STUDIES ON NON-AQUEOUS EMULSIONS

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This thesis describes research conducted in the School of Pharmacy, University of London between October 2002 and October 2006 under the supervision of Prof. Alexander T. Florence. I certify that the research described is original and that any parts of the work that have been conducted by collaboration are clearly indicated. I also certify that I have written all the text herein and have clearly indicated by suitable citation any part of this dissertation that has already appeared in publication.

Signature  
Orawan Suithimsathregon

Date  
22 Dec 2006
Dedicated to my beloved Kun' Mae
I never think of the future - it comes soon enough.

Albert Einstein
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ABSTRACT

This thesis explores a neglected field in pharmacy, that of non-aqueous (o/o) emulsions and of multiple emulsions of the form o/o/w. Non-aqueous, oil-in-oil, or anhydrous emulsions composed of 'oil' droplets dispersed in another immiscible oil phase were formulated using different non-aqueous solvent and natural oils. Various types of surfactants were used to stabilise such systems including non-ionic, poloxamer, and silicone surfactants. Among all formulations studied, the most stable emulsions could be formed with castor oil as the disperse phase and dimethicone or cyclopentasiloxane as the continuous phase. Only silicone surfactants which were miscible in silicone oil stabilised such systems. Emulsions formulated using these surfactants were found to be stable against phase separation and exhibited least globule growth over 168 h.

An oil-in-oil-in-water (o/o/w) emulsion was formulated using a castor oil-in-silicone oil (co/so) emulsion as the disperse phase and nonylphenol ethoxy 7 as hydrophilic surfactant. Slow release patterns of $^3$H-dehydroepiandrosterone and $^3$H-dexamethasone solubilised in the disperse castor oil phase into an aqueous dialyzing medium were observed up to 48 h in in vitro. Intramuscular administration of $^3$H-dexamethasone in a co/so non-aqueous emulsion in rats confirmed slower absorption than from co/w emulsions with a longer $T_{\text{max}}$ and lower $C_{\text{max}}$, together with an increase of MRT and $t_{1/2}$. In addition, slow clearance of the drug from the muscle injection site was observed. Non-aqueous emulsions may be considered as depot formulations for sustained release delivery, but further studies of these formulations are necessary for optimal effect.

Magnetic nanoparticles were synthesized and characterised using TEM and powder X-ray crystallography. When the disperse phase is loaded with 10 nm magnetic nanoparticles, magnetic non-aqueous emulsions could be formulated. The use of an external magnetic field to immobilize or control the movement of magnetite carrying globules was also studied. Finally, flow behaviour non-aqueous emulsions in the microchannels of microfluidic devices was investigated. The phenomenological results of flow behaviour such as chain-like aggregation, oscillatory movement of droplets as well as the breakup of droplets were observed. These findings may provide a better understanding in the stabilisation and production of emulsion systems.
### ABBREVIATIONS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AUC$</td>
<td>Area under plasma concentration-time curve</td>
</tr>
<tr>
<td>$AUC_{\infty}$</td>
<td>Area under plasma concentration-time curve from time zero to infinity</td>
</tr>
<tr>
<td>BB</td>
<td>Benzyl benzoate</td>
</tr>
<tr>
<td>$CL$</td>
<td>Clearance</td>
</tr>
<tr>
<td>$C_{\max}$</td>
<td>Maximum plasma concentration</td>
</tr>
<tr>
<td>CP</td>
<td>Cloud point</td>
</tr>
<tr>
<td>cmc</td>
<td>Critical micellar concentration</td>
</tr>
<tr>
<td>$co/so$</td>
<td>Castor oil/silicone oil</td>
</tr>
<tr>
<td>$^3$H-DEXA</td>
<td>$^3$H-dexamethasone</td>
</tr>
<tr>
<td>$^3$H-DHEA</td>
<td>$^3$H-dehydroepiandrostosterone</td>
</tr>
<tr>
<td>DLVO</td>
<td>Deryagin, Landau, Verwey, and Overbeek</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-dimethylformamide</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethylsulphoxide</td>
</tr>
<tr>
<td>HLB</td>
<td>Hydrophile - lipophile balance</td>
</tr>
<tr>
<td>IPM</td>
<td>Isopropyl myristate</td>
</tr>
<tr>
<td>$k_e$</td>
<td>Elimination rate constant</td>
</tr>
<tr>
<td>$MRT$</td>
<td>Mean residence time</td>
</tr>
<tr>
<td>NMF</td>
<td>N-methylformamide</td>
</tr>
<tr>
<td>PCS</td>
<td>Photon correlation spectroscopy</td>
</tr>
<tr>
<td>PDMS</td>
<td>Polydimethylsiloxane</td>
</tr>
<tr>
<td>PEG</td>
<td>Polyethylene glycol</td>
</tr>
<tr>
<td>PEO</td>
<td>Polyethylene oxide</td>
</tr>
<tr>
<td>PG</td>
<td>Propylene glycol</td>
</tr>
<tr>
<td>PPO</td>
<td>Polypropylene oxide</td>
</tr>
<tr>
<td>$t_{1/2}$</td>
<td>Elimination half-life</td>
</tr>
<tr>
<td>VSM</td>
<td>Vibrating sample magnetometer</td>
</tr>
</tbody>
</table>
# TABLE OF CONTENTS

