over the 6-month period. The change may, in part, be caused by instrumental error (*i.e.* up to 10% bias in the HPLC analysis), or it could be due to instability of CEL, considering that some drugs were adsorbed onto silica without being fully solubilised in lipids. Sanganwar and Gupta [41] who studied the adsorption of fenofibrate onto silica materials have found a time-dependent increase in drug crystallisation upon storage; however the authors did not conclude any chemical degradation of CEL in their silica-based systems. Of important note is that, all other SLH microcapsules have demonstrated reasonable real-time chemical stability, hence potentially meeting the aim of improving the shelf-life of a lipidic formulation via a suitable drying technology.

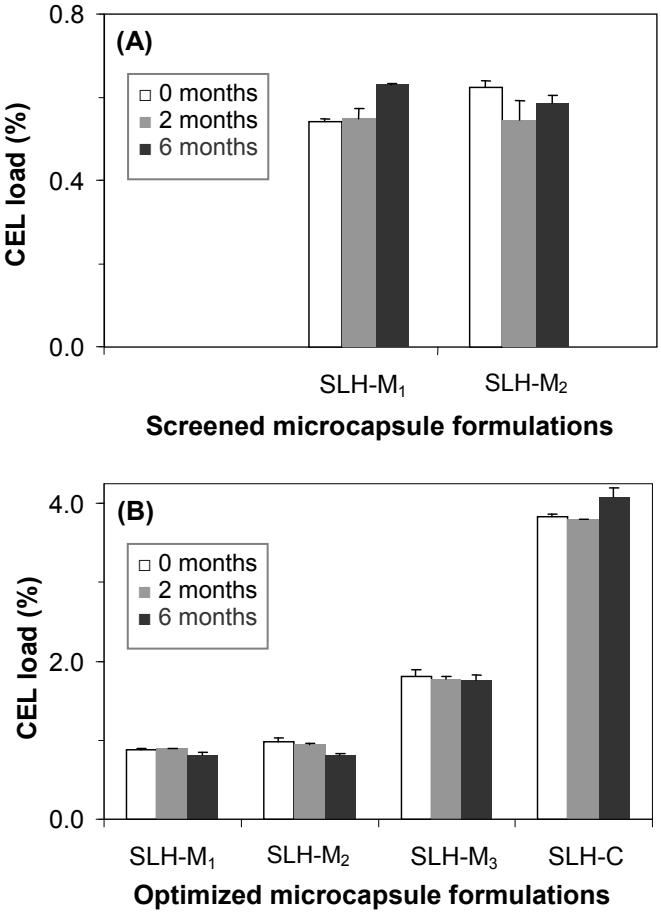


Figure 2.10 The celecoxib content of various SLH microcapsules stored at ambient temperature for up to 6 months: (A) screened microcapsule formulations and (B) optimised microcapsule formulations (mean ± S.E.M., n = 3).

(b) Drug crystallinity. DSC thermograms of pure CEL and various powdery CEL formulations are depicted in **Figure 2.11**, and compared to the data previously reported by Lu *et al*. [37]. It can be seen that pure CEL exhibited a sharp melting endotherm at 164 °C which reflects its original existence in the stable Form I–III crystalline structures. In contrast, Celebrex® capsule materials presented double melting peaks at 165 °C and 149 °C, identifying the co-existence of the thermodynamically stable Form I–III and the novel Form IV crystals according to the description by Lu *et al.* [37]. When pure CEL was physically mixed with silica (at 1% dose), the melting peak at 163 °C (hence presence of polymorph Form I) was retained in addition to the broad endotherm centralised at 100 °C which resulted from evaporation of the moisture content of the physical mixture. The absence of an endothermic peak for all four SLH microcapsule powders (containing 1–4% CEL)

strongly suggested that the encapsulated drugs were molecularly well dispersed in the silica-lipid matrices.

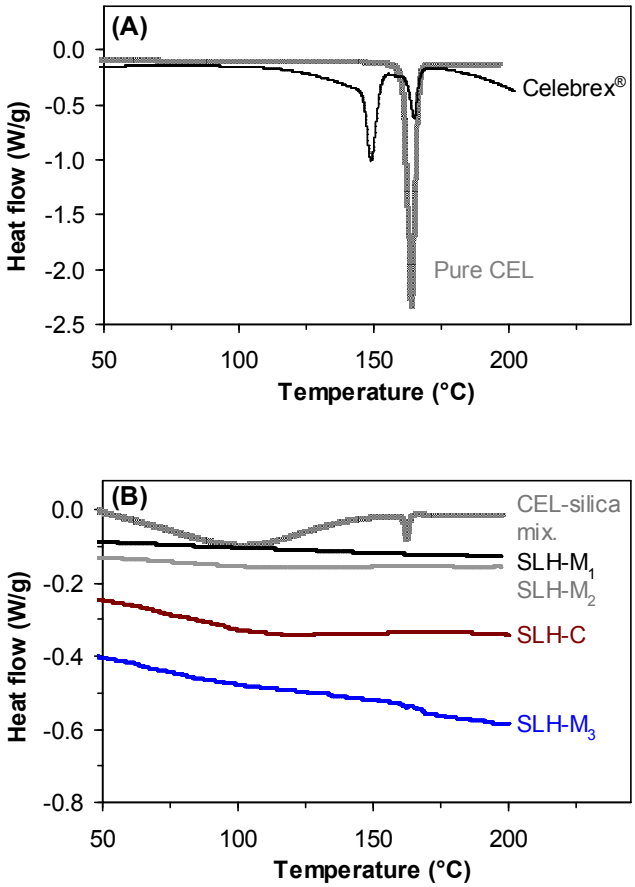


Figure 2.11 DSC thermograms of (A) Celebrex® and pure celecoxib powder, and (B) celecoxib (1%)-silica physical mixture and celecoxib-loaded SLH microcapsules (drug loading levels are approximately 1, 2 and 4% for SLH-M_1/-M_2, SLH-M_3 and SLH-C, respectively) after 6 months of storage at ambient temperature.

The absence of CEL crystals in the microcapsule powder was more specifically confirmed by XRD analysis. As depicted in **Figure 2.12**, the sharp diffraction peaks of crystalline CEL, e.g. at 18.7° and 25.0° (2θ), were not observed in the XRD patterns for the CEL-loaded SLH microcapsules. Therefore, complete amorphisation of CEL was attained in the silica-lipid matrices and this was confirmed for the SLH formulations stored at ambient temperature for at least 6 months.

The DSC and XRD data are useful in predicting the dissolution rate of the various CEL formulations studied, in which the amorphous CEL (present in the SLH formulations) is

expected to dissolve relatively faster than the crystalline CEL; and Form IV crystals (constituting part of Celebrex®) are anticipated to dissolve relatively faster (>2 times as reported by Lu *et al.* [37]) than the thermodynamically stable Form I–III polymorphs (*i.e.* pure CEL). This hypothesized dissolution trend will be proven by the experimental dissolution data presented in **Chapter 3**.

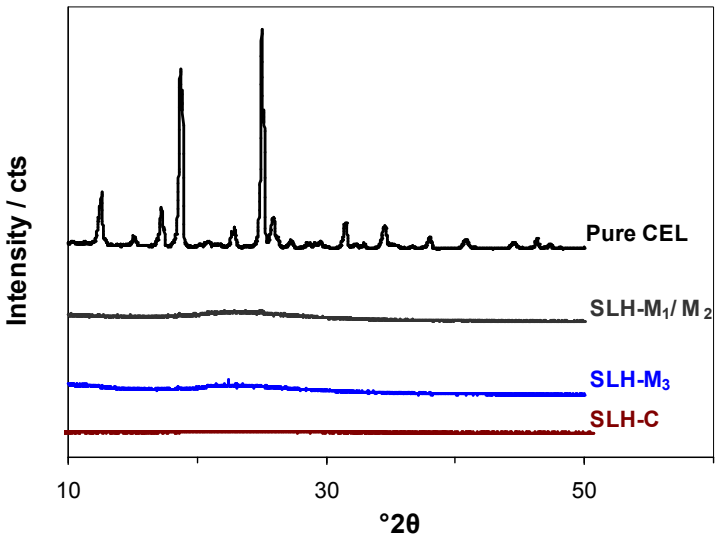


Figure 2.12 XRD profiles of pure celecoxib powder and celecoxib-loaded SLH microcapsules (drug loading levels are approximately 1, 2 and 4% for SLH-M_1/-M_2, SLH-M_3 and SLH-C, respectively) after 6 months of storage at ambient temperature.

2.5.5 Characterisation of control formulation systems

In addition to pure CEL, two other modified formulations of CEL were employed as positive controls for comparison with the newly fabricated SLH microcapsule formulations: (*i*) the marketed product Celebrex® capsules and (*ii*) a dry emulsion system stabilised by water-soluble polysaccharides (*i.e.* maltodextrin).

The product information for Celebrex® lists the following excipients in the capsules: croscarmellose sodium, edible inks, gelatine, lactose monohydrate, magnesium stearate, povidone, sodium lauryl sulphate and titanium dioxide [222]. By using the extraction and HPLC method similar to the one described in **Section 2.4.5.2**, the CEL content of the 100 mg strength capsules was determined to be 36.8 ± 0.14 % (c.f. 40% calculated from the

labelled content). The CEL content of Celebrex® was found to be chemically stable during storage at ambient conditions for 12 months (*i.e.* 36.3 ± 0.16 %).

The maltodextrin-stabilised dry emulsion (designated as MaltoDE) was loaded with an equivalent amount of CEL, Miglyol oil and lecithin as the SLH-M_1 microcapsules, and stabilised at a oil:maltodextrin mass ratio of 1:3 (c.f. oil:silica ratio of 1:0.5) prior to spray-drying under the conditions same as before (**Section 2.4.3**). This produced spherical core-shell dry emulsions (possibly with some small pores present in the dense carrier wall) in which the lipid cores were encapsulated by the water-soluble carrier as depicted in previous literature [154, 155, 160]. The MaltoDE formulation was characterised for the redispersed colloidal properties and drug content as a function of time (**Table 2.8)**. The MaltoDE system was found to be stable when stored at ambient conditions. Comparable to the SLH microcapsules, there were no significant changes in the particle sizes, zeta potentials and CEL content during the 6-month stability analyses. However, the CEL loading level for the MaltoDE system, *i.e.* <0.3%, was significantly lower than that of the SLH microcapsules (0.9–4%). This can be explained by the relatively lower lipid load for the MaltoDE system (24%) compared to that of the SLH formulations (40–60%), because a much higher maltodextrin level is required to form the closed shell microcapsule structure.

Table 2.8 Physical and chemical stability of redispersed maltodextrin-stabilised dry emulsion (MaltoDE) stored at ambient conditions (mean ± S.E.M., *n* = 3)

Storage	Particle size		Zeta potential	Lipid load	Drug load
(months)	*z*-average (d.nm)	PDI	(mV)	(% w/w)	(% w/w)
0	448.7 ± 180	0.408 ± 0.12	-66.3 ± 7.2	24.0 ± 0.1	0.25 ± 0.00
2	593.9 ± 245	0.745 ± 0.22	-53.5 ± 2.6	NA	0.24 ± 0.01
6	877.7 ± 347	0.707 ± 0.19	-49.7 ± 14.7	NA	0.22 ± 0.02

These selected control systems allow a systematic evaluation of various formulation parameters that potentially affect the solubilisation and absorption profiles of CEL in the subsequent performance studies. As summarised in **Table 2.9**, the variables of interest incorporated into the studied CEL formulations include the effect of non-lipidic solubiliser (such as SLS present in the Celebrex® capsules) in comparison with lipid excipients, as well as the effect of hydrophilic silica nanoparticles in manipulating the characteristics of drug release, lipid vehicle digestion and drug absorption.

Table 2.9 Comparison of the variables of interest in the studied celecoxib formulations

CEL formulation	Variables of interest		
	Non-lipidic solubiliser	Lipids/ oils	Silica
Pure CEL	✗	✗	✗
Celebrex® capsules	✓	✗	✗
MaltoDE	✗	✓	✗
SLH microcapsules	✗	✓	✓

2.6 Summary

A novel solid dosage form, silica-lipid hybrid (SLH) microcapsule system with a highly porous internal matrix, has been successfully developed from o/w emulsion templates using a simple two-step approach: high pressure homogenisation followed by spray-drying. The SLH microcapsules were formulated based on medium-chain glycerides (either TG or a MG/DG blend) as the oil phase, soybean lecithin as the emulsifier, and Aerosil silica nanoparticles as the stabiliser and solid carrier; all of which are GRAS-listed substances. Each SLH formulation (2–5 μm in the redispersed state) is comprised of 40–60% (w/w) oil encapsulated by or embedded in a porous Aerosil silica-based matrix at a oil:silica mass ratio of approximately 1:0.5 (w/w). The formation of the macro-porous (50–200 nm), homogenous silica-lipid matrices was not significantly affected by the initial location of silica nanoparticles in the precursor emulsions (*i.e.* aqueous or aqueous-and-oil phases).

The readily redispersible SLH formulations have demonstrated excellent physicochemical stability at ambient conditions for a study period of 6–12 months, in which subtle changes in the redispersed colloidal properties and drug content were evident. DSC and XRD analyses confirmed stable encapsulation of the model BCS Class II drug celecoxib (CEL) in a molecularly dispersed/ amorphous form when stored in airtight glass containers at ambient temperature. In comparison with the ordinary core-shell structured dry emulsions (encapsulated by maltodextrin in the current investigation), porous SLH microcapsules comprised of a high surface area network has offered relatively higher lipid and drug loading levels. The current investigation highlights the potential of the SLH microcapsule system to act as a stable and efficient encapsulating agent for poorly water-soluble drugs.

CHAPTER THREE

In Vitro Assessment of SLH Microcapsules (I): Drug Dissolution/ Release Studies

3.1 Introduction

3.1.1 An overview of drug dissolution/ release studies

Dissolution is the primary requirement for a drug compound to become readily absorbable across the GI membranes and to reach the cellular receptor site for triggering a desirable pharmacological response [15, 59]. However, the process of dissolution is a great challenge for many hydrophobic compounds (or the so-called 'brick dust molecules') when administered orally. Compounds that are practically insoluble in water (*i.e.* aqueous solubility of <0.1 mg/ml) often present dissolution-limited absorption in the gut and this is the key issue hampering the therapeutic use of many novel chemical entities, which mostly fall into the BCS Class II category (poor solubility, high permeability). For BCS Class II drugs, dissolution (or drug release) study is a major component in the process of formulation development. In the context of lipid-based dosage forms where the drugs are often presented in the dissolved state, drug dispersion test is considered to be a more appropriate descriptive term for such analysis [2, 54]. From the regulatory perspective, dissolution tests play an important role as a quality control test for oral solid dosage forms (as officially adopted by the FDA since 1970), and as a predictive tool for bioavailability and bioequivalence justification for modified drug formulations [16]. The guidelines for dissolution tests are documented in the U.S. Pharmacopoeia (UPS) 23 and the later editions, which are regarded as equivalent to the European Pharmacopoeia (EP) and the Japanese Pharmacopoeia (JP) [16].

The USP guideline has listed seven dissolution apparatus comprised of either a beaker-like closed system, a flow-through open system, or a two compartmental system based on dialysis. The application of each apparatus specified for various novel dosage forms (e.g. oral suspensions, chewable tablets, liquid-filled capsules) has been summarised by Siewert *et al.* [245]. Amongst all apparatus, the closed system Apparatus I (the rotating basket designed for floating dosage forms) and Apparatus II (the paddle system intended for both immediate- and modified-release formulations) are the most commonly used [16].

Conventionally, a volume of 500–1000 ml of one of the following dissolution media is recommended for simulating the GI fluids available for dissolving a drug substance (after considering both ingested volume and the paragastrointestinal organ secretions): (*i*) 0.1 N HCl or simulated gastric fluid USP without enzymes; (*ii*) pH 4.5 sodium acetate buffer; and (*iii*) pH 6.8 phosphate buffer or simulated intestinal fluid USP without enzymes [18]. However, these buffer solutions have often failed to provide enough sink conditions for complete dissolution of most poorly water-soluble drugs. As a result, modified simulation of the fasted and fed state environment by adding synthetic or naturally occurring surfactants, milk and exogenous fat has been attempted with significant success in improving the dissolution results of BCS Class II drugs [246]. These fasted and fed state simulated intestinal fluids (*i.e.* FaSSIF and FeSSIF, respectively) have shown to produce better *in vivo* prediction for some modified formulations [247].

In order to develop a meaningful dissolution test, Noory *et al.* [248] suggested that a stepwise trial with different surfactants added at various concentrations is warranted to achieve a dissolution medium with a suitable sink effect (*i.e.* biorelevant) and reasonable discriminating power for the dissolution outcomes. This systematic strategy has served as an important basis for dissolution testings in the FDA field laboratories [16, 248]. Examples of high-purity synthetic surfactants that are regarded as physiologically relevant to the naturally occurring surfactants (e.g. lecithins and bile salts) include sodium lauryl sulphate (SLS), cetyltriammonium bromide (CTAB), and polysorbate (or Tween), which are anionic, cationic and non-ionic in character, respectively [248]. There is no strict rule with respect to the choice of surfactants because various drugs react differently to a given surfactant; but ultimately a medium containing the lowest possible concentration of surfactants (recommended to be ≤2% w/v) that can produce the intended drug dissolution or release profiles (e.g. ≥85% dissolution in 120 min or less) is desirable. The use of hydroalcoholic media sometimes may provide encouraging dissolution results but their *in vivo* relevancy is questionable, which may further complicate the *in vitro-in vivo* correlations [16].

In the case of modified formulations of CEL, the literature gives a wide variation of dissolution methodology, in which blank buffer solutions at both acidic and neutral pH [249], buffered media containing SLS [208, 250] or Tween 80 [39, 251], as well as alcohol aqueous solutions [37] have been used. As shown in **Table 3.1**, the FDA has even recommended an unusual adjustment of the media to pH 12 (*i.e.* out of the gastrointestinal

pH range) in addition to the employment of SLS to produce an acceptable dissolution profile for the CEL capsule products [252].

Table 3.1 FDA recommended dissolution method for celecoxib [252]

Dosage Form	USP Apparatus	Speed	Medium	Volume	Recommended sampling time	Date updated
Capsule	II (paddle)	50, 100, 200 mg: 50 rpm; 400 mg: 75 rpm	Tier 1 medium: 0.04 M tribasic sodium phosphate (pH 12) with 1% SLS Tier 2 medium: initially 750 ml of simulated gastric fluid USP (includes pepsin); at 20 min, while stirring, add 180 ml of appropriate [SLS] solution. Add 70 ml of 1.2 N NaOH to adjust the pH to 12	Tier 1: 1000 ml Tier 2: 750ml (initial) 1000 ml (final)	15, 30, 45 and 60 min	07/15/2009

In this work, we employed the conventional pharmacopoeial dissolution method to investigate the *in vitro* drug dissolution or release (*i.e.* dispersion) performance of various CEL formulations (with or without lipids), then compared to that evaluated using the novel enzyme-mediated drug release study (*i.e.* the lipolysis study as detailed in **Chapter 4**). The current dissolution model uses the simplest possible dissolution medium (containing SLS) which is reasonably close to the original description of the simulated intestinal fluid USP without enzymes (at neutral pH) to characterise the dissolution profiles of various CEL formulations.

3.1.2 Specific aims and hypotheses

The major aims of the present dissolution study were to characterise and rank order the dissolution performance of various CEL formulations, as well as to identify the formulation parameters that potentially affect the dissolution/ release characteristics of CEL: the presence and absence of lipid excipients, the types of solid carriers incorporated (*i.e.* water-soluble maltodextrin vs. insoluble silica nanoparticles), and the degree of crystallinity of

CEL. The lipid-based formulations investigated are the MaltoDE systems, SLH-M_1 and SLH-M_2 microcapsules; whereas the non-lipid formulations tested are pure CEL and the Celebrex® capsule materials.

The hypotheses specifically addressed in this chapter are:

(*i*) CEL in varying polymorphic forms exhibit different dissolution kinetics; and

(*ii*) the SLH microcapsules offer an enhanced drug dissolution profiles due to the solubilising effect of lipids and increased dispersion effect of hydrophilic silica nanoparticles.

3.2 Materials

Sodium lauryl sulphate, SLS (≥99.0% purity) was purchased from Sigma-Aldrich, Australia with the following specifications: M.W. 288.38, aggregation number 62, critical micellar concentration (CMC) 7–10 mM (20–25 °C) and micellar average M.W. 18,000. Other chemicals used for the preparation of buffer solutions (or dissolution media) and in the HPLC analytical work are specified in **Section 2.2** (Chapter 2).

3.3 Methods

3.3.1 Solubility assessment and determination of sink conditions

The equilibrium solubility (C_s) of CEL in phosphate buffer (0.05 M, pH 7.2) containing 0–1% (w/v) SLS was determined by adding an excess amount of CEL (~10 mg/ml) to 10 ml of each medium. The mixture was shaken in a thermostated mixer chamber (37 °C) for 48 h. The equilibrated samples were centrifuged at 9400 *g* for 15 min to remove undissolved materials (*i.e.* excess CEL) and assayed for dissolved [CEL] by using the HPLC method outlined in **Section 2.4.1(a)**. The HPLC calibration curves were re-generated according to the procedures detailed in **Section 2.4.1(b)** by replacing the mobile phase solution (acetornitrile:methanol:water, 50:10:40 v/v, pH 3.5) with acetonitrile:phosphate buffer (50:50 v/v, pH 3.5) in the serial dilution steps. The new buffer solution-based calibration equation was $y = 470532x - 21235$ ($R^2 = 0.9998$) and the analytes were diluted to the calibration concentration range (0.08–10 μg/ml) with acetonitrile, if necessary.

Considering that a 2–5 mg pure CEL sample will be used for the subsequent dissolution studies, the presence of sink conditions was assessed based on the number of folds of saturation volume (FSV) provided by a 900 ml of medium with specified SLS concentrations [253]:

$$\mathrm{FSV} = \frac{C_\mathrm{S}}{[\mathrm{CEL}]\,\text{in the dissolution medium at}\,100\%\,\text{dissolution}} \qquad (5)$$

Sink conditions are achieved if drug concentrations are maintained at or below one-third of the C_s value, *i.e.* FSV ≥ 3 [253-255].

3.3.2 Comparative dissolution studies

Following determination of the FSV values, the dissolution of pure CEL and various CEL formulations were tested under both sink (0.5% SLS, 900 ml) and non-sink (0% and 0.05% SLS, 900 ml) conditions, by using the USP 23 type II apparatus (paddle method) operating at 75 ± 0.02 rpm. Prior to each dissolution test, phosphate buffer solutions (0.05 M, pH 7.2) containing fixed concentrations of SLS were degassed by ultrasonication (set to a degas mode) for 1 h to minimise the formation of bubbles during the dissolution experiments. With the dissolution medium equilibrated and maintained at 37 ± 0.5 °C, each formulation sample containing ~2 mg of CEL (equivalent to the amount used in the *in vivo* absorption studies at a 5 mg/kg dose) was added into the medium and the dissolution process was timed immediately. Aliquots of 3 ml were drawn at fixed time points (up to 4 h) and replaced with an equal volume of fresh (*i.e.* drug free), equilibrated dissolution medium. As before, the drawn samples were centrifuged at 9400 *g* for 15 min to remove undissolved materials (including solid CEL and formulation excipients). The supernatant was subjected to another cycle of centrifugation under the same conditions. An aliquot of 100 µl was taken from the centrifuged samples prior to a two-fold dilution with acetonitrile. Centrifugation was selected for phase separation of the samples because some preliminary experiments showed that most (Millipore) filters adsorb CEL, thus rendering an inaccurate and imprecise result. The amount of CEL dissolved in the dissolution media was analysed by the same HPLC method.

Two dissolution parameters were evaluated for each formulation (without the use of a specific mathematical modelling method): (*i*) the dissolution efficiency (%DE) and (*ii*) the

time to achieve 50% of drug dissolution ($t_{50\%}$). %DE is the area under the dissolution curve between two specified time points expressed as a percentage of the area of the rectangle described by 100% dissolution in the same time intervals, which can be calculated using the following equation [256]:

$$\%DE = \frac{\int_{t_1}^{t_2} y\,dt}{y_{100}(t_2 - t_1)} \times 100 \qquad (6)$$

where y is the percentage of drug dissolved at time t. The $t_{50\%}$ values were inferred from the dissolution-time curves.

3.4 Results and Discussion

3.4.1 Aqueous solubility of celecoxib and selection of dissolution medium

The equilibrium solubility of pure CEL in the presence and absence of surfactants (*i.e.* SLS) is presented in **Table 3.2**. The solubility of CEL increased in a non-linear but seemingly exponential ($R^2 = 0.9494$) fashion with the addition of SLS: a 4-fold higher solubility from 0 to 0.5% SLS, followed by another 15-fold enhancement in solubility with the next 0.5% [SLS] increment.

Table 3.2 Equilibrium solubility (C_s) of pure celecoxib and the number of folds of saturation volume (FSV) in 900 ml of phosphate buffer (pH 7.2) containing different amount of sodium lauryl sulphate (SLS) at 37 °C (mean ± S.E.M., n = 2). The dashed line indicates the position of the reported CMC level of SLS in water

Concentration of SLS (% w/v)	**C_s (µg/ml)**	**FSV**	
		2 mg CEL	5 mg CEL
0	2.1 ± 0.0	0.9	0.4
0.05	2.3 ± 0.1	1.0	0.4
0.1	3.7 ± 0.2	1.7	0.7
0.25	3.2 ± 0.2	1.4	0.6
0.5	8.0 ± 0.4	3.6	1.4
1.0	119.0 ± 29.0	53.6	21.4

A minimum level of 0.5% SLS was sufficient to provide sink conditions for a 2 mg CEL dose (*i.e.* FSV > 3), but not for a 5 mg CEL dose which required higher SLS content to provide sufficient solubilising capacity. Although concentrations of up to 2% SLS have been used for the USP dissolution testing of some highly lipophilic drugs (e.g. flutamide tablets [257]), Noory *et al.* [248] recommended that a medium containing a SLS concentration nearest to the surfactant's CMC values of 7–10 mM (or 0.2–0.3% at 20 to 25 °C) is sufficient for achieving appropriate sink conditions. In the following dissolution tests, media containing up to 0.5% SLS were employed for probing the dissolution profiles of CEL samples at a 2 mg dose.

3.4.2 Dissolution profiles of various celecoxib formulations

Generally, SLS provides much higher micellar solubilisation capacity (or micellar association constant) for the unionised species compared to ionised species for some weakly acidic drugs, including carbamazepine [33], piroxicam [258], and ketoprofen [259]. For this reason, SLS appeared to be a suitable wetting and solubilising agent for maintaining a sink effect for the dissolution of CEL compounds in view of its unionised character (pK_a ~11) at the gastrointestinal pH.

The dissolution profiles of CEL from various formulations were examined in a blank aqueous medium, as well as in the presence of SLS below and above the CMC level (representing non-sink and sink conditions, respectively). As illustrated in **Figure 3.1**, pure CEL powder exhibited the lowest rate and extent of dissolution at all times under both sink and non-sink conditions. This is mainly related to its poor wettability and crystalline poorly soluble character. The addition of SLS (both below and above the CMC level) significantly improved the dissolution profiles of all studied formulations, and effectively differentiating the *in vitro* dissolution performance between pure CEL compound and Celebrex®, and against that of the lipid-based formulations (including MaltoDE and SLH microcapsules). Celebrex® capsules, which contains an unknown amount of solubilising agents (such as SLS as claimed in the product information), has demonstrated an initial fast dissolution in the first 10–30 min followed by a slow dissolution phase propagating to a plateau stage. This dissolution pattern is likewise for the MaltoDE and SLH microcapsule systems, but at a different magnitude depending on the concentrations of SLS. Significantly, the studied

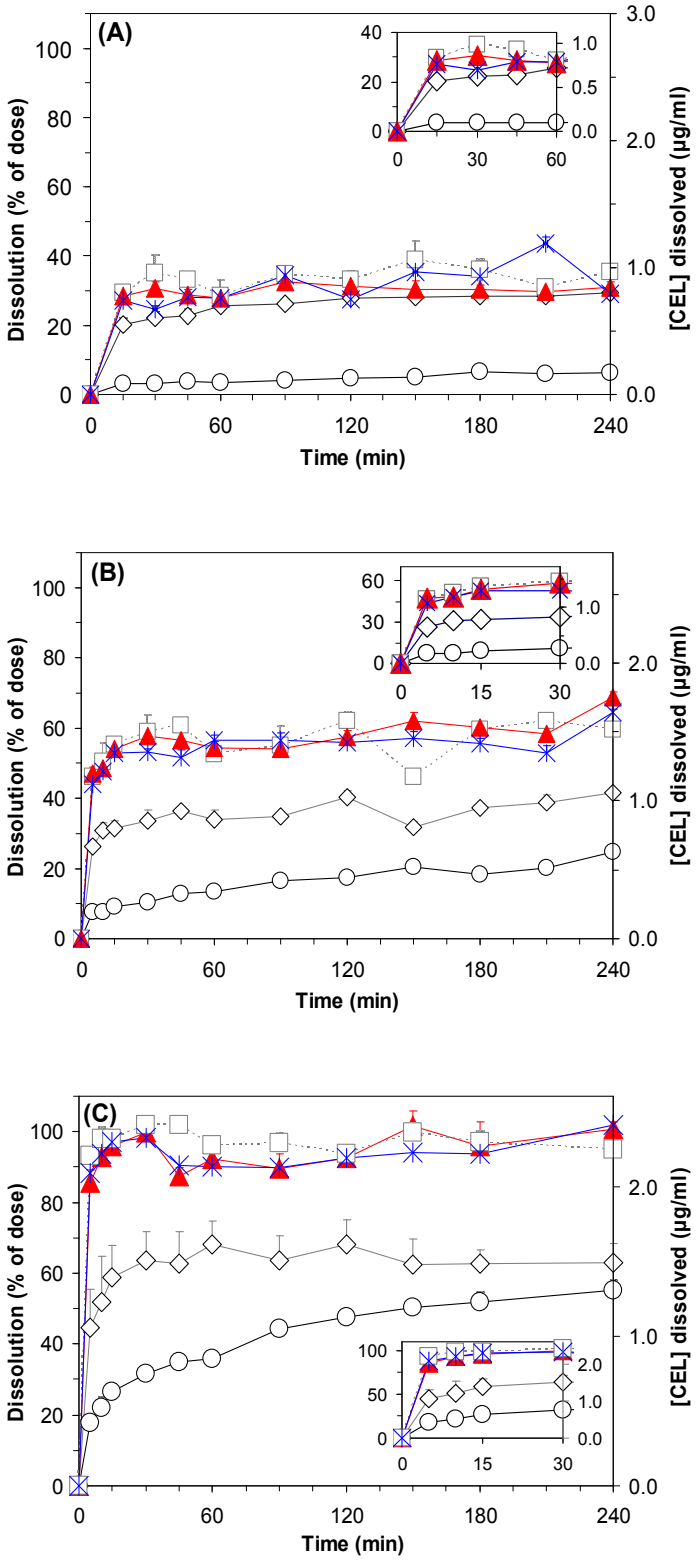


Figure 3.1 Mean dissolution profiles of CEL (2 mg) in phosphate buffer (0.05 M, pH 7.2) containing (A) 0% SLS, (B) 0.05% SLS and (C) 0.5% SLS at 37 °C (mean ± S.E.M., *n* = 3): Pure CEL (○), Celebrex® (◇), MaltoDE (□), SLH-M_1 (▲) and SLH-M_2 (✳). The inset shows the initial phase of dissolution at an enlarged scale for each formulation.

lipid-based formulations, *viz.* MaltoDE and SLH microcapsules were found to produce higher rate and extent of dissolution in comparison with pure CEL and Celebrex®, in which complete drug dissolution was achieved in the former cases under simulated sink conditions.

The dissolution profiles (under sink conditions) were specifically characterised and compared in terms of %DE (at 5, 15 and 180 min) and $t_{50\%}$ values (**Table 3.3**). Taking pure CEL as the primary reference, SLH microcapsules and MaltoDE was found to produce a four- to five-fold increase in %DE at the initial stage (*i.e.* in the first 5 and 15 min), and a two-fold enhancement at the plateau phase of the dissolution process (determined at 180 min), double that observed for Celebrex®. Similarly, the values of $t_{50\%}$ derived from the dissolution curves were substantially reduced for the lipid-based formulations (<3 min); this is followed by Celebrex® (9 min) and pure CEL (145 min). Apparently, both MaltoDE and SLH microcapsules were not significantly different in their drug release profiles regardless of the water-soluble or insoluble character of the solid carriers, which produced different internal morphologies after spray-drying (*i.e.* core-shell versus porous matrix structures). This suggests that the ease of dispersion or hydrophilicity of the lipid carrier system is of prime importance in governing a fast drug release in an aqueous environment. It should be emphasised that both SLH microcapsules and MaltoDE successfully attained more than 85% of drug release within 30 min under appropriately simulated sink conditions, hence meeting the FDA criteria for classification as immediate-release dosage forms [19, 260].

Table 3.3 Dissolution characteristics (with the number of fold of improvement compared to pure CEL) of various CEL formulations in phosphate buffer (pH 7.2) containing 0.5% SLS at 37 °C (mean ± S.E.M., n = 3)

CEL formulation	**Dissolution efficiency (%DE)***			**$t_{50\%}$ (min)***
	t = 5 min	t = 15 min	t = 180 min	
Pure CEL	8.9 ± 1	24.3 ± 5	48.9 ± 3	144.8
Celebrex	22.4 ± 9 (2.5)	55.3 ± 19 (2)	65.3 ± 12 (1)	8.8 (16)
MaltoDE	46.6 ± 1 (5)	98.0 ± 2 (4)	96.6 ± 1 (2)	2.7 (54)
SLH-A_1	42.8 ± 3 (5)	94.4 ± 7 (4)	97.0 ± 5 (2)	2.9 (50)
SLH-A_2	44.2 ± 1 (5)	95.3 ± 7 (4)	93.3 ± 2 (2)	2.8 (52)

* $p < 0.05$ (analysed by one-way ANOVA using the SPSS version 15.0 software)

Furthermore, these results clearly highlight the important roles of lipids and hydrophilic carriers in enhancing drug dissolution or release kinetics, which potentially result in an improved rate and extent of CEL absorption in the intestinal milieu. Two mechanisms were deduced from the dissolution data that accounted for the superior CEL dissolution/ release profiles observed for the SLH and MaltoDE formulations, as compared to the non-lipidic control systems. First, the hydrophilic carriers (explicitly Aerosil silica particles and maltodextrin) facilitated the dispersion of drug-containing lipid droplets in the aqueous medium; this step is probably comparable to Celebrex® in which the dispersion or wettability of CEL was enhanced by the solubilising excipients (e.g. SLS) via a decreased interfacial tension between the drug particles and the dissolution medium [59]. Second, a critical factor distinguishing the dissolution performance of the studied formulations is the molecular state of the encapsulated drugs – either crystalline or amorphous forms [38, 177]. SLH and MaltoDE which encapsulated CEL in a molecularly dissolved state did not require the initial dissolution step as in the case of pure drug and Celebrex®. The initial burst drug release from the lipid-based vehicles (indicated by relatively higher $\%DE_5$ and $\%DE_{15}$) was plausibly a result of the low resistances encountered by the drug molecules when diffused into the surrounding medium across the droplet interface. Complete drug transfer from the oil cores into the aqueous surroundings was driven by the presence of SLS clusters which formed highly solubilising micellar structures capable of hosting the lipophilic drug compounds. Under non-sink conditions, CEL could not fully release from the lipid-based formulations due partly to insufficient micellar formation as a result of possible surfactant consumption by the lipid droplets [18], as well as preferred drug partitioning in the lipid cores. The latter was verified by the distribution coefficient (log *D*) of CEL in Miglyol oil and blank phosphate buffer, which was determined by a simple shake-flask experiment conducted according to the Organisation for Economic Cooperation and Development (OECD) Test Guideline [20]. The analytical log *D* value of 3.7 was close to the reported log *P* value of 3.5 for CEL [208].

It should not be ruled out that different polymorphic forms also contributed to different drug intrinsic solubility which in turn has a significant impact on the dissolution kinetics [16]. In agreement with the findings of Lu *et al.* [37], the 2–3 times faster dissolution rate observed for Celebrex® was possibly an outcome of the metastable Form IV CEL crystals detected in the commercial capsule materials, in comparison with pure CEL primarily consists of thermodynamically stable Form I-III polymorphs. The apparent dissolution trend of SLH

microcapsules ≈ MaltoDE > Celebrex® > pure CEL, which is in accordance with the DSC and XRD profiles, was further evaluated using animal models (**Chapter 5**) and critically analysed for *in vitro-in vivo* correlations (**Chapter 6**).

3.5 Summary

The *in vitro* drug dissolution/ release profiles of various CEL formulations were successfully established using a conventional dissolution model without the use of enzymes. Under simulated intestinal sink conditions, the rate and extent of CEL dissolution/ release could be ranked as follows: SLH microcapsules ≈ MaltoDE > Celebrex® > pure CEL. These results highlight the significant roles of lipids and hydrophilic carriers (*i.e.* Aerosil silica nanoparticles and maltodextrin) in improving the dissolution kinetics of CEL via:

(*i*) an enhanced lipid and drug dispersion in the aqueous medium in which the pre-dissolved drug substance can easily diffuse out of the carrier systems; and

(*ii*) preservation of the encapsulated drugs in a molecularly dispersed/ amorphous form which exhibits a higher intrinsic solubility in comparison with the crystalline forms.

The current dissolution test has provided a reasonable discriminating power between pure CEL, Celebrex® and the lipid-based formulations (including the SLH microcapsules and the MaltoDE system). Further investigation using the novel enzyme-mediated lipolysis method is warranted to verify the superiority between the SLH microcapsules and the MaltoDE system with respect to their drug solubilising capacities in the GI lumen.

CHAPTER FOUR

In Vitro Assessment of SLH Microcapsules (II): Enzymatic Digestion of Various Lipid-based Formulations

4.1 Introduction

4.1.1 An overview of lipolysis study

The application of dissolution models, whether conventional or modified by incorporating various endogenous solubilising species (e.g. bile salts and lecithin), has been limited in predicting the *in vivo* outcome of digestible lipid dosage forms. The main reason is that drug release (and hence intestinal absorption) from a lipid-based formulation is not simply governed by the dispersion or miscibility of the lipid phase with the aqueous medium, but also by the endogenous processing of the lipid excipients which further influences the state of solubilisation and intraluminal transport of the lipophilic drugs [60].

In recent years, studies on the kinetics and dynamics of lipid digestion have become increasingly important for characterising the intraluminal fate of drugs resulting from the digestion of lipid-based dosage forms. The typical experimental set-up of a lipolysis model is described in **Section 1.3.5.2** (Chapter 1). The use of an *in vitro* lipolysis model to probe the intestinal solubilisation patterns of a lipophilic drug (*i.e.* phase partition behaviour of cyclosporine) was first described by Reymond and Sucker about two decades ago [3, 191]. At present, the lipolysis analytical techniques used for characterising the digestion process of various formulation lipids may encompass one or more of the following procedures: (*i*) titrimetric or spectroscopic assay of the free fatty acids (FFA) generated during lipolysis; (*ii*) chromatographic measurement of the digested lipid components such as FFA, MG, DG and TG; (*iii*) drug quantification in the separated digestion phases (*i.e.* oil, aqueous and pellet phases); and (*iv*) microscopic analysis of the sequential changes of the digesting lipids [10-12, 14, 206, 261, 262]. These methodologies have fostered more thorough investigations into the physical and chemical changes of various formulation lipids digested under simulated GI conditions, hence providing knowledge on the selection of appropriate lipid excipients for successful formulation development.

The intestinal phase behaviour of lipid digests is well understood: the biliary secretions (e.g. BS, PL) interact with the lipid digestion products (*i.e.* FA and MG) to generate a series of colloidal species (lamellar/ hexagonal/ cubic liquid crystals, uni-/ multi-lamellar vesicles,

simple/ mixed micelles) that provide varying degrees of solubilising capacity to drugs according to their lipophilicity [61]. These lipophilic microenvironments are important in facilitating drug diffusion across the non-stirred aqueous layer and create a concentration gradient at the absorptive site to promote drug absorption [8, 60] (refer to **Section 1.3.2** for detailed description of these processes). The following formulation parameters have been known to affect the intraluminal solubilisation capacity for drugs: (*i*) lipid properties (including digestibility, acyl chain length, degree of unsaturation and the class of lipid), (*ii*) solubilising excipients (such as surfactants and cosolvents), and (*iii*) the types of formulation (e.g. solution, suspension, emulsion and self-emulsifying system); these have recently been reviewed by Porter *et al* [8]. Due to the diversity of lipid (and surfactant) excipients, as well as their specific and differential effects on gastric emptying, drug solubilisation and absorption pathways, it is difficult to establish distinct criteria for the use of certain lipid formulations for a given drug. Based on many previous publications on drug bioavailability resulting from lipid-based formulations [8, 60], the effect of lipid digestion on bioavailability is concluded to be 'drug-specific', although it has previously been thought that digestion of the lipid vehicles is essential to promote drug release and absorption in the gut. Myers and Stella [148] have suggested the prime importance of emulsification in governing drug release and absorption from lipidic carriers, regardless of the digestibility nature of the formulation lipids. The reason for this is that an emulsified system can either provide an increased surface area available for digestion in the case of digestible lipids, or for drug release in the context of non-digestible lipids. With respect to the effect of lipid acyl chain length, it has generally been assumed that LCT, which is digested more slowly, may be more appropriate for encapsulating drugs with higher lipophilicity ($\log P > 5$), mainly due to slower drug transfer into the aqueous environment, greater swelling capacity associated with the long-chain lipolysis products, and their potential in stimulating lymphatic drug transport [8, 60]. For drugs exhibiting lower lipophilicity ($2 < \log P < 4$), MCT is considered as a more suitable choice of lipids; this is because MCT is digested relatively faster than LCT, which leads to more rapid formation of the medium-chain solubilising species that potentially support drugs of lower $\log P$ characters in a supersaturated state [8, 60].

Considering our current model drug, CEL, which is an intermediate lipophilic compound ($\log P = 3.5$), medium-chain digestion products are expected to provide an adequately solubilising environment for the drug molecules with a minimised risk of precipitation. In

the current lipolysis study, we employed a rigorous lipolysis model to investigate the digestion patterns of a series of medium-chain lipid vehicles, namely lipid solutions, submicron lipid emulsions (in the presence and absence of silica nanoparticles), the MaltoDE capsules, and the four types of SLH microcapsules fabricated from different precursor emulsions (**Chapter 2**). The current lipolysis kinetics and phase partition studies are essential to provide a comparative solubilisation result in addition to the dissolution (or drug release) data obtained in **Chapter 3**. By combining the dissolution and lipolysis results, we are able to gain further understanding on the mechanisms of drug release from the SLH microcapsule systems, as well as to predict the *in vivo* outcome of the studied formulations.

In this chapter, we report the first conclusive results on the effect of (hydrophilic) silica nanoparticles on the enzyme-mediated digestion of various emulsified lipid-based systems. It has long been known that silica particles are chemically stable and act "stealthy" in biological environments [174, 183]. Therefore, colloidal silica have found great applications in physically stabilising microemulsion systems, and the adsorption behaviour of silica nanoparticles at the oil-water interfaces have been well characterised previously [217, 218, 241, 263, 264] (see **Section 2.1.1.3**). Yet the questions remain whether (*i*) the presence of silica nanoparticles can confer chemical stability to lipid emulsions under digesting conditions, and (*ii*) how different silica distributions at the oil-water interface can affect the stability of lipid emulsions against enzymatic degradation. Due to the hydrophilic and "stealthy" properties of Aerosil silica nanoparticles, it was expected that (either weak or strong) adsorption of silica nanoparticles at the oil-water interface could provide a physical shield around the lipid droplets (or reduce the area of the bare oil-water interface [265]), thereby reducing the potential of interfacial adsorption and hydrolytic activity of the lipase enzymes. Such control in lipid digestion is essential in manipulating the release of drugs into the GI aqueous environment with a minimised risk of precipitation.

4.1.2 Specific aims and hypotheses

The current lipolysis experiments involve two parts: analysis of the lipolysis kinetics and determination of the phase partition behaviour of CEL during lipid digestion. In the lipolysis kinetics study, the key focus was to investigate the various factors that potentially control the digestion pattern of a series of medium-chain lipid vehicles: the types of oils (*i.e.*

MG/DG mixture versus the corresponding TG analogue), the effect of emulsification, and the inhibitory/ enhancing effect of various emulsion stabilisers (*i.e.* lecithin, silica nanoparticles which are water-insoluble, and maltodextrin which is water-soluble). The lipolysis kinetic data in conjunction with the drug phase partition results are useful in indicating the role of lipid digestibility in enhancing intestinal solubilisation or precipitation of CEL.

The specific hypotheses of the current investigation are:

(*i*) emulsified lipid systems are digested relatively faster than the non-emulsified lipid substrates;

(*ii*) the presence of the water-insoluble Aerosil silica nanoparticles exerts an inhibitory effect on the lipolysis of lipid emulsions, hence controlling the release of CEL into the lipolysis medium;

(*iii*) the presence of the water-soluble maltodextrin exerts a negligible effect on the digestibility of lipid formulations;

(*iv*) the SLH microcapsules facilitate a more controllable rate and extent of lipolysis in comparison with the lipid solutions and emulsions, hence producing a controlled release of drugs into the aqueous medium; and

(*v*) the lipolysis test (including both lipolysis kinetics and phase partition studies) is a useful tool for probing the mechanisms of drug release from the SLH microcapsule systems.

4.2 Materials

Materials used for the preparation of various test formulations and for the HPLC drug assay are detailed in **Section 2.3**. Chemicals used for the lipolysis study include sodium taurodeoxycholate (NaTDC) 99%, Trizma maleate, type X-E L-α-lecithin (approximately 60% pure phosphatidylcholine, from dried egg yolk), porcine pancreatin extract (activity equivalent to 8 x USP specification), 4-bromophenylboronic acid (4-BPB), calcium chloride dehydrate and sodium hydroxide pellets, which were purchased from Sigma-Aldrich (Australia). The titration solution of 0.6 M NaOH was diluted from a 1 M NaOH stock

solution (Titrisol, Merck, Germany). All other chemicals were of analytical grade and used as received. High purity (Milli-Q) water was used throughout the study.

4.3 Methods

4.3.1 Preparation of the lipolysis medium

The fasted state lipolysis medium was prepared according to the protocols developed by previous researchers [12, 13, 93]. The procedures include the preparation of a standard buffer solution (pH ~7.5), a BS/PL mixed micellar solution, and a pancreatic enzyme solution. The level of each lipolysis component in the experiments was chosen according to the likely concentration range encountered in the human fasted state duodenal contents (**Table 4.1**).

Table 4.1 The experimental level of each lipolysis component in comparison with the likely concentration range encountered in the fasted state human duodenal contents

Lipolysis component	Concentration/ level	
	Experimental value	**Physiological range**
Bile salts	5 mM	5.9 ± 1.8 mM [80]
Phospholipid: bile salt molar ratio	1: 4	1: 4 [266]
Pancreatic lipase/colipase activity	1000 TBU/ml*	500 TBU/ml [80]
Ca^{2+}	5 mM**	0.5 ± 0.2 mM [267]
Na^{+}	150 mM	142 ± 13 mM [267]
pH	7.5***	7.1 ± 0.6 [267]

* Tributyrin units (TBU) are defined as the amount of enzymes required to produce 1 μmole of titratable fatty acid per min. Lipase/colipase was added in excess (but within the physiological range) to ensure efficient lipid digestion without being restricted by the enzyme activity

** A high calcium level was employed to activate the enzymes and to remove lipolytic products from the droplet surfaces via the formation of Ca-fatty acid soaps, hence favoring the equilibrium towards the ionised fatty acids which allows lipolysis to continue [10]

*** The chosen experimental pH was slightly higher than the reported physiological value to ensure a high degree of fatty acid ionisation (pK_a 6.8 for medium chain fatty acids [268]) for efficient titration throughout the lipolysis process

(a) Standard lipolysis buffer solution. The standard buffer solution (pH 7.50 ± 0.05) consisted of the following components, which were sequentially dissolved in Milli-Q water using a magnetic stirrer in a standard volumetric flask:

Trizma maleate	50 mM (or 11.860 g)
$CaCl_2.2H_2O$	5 mM (or 0.735 g)
NaCl	150 mM (or 8.766 g)
NaOH	adjusted to give pH 7.5 (or 2.5–2.6 g)
Milli-Q water	up to volume (1000 ml)

(b) Fasted state mixed micellar solution. The fasted state mixed micellar solution was prepared in the following sequence in order to produce a resultant BS:PL concentration of 5 mM:1.25 mM in the reaction mixture after enzyme additions: (*i*) egg lecithin (80.7 mg) was dissolved in chloroform (3–4 ml), and the chloroform was evaporated under vacuum (Rotavapor-RE, Buchi, Switzerland) to form a thin film of lecithin on the walls of a 50 ml round-bottom flask; (*ii*) NaTDC (130.4 mg) and the standard lipolysis buffer (45 ml) were added to the flask; and (*iii*) the mixture was stirred at ambient temperature for ~12 h (using a magnetic stirrer) to produce a transparent, light yellowish micellar solution. The mixed micellar solutions were kept refrigerated (2–8 °C) and used within two weeks from the day of preparation.

(c) Pancreatin extract. Pancreatin extracts (containing pancreatic lipase, colipase and other non-specific lipolytic enzymes such as phospholipase A_2) were freshly prepared each day by stirring 1 g of porcine pancreatin powder in 5 ml of standard lipolysis buffer for 15 min (using a magnetic stirrer), followed by centrifugation at 1600 *g* (5 °C) for 15 min. The supernatant phase was collected and stored on ice until use.

4.3.2 Lipolysis studies

The lipolysis experiments were conducted according to the titrimetric and digestion phase separation methods developed by Sek *et al.* [12, 13], which were based on previous methodologies [93, 269].

(a) Lipolysis kinetic studies by pH-stat titration. The progress of lipid digestion was monitored for 45–60 min by using a TitraLab® 854 pH-stat titration apparatus (Radiometer Analytical, France), which consists of a jacketed glass reaction vessel (X51T030, 15–45 ml) connected to a water bath regulated by a thermoregulator heater and pump, a pH electrode (pHC2401-8) and an auto-burette (TIM854) (see **Figure 1.7** in Chapter 1 for the instrumental set-up).

In each lipolysis experiment, a known quantity of lipid formulation (at a dose of 100 mg oil per 10 ml of lipolysis volume) was initially dispersed in the buffered micellar solution by stirring continuously (using a magnetic stirrer) for 10 min in the jacketed glass reaction vessel thermostatted at 37 °C. The pH of the lipolysis medium was re-adjusted to 7.50 ± 0.01 with either 0.1 M NaOH or HCl solution. With the reaction mixture kept under constant stirring throughout the experiment, lipolysis was initiated by adding 1 ml of pancreatin extract per 10 ml of lipolysis volume to produce ~1000 TBU of pancreatic lipase activity. As lipolysis proceeded, FFA produced in the reaction vessel was immediately titrated with 0.6 M NaOH via an auto-burette to maintain a constant pH in the digestion medium at the pre-set value. The associated TitraMaster 85 Software recorded the cumulative volume of NaOH titrant dispensed from the auto-burette (at a 5 s intervals), and also monitored the pH profiles throughout the lipolysis experiments. The data was exported into a Microsoft® Excel spreadsheet for further analysis.

Blank lipolysis experiments (for micellar solutions) were performed in the same way without the addition of exogenous formulation lipids. To compensate for the pH changes resulting from environmental factors (e.g. absorption of atmospheric carbon dioxide) and additional FFA produced by components other than the studied formulation lipids (e.g. TG/ DG content present in PL, and degradation of the pancreatin extracts), the titrant volume for micelle samples was subtracted from that of the studied lipidic samples. The consumption of NaOH (after background correction) was used to calculate the number of moles (n) of FFA liberated based on a 1:1 stoichiometric reaction ratio:

$$n(\text{FFA})\ (\text{mole}) = \text{volume}\ (\text{NaOH})\ (\text{L}) \times \text{concentration}\ (\text{NaOH})\ (\text{M}) \qquad (7)$$

The values of n(FFA) were then used to quantify the magnitude of lipolysis, with the assumption that one mole of TG is completely hydrolysed to form 3 moles of FFA in the presence of high enzyme concentrations (~1000 TBU) during the 60 min lipolysis period

(c.f. 100% FFA titrated for MCT in 30 min by assuming 2 moles of FFA and 1 mole of MG produced from the hydrolysis of a TG molecule [262]):

$$\text{Lipid hydrolysis, H (\%)} = \frac{\text{n(FFA)}}{3 \times \text{n(TG)}} \times 100 \qquad (8)$$

Whilst most published work has focused on comparison of the extent of lipolysis or the post-digestive drug phase partitioning ratios [93, 104, 170, 197, 270, 271], reports on detailed kinetic analysis of the lipolysis process of formulation lipids are scarce [14, 71, 272, 273]. The enzyme-mediated lipolysis can be described as a pseudo first-order reaction because the reaction rate is strongly dependent on the concentration of the lipid substrates, while the concentration (*i.e.* activity) of the lipase-colipase enzymes is expected to remain constant throughout the reaction due to their presence in excess. Therefore,

$$[\text{Lipolysis product}] = k_0 [\text{lipid substrates}] [\text{enzymes}] = k [\text{lipid substrates}] \qquad (9)$$

where k_0 is the first-order reaction rate constant, and $k = k_0$ [enzymes] is the pseudo first-order reaction rate constant.

The lipolysis processes were determined to be mono-, bi- or tri-phasic reactions depending on the types of lipid formulation; these are presented in **Section 4.4**. Correspondingly, mono-, bi- or tri-exponential equations [equation (10)] were fitted to the data as appropriate using curve stripping and an iterative procedure to determine the best exponential fit.

$$y = y_1 e^{-k_1 t} + y_2 e^{-k_2 t} + \ldots \qquad (10)$$

From this, the rate constants (k_1, k_2 …) characterising the lipolysis processes were obtained.

(b) Phase partition studies by ultracentrifugation and HPLC analysis. The phase distribution of CEL was examined during the lipolysis process of selected formulations (particularly SLH-M_1, SLH-M_3 and SLH-C) and compared to that of the blank micelle samples. At time 1, 5, 15, 30 and 60 min, aliquots of 1 ml of lipolysis samples were collected into individual Beckman polyallomer tubes prefilled with 10 µl of 4-BPB (0.5 M in methanol) equilibrated at 37 °C. 4-BPB was used as an enzyme inhibitor to stop the lipolysis process in the collected samples. Each collected sample was separated into an upper undigested oil layer, a dispersed aqueous phase and a pellet phase by ultracentrifugation (Beckman XL-80 Ultracentrifuge coupled with a 50 Ti rotor, USA) at the maximum achievable 181,000 *g* for 45 min (37 °C). The separated lipolysis samples

were processed as follows: the aqueous phase was diluted with acetonitrile (to meet the CEL calibration concentration range of 0.08–10 μg/ml) and centrifuged at 1600 *g* for 15 min prior to HPLC analysis as described in **Section 2.4.1(a)**; the pellet fraction was dissolved in a chloroform:methanol (2:1 v/v) mixture and acidified with 100 μl of 1 M HCl before being transferred into a 5 ml volumetric flask, followed by the same dilution and centrifugation steps prior to HPLC analysis. It is noted that isolation of the undigested oil film was not practically feasible due to the low oil content of <10 mg per ml of the digestion sample, therefore the drug content in the oil phase was not determined.

4.3.3 Morphological examination of the digested samples

The structural integrity and external morphology of the post-digested samples of SLH microcapsules, namely SLH-M_1 and SLH-C, have been evaluated from the images obtained using a freeze-fracture scanning electron microscope (Philips XL 30 FEG-SEM with an Oxford CT 1500 cryo transfer system). Prior to imaging, each sample was processed in the following sequential steps: cryo-fixation, fracturing, etching and platinum coating. Each post-digested sample was deposited onto a flat copper specimen holder mounted with a "split" brass tube, and cryo-fixed by rapid cooling with liquid nitrogen (-196 °C). The frozen specimen was then transferred into the specimen exchange chamber of the system (-150 °C and 10^{-6} Torr) in which the brass tube was splitted with a single-edge scalpel blade pre-cooled to -150 °C. The ice on the sample surface was removed by sublimation (with the temperature increased to -95 °C for ~2 min). The fractured and etched sample was sputter-coated with platinum (~2 nm) for conductivity prior to SEM imaging.

4.4 Results and Discussion

4.4.1 Comparative lipolysis kinetics of various lipid-based vehicles

Lipase-mediated digestion is known to be self-catalytic due to continuous interfacial binding of the lipase enzyme promoted by the paired amphiphilic cofactor (colipase), despite the desorption effect caused by bile salts [8]. Theoretically, both the rate and extent of lipolysis are greatly dependent on the accessibility of the enzymes to the substrates, or the binding affinity of a lipid substrate to the enzymes at the open active or catalytic site [274, 275]. The susceptibility to digestion is therefore significantly affected by the surface

properties of a lipid substrate. The current work investigates the influences of four formulation parameters on the lipolysis characteristics of various medium-chain lipid vehicles: (*i*) the types of oils (TG versus MG/DG mixtures), (*ii*) the significance of emulsification, (*iii*) the effect of a lipidic excipient (*i.e.* lecithin), and (*iv*) the effect of two non-lipidic excipients (silica nanoparticles vs. maltodextrin). Out of these variables, our major interest was to investigate the role of Aerosil silica nanoparticles in influencing the digestibility of lipid-based formulations. This information provides further mechanistic knowledge on the enhancement effect of SLH microcapsules in drug release and absorption.

4.4.1.1 Lipolysis of Miglyol-based systems

(a) Lipolysis-time profiles

Figure 4.1(A) depicts the percentage of lipid digestion as a function of time for the control systems: pure Miglyol oil, lipid solution (in which lecithin was pre-dispersed at a lipid:lecithin mass ratio of 1:0.06, similar to all other formulations except SLH-M_3), homogenised lipid emulsion (mean hydrodynamic diameter = 0.17 ± 0.01 μm), and the MaltoDE system (mean hydrodynamic diameter = 0.88 ± 0.35 μm). The inset shows the pseudo first-order kinetic fits of the lipid hydrolysis process for each studied system.

Under simulated fasted intestinal conditions, pure Miglyol oil and lipid solution were digested near to completion in 60 min at a relatively slow and constant manner, in which the entire process can be well described by a monophasic first-order kinetic model ($R^2 > 0.9$). In contrast, the emulsified systems (*i.e.* bare lipid emulsion and MaltoDE) have demonstrated a biphasic reaction, initiated with a fast lipolysis phase followed by a time-dependent reduction in the lipolysis rate after the first few minutes. Despite the differences in the initial lipolysis rates, the bare emulsion and lipid solution were not significantly different in the ultimate extent of lipolysis (*i.e.* almost 100% at 60 min). However, the MaltoDE system was digested up to only ~70%.

When compared to lipid emulsions stabilised by Aerosil silica either from the aqueous or lipid phase (mean hydrodynamic diameter = 0.17 ± 0.01 μm) (**Figure 4.1B**), the biphasic kinetics was also detectable as in the case of the bare emulsion, but a reduction in the extent

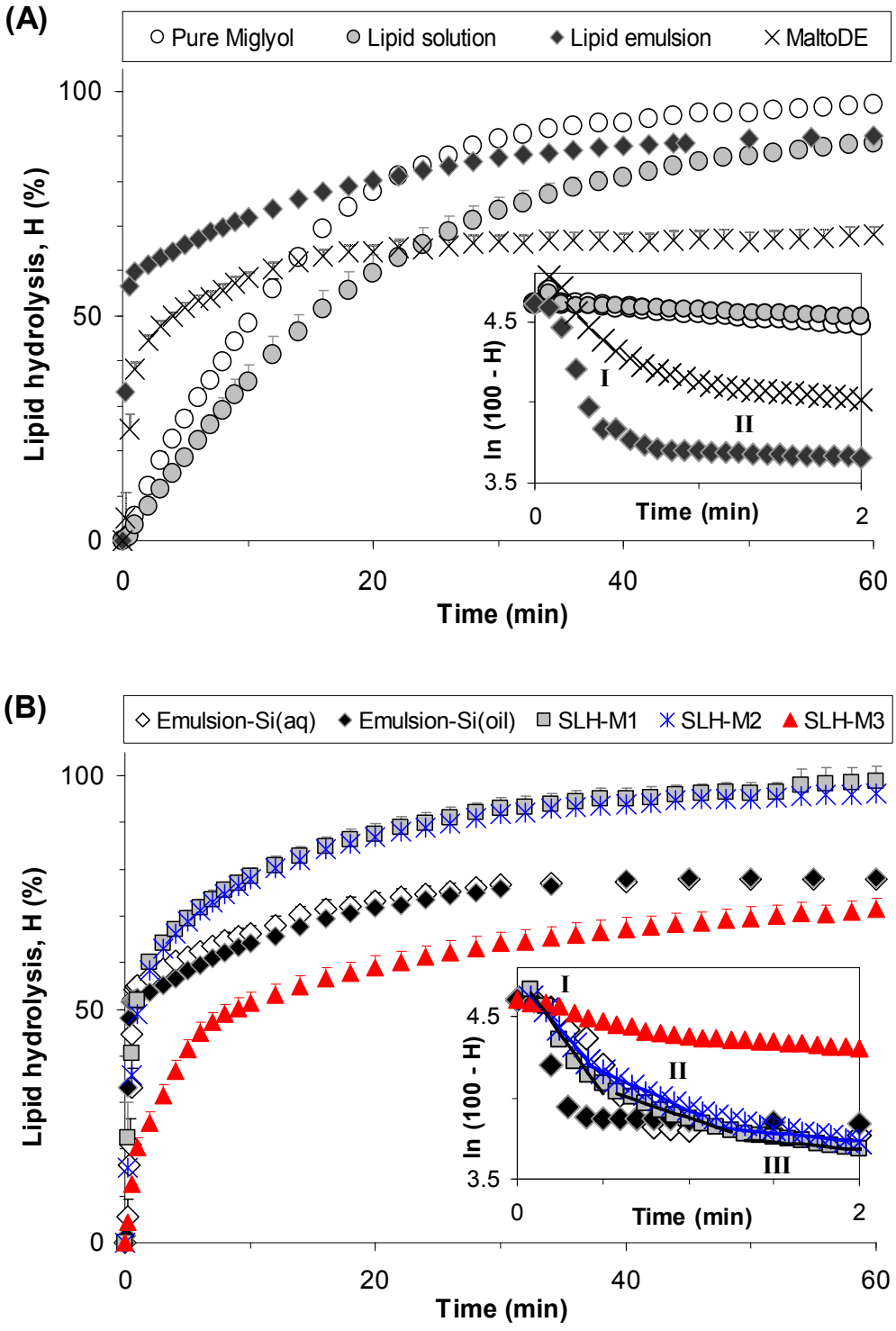


Figure 4.1 Time dependence of the enzyme-mediated lipolysis for various Miglyol oil-based systems under simulated fasted intestinal conditions at 37 °C (mean ± S.E.M., *n* = 3). The insets show the pseudo first-order descriptions of the lipolysis process for each formulation which can be described by monophasic, biphasic or triphasic kinetics.

of lipolysis was noticeable for the silica-stabilised emulsions. Surprisingly, such inhibitory effect exerted by silica in the submicron emulsion systems was not observed for SLH-M_1 and SLH-M_2 microcapsules. Regardless of the larger particle sizes (mean hydrodynamic diameter = 2–3 μm), both SLH-M_1 and SLH-M_2 systems exhibited an equally fast lipolysis with significantly higher magnitude (~100% at 60 min) than the silica-stabilised submicron emulsions. The inset in **Figure 4.1B** clearly revealed a triphasic reaction in the early phase for these SLH formulations. In contrast, the SLH-M_3 microcapsules, which contain a higher lecithin level in the oil phase, demonstrated a greater retardation in both the rate and extent of lipolysis, in comparison with the silica-stabilised emulsions. The contribution of various factors towards an enhanced or inhibited lipolysis of the studied formulations is discussed in further detail based on the lipolysis characteristics derived below.

(b) Lipolysis characteristics

The lipolysis characteristics of each formulation are evaluated based on (*i*) the lag time, *i.e.* the period of time elapsed between the time of enzyme addition and the time of detecting lipid hydrolysis; (*ii*) the pseudo first-order hydrolysis rate constant (*k*), which was calculated employing data points of every 5 s up to 10 min; and (*iii*) the maximum percentage of lipid hydrolysis obtained at the end of each experiment (*i.e.* H_{60min}). These parameters are used as an indirect measure of the susceptibility of each formulation to enzymatic degradation. The results are summarised in **Table 4.2**.

The detection of a lag phase is common for lipase/colipase-mediated hydrolytic reactions, mainly due to the required activation processes involving enzyme interfacial penetration and adsorption before they become catalytically active at the exterior of the insoluble substrates [269, 275]. The comparison of the lag phase duration data has highlighted the importance of emulsification (*i.e.* reduction in the initial droplet sizes) in increasing the substrate susceptibility to enzymatic degradation: the emulsified systems such as the lipid emulsions and SLH microcapsules (with the exception of MaltoDE system) have shown a markedly reduced lag time (*i.e.* 2–4 fold lower) than the non-emulsified systems, including oil (and lecithin) solutions. There are also several other findings [271, 275, 276] confirming that the rate of lipid digestion increases as a function of decreasing droplet sizes of lipid substrates. The role of initial emulsification in facilitating gastric and duodenal lipolysis rate has been proven physiologically by Armand et al. [90] who pointed out that fine emulsions (0.7 μm) offer a ~5 times higher total surface area accessible to lipase than coarse emulsions

(10 μm) in the human stomach; this renders a relatively higher overall rate and extent of lipolysis for the fine emulsions (~73%) compared to the coarse emulsions (~46%) over a 4 h period. However, the overall fat absorption measured over a 7 h period was insignificantly different for these two emulsions. This indicates that a faster digestion may not necessarily lead to a faster absorption of the lipid products, therefore the effect of lipolysis rate on drug absorption needs to be interpreted with care.

Table 4.2 Lipolysis characteristics of various Miglyol oil-based systems: lag phase duration, initial hydrolysis rate constant (*k*), and the maximum percentage of hydrolysis (H at 60 min) (mean ± S.E.M., *n* = 3)

Lipid systems	Lag phase duration (s)	*k* (min^{-1} x 10^{-1})			H_{60min} (%)
		Phase I	Phase II	Phase III	
Miglyol oil	**17.5 ± 7.5**	**0.75** (R^2 = 0.996)	—	—	**97.1 ± 0.8**
Lipid solution (oil:lecithin = 1:0.06)	**22.5 ± 7.5**	**0.45** (R^2 = 0.998)	—	—	**88.5 ± 0.4**
Lipid emulsion (170 ± 15 nm)	**5.1 ± 0.0***	**20.9** (R^2 = 0.970)	**0.41** (R^2 = 0.999)	—	**90.0 ± 0.2**
MaltoDE (878 ± 347 nm)	**15.0 ± 2.9**	**8.6** (R^2= 0.954)	**0.40** (R^2 = 0.951)	—	**68.0 ± 2.1****
Silica(aq)-stabilised emulsion (172 ± 14 nm)	**10.0 ± 2.9***	**12.2** (R^2 = 0.968)	**0.33** (R^2 = 0.988)	—	**77.8 ± 0.5****
Silica(oil)-stabilised emulsion (168 ± 15 nm)	**5.1 ± 0.0***	**31.7** (R^2 = 0.985)	**0.32** (R^2 = 0.998)	—	**81.7 ± 0.0****
SLH-M_1 (2384 ± 222 nm)	**10.0 ± 0.0***	**13.1** (R^2 = 0.967)	**3.0** (R^2 = 0.978)	**0.82** (R^2 = 0.991)	**98.8 ± 3.3**
SLH-M_2 (1955 ± 422 nm)	**6.7 ± 1.7***	**11.0** (R^2 = 0.994)	**3. 5** (R^2 = 0.986)	**0.89** (R^2 = 0.980)	**96.2 ± 1.5**
SLH-M_3 (1981 ± 206 nm)	**7.5 ± 2.5***	**2.6** (R^2 = 0.964)	**0.58** (R^2 = 0.963)	—	**71.5 ± 2.3****

* statistically lower than lipid solution ($p < 0.05$, one-way ANOVA)

** statistically lower than lipid solution ($p < 0.01$, one-way ANOVA)

Analysis of the initial rate constants (*k*) and the maximum extent of lipolysis (H_{60min}) evidenced clearly the inhibitory role of lecithin and maltodextrin in the lipolysis process. It can be seen that even a low level of lecithin (i.e oil:lecithin = 1:0.06 w/w) caused significant

reductions in the *k* values (from 0.075 min^{-1} to 0.045 min^{-1}) and the H_{60min} value (from 97% to 89%) in comparison with the pure oil (**Table 4.2**). The lipid emulsion was digested much faster initially ($k_{phase\ I}$ = 2.09 min^{-1}), most probably because of the greater interfacial area available for lipase/coliapse binding; but the reaction was quickly retarded to a rate and extent resembling that of a lipid solution after reaching 50–60% of lipolysis. The fact that lecithin and other phospholipids function as potent inhibitors for lipases of diverse origin (e.g. pancreas, plasma) has been proven at least four decades ago [277, 278]. It was specified in the later research [274, 279] that lipase/colipase complexes have a greater selectivity towards TG and simple/ mixed micelles formed from lipids and BS, but less activity towards liquid crystalline bilayers or vesicles formed by lecithin. Another significant finding is that, maltodextrin (which is a reducing sugar) may act as a lipase inhibitor similar to some basic polysaccharides (e.g. chitosan, modified polydextrose), which have been shown to inhibit lipase activities in a dose-dependent manner [280]. For this reason, the MaltoDE system was found to produce the lowest extent of lipolysis among all the tested formulations, although it was initially emulsified at a low lecithin level (oil:lecithin = 1:0.06 w/w) and produced reasonably small particle sizes (<1 μm) upon redispersion in water.

Based on the fact that silica nanoparticles are relatively 'inert' or stable against biological reactions, we hypothesized a protective effect against enzyme-mediated lipolysis for the silica-stabilised systems. This was confirmed in the case of submicron emulsions stabilised by silica from both aqueous (oil:silica = 1:0.5 w/w) and oil phases (oil:silica = 1:0.06 w/w), in which a ~10% reduction in the H_{60min} data was detected with subtle changes in the reaction kinetics (*k*) (**Table 4.2**). However, the SLH-M_1 and SLH-M_2 systems prepared at an equivalent oil:lecithin:silica ratio of approximately 1:0.06:0.5 (w/w) have shown a different lipolysis behaviour in which a complete digestion was observed. Both SLH-M_1 and SLH-M_2 were digested in a three-phase manner comprised of 'phase I' – an intermediate *k* value slightly lower than that of the lipid emulsions; followed by 'phase II' – a short 'transition rate' when lipolysis reached ~50%; and 'phase III' – where the reaction proceeded to completion with a *k* value similar to that of pure Miglyol oil. It is clear that SLH-M_1 and SLH-M_2 were not significantly different from one another in their lipolysis characteristics despite the different initial locations of silica nanoparticles; likewise for their physicochemical and dissolution properties as presented in **Chapter 2** and **Chapter 3**, respectively. Our interpretation of these findings is that silica nanoparticles in the dispersed

state may (weakly or strongly) self-assemble at the oil-water interface and provide a physical shield around the lipid droplets [218]. As such, silica nanoparticles impede the access of enzymes for lipolytic activity. When silica nanoparticles are processed into a porous cross-linked network with a high surface area (*i.e.* 184 m^2/g as detailed in **Section 2.5.3.1**), it is plausible that the lipid substrates are adsorbed onto the silica matrices in an orderly manner with an 'open access' for lipid release or enzyme entry. This underlines the need for specific investigations on the influence of different porosities and internal structures on the lipolysis of SLH microcapsules to be carried out in the future. Recently, Aranberri *et al.* [281] have investigated the factors involved in controlling the porosity of macroporous silica materials (pore sizes ranging from 5 to 87 μm) generated by evaporation of solid-stabilised emulsion templates: the volatility and content of oil, the hydrophilicity and concentration of particles, and the emulsion type of the initial template. It is of our further interest to explore the effectiveness of various approaches (e.g. pre-controlling the initial particle sizes prior to drying [281]) in producing SLH microcapsules with more well defined and controllable porosity by using the spray-drying technique.

On the other hand, the SLH-M_3 microcapsules have shown different lipolysis kinetics to that of the silica-stabilised emulsions, and that of the SLH-M_1 and SLH-M_2 microcapsules. In this case, the lipids were digested at a slower pace in which the 'phase I' observed for SLH-M_1 and SLH-M_2 microcapsules could not be identified. We propose that such a reduced rate and extent of lipolysis is a consequence of the high lecithin content in this formulation (oil:lecithin:silica = 1:0.5:0.5 w/w). Previously, Pieroni and Verger [279] have systematically investigated the inhibitory effect of lecithin in mixed triglyceride-lecithin films and confirmed that lipase shows specific activity towards triglycerides, but the lipase activity decreases linearly with increasing lecithin levels. They also reported that in the presence of colipase, lipase can bind to lecithin films but do not exert its normal hydrolytic activity. These findings provide a reason to elucidate the retarded lipolysis of SLH-M_3 microcapsules, in which a high amount of lecithin that resides at the oil-water interface has potentially formed a competitive binding site for the lipase/colipase complexes; this consequently reduced the accessibility of the enzyme complexes to the 'hidden' triglyceride cores. This is an interesting mechanism in which the inhibitory role of lecithin dominates the positive porosity effect of the SLH microcapsule systems in producing a more controllable rate and extent of lipolysis.

4.4.1.2 Dose-dependent inhibitory effect of Aerosil silica on lipolysis

The dose-dependency of lecithin inhibition on the lipolysis of lipid droplets is well understood [279]. In order to increase our knowledge of the lipolysis inhibitory effect of Aerosil silica in a dose-dependent manner, we examined the digestion kinetics of silica-stabilised lipid emulsions by varying the concentration and initial location of the dispersed silica nanoparticles. Homogenised submicron emulsions (mean hydrodynamic diameter ~200 nm) were freshly prepared at the same oil:lecithin mass ratio as before (1:0.06), with Aerosil silica dispersed either in the aqueous phase (0–80% relative to the oil content) or lipid phase (0–6% relative to the oil content). Each homogenised and silica-containing system was equilibrated for at least 3 h prior to lipolysis kinetic studies.

(a) Incorporation of silica nanoparticles from the aqueous phase

The lipolysis-time profiles for the emulsions stabilised by silica nanoparticles incorporated from the aqueous phase are presented in **Figure 4.2**, with the corresponding lipolysis characteristics (H values) summarised in **Table 4.3**.

As the concentration of silica nanoparticles is increased from 0 to 50% (relative to the oil content), there is a small but systematic reduction (5–10%) in the H_{45min} values. Theoretically, hydrophilic silica nanoparticles are weakly adsorbed at the interface and favourably remained in the aqueous phase when initially dispersed in water [241, 242]. As a result, the water-soluble enzymes may gain access to the lipid interface relatively easily at the beginning, regardless of the [silica] present in the system; this provides an explanation for the subtle changes in the H_{5min} values (*i.e.* a ≤5% change). As lipolysis proceeds, the sizes of the lipid droplets are expected to be reduced and hence, the shielding effect of silica nanoparticles may become more noticeable in the latter phases of lipolysis, rendering a more significant retardation in the final lipolysis extent. It was found that further increment in the concentration of silica (*i.e.* to 80% relative to oil or 8% of the emulsion) did not further inhibit the extent of lipolysis. This can be reasoned by the strong inter-particle interactions of the dispersed silica via the surface silanol groups. In previous work [264, 265], it has been shown that silica nanoparticles tend to form aggregates in water at higher concentrations (*i.e.* from 0.5 to 5% of the emulsion) without further adsorbing to the droplet interface. Consequently, the droplet stability to creaming was further enhanced via an increase in the viscosity of the continuous aqueous phase. However, the current results

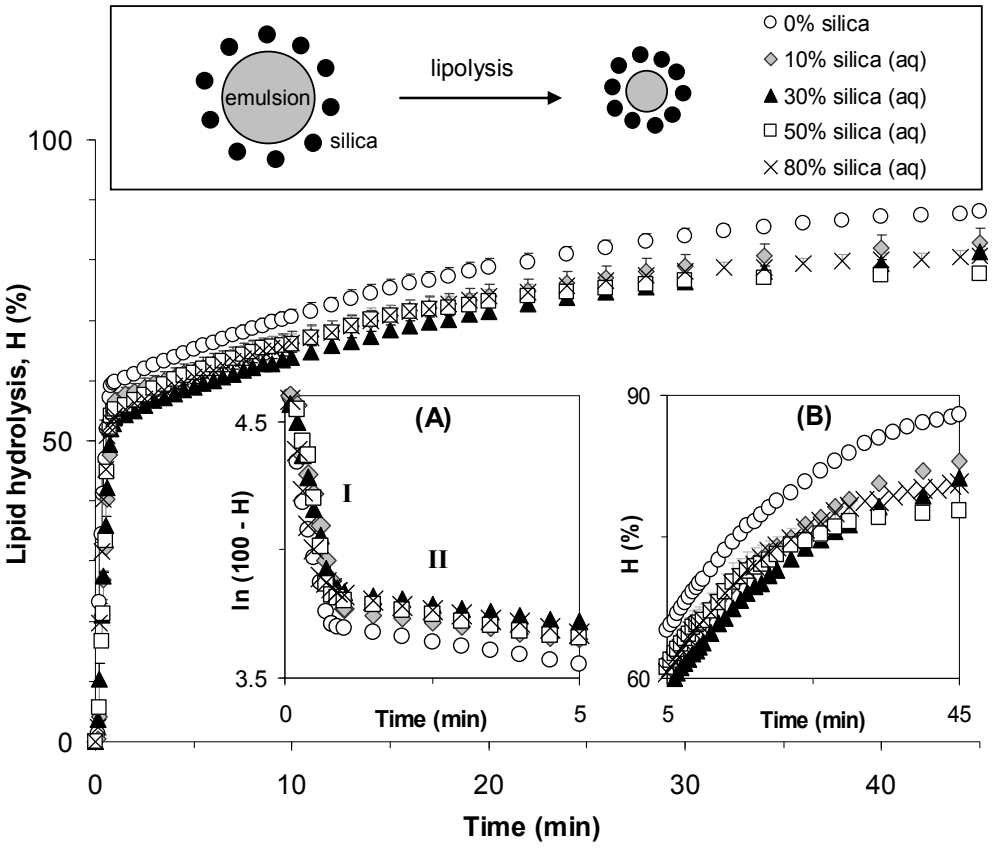


Figure 4.2 The lipolysis profiles of Miglyol oil-based emulsions stabilised by Aerosil silica from the aqueous phase at various concentrations (mean ± S.E.M., *n* = 3): (Inset A) pseudo first-order descriptions of the lipolysis process and (Inset B) the slow phase of lipolysis at an enlarged scale for each formulation.

Table 4.3 Lipolysis characteristics of Miglyol oil-based emulsions stabilised by Aerosil silica from the aqueous phase: the percentages of hydrolysis (H) at 5 min and 45 min post-lipolysis (mean ± S.E.M., *n* = 3)

% Aerosil silica	H (%)	
	H_{5min}*	H_{45min}*
0	65 ± 0.9	88 ± 0.5
10	62 ± 1.6	83 ± 2.4
30	59 ± 0.9	81 ± 1.0
50	61 ± 1.1	78 ± 0.5
80	61 ± 0.7	81 ± 1.1

* $p < 0.05$ (one-way ANOVA)

show that the formation of such highly viscous silica network has no significant impact on the enzymatic degradation behaviour of the lipid droplets.