**Acknowledgements** ................................................................................................................... 5  
**Abstract** ...................................................................................................................................... 6  
**Abbreviations** ............................................................................................................................. 7  
**Table of contents** ....................................................................................................................... 8  
**List of figures** ............................................................................................................................. 13  
**List of tables** ............................................................................................................................. 19  

## Chapter I

**INTRODUCTION** ....................................................................................................................... 21  

1.1 Description of emulsions ......................................................................................................... 21  
1.2 Non-aqueous emulsions ......................................................................................................... 22  
1.3 Emulsion stability ................................................................................................................... 23  
1.3.1 Basic concept ....................................................................................................................... 23  
1.3.2 DLVO and steric stabilisation theory .................................................................................... 26  
1.3.3 Stability of o/w and w/o emulsions .................................................................................... 28  
1.3.4 Stabilisation of non-aqueous systems .................................................................................. 31  
1.4 Emulsion formation ................................................................................................................. 33  
1.5 Emulsion applications ............................................................................................................. 33  
1.5.1 Emulsions as drug delivery system ..................................................................................... 33  
1.5.2.1 Conventional emulsions ............................................................................................... 34  
1.5.2.2 Multiple emulsions ........................................................................................................ 36  
1.5.2.3 Microemulsions .............................................................................................................. 37  
1.5.2.4 Magnetic emulsions ........................................................................................................ 37  
1.5.2.5 Non-aqueous emulsions ............................................................................................... 37  
1.5.2 Non-pharmaceutical applications ......................................................................................... 38  
1.6 Emulsification techniques ........................................................................................................ 39  
1.6.1 Membrane emulsification ................................................................................................... 40  
1.6.2 Microchannel emulsification ............................................................................................... 43  
1.7 Microfluidics ........................................................................................................................... 44  
1.7.1 Background ........................................................................................................................ 44  
1.7.2 Applications ......................................................................................................................... 46  
1.8 Aims and outline of the work in thesis ..................................................................................... 50  

## Chapter II

**FORMULATION OF NON-AQUEOUS EMULSIONS** ..................................................................... 51  

2.1 Introduction .............................................................................................................................. 51  
2.2 Materials and methods ........................................................................................................... 52
Chapter III

FORMULATION AND PHYSICOCHEMICAL PROPERTIES OF CASTOR OIL AND SILICONE OIL EMULSIONS

3.1 Introduction .................................................. 75
3.2 Materials and methods ...................................... 76
  3.2.1 Materials .................................................. 76
  3.2.2 Determination surfactant miscibility in liquid phases ...... 76
  3.2.3 Preparation of emulsions ............................... 76
  3.2.4 Preparation of multiple emulsions ......................... 77
  3.2.5 Assessment of emulsion stability ......................... 77
    3.2.5.1 Physical stability of coarse emulsions ................. 77
    3.2.5.2 Determination of particle size and size distribution ... 78
  3.2.6 Determination of interfacial tension .................... 78
  3.2.7 Determination of viscosity ............................. 79
3.3 Results and discussion ....................................... 80
  3.3.1 Determination of surfactant miscibility in liquid phases ...... 80
  3.3.2 Stability assessment for selection of suitable surfactants ...... 82
  3.3.3 Determination of interfacial tension .................... 87
  3.3.4 Determination of particle size and size distribution .......... 91
    3.3.4.1 Particle size ........................................... 91
    3.3.4.2 Particle size distributions ........................... 93
  3.3.5 Rheological behaviour of emulsions ..................... 95
  3.3.6 Multiple emulsions ..................................... 99
    3.3.6.1 Oil-in-oil-in-water emulsions .......................... 99
    3.3.6.2 oil-in-oil-in-oil anhydrous multiple emulsions .......... 100
3.4 Summary ..................................................... 101
Chapter IV

IN VITRO STUDIES: RELEASE OF TWO MODEL DRUGS FROM NON-AQUEOUS EMULSIONS ............................................................................................................. 102