(b) Incorporation of silica nanoparticles from the oil phase

The time course of lipolysis for the emulsions stabilised by silica nanoparticles incorporated from the oil phase is plotted in **Figure 4.3**, with the corresponding lipolysis characteristics (H values) summarised in **Table 4.4**. The data reveals that a similar protection effect (*i.e.* reduced extent of lipolysis) could be achieved when silica nanoparticles are pre-dispersed in the oil phase, but at ~10 times lower silica levels (with a miscible concentration limit of 6% silica relative to Miglyol oil). Surprisingly, the systematic trend of reduced H_{5min} values with increasing [silica] (*i.e.* a dose-dependent inhibition) is less clear when the emulsions are stabilised by silica from the oil phase.

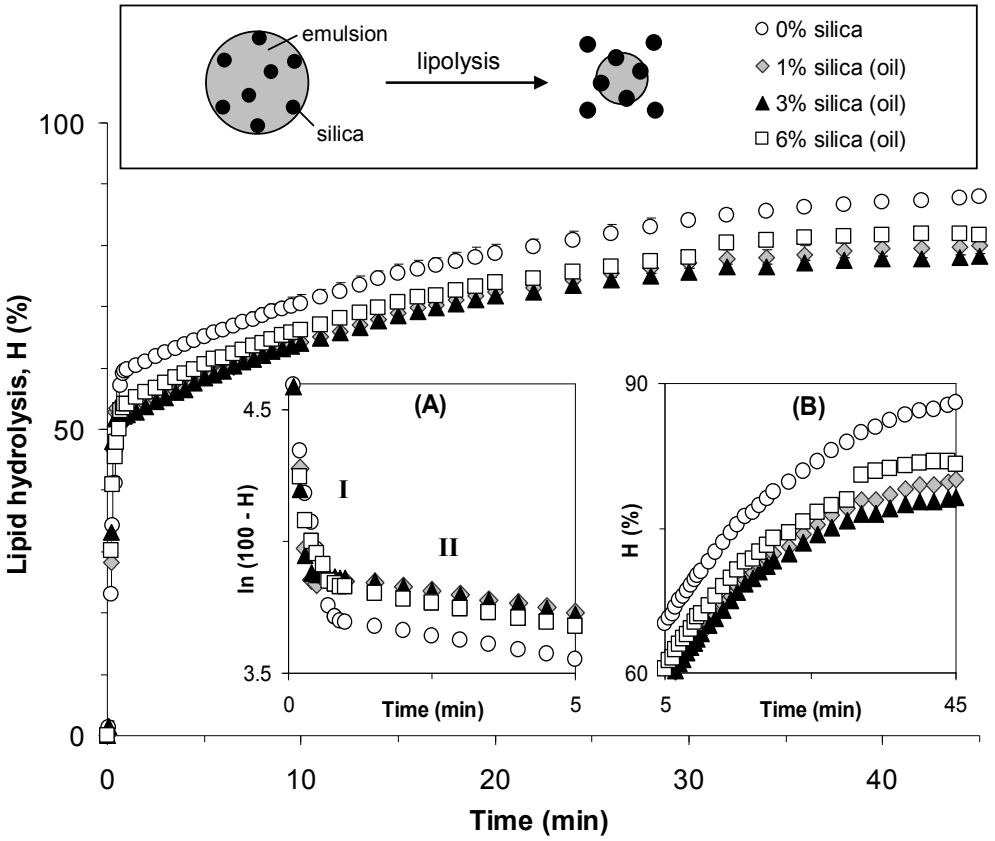


Figure 4.3 The lipolysis behaviour of Miglyol oil-based emulsions stabilised by Aerosil silica from the oil phase at various concentrations (mean ± S.E.M., *n* = 3): (Inset A) pseudo first-order descriptions of the lipolysis process and (Inset B) the slow phase of lipolysis at an enlarged scale for each formulation.

Table 4.4 Lipolysis characteristics of Miglyol oil-based emulsions stabilised by Aerosil silica from the oil phase: the percentages of hydrolysis (H) at 5 min and 45 min post-lipolysis (mean ± S.E.M., n = 3)

% Aerosil silica	H (%)	
	H_{5min}*	H_{45min}*
0	65 ± 0.9	88 ± 0.5
1	58 ± 1.0	80 ± 1.6
3	58 ± 0.6	78 ± 0.4
6	61 ± 0.8	82 ± 0.0

* $p < 0.01$ (one-way ANOVA)

Several explanations are proposed for this observation. First, the small range of the investigated concentrations of silica nanoparticles (*i.e.* 1–6% relative to the oil content) may not be discriminative enough to produce a clear dose-dependent lipolysis inhibition trend. Second, silica nanoparticles are known to partition slowly from the oil bulk phase to the interface [218], therefore a longer equilibration time may be required when higher concentrations of silica nanoparticles are dispersed initially in the oil phase. Third, considering that the accommodation of silica nanoparticles in the oil bulk phase or at the interface is strongly dependent on the amphiphilic lecithin component [218], there is a likelihood for the hydrophilic silica nanoparticles to partition into the aqueous phase during digestion. The presence of various hydrolytic enzymes in the pancreatin extract implies that lecithin digestion by other enzymes, particularly phospholipase A_2, is possible; this would cause a continuous reduction in the lecithin level in the oil phase as lipolysis progresses. Thus, it may be worth conducting further experiments by incorporating higher levels of lecithin into the oil phase to determine if more silica nanoparticles can be dispersed in the oil. This would in turn provide an insight into the synergistic role of lecithin and silica nanoparticles in inhibiting the lipase/colipase activity. Such synergistic inhibition mechanism can be exploited to achieve a more controllable lipolysis and thus, a sustained drug release pattern.

Based on the current lipolysis results of various Miglyol-based systems containing different concentrations of silica nanoparticles or lecithin as the emulsion stabiliser (*i.e.* silica-stabilised emulsions, SLH-M_1/-M_2 and SLH-M_3), we propose that Aerosil silica

nanoparticles are less potent inhibitors than lecithin against lipase/colipase hydrolytic activity.

4.4.1.3 Lipolysis of Capmul-based systems

The lipolysis work was extended to Capmul-based vehicles in order to understand the effect of the types of oil on the digestion behaviour of the SLH microcapsules. As can be seen from **Figure 4.4**, all tested systems including pure Capmul oil, lipid solution (oil:lecithin 1:0.06), lipid emulsion and SLH-C microcapsules, were digested in a biphasic manner, *i.e.* different from the observations for the Miglyol-based vehicles.

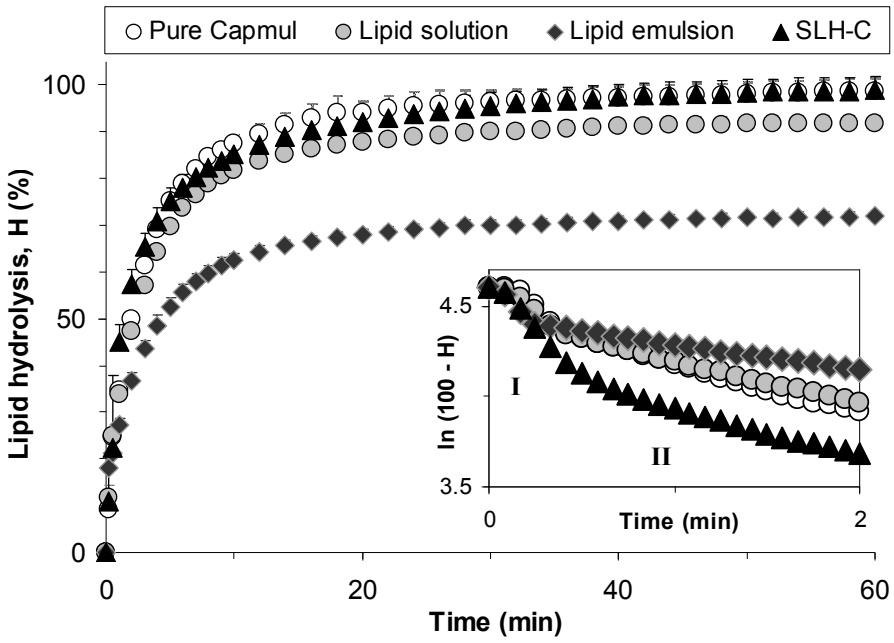


Figure 4.4 Time dependence of the lipase-mediated lipolysis for various Capmul oil-based systems under simulated fasted intestinal conditions at 37 °C (mean ± S.E.M., n = 3). The inset shows the pseudo first-order descriptions of the lipolysis process for each formulation.

Table 4.5 Lipolysis characteristics of various Capmul oil-based systems: lag phase duration, initial hydrolysis rate constant (k), and the maximum percentage of hydrolysis (H at 60 min) (mean ± S.E.M., n = 3)

Lipid systems	Lag phase duration (s)	k (min^{-1} x 10^{-1})			H_{60min} (%)*
		Phase I	Phase II	Phase III	
Capmul oil	**5.1 ± 0.0**	**7.1** (R^2 = 0.987)	**2.2** (R^2 = 0.992)	—	**99 ± 2.7**
Lipid solution (oil:lecithin = 1:0.06)	**6.8 ± 1.7**	**6.2** (R^2 = 0.981)	**1.9** (R^2 = 0.993)	—	**92 ± 1.7**
Lipid emulsion (217 ± 10 nm)	**5.1 ± 0.0**	**7.3** (R^2 = 0.971)	**1.0** (R^2 = 0.983)	—	**72 ± 1.0**
SLH-C (2548 ± 705 nm)	**5.1 ± 0.0**	**7.4** (R^2 = 0.966)	**1.8** (R^2 = 0.987)	—	**99 ± 2.8**

* $p < 0.01$ (analysed by one-way ANOVA)

In all cases, there was no significant lag time (5–7 s) for the initiation of lipolysis upon the addition of pancreatic enzymes (**Table 4.5**), note that the experimental data was collected at every 5 s. This signifies that Capmul oils, being surface active and relatively more polar than its TG analogue [207], are highly susceptible to digestion by lipase/colipase complexes. In agreement with a previous finding [262], Capmul oil which consists of partially hydrolysed lipids (MG/DG), was digested more quickly ($k_{phase\ I}$ = 0.71 min^{-1} followed by $k_{phase\ II}$ = 0.22 min^{-1}) than Miglyol oil (k = 0.075 min^{-1}); likewise for the lipid solutions.

When comparing the lipolysis characteristics of SLH-C microcapsules to that of pure Capmul oil (**Table 4.5**), there is no significant difference observed in both the rate and extent of lipolysis. The contributing factors described previously for the Miglyol-based systems, such as initial emulsification of oils (*i.e.* reduced initial droplet sizes) and increased porosity of the lipid carrier (*i.e.* further enhancement of the total surface area), are apparently of negligible importance for the digestion of Capmul oil. Unexpectedly, the digestion of Capmul-based lipid emulsion was dramatically reduced at early time points and achieved only up to 72% lipolysis at 60 min. This indicates that lecithin plays a major role in controlling the digestion of Capmul oil and emulsion, as suggested previously for the Miglyol-based systems. It is assumed that lipolysis of the Capmul-based emulsion was inhibited via the same mechanism, *i.e.* lecithin formed a competitive binding site for the

enzyme complexes, thereby reducing the accessibility of enzymes to the glyceride components during lipolysis.

Overall, the current results highlight various formulation factors that potentially lead to different lipolysis characteristics: oil polarity and surface activity, emulsification, and the presence of an enzyme inhibitor (e.g. lecithin, maltodextrin and silica nanoparticles as investigated in the present work).

4.4.2 Mechanisms of digestion of SLH microcapsules

It is now recognised that formulation of lipids in the form of SLH microcapsules leads to enhanced lipid digestibility (especially in the presence of low lecithin levels), in conjunction with increased drug dissolution (or dispersion) as reported previously in **Chapter 3**. The SLH microcapsules were prepared from homogenised submicron emulsions (<0.2 μm), but they do not re-disperse to form back the original emulsions. The spray-drying process has resulted in hybridisation of the lipids with fumed silica, hence the formation of the micron-sized (2–5 μm) capsule carriers with internal nano-structured network. Despite the relatively large particle sizes, lipids from SLH microcapsules were digested rather quickly with complete hydrolysis within 1 h, which are clearly superior to lipid solutions and emulsions. This does not support our initial hypothesis that silica nanoparticles present in the SLH microcapsules may inhibit lipolysis by blocking the access of the digesting enzymes to the droplet interface. The increased digestibility of the SLH microcapsules is attributed to the highly porous internal matrix which presents an extremely high surface area-to-volume ratio that facilitates lipid vehicle digestion and drug release/ solubilisation. The lipid substrates could have been presented in multiple ways for the enzymes to bind and digest. Without specifying the types of lipids or the level of lecithin employed, we illustrate in **Figure 4.5** the possible fate of SLH microcapsules under simulated intestinal digesting conditions. These proposed mechanisms are supported by the laser diffraction analysis (method described in **Section 2.4.4.2a)** of the dispersed particle sizes as a function of time (in the absence of enzymes), and visualisation of the resultant microcapsule structure after 1 h of lipolysis by using freeze-fracture SEM (**Figure 4.6**).

In the first situation (labeled as ① in **Figure 4.5**), the SLH microcapsules with an inert porous matrix potentially retain their structural integrity after being redispersed in the aqueous medium. The high mechanical strength is provided by the formation of a strong

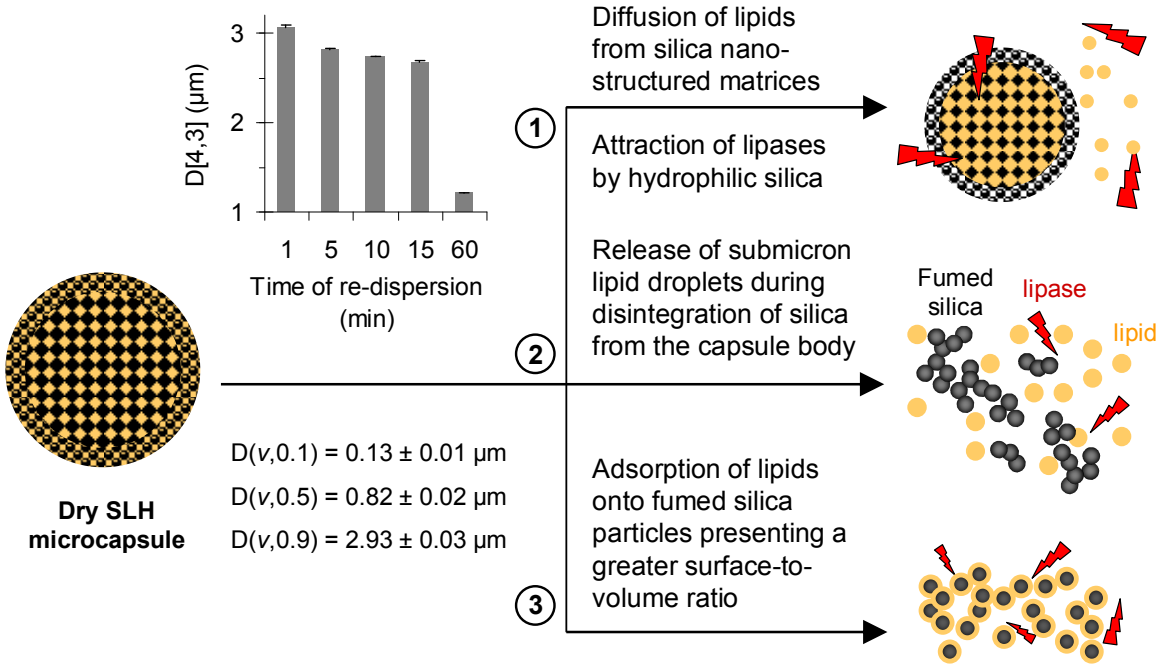


Figure 4.5 Schematic representation of the possible fate of SLH microcapsules under simulated intestinal digesting conditions (drawing not according to scale). In the particle sizing results, D[4,3] is the volume-weighted mean diameter; D(*v*,0.1), D(*v*,0.5) and D(*v*,0.9) are the particle diameters at 10, 50 and 90% cumulative size, respectively, after 60 min of redispersion in the lipolysis medium (5 mM BS:1.25 mM PL).

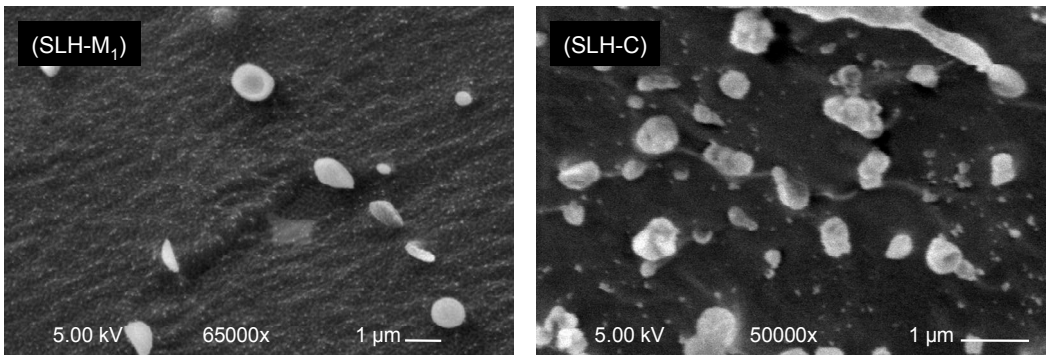


Figure 4.6 Freeze-fracture SEM images for a Miglyol-based and a Capmul-based microcapsule system after 60 min of lipolysis under simulated fasted intestinal conditions (5 mM BS:1.25 mM PL).

cross-linked network via the surface silanol groups of silica nanoparticles during the spray-drying process. Therefore, the enzymatic reaction is expected to be highly dependent on the rate of oil droplet leakage from the internal pores (50–200 nm as determined by BET analysis in **Chapter 2**). Furthermore, silica nanoparticles have been extensively studied for surface immobilisation of enzymes (without causing denaturation) for improved catalytic activities [282-286]. Considering that silica may have specific adhesive properties towards cellular surfaces and protein macromolecules through the hydroxyl groups, the silica network may also act as a strong adsorption template for the digesting enzymes. This would reduce the penetration barrier of the enzymes to the substrate interfaces, and provide control over the propagation of enzymes from one substrate to another, both along and within the microcapsule network. This possibly explains the consistently high lipid digestibility (>90%) of the SLH microcapsules, which is unaffected by the types of oils loaded, when compared to the corresponding bare lipid emulsions.

The freeze-fracture SEM images presented in **Figure 4.6** show that the post-digested SLH-M_1 and SLH-C microcapsules have a mixed appearance of spherical, indented, elongated and aggregated structures that are smaller in particle sizes (*i.e.* ~1 μm and less in diameters) than the original undigested microcapsules (2–5 μm), along with traces of dispersed silica fragments or particles after 60 min of lipolysis. From this observation, it is deduced that Aerosil silica, by virtue of its hydrophilic character, becomes partially disintegrated from the microcapsule body when subjected to constant stirring or agitation in the aqueous medium or in the GI tract (labeled as ② in **Figure 4.5**). Further evidence is provided by the particle sizing results which show the presence of smaller particle size fractions, *i.e.* D(v,10) of <0.2 μm (comparable to the original emulsion droplet sizes) and D(v,50) of <1 μm. The time-dependent analysis also revealed a continuous reduction in the microcapsule particle sizes as a function of redispersion time (depicted by the bar graph in **Figure 4.5**). The disintegrated fumed silica fragments, known to posses extremely high specific surface area capable of adsorbing organic molecules [286], may serve as an adsorbent for lipids, thus presenting a greater surface area-to-volume ratio facilitating the substrate-enzyme interactions (labeled as ③ in **Figure 4.5**). Based on the high polydispersity of the redispersed SLH microcapsules, *i.e.* a size span of >3 and a uniformity of >3, we propose the possibility of a combination of these three illustrated mechanisms.

4.4.3 Phase partition of celecoxib during lipolysis

The distribution behaviour of CEL between the aqueous and pellet phases was determined for selected formulations to verify the role of lipid digestibility on drug solubilisation. The drug phase partition data for blank micelles (without the addition of exogenous lipids) and three types of SLH microcapsules that vary in their compositions is presented in **Figure 4.7**.

Based on the percentage of drug present in the aqueous and pellet phases (panel A), it can be inferred that continuous lipolysis of the SLH microcapsules (containing 50–60% w/w of medium-chain glycerides) provides a significantly higher solubilising environment for CEL in comparison to the fasted state blank micellar solution which is composed of 5 mM BS:1.25 mM PL. This observation is in good agreement with several previous findings for different lipophlic drugs in liquid lipid carriers [61, 93, 206]. The mechanisms behind the enhanced solubilisation capacity in the presence of exogenous lipids have been well described. MCT is quickly digested by lipase/colipase complexes to form a lipid-rich (mostly vesicular) phase in which drugs can be solubilised in a supersaturated state without being affected by dilution of the system for a considerable period of time (~30 min) [61, 206]. The lipolytic products also result in swelling of the endogenous BS/PL structures via intercalation into the micelle interface (but at a lower capacity than LCT [61, 270]), as well as generating simple micelles as the colloidal solubilising species in addition to the production of mixed micelles [61]. All these events contribute to a higher solubilising capacity of the medium, thereby reducing drug precipitation in comparison with the control blank micellar medium.

An important note to the current phase partition data is that the CEL loading level of each studied SLH microcapsule system is different: SLH-M_1 0.64%, SLH-M_3 1.81%, and SLH-C 3.84% (w/w). In this study, the lipid dose is fixed at 10 mg/ml in the lipolysis medium; this corresponds to a maximum achievable CEL concentration of approximately 100 µg/ml, 370 µg/ml and 620 µg/ml for SLH-M_1, SLH-M_3 and SLH-C, respectively. According to the partition results shown in **Figure 4.7A**, there is a higher percentage of CEL solubilised into the aqueous phase than precipitated for both Miglyol-based microcapsule systems; but there is a higher tendency of drug precipitation than solubilisation for the Capmul-based microcapsules, most probably due to the much higher CEL dose in the latter case. Therefore, it is more appropriate to compare the solubilisation results in term of the absolute concentrations (panel B) rather than the percentage of CEL solubilised (panel A).

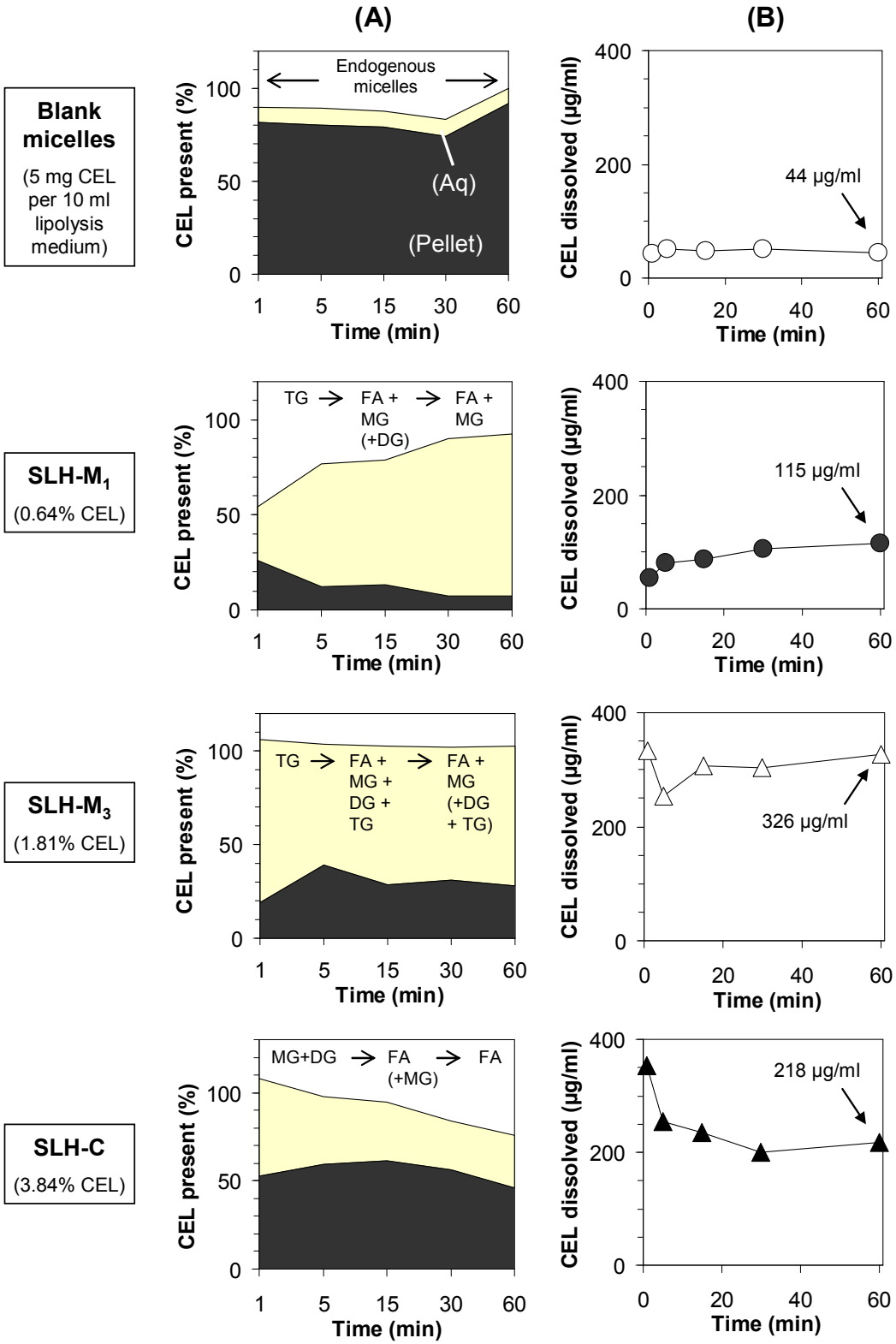


Figure 4.7 Phase partition results for celecoxib in the simulated fasted intestinal medium (5 mM BS:1.25 mM PL) at 37 °C (mean ± S.E.M., *n* = 2): (A) percentage of celecoxib distributed in the aqueous and pellet phases, and the likely species of lipid products generated [262], and (B) concentration of celecoxib solubilised in the lipolysis aqueous phase.

For SLH-M_1 microcapsules (which contain the lowest CEL dose), there is a clear trend of increasing % CEL in the aqueous phase together with a decreasing % CEL in the pellet phase as lipolysis proceeded (panel A and B). Combined with the lipolysis-time profile in **Figure 4.1B**, a complete lipolysis of a 10 mg/ml lipid dose could produce a final solubilised [CEL] of 115 μg/ml, which is approximately 2.5-fold higher than that of the blank micelle medium (44 μg/ml), and 16-fold greater than the solubility of CEL in water (*i.e.* 7 μg/ml [220]). However, this is not the maximum solubility limit for CEL in the post-digested medium. When compared to the SLH-M_3 microcapsules (which contain similar oil content but a higher lecithin level than SLH-M_1), even though the lipid digestion was slower and incomplete (~70% lipolysis at 60 min), the solubilisation curve (panel B) reveals a significantly higher [CEL] dissolved ranging from 250–350 μg/ml. This observation could be reasoned by: (*i*) the presence of higher CEL dose in the SLH-M_3 microcapsules, in which a greater concentration gradient was created in the lipolysis medium that drove partition of the drug molecules from the solution into the solubilising colloidal phases, and (*ii*) the higher content of the swelling amphiphiles, lecithin, in the formulation which potentially assists in drug solubilisation.

Whilst the equilibrium solubility of CEL in Capmul oil (93 mg/g) was determined to be significantly higher than that in Miglyol oil (11 mg/ml), the lipolytic products of SLH-C microcapsules were found to provide less solubilising capacity than the SLH-M_3 system, as evidenced by a massive drug precipitation (>70%) and a lower [CEL] solubilised in the case of post-digested SLH-C microcapsules (**Figure 4.7B**). We attribute this as a consequence of different lipolytic products generated from the digestion of Capmul (DG/MG mixture) and Miglyol (TG) oils. According to Sek *et al*. [262], medium-chain TG is expected to be hydrolysed to form MG (15%) and FA (70%) in the aqueous phase, with some FA (15%) pelleted out after 30 min; DG is also possibly accumulated in the first 5 min of lipolysis. In contrast, medium-chain MG/DG mixture is completely hydrolysed in 30 min to produce only FA as the end products, in which 80% is present in the aqueous phase and the remaining 20% in the pellet phase. Based on this information, we deduced that the partially digested and absorbable glyceride forms, *i.e.* MG and possibly DG, are important lipid species in forming highly solubilising colloidal structures to host the unionised drug molecules, such as CEL. Full lipid hydrolysis may not be necessary to induce drug release and solubilisation. This inherently elucidates the higher initial [CEL] solubilised for the SLH-C system because of the presence of MG/DG mixtures. As lipolysis progresses, the

concentration of the MG/DG species declines rapidly because they are efficiently digested by the lipase/colipase complexes, producing the titratable FFA monomers which do not favour solubilisation of the CEL molecules. Hence, a declining solubilisation curve was observed for the SLH-C microcapsules under digesting conditions. In the case of SLH-M_3 microcapsules, the continuous gain of the MG and DG species with incomplete conversion into the titratable FFA has adequately maintained CEL dispersion in the lipolysis aqueous phase.

In spite of the high recovery of CEL in the pellet phase, especially for SLH microcapsules with higher drug loading levels, the final solubilising capacity generated by lipolysis for CEL is considerably higher than that observed in the previous dissolution (or dispersion) study. It is noteworthy that CEL could be solubilised at >40 μg/ml in 1 h in the lipolysis medium, which is significantly higher compared to <3 μg/ml in 4 h in the SLS-containing (0.5%) medium (**Figure 3.1** in Chapter 3). Further *in vitro-in vivo* correlations (IVIVCs) are clearly warranted to fully assess the predicting power of the lipolysis model in comparison with the dissolution test in reflecting the *in vivo* drug absorption kinetics and dynamics. This prompted the subsequent *in vivo* absorption studies of CEL in various non-lipid and lipid-based formulations (**Chapter 5**), followed by the IVIVC analysis (**Chapter 6**).

4.5 Summary

A dynamic *in vitro* lipolysis model was employed to effectively highlight several important factors contributing to an enhanced or inhibited lipolysis of various medium-chain lipid vehicles: lipid solutions, bare lipid emulsions, silica-stabilised emulsions, a dry emulsion (MaltoDE) system and the SLH microcapsules. The importance of emulsification on lipid digestibility is evidenced in the case of Miglyol-based systems, in which significantly higher initial lipolysis rate constants were determined for SLH microcapsules and lipid emulsions in comparison with lipid solutions. Capmul MCM, owing to its more polar and surface active properties, has shown high susceptibility to enzymatic lipolysis without requiring the pre-emulsification step; this renders an equally fast and complete lipolysis profile for Capmul-based solutions and microcapsules.

In this chapter, we have identified two potent inhibitors to the lipase/colipase-mediated digestion of lipid-based vehicles, *i.e.* lecithin and maltodextrin. Aerosil silica, in the

dispersed state, exerted an inhibitory effect on the digestion of submicron lipid emulsions regardless of their initial locations, *i.e.* in the aqueous or lipid phase. This inhibitory effect, however, was not observed for SLH-microcapsules fabricated from both Miglyol and Capmul oils in the presence of low lecithin level (oil:lecithin 1:0.06 w/w). The highly porous matrix structure of the SLH microcapsules has contributed towards an enhanced lipid digestibility by providing a high surface area that facilitates the enzyme-substrate interactions.

The lipolysis phase partition data indicates that at a fixed lipid dose, the total fraction of CEL solubilised in the post-digestive state declines with an increasing drug load. The initial solubility of the drug in the lipid vehicles does not directly correlate to the medium solubilisation capacity after lipolysis. We propose that the types of the end lipolysis products, either in the partially digested but absorbable forms (MG and probably DG) or in the completely hydrolysed and titratable form (FFA), significantly influence the solubilising power of a lipolysis medium. Furthermore, we emphasise that the lipolysis model (both kinetic and phase partition studies) is a better tool in probing the mechanisms of drug release and providing more discriminative results between the various lipid-based formulations in comparison with the classic dissolution (or dispersion) test.

CHAPTER FIVE

In Vivo Assessment of SLH Microcapsules: Oral Absorption of Celecoxib from Non-lipid and Lipid-based Formulations

5.1 Introduction

Based on the *in vitro* drug dissolution (or release) and lipolysis (or drug phase partition) data presented in the previous chapters, it is proposed that the SLH microcapsules may improve the oral absorption of CEL via several mechanisms: (*i*) pre-dissolution of drug in the lipid phase, which eliminates the rate-limiting dissolution step *in vivo*, (*ii*) interfacial transfer of drug via concentration gradient-driven diffusion from the formulation (lipid) phase into the bulk (aqueous) phase, and (*iii*) increased lipid carrier digestibility, which produces various amphiphiles facilitating solubilisation of the released drugs in the GI fluids (to a varying degree depending on the types of formulation lipids and the drug loading levels). These mechanisms are typical theoretical assumptions for many lipid-based systems formulated with digestible lipids, but in practice an improved oral bioavailability may not be occurring *in vivo* [139]. To confirm whether increased drug dissolution (or release) and post-lipolysis solubilisation can lead to an improved pharmacokinetic (PK) properties of CEL, a concurrent *in vivo* absorption study of various CEL formulations using an intact animal model has been conducted and is presented in this chapter.

5.1.1 Pharmacokinetic properties of celecoxib

The collective PK properties of CEL reported in humans, dogs and rats are summarised in **Table 5.1**. The metabolism of CEL in several animal species (*i.e.* mouse, rat, rabbit, dog and monkey) has been found to be similar to the human hepatic metabolism pathway, which involves hydroxylation followed by oxidation and glucuronidation of the drug molecules prior to excretion [227-229]. When CEL (Celebrex®, Pfizer/Pharmacia) is administered orally, the rate of absorption is moderate with the maximum plasma concentration (C_{max}) achieved in between 2–4 h (t_{max}) in healthy human subjects; this reflects a moderate onset of therapeutic action (*i.e.* anti-inflammation and pain relief). An intravenous dosage form of CEL is not readily available mainly because of its poor aqueous solubility; therefore the

Table 5.1 Pharmacokinetic properties of celecoxib in various healthy subjects (reported mean values)

PK parameter	Subject				
	Human	Dog		Rat	
	(Oral)[a]	(I.V.)[b]	(Oral)[b]	(I.V.)[c]	(Oral)[d]
$AUC_{0\rightarrow\infty}$ (h.μg/ml)	6.0–10.1	4.0–11.5	2–10 (solution); 1–4 (capsule)	2.1; 9.3[d] (male) 8.4 (female)	4.6
$t_{1/2}$ (h)	~11	1.3–5.1	NA	2.8; 3.7[d] (male) 14.0 (female)	3.1
V_{ss} (L/kg)	5.7–7.1 (or 455±166 L)	1.9–2.3	NA	2.3[d]; 2.5 (male) 2.4 (female)	5.2
t_{max} (h)	2–4	—	0.5–1 (solution); 2–3 (capsule)	—	1.6
C_{max} (μg/ml)	0.70–1.06	—	0.8–1.3 (solution); 0.2–0.6 (capsule)	—	0.94
F (%)	NA	—	64–88 (solution); 22–40 (capsule)	—	49

[a] Based on an oral capsule dose of 200 mg in the fasted state (Ref: [208, 222, 229, 287])
[b] Based on an oral dose of 5 mg/kg in the fasted state (Ref: [229])
[c] Based on a dose of 1 mg/kg (Ref: [228])
[d] Based on a dose of 5 mg/kg (Ref: [288])

absolute bioavailability (F) has not been reported in the literature for humans [222, 287]. Even so, changes in the rate and extent of drug absorption can be inferred from the area under the plasma concentration-time curve (AUC) and the C_{max} values [289]. These two PK parameters, *viz.* $AUC_{0\rightarrow24}$ and C_{max}, have been found to be linearly related to CEL doses within the range of 100–800 mg in healthy adults [287, 290]. Currently, there is no published data relating the plasma concentrations of CEL to the adverse effects normally encountered for conventional NSAIDs (particularly gastric ulceration and bleeding) [287]. It is known that platelet aggregation (which is related to inhibition of the COX-1 pathway) is not inhibited by up to 800 mg of CEL [290]. Since CEL is generally well tolerated but poorly absorbed, it has been extensively studied as a model drug for the development of

novel formulations for BCS Class II drugs. As described in the following section, most of the oral formulation strategies developed for CEL have been tailored to increase the rate and extent of dissolution for achieving faster onset of action and improved bioavailability for various therapeutic applications (such as anti-inflammatory and chemo-preventive use).

5.1.2 An overview of formulation strategies for celecoxib

Although some researchers have proposed that topical/ transdermal [209, 291, 292] and intra-articular/ intravenous [251, 293] delivery of CEL are more effective than oral dosing, the oral dosage forms remain the most convenient route of administration that is associated with better patient compliance [18]. Most of the formulation studies for CEL have shown improved drug solubility and dissolution rate, which eventually increased the F and/ or C_{max} responses. However, a change in t_{max} or the drug release duration has rarely been reported. These formulation strategies include:

(*i*) manipulation of the solid state of CEL, e.g. conversion into a fast-dissolving polymorphic form [37];

(*ii*) nanosuspensions [294] and solid dispersions formulated with superdisintegrants (such as croscarmellose and pre-gelatinised starch) or compression stabilisers (e.g. polyvinyl pyrrollidone) [39, 40, 295];

(*iii*) prodrug approach [50, 51];

(*iv*) formation of soluble crystalline salt with precipitation inhibitors [48];

(*v*) complexation with β-cyclodextrins [296, 297];

(*vii*) a gastro-retentive polymer-based floating capsules [250]; and

(*viii*) enhanced dispersion in lipid/ surfactant-containing SMEDDS [208], microemulsions (*i.e.* oil-based concentrates) [298] and proniosomes (*i.e.* dry particles that readily disperse in water to form niosomes, which are uni- or multilamellar vesicles made up of non-ionic surfactants) [299].

The conceptual details of each formulation technology are described in **Section 1.2.2** (Chapter 1). Overall, the number of reports on CEL oral delivery by using lipid-based

carriers is rather limited, whilst there is a myriad of research investigating the use of lipid-based formulations in improving the bioavailability of poorly water-soluble drugs [3, 8, 60, 133]. Some other non-oral lipid-based formulations of CEL that have been investigated include a nanostructured lipid carrier (NLC)-based topical gel [210], and transdermally applied microemulsions [209] or nanoemulsions [291, 300]. Importantly, the effect of "high fat meals" (e.g. containing butter, peanut butter, eggs fried with butter, bacon) on the bioavailability enhancement of CEL has been found to be pronounced in the animal model (*i.e.* 3–5 fold increase in dogs [229]), but less clinically significant with respect to safety or efficacy in humans (*i.e.* co-administration of Celebrex® capsules with a meal containing 75 g fat resulted in only a 10–20% increase in the *AUC* values) [222, 229]. Specific investigations have yet to be reported to illuminate the specific role of various formulation lipids (e.g. TG/ DG/ MG with different acyl chain length) in enhancing the oral absorption of CEL at various dose strengths.

In the current oral absorption study, we assessed the PK properties of CEL delivered orally from various non-lipid and lipid-based formulations in healthy, fasted rats. At the early stage of formulation development, pre-clinical pharmacokinetic assessments using rodent models is common to guide the process of product optimisation [301].

5.1.3 Specific aims and hypotheses

The major aim of the current work was to determine the absorption profiles of CEL resulting from various oral formulations in a fasted rat model: pure CEL, Celebrex® (which is thought to be 'surfactant-based'), as well as a series of simple lipid solutions/ emulsions and the solid dosage lipid vehicles, including MaltoDE and SLH microcapsules (in the redispersed form). Three PK parameters, specifically t_{max}, C_{max} and F are used to interpret the changes in the rate and extent of CEL absorption. A dose proportionality study was also carried out to determine if the SLH microcapsule technology produces a linear dose-absorption response rendering a more predictable absorption outcome.

As inferred from the previous *in vitro* data, we hypothesize that:

(*i*) the SLH microcapsules produce better CEL absorption profiles than the pure drug (administered as an aqueous suspension) and the Celebrex® capsule materials (as an

aqueous suspension), due to enhanced drug release and intraluminal solubilisation by the lipolysis products;

(*ii*) the extent of CEL absorption from the SLH microcapsules follow a linear increment with the administration dose, whereas the pure drug has a lack of dose linearity in the bioavailability due to its poor intrinsic aqueous solubility and dissolution.

5.2 Materials

Materials and chemicals used for the preparation of various CEL formulations and for the HPLC drug assay are described in **Section 2.3**. Chemicals used in the animal experiments include normal saline (NS) solutions, heparinised saline solutions (50 units per 5 ml), heparin solutions (5000 units per ml diluted to 1000 units per ml with NS), and the anaesthetics isoflurane (AstraZeneca Limited, Auckland). Healthy male Sprague-Dawley (S.D.) rats were purchased from the Institute of Medical and Veterinary Science (IMVS), Adelaide, prior to February 2008. Later, the S.D. rats were sourced from the Lab Animal Services, University of Adelaide.

5.3 Methods

5.3.1 Animal study protocol

All animal experiments were conducted according to the protocol [302] approved by the Animal Ethics Committee, IMVS (Australia). Groups of five healthy male S.D. rats weighing from 300 to 360 g were used for each treatment. The individually caged rats were allowed to acclimatise to the environment for at least 2 days prior to the experiment and fed with normal diet and water. One to two days prior to CEL dosing, each rat was cannulated in the right jugular vein under inhaled isoflurane, and then allowed to recover. The orally dosed treatment groups were fasted overnight (14 ± 1 h) prior to the day of dosing and were given access to food 4 h post-dose. Water was accessible to all treatment groups throughout the study.

5.3.2 Preparation and oral administration of celecoxib formulations

The oral CEL formulations tested at various doses (*i.e.* 5, 20 and 50 mg/kg) and the corresponding formulation compositions are listed in **Table 5.2**.

Table 5.2 The composition of oral CEL formulations tested in rats

CEL formulation	Compositions (mg)*		Total powder mass administered (mg) *	Total volume administered (ml)
	CEL	Lipid		
Low dose: 5 mg/kg **				
Aqueous suspension	1.75	—	—	1.75
Celebrex®	1.75	—	4.7	2
Lipid solution	1.75	175	—	0.18
Lipid emulsion	1.75	175	—	1.75
MaltoDE	1.75	168	700	2
SLH-M_1	1.75	168	324	2
SLH-M_2	1.75	121	282	2
Medium dose: 20 mg/kg ***				
Aqueous suspension	7.0	—	—	2.8
Celebrex®	7.0	—	18	3
SLH-M_1	7.0	409	787	3
SLH-M_3	7.0	241	387	3
SLH-C	7.0	111	182	3
High dose: 50 mg/kg ***				
Aqueous suspension	17.5	—	—	3
Celebrex®	17.5	—	47	3
SLH-C	17.5	278	455	3

* The total powder mass includes the active drug, lipid excipients (if any) and other non-lipid inactive ingredients (such as Aerosil silica in the SLH microcapsules, and maltodextrin in the MaltoDE formulation); each total mass of powder administered was estimated based on a rat weighing 350 g

** The rats used for the low dose experiments were supplied by the IMVS

*** The rats used for the medium and high dose experiments were supplied by the Lab Animal Services, University of Adelaide

Pure CEL powder and the Celebrex® powder materials were suspended in 0.25% w/v sodium carboxymethylcellulose by ultrasonication (for 30–45 min). The homogenised lipid emulsion (mean hydrodynamic diameters ~170 nm) was prepared as previously described (**Section 2.4.3**). The liquid samples including pure CEL aqueous suspension, Celebrex® capsule material suspension, lipid solutions and emulsions were used for dosing within five days after preparation. The powdery formulations, *i.e.* MaltoDE and the SLH microcapsules, were redispersed in Milli-Q water on the day of dosing by brief vortex-mixing (10–20 s) and hand-held shaking; the SLH-M_3 powder required additional ultrasonication (10 s) before a homogenous suspension could be formed. Each cannulated and fasted rat was administered orally with one of the listed formulations via an oral gavage under light inhaled isoflurane. Time was recorded immediately after formulation administration for subsequent blood sampling.

5.3.3 Blood sample collection and analysis

After dosing, all rats were kept conscious in individual cages during the process of blood sampling. Blood samples of 0.2 ml were collected from the cannulated jugular vein at designated time intervals and transferred into Eppendorf tubes prefilled with 10 µl of heparin solution (1000 units per ml). The cannula was flushed with an equal volume (0.2 ml) of heparinised normal saline (50 units per 5 ml) to prevent the formation of blood clots.

The collected blood samples were centrifuged at 9400 *g* for 5 min to separate the plasma from the whole blood. An aliquot of 100 µl plasma was vortex-mixed with 100 µl acetonitrile and centrifuged at 3500 *g* for 10 min to remove proteins prior to HPLC analysis as described before (**Section 2.4.1**). CEL content in the plasma samples was determined from the calibration curves generated using CEL dissolved in the mobile phase solutions without the use of plasma, due to acceptably high extraction recoveries of CEL from the rat plasma samples (*i.e.* 102–108% detection of the known CEL content as validated in **Section 2.5.1**). The use of an internal standard is not required because of the high specificity and reproducibility of the assay: the intra- and inter-day assay precision was assessed by the coefficient of variance (≤3% and ≤6%, respectively) and the accuracy was expressed as the percentage bias (≤9%) (**Section 2.5.1**). External controls have been run at the time of

analysis of rat plasma samples to ensure that no systematic change has occurred between assay validation and sample analysis.

5.3.4 Pharmacokinetic and statistical analysis

The CEL plasma concentration (C_p) results obtained were dose-normalised to 5, 20 and 50 mg/kg accordingly to allow easy comparison between rats. The mean C_p-time curves were constructed based on the mean dose-normalised data. The PK parameters were determined using the PC software, WinNonlin® Standard Edition Version 4.1 (Pharsight Corporation, CA), based on a non-compartmental model. The t_{max} and C_{max} values were obtained from the individual CEL C_p-time profile. The $AUC_{0\rightarrow\infty}$ values were calculated using the linear trapezoidal rule and used to estimate the relative bioavailability ($F_{rel.}$) of the test formulations with reference to that of the CEL aqueous suspension, according to equation (11) [289, 303]:

$$F_{rel.} = \frac{AUC_{0\rightarrow\infty}(\text{test})}{AUC_{0\rightarrow\infty}(\text{reference})} \times \frac{\text{Dose (reference)}}{\text{Dose (test)}} \qquad (11)$$

The PK data was statistically analysed based on one-way analysis of variance (ANOVA) with a Least Significant Difference (LSD) post-hoc test by using the statistical package for social sciences (SPSS version 15.0) software. The level of significance was set at $p < 0.05$.

5.4 Results and Discussion

5.4.1 Comparative oral pharmacokinetics of celecoxib from non-lipid and lipid-based formulations

A comprehensive comparison of the oral PK properties of CEL resulting from various non-lipid formulations (*i.e.* aqueous suspensions of pure CEL and Celebrex®) and lipid-based formulations (*i.e.* lipid solution, emulsion, redispersed MaltoDE and SLH microcapsules) was conducted in a fasted rat model. The dose employed, *i.e.* 5 mg/kg, is equivalent to a 400 mg daily dose in human assuming an average weight of 70–80 kg. The mean C_p-time curves of CEL following a single dose of each oral formulation are presented in **Figure 5.1**. The corresponding non-compartmental PK data is summarised in **Table 5.3**.

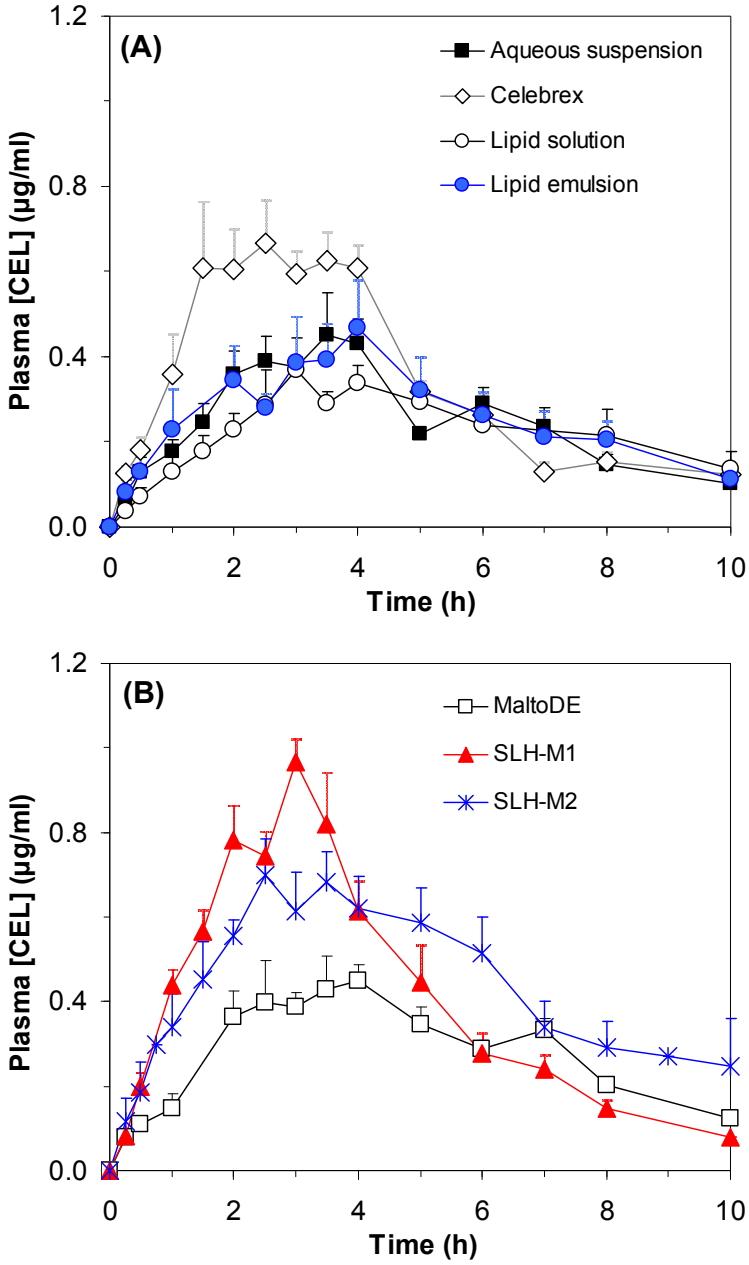


Figure 5.1 Plasma concentration–time profiles of celecoxib in male S.D. rats following a single oral dose of various formulations equivalent to 5 mg/kg celecoxib (mean ± S.E.M., *n* = 5).

The absolute bioavailability of CEL was not determined in this study because there are no commercial intravenous CEL formulations available to ensure accurate dosing. It is noted that a dose-dependent intravenous PK assessment of CEL has been conducted in our laboratory. The results have shown a non-proportional drug systemic clearance (CL) between the two treatment groups dosed at 5 mg/kg and 10 mg/kg CEL in a PEG 400:saline (2:1 v/v) solution (**Appendix I**). The reasons for such non-proportional PK responses are

yet to be elucidated (not a major focus of this study), but it is suggested that the reduced drug metabolism and clearance at a higher CEL dose may be caused by the administered PEG 400 content [304] or due to saturation of the drug metabolising enzymes. For easy comparison, the relative bioavailbility ($F_{rel.}$) in respect to the CEL aqueous suspension was used to compare the oral absorption efficiency of various CEL formulations.

Table 5.3 Non-compartmental pharmacokinetic parameters of celecoxib following a single oral dose (5 mg/kg) of various celecoxib formulations to male S.D. rats (mean ± S.E.M., n = 5)

CEL formulation	PK parameters			
	t_{max} (min)	C_{max} (μg/ml)	$AUC_{0\rightarrow\infty}$ (min.μg/ml)	$F_{rel.}$ (%)
(Control systems)				
Aqueous suspension	202 ± 28	0.50 ± 0.08	167 ± 11	100 ± 7
Celebrex®	162 ± 20	0.63 ± 0.08	223 ± 16	133 ± 9
Lipid solution	158 ± 14	0.44 ± 0.06	170 ± 17	101 ± 10
Lipid emulsion (~170 nm)	240 ± 50	0.49 ± 0.10	197 ± 35	117 ± 21
MaltoDE (~800 nm)	233 ± 31	0.51 ± 0.06	194 ± 6	116 ± 4
(Novel systems)				
SLH-M_1 (~2500 nm)	174 ± 15	1.00 ± 0.07[a, b, c]	250 ± 15[a]	150 ± 9[a]
SLH-M_2 (~2000 nm)	144 ± 24[b]	0.74 ± 0.07[a]	270 ± 27[a, b]	170 ± 16[a, b]

[a] statistically higher than aqueous suspension ($p < 0.05$)

[b] statistically higher than MaltoDE ($p < 0.05$)

[c] statistically higher than Celebrex® ($p < 0.05$)

Among all tested formulations, CEL in the aqueous suspension form produced the lowest bioavailability (*i.e.* the lowest $AUC_{0\rightarrow\infty}$ value). A modest improvement in the absorption was observed for the suspended Celebrex® materials, which showed an increased C_{max} and

$F_{rel.}$ (~133%) in comparison with the CEL aqueous suspension. This is probably attributed to the surfactant (e.g. SLS) and superdisintegrant (e.g. croscarmellose sodium and povidone) contents of the capsule product [222] which facilitate the dispersion of drugs in the intestinal milieu. Interestingly, the simple lipid solution, submicron emulsion, and the dry emulsion system (MaltoDE) had negligible influence on the CEL absorption profile. Similar findings have also been presented by Takeuchi *et al.* [151] who observed only subtle differences in the t_{max}, C_{max}, and AUC of vitamin E acetate formulated in a lipid solution, an o/w emulsion and a silica-adsorbed dry emulsion system. Statistical analysis reveals that the PK data obtained for Celebrex®, lipid solution, lipid emulsion and MaltoDE was not significantly different from that of the pure CEL aqueous suspension.

In contrast, the Miglyol-based SLH microcapsules (SLH-M_1 and SLH-M_2) have substantially increased the fasted state absorption of CEL. Although in the *in vitro* tests, the SLH microcapsules redispersed to form relatively larger particles (*i.e.* mean hydrodynamic diameters 2–3 μm at pH 7) in comparison with lipid emulsions (<0.2 μm) and the MaltoDE system (<1 μm), the SLH formulations have exhibited a superior bioavailability (*i.e.* $F_{rel.}$ = 150–170%) in conjunction with a statistically higher C_{max} compared to the examined control systems ($p < 0.05$). It is noted that the differences in the bioavailability of the SLH formulations and Celebrex® are less obvious from the current study, possibly due to a relatively low dose of CEL administered, *i.e.* 5 mg/kg (c.f. reported CEL minimum efficacious dose of 10 mg/kg in rats [305]). The t_{max} values have remained constant (*i.e.* 2–4 h) across the various treatment groups notwithstanding the differences observed in other PK parameters. Thus, it is inferred that the gastric emptying time is not significantly influenced by the exogenous MCT excipients (at the current 'low' dose) and the total mass (or density) of formulation administered. It has been reported earlier in humans that administration of a low dose of MCT (*i.e.* 2 g) does not induce significant changes in the gastric emptying rate and biliary secretions; in contrast, a similar dose of LCT was shown to cause a noticeable delay in gastric emptying and stimulate the gall bladder contraction, which resulted in an elevation in the intestinal BS, PL and cholesterol levels [85].

Overall, the *in vivo* data does not conform to the previous *in vitro* dissolution study that shows improving dissolution profiles in the rank order of pure CEL < Celebrex® < MaltoDE ≈ SLH-M_1/-M_2 (**Chapter 3**). Furthermore, the bioavailability data is not in agreement with the lipolysis results which reveal significantly different digestion rate and extent between lipid solution, emulsion, MaltoDE and the SLH microcapsules (**Chapter 4**). Two factors

which were considered important in facilitating greater CEL absorption were not evident from these *in vivo* results: firstly, the solubilising effect of lipids/ surfactants; and secondly, the role of emulsification in facilitating drug release or lipid digestibility.

In fact, rats do not possess a gallbladder as do humans, therefore there is always a continuous secretions of BS into the GI fluids even without stimulation by lipids or food [74, 200]. Thus, it is anticipated that there is a sufficient level of micelles present in the rat intestinal content to promote drug absorption, whether in the fasted or fed state. The 'post-prandial' effect produced by the low dose of exogenous lipids (*i.e.* ≤175 mg in the present formulations) or the enhanced dispersion effect of surfactants (in the case of Celebrex®) may lack significance in promoting higher drug absorption in rats due to the pre-existence of such solubilising colloids. This explains the subtle bioavailability differences between the pure CEL suspension, Celebrex® material suspension, lipid solution, lipid emulsion and the MaltoDE formulations.

With respect to the particle size effects in absorption, controversial results have been published – some studies provided evidence that reduced particle sizes of the lipid-based carriers can lead to an increased drug bioavailability (e.g. cyclosporin [306] and penclomedine [276]), while some others reported no differences between crude oils and emulsified systems (e.g. vitamin E acetate [151] and danazol [170]). The present results are in good agreement with the latter scenario, *i.e.* variation in the initial particle sizes of the lipid vehicles is not a major determinant of the rate and extent of CEL absorption. In the GI tract, the ingested lipids are prone to emulsification starting from the stomach as promoted by the partially digested lipid products (DG, MG and FA) in conjunction with the shear forces resulting from the gastric movements; this is further enhanced by the biliary and pancreatic secretions in the duodenum, in which the endogenous amphiphiles (BS, PL) are capable of reducing or stabilising the lipid droplet sizes [60, 90].

Bearing in mind that all tested lipid-based formulations are composed of comparable amount of lipids (120–175 mg) and emulsifier (approximately 7.2–10.5 mg lecithin), the variation in the absorption results is expected to lie in the types of the stabiliser employed in the formulations (*i.e.* silica nanoparticles). We propose that the presence of Aerosil silica in the form of highly porous microcapsule structure (as characterised in **Chapter 2**) have played a vital role in increasing the absorption of CEL from the SLH-M_1/-M_2 formulations via improved drug dispersion (as confirmed in **Chapter 3**) in combination with enhanced

intraluminal processing of the lipid excipients (via several mechanisms as detailed in **Chapter 4**). In the following dose-dependent study, the SLH formulations were further evaluated at two higher CEL doses in order to determine if the absorption increment follows a linear trend according to the CEL dose administered.

5.4.2 Dose-dependent oral pharmacokinetics of celecoxib from non-lipid and lipid-based formulations

It is generally known that the PK parameters of CEL (particularly C_{max} and AUC) show a lack of linearity at higher doses, mainly because of its poor aqueous solubility and erratic absorption [50, 222]. There have been few studies reporting dose proportionality assessment of CEL when administered in various dosage forms. Some examples are the findings of McAdam *et al.* [290], who reported reasonably linear dose-PK relationship for up to 800 mg CEL (Celebrex® capsules) in humans; and Mamidi *et al.* [50] who found proportional dose-PK responses in rats for their CEL prodrug formulation, but not for the CEL conventional (suspension) formulation over the dose range of 10–100 mg/kg.

In this study, the dose-dependent PK of CEL is systematically investigated for various oral formulations at three dosage levels (*i.e.* 5, 20 and 50 mg/kg CEL) in a fasted rat model. The formulations of interest include CEL aqueous suspension, Celebrex® capsule materials and the SLH microcapsule formulations. The mean C_p-time profile of each studied formulation is presented in **Figure 5.2**. The corresponding PK data is summarised in **Table 5.4**.

When CEL is administered at higher doses (*i.e.* 20–50 mg/kg), all the modified formulations of CEL, including Celebrex® and the three SLH microcapsules examined (*i.e.* SLH-M_1, SLH-M_2 and SLH-C), exhibited approximately 2 to 2.5-fold higher bioavailability (*i.e.* $F_{rel.}$ = 191–243%) as compared to the pure CEL aqueous suspension ($p < 0.05$). Such bioavailability improvements (*i.e.* >20%) are regarded as clinically significant [289]. It is interesting to note that for Celebrex®, the bioavailability enhancement was found to be statistically significant only at doses greater than 20 mg/kg, whereas the SLH formulations demonstrated statistically greater bioavailability at all three dosage levels, ranging from 5 to 50 mg/kg. This indicates the superior dispersion-enhancing effect of the lipid-based carriers as compared to the non-lipid systems. When a higher dose of CEL is administered, the endogenous solubilising capacity for CEL may become limited, potentially due to saturation

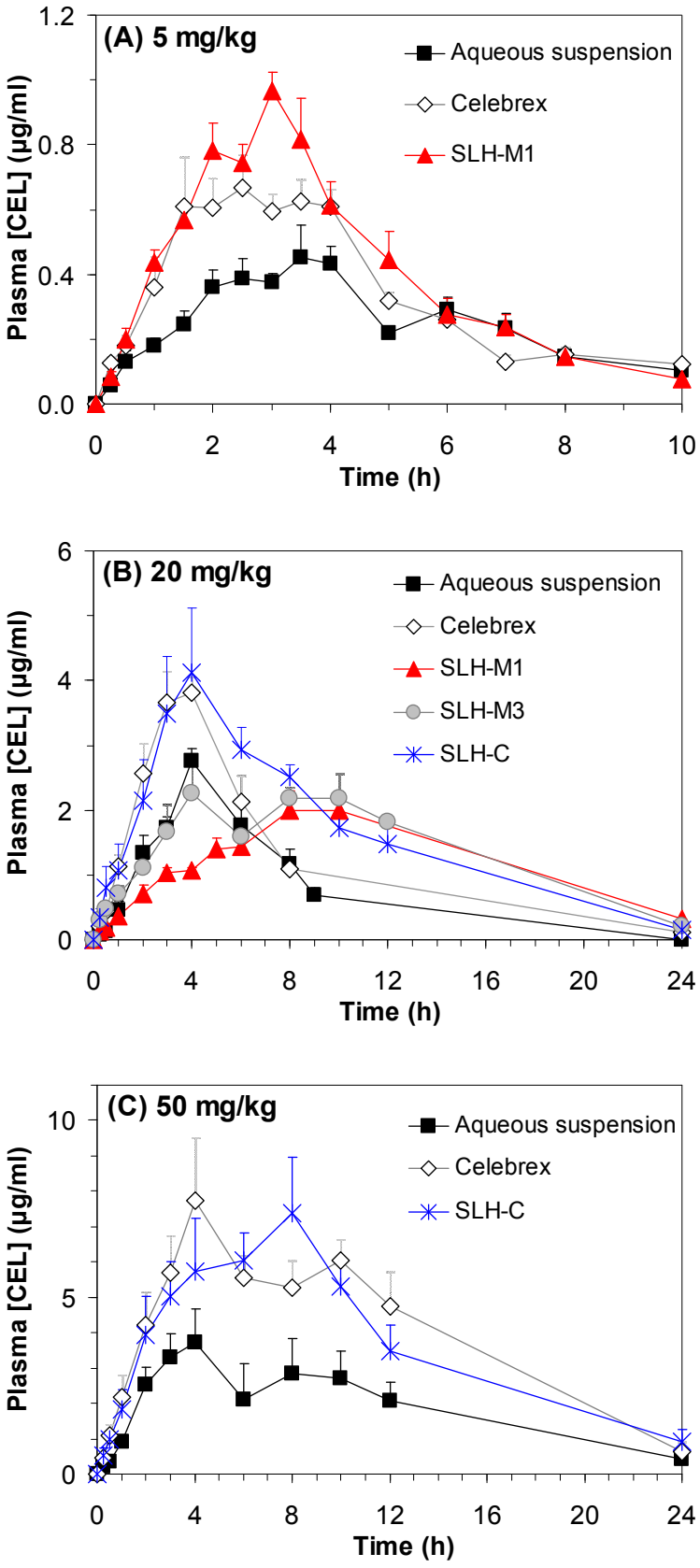


Figure 5.2 Plasma concentration–time profiles of celecoxib in male S.D. rats following a single oral dose of various formulations equivalent to (A) 5 mg/kg (*n* = 5), (B) 20 mg/kg (*n* = 5), and (C) 50 mg/kg of celecoxib (*n* = 4) (mean ± S.E.M.).

Table 5.4 Dose-dependent pharmacokinetic parameters of celecoxib following a single oral dose of various celecoxib formulations to male S.D. rats based on non-compartmental calculation (mean ± S.E.M., n = 5 unless otherwise specified)

CEL formulation	PK parameters			
	t_{max} (min)	C_{max} (μg/ml)	$AUC_{0\rightarrow\infty}$ (min.μg/ml)	$F_{rel.}$ (%)
Low dose: 5 mg/kg				
Aqueous suspension	202 ± 28	0.50 ± 0.08	167 ± 11	100 ± 7
Celebrex®	162 ± 20	0.63 ± 0.08	223 ± 16	133 ± 9
SLH-M_1	174 ± 15	1.00 ± 0.07 [a, b]	250 ± 15[a]	150 ± 9[a]
Medium dose: 20 mg/kg				
Aqueous suspension	270 ± 30	2.92 ± 0.37	951 ± 153	100 ± 16
Celebrex®	252 ± 29	4.18 ± 0.28	1882 ± 177[a]	198 ± 19[a]
SLH-M_1*	560 ± 40[a, b]	2.16 ± 0.29	1859 ± 109[a]	195 ± 12[a]
SLH-M_3	456 ± 59[a, b]	3.21 ± 0.44	2036 ± 187[a]	214 ± 20[a]
SLH-C	264 ± 24	4.33 ± 0.89	2316 ± 259[a]	244 ± 27[a]
High dose: 50 mg/kg				
Aqueous suspension*	285 ± 66	3.88 ± 0.96	3089 ± 546	100 ± 18
Celebrex®*	300 ± 60	8.83 ± 0.85[a]	5975 ± 525[a]	193 ± 17[a]
SLH-C*	360 ± 69	9.10 ± 0.74[a]	5895 ± 565[a]	191 ± 18[a]

* $n = 4$

[a] statistically higher than aqueous suspension ($p < 0.05$)

[b] statistically higher than Celebrex® ($p < 0.05$)

of the endogenous micellar systems with drug molecules. As a result, the solubilising effect contributed by the exogenous lipid and surfactant excipients becomes more prominent in preventing intraluminal drug precipitation, which eventually promotes a substantially greater extent of drug absorption at higher CEL doses.

In the previous chapters, it has been shown that SLH-M_1 is not significantly different from SLH-M_2 in terms of physicochemical characteristics (**Chapter 2**), dissolution (**Chapter 3**)

and lipolysis properties (**Chapter 4**); likewise for their oral absorption profiles at a 5 mg/kg CEL dose (**Section 5.4.1**). Therefore, the 'medium dose' experiments employing 20 mg/kg CEL was undertaken only for the formulation of SLH-M_1. The SLH-M_3 system is essentially similar to the SLH-M_1 system in their compositions except for a higher lecithin level and thus an increased CEL loading level in the former system. Parallel examination of the formulations of SLH-M_1, SLH-M_3 and SLH-C at the same CEL dosage level (*viz.* 20 mg/kg) allows us to distinguish the effect of different lipid dose on the absorption of CEL. From **Table 5.4**, it can be seen that both SLH-M_1 and SLH-M_3 showed significant delay in the absorption process as evidenced by the statistically higher t_{max} values (7–9 h), presumably resulting from the higher lipid mass of ~400 mg and ~240 mg Miglyol oil administered, respectively (**Table 5.2**). In contrast, SLH-C which contains ~110 mg Capmul oil per dose showed a t_{max} value of ~4 h, which is similar to that observed for the CEL aqueous suspension and Celebrex®. Such findings indicate that co-administration of a high amount of lipids (*i.e.* ≥240 mg Miglyol oil in the present case) potentially extend CEL absorption without affecting the overall bioavailability, in which the $AUC_{0\rightarrow\infty}$ values of SLH-M_1 and SLH-M_3 are statistically higher than that of the pure CEL suspension, but comparable to other tested formulations (including Celebrex® and SLH-C). Previously, Friedman *et al.* [307] have described that fat emulsions (e.g. Intralipid) tend to partition in the stomach into an aqueous phase which empties much faster than the retained oil phase. The delayed/ extended CEL absorption observed for SLH-M_1 and SLH-M_3 may be caused by several mechanisms: (*i*) a delay in gastric emptying (as estimated by Friedman *et al.*, the gastric emptying rate for fat calories is 0.03–0.08 kcal/min in rats [307]), (*ii*) an extended intestinal transit time [18, 82], or simply due to (*iii*) a longer digestion time required for the higher amount of lipids prior to drug absorption.

In contrast to the lipolysis data which illustrates different lipolysis profiles between the formulations of SLH-M_1/-M_2 and SLH-M_3, these three Miglyol-based formulations have been found to be equivalent in their *in vivo* performances. The values of $AUC_{0\rightarrow\infty}$ or $F_{rel.}$ obtained across the Miglyol- and Capmul-based SLH formulations (SLH-M_1, SLH-M_3 and SLH-C) at the medium dose level are essentially the same (**Table 5.4**). Therefore, the phase partition data which showed an increasing drug precipitation trend of SLHM_1 ≈ SLH-M_3 < SLH-C (**Section 4.4.3**) does not accurately depict the *in vivo* behaviour of the formulations. In the *in vitro* lipolysis tests, the digestion was conducted over a prolonged time period (*i.e.* 60 min rather than 30 min or less as reported by others using the same lipolysis model

[12, 170, 197, 206]), and in the presence of an excess amount of enzymes to ensure complete hydrolysis of the fat molecules. This means that the end lipolysis products accounted for solubilising the drug molecules in the *in vitro* medium are mainly the glycerol and FA species. While in the *in vivo* conditions, the partially digested lipids in the forms of MG and FA can undergo immediate absorption across the intestinal membrane without the need of a complete hydrolysis [79]. Therefore, the massive drug precipitation detected for SLH-C in the *in vitro* 'non-sink' model was not an accurate depiction of the intestinal situations, in which the colloidal species composed of MG and FA readily solubilised the CEL molecules and promoted an immediate absorption across the intestinal wall before further hydrolysis occurred.

It is now confirmed that both Miglyol- and Capmul-based microcapsule formulations produce a similar *in vivo* absorption performance. Considering a reasonable total feed dose size (*i.e.* total mass of administration <1 g per rat), the 'high dose' experiments conducted at 50 mg/kg CEL was undertaken with the SLH-C formulations because of its higher CEL loading level and hence, lower mass of administration required. The aqueous suspensions of pure CEL and Celebrex® were used as the positive controls. From **Table 5.4**, the PK results clearly reveal that SLH-C is capable of increasing the absorption of CEL at both medium and high doses, in which the absorption profiles are comparable to that of the suspended Celebrex® materials (**Figure 5.2C**). The increase in C_{max} (~9 μg/ml) and $AUC_{0\rightarrow\infty}$ (~6000 min.μg/ml) for SLH-C and Celebrex® is almost double that of the CEL aqueous suspension (*i.e.* ~4 μg/ml and ~3000 min.μg/ml, respectively); this was also seen at the medium dose level. It is noted that there is no significant delay in the t_{max} for SLH-C even though the mass of lipid administered is considered high (~280 mg Capmul oil). The unchanged rate of absorption for the SLH-C formulation may be rationalised by the composition of Capmul oil, which consists primarily of MG (58%) that is readily absorbable across the intestinal membrane, and DG (36%) that hydrolyses quickly into the absorbable forms (MG and FA) (**Section 4.4.1.3**) [206]. By also taking into account the amphiphilic character of Capmul oil, it is anticipated that oil retention in the stomach (as suggested for SLH-M_1 and SLH-M_3) was negligible in the case of SLH-C.

In order to clearly illustrate the dose-PK relationship, the values of $AUC_{0\rightarrow\infty}$ and C_{max} obtained for CEL aqueous suspension, Celebrex® and the SLH-microcapsules are plotted against the dose of CEL administered. The formulations of SLH-M_1/-M_2/-M_3 and SLH-C were grouped as the same lipid-based system because only subtle differences have been

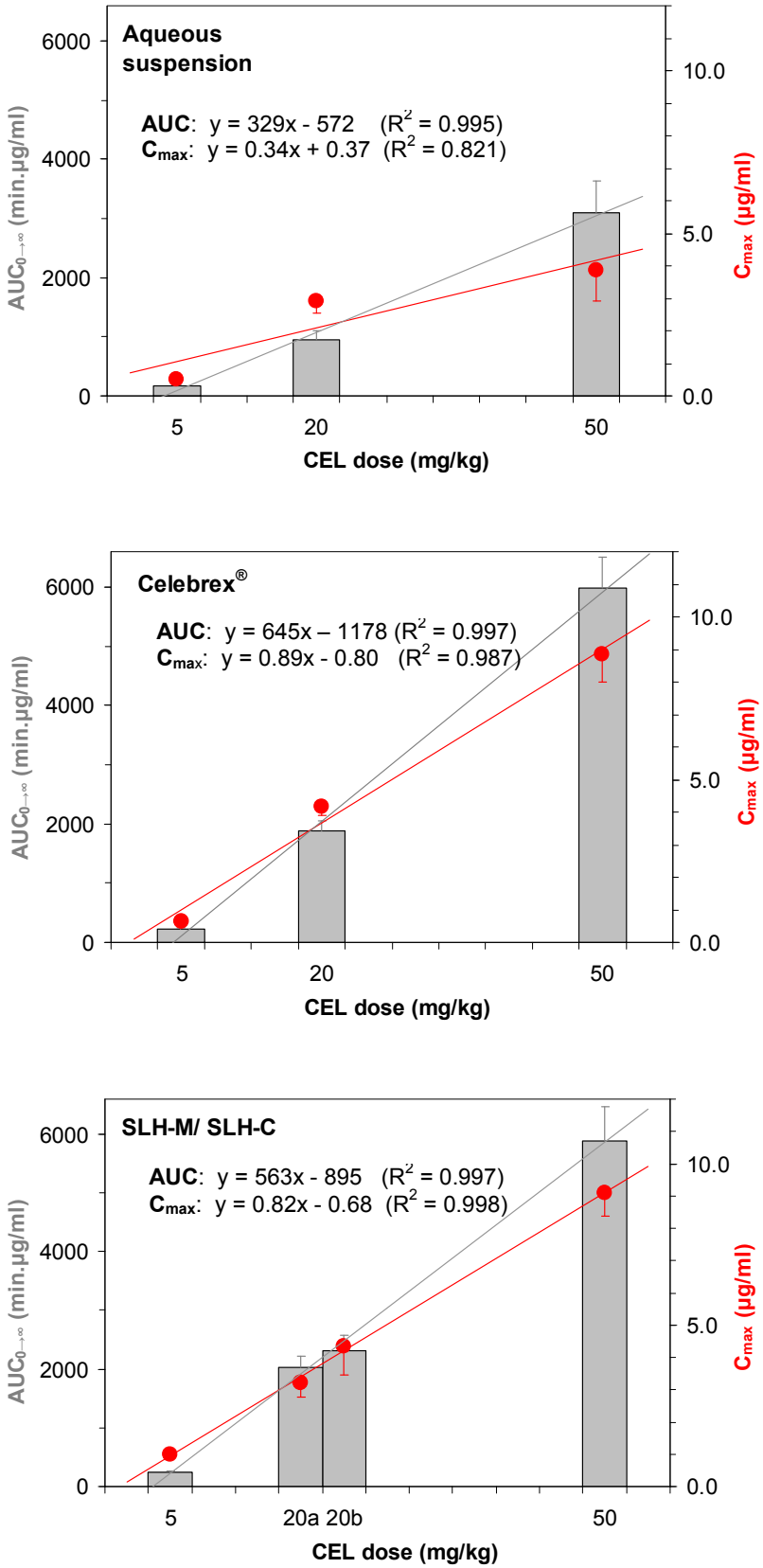


Figure 5.3 Linearity in pharmacokinetics of celecoxib over the dose range of 5–50 mg/kg following a single oral administration of various celecoxib formulations to male S.D. rats: $AUC_{0\rightarrow\infty}$ (grey bars) and C_{max} (closed circles).

observed in the bioavailability between these treatment groups. As depicted in **Figure 5.3**, the $AUC_{0\rightarrow\infty}$ and C_{max} values increase linearly between the three selected CEL doses (*i.e.* 5, 20 and 50 mg/kg) for all three studied formulations. The dose-PK relationships are reasonably linear in all scenarios ($R^2 \geq 0.99$), except for the C_{max} increment for CEL aqueous suspension ($R^2 = 0.82$).

Since the slope of the plots represents the rate of change in $AUC_{0\rightarrow\infty}$ or C_{max} values as a function of CEL dose, the slope results obtained for Celebrex® and the SLH formulations imply that these formulations could provide a two-fold higher CEL bioavailability or C_{max} than the pure CEL within the dose range of 5–50 mg/kg. However, it has previously been shown that the usual recommended CEL daily dose (*i.e.* ≤5 mg/kg or 200–400 mg daily for humans) is already at the upper limit of the sigmoidal dose-response curve [287]. Therefore, the observed proportional increase in the $AUC_{0\rightarrow\infty}$ and C_{max} values may not necessarily correlate to a greater anti-inflammatory response or efficacy. Importantly, the predicted dose-PK linearity can be valuable in assessing the anti-cancer properties of CEL, as previous research has reported that a CEL daily dose of 500–1000 mg/kg is safe and efficacious in rodent models for inhibiting tumour growth [308].

The therapeutic application of the SLH microcapsule system for the oral dosing of CEL is to some degree limited by the current drug loading level, *i.e.* a large unit dose of the carrier would be required to achieve desirable drug efficacy. Nevertheless, the present results provide clear impetus for similar studies on a wide range of other poorly water-soluble drugs that require more predictable oral absorption to minimise dose-related side effects.

5.5 Summary

The oral absorption profiles of CEL were determined in a fasted rat model following a single administration of various non-lipid and lipid-based formulations. At a dose level of 5mg/kg CEL, there was no significant difference observed across the treatment groups dosed with the following CEL formulations: pure CEL aqueous suspension, lipid solution, submicron emulsion, Celebrex® and the redispersed MaltoDE system. These results suggest that the dispersion-enhancing effect of lipids/ surfactants and the difference in the initial particle sizes (*i.e.* the role of emulsification) are not the major determinants of the absorption of CEL at this low dose level.

Interestingly, all four redispersed SLH microcapsules fabricated from either Milgyol or Capmul oil have exhibited significantly improved fasted state absorption of CEL, as evidenced by statistically higher $AUC_{0\rightarrow\infty}$ and C_{max} values ($p < 0.05$). The superiority in the pharmacokinetics of CEL formulated as the SLH microcapsules clearly points out the key role of the silica-based porous microcapsule structure in enhancing the absorption of CEL, presumably facilitated by a more controllable intraluminal processing of the formulation lipids. The four types of SLH microcapsules, *i.e.* SLH-M_1/-M_2/-M_3 and SLH-C, are essentially equivalent to one another in their bioavailability enhancing effects as demonstrated for CEL.

The dose-PK proportionality study of CEL was examined at three dosage levels: 5, 20 and 50 mg/kg. Dose linearity in the $AUC_{0\rightarrow\infty}$ and C_{max} values was confirmed for SLH microcapsules, Celebrex®, and the pure CEL. At these three dosage levels, the SLH microcapsules were found to be comparable to Celebrex®, and significantly better than the pure CEL administered as an aqueous suspension. Specifically, the $AUC_{0\rightarrow\infty}$ and C_{max} results produced by the SLH formulations and Celebrex® were 2-fold higher than the pure drug. Such improvement was found to be linearly related to the dose of administration within the range of 5–50 mg/kg.