4.1 Introduction ............................................................................................................ 102
4.2 Materials and methods ....................................................................................... 103
  4.2.1 Materials ....................................................................................................... 103
  4.2.2 The assessment of the particle size in the presence of water .................. 103
  4.2.3 In vitro release ............................................................................................... 104
  4.2.4 Determination of the partition coefficient of drug .................................... 104
4.3 Results and discussion .......................................................................................... 105
  4.3.1 The assessment of particle size in the presence of aqueous phase 105
  4.3.2 Measurement of release rate of entrapped drug ........................................ 106
    4.3.2.1 The release profile of simple o/o emulsions .......................................... 106
    4.3.2.2 The release profile from multiple emulsions ......................................... 111
  4.3.3 Determination of partition coefficient ........................................................ 113
  4.3.4 Release profile mechanism considerations ................................................ 114
4.4 Summary ................................................................................................................. 119

Chapter V

IN VIVO STUDIES: INTRAMUSCULAR ABSORPTION AND BIODISTRIBUTION OF DRUG FROM NON-AQUEOUS EMULSIONS .......... 120

5.1 Introduction ............................................................................................................ 120
5.2 Materials and methods ......................................................................................... 122
  5.2.1 Materials ....................................................................................................... 122
  5.2.2 Preparation of $^3$H-dexamethasone emulsions ........................................ 122
  5.2.3 Animals ......................................................................................................... 122
  5.2.4 Intramuscular injection ................................................................................ 123
  5.2.5 Blood and organ sampling ........................................................................... 123
  5.2.6 Preparation for liquid scintillation counting .............................................. 124
5.3 Results and discussion ........................................................................................... 125
  5.3.1 The $^3$H-dexamethasone plasma concentration ........................................ 125
  5.3.2 The absorption of $^3$H-dexamethasone from the muscle injection site ... 128
  5.3.3 The tissue distribution of $^3$H-dexamethasone from emulsions .......... 133
5.4 Summary ................................................................................................................. 141

Chapter VI

MAGNETIC NON-AQUEOUS EMULSIONS ............................................................................................................. 142

6.1 Introduction ............................................................................................................ 142
6.2 Materials and methods ....................................................................................... 146
Chapter VII

FLOW BEHAVIOUR OF EMULSION DROPLETS IN MICROFLUIDIC DEVICES

7.1 Introduction ................................................................. 171
7.2 Materials and methods .................................................. 172
  7.2.1 Materials .............................................................. 172
  7.2.2 Microfluidic devices .................................................. 173
  7.2.3 Preparation of emulsions .......................................... 174
  7.2.4 Image capturing ...................................................... 174
  7.2.5 Experimental set up ............................................... 174
7.3 Results and discussion .................................................. 176
  7.3.1 Chaining linear or quasi-linear association of emulsion droplets 176
  7.3.2 Oscillatory movement of emulsion droplets ................. 183
  7.3.3 Formation of droplets .............................................. 188
  7.3.4 Break-up of emulsion droplet ................................... 193
    7.3.4.1 Continuous phase flow induced break-up ............... 194
    7.3.4.2 Disperse phase induced droplet break-up ............... 196
7.4 Summary ........................................................................ 204
Chapter VIII

CONCLUSIONS AND FUTURE PERSPECTIVES.................................................... 205

8.1 Conclusions................................................................................................. 205
8.2 Future perspectives..................................................................................... 207

REFERENCES.....................................................................................................209
LIST OF FIGURES

Chapter I

Figure 1.1 Photomicrograph of a non-aqueous emulsion .................................................... 22
Figure 1.2 A schematic diagram representing the emulsion instability ................................. 25
Figure 1.3 The model represents the steric stabilisation between two emulsion droplets. 27
Figure 1.4 A schematic representing the interaction between two emulsion droplets ...... 29
Figure 1.5 Schematic representing of the membrane emulsification method ....................... 40
Figure 1.6 A schematic diagram showing the membrane emulsification method .......... 41
Figure 1.7 Examples of products produced by membrane emulsification method .......... 42
Figure 1.8 A schematic diagram of microchannel emulsification method ......................... 43
Figure 1.9 A comparison of different scales representing size dependent diffusion time 46
Figure 1.10 Schematic representing the process of forming droplets for screening purposes in protein crystallization ................................................................. 47
Figure 1.11 Schematic representing tissue organization, culture and analysis in microsystems ........................................................................................................... 47
Figure 1.12 Schematic diagram of the formation of w/o/w emulsions in a microfluidic cell ................................................................................................................ 48

Chapter II

Figure 2.1 The effect of the HLB value of the surfactant mixture on the stability of BB/W emulsions ............................................................................................................ 63
Figure 2.2 Photomicrographs of benzyl benzoate-in-water/propylene glycol emulsions taken 2h after preparation ................................................................. 66
Figure 2.3 Photomicrographs of benzyl benzoate-in-water/propylene glycol emulsions taken 3 days after preparation ................................................................. 67
Figure 2.4 The effect of propylene glycol on the cloud point of Triton X-100 and Triton X-114 solutions .............................................................................................. 69
Figure 2.5 The effect of propylene glycol concentration on the cloud point of aqueous Triton X-100 solutions .................................................................................... 71
Figure 2.6 An extrapolation of the effect of propylene glycol concentration on the cloud point of Triton X-100 aqueous solutions ............................................................ 72

Chapter III

Figure 3.1 Picture of the shape of a pendant drop of castor oil in dimethicone .............. 79
Figure 3.2 Physical appearance of non-aqueous showing the stability ............................ 82
Figure 3.3 A schematic illustration of the assumed orientation of a silicone surfactant copolymer at the castor oil and silicone oil interface .............................................. 86
Figure 3.4 The interfacial tension of castor oil in two silicone oils with three silicone surfactants: DC 3225C, DC 5225C and DC 9011 ......................................................... 88
Figure 3.5 The interfacial tension of castor oil-silicone oil systems plotted as a function of the logarithm of the silicone surfactant concentration (DC 190) ............... 89
Figure 3.6 Particle size distributions of castor oil-in-silicone oil emulsions stabilised by three silicone surfactants ................................................................................. 93
Figure 3.7 Particle size distributions of castor oil-in-dimethicone emulsions varying in the concentration (% w/v) ................................................................................. 94
Figure 3.8 Mean particle size of castor oil-in-dimethicone emulsions as a function of silicone surfactant concentration as well as different type of silicone surfactant ................................................................................. 95
Figure 3.9 A plot of log [apparent viscosity] versus shear rate for castor oil-in-dimethicone emulsions using three silicone surfactants ................................................. 97
Figure 3.10 Photomicrographs of castor oil-in-dimethicone emulsions formulated with three silicone surfactants: DC 3225C, DC 5225C and DC 9011 ..................... 98
Figure 3.11 Photomicrographs of o/o/w emulsions formulated with Tergitol NP-7 as the secondary emulsifier ......................................................................................... 99
Figure 3.12 Photomicrograph of dimethicone-in-castor oil-in-dimethicone o/o/o emulsions. .............................................................................................................. 100

Chapter IV

Figure 4.1 Comparison of the mean particle size of the castor oil/dimethicone and castor oil/cyclopentasiloxane emulsions with and without contact with water ...... 106
Figure 4.2 *In vitro* release of two lipophilic model drugs from the disperse phase of castor oil-in-dimethicone emulsions ........................................................................................................ 108

Figure 4.3 *In vitro* release of $^3$H-DEXA from castor oil-in-dimethicone emulsions and castor oil-in-water emulsions ........................................................................................ 111

Figure 4.4 *In vitro* release of $^3$H-DEXA from castor oil-in-dimethicone-in-water (o/o/w) emulsions .................................................................................................................. 112

Figure 4.5 Photomicrograph of castor oil-in-dimethicone-in-water (o/o/w) multiple emulsions ...................................................................................................................... 113

Figure 4.6 Schematic representation of drug release from castor oil-in-dimethicone emulsions ....................................................................................................................... 115

Figure 4.7 *In vitro* release profiles of two lipophilic model drugs from castor oil-in-dimethicone emulsions at phase volume of 0.25 and 0.50 ........................................ 118

Chapter V

Figure 5.1 A semilog plot of $^3$H-dexamethasone plasma concentration as a function of time following the intramuscular injection ...................................................... 125

Figure 5.2 A plot of dexamethasone plasma concentration as a function of time following intramuscular injection .......................................................................................... 127

Figure 5.3 Clearance of $^3$H-dexamethasone from the injection site after intramuscular injection of emulsions ............................................................................................ 128

Figure 5.4 Relationship between clearance and plasma level of $^3$H-dexamethasone. A) co/so emulsions and B) co/w emulsions. ................................................................. 129

Figure 5.5 A semilog plot of $^3$H-dexamethasone remaining at the injection site versus time. ......................................................................................................................... 130

Figure 5.6 Schematic diagram representing the sequence of drug absorption from emulsion system ................................................................................................. 132

Figure 5.7 The thigh of a rat autopsied at 48 h after a single injection of $^3$H-dexamethasone-co/so emulsion ......................................................................................... 133

Figure 5.8 Tissue distribution of $^3$H-dexamethasone administered intramuscularly as co/so emulsion to rats .................................................................................. 136
Figure 5.9 Tissue distribution of $^3$H-dexamethasone administered intramuscularly as co/w emulsion to rats ................................................................. 137

Figure 5.10 The percentage of $^3$H-dexamethasone distributed in A: the liver and B: the kidneys .................................................................................................................. 140