Overall, the *in vitro* dissolution and lipolysis data did not accurately correlate to the *in vivo* absorption rank order. The dissolution results ranked the performance of the various systems as pure CEL < Celebrex® < MaltoDE ≈ SLH microcapsules; whereas the lipolysis data showed considerably different lipolysis kinetics and dynamics across the various lipid-based systems (*i.e.* lipid solution ≠ submicron emulsion ≠ MaltoDE ≠ SLH-M ≠ SLH-C). The *in vivo* results by contrast, reveal equivalent performance between pure drug, lipid solution, submicron emulsion and MaltoDE, and in general, better absorption profiles for Celebrex® and the SLH microcapsules (in which SLH-M ≈ SLH-C regardless of their different lipid and drug loading levels). A closer assessment of the IVIVC was undertaken for the individual formulation, and this is discussed in the next chapter.

CHAPTER SIX

In vitro-in vivo Correlations for Various Celecoxib Oral Dosage Forms

6.1 Introduction

6.1.1 An overview of in vitro-in vivo correlation studies

The concept of *in vitro–in vivo* correlations (IVIVCs), which is to use a relevant *in vitro* test to predict the *in vivo* outcome of a new or modified dosage form, was introduced at least four decades ago [309, 310]. The U.S. FDA defines IVIVC as "a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form and a relevant *in vivo* response" [311]. A predictive and reliable IVIVC (with a mean prediction error of <10% for bioavailability parameters) is useful for minimising or avoiding human studies (*i.e.* supporting biowaivers in bioequivalence studies) in dosage form development and optimisation; it can also serve as a product quality control tool for any post-approval changes [310, 312].

As documented in the U.S. FDA-IVIVC guidance [311, 312] and USP [312, 313], IVIVC can be divided into four major levels:

(*i*) Level A, the highest level of correlation representing a point-to-point relationship between the entire *in vitro* and *in vivo* profiles;

(*ii*) Level B, a statistical moment analysis which compares the average parameters derived from the *in vitro* and *in vivo* data (but not useful for regulatory purposes);

(*iii*) Level C, a single-point correlation based on a chosen *in vitro* and *in vivo* parameter (for at least three different formulations) which allows qualitative ranking and selection of pilot formulations in the early stage of formulation development; and

(*iv*) Multiple Level C, which relates more than one *in vivo* parameters to the *in vitro* data at several (*i.e.* at least three) time points covering the early, middle and late stages of the *in vitro* profile; this can be as useful as the Level A correlations.

The various parameters that are frequently used for establishing IVIVC of different levels are summarised in **Table 6.1**.

Table 6.1 Various parameters used in the IVIVC studies of poorly water-soluble drugs

Level of IVIVC	Parameters		References
	In vitro	*In vivo*	
A	Fraction or % of drug dissolved/ permeated (from dissolution/ permeation curve)	Fraction or % of drug absorbed (from deconvoluted absorption curve)	[314-319]
B	Mean dissolution time (MDT)	Mean residence time (MRT), mean *in vivo* dissolution time	*
C (single and multiple)	Disintegration time, $t_{10\%}$ or $t_{50\%}$ or $t_{90\%}$, dissolution rate, %DE, % permeation or predicted % absorption, % solubilisation	*AUC* (total or cumulative), C_{max}, t_{max}, absorption rate, time to have 10, 50, 90% absorbed	[48, 104, 188, 197, 200, 320-322]

* Level B correlations have been more commonly reported for modified formulations of water-soluble compounds [323, 324]

For BCS Class II drugs, dissolution or drug release tests are generally regarded as the most appropriate *in vitro* tool for predicting absorption profiles, as dissolution is the major rate-limiting factor to drug absorption [15, 312, 319]. According to Emami's review on IVIVC [310], linear correlations (or relationships) are considered the simplest to interpret and thus far, the most commonly reported; whilst reports on non-linear but predictive correlations are scarce. Various mathematical approaches have been developed to guide the establishment of dissolution-based IVIVC. As detailed discussion of the various mathematical models is not the major focus of this chapter, the interested readers are referred to the textbooks [5, 312].

IVIVC has been successfully developed for many model poorly water-soluble compounds using simple buffered dissolution media [48, 188, 315, 319, 320]. However, the complexity of the intra-luminal processes (e.g. the presence of endogenous surfactants and enzymes, gastrointestinal motility and ionic strength) has limited the utility of simple pharmacopoeial dissolution models for achieving adequate IVIVC for modified formulations of Class II drugs. This led to an extensive exploration of various biorelevant dissolution media to improve the *in vivo* predictability of the dissolution tests. Most of the biorelevant dissolution models were modified by incorporating BS and lecithin as a simulation of the intraluminal colloidal species under fasted or fed states, in order to provide better drug wetting and solubilisation capacity of the test media [5, 314, 316, 318, 322, 325].

Yet, the applicability of these modified dissolution models to lipid dosage forms is somewhat limited because drug release from the lipid carriers (and hence intestinal absorption) is not simply governed by the dispersion or miscibility of the lipid phase with the aqueous medium, but also by endogenous processing (*i.e.* digestion) of the lipid excipients, which subsequently changes the solubilisation state of the encapsulated drugs [8, 9]. Therefore, the use of dynamic lipolysis models to fully characterise the post-digestive state of the formulation lipids and drug solubilisation has become increasingly well recognised and effectively employed to assess (or rank) the *in vivo* absorption of many lipid-based formulations [197, 198, 317, 321]. Dahan and Hoffman systematically analysed the utility of a dynamic lipolysis model in interpreting the *in vivo* absorption of different Class II compounds formulated as lipid suspensions, including dexamethasone (log *P* = 1.7), griseofulvin (log *P* = 2.2), progesterone (log *P* = 4.0) and vitamin D_3 (log *P* = 9.1) [104, 197]. Their findings suggested that the post-digestion drug phase partition results could accurately predict the *in vivo* bioavailability provided that: (*i*) the drug is not absorbed significantly via the lymphatic pathway, and (*ii*) the intra-enterocyte metabolism, if any, obeys linear kinetics such that there is a constant ratio between the amount of drug absorbed and that metabolised.

As reviewed in Chapter 1 (**Table 1.9**), most of the previous IVIVC studies reported for lipid-based dosage forms [197, 198, 317, 321, 322] have demonstrated single-point correlations (or rank order correlations) between the rate or concentration of drug solubilised in the lipolysis aqueous phase and the *in vivo* t_{max} or *AUC* values. An exception is the study conducted by Fatouros *et al.* [321], in which the authors employed a mathematical modelling approach (*i.e.*, the 'neuro-fuzzy network') to predict the entire plasma concentration-time profiles from the *in vitro* drug solubilisation data. Thus far, correlations based directly on lipolysis kinetics (instead of drug phase partitioning) and drug absorption data have not been presented. For Class II drugs encapsulated in digestible lipids (which is the current formulation approach), the process of drug release and subsequent absorption is largely dependent on the digestion rate of the lipid carriers [322]. For this reason, it is anticipated that some extent of correlation should exist between % lipid digestion and % drug absorption, especially for drugs demonstrating appreciably high intestinal permeability. This forms the basis of the current IVIVC studies, which is to compare the dissolution-based and lipolysis-based approaches (including the lipolysis kinetic and drug phase partition studies) in establishing higher levels of IVIVC for the

various test formulations of CEL (including the non-lipid and lipid-based systems). The potential and limitation of each *in vitro* study in predicting the *in vivo* parameters are discussed.

6.1.2 Specific aims and hypotheses

In the current IVIVC assessments, the development of Level B correlations was not considered due to the lack of regulatory relevance [311, 312]. Level C (single-point) correlations were established to rank the *in vivo* performance of the studied CEL formulations based on the dissolution and lipolysis characteristics previously determined (refer to **Chapter 4** and **Chapter 5**, respectively). The most informative Level A (point-to-point) correlations and the Multiple Level C correlations were established to determine if the *in vitro* dissolution and lipolysis models employed are appropriate to represent the *in vivo* absorption outcomes. This is important in guiding further refinement of the *in vitro* models.

The hypotheses for this chapter are that:

(*i*) *in vitro* dissolution model is a suitable predictive tool for non-lipid formulations (*i.e.* pure CEL and Celebrex®), which can be used to establish multiple-point IVIVC for CEL; and

(*ii*) *in vitro* lipolysis model is an appropriate predictive tool for fully characterising the drug solubilisation and absorption process resulting from various lipid-based dosage forms, including lipid solution and emulsion, the MaltoDE system and the SLH microcapsules.

6.2 Theory for *in vitro-in vivo* correlations

6.2.1 Dissolution-based IVIVC

The concept of dissolution efficiency (%DE) has been introduced by Khan [256] as a useful dissolution parameter to be correlated with the corresponding *in vivo* bioavailability parameters (either *AUC* or *F*), because both *in vitro* and *in vivo* variables are estimated by the integration of the area under the curves. As the first step, single-point Level C correlations were established based directly on the *in vitro* parameters (%DE) and the *in*

vivo PK data (including $AUC_{0\rightarrow\infty}$ and C_{max}) to rank the dissolution-absorption performance of the studied formulations.

In principle, an ideal dissolution-based Level A IVIVC can be obtained by plotting the *in vivo* fraction of drug absorbed ($F_{absorbed}$) against the *in vitro* fraction of drug dissolved ($F_{dissolved}$) by including a time scaling factor to make the two curves superimposable [312, 314-319]. The $F_{absorbed}$-time profile is usually derived by deconvolution of the C_p-time curve using one of the following mass balance methods: Wagner-Nelson method (for one-compartmental model) [18, 312, 326] or Loo-Riegelman method (for a two-compartmental model) [18, 312]; alternatively, model-independent numerical methods can also be used [18, 312]. Considering that the Loo-Riegelman method and the numerical deconvolution require data (of observations spaced at equal intervals limits) obtained for both oral (test formulation) and intravenous (reference) treatment in the same subject, these methods are not applicable in the current work and therefore, are not discussed in detail in this chapter. The one-compartmental Wagner-Nelson deconvolution method was employed with the assumption of a linear, causal and time-invariant system [18, 312, 326]. Level A IVIVCs were developed according to the typical procedures as described below [18, 312, 326]:

(Step 1) Deconvolution of the C_p-time profiles

The cumulative amount of drug absorbed at each time point (A_T) was calculated by deconvolution of the C_p-time profile according to the following equation developed by Wagner and Nelson [326]:

$$\frac{A_T}{V_d} = C_T + K_{el} \times AUC_{0\rightarrow T} \qquad (12)$$

where V_d is the apparent volume of distribution, C_T is the plasma drug concentration at time T, K_{el} is the first-order elimination rate constant, and $AUC_{0\rightarrow T}$ is the area-under-the-curve of the C_p-time plot from time 0 to T. For CEL, a K_{el} value of 0.0037 min^{-1} was obtained from the slope of the least square fitted log linear plot of C_p versus time for CEL administered as an intravenous dose (**Appendix I**). It is noted that the K_{el} value obtained (which can be calculated as $\ln 2/t_{1/2}$) is comparable to the literature values of 0.0031 min^{-1} [228] and 0.0037 min^{-1} [288].

Typically, the values of A_T/V_d progressively increase until a maximum or asymptotic value is reached. When the individual A_T/V_d values are expressed as fractions of the maximum or

asymptotic value, the results are the fraction of drug absorbed ($F_{absorbed}$) at various time T. It is important to note that the $F_{absorbed}$ so determined is a fraction of the total amount of drug which is absorbed, not as a fraction of the dose administered.

(Step 2) Weibull fitting and time scaling

The Weibull function (equation 13) was used to scale the *in vitro* dissolution profiles to enable direct correlation with the *in vivo* absorption profiles [18, 312]:

$$M_t = M_{\max} \cdot \left(1 - e^{[(t+t_0)/\alpha]^{\beta}}\right) \qquad (13)$$

where M_t is the mass of drug dissolved at time t, M_{max} is the maximum cumulative mass dissolved, t_0 is the lag time before the onset of dissolution (which is generally assumed to be insignificant), α is a time parameter that represents the overall rate of the process, and β is the 'shape' factor of the distribution. The *in vitro* cumulative dissolved and *in vivo* cumulative absorbed fractions were fitted to the Weibull model. A perfect correlation was considered to be achieved if the curves were made superimposable by incorporating a time scaling factor determined as follows:

$$t_{in\ vivo} = a + b \cdot t_{in\ vitro} \qquad (14)$$

where $t_{in\ vivo}$ is the time *in vivo*, $t_{in\ vitro}$ is the time *in vitro*, a is the lag time *in vivo*, and b is the time scale factor. The time scale factor was calculated as the ratio of the time factors (α) of *in vitro* and *in vivo* Weibull fitted profiles (provided both profiles have an infinite dissolution or asymptotic value of 100%):

$$b = \frac{\alpha_{in\ vivo}}{\alpha_{in\ vitro}} \qquad (15)$$

(Step 3) Correlation between the time-scaled *in vitro* and *in vivo* profiles

The $F_{absorbed}$ values were plotted against the time-scaled $F_{dissolved}$ data to determine the linearity of the absorption-dissolution relationship.

6.2.2 Lipolysis-based IVIVC

A systematic guide for developing a lipolysis-based IVIVC is not yet available. For this reason, the current correlation approaches did not employ any specific mathematical models. Level C (single-point and multiple-point) correlations were established based directly on the *in vitro* parameters obtained (specifically the % lipolysis and the % CEL solubilised in the aqueous phase at specified time points) and the *in vivo* PK data ($AUC_{0\rightarrow\infty}$ and C_{max}).

6.3 Results and Discussion

6.3.1 Dissolution-based IVIVC

The dissolution-based IVIVC analyses were conducted for various CEL formulations that have previously been investigated for the *in vitro* dissolution (or drug release) profiles in **Chapter 3**. The non-lipid formulations include pure CEL and Celebrex®, while lipid-based formulations include the maltodextrin-based dry emulsion (MaltoDE) and two Miglyol oil-based SLH microcapsules (*i.e.* SLH-M_1 and SLH-M_2).

(a) Level C Correlations. To assess the *in vivo* predictability of the current dissolution model employing SLS as the solubilising agent, the %DE data obtained from the dissolution profiles under sink conditions (**Chapter 3**) is correlated with the *in vivo* PK parameters for CEL obtained from an orally dosed rat absorption study (**Chapter 5**). It is noted that both *in vitro* and *in vivo* data used for IVIVC development is based on the same dose of drug, *viz.* approximately 2 mg CEL. Interestingly, linear single-point IVIVC were observed across four studied formulations of CEL, *i.e.* in the rank order of pure CEL < Celebrex® < SLH-M_1 or SLH-M_2 based on the mean %DE values and the mean $AUC_{0\rightarrow\infty}$ or C_{max} data. As shown in **Figure 6.1**, the %DE obtained over the initial dissolution stage (*i.e.* $\%DE_{15}$) was found to be linearly correlated with the $AUC_{0\rightarrow\infty}$ values (Panel A, $R^2 > 0.9$). This indicates that the rate of dissolution or drug release has a significant impact on the bioavailability of CEL. Furthermore, the $\%DE_{180}$ was well correlated with the values of C_{max} (Panel B, $R^2 > 0.9$), which was estimated to be reached at the same time point (t_{max} = 3 to 4 h as reported in **Chapter 5**).

It is noted that the MaltoDE system was not effectively fitted into the correlations obtained, since the *in vitro* dissolution results (~100% release) were not in accord with the relatively

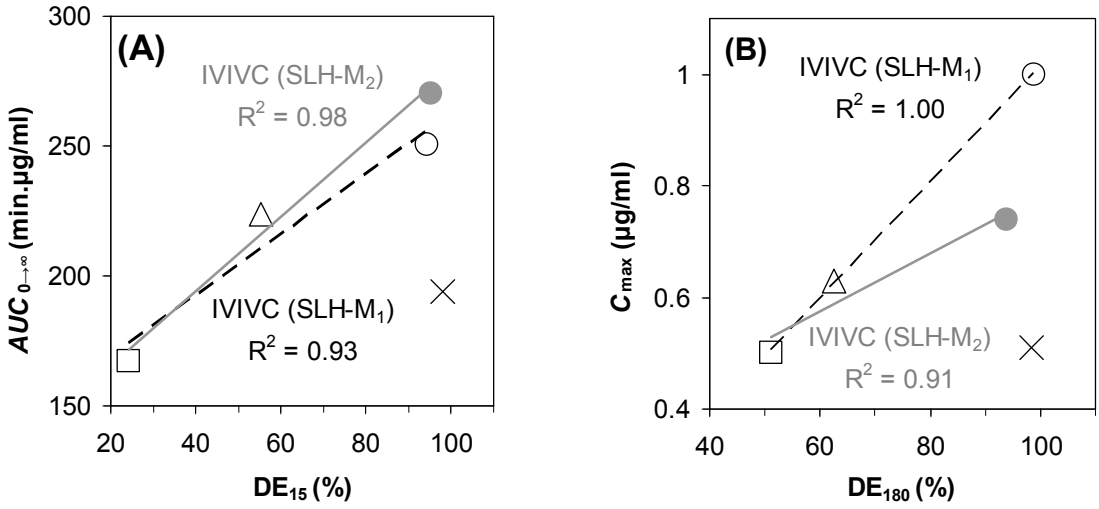


Figure 6.1 Single-point correlations between pure CEL (□), Celebrex® (△) and SLH-M_1 (○) or SLH-M_2 (●): (A) DE_{15} correlated to the bioavailability ($AUC_{0\rightarrow\infty}$) and (B) DE_{180} correlated to the maximum plasma concentration (C_{max}). The MaltoDE formulation (×) is an outlier which is not fitted into the least squares regression lines. The dashed lines correspond to the linear correlations between pure CEL, Celebrex® and SLH-M_1, whereas the solid lines correspond to the linear correlations between pure CEL, Celebrex® and SLH-M_2.

poor *in vivo* performance (*i.e.* a relatively low $F_{rel.}$ and C_{max}). This suggests that the actual process of CEL absorption from the lipid-based carriers is not solely governed by the dissolution process, but it involves other mechanisms, such as lipolysis of the formulation lipids (a detailed discussion was presented in **Chapter 4** and **Chapter 5**).

(b) Level A correlations. The $F_{absorbed}$-time profiles obtained by the method of Wagner-Nelson deconvolution and the corresponding cumulative dissolution curves (*i.e.* $F_{dissolved}$ vs. time) are presented in **Figure 6.2**. The plots (left-hand figures) clearly show that the *in vitro* drug dissolution occurred substantially faster than the *in vivo* absorption, particularly for the lipid-based formulations (*i.e.* MaltoDE, SLH-M_1 and SLH-M_2). This is because the encapsulated CEL has already been solubilised in the lipid phase for these formulations. As such, the *in vitro* drug release is solely a process of simple diffusion involving rapid drug transfer from the lipid cores into the aqueous surroundings driven by the presence of SLS micellar clusters.

The fast burst release of CEL resulting from these lipid-based systems has complicated the linear fitting with the Weibull function and consequently, a point-to-point correlation

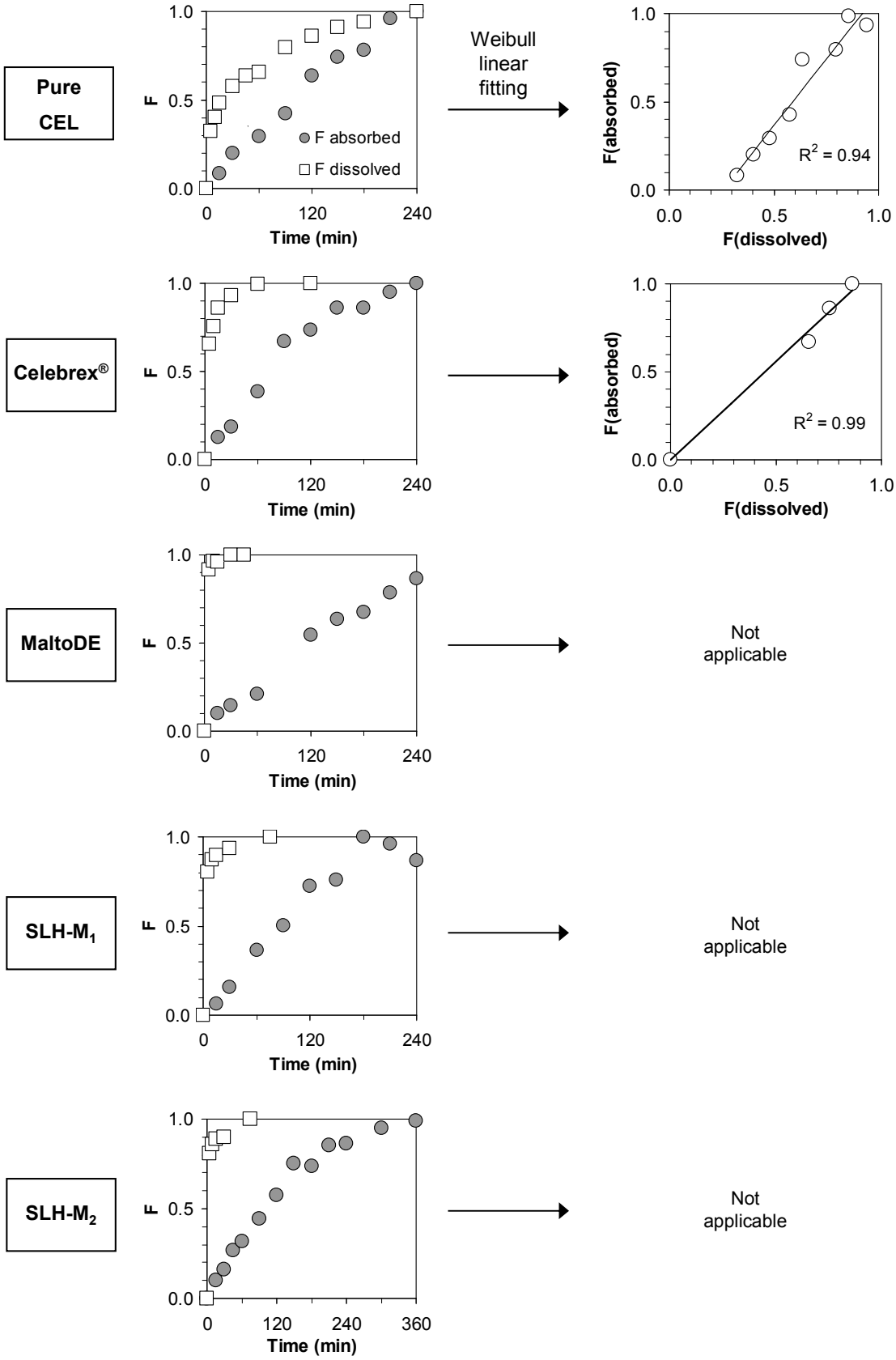


Figure 6.2 Point-to-point correlations between the fraction of drug dissolved and the fraction of drug absorbed.

between the entire dissolution and absorption profiles was not feasible for the formulations of MaltoDE, SLH-M_1 and SLH-M_2. In contrast, pure CEL and Celebrex® are lipid-free formulations containing drugs in the crystalline forms (as reported in **Chapter 2**). Therefore, a higher correlation between drug dissolution (which is the major barrier to drug absorption) and the bioavailability parameters is anticipated. Following Weibull linear fitting, the time scale factor, *b*, was calculated to be 3.1 for pure CEL and 17.6 for Celebrex®. A reasonably linear relationship between $F_{dissolved}$ and $F_{absorbed}$ was obtained for pure CEL ($R^2 = 0.94$), but with a non-zero intercept (right-hand figures of **Figure 6.2**). As for Celebrex®, only three data points could be directly correlated, in which an extrapolation through the origin produced good linearity ($R^2 = 0.99$).

A major limitation of the current dissolution model is the use of a non-biorelevant medium which contains a reasonably small quantity of SLS to mimic the *in vivo* sink conditions. In order to obtain a better IVIVC, Lue *et al.* [318] have suggested the use of a physiologically relevant dissolution medium, for example, a fed state medium containing appropriate amount of BS and lipolysis products (such as lecithin, oleic acid and glycerol monooleate). A future challenge is to establish a more rigorous dissolution or drug release method to enable the development of higher IVIVC for various lipid-based systems. A recent study has compared various dissolution test methods (*i.e.* using different USP Apparatus in the presence of varying media) for the *in vivo* prediction of a weakly acidic drug RZ-50 (*i.e.* an experimental Roche compound) formulated as lipid suspensions filled in soft gelatin capsules [317]. Based on the IVIVC results obtained in this study, the authors concluded that the USP Apparatus 3 (reciprocating cylinder, Bio-Dis) is more effective than the USP Apparatus 2 (paddle system) in predicting the *in vivo* drug absorption in the presence of a biorelevant medium. The highest correlation was obtained with a fed state simulated gastric fluid (pH 5.0, containing glacial acetic acid, sodium acetate and sodium chloride), followed by a fasted state simulated intestinal fluid (pH 6.5, containing BS, lecithin and pancreatin). However, the utility of various USP apparatus in improving the IVIVC results could not be assessed in our laboratory as only the USP Apparatus 2 is available.

6.3.2 Lipolysis-based IVIVC

The lipolyisis-based IVIVC analyses were conducted for various CEL formulations that have previously been investigated for the *in vitro* lipolysis kinetics and/or CEL phase

partition profiles in **Chapter 4**. The non-lipid formulations include pure CEL, while lipid-based formulations include MaltoDE and four types of SLH microcapsules, *i.e.* the Miglyol oil-based SLH-M_1, SLH-M_2 and SLH-M_3 microcapsules, and the Capmul oil-based SLH-C microcapsules.

(a) Level C correlations. Direct single-point correlations were investigated in two ways. In the first approach, the *in vivo* $AUC_{0\rightarrow\infty}$ or C_{max} values were correlated with the % hydrolysis of the lipid vehicles (H). As shown in **Figure 6.3**, a linear rank order of lipid solution < lipid emulsion < SLH-M_1 or SLH- M_2 was obtained ($R^2 > 0.8$). It is essential to note that the H values (up to 45 min) were reasonably well correlated with the F and C_{max} values for all these four studied lipid systems ($R^2 > 0.7$); only a representative correlation data indicating the best linear regression is presented in **Figure 6.3**. There results are suggestive of the prime importance of lipid digestibility to the absorption of CEL in the intestinal milieu.

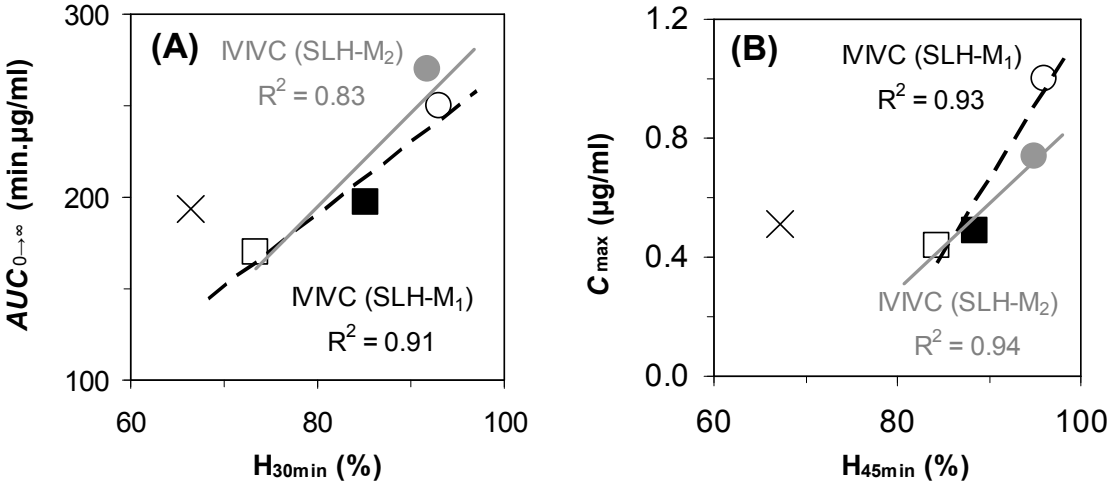


Figure 6.3 Single-point correlations between lipid solution (□), lipid emulsion (■) and SLH-M_1 (○) or SLH-M_2 (●): (A) percentage lipolysis (H) correlated to the bioavailability ($AUC_{0\rightarrow\infty}$) and (B) percentage lipolysis (H) correlated to the maximum plasma concentration (C_{max}). MaltoDE (×) is an outlier which is not fitted into the least squares regression lines.

Similar to the dissolution-based Level C IVIVC reported in **Section 6.3.1(a)**, the MaltoDE system was not fitted into the correlations observed. In the *in vitro* lipolysis model (**Section 4.4.1.1**), the MaltoDE system was found to produce a significantly lower % of lipolysis (<70%) in comparison with the lipid solution and emulsion (~90% lipolysis). However, the *in vivo* absorption study (**Section 5.4.1**) shows that the MaltoDE formulation produced almost the same $AUC_{0\rightarrow\infty}$ and C_{max} as the lipid solution and emulsion. Therefore,

the inhibitory effect of maltodextrin as inferred from the lower *in vitro* lipolysis extent for the MaltoDE system may not be accurately reflective of the *in vivo* situation. This requires a more systematic investigation into the proposed inhibitory effect of maltodextrin on lipolysis mediated by the pancreatic enzymes, *i.e.* a future focus of studies in our laboratory.

In the second approach, the *in vivo* $AUC_{0\rightarrow\infty}$ or C_{max} values were correlated with: (*i*) the final concentration of CEL solubilised in the post-lipolysis aqueous phase at 60 min (*i.e.* $[CEL]_{aq\ (60\ min)}$) and (*ii*) the maximum concentration of CEL solubilised in the aqueous phase during lipolysis (*i.e.* $[CEL]_{aq\ (max)}$). This was conducted using the PK results obtained at an oral CEL dose of 20 mg/kg, because the amount of CEL administered to the rats (*i.e.* 6–7.2 mg CEL per rat) is in the middle range of the CEL dose incorporated into the *in vitro* lipolysis medium (*i.e.* 2–12.4 mg CEL per 20 ml lipolysis medium). The results are shown in **Figure 6.4**.

The plots in panel (A) and (B) clearly depict a linear IVIVC for pure CEL, SLH-M_1 and SLH-M_3 ($R^2 > 0.9$ with a zero intercept), in which case the $[CEL]_{aq}$ in the post-digestive state (*i.e.* after 60 min of lipolysis) was considered. However, SLH-C was not fitted into the correlations. Previously, the lipolysis phase partition results (**Section 4.4.3**) have shown that a complete hydrolysis of the SLH-C microcapsules led to a significant drug precipitation (*i.e.* >50% of the CEL dose of 620 μg/ml lipolysis medium). The post-lipolysis CEL solubilisation capacity given by SLH-C (218 μg/ml) was significantly lower than that produced by SLH-M_3 (326 μg/ml). Notwithstanding these results, the *in vivo* $AUC_{0\rightarrow\infty}$ and C_{max} obtained for SLH-C (*i.e.* 2316 min.μg/ml and 4.33 μg/ml, respectively) were apparently higher than that for SLH-M_3 (2036 min.μg/ml and 3.21 μg/ml, respectively), although these differences did not reach statistical significance. Such discrepancies in the *in vitro* and *in vivo* findings can be explained by the fact that fat molecules do not need to undergo complete hydrolysis for complete absorption to occur [79]; the 58% MG component present in the Capmul oil (of SLH-C) is sufficiently solubilising for CEL and is assumed to be readily absorbable in the GI tract. Therefore, the high percentage of CEL precipitation observed in the *in vitro* lipolysis medium is not reflective of the *in vivo* solubilisation state for the SLH-C formulation.

In an alternative approach, the $[CEL]_{aq\ (max)}$ values were taken to correlate with the PK data. This approach is considered based on the concept of drug supersaturation effect as

suggested by Guzman *et al.* [48] and Brouwers *et al.* [42]. The authors proposed that the creation of a highly supersaturated system in the GI lumen (even for a limited time period)

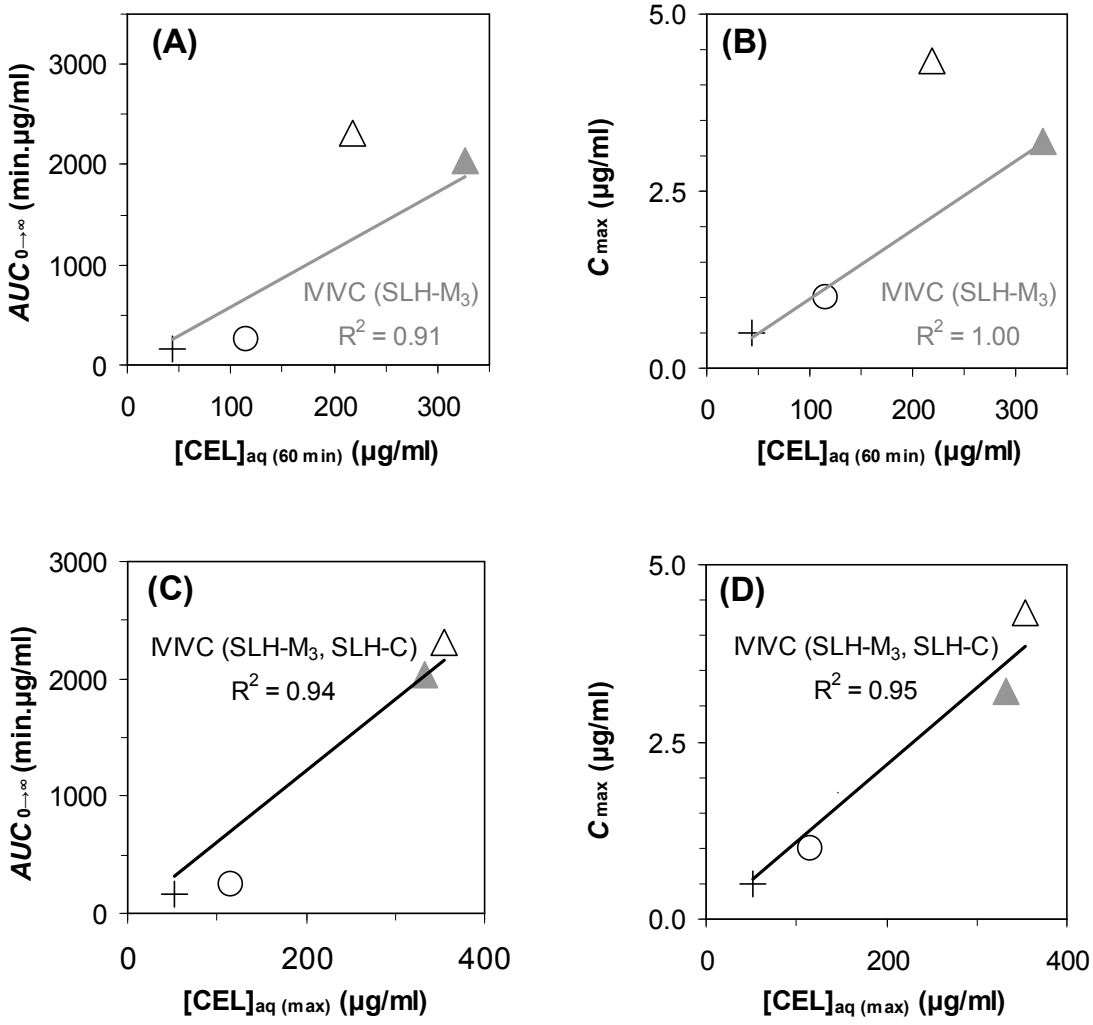


Figure 6.4 Single-point correlations for various CEL formulations, including pure CEL (+), SLH-M_1 (○), SLH-M_3 (▲), and SLH-C (△). The final concentration of CEL solubilised in the post-lipolysis aqueous phase at 60 min ($[CEL]_{aq\ (60\ min)}$) was correlated to: (A) the bioavailability ($AUC_{0\rightarrow\infty}$) and (B) the maximum plasma concentration (C_{max}). The maximum concentration of CEL solubilised in the aqueous phase during lipolysis ($[CEL]_{aq\ (max)}$) was correlated to (C) $AUC_{0\rightarrow\infty}$ and (D) C_{max}. Note that in panel (A) and (B), SLH-C is an outlier which is not fitted into the least squares regression lines.

is an important mechanism that facilitates the intestinal absorption of highly permeable drugs, such as CEL. As shown in **Figure 6.4** (panels C and D), there is a strong correlation between the $AUC_{0\rightarrow\infty}$ or C_{max} values and the $[CEL]_{aq\ (max)}$ data for all four studied formulations: pure CEL < SLH-M_1 < SLH-M_3 < SLH-C ($R^2 > 0.9$ with a zero intercept in

both plots). These IVIVC results effectively support the 'supersaturation' concept in which the maximum achievable aqueous solubilisation level in the lipolysis medium adequately depicts the *in vivo* bioavailability of pure CEL and the SLH formulations loaded with different CEL content (*i.e.* 0.64% w/w for SLH-M_1, 1.81% for SLH-M_3 and 3.84% for SLH-C). This correlation approach represents a significant novelty in the studies of lipolysis-based IVIVC and therefore, will be further investigated in our laboratory using different types of lipid excipients and model lipophilic drugs.

(b) Multiple Level C correlations based on lipolysis kinetics. The lipolysis-based correlation is extended to a multiple-point correlation for the individual lipid-based formulations. Specifically, the extent of lipolysis (H) obtained at the early, middle and late stages (covered by t = 1, 5, 15, 30 and 60 min) was correlated with the C_p data when the absorption rate dominated over the elimination rate, *i.e.* at times prior to reaching C_{max} (t = 15, 30, 60, 120 and 180 min). **Figure 6.5** provides evidence of an exponential (left-hand figures) or a log-linear (right-hand figures) relationship between %H and the C_p data obtained at the specified time points. A strong multiple-point correlation was obtained for each of the tested formulations ($R^2 \geq 0.8$): lipid solution, lipid emulsion, MaltoDE, SLH-M_1/-M_2/-M_3 and SLH-C. This supports our argument that the percentage of drug release and absorption is highly dependent on the percentage of digestion of the lipid vehicles when a digestible lipid is used for drug encapsulation. This makes possible the estimation of CEL absorption based solely on the extent of lipid digestion for formulations ranging from a simple lipid solution and emulsion, to the more advanced systems such as the MaltoDE and SLH microcapsules.