Chapter VI

Figure 6.1 A typical hysteresis loop for a magnetic material showing the magnetic parameters ........................................................................................................ 143

Figure 6.2 Schematics of magnetic nanoparticles coated with a polymeric layer which can be further conjugated with affinity molecules such as antibodies ............... 145

Figure 6.3 A Schematic showing the synthesis of magnetic iron oxide using method I. 147

Figure 6.4 Schematic showing the synthesis of magnetic iron oxide using method II. 149

Figure 6.5 This schematic diagram represents the vibrating sample magnetometer showing the vibration unit and the sample chamber .................. 152

Figure 6.6 The experimental imaging set up, showing an inverted microscope to which is attached a micromanipulator, placed on an anti-vibration table .......... 153

Figure 6.7 The electromagnet used to generate the magnetic field in the experiment .. 154

Figure 6.8 Schematic diagram representing the experimental set up to obtain the sequence of images .......................................................................................................... 155

Figure 6.9 TEM images of magnetic nanoparticles synthesized ................................................................. 156

Figure 6.10 The mean particle size distribution of the magnetic nanoparticles .......... 157

Figure 6.11 The X-ray diffraction patterns of synthesized magnetic nanoparticles ...... 158

Figure 6.12 The plot of magnetization and applied field of magnetic nanoparticles ..... 160

Figure 6.13 The photomicrograph of a sample of a magnetic non-aqueous emulsions. 161

Figure 6.14 Schematic representation of the alignment of magnetic particles inside the castor oil droplet under an external magnetic field ...................... 162

Figure 6.15 Photomicrograph of water-in-dimethicone emulsions represent an effect of the direction of an applied field ................................................................. 163

Figure 6.16 Photomicrographic sequences of emulsion droplets moving under an external magnetic field ......................................................................................... 164

Figure 6.17 Photomicrographic sequence of the movement of magnetic non-aqueous emulsions ........................................................................................................... 165
Figure 6.18 Image sequence (centimeter sized) of the movement of magnetic non-aqueous emulsions ................................................................. 167

Figure 6.19 A plot of the velocity of emulsion samples as a function of the distance from the iron core representing the exponential decay ........................................... 168

Figure 6.20 A plot of the velocity of emulsion samples as a function of magnetic field strength generated by electromagnet. .................................................. 169

Chapter VII

Figure 7.1 The illustration of the ‘Snake mixer’ slide .......................................................... 173

Figure 7.2 The diagram of the second microfluidic chip ..................................................... 174

Figure 7.3 Schematic diagram representing the experimental set up of the first method. .......................................................................................................................... 175

Figure 7.4 Schematic diagram showing the experimental set up of the second method. .......................................................................................................................... 176

Figure 7.5 Chaining behaviour of magnetic emulsion droplets under a magnetic field. 177

Figure 7.6 A) Photomicrograph of glass beads suspended in silicone oil under an external electric field B) Photomicrograph of a nematic multiple emulsion. ............. 178

Figure 7.7 Photomicrographic sequence of chain formation of droplets of castor oil in the continuous phase of dimethicone containing silicone surfactant ............. 179

Figure 7.8 Photomicrographic sequence showing chain formation of castor oil emulsion droplets in dimethicone containing silicone surfactant .......................... 180

Figure 7.9 Diagram representing the flow behaviour of fluids in a cylindrical tube. A) shows turbulent flow B) shows laminar flow ..................................................... 181

Figure 7.10 Diagram representing the shear stress exerted on castor oil droplets at each layer of the liquid with different viscosities .................................................. 182

Figure 7.11 Photomicrographic sequence of the oscillation of an emulsion droplet ......... 183

Figure 7.12 Diagram representing the oscillation movement of droplet in one cycle. The lower graph plotted between the velocity of droplet and time ......................... 184

Figure 7.13 Images of water droplets on 7.1 μm glass fibres, recorded at an air flow velocity of 1 m s⁻¹ from the left to right ......................................................... 185

Figure 7.14 Oscillation and chaining of propylene glycol-in-silicone oil emulsions ....... 186

17
Figure 7.15 Represent of the formation of castor oil droplets at the Y-junction of a microchannel driven by the flow of the continuous phase. .............................. 189
Figure 7.16 Photomicrographic sequence showing the serial stages of the formation of the primary and secondary (satellite) droplet. ................................................................. 190
Figure 7.17 An image sequence showing the formation of the third droplet with a length of 100 µm .................................................................................................................. 191
Figure 7.18 Disjoining pressure profiles of thin film .................................................. 192
Figure 7.19 The breakup of a droplet by the continuous phase flow at T-junction...... 194
Figure 7.20 Schematic of the flow of a droplet inside a channel towards a T-junction . 195
Figure 7.21 The breakup of a droplet by a disperse phase droplet at a T-junction....... 197
Figure 7.22 Different condition for breaking and non-breaking drops at T-junction.... 198
Figure 7.23 Breakup of a droplet by disperse phase flow at a T-junction..................... 199
Figure 7.24 Breakup of a droplet by continuous phase flow at a T-junction............... 200
Figure 7.25 A schematic diagram representing the break-up of one large drop into smaller droplets.................................................................................................................. 201
Figure 7.26 The breakup of droplet by the geometry of the microchannel at a T-junction.. ....................................................................................................................... 202
LIST OF TABLES

Chapter I

Table 1.1 Commercial formulations of emulsions based on Intralipids™ ......................... 35

Chapter II

Table 2.1 A list of conventional surfactants (hydrocarbon-based) explored in this work 54  
Table 2.2 List of block copolymer surfactants investigated in this work ....................... 55  
Table 2.3 List of silicone surfactants used in this work ................................................. 55  
Table 2.4 Apparent miscibility between two non-aqueous phases ................................. 59  
Table 2.5 The range of HLB values obtained by mixing surfactants in different proportions ................................................................. 63

Chapter III

Table 3.1 Miscibility of the surfactants in each oil phase............................................. 81  
Table 3.2 Stability of castor oil in silicone oil emulsions in the presence of a range of conventional and silicone surfactants ......................................................... 83  
Table 3.3 The interfacial tension of different silicone surfactants at the castor oil/dimethicone interface ................................................................. 90  
Table 3.4 The interfacial tension of different silicone surfactants at the castor oil/cyclopentasiloxane interface ................................................................. 90  
Table 3.5 Mean particle size of castor oil-in-dimethicone emulsions and castor oil-in-cyclopentasiloxane emulsions with DC 3225C ........................................ 91  
Table 3.6 Mean particle size of castor oil-in-dimethicone emulsions and castor oil-in-cyclopentasiloxane emulsions with DC 5225C ........................................ 92  
Table 3.7 Mean particle size of castor oil-in-dimethicone emulsions and castor oil-in-cyclopentasiloxane emulsions with DC 9011 ............................................. 92

Chapter IV

Table 4.1 The partition coefficient of $^3$H-DHEA and $^3$H-DEXA between two phases .. 113
Chapter V

Table 5.1 Plasma pharmacokinetic parameters following the intramuscular injection of rats with $^3$H-dexamethasone in co/o emulsions and co/w emulsions.......... 126

Table 5.2 Tissue distribution of $^3$H-dexamethasone administered as co/o emulsions to rats........................................................................................................................134

Table 5.3 Tissue distribution of $^3$H-dexamethasone administered as co/w emulsions to rats........................................................................................................................135

Chapter VI

Table 6.1 The mean particle sizes of magnetic nanoparticles. ........................................... 156

Table 6.2 X-ray diffraction data expressed as the position of peak (2θ) and d-spacing (Å) for magnetic nanoparticles Fe$_3$O$_4$. ................................................................. 159

Chapter VII

Table 7.1 The flow behaviour of emulsions in microfluidic devices .................... 187
Chapter I

INTRODUCTION

This thesis addresses aspects of non-aqueous dispersions of various forms: oil-in-oil emulsions and multiple emulsions, their formulation and stabilisation and their behaviour in flow conditions as well as their potential use in drug delivery. This introduction gives the background and context of the work.

A colloidal dispersion can be defined as a heterogeneous system that is mainly composed of two components, a disperse and continuous phase. Depending on the nature of the disperse phase e.g. a gas, liquid, or solid with a liquid as the continuous phase the system is termed respectively as a foam, an emulsion, or a suspension. The present work is of the interest as emulsions as dosage forms have been studied and used for many decades. Even though there has been emergence of many new types of colloidal systems such as liposomes, microparticles and nanoparticles, emulsions still continue to have a place in pharmacy as well as having a wide range of industrial applications including advanced materials processing, waste treatment, enhanced oil recovery, food processing and in cosmetics.

1.1 Description of emulsions
An emulsion is composed of two immiscible liquids (traditionally oil and water), one of which is dispersed as droplets distributed throughout the other. The types of emulsion are
classified by the combination of all the phases, for example water-in-oil (w/o), oil-in-water (o/w), multiple or double (w/o/w or o/w/o) emulsions. These are all intrinsically thermodynamically unstable. Microemulsions on the other hand are thermodynamically stable with a very small droplet size (Becher, 2001; Florence and Attwood, 2006). In this thesis, a neglected type of emulsions has been investigated, “non-aqueous”, “oil-in-oil”, or “anhydrous” emulsions. These emulsions have attracted less attention than conventional systems but are of the interest here as they can be used as water free systems. Their potential and properties will be addressed in this thesis.