The non-zero intercepts in each of the log-linear plot (right-hand figures) suggest that drug release via diffusion can occur before digestion of the lipid vehicles is initiated. One may infer that the intercept of each plot represent the solubilisation level of CEL in the intestinal milieu under a non-digesting condition (*i.e.* H = 0%). The highest intercept value was obtained for SLH-M_3 (5.08), followed by SLH-C (3.99) and lipid solution (3.63). This may be related either to (*i*) the higher CEL loading levels for SLH-M_3 and SLH-C, which potentially created a concentration gradient in the intestinal milieu to promote CEL diffusion and intestinal permeation, and/or (*ii*) an enhanced drug dispersion effect as facilitated by the surface active, amphiphilic components of these systems, *i.e.* high lecithin levels in SLH-M_3 and the content of Capmul oil in SLH-C. For the lipid solution, it is assumed that a slower CEL diffusion from the lipid phase towards the intestinal absorption

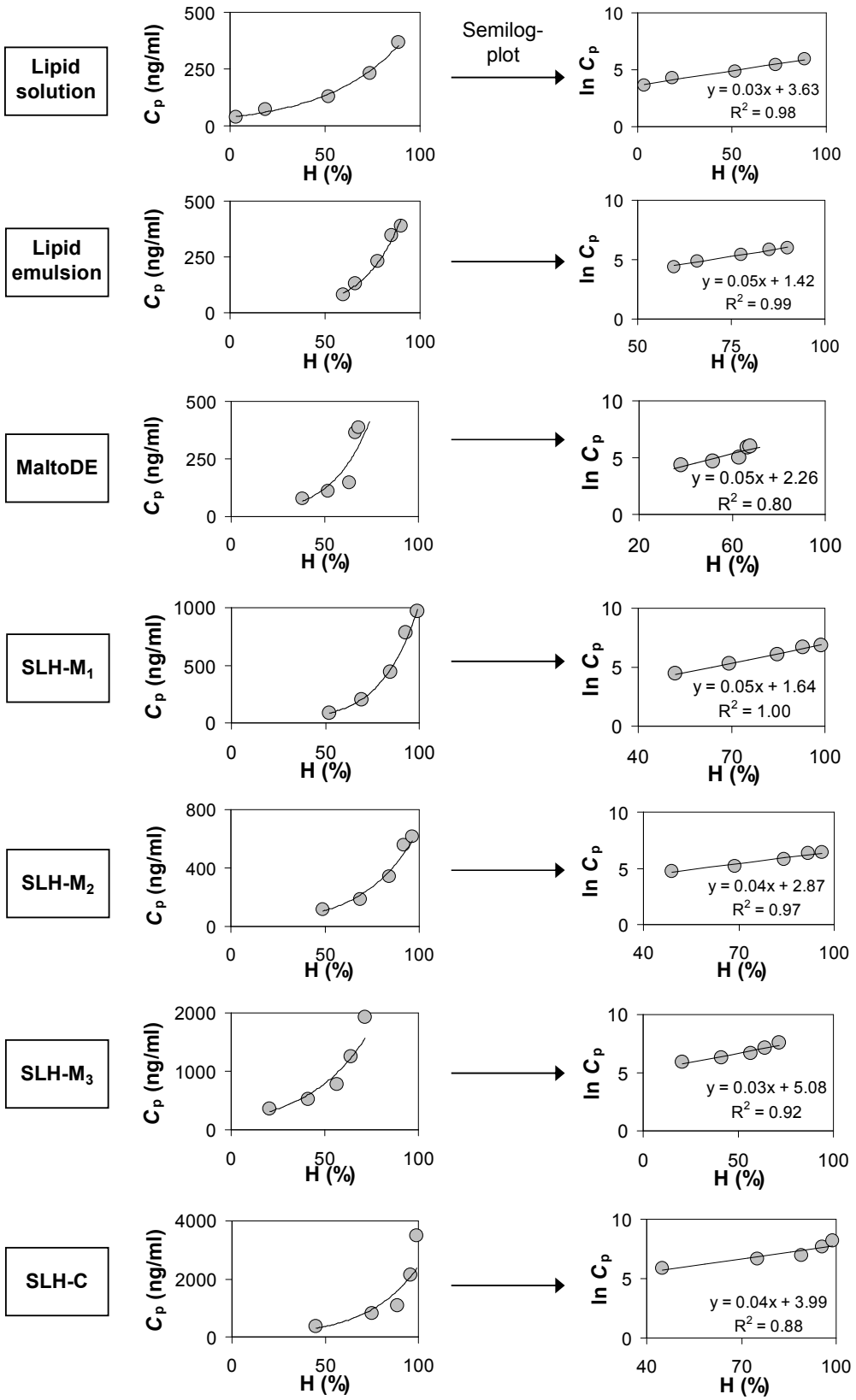


Figure 6.5 Multiple-point correlations between the percentage of *in vitro* lipid hydrolysis (H) obtained at t = 1, 5, 15, 30 and 60 min, and the *in vivo* plasma concentration of celecoxib (C_p) at t = 15, 30, 60, 120 and 180 min resulted from oral administration of the corresponding lipid dosage forms.

wall was involved prior to absorption. Other lipid vehicles including lipid emulsion, MaltoDE, SLH-M_1 and SLH-M_2 have each given a relatively lower intercept value (*i.e.* <3). This possibly means that digestion of these lipid vehicles is crucial to effectively release the encapsulated drug molecules for absorption to occur. The dependence of drug absorption on the % digestion of the lipid vehicles may be inferred from the slope of each plot (which explains how % digestion influences the increment in C_p). A lower slope value would indicate a lower degree of such lipolysis-absorption dependence effect; as an example, SLH-M_3 which has the highest intercept value (and thus, the highest pre-digestion solubilisation level of CEL) also has the lowest slope value (*i.e.* less dependence on the vehicle digestion to release CEL molecules).

The logarithmic relationship observed between the C_p and %H values could be attributed to a number of time-dependent *in vivo* events. Firstly, in the current *in vitro* lipolysis test, the %H value is calculated based on the titratable [FFA] produced in the lipolysis medium, with the assumption that one TG molecule is completely hydrolysed to produce 1 glycerol and 3 FA monomers. This does not take into account the real physiological situation (as mentioned previously) that digested glycerides (in the form of MG and FA species) can be completely absorbed through the intestinal membrane at only 66% hydrolysis [79]. It is also important to consider that the *in vitro* simulation of the human fasted state intestinal conditions may not be equivalent to the *in vivo* rat fasted state intestinal contents [200].

Secondly, the current method also does not consider the lipolytic effect contributed by other fat digesting enzymes that are present in the GI tract. A specific example is the gastric lipase, which is known to account for 10–20% TG hydrolysis in the stomach, and ~10% lipolysis in the intestines (in which its lipolysis role becomes increasingly important at the late phase of the *in vivo* intraduodenal lipolysis) [76, 79].

Thirdly, the potential of lymphatic transport for CEL is not clear from the published literature. Although the lymphatic absorption of CEL is assumed to be of little significance due to its intermediate lipophilicity (log P < 5), the lymphatic transport of medium-chain FA (~2%) has been reported [126]. It is typically known that both SCT and MCT (carbon chain length <12) are transported into the systemic circulation predominantly via the hepatic portal system, but the relationship of the lipid transport pathway to the FA chain length is not absolute [5, 126].

Overall, the multiple-point correlations based on the lipolysis kinetics confirm the significance of lipid carrier digestibility to drug absorption, in which the generated lipid digestion products have crucial influences on the physiological conditions (such as the formation of highly solubilising vesicular and micellar phases in the intestinal milieu) and biochemical changes (e.g. increased intestinal permeability) [61, 62]. Thus far, this correlation method has been attempted for CEL with one selected lipid dose, lipid type (*i.e.* medium-chain lipids), enzyme level, and BS/PL concentration (which simulates the fasted-state), all of which if modified, would change the results of the lipolysis kinetics and correlation patterns. It is stated in the U.S. FDA guidance that a biowaiver can only be justified if a correlation covering at least three data points from the *in vitro* profiles with any relevant PK parameters is obtained at the same time points [310]. Obviously, the limitation for the current correlation is the difference in the time scale of the *in vitro* and *in vivo* data. If the selected time points (t) for both *in vitro* and *in vivo* data are plotted against one another according to the concept of a Levy plot (*i.e.* $t_{in\ vivo}$ vs. $t_{in\ vitro}$) [18], it yields a slope of approximately 3 which can be regarded as the time scaling factor (**Figure 6.6**). One would infer that the *in vivo* absorption occurs three-fold slower than the *in vitro* lipolysis under the simulated intestinal conditions. Further evaluation is warranted to clearly establish the general applicability of the current correlation approach.

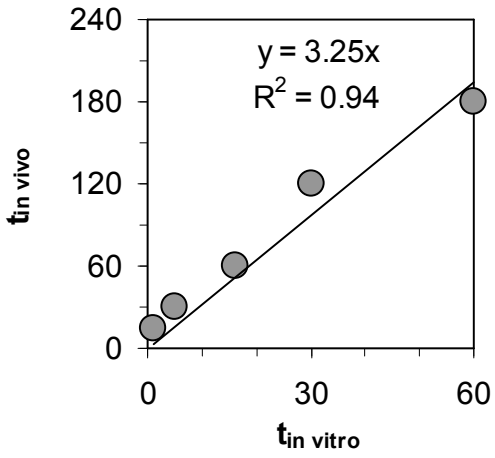


Figure 6.6 The time points selected from the *in vivo* absorption study ($t_{in\ vivo}$) against the time points chosen from the *in vitro* lipolysis study ($t_{in\ vitro}$) for the development of IVIVC (resembling the Levy plot).

(c) Multiple Level C correlations based on drug phase partition. According to previous reports [197, 198, 317, 321, 322], it is a common approach to develop correlations between the percentage/ fraction/ concentration of drug solubilised in the post-digestive aqueous phase and the *in vivo AUC* values. In the current investigation, we correlate the *in vitro AUC* values of drug solubilised in the lipolysis aqueous phase with the *in vivo AUC* values derived from the C_p-time profiles at various time points (for rats dosed at a 20 mg/kg CEL dose). **Table 6.2** summarises the *in vitro AUC* values of CEL solubilised in the lipolysis aqueous phase, which were derived from the results in **Figure 4.7** (Chapter 4).

Table 6.2 The values of area-under-the curve (with the number of fold of increment compared to blank micelles) derived from the CEL solubilisation plots for various CEL formulations during digestion under simulated fasted intestinal conditions at 37 °C (n = 2)

Lipid systems	$AUC_{1\rightarrow t}$ **(min.mg/ml)**			
	t = 5 min	t = 15 min	t = 30 min	t = 60 min
Blank micelles	0.190	0.688	1.427	2.844
SLH-M_1	0.270 (1.4)	1.108 (1.6)	2.554 (1.8)	5.866 (2.1)
SLH-M_3	1.173 (6.2)	3.977 (5.8)	8.557 (6.0)	18.01 (6.3)
SLH-C	1.216 (6.4)	3.659 (5.3)	6.914 (4.8)	13.18 (4.6)

By employing the same time scaling factor as specified in **Section 6.3.2(b),** we are able to establish multiple-point correlations between the *in vitro* and *in vivo AUC* values for each of the system examined. As depicted in **Figure 6.7**, linear IVIVCs were obtained for pure CEL (in blank micelles or in the absence of formulation lipids), SLH-M_1, SLH-M_3 and SLH-C ($R^2 > 0.9$ with a zero intercept). Therefore, the *AUC* data of CEL solubilised (*in vitro*) and absorbed (*in vivo*) represents another parameter that can be used for IVIVC development.

In conclusion, more extensive investigations are required to assess the versatility of the various correlation approaches presented in this chapter for the assessment of various lipid dosage forms of BCS Class II drugs. The inclusion of more formulation variables, such as different types and doses of lipids, different drugs possessing varying lipophilicity and ionisation constants, and different simulation of the intestinal fluids, would permit further refinement of the correlation approaches.

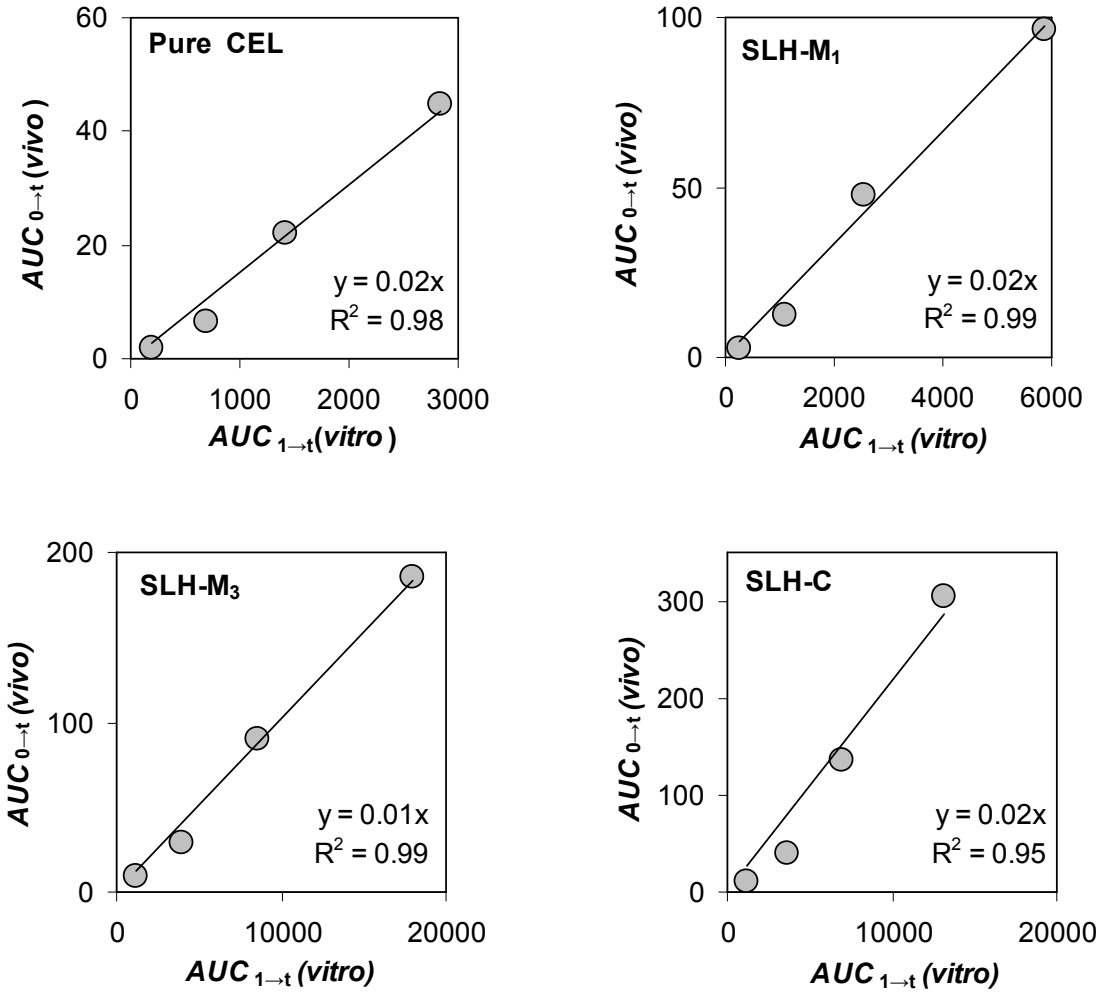


Figure 6.7 Multiple-point correlations between the *AUC* of CEL solubilised in the *in vitro* lipolysis aqueous phase obtained at t = 5, 15, 30 and 60 min, and the *in vivo* *AUC* data of celecoxib obtained at t = 30, 60, 120 and 180 min resulted from oral administration of the corresponding lipid dosage forms. All *AUC* values are expressed in the unit of min.μg/ml.

6.3.3 Proposed mechanisms of drug release, solubilisation and absorption from the SLH microcapsules

A combined IVIVC analysis of the dissolution and lipolysis characteristics has provided an improved understanding of the mechanisms of drug release, solubilisation and absorption from the SLH microcapsule system. The following mechanisms are proposed for the enhancement of CEL oral absorption resulting from the SLH microcapsule formulations (**Figure 6.8**):

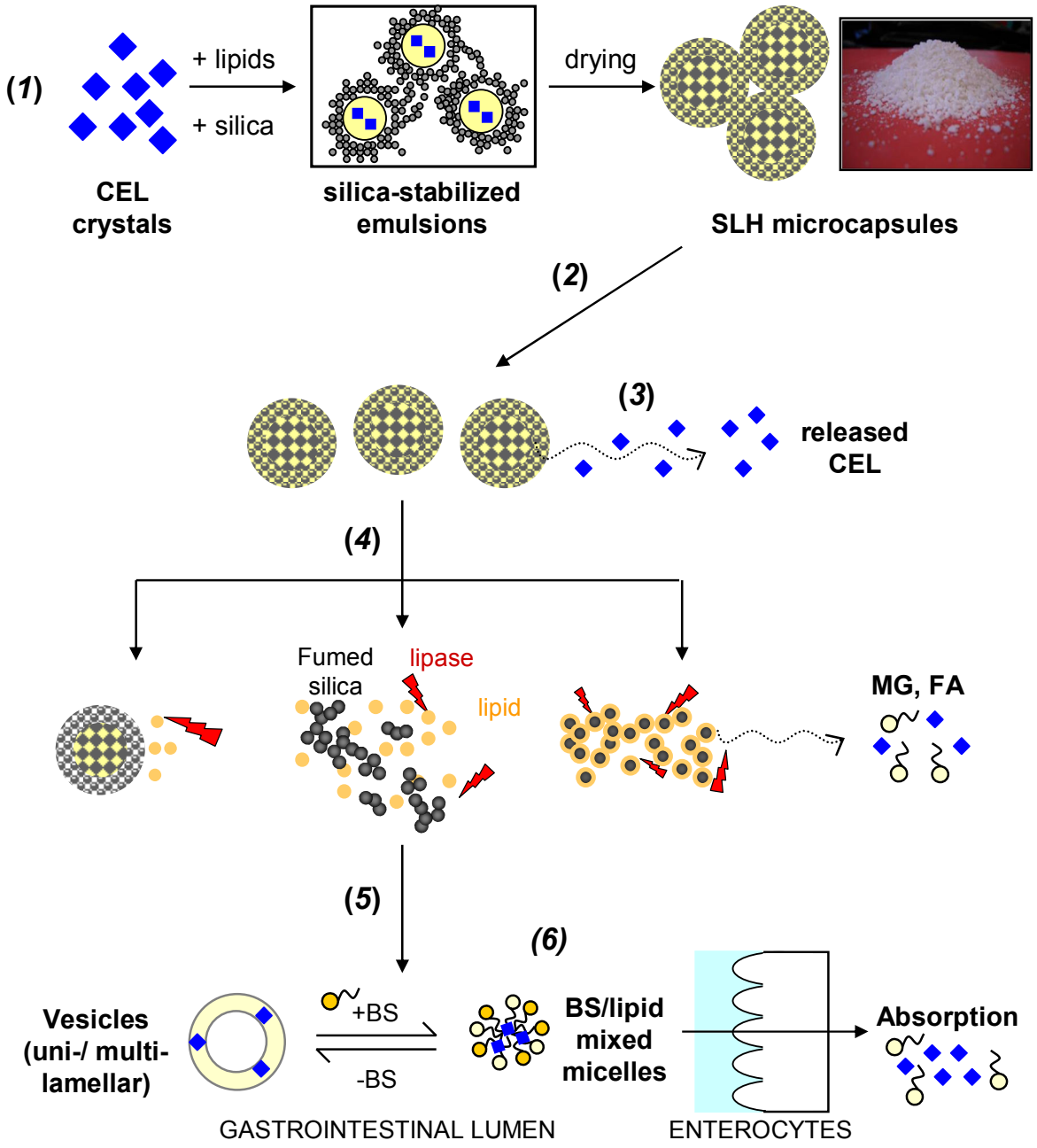


Figure 6.8 Schematic representation (not to scale) of the proposed mechanisms for the enhancement of CEL oral absorption resulting from the SLH microcapsule system: (*1*) CEL pre-dissolution in the lipid phase, (*2*) enhanced aqueous dispersion of the SLH microcapsules, (*3*) rapid CEL release from the high-surface-area porous microcapsule carrier, (*4*) enhanced digestion of the embedded, adsorbed or released lipid droplets, (*5*) formation of the amphiphilic colloidal species, and (*6*) supersaturated solubilisation of CEL in the colloidal phases leading to an enhanced absorption.

(*1*) CEL molecules are pre-dissolved in the lipid phase of the porous SLH microcapsules (in which the drug molecularly dispersed/ amorphous state is well preserved in the solid formulation);

(*2*) the SLH microcapsules are readily redispersed in the aqueous medium, which is facilitated by the wetting effect of the hydrophilic silica component of the microcapsule carrier;

(*3*) upon redispersion in an aqueous medium, the encapsulated CEL molecules undergo a rapid interfacial transfer from the microcapsule lipid phase into the aqueous surroundings via a concentration gradient-driven diffusion mechanism, which is facilitated by the high surface area of the porous microcapsule carrier;

(*4*) the emulsified lipid phase embedded in or adsorbed on the high-surface-area microcapsule carrier is enhanced in its digestibility, in which the 'inert' silica macroporous network potentially act as a substrate/ enzyme immobilisation template for improved hydrolytic activity of the digesting enzymes;

(*5*) the rapid generation of the lipid digestion products (mainly MG and FA) subsequently result in an efficient formation of the amphihilic colloidal species in the presence of the endogenous BS/PL species; and

(*6*) the released CEL molecules are solubilised in these colloidal phases (plausibly in a supersaturated state) and trafficked across the barrier of the unstirred water layer to reach the intestinal absorptive cells. Hydrophilic silica nanoparticles may also play a role as a precipitation inhibitor [42] to maintain the supersaturation state of CEL in the intestinal lumen, which eventually promote efficient absorption of the highly permeable CEL molecules into the systemic circulation.

6.4 Summary

Level C (single-point and multiple-point) and Level A (direct point-to-point) correlations were investigated for various non-lipid and lipid-based CEL formulations based on *in vitro* dissolution and lipolysis models. By using appropriate parameters, both *in vitro* dissolution and lipolysis tests can be used for ranking the *in vivo* bioavailability of the studied CEL formulations. The single-point Level C correlations established are regarded as useful for

formulation screening purposes at the pre-clinical stage. Overall, the dissolution test is more appropriate for characterising the absorption profiles of non-lipid CEL formulations (*i.e.* pure CEL and Celebrex® in the current investigation), while the development of Level A IVIVC based on the dissolution results is limited for the lipid-based systems.

The lipolysis-based IVIVC was established based on two aspects of the lipolysis models, one employing the lipolysis kinetics and the other based on the drug phase partition results. With significantly linear correlations (both Level C and Multiple Level C IVIVCs) obtained for various lipid dosage forms (including the simple lipid solution and emulsion, and the novel MaltoDE and SLH microcapsule systems), the current findings provide the first conclusive results that support the use of lipolysis kinetics (in addition to drug phase partitioning) to correctly interpret the *in vivo* drug absorption profiles. In agreement with a number of previous publications, drug phase partition data is suitable for IVIVC development since both the *in vitro* and *in vivo* parameters illustrate the state of drug solubilisation during or after lipolysis. However, time-dependent drug precipitation observed in the *in vitro* lipolysis model may not be reflective of the *in vivo* situation. This argument strongly holds particularly for drugs that are highly permeable to the intestinal membrane, in which the drugs potentially undergo rapid absorption once they are solubilised in a supersaturated state.

The IVIVC analyses undertaken based on both dissolution and lipolysis results have provided a significant insight into the mechanisms behind the improved oral absorption of CEL promoted by the SLH microcapsules. By knowing the *in vitro* parameters that are closely correlated to the *in vivo* PK data, the absorption performance of the SLH formulations can be further optimised by controlling the relevant formulation parameters.

CHAPTER SEVEN

Summary and Implications of Research Findings

7.1 Summary of Research Findings

This thesis describes a systematic development strategy for the porous silica-lipid hybrid (SLH) microcapsules as a novel lipid-based solid dosage form for effective oral delivery of poorly water-soluble (and lipophilic) compounds. The COX-2 selective non-steroidal anti-inflammatory drug celecoxib (CEL) was used as a paradigmatic example of BCS Class II drugs. The various *in vitro* and *in vivo* assessments undertaken in this research revealed a number of attractive biopharmaceutical advantages of the SLH microcapsule system over the conventional suspension and emulsion dosage forms.

7.1.1 Fabrication and physicochemical properties of SLH microcapsules

The fabrication method of the SLH microcapsules is presented in **Chapter 2**. The SLH microcapsules were developed from o/w emulsion templates using a simple two-step approach: high pressure homogenisation of the emulsions followed by spray-drying. The optimised SLH formulations of CEL were composed of medium-chain glycerides (either triglycerides or a mono-/diglyceride blend) as the oil phase, soybean lecithin as the emulsifier, and hydrophilic (Aerosil) silica nanoparticles as the emulsion stabiliser and solid carrier; all of which are FDA GRAS-listed substances. The formation of the highly porous (50–200 nm), homogenous silica-lipid matrices was effectively achieved by spray-drying of the silica-stabilised lipid emulsions, in which the silica nanoparticles re-assembled upon water removal to form a microcapsule network capable of adsorbing or embedding the lipid droplets. Each spray-dried SLH formulation (2–5 μm in the redispersed state) is comprised of 40–60% (w/w) oil encapsulated by or embedded in the porous Aerosil silica-based matrix at a oil:silica mass ratio of approximately 1:0.5 (w/w).

The readily redispersible SLH formulations demonstrated excellent physicochemical stability at ambient conditions for a study period of 6–12 months. DSC and XRD analyses confirmed stable encapsulation of CEL in a molecularly dispersed/ amorphous form when stored in airtight glass containers at ambient temperature. In comparison with an ordinary core-shell structured dry emulsion encapsulated by maltodextrin (which is designated as

MaltoDE), the porous SLH microcapsules comprised of a high surface area network (BET surface area = 184 m^2/g) has offered relatively higher lipid and CEL loading levels.

7.1.2 In vitro performance of SLH microcapsules

The *in vitro* dissolution results presented in **Chapter 3** confirm an enhanced CEL release (or dispersion) from the SLH microcapsules. Under simulated intestinal sink conditions, the rate and extent of CEL dissolution/ release was ranked as follows: SLH microcapsules ≈ MaltoDE > Celebrex® (the marketed CEL product) > pure CEL. The dissolution efficiency (%DE) obtained for the SLH formulations is 2- to 5-fold higher than Celebrex® and pure CEL.

Due to the digestible nature of the lipids employed in formulating the SLH microcapsules, a dynamic *in vitro* lipolysis model was conducted (in **Chapter 4**) to investigate: (*i*) the lipolysis characteristics of the SLH microcapsules in comparison with several medium-chain lipid control systems (*i.e.* lipid solutions, bare lipid emulsions, silica-stabilised liquid emulsions and MaltoDE); and (*ii*) the phase partition behaviour of CEL resulting from lipolysis of the SLH microcapsule carriers. Based on the lipolysis kinetics data, two potent inhibitors for pancreatic enzyme-mediated lipolysis were proposed, *i.e.* lecithin and maltodextrin. Aerosil silica, in the dispersed state, exerted an inhibitory effect on the digestion of submicron lipid emulsions regardless of their initial locations, *i.e.* in the aqueous or lipid phase. This inhibitory effect, however, was not observed for the SLH-microcapsules fabricated in the presence of low lecithin level (oil:lecithin = 1:0.06 w/w). Such findings highlight the importance of the highly porous matrix structure of the SLH microcapsules in facilitating a more predictable and enhanced lipid digestibility (*i.e.* ~100% lipolysis with up to 20-fold higher initial digestion rate constants) as compared to lipid solutions, submicron emulsions and MaltoDE. In parallel to this, the phase partition results reveal a significantly higher aqueous solubilisation level (*i.e.* 2.5- to 7.5-fold higher) for CEL resulting from the digested SLH microcapsules in comparison with a fasted state micellar solution.

7.1.3 In vivo performance of SLH microcapsules

Chapter 5 describes the *in vivo* absorption performance of the various non-lipid and lipid-based formulations of CEL in an orally dosed fasted rat model. At a dose level of 5 mg/kg CEL, there was no statistically significant difference observed across the treatment groups dosed with the following CEL formulations: pure CEL aqueous suspension, lipid solution, submicron emulsion, Celebrex® and the redispersed MaltoDE system. In contrast, the SLH formulations produced a statistically higher relative bioavailability, $F_{rel.}$ (150 to 240%) in reference to the pure drug administered as an aqueous suspension at three CEL dose levels, *i.e.* 5, 20 and 50 mg/kg ($p < 0.05$). Overall, the pharmacokinetic data, particularly $F_{rel.}$ (or $AUC_{0\rightarrow\infty}$) and C_{max}, indicates a rank order of SLH microcapsules ≈ Celebrex® > MaltoDE ≈ submicron emulsion ≈ lipid solution ≈ pure CEL (as an aqueous suspension). The dose-dependent pharmacokinetic studies indicate that the SLH formulations and Celebrex® consistently produced a 2-fold higher $AUC_{0\rightarrow\infty}$ and C_{max} values than the pure drug within the dose range of 5–50 mg/kg CEL.

7.1.4 In vivo-in vivo correlations to probe the drug absorption mechanisms

In **Chapter 6**, the *in vitro-in vivo* correlations (IVIVCs) were investigated for various non-lipid and lipid-based CEL formulations based on the dissolution and lipolysis data. Overall, the dissolution model is more appropriate for characterising the absorption profiles of non-lipid CEL formulations, *i.e.* pure CEL and Celebrex® in the current investigation. A rank order correlation based on the %DE results was obtained for pure CEL, Celebrex® and the SLH microcapsules. A multiple-point correlation between the dissolution and absorption profiles is not practical for the SLH microcapsules. On the other hand, the lipolysis model enables more qualitative IVIVCs to be developed for the lipid-based systems, *i.e.* blank micellar solutions, lipid solutions, emulsions, MaltoDE and SLH microcapsules. Both rank order and Multiple Level C correlations ($R^2 > 0.8$) were successfully established based on lipolysis kinetics and CEL phase partition data.

A parallel analysis of the dissolution- and lipolysis-based IVIVC provides a significant insight into the mechanisms of drug release, solubilisation and absorption promoted by the SLH microcapsule system. The improved oral absorption of CEL delivered using the SLH microcapsules is likely to be facilitated by an enhanced drug dissolution (or dispersion), and more importantly by an enhanced or better controlled digestion of the lipid vehicle. We

propose that Aerosil silica nanoparticles play an important role in the SLH microcapsule system by:

(*i*) stabilising the emulsified lipid phase in the solid state, in which a lipophilic drug can be encapsulated and well preserved in the amorphous/ molecularly dispersed state;

(*ii*) providing a high surface area for enhancing lipid/ drug dispersion and facilitating the interfacial interactions between the digesting enzymes and the lipid substrates in an aqueous medium; and

(*iii*) creating and possibly maintaining the supersaturated solubilisation state of CEL in the intestinal milieu which eventually leads to an enhanced absorption of the highly permeable drug molecules.

These explain the superiority of the SLH formulations in promoting better CEL absorption in comparison with the pure drug, simple lipid solution, submicron emulsion and the MaltoDE system.

7.2 Conclusions and future directions

Some major challenges to the successful development of lipid-based formulations (reviewed in **Chapter 1**) include: (*i*) the lack of clear guidelines on their formulation design, (*ii*) a poor understanding of the absorption mechanisms of the formulations, and (*iii*) the lack of standard *in vitro* tools for predicting their *in vivo* performance. Extensive work has been undertaken by many formulation scientists to optimise the test methods for lipid-based systems, which also improve the understanding of the digestion patterns and solubilising capacity of various types of lipid excipients. However, at present there is still little evidence to support the *in vivo* predictability of the published digestion test methods. Consequently, such novel models are yet to be proven before they could be used in the regulatory approval process for establishing reliable IVIVCs for various lipid-based formulations containing poorly water-soluble drugs.

The current research has thus contributed to the area of lipid-based formulation development by:

(*i*) describing a systematic development strategy for the porous SLH microcapsules as a novel solid-state lipid-based formulation and hence, providing a useful foundation for future formulation design employing colloidal and nanoparticle technologies;

(*ii*) successfully employing the compendial dissolution model and the novel lipolysis model to probe the drug release mechanisms of the SLH formulations. A careful comparison between these two test models has proved the suitability of a lipolysis model for the evaluation of lipid-based dosage forms;

(*iii*) elucidating the feasibility of developing single-point and multiple-point IVIVCs based on two aspects of the lipolysis models: one employing the *lipolysis kinetics* and the other based on the *drug phase partition* results. This is a significant step towards establishing a systematic protocol for developing a lipolysis-based IVIVC, which is not yet available at present.

(*iv*) presenting a comprehensive *in vitro* and *in vivo* evaluation on silica-lipid hybrid systems for effective oral delivery of poorly water-soluble drugs. The research findings have provided strong evidence for the synergistic role of lipid and silica excipients in enhancing the oral bioavailability of poorly water-soluble drugs when compared to conventional lipid suspension and emulsion formulations. Such bioavailability enhancement effect has been demonstrated in a dose-dependent manner for celecoxib when encapsulated in the SLH microcapsules.

Significantly, the research presented in this thesis provides a sound platform on which silica-lipid hybrid carriers can be tailored to enhance the bioavailability of a myriad of poorly water-soluble chemical entities through a versatile selection of lipid excipients. The potential of the SLH microcapsules in enhancing the bioavailability of poorly water-soluble and lipophilic compounds has thus far been demonstrated for two intermediate lipophilic drugs. One is CEL (log P = 3.5, pK_a = 11.1) as investigated in the current thesis, and the other is the non-selective anti-inflammatory drug, indomethacin (log P = 3.6, pK_a = 4.5) [327, 328]. Some extended work is underway to further explore the versatility of the SLH microcapsules in encapsulating and delivering drugs which posses a higher lipophilicity such as the anti-fungal agent itraconazole (log P = 5.7, pK_a = 3.7).

The work so far has also revealed a number of limitations associated with the current formulation design. The following main points have been identified where improvements

can be achieved: drug loading efficiency, control over the kinetics of drug release and lipid digestion, as well as biopharmaceutical advantage over the marketed products (*i.e.* Celebrex® in this project). These outline several possible directions towards improving the viability of the SLH microcapsules for human administration, such as:

(*i*) further optimisation of the *drug loading level* of the microcapsule carriers. This can be done by varying the formulation parameters such as using different types of lipids, incorporating multiple lipid excipients (or micellar systems), minimising the mass ratio of silica:lipid, using a different type of emulsifier, as well as attempting a different fabrication method which has a minimised risk of excipient loss during preparation (e.g. vacuum filtration or a simple evaporation method);

(*ii*) development of an effective and reproducible approach to further control the *pore size distribution* of the microcapsule carriers. A tunable porous structure may eventually produce a better controlled drug release or lipid digestion property. This is a challenging process for microcapsules produced by spray-drying because of the random and unpredictable redistribution of the oily excipients and hydrophilic silica nanoparticles under the high air-flow conditions. However, such control may be eventually achieved using a filtration or an evaporation drying method;

(*iii*) employment of a different *in vitro* model to further elucidate the *drug absorption mechanisms* resulting from the SLH microcapsule systems. The digestion-permeation model which accounts for both pre-enterocyte and intra-cellular events is a promising candidate;

(*iv*) implementation of an *in vivo GI toxicological testing* with the SLH microcapsules to determine the absence or presence of a local irritation effect resulting from the use of multiple excipients; and

(*v*) performance of the pharmacokinetic studies in an *alternative animal model*, such as dogs, which can provide a valuable comparison of the drug absorption profiles under fasted and fed conditions.

References

1. Wu, C.-Y.,Benet, L. Z., Predicting drug disposition via application of BCS: transport/absorption/ elimination interplay and development of a biopharmaceutics drug disposition classification system. *Pharmaceutical Research* **2005,** 22 (1), 11-23.
2. Pouton, C. W., Formulation of poorly water-soluble drugs for oral administration: physicochemical and physiological issues and the lipid formulation classification system. *European Journal of Pharmaceutical Sciences* **2006,** 29 (3-4), 278-287.
3. Porter, C. J. H.,Trevaskis, N. L.,Charman, W. N., Lipids and lipid-based formulations: optimizing the oral delivery of lipophilic drugs. *Nature Reviews Drug Discovery* **2007,** 6 (3), 231-248.
4. Hauss, D. J., Oral lipid-based formulations. *Advanced Drug Delivery Review* **2007,** 59 (7), 667-676.
5. Hauss, D. J., *Oral lipid-based formulations: enhancing the bioavailability of poorly water-soluble drugs*. Informa Healthcare USA: New York, **2007**; Vol. 170.
6. Kasim, N. A.,Whitehouse, M.,Ramachandran, C.,Bermejo, M.,Lennernäs, H.,Hussain, A. S.,Junginger, H. E.,Stavchansky, S. A.,Midha, K. K.,Shah, V. P.,Amidon, G. L., Molecular properties of WHO essential drugs and provisional biopharmaceutical classification. *Molecular Pharmaceutics* **2004,** 1 (1), 85-96.
7. Lindenberg, M.,Kopp, S.,Dressman, J. B., Classification of orally administered drugs on the World Health Organization Model list of Essential Medicines according to the biopharmaceutics classification system. *European Journal of Pharmaceutics and Biopharmaceutics* **2004,** 58 (2), 265-278.
8. Porter, C. J. H.,Pouton, C. W.,Cuine, J. F.,Charman, W. N., Enhancing intestinal drug solubilisation using lipid-based delivery systems. *Advanced Drug Delivery Review* **2008,** 60 (6), 673-691.
9. O'Driscoll, C. M.,Griffin, B. T., Biopharmaceutical challenges associated with drugs with low aqueous solubility — the potential impact of lipid-based formulations *Advanced Drug Delivery Review* **2008,** 60 (6), 617-624.
10. Zangenberg, N. H.,Mullertz, A.,Kristensen, H. G.,Hovgaard, L., A dynamic in vitro lipolysis model: I. Controlling the rate of lipolysis by continuous addition of calcium. *European Journal of Pharmaceutical Sciences* **2001,** 14 (2), 115-122.
11. Zangenberg, N. H.,Mullertz, A.,Gjelstrup Kristensen, H.,Hovgaard, L., A dynamic in vitro lipolysis model: II. Evaluation of the model. *European Journal of Pharmaceutical Sciences* **2001,** 14 (3), 237-244.
12. Sek, L.,Porter, C. J. H.,Charman, W. N., Characterisation and quantification of medium chain and long chain triglycerides and their in vitro digestion products, by HPTLC coupled with in situ densitometric analysis. *Journal of Pharmaceutical and Biomedical Analysis* **2001,** 25 (3-4), 651-661.
13. Sek, L. An in-vitro model of lipid digestion for assessing the oral bioavailability enhancement potential of lipidic formulations. Ph.D. thesis, Monash University, Melbourne, **2002**.
14. Brogård, M.,Troedsson, E.,Thuresson, K.,Ljusberg-Wahren, H., A new standardized lipolysis approach for characterization of emulsions and dispersions. *Journal of Colloid and Interface Science* **2007,** 308 (2), 500-507.
15. Amidon, G. L.,Lennernas, H.,Shah, V. P.,Crison, J. R., A theoretical basis for a biopharmaceutic drug classification: the correlation of in vitro drug product dissolution and in vivo bioavailability. *Pharmaceutical Research* **1995,** 12 (3), 413-420.
16. Adeyeye, M. C.,Brittain, H. G., *Preformulation in solid dosage form development*. Informa Healthcare: New York, **2008**; Vol. 178.
17. Chakraborty, S.,Shukla, D.,Mishra, B.,Singh, S., Lipid - An emerging platform for oral delivery of drugs with poor bioavailability. *European Journal of Pharmaceutics and Biopharmaceutics* **2009,** 73 (1), 1-15.