1.2 Non-aqueous emulsions

In majority of papers on emulsions published over the last century, the two immiscible components have been a water phase and an oil phase. However, without water or an aqueous phase, emulsions can be formulated. Such systems, comprise oil droplets dispersed in another immiscible oil phase. Photomicrographs of such systems (Fig 1.1) are unremarkable, but the systems themselves provide considerable challenge in design and formulation. In Figure 1.1 there is evidence of the linear or quasi-linear formation of the droplets (“chaining”) a topic to which we will return later in the thesis.

![Figure 1.1 Photomicrograph of a non-aqueous emulsion composed of castor oil-in-dimethicone stabilised with a silicone surfactant (DC 3225C: cyclomethicone and PEG/PPG-18/18 Dimethicone) 5 % (Bar = 10 μm).](image-url)
There are relatively few publications on the subject of non-aqueous emulsions. One of the earliest was that of McMahon et al., (1963) who found that certain ionic surfactants could stabilise the non-aqueous system of olive oil and glycerine. Instead of ionic emulsifying agents, Petersen et al. (1964) investigated the same system using as emulsifiers non-ionic surfactants, which are less toxic and have wider pharmaceutical applicability. The physicochemical properties such as droplet size distribution and the rheological data were also studied in the selected olive oil-in-glycerine emulsions stabilised with anionic amine surfactants and their admixtures (Hamill et al., 1965; Hamill and Petersen, 1966a; 1966b). Aging effects and the influence of temperature change on the droplet size and viscosity were also evaluated with a similar system of glycerine-in-mineral oil emulsions, varying the ionic surfactant used (Reichmann and Petersen, 1973).

Some authors have used polar organic liquids such as acetonitrile, dimethylsulfoxide (DMSO), N,N-dimethylformamide (DMF), formamide and N-methylformamide (NMF) to formulate non-aqueous emulsions with a non-polar organic liquid (e.g. alkanes) using conventional non-ionic or polymeric surfactants as a surface active agents (Periard et al., 1970; Cameron and Sherrington, 1996; Imhof and Pine, 1997b). The use of such polar liquids as the continuous phase allows a rather simpler approach to selection of an emulsifying agent to stabilise the system. For example, a conventional non-ionic surfactant with a typical non-polar hydrocarbon chain and a polar polyoxyethylene group which will be soluble in the continuous phase in a non-aqueous emulsion may behave in an anticipated manner as in an o/w type in terms of stabilisation.

1.3 Emulsion stability
1.3.1 Basic concept
A stable emulsion is considered to be one in which the dispersed droplets maintain their initial character and remain uniformly distributed throughout the continuous phase over the desired shelf life (Aulton, 2002). It is well known that the emulsions are inherently unstable systems and eventually revert to the original state of two separate liquid phases. The instability of emulsions could be explained by the fact that in the case of a disperse phase (as in droplets) there is an increase in the total interfacial area, which causes an
increase in the interfacial free energy of the system. Consequently an unstable system will result, even in the presence of emulsifiers, which can, however, prolong the lifetime of emulsion. The thermodynamic instability results from the emulsion attempting to reduce the surface area of the dispersed phase through droplet coalescence. There are many approaches used to address the stabilisation issue of emulsion systems as described below.

Changes in the stability could occur through either physical and/or chemical changes in the emulsion system. The chemical instabilities could be exemplified by oxidation and hydrolysis of emulsion components and may accompany with the change in emulsion pH and rancidity of the oil. Whereas the physical instabilities normally result from changes in the dispersed droplet size via several distinct processes which are reversible, such as creaming, sedimentation and flocculation and others which are irreversible such as coalescence and Ostwald ripening (Eccleston, 2002) as seen in Figure 1.2.

One approach which can be applied to minimize the physical instability is to reduce the rate of creaming or sedimentation using the concepts of the Stokes’ law which states that the rate of creaming or sedimentation \( (v) \) is inversely proportional to the viscosity of the continuous phase and could be expressed as

\[
  v = \frac{2a^3 g (\sigma - \rho)}{9\eta}
\]

(1.1)

where \( g \) is the acceleration due to gravity, \( a \) is the droplet radius, \( \sigma \) is the density of the sphere (i.e. droplet of the dispersed phase), \( \rho \) is the density of the liquid (i.e. the continuous phase) and \( \eta \) is the viscosity of the continuous phase (Aulton, 2002). A reduction of the creaming or sedimentation rate is achieved by reducing the droplet size, increasing the viscosity of the continuous phase and decreasing the phase density difference. Even though the frequency of the particle collisions can be minimized, collisions will occur due to the random movement of the particles.
The principal approach to attain a stable system is the addition of third component, surface active agents (surfactants or emulsifiers). The adsorption of surfactant molecules can ensure the stability of the emulsion against coalescence by reducing the surface free energy at the boundary between the two phases, forming a physical barrier as well as modifying the interaction forces between two neighbouring particles.