18. Dressman, J. B.,Lennernas, H., *Oral drug absorption: prediction and asessment*. Marcel Dekker: New York, **2000**; Vol. 106.
19. US Food and Drug Administration. *Guidance for Industry: waiver of in vivo bioavailability and bioequivalence studies for immediate-release solid oral dosage forms based on a Biopharmaceutics Classification System*. Food and Drug Administration, Rockville, MD, **2000**. Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070246.pdf.
20. Organization for Economic Cooperation and Development. *Guidelines for The Testing of Chemicals, Test Guideline 107, Partition Coefficient (n-Octanol/Water)*. OECD, Paris, France, **1995**.
21. Prabhu, S.,Ortega, M.,Ma, C., Novel lipid-based formulations enhancing the in vitro dissolution and permeability characteristics of a poorly water-soluble model drug, piroxicam. *International Journal of Pharmaceutics* **2005,** 301 (1-2), 209-216.
22. Custodio, J. M.,Wu, C.-Y.,Benet, L. Z., Predicting drug disposition, absorption/elimination/transporter interplay and the role of food on drug absorption. *Advanced Drug Delivery Review* **2008,** 60 (6), 717-733.
23. Das, N. G.,Das, S. K., Formulation of poorly soluble drugs. *Technology Overviews* **2006,** Drug Delivery Report Spring/Summer 2006, 52-55.
24. Fahr, A.,Liu, X., Drug delivery strategies for poorly water-soluble drugs. *Expert Opinion on Drug Delivery* **2007,** 4 (4), 403-416.
25. Liu, R., *Water-insoluble drug formulation*. 2nd ed.; CRC Press: Boca Raton, FL, **2008**.
26. Simões, S.,Sousa, A.,Figueiredo, M., Dissolution rate studies of pharmaceutical multisized powders -a practical approach using the Coulter method. *International Journal of Pharmaceutics* **1996,** 127 (2), 283-291.
27. Hintz, R. J.,Johnson, K. C., The effect of particle size distribution on dissolution rate and oral absorption. *International Journal of Pharmaceutics* **1989,** 51 (1), 9-17.
28. Vogt, M.,Kunath, K.,Dressman, J. B., Dissolution enhancement of fenofibrate by micronization, cogrinding and spray-drying: Comparison with commercial preparations. *European Journal of Pharmaceutics and Biopharmaceutics* **2008,** 68 (2), 283-288.
29. Tandya, A.,Dehghani, F.,Foster, N. R., Micronization of cyclosporine using dense gas techniques. *The Journal of Supercritical Fluids* **2006,** 37 (3), 272-278.
30. Chiou, A. H.-J.,Yeh, M.-K.,Chen, C.-Y.,Wang, D.-P., Micronization of meloxicam using a supercritical fluids process. *The Journal of Supercritical Fluids* **2007,** 42 (1), 120-128.
31. Perrut, M.,Jung, J.,Leboeuf, F., Enhancement of dissolution rate of poorly-soluble active ingredients by supercritical fluid processes: Part I: Micronization of neat particles. *International Journal of Pharmaceutics* **2005,** 288 (1), 3-10.
32. Rasenack, N.,Müller, B. W., Dissolution rate enhancement by *in situ* micronization of poorly water-soluble drugs. *Pharmaceutical Research* **2002,** 19 (12), 1894-1900.
33. Crison, J. R.,Shah, V. P.,Skelly, J. P.,Amidon, G. L., Drug dissolution into micellar solutions: development of a convective diffusion model and comparison to the film equilibrium model with application to surfactant-facilitated dissolution of carbamazepine. *Journal of Pharmaceutical Sciences* **1996,** 85 (9), 1005-1011.
34. Kawakami, K.,Oda, N.,Miyoshi, K.,Funaki, T.,Ida, Y., Solubilization behavior of a poorly soluble drug under combined use of surfactants and cosolvents. *European Journal of Pharmaceutical Sciences* **2006,** 28 (1-2), 7-14.
35. Aguiar, A. J.,Krc Jr., J.,Kinkel, A. W.,Samyn, J. C., Effect of polymorphism on the absorption of chloramphenicol from chloramphenicol palmitate. *Journal of Pharmaceutical Sciences* **1967,** 56 (7), 847-853.
36. Bauer, J.,Spanton, S.,Henry, R.,Quick, J.,Dziki, W.,Porter, W.,Morris, J., Ritonavir: An extraordinary example of conformational polymorphism. *Pharmaceutical Research* **2001,** 18 (6), 859-866.
37. Lu, G. W.,Hawley, M.,Smith, M.,Geiger, B. M.,Pfund, W., Characterization of a novel polymorphic form of celecoxib. *Journal of Pharmaceutical Sciences* **2006,** 95 (2), 305-317.

38. Nakagami, H., Solid dispersions of indomethacin and griseofulvin in non-porous fumed silicon dioxide, prepared by melting. *Chemical and Pharmaceutical Bulletin* **1991,** 39 (9), 2417-2421.
39. Dixit, R. P.,Nagarsenker, M. S., In vitro and in vivo advantage of celecoxib surface solid dispersion and dosage form development. *Indian Journal of Pharmaceutical Sciences* **2007,** 69 (3), 370-377.
40. Nagabhushanam, M. V., Formulation studies on solid dispersions of celecoxib in superdisintegrants alone and with PVP. *Rasayan Journal of Chemistry* **2009,** 2 (3), 691-698.
41. Sanganwar, G. P.,Gupta, R. B., Dissolution-rate enhancement of fenofibrate by adsorption onto silica using supercritical carbon dioxide. *International Journal of Pharmaceutics* **2008,** 360 (1-2), 213–218.
42. Brouwers, J.,Brewster, M. E.,Augustijns, P., Supersaturating drug delivery systems: The answer to solubility-limited oral bioavailability? *Journal of Pharmaceutical Sciences* **2009,** 98 (8), 2549-2572.
43. Serajuddln, A. T. M., Solid dispersion of poorly water-soluble drugs: Early promises, subsequent problems, and recent breakthroughs. *Journal of Pharmaceutical Sciences* **1999,** 88 (10), 1058-1066.
44. Leuner, C.,Dressman, J., Improving drug solubility for oral delivery using solid dispersions. *European Journal of Pharmaceutics and Biopharmaceutics* **2000,** 50 (1), 47-60.
45. Hirayama, F.,Uekama, K., Cyclodextrin-based controlled drug release system. *Advanced Drug Delivery Review* **1999,** 36 (1), 125-141.
46. Brewster, M. E.,Loftsson, T., Cyclodextrins as pharmaceutical solubilizers. *Advanced Drug Delivery Reviews* **2007,** 59 (7), 645-666.
47. Fini, A.,Fazio, G.,Rapaport, I., Diclofenac/N-(2-hydroxyethyl)pyrrolidine: A new salt for an old drug. *Drugs under Experimental and Clinical Research* **1993,** 19 (3), 81-88.
48. Guzman, H. R.,Tawa, M.,Zhang, Z.,Ratanabanangkoon, P.,Shaw, P.,Gardner, C. R.,Chen, H.,Moreau, J.-P.,Almarsson, O.,Remenar, J. F., Combined use of crystalline salt forms and precipitation inhibitors to improve oral absorption of celecoxib from solid oral formulations. *Journal of Pharmaceutical Sciences* **2007,** 96 (10), 2686-2702.
49. Dewland, P. M.,Reader, S.,Berry, P., Bioavailability of ibuprofen following oral administration of standard ibuprofen, sodium ibuprofen or ibuprofen acid incorporating poloxamer in healthy volunteers. *BMC Clinical Pharmacology* **2009,** 9, art. no. 19.
50. Mamidi, R. N. V. S.,Mullangi, R.,Kota, J.,Bhamidipati, R.,Khan, A. A.,Katneni, K.,Datla, S.,Singh, S. K.,Rao, K. Y.,Rao, C. S.,Srinivas, N. R.,Rajagopalan, R., Pharmacological and pharmacokinetics evaluation of celecoxib prodrugs in rats. *Biopharmaceutics and Drug Disposition* **2002,** 23 (7), 273-282.
51. Singh, S. K.,Vobbalareddy, S.,Kalleda, S. R.,Casturi, S. R.,Datla, S. R.,Mamidi, R. N. V. S.,Mullangi, R.,Ramanujam, R.,Yeleswarapua, K. R.,Iqbal, J., Identification of 2-hydroxymethyl-4-[5-(4-methoxyphenyl)-3-trifluoromethyl-pyrazol-1-yl]-N-propionylbenzenesulfonamide sodium as a potential COX-2 inhibitor for oral and parenteral administration. *Bioorganic and Medicinal Chemistry* **2006,** 14 (24), 8626-8634.
52. Stella, V. J.,Nti-Addae, K. W., Prodrug strategies to overcome poor water solubility. *Advanced Drug Delivery Reviews* **2007,** 59 (7), 677-694.
53. Müller, C. E., Prodrug approaches for enhancing the bioavailability of drugs with low solubility. *Chemistry and Biodiversity* **2009,** 6 (11), 2071-2083.
54. Pouton, C. W.,Porter, C. J. H., Formulation of lipid-based delivery systems for oral administration: materials, methods and strategies. *Advanced Drug Delivery Review* **2008,** 60 (6), 625-637
55. Miyako, Y.,Zhao, Y.,Takeshima, K.,Kataoka, T.,Handa, T.,Pinal, R., Solubility of hydrophobic compounds in water-cosolvent mixtures: Relation of solubility with water-cosolvent interactions. *Journal of Pharmaceutical Sciences* **2010,** 99 (1), 293-302.
56. Prajapati, B. G.,Patel, M. M., Conventional and alternative pharmaceutical methods to improve oral bioavailability of lipophilic drugs. *Asian Journal of Pharmaceutics* **2007,** 1 (1), 1-8.

57. Pudipeddi, M.,Serajuddin, A. T. M., Trends in solubility of polymorphs. *Journal of Pharmaceutical Sciences* **2005,** 94 (5), 929-939.
58. Netterwald, J., Screened Gems. *Drug Discovery & Development magazine* **2007,** 9 (9), 22-26. Available at http://www.dddmag.com/screened-gems.aspx.
59. Jackson, K.,Young, D.,Pant, S., Drug-excipient interactions and their affect on absorption. *Pharmaceutical Science and Technology Today* **2000,** 3 (10), 336-345.
60. Humberstone, A. J.,Charman, W. N., Lipid-based vehicles for the oral delivery of poorly water soluble drugs. *Advanced Drug Delivery Review* **1997,** 25 (1), 103-128.
61. Kossena, G. A.,Boyd, B. J.,Porter, C. J. H.,Charman, W. N., Separation and characterization of the colloidal phases produced on digestion of common formulation lipids and assessment of their impact on the apparent solubility of selected poorly water-soluble drugs. *Journal of Pharmaceutical Sciences* **2003,** 92 (3), 634-648.
62. Lindmark, T.,Kimura, Y.,Artursson, P., Absorption enhancement through intracellular regulation of tight junction permeability by medium chain fatty acids in Caco-2 cells. *Journal of Pharmacology and Experimental Therapeutics* **1998,** 284 (1), 362-369.
63. Monographs: Medicinal and pharmaceutical substances. *British Pharmacopoeia*, H.M.S.O.:London, England. **2009**.
64. US Food and Drug Administration. *Inactive Ingredient Search for Approved Drug Products.* Food and Drug Administration, Rockville, MD, **2009**. Available at http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm.
65. Venkataram, S.,Rogers, J. A., Controlled release of griseofulvin from coprecipitates with phospholipids. *Drug Development and Industrial Pharmacy* **1985,** 11 (2-3), 223-238.
66. Vudathala, G. K.,Rogers, J. A., Dissolution of fludrocortisone from phospholipid coprecipitates. *Journal of Pharmaceutical Sciences* **1992,** 81 (3), 282-286.
67. Biswas, M.,Akogyeram, C. O.,Scott, K. R.,Potti, G. K.,Gallelli, J. F.,Habib, M. J., Development of carbamazepine:phospholipid solid dispersion formulations. *Journal of Controlled Release* **1993,** 23 (3), 239-245.
68. Vudathala, G. K.,Rogers, J. A., Effect of cholesterol on the aging of griseofulvin-phospholipid coprecipitates. *International Journal of Pharmaceutics* **1991,** 69 (1), 13-19.
69. Vudathala, G. K.,Rogers, J. A., Oral bioavailability of griseofulvin from aged griseofulvin:lipid coprecipitates: In vivo studies in rats. *Journal of Pharmaceutical Sciences* **1992,** 81 (12), 1166-1169.
70. Small, D. M., A classification of biologic lipids based upon their interaction in aqueous systems. *Journal of the American Oil Chemists Society* **1968,** 45 (3), 108-119.
71. Carey, M. C.,Small, D. M.,Bliss, C. M., Lipid digestion and absorption. *Annual Review of Physiology* **1983,** 45, 651-677.
72. Nutting, D. F.,Kumar, N. S.,St. Hilaire, R. J.,Mansbach II, C. M., Nutrient absorption. *Current Opinion in Clinical Nutrition and Metabolic Care* **1999,** 2 (5), 413-419.
73. Charman, W. N., Lipids, lipophilic drugs, and oral drug delivery - Some emerging concepts. *Journal of Pharmaceutical Sciences* **2000,** 89 (8), 967-978.
74. O'Driscoll, C. M., Lipid-based formulations for intestinal lymphatic delivery *European Journal of Pharmaceutical Sciences* **2002,** 15 (5), 405-415.
75. Trevaskis, N. L., Charman, W.N., Porter, C.J.H. , Lipid-based delivery systems and intestinal lymphatic drug transport: A mechanistic update. *Advanced Drug Delivery Review* **2008,** 60 (6), 702-716.
76. Embleton, J. K.,Pouton, C. W., Structure and function of gastro-intestinal lipases. *Advanced Drug Delivery Review* **1997,** 25 (1), 15-32.
77. Wasan, K. M., Formulation and physiological and biopharmaceutical issues in the development of oral lipid-based drug delivery systems. *Drug Development and Industrial Pharmacy* **2001,** 27 (4), 267-276.
78. Hermoso, J.,Pignol, D.,Penel, S.,Roth, M.,Chapus, C.,Fontecilla-Camps, J. C., Neutron crystallographic evidence of lipase-colipase complex activation by a micelle. *EMBO Journal* **1997,** 16 (18), 5531-5536.

79. Carriere, F.,Rogalska, E.,Cudrey, C.,Ferrato, F.,Laugier, R.,Verger, R., In vivo and in vitro studies on the stereoselective hydrolysis of tri- and diglycerides by gastric and pancreatic lipases. *Bioorganic and Medicinal Chemistry* **1997,** 5 (2), 429-435.
80. Armand, M.,Borel, P.,Pasquier, B.,Dubois, C.,Senft, M.,Andre, M.,Peyrot, J.,Salducci, J.,Lairon, D., Physicochemical characteristics of emulsions during fat digestion in human stomach and duodenum. *American Journal of Physiology* **1996,** 271 (1 Part 1), G184-G191.
81. Pafumi, Y.,Lairon, D.,de la Porte, P. L.,Juhel, C.,Storch, J.,Hamosh, M.,Armand, M., Mechanisms of inhibition of triacylglycerol hydrolysis by human gastric lipase. *Journal of Biological Chemistry* **2002,** 277 (31), 28070-28079.
82. Porter, C. J. H.,Charman, W. N., In vitro assessment of oral lipid based formulations. *Advanced Drug Delivery Review* **2001,** 50 (SUPPL. 1), S127-S147.
83. Hunt, J. N.,Knox, M. T., A relation between the chain length of fatty acids and the slowing of gastric emptying. *Journal of Physiology* **1968,** 194 (2), 327-336.
84. Raybould, H. E.,Meyer, J. H.,Tabrizi, Y.,Liddle, R. A.,Tso, P., Inhibition of gastric emptying in response to intestinal lipid is dependent on chylomicron formation. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology* **1998,** 274 (6 43-6), R1834-R1838.
85. Kossena, G. A.,Charman, W. N.,Wilson, C. G.,Mahony, B. O.,Lindsay, B.,Hempenstall, J. M.,Davison, C. L.,Crowley, P. J.,Porter, C. J. H., Low dose lipid formulations: Effects on gastric emptying and biliary secretion. *Pharmaceutical Research* **2007,** 24 (11), 2084-2096.
86. Bates, T. R.,Sequeira, J. A., Bioavailability of micronized griseofulvin from corn oil in water emulsion, aqueous suspension, and commercial tablet dosage forms in humans. *Journal of Pharmaceutical Sciences* **1975,** 64 (5), 793-797.
87. Chakrabarti, S.,Belpaire, F. M., Bioavailability of phenytoin in lipid containing dosage forms in rats. *Journal of Pharmacy and Pharmacology* **1978,** 30 (5), 330-331.
88. Patton, J. S.,Carey, M. C., Watching fat digestion. The formation of visible product phases by pancreatic lipase is described. *Science* **1979,** 204 (4389), 145-148.
89. Sunesen, V. H.,Vedelsdal, R.,Kristensen, H. G.,Christrup, L.,Müllertz, A., Effect of liquid volume and food intake on the absolute bioavailability of danazol, a poorly soluble drug. *European Journal of Pharmaceutical Sciences* **2005,** 24 (4), 297-303.
90. Armand, M.,Pasquier, B.,André, M.,Borel, P.,Senft, M.,Peyrot, J.,Salducci, J.,Portugal, H.,Jaussan, V.,Lairon, D., Digestion and absorption of 2 fat emulsions with different droplet sizes in the human digestive tract. *American Journal of Clinical Nutrition* **1999,** 70 (6), 1096-1106.
91. Olbrich, C.,Muller, R. H., Enzymatic degradation of SLN - effect of surfactant and surfactant mixtures. *International Journal of Pharmaceutics* **1999,** 180 (1), 31-39.
92. Hernell, O.,Staggers, J. E.,Carey, M. C., Physical-chemical behavior of dietary and biliary lipids during intestinal digestion and absorption. 2. Phase analysis and aggregation states of luminal lipids during duodenal fat digestion in healthy adult human beings. *Biochemistry®* **1990**, 2041-2056.
93. MacGregor, K. J.,Embleton, J. K.,Lacy, J. E.,Perry, E. A.,Solomon, L. J.,Seager, H.,Pouton, C. W., Influence of lipolysis on drug absorption from the gastro-intestinal tract. *Advanced Drug Delivery Review* **1997,** 25 (1), 33-46.
94. Shiau, Y.-F., Mechanism of intestinal fatty acid uptake in the rat: The role of an acidic microclimate. *Journal of Physiology* **1990,** 421, 463-474.
95. Mithani, S. D.,Bakatselou, V.,TenHoor, C. N.,Dressman, J. B., Estimation of the increase in solubility of drugs as a function of bile salt concentration. *Pharmaceutical Research* **1996,** 13 (1), 163-167.
96. Cai, X.,Grant, D. J. W.,Wiedmann, T. S., Analysis of the solubilization of steroids by bile salt micelles. *Journal of Pharmaceutical Sciences* **1997,** 86 (3), 372-377.
97. Kossena, G. A.,Charman, W. N.,Boyd, B. J.,Dunstan, D. E.,Porter, C. J. H., Probing drug solubilization patterns in the gastrointestinal tract after administration of lipid-based delivery systems: a phase diagram approach *Journal of Pharmaceutical Sciences* **2004,** 93 (2), 332-348.

98. Ockner, R. K.,Manning, J. A., Fatty acid binding protein in small intestine. Identification, isolation, and evidence for its role in cellular fatty acid transport. *Journal of Clinical Investigation* **1974,** 54 (2), 326-338.
99. Stremmel, W., Uptake of fatty acids by jejunal mucosal cells is mediated by a fatty acid binding membrane protein. *Journal of Clinical Investigation* **1988,** 82 (6), 2001-2010.
100. Schwenk, R. W.,Holloway, G. P.,Luiken, J. J. F. P.,Bonen, A.,Glatz, J. F. C., Fatty acid transport across the cell membrane: Regulation by fatty acid transporters. *Prostaglandins Leukotrienes and Essential Fatty Acids* **2010,** (in press).
101. Besnard, P.,Niot, I.,Poirier, H.,Clément, L.,Bernard, A., New insights into the fatty acid-binding protein (FABP) family in the small intestine. *Molecular and Cellular Biochemistry* **2002,** 239 (1-2), 139-147.
102. Wacher, V. J.,Wu, C.-Y.,Benet, L. Z., Overlapping substrate specificities and tissue distribution of cytochrome p450 3A and P-glycoprotein: Implications for drug delivery and activity in cancer chemotherapy. *Molecular Carcinogenesis* **1995,** 13 (3), 129-134.
103. Benet, L. Z.,Cummins, C. L., The drug efflux-metabolism alliance: Biochemical aspects. *Advanced Drug Delivery Reviews* **2001,** 50 (SUPPL. 1), S3-S11.
104. Dahan, A.,Hoffman, A., The effect of different lipid based formulations on the oral absorption of lipophilic drugs: the ability of in vitro lipolysis and consecutive ex vivo intestinal permeability data to predict in vivo bioavailability in rats. *European Journal of Pharmaceutics and Biopharmaceutics* **2007,** 67 (1), 96-105.
105. Anderberg, E. K.,Nystrom, C.,Artursson, P., Epithelial transport of drugs in cell culture. VII: Effects of pharmaceutical surfactant excipients and bile acids on transepithelial permeability in monolayers of human intestinal epithelial (Caco-2) cells. *Journal of Pharmaceutical Sciences* **1992,** 81 (9), 879-887.
106. Swenson, E. S.,Milisen, W. B.,Curatolo, W., Intestinal permeability enhancement: Efficacy, acute local toxicity, and reversibility. *Pharmaceutical Research* **1994,** 11 (8), 1132-1142.
107. Washington, N.,Washington, C.,Wilson, C. G., *Physiological pharmaceutics: barriers to drug absorption*. 2nd ed.; Taylor and Francis: London, **2001**.
108. Dintaman, J. M.,Silverman, J. A., Inhibition of P-glycoprotein by D-α-tocopheryl polyethylene glycol 1000 succinate (TPGS). *Pharmaceutical Research* **1999,** 16 (10), 1550-1556.
109. Yu, L.,Bridgers, A.,Polli, J.,Vickers, A.,Long, S.,Roy, A.,Winnike, R.,Coffin, M., Vitamin E-TPGS increases absorption flux of an HIV protease inhibitor by enhancing its solubility and permeability. *Pharmaceutical Research* **1999,** 16 (12), 1812-1817.
110. Collnot, E.-M.,Baldes, C.,Wempe, M. F.,Hyatt, J.,Navarro, L.,Edgar, K. J.,Schaefer, U. F.,Lehr, C.-M., Influence of vitamin E TPGS poly(ethylene glycol) chain length on apical efflux transporters in Caco-2 cell monolayers. *Journal of Controlled Release* **2006,** 111 (1-2), 35-40.
111. Sachs-Barrable, K.,Thamboo, A.,Lee, S. D.,Wasan, K. M., Lipid excipients Peceol and Gelucire 44/14 decrease P-glycoprotein mediated efflux of rhodamine 123 partially due to expression within Caco-2 cells. *Journal of Pharmacy and Pharmaceutical Sciences* **2007,** 10 (3), 319-331.
112. Ingels, F.,Deferme, S.,Destexhe, E.,Oth, M.,Van Den Mooter, G.,Augustijns, P., Simulated intestinal fluid as transport medium in the Caco-2 cell culture model. *International Journal of Pharmaceutics* **2002,** 232 (1-2), 183-192.
113. Konishi, T.,Satsu, H.,Hatsugai, Y.,Aizawa, K.,Inakuma, T.,Nagata, S.,Sakuda, S.-H.,Nagasawa, H.,Shimizu, M., A bitter melon extract inhibits the P-glycoprotein activity in intestinal Caco-2 cells: Monoglyceride as an active compound. *BioFactors* **2005,** 22 (1-4), 71-74.
114. Wagner, D.,Spahn-Langguth, H.,Hanafy, A.,Koggel, A.,Langguth, P., Intestinal drug efflux: Formulation and food effects. *Advanced Drug Delivery Reviews* **2001,** 50 (SUPPL. 1), S13-S31.
115. Mountfield, R. J.,Senepin, S.,Schleimer, M.,Walter, I.,Bittner, B., Potential inhibitory effects of formulation ingredients on intestinal cytochrome P450. *International Journal of Pharmaceutics* **2000,** 211 (1-2), 89-92.

116. Porter, C. J. H.,Charman, W. N., Intestinal lymphatic drug transport: An update. *Advanced Drug Delivery Reviews* **2001,** 50 (1-2), 61-80.
117. Hyun, S. A.,Vahouny, G. V.,Treadwell, C. R., Portal absorption of fatty acids in lymph- and portal vein-cannulated rats. *Biochimica et Biophysica Acta (BBA)/Lipids and Lipid Metabolism* **1967,** 137 (2), 296-305.
118. McDonald, G. B.,Weidman, M., Partitioning of polar fatty acids into lymph and portal vein after intestinal absorption in the rat. *Quarterly Journal of Experimental Physiology* **1987,** 72 (2), 153-159.
119. Mansbach II, C. M.,Dowell, R. F.,Pritchett, D., Portal transport of absorbed lipids in rats. *American Journal of Physiology - Gastrointestinal and Liver Physiology* **1991,** 261 (3 24-3), G530-G538.
120. Nordskog, B. K.,Phan, C. T.,Nutting, D. F.,Tso, P., An examination of the factors affecting intestinal lymphatic transport of dietary lipids. *Advanced Drug Delivery Reviews* **2001,** 50 (1-2), 21-44.
121. Charman, W. N. A.,Stella, V. J., Estimating the maximal potential for intestinal lymphatic transport of lipophilic drug molecules. *International Journal of Pharmaceutics* **1987,** 34 (1-2), 175-178.
122. Charman, W. N. A.,Stella, V. J., Effects of lipid class and lipid vehicle volume on the intestinal lymphatic transport of DDT. *International Journal of Pharmaceutics* **1986,** 33 (1-3), 165-172.
123. Porter, C. J. H.,Charman, S. A.,Humberstone, A. J.,Charman, W. N., Lymphatic transport of halofantrine in the conscious rat when administered as either the free base or the hydrochloride salt: Effect of lipid class and lipid vehicle dispersion. *Journal of Pharmaceutical Sciences* **1996,** 85 (4), 357-361.
124. Nankervis, R.,Davis, S. S.,Day, N. H.,Shaw, P. N., Intestinal lymphatic transport of three retinoids in the rat after oral administration: Effect of lipophilicity and lipid vehicle. *International Journal of Pharmaceutics* **1996,** 130 (1), 57-64.
125. Charman, W. N.,Porter, C. J. H., Lipophilic prodrugs designed for intestinal lymphatic transport. *Advanced Drug Delivery Reviews* **1996,** 19 (2), 149-169.
126. Mu, H.,Porsgaard, T., The metabolism of structured triacylglycerols. *Progress in Lipid Research* **2005,** 44 (6), 430-448.
127. Holm, R.,Porter, C. J. H.,Müllertz, A.,Kristensen, H. G.,Charman, W. N., Structured triglyceride vehicles for oral delivery of halofantrine: Examination of intestinal lymphatic transport and bioavailability in conscious rats. *Pharmaceutical Research* **2002,** 19 (9), 1354-1361.
128. Holm, R.,Porter, C. J. H.,Edwards, G. A.,Müllertz, A.,Kristensen, H. G.,Charman, W. N., Examination of oral absorption and lymphatic transport of halofantrine in a triple-cannulated canine model after administration in self-microemulsifying drug delivery systems (SMEDDS) containing structured triglycerides. *European Journal of Pharmaceutical Sciences* **2003,** 20 (1), 91-97.
129. Colburn, W. A.,Gibson, D. M.,Wiens, R. E.,Hanigan, J. J., Food increases the bioavailability of isotretinoin. *Journal of Clinical Pharmacology* **1983,** 23 (11-12), 534-539.
130. Humberstone, A. J.,Porter, C. J. H.,Charman, W. N., A physicochemical basis for the effect of food on the absolute oral bioavailability of halofantrine. *Journal of Pharmaceutical Sciences* **1996,** 85 (5), 525-529.
131. Armstrong, N. A.,James, K. C., Drug release from lipid-based dosage forms. II. *International Journal of Pharmaceutics* **1980,** 6 (3-4), 195-204.
132. Pouton, C. W., Lipid formulations for oral administration of drugs: non-emulsifying, self-emulsifying and 'self-microemulsifying' drug delivery systems. *European Journal of Pharmaceutical Sciences* **2000,** 11 (Supplement 2), S93-S98.
133. Gershanik, T.,Benita, S., Self-dispersing lipid formulations for improving oral absorption of lipophilic drugs. *European Journal of Pharmaceutics & Biopharmaceutics* **2000,** 50 (1), 179-188.
134. Mehnert, W.,Mäder, K., Solid lipid nanoparticles: Production, characterization and applications. *Advanced Drug Delivery Review* **2001,** 47 (2-3), 165-196.

135. Chen, Y.,Chen, J.-M.,Chen, M.,Gao, S.,Zhong, Y.-Q., Cubosomes as drug delivery system: Recent advance. *Academic Journal of Second Military Medical University* **2009,** 30 (7), 834-839.
136. Fricker, G.,Kromp, T.,Wendel, A.,Blume, A.,Zirkel, J.,Rebmann, H.,Setzer, C.,Quinkert, R.-O.,Martin, F.,Müller-Goymann, C., Phospholipids and lipid-based formulations in oral drug delivery. *Pharmaceutical Research* **2010,** 27 (8), 1469-1486.
137. Wasan, K. M., *Role of lipid excipients in modifying oral and parenteral drug delivery: Basic principles and biological examples*. John Wiley & Sons, Inc.: Hoboken, New Jersey, **2007**.
138. Constantinides, P. P., Lipid microemulsions for improving drug dissolution and oral absorption: Physical and biopharmaceutical aspects. *Pharmaceutical Research* **1995,** 12 (11), 1561-1572.
139. Fatouros, D. G.,Karpf, D. M.,Nielsen, F. S.,Mullertz, A., Clinical studies with oral lipid based formulations of poorly soluble compounds. *Therapeutics and Clinical Risk Management* **2007,** 3 (4), 591-604.
140. Tokumura, T.,Tsushima, Y.,Tatsuishi, K., Enhancement of the oral bioavailability of cinnarizine in oleic acid in beagle dogs. *Journal of Pharmaceutical Sciences* **1987,** 76 (4), 286-288.
141. Hargrove, J. T.,Maxson, W. S.,Colston Wentz, A., Absorption of oral progesterone is influenced by vehicle and particle size. *American Journal of Obstetrics and Gynecology* **1989,** 161 (4), 948-951.
142. Yamaoka, Y.,Roberts, R. D.,Stella, V. J., Low-melting phenytoin prodrugs as alternative oral delivery modes for phenytoin: A model for other high-melting sparingly water-soluble drugs. *Journal of Pharmaceutical Sciences* **1983,** 72 (4), 400-405.
143. Yamahira, Y.,Noguchi, T.,Takenaka, H.,Maeda, T., Biopharmaceutical studies of lipid-containing oral dosage forms: Relationship between drug absorption rate and digestibility of vehicles. *International Journal of Pharmaceutics* **1979,** 3 (1), 23-31.
144. Palin, K. J.,Wilson, C. G., The effect of different oils on the absorption of probucol in the rat. *Journal of Pharmacy and Pharmacology* **1984,** 36 (9), 641-643.
145. Gallo-Torres, H. E.,Ludorf, J.,Brin, M., The effect of medium-chain triglycerides on the bioavailability of vitamin E. *International Journal for Vitamin and Nutrition Research* **1978,** 48 (3), 240-249.
146. Nakano, M., Places of emulsions in drug delivery. *Advanced Drug Delivery Review* **2000,** 45 (1), 1-4.
147. Pouton, C. W., Formulation of self-emulsifying drug delivery systems. *Advanced Drug Delivery Review* **1997,** 25 (1), 47-58.
148. Myers, R. A.,Stella, V. J., Systemic bioavailability of penclomedine (NSC-338720) from oil-in-water emulsions administered intraduodenally to rats. *International Journal of Pharmaceutics* **1992,** 78 (2-3), 217-226.
149. Serajuddin, A. T.,Sheen, P. C.,Mufson, D.,Bernstein, D. F.,Augustine, M. A., Physicochemical basis of increased bioavailability of a poorly water-soluble drug following oral administration as organic solutions. *Journal of Pharmaceutical Sciences* **1988,** 77 (4), 325-329.
150. Araya, H.,Tomita, M.,Hayashi, M., The novel formulation design of O/W microemulsion for improving the gastrointestinal absorption of poorly water soluble compounds. *International Journal of Pharmaceutics* **2005,** 305 (1-2), 61-74.
151. Takeuchi, H.,Sasaki, H.,Niwa, T.,Hino, T.,Kawashima, Y.,Uesugi, K.,Ozawa, H., Redispersible dry emulsion system as novel oral dosage form of oily drugs: in vivo studies in beagle dogs. *Chemical and Pharmaceutical Bulletin* **1991,** 39 (12), 3362-3364.
152. Dollo, G.,Le Corre, P.,Guerin, A.,Chevanne, F.,Burgot, J. L.,Leverge, R., Spray-dried redispersible oil-in-water emulsion to improve oral bioavailability of poorly soluble drugs. *European Journal of Pharmaceutical Sciences* **2003,** 19 (4), 273-280.
153. Jang, D. J.,Jeong, E. J.,Lee, H. M.,Kim, B. C.,Lim, S. J.,Kim, C. K., Improvement of bioavailability and photostability of amlodipine using redispersible dry emulsion. *European Journal of Pharmaceutical Sciences* **2006,** 28 (5), 405-411.

154. Hansen, T.,Holm, P.,Schultz, K., Process characteristics and compaction of spray-dried emulsions containing a drug dissolved in lipid. *International Journal of Pharmaceutics* **2004,** 287 (1-2), 55-66.
155. Soottitantawat, A.,Takayama, K.,Okamura, K.,Muranaka, D.,Yoshii, H.,Furuta, T.,Ohkawara, M.,Linko, P., Microencapsulation of l-menthol by spray drying and its release characteristics. *Innovative Food Science and Emerging Technologies* **2005,** 6 (2), 163-170.
156. Christensen, K. L.,Pedersen, G. P.,Kristensen, H. G., Preparation of redispersible dry emulsions by spray drying. *International Journal of Pharmaceutics* **2001,** 212 (2), 187-194.
157. Christensen, K. L.,Pedersen, G. P.,Kristensen, H. G., Physical stability of redispersible dry emulsions containing amorphous sucrose. *European Journal of Pharmaceutics & Biopharmaceutics* **2002,** 53 (2), 147-153.
158. Ragno, G.,Cione, E.,Garofalo, A.,Genchi, G.,Ioele, G.,Risoli, A.,Spagnoletta, A., Design and monitoring of photostability systems for amlodipine dosage forms *International Journal of Pharmaceutics* **2003,** 265 (1-2), 125-132.
159. Hansen, T.,Holm, P.,Rohde, M.,Schultz, K., In vivo evaluation of tablets and capsules containing spray-dried o/w-emulsions for oral delivery of poorly soluble drugs. *International Journal of Pharmaceutics* **2005,** 293 (1-2), 203-211.
160. Pedersen, G. P.,Fäldt, P.,Bergenståhl, B.,Kristensen, H. G., Solid state characterisation of a dry emulsion: a potential drug delivery system *International Journal of Pharmaceutics* **1998,** 171 (2), 257-270.
161. Dixit, R. P.,Nagarsenker, M. S., Dry adsorbed emulsion of simvastatin: Optimization and in vivo advantage. *Pharmaceutical Development and Technology* **2007,** 12 (5), 495-504.
162. Yi, T.,Wan, J.,Xu, H.,Yang, X., A new solid self-microemulsifying formulation prepared by spray-drying to improve the oral bioavailability of poorly water soluble drugs. *European Journal of Pharmaceutics and Biopharmaceutics* **2008,** 70 (2), 439-444.
163. Nekkanti, V.,Karatgi, P.,Prabhu, R.,Pillai, R., Solid self-microemulsifying formulation for candesartan cilexetil. *AAPS PharmSciTech* **2009**, 1-9.
164. Wang, Z.,Sun, J.,Wang, Y.,Liu, X.,Liu, Y.,Fu, Q.,Meng, P.,He, Z., Solid self-emulsifying nitrendipine pellets: Preparation and in vitro/in vivo evaluation. *International Journal of Pharmaceutics* **2010,** 383 (1-2), 1-6.
165. Shah, N. H.,Carvajal, M. T.,Patel, C. I.,Infeld, M. H.,Malick, A. W., Self-emulsifying drug delivery systems (SEDDS) with polyglycolyzed glycerides for improving in vitro dissolution and oral absorption of lipophilic drugs. *International Journal of Pharmaceutics* **1994,** 106 (1), 15-23.
166. Julianto, T.,Yuen, K. H.,Noor, A. M., Improved bioavailability of vitamin E with a self emulsifying formulation. *International Journal of Pharmaceutics* **2000,** 200 (1), 53-57.
167. Mueller, E. A.,Kovarik, J. M.,Van Bree, J. B.,Tetzloff, W.,Grevel, J.,Kutz, K., Improved dose linearity of cyclosporine pharmacokinetics from a microemulsion formulation. *Pharmaceutical Research* **1994,** 11 (2), 301-304.
168. Trull, A. K.,Tan, K. K. C.,Tan, L.,Alexander, G. J. M.,Jamieson, N. V., Enhanced absorption of new oral cyclosporin microemulsion formulation, neoral, in liver transplant recipients with external biliary diversion. *Transplantation Proceedings* **1994,** 26 (5), 2977-2978.
169. Iwanaga, K.,Kushibiki, T.,Miyazaki, M.,Kakemi, M., Disposition of lipid-based formulation in the intestinal tract affects the absorption of poorly water-soluble drugs. *Biological and Pharmaceutical Bulletin* **2006,** 29 (3), 508-512.
170. Porter, C. J. H.,Kaukonen, A. M.,Boyd, B. J.,Edwards, G. A.,Charman, W. N., Susceptibility to lipase-mediated digestion reduces the oral bioavailability of danazol after administration as a medium-chain lipid-based microemulsion formulation. *Pharmaceutical Research* **2004,** 21 (8), 1405-1412.
171. Grove, M.,Müllertz, A.,Nielsen, J. L.,Pedersen, G. P., Bioavailability of seocalcitol. II: Development and characterisation of self-microemulsifying drug delivery systems (SMEDDS) for oral administration containing medium and long chain triglycerides. *European Journal of Pharmaceutical Sciences* **2006,** 28 (3), 233-242.

172. Mueller, E. A.,Kovarik, J. M.,Van Bree, J. B.,Grevel, J.,Lucker, P. W.,Kutz, K., Influence of a fat-rich meal on the pharmacokinetics of a new oral formulation of cyclosporine in a crossover comparison with the market formulation. *Pharmaceutical Research* **1994,** 11 (1), 151-155.
173. Kovarik, J. M.,Mueller, E. A.,Van Bree, J. B.,Tetzloff, W.,Kutz, K., Reduced inter- and intraindividual variability in cyclosporine pharmacokinetics from a microemulsion formulation. *Journal of Pharmaceutical Sciences* **1994,** 83 (3), 444-446.
174. Barbé, C.,Bartlett, J.,Kong, L.,Finnie, K.,Lin, H. Q.,Larkin, M.,Calleja, S.,Bush, A.,Calleja, G., Silica particles: a novel drug-delivery system. *Advanced Materials* **2004,** 16 (21), 1959-1966.
175. Jacobson, G. B.,Shinde, R.,Contag, C. H.,Zare, R. N., Sustained release of drugs dispersed in polymer nanoparticles. *Angewandte Chemie - International Edition* **2008,** 47 (41), 7880-7882.
176. Wu, Z.,Jiang, Y.,Kim, T.,Lee, K., Effects of surface coating on the controlled release of vitamin B1 from mesoporous silica tablets. *Journal of Controlled Release* **2007,** 119 (2), 215-221.
177. Mellaerts, R.,Mols, R.,Jammaer, J. A. G.,Aerts, C. A.,Annaert, P.,Van Humbeeck, J.,Van den Mooter, G.,Augustijns, P.,Martens, J. A., Increasing the oral bioavailability of the poorly water soluble drug itraconazole with ordered mesoporous silica. *European Journal of Pharmaceutics and Biopharmaceutics* **2008,** 69 (1), 223-230.
178. Quintanar-Guerrero, D.,Ganem-Quintanar, A.,Nava-Arzaluz, M. G.,Piñón-Segundo, E., Silica xerogels as pharmaceutical drug carriers. *Expert Opinion on Drug Delivery* **2009,** 6 (5), 485-498.
179. Manzano, M.,Colilla, M.,Vallet-Regí, M., Drug delivery from ordered mesoporous matrices. *Expert Opinion on Drug Delivery* **2009,** 6 (12), 1383-1400.
180. Zhu, Y.,Shi, J.,Shen, W.,Chen, H.,Dong, X.,Ruan, M., Preparation of novel hollow mesoporous silica spheres and their sustained-release property. *Nanotechnology* **2005,** 16 (11), 2633-2638.
181. Czarnobaj, K.,Łukasiak, J., In vitro release of cisplatin from sol-gel processed organically modified silica xerogels. *Journal of Materials Science: Materials in Medicine* **2007,** 18 (10), 2041-2044.
182. Maver, U.,Godec, A.,Bele, M.,Planinšek, O.,Gaberšček, M.,Srčič, S.,Jamnik, J., Novel hybrid silica xerogels for stabilization and controlled release of drug. *International Journal of Pharmaceutics* **2007,** 330 (1-2), 164-174.
183. Contessotto, L.,Ghedini, E.,Pinna, F.,Signoretto, M.,Cerrato, G.,Crocellè, V., Hybrid organic-inorganic silica gel carriers with controlled drug-delivery properties. *Chemistry - A European Journal* **2009,** 15 (44), 12043-12049.
184. Thomas, M. J. K.,Slipper, I.,Walunj, A.,Jain, A.,Favretto, M. E.,Kallinteri, P.,Douroumis, D., Inclusion of poorly soluble drugs in highly ordered mesoporous silica nanoparticles. *International Journal of Pharmaceutics* **2010,** 387 (1-2), 272-277.
185. Zhu, Y.-F., Shi, J.-L., Li, Y.-S., Chen, H.-R., Shen, W.-H., Dong, X.-P. , Storage and release of ibuprofen drug molecules in hollow mesoporous silica spheres with modified pore surface *Microporous and Mesoporous Materials* **2005,** 85 (1-2), 75-81.
186. Llabrés, M.,Vila, J. L.,Martínez-Pacheco, R., Quantification of the effect of excipients on bioavailability by means of response surfaces. I: amoxicillin in fat matrix. *Journal of Pharmaceutical Sciences* **1982,** 71 (8), 924-927.
187. Llabrés, M.,Vila, J. L.,Martínez-Pacheco, R., Quantification of the effect of excipients on bioavailability by means of response surfaces. II: amoxicillin in fat-silica matrix. *Journal of Pharmaceutical Sciences* **1982,** 71 (8), 927-930.
188. Llabrés, M.,Vila, J. L.,Martínez-Pacheco, R., Quantification of the effect of excipients on bioavailability by means of response surfaces. III: in vivo-in vitro correlations. *Journal of Pharmaceutical Sciences* **1982,** 71 (8), 930-932.
189. Fatouros, D. G.,Mullertz, A., In vitro lipid digestion models in design of drug delivery systems for enhancing oral bioavailability. *Expert Opinion on Drug Metabolism and Toxicology* **2008,** 4 (1), 65-76.

190. Hultin, M.,Mullertz, A.,Zundel, M. A.,Olivecrona, G.,Hansen, T. T.,Deckelbaum, R. J.,Carpentier, Y. A.,Olivecrona, T., Metabolism of emulsions containing medium- and long-chain triglycerides or interesterified triglycerides. *Journal of Lipid Research* **1994,** 35 (10), 1850-1860.
191. Reymond, J.-P.,Sucker, H., In vitro model for ciclosporin intestinal absorption in lipid vesicles. *Pharmaceutical Research* **1988,** 5 (10), 673-676.
192. Rigler, M. W.,Honkanen, R. E.,Patton, J. S., Visualization by freeze fracture, in vitro and in vivo, of the products of fat digestion. *Journal of Lipid Research* **1986,** 27 (8), 836-857.
193. Fatouros, D. G.,Bergenstahl, B.,Mullertz, A., Morphological observations on a lipid-based drug delivery system during in vitro digestion. *European Journal of Pharmaceutical Sciences* **2007,** 31 (2), 85-94.
194. Chen, M.-L., Lipid excipients and delivery systems for pharmaceutical development: a regulatory perspective. *Advanced Drug Delivery Review* **2008,** 60 (6), 768-777.
195. US Food and Drug Administration. *GRAS Substances (SCOGS) Database*. Food and Drug Administration, Rockville, MD, **2000**. Available at http://www.fda.gov/Food/FoodIngredientsPackaging/GenerallyRecognizedasSafeGRAS/GRASSubstancesSCOGSDatabase/default.htm.
196. US Food and Drug Administration. *Inactive Ingredient Search for Approved Drug Products*. Food and Drug Administration, Rockville, MD, **2000**. Available at http://www.accessdata.fda.gov/scripts/cder/iig/index.cfm.
197. Dahan, A.,Hoffman, A., Use of a dynamic in vitro lipolysis model to rationalize oral formulation development for poor water soluble drugs: correlation with in vivo data and the relationship to intra-enterocyte processes in rats. *Pharmaceutical Research* **2006,** 23 (9), 2165-2174.
198. Porter, C. J. H.,Kaukonen, A. M.,Taillardat-Bertschinger, A.,Boyd, B. J.,O'Connor, J. M.,Edwards, G. A.,Charman, W. N., Use of in vitro lipid digestion data to explain the in vivo performance of triglyceride-based oral lipid formulations of poorly water-soluble drugs: studies with halofantrine. *Journal of Pharmaceutical Sciences* **2004,** 93 (5), 1110-1121.
199. Reymond, J.-P.,Sucker, H.,Vonderscher, J., In vivo model for ciclosporin intestinal absorption in lipid vehicles. *Pharmaceutical Research* **1988,** 5 (10), 677-679.
200. Buch, P.,Langguth, P.,Kataoka, M.,Yamashita, S., IVIVC in oral absorption for fenofibrate immediate release tablets using a dissolution/permeation system. *Journal of Pharmaceutical Sciences* **2009,** 98 (6), 2001-2009.
201. Hunter, R. J., *Zeta potential in colloid science: principles and applications*. Academic Press: London, **1981**.
202. Adamovics, J. A., *Chromatographic analysis of pharmaceuticals*. M. Dekker: New York, **1990**.
203. Stanley-Wood, N. G.,Lines, R. W., *Particle Size Analysis*. Royal Society of Chemistry: Cambridge, England, **1992**.
204. Rietdorf, J.,Gadella, T. W. J., *Microscopy techniques*. Springer: Heidelberg, **2005**.
205. Mattson, F. H.,Volpenhein, R. A., Synthesis and properties of glycerides. *Journal of Lipid Research* **1962,** 3, 281-296.
206. Kaukonen, A. M.,Boyd, B. J.,Porter, C. J. H.,Charman, W. N., Drug solubilization behavior during in vitro digestion of simple triglyceride lipid solution formulations. *Pharmaceutical Research* **2004,** 21 (2), 245-253.
207. Constantinides, P. P.,Scalart, J.-P.,Lancaster, C.,Marcello, J.,Marks, G.,Ellens, H.,Smith, P. L., Formulation and intestinal absorption enhancement evaluation of water-in-oil microemulsions incorporating medium-chain glycerides. *Pharmaceutical Research* **1994,** 11 (10), 1385-1390.
208. Subramanian, N.,Ray, S.,Ghosal, S. K.,Bhadra, R.,Priya, S., Formulation design of self-microemulsifying drug delivery systems for improved oral bioavailability of celecoxib. *Biological and Pharmaceutical Bulletin* **2004,** 27 (12), 1993-1999.

209. Subramanian, N., Ghosal, S.K., Moulik, S.P., Enhanced in vitro percutaneous absorption and in vivo anti-inflammatory effect of a selective cyclooxygenase inhibitor using microemulsion. *Drug Development and Industrial Pharmacy* **2005,** 31 (4-5), 405-416.
210. Joshi, M.,Patravale, V., Nanostructured lipid carrier (NLC) based gel of celecoxib. *International Journal of Pharmaceutics* **2008,** 346 (1-2), 124-132.
211. Park, M.-J.,Ren, S.,Lee, B.-J., In vitro and in vivo comparative study of intraconazole bioavailability when formulated in highly soluble self-emulsifying system and in solid dispersion. *Biopharmaceutics and Drug Disposition* **2007,** 28 (4), 199-207.
212. Washington, C., Stability of lipid emulsions for drug delivery. *Advanced Drug Delivery Review* **1996,** 20 (2-3), 131-145.
213. Nagasaka, Y.,Ishii, F., Interaction between erythrocytes from three different animals and emulsions prepared with various lecithins and oils. *Colloids and Surfaces B - Biointerfaces* **2001,** 22 (2), 141-147.
214. Therapeutic Good Administration. *Complementary Medicines Evaluation Committee (CMEC) extracted ratified minutes, sixteenth meeting*, Sydney, **1999.** Available at http://www.tga.gov.au/docs/pdf/cmec/cmecmi16.pdf.
215. Barnes, T. J. Emulsion droplets of controlled deformability: electrokinetics, colloid stability and polymer adsorption. PhD thesis, University of South Australia, Adelaide, 2003.
216. Prestidge, C. A.,Simovic, S., Nanoparticle encapsulation of emulsion droplets. *International Journal of Pharmaceutics* **2006,** 324 (1), 92-100.
217. Simovic, S.,Prestidge, C. A., Colloidosomes from the controlled interaction of submicrometer triglyceride droplets and hydrophilic silica nanoparticles. *Langmuir* **2008,** 24 (14), 7132-7137.
218. Ghouchi Eskandar, N.,Simovic, S.,Prestidge, C. A., Synergistic effect of silica nanoparticles and charged surfactants in the formation and stability of submicron oil-in-water emulsions. *Physical Chemistry Chemical Physics* **2007,** 9 (48), 6426-6434.
219. Aveyard, R.,Clint, J. H.,Horozov, T. S., Solid particles as emulsion stabilizers. *Progress in Colloid and Polymer Science* **2002,** 121, 11-18.
220. Seedher, N.,Bhatia, S., Solubility enhancement of COX-2 inhibitors using various solvent systems. *AAPS PharmSciTech* **2003,** 4 (3), 1-9.
221. Chawla, G.,Gupta, P.,Thilagavathi, R.,Chakraborti, A. K.,Bansal, A. K., Characterization of solid-state forms of celecoxib *European Journal of Pharmaceutical Sciences* **2003,** 20 (3), 305-317.
222. US Food and Drug Administration. *Celebrex® (celecoxib) capsules*. Food and Drug Administration, Rockville, MD, **1998**. Available at http://www.fda.gov/cder/foi/label/2005/020998s018,019lbl.pdf.
223. US Food and Drug Administration. *COX-2 selective (includes Bextra, Celebrex, and Vioxx) and non-selective non-steroidal anti-Inflammatory drugs (NSAIDs)*. Food and Drug Administration, Rockville, MD, **2009**. Available at http://www.fda.gov/Drugs/DrugSafety/PostmarketDrugSafetyInformationforPatientsandProviders/ucm103420.htm.
224. Wang, D.,Dubois, R. N., Prostaglandins and cancer. *Gut* **2006,** 55 (1), 115-122.
225. de Souza Pereira, R., Selective cyclooxygenase-2 (COX-2) inhibitors used for preventing or regressing cancer. *Recent Patents on Anti-Cancer Drug Discovery* **2009,** 4 (2), 157-163.
226. Hirohata, M.,Ono, K.,Yamada, M., Non-steroidal anti-inflammatory drugs as anti-amyloidogenic compounds. *Current Pharmaceutical Design* **2008,** 14 (30), 3280-3294.
227. Paulson, S. K.,Hribar, J. D.,Liu, N. W. K.,Hajdu, E.,Bible Jr., R. H.,Piergies, A.,Karim, A., Metabolism and excretion of [14C]celecoxib in healthy male volunteers. *Drug Metabolism and Disposition* **2000,** 28 (3), 308-314
228. Paulson, S. K.,Zhang, J. Y.,Breau, A. P.,Hribar, J. D.,Liu, N. W. K.,Jessen, S. M.,Lawal, W. M.,Cogburn, J. N.,Gresk, C. J.,Markos, C. S.,Maziasz, T. J.,Schoenhard, G. L.,Burton, E. G., Pharmacokinetics, tissue distribution, metabolism, and excretion of celecoxib in rats. *Drug Metabolism and Disposition* **2000,** 28 (5), 514-521
229. Paulson, S. K.,Vaughn, M. B.,Jessen, S. M.,Lawal, Y.,Gresk, C. J.,Yan, B.,Maziasz, T. J.,Cook, C. S.,Karim, A., Pharmacokinetics of celecoxib after oral administration in dogs

and humans: Effect of food and site of absorption. *Journal of Pharmacology and Experimental Therapeutics* **2001,** 297 (2), 638-645

230. Zarghi, A.,Shafaati, A.,Foroutan, S. M.,Khoddam, A., Simple and rapid high-performance liquid chromatographic method for determination of celecoxib in plasma using UV detection: application in pharmacokinetic studies. *Journal of Chromatography B* **2006,** 835 (1-2), 100-104.
231. Malvern Instruments Ltd, *Zetasizer Nano Series*, Malvern Instruments - Zetasizer **2009**. Available at http://www.malvern.com/LabEng/products/zetasizer/zetasizer_nano.htm.
232. Urum, K.,Pekdemir, T., Evaluation of biosurfactants for crude oil contaminated soil washing. *Chemosphere* **2004,** 57 (9), 1139-1150.
233. Shchipunov, Y. A., Lecithin organogel: a micellar system with unique properties. *Colloids and Surfaces A: Physicochemical and Engineering Aspects* **2001,** 183-185, 541-554.
234. Puranajoti, P.,Patil, R. T.,Sheth, P. D.,Bommareddy, G.,Dondeti, P.,Egbaria, K., Design and development of topical microemulsion for poorly water-soluble antifungal agents. *Journal of Applied Research* **2002,** 2 (1), XXVII-XXVIII.
235. Sari, P.,Razzak, M.,Tucker, I. G., Isotropic medium chain mono–diglyceride/oil/water formulations for solubilization of lipophilic and hydrophilic drugs. *International Journal of Pharmaceutics* **2004,** 270 (1-2), 287-296.
236. Chambin, O., Bérard, V., Rochat-Gonthier, M.H., Pourcelot, Y. , Dry adsorbed emulsion: 2. Dissolution behaviour of an intricate formulation. *International Journal of Pharmaceutics* **2002,** 235 (1-2), 169-178.
237. Steckel, H.,Brandes, H. G., A novel spray-drying technique to produce low density particles for pulmonary delivery. *International Journal of Pharmaceutics* **2004,** 278 (1), 187-195.
238. Samanta, B.,Patra, D.,Subramani, C.,Ofir, Y.,Yesilbag, G.,Sanyal, A.,Rotello, V. M., Stable magnetic colloidosomes via click-mediated crosslinking of nanoparticles at water-oil interfaces. *Small* **2009,** 5 (6), 685-688.
239. Wang, X.,Wenk, E.,Matsumoto, A.,Meinel, L.,Li, C.,Kaplan, D. L., Silk microspheres for encapsulation and controlled release. *Journal of Controlled Release* **2007,** 117 (3), 360-370.
240. Li, Y.,Wong, H. L.,Shuhendler, A. J.,Rauth, A. M.,Wu, X. Y., Molecular interactions, internal structure and drug release kinetics of rationally developed polymer-lipid hybrid nanoparticles. *Journal of Controlled Release* **2008,** 128 (1), 60-70.
241. Binks, B. P.,Kirkland, M., Interfacial structure of solid-stabilised emulsions studied by scanning electron microscopy. *Physical Chemistry Chemical Physics* **2002,** 4 (15), 3727-3733.
242. Ghouchi Eskandar, N.,Simovic, S.,Prestidge, C. A., Nanoparticle coated submicron emulsions: sustained in-vitro release and improved dermal delivery of all-trans-retinol. *Pharmaceutical Research* **2009,** 26 (7), 1764-1775.
243. Binks, B. P.,Horozov, T. S., *Colloidal particles at liquid interfaces*. Cambridge University Press: Cambridge, **2006**.
244. Binks, B. P., Particles as surfactants - similarities and differences. *Current Opinion in Colloid and Interface Science* **2002,** 7 (1-2), 21-41.
245. Martin Siewert,Jennifer Dressman,Cynthia Brown,Shah, V., FIP/AAPS guidelines for dissolution/ in vitro release testing of novel/ special dosage forms. *Dissolution Technologies* **2003**, 6-15.
246. Galia, E.,Nicolaides, E.,Hörter, D.,Löbenberg, R.,Reppas, C.,Dressman, J. B., Evaluation of various dissolution media for predicting In vivo performance of class I and II drugs. *Pharmaceutical Research* **1998,** 15 (5), 698-705.
247. Dressman, J. B.,Reppas, C., In vitro–in vivo correlations for lipophilic, poorly water-soluble drugs. *European Journal of Pharmaceutical Sciences* **2000,** 11 (2), S73–S80.
248. Noory, C.,Tran, N.,Ouderkirk, L.,Shah, V., Steps for development of a dissolution test for sparingly water-soluble drug products. *American Pharmaceutical Review* **2002,** 5 (4), 16-20.
249. Rawat, S.,Jain, S. K., Solubility enhancement of celecoxib using [beta]-cyclodextrin inclusion complexes. *European Journal of Pharmaceutics and Biopharmaceutics* **2004,** 57 (2), 263-267.

250. Ali, J.,Arora, S.,Ahuja, A.,Babbar, A. K.,Sharma, R. K.,Khar, R. K., Formulation and development of floating capsules of celecoxib: in vitro and in vivo evaluation. *AAPS PharmSciTech* **2007,** 8 (4), art. no. 119, pp. E1-E8
251. Thakkar, H.,Sharma, R. K.,Mishra, A. K.,Chuttani, K.,Murthy, R. S. R., Celecoxib incorporated chitosan microspheres: in vitro and in vivo evaluation. *Journal of Drug Targeting* **2004,** 12 (9-10), 549-557.
252. US Food and Drug Administration. *Dissolution methods: celecoxib*. Food and Drug Administration, U.S. Department of Health and Human Services, Rockville, MD, **2009**. Available at http://www.accessdata.fda.gov/scripts/cder/dissolution/dsp_SearchResults_Dissolutions.cfm.
253. El-Massik, M. A., Abdallah, O.Y., Galal, S., Daabis, N.A. , Towards a universal dissolution medium for carbamazepine. *Drug Development and Industrial Pharmacy* **2006,** 32 (7), 893-905.
254. Brown, W.,Marques, M., February 2006 Questions and Answers. *Dissolution Technologies* **2006,** 13 (1).
255. Rohrs, B. R., Dissolution method development for poorly soluble compounds. *Dissolution Technologies* **2001,** 8 (3), 1-5.
256. Khan, K. A., The concept of dissolution efficiency. *Journal of Pharmacy and Pharmacology* **1975,** 27 (1), 48-49.
257. Zhang, H.,Yu, L. X., Dissolution testing for solid oral drug products: theoretical considerations. *American Pharmaceutical Review* **2004,** 7 (5), 26-30.
258. Jinno, J.,Oh, D.-M.,Crison, J. R.,Amidon, G. L., Dissolution of ionizable water-insoluble drugs: the combined effect of pH and surfactant. *Journal of Pharmaceutical Sciences* **2000,** 89 (2), 268-274.
259. Sheng, J. J.,Kasim, N. A.,Chandrasekharan, R.,Amidon, G. L., Solubilization and dissolution of insoluble weak acid, ketoprofen: effects of pH combined with surfactant. *European Journal of Pharmaceutical Sciences* **2006,** 29 (3-4 Spec. Iss.), 306-314.
260. US Food and Drug Administration. *Guidance for Industry: dissolution testing of immediate release solid oral dosage forms*. Food and Drug Administration, Rockville, MD, **1997**. Available at http://www.fda.gov/cder/guidance.htm.
261. Olbrich, C.,Kayser, O.,Muller, R. H., Lipase degradation of Dynasan 114 and 116 solid lipid nanoparticles (SLN) - effect of surfactants, storage time and crystallinity. *International Journal of Pharmaceutics* **2002,** 237 (1-2), 119-128.
262. Sek, L.,Porter, C. J. H.,Kaukonen, A. M.,Charman, W. N., Evaluation of the in-vitro digestion profiles of long and medium chain glycerides and the phase behaviour of their lipolytic products. *Journal of Pharmacy & Pharmacology* **2002,** 54 (1), 29-41
263. Horozov, T. S.,Binks, B. P., Particle-stabilized emulsions: a bilayer or a bridging monolayer? *Angewandte Chemie International Edition* **2006,** 45 (5), 773-776.
264. Binks, B. P.,Philip, J.,Rodrigues, J. A., Inversion of silica-stabilized emulsions induced by particle concentration. *Langmuir* **2005,** 21 (8), 3296-3302.
265. Binks, B. P.,Whitby, C. P., Silica particle-stabilized emulsions of silicone oil and water: Aspects of emulsification. *Langmuir* **2004,** 20 (4), 1130-1137.
266. Duane, W. C.,Ginsberg, R. L.,Bennion, L. J., Effects of fasting on bile acid metabolism and biliary lipid composition in man. *Journal of Lipid Research* **1976,** 17 (3), 211-219.
267. Lindahl, A.,Ungell, A.-L.,Knutson, L.,Lennernäs, H., Characterization of fluids from the stomach and proximal jejunum in men and women. *Pharmaceutical Research* **1997,** 14 (4), 497-502.
268. Cistola, D. P.,Hamilton, J. A.,Jackson, D.,Small, D. M., Ionization and phase behavior of fatty acids in water: Application of the Gibbs phase rule. *Biochemistry* **1988,** 27 (6), 1881-1888.
269. Alvarez, F. J.,Stella, V. J., The role of calcium ions and bile salts on the pancreatic lipase-catalyzed hydrolysis of triglyceride emulsions stabilized with lecithin. *Pharmaceutical Research* **1989,** 6 (6), 449-457.

270. Christensen, J. O.,Schultz, K.,Mollgaard, B.,Kristensen, H. G.,Mullertz, A., Solubilisation of poorly water-soluble drugs during in vitro lipolysis of medium- and long-chain triacylglycerols. *European Journal of Pharmaceutical Sciences* **2004,** 23 (3), 287-296.
271. Yap, S. P.,Yuen, K. H., Influence of lipolysis and droplet size on tocotrienol absorption from self-emulsifying formulations. *International Journal of Pharmaceutics* **2004,** 281 (1-2), 67-78.
272. Nini, L.,Sarda, L.,Comeau, L. C.,Boitard, E.,Dube?s, J. P.,Chahinian, H., Lipase-catalysed hydrolysis of short-chain substrates in solution and in emulsion: a kinetic study. *Biochimica et Biophysica Acta - Molecular and Cell Biology of Lipids* **2001,** 1534 (1), 34-44.
273. Ljusberg-Wahren, H.,Seier Nielsen, F.,Brogard, M.,Troedsson, E.,Mullertz, A., Enzymatic characterization of lipid-based drug delivery systems. *International Journal of Pharmaceutics* **2005,** 298 (2), 328-332.
274. Pignol, D.,Ayvazian, L.,Kerfelec, B.,Timmins, P.,Crenon, I.,Hermoso, J.,Fontecilla-Camps, J. C.,Chapus, C., Critical role of micelles in pancreatic lipase activation revealed by small angle neutron scattering. *Journal of Biological Chemistry* **2000,** 275 (6), 4220–4224.
275. Wickham, M.,Garrood, M.,Leney, J.,Wilson, P. D. G.,Fillery-Travis, A., Modification of a phospholipid stabilized emulsion interface by bile salt: effect on pancreatic lipase activity. *Journal of Lipid Research* **1998,** 39 (3), 623-632.
276. De Smidt, P. C., Campanero, M.A., Trocóniz, I.F., Intestinal absorption of penclomedine from lipid vehicles in the conscious rat: contribution of emulsification versus digestibility. *International Journal of Pharmaceutics* **2004,** 270 (1-2), 109-118.
277. Klein, E.,Lyman Jr., R. B.,Peterson, L.,Berger, R. I., The effect of lecithin on the activity of pancreatic lipase. *Life Sciences* **1967,** 6 (12), 1305-1307.
278. Berger, R. I.,Klein, E.,Peterson, L.,Hunt, M.,Lever, W. F., The effect of phospholipids on the lipolytic activity of heparin-induced plasma lipase. *Life Sciences* **1968,** 7 (12 Part 1), 647-651.
279. Pieroni, G.,Verger, R., Hydrolysis of mixed monomolecular films of triglyceride/lecithin by pancreatic lipase. *Journal of Biological Chemistry* **1979,** 254 (20), 10090-10094.
280. Tsujita, T.,Takaichi, H.,Takaku, T.,Sawai, T.,Yoshida, N.,Hiraki, J., Inhibition of lipase activities by basic polysaccharide. *Journal of Lipid Research* **2007,** 48 (2), 358-365.
281. Aranberri, I.,Binks, B. P.,Clint, J. H.,Fletcher, P. D. I., Synthesis of macroporous silica from solid-stabilised emulsion templates. *Journal of Porous Materials* **2009,** 16 (4), 429-437.
282. Vertegel, A. A.,Siegel, R. W.,Dordick, J. S., Silica nanoparticle size influences the structure and enzymatic activity of adsorbed lysozyme. *Langmuir* **2004,** 20 (16), 6800-6807.
283. Bal, R.,Tope, B. B.,Das, T. K.,Hegde, S. G.,Sivasanker, S., Alkali-loaded silica, a solid base: investigation by FTIR spectroscopy of adsorbed CO2 and its catalytic activity. *Journal of Catalysis* **2001,** 204 (2), 358-363.
284. Badr, Y.,Abd El-Wahed, M. G.,Mahmoud, M. A., Photocatalytic degradation of methyl red dye by silica nanoparticles. *Journal of Hazardous Materials* **2008,** 154 (1-3), 245-253.
285. Barik, T. K.,Sahu, B.,Swain, V., Nanosilica - from medicine to pest control. *Parasitology Research* **2008,** 103 (2), 253-258.
286. Cruz, J. C.,Pfromm, P. H.,Rezac, M. E., Immobilization of Candida antarctica Lipase B on fumed silica. *Process Biochem.* **2009,** 44 (1), 62-69.
287. Davies, N. M.,McLachlan, A. J.,Day, R. O.,Williams, K. M., Clinical pharmacokinetics and pharmacodynamics of celecoxib: a selective cyclo-oxygenase-2 inhibitor. *Clinical Pharmacokinetics* **2000,** 38 (3), 225-242.
288. Guirguis, M. S.,Sattari, S.,Jamali, F., Pharmacokinetics of celecoxib in the presence and absence of interferon-induced acute inflammation in the rat: application of a novel HPLC assay. *Journal of Pharmacy and Pharmaceutical Sciences* **2001,** 4 (1), 1-6.
289. Schoenwald, R. D., *Pharmacokinetics in drug discovery and development*. CRC Press: Boca Raton, **2002**.
290. McAdam, B. F.,Catella-Lawson, F.,Mardini, I. A.,Kapoor, S.,Lawson, J. A.,Fitzgerald, G. A., Systemic biosynthesis of prostacyclin by cyclooxygenase (COX)-2: The human pharmacology of a selective inhibitor of COX-2. *Proceedings of the National Academy of Sciences of the United States of America* **1999,** 96 (1), 272-277.

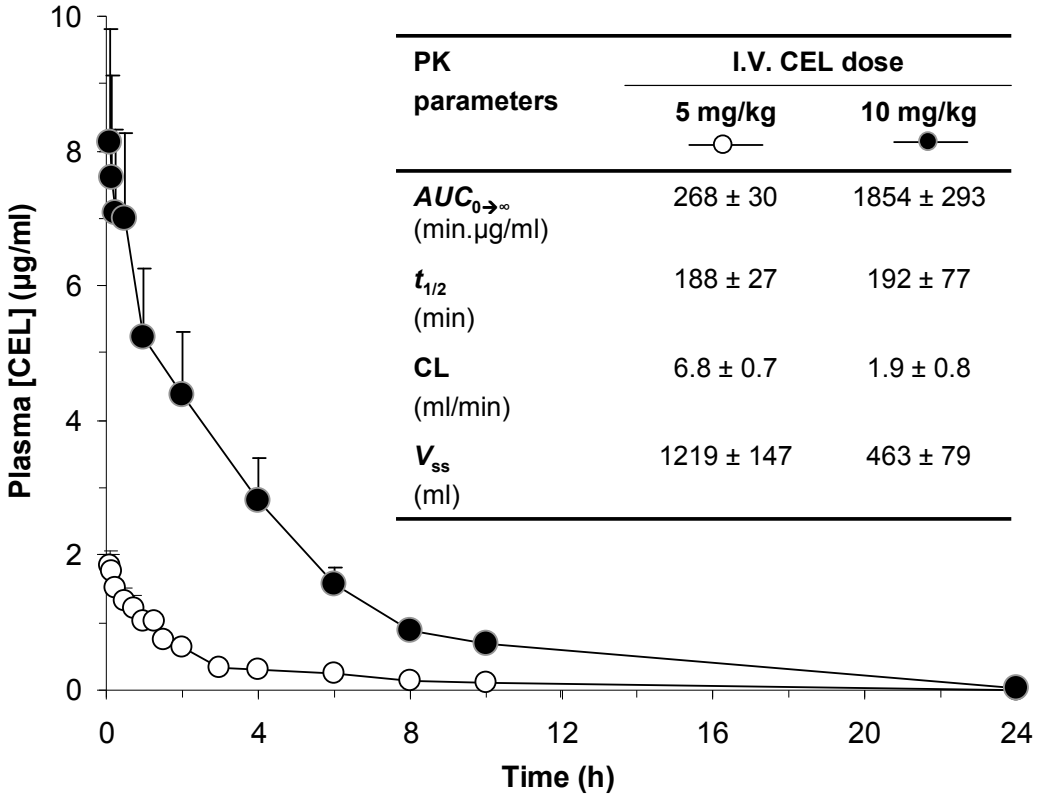
291. Shakeel, F.,Baboota, S.,Ahuja, A.,Ali, J.,Shafiq, S., Skin permeation mechanism and bioavailability enhancement of celecoxib from transdermally applied nanoemulsion. *Journal of Nanobiotechnology* **2008,** 6 (8).
292. Gupta, S.,Moulik, S. P., Biocompatible microemulsions and their prospective uses in drug delivery. *Journal of Pharmaceutical Sciences* **2008,** 97 (1), 22-45.
293. Thakkar, H.,Sharma, R. K.,Mishra, A. K.,Chuttani, K.,Murthy, R. R., Albumin microspheres as carriers for the antiarthritic drug celecoxib. *AAPS PharmSciTech* **2005,** 6 (1, art. no. 12), E65-E73.
294. Dolenc, A.,Kristl, J.,Baumgartner, S.,Planinšek, O., Advantages of celecoxib nanosuspension formulation and transformation into tablets. *International Journal of Pharmaceutics* **2009,** 376 (1-2), 204-212.
295. Dhumal, R. S.,Shimpi, S. L.,Paradkar, A. R., Development of spray-dried co-precipitate of amorphous celecoxib containing storage and compression stabilizers. *Acta Pharmaceutica* **2007,** 57 (3), 287-300.
296. Nagarsenker, M. S., Joshi, M.S., Celecoxib-cyclodextrin systems: Characterization and evaluation of in vitro and in vivo advantage. *Drug Development and Industrial Pharmacy* **2005,** 31 (2), 169-178.
297. Perrut, M.,Jung, J.,Leboeuf, F., Enhancement of dissolution rate of poorly soluble active ingredients by supercritical fluid processes: Part II: Preparation of composite particles. *International Journal of Pharmaceutics* **2005,** 288 (1), 11-16.
298. Garti, N.,Avrahami, M.,Aserin, A., Improved solubilization of celecoxib in U-type nonionic microemulsions and their structural transitions with progressive aqueous dilution. *Journal of Colloid and Interface Science* **2006,** 299 (1), 352-365.
299. Nasr, M., In Vitro and In Vivo Evaluation of Proniosomes Containing Celecoxib for Oral Administration. *AAPS PharmSciTech* **2009**, 1-5
300. Baboota, S.,Shakeel, F.,Ahuja, A.,Ali, J.,Shafiq, S., Design, development and evaluation of novel nanoemulsion formulations for transdermal potential of celecoxib. *Acta Pharmaceutica* **2007,** 57 (3), 315-332.
301. Smith, D. A.,Waterbeemd, H. v. d.,Walker, D. K., *Pharmacokinetics and metabolism in drug design*. 2nd ed.; Wiley-VCH: Weinheim, **2006**.
302. National Health and Medical Research Council. *Guidelines to promote the wellbeing of animals used for scientific purposes: The assessment and alleviation of pain and distress in research animals*. Australian Government, **2008**. Available at http://www.nhmrc.gov.au/health_ethics/animal/index.htm.
303. Ritschel, W. A.,Kearns, G. L., *Handbook of basic pharmacokinetics - including clinical applications*. 5th ed. ed.; American Pharmaceutical Association, Washington: **1999**.
304. Webster, L. K.,Jones, D. B.,Mihaly, G. W.,Smallwood, R. A., Effect of omeprazole and polyethylene glycol-400 on antipyrine elimination by the isolated perfused rat liver. *Journal of Pharmacy and Pharmacology* **1984,** 36 (7), 470-472.
305. Whiteside, G. T.,Adedoyin, A.,Leventhal, L., Predictive validity of animal pain models? A comparison of the pharmacokinetic-pharmacodynamic relationship for pain drugs in rats and humans. *Neuropharmacology* **2008,** 54 (5), 767-77.
306. Tarr, B. D., Yalkowsky, S.H., Enhanced intestinal absorption of cyclosporin in rats through the reduction of emulsion droplet size. *Pharmaceutical Research* **1989,** 6 (1), 40-43.
307. Friedman, M. I.,Ramirez, I.,Tordoff, M. G., Gastric emptying of ingested fat emulsion in rats: Implications for studies of fat-induced satiety. *American Journal of Physiology - Regulatory Integrative and Comparative Physiology* **1996,** 270 (3 39-3), R688-R692.
308. Grubbs, C. J.,Lubet, R. A.,Koki, A. T.,Leahy, K. M.,Masferrer, J. L.,Steele, V. E.,Kelloff, G. J.,Hill, D. L.,Seibert, K., Celecoxib inhibits N-butyl-N-(4-hydroxybutyl)-nitrosamine-induced urinary bladder cancers in male B6D2F1 mice and female Fischer-344 rats. *Cancer Research* **2000,** 60 (20), 5599-5602.
309. Levy, G.,Leonards, J. R.,Procknal, J. A., Development of in vitro dissolution tests which correlate quantitatively with dissolution rate-limited drug absorption in man. *Journal of Pharmaceutical Sciences* **1965,** 54 (12), 1719 - 1722.

310. Emami, J., In vitro - in vivo correlation: from theory to applications. *Journal of Pharmacy & Pharmaceutical Sciences* **2006,** 9 (2), 31-51.
311. US Food and Drug Administration. *Guidance for industry, extended release oral dosage forms: development, evaluation and application of an in vitro/in vivo correlation*. FDA Center for Drug Evaluation and Research (CDER), Rockville, MD, **1997**. Available at http://www.fda.gov/downloads/Drugs/GuidanceComplianceRegulatoryInformation/Guidances/UCM070246.pdf.
312. Chilukuri, D. M.,Sunkara, G.,Young, D., *Pharmaceutical product development: in vitro-in vivo correlation*. Informa Healthcare: New York, **2007**; Vol. 165.
313. United States Pharmacopoeia. *In vitro and in vivo evaluations of dosage forms*. 27th ed., Revision. Mack Publishing Co.: Easton, PA., **2004**.
314. Sunesen, V. H.,Pedersen, B. L.,Kristensen, H. G.,Müllertz, A., In vivo in vitro correlations for a poorly soluble drug, danazol, using the flow-through dissolution method with biorelevant dissolution media. *European Journal of Pharmaceutical Sciences* **2005,** 24 (4), 305-313.
315. Donato, E. M.,Martins, L. A.,Fröehlich, P. E.,Bergold, A. M., Development and validation of dissolution test for lopinavir, a poorly water-soluble drug, in soft gel capsules, based on in vivo data. *Journal of Pharmaceutical and Biomedical Analysis* **2008,** 47 (3), 547-552.
316. Okumu, A.,DiMaso, M.,Löbenberg, R., Dynamic dissolution testing to establish in vitro/in vivo correlations for montelukast sodium, a poorly soluble drug. *Pharmaceutical Research* **2008,** 25 (12), 2778-2785.
317. Jantratid, E.,Janssen, N.,Chokshi, H.,Tang, K.,Dressman, J. B., Designing biorelevant dissolution tests for lipid formulations: case example – lipid suspension of RZ-50. *European Journal of Pharmaceutics and Biopharmaceutics* **2008,** 69 (2), 776-785.
318. Lue, B.-M.,Nielsen, F. S.,Magnussen, T.,Schou, H. M.,Kristensen, K.,Jacobsen, L. O.,Müllertz, A., Using biorelevant dissolution to obtain IVIVC of solid dosage forms containing a poorly-soluble model compound. *European Journal of Pharmaceutics and Biopharmaceutics* **2008,** 69 (2), 648-657.
319. Kovačević, I.,Parojčić, J.,Homšek, I.,Tubić-Grozdanis, M.,Langguth, P., Justification of biowaiver for carbamazepine, a low soluble high permeable compound, in solid dosage forms based on IVIVC and gastrointestinal simulation. *Molecular Pharmaceutics* **2009,** 6 (1), 40-47.
320. Vogt, M.,Vertzoni, M.,Kunath, K.,Reppas, C.,Dressman, J. B., Cogrinding enhances the oral bioavailability of EMD 57033, a poorly water soluble drug, in dogs. *European Journal of Pharmaceutics and Biopharmaceutics* **2008,** 68 (2), 338-345.
321. Fatouros, D. G.,Nielsen, F. S.,Douroumis, D.,Hadjileontiadis, L. J.,Mullertz, A., In vitro-in vivo correlations of self-emulsifying drug delivery systems combining the dynamic lipolysis model and neuro-fuzzy networks. *European Journal of Pharmaceutics and Biopharmaceutics* **2008,** 69 (3), 887-898.
322. Schamp, K.,Schreder, S.-A.,Dressman, J., Development of an in vitro/in vivo correlation for lipid formulations of EMD 50733, a poorly soluble, lipophilic drug substance. *European Journal of Pharmaceutics and Biopharmaceutics* **2006,** 62 (3), 227-234.
323. Domínguez, A. R.,Medina, R. L.,Hurtado, M. P., Bioequivalence study of paracetamol tablets: In vitro-in vivo correlation. *Drug Development and Industrial Pharmacy* **2000,** 26 (8), 821-828.
324. Kortejärvi, H.,Mikkola, J.,Bäckman, M.,Antila, S.,Marvola, M., Development of level A, B and C in vitro - In vivo correlations for modified-release levosimendan capsules. *International Journal of Pharmaceutics* **2002,** 241 (1), 87-95.
325. Löbenberg, R.,Krämer, J.,Shah, V. P.,Amidon, G. L.,Dressman, J. B., Dissolution testing as a prognostic tool for oral drug absorption: dissolution behavior of glibenclamide. *Pharmaceutical Research* **2000,** 17 (4), 439-444.
326. Wagner, J. G.,Nelson, E., Percent absorbed time plots derived from blood level and/or urinary excretion data. *Journal of Pharmaceutical Sciences* **1963,** 52 (6), 610 - 611.
327. Simovic, S.,Heard, P.,Hui, H.,Song, Y.,Peddie, F.,Davey, A. K.,Lewis, A.,Rades, T.,Prestidge, C. A., Dry hybrid lipid-silica microcapsules engineered from submicron lipid

droplets and nanoparticles as a novel delivery system for poorly soluble drugs. *Molecular Pharmaceutics* **2009,** 6 (3), 861-872.

328. Simovic, S.,Hui, H.,Song, Y.,Davey, A. K.,Rades, T.,Prestidge, C. A., An oral delivery system for indomethicin engineered from cationic lipid emulsions and silica nanoparticles. *Journal of Controlled Release* **2010,** 143 (3), 367-373.

Appendix I



PK parameters	I.V. CEL dose	
	5 mg/kg	10 mg/kg
$AUC_{0\rightarrow\infty}$ (min.µg/ml)	268 ± 30	1854 ± 293
$t_{1/2}$ (min)	188 ± 27	192 ± 77
CL (ml/min)	6.8 ± 0.7	1.9 ± 0.8
V_{ss} (ml)	1219 ± 147	463 ± 79

Figure: The mean plasma concentration–time curves and the summarised pharmacokinetic data (inset) of celecoxib in male S.D. rats following a single intravenous dose of celecoxib in PEG 400/saline (2:1 v/v) solution (mean ± S.E.M., n = 5).