Figure 1.2 A schematic diagram which represents the emulsion instability by various processes. Creaming or sedimentation and flocculation are reversible processes and can lead to droplet coalescence and finally phase separation. An increase in the emulsion droplet size may also occur through Ostwald ripening, which involves the diffusion of material from smaller droplets to larger droplets. Adapted from Taylor, 1998.
1.3.2 DLVO and steric stabilisation theory

One of the early theories to explain emulsion stability is based on the electrostatic repulsion and van der Waals' forces of attraction derived by Deryagin, Landau, Verwey, and Overbeek (DLVO) (Verwey and Overbeek, 1948). Known as the DLVO theory of stabilisation, it describes the total potential energy of interaction ($V_T$) as a combination of the electrostatic repulsive energy ($V_R$) and attractive potential energy ($V_A$), namely

$$V_T = V_A + V_R$$  \hspace{1cm} (1.2)

Apart from electrostatic and dispersive interactions, another force involved is the steric repulsion occurring for example when emulsions are stabilised by adsorbed non-ionic polymeric surfactants. Steric interactions occur between the adsorbed molecules caused by the close approach of two emulsion particles with adsorbed polymer layers, hence an additional term has to be included in the potential energy of interaction to account for this effect-called steric stabilisation ($V_S$). Therefore the total potential energy of interaction ($V_T$) in the absence of electrostatic repulsion, is given by

$$V_T = V_A + V_S$$  \hspace{1cm} (1.3)

and in the presence of electrostatic repulsion is given by

$$V_T = V_A + V_R + V_S$$  \hspace{1cm} (1.4)

There are at least three parameters to assess quantitatively for the steric effect which are i) chain length of polymer, ii) interaction between the chains and continuous phase and iii) the amount of chains per unit area of the interface. The steric interactions between two approaching particles occur when the distance between two emulsion droplets is within twice the length of the polymer stabilising chains ($H = 2\delta$) (Fig 1.3) (Florence and Attwood, 2006). For two emulsions with the droplets of same size, the effective thickness of polymer ($\delta_0$) on each droplet can be determined by the following equation (Morrison and Ross, 2002),
where $a$ is the radius of emulsion particle, $A_{121}$ is the Hamaker constant of particles (material 1) dispersed in a medium (material 2) and $k$ is the Boltzmann constant. However, some factors must be taken into account to apply this concept for example, a change in conformation or solvation of the polymer chain during interaction and the specific interaction between them, as well as desorption of the polymeric species (March and Napper, 1977). They found that steric stabilisation could be achieved only when the stabilising polymer chains were firmly attached to the emulsion droplet.

\[ \delta_0 = \frac{a A_{121}}{24 k T} \]  

\[ (1.5) \]

\[ \text{Figure 1.3} \] The model represents the steric stabilisation between two emulsion droplets to which the non-ionic polymeric surfactants are adsorbed. Both the droplets of radius ($a$) with adsorbed layers of thickness ($\delta$) move towards each other reaching the point where the distance between two droplets ($H$) is within $2\delta$ (twice the thickness of polymer chains), at this point the steric stabilising forces come into play.

Steric repulsion is explained by the free energy changes which take place when two polymer-surrounded particles interact. According to the Gibbs’-Helmholtz equation a positive value of $\Delta G$ is required for the dispersion stability, a negative value resulting in particle aggregation. The equation is written in the form,

\[ \Delta G = \Delta H - T \Delta S \]  

\[ (1.6) \]

From this equation, an increase in the positive value of $\Delta G$ can be discussed in different ways. Entropic stabilisation occur when both $\Delta H$ and $\Delta S$ are negative but $T \Delta S > \Delta H$. 

27
Negative entropy changes occur through interpenetration and compression of the polymer chains between two nearby particles as these chains become more ordered. Stabilisation by these effects generally occurs in non-aqueous dispersions. Another way leading to a positive $\Delta G$ is if $\Delta H$ and $\Delta S$ are both positive but $\Delta H > \Delta S$. Here entropy aids aggregation, whereas enthalpy aids for stabilisation which is termed as enthalpic stabilisation. This latter effect is common with aqueous dispersions, predominantly where polymer consists of polyoxyethylene chains. The ether oxygen groups of PEO chain contribute to H-bonding with water molecules. When the interpenetration and compression of PEO chain occur, there is an increased probability of contact between PEO group resulting in some of the bound water molecules being released, leading to a positive enthalpy change (Aulton, 2002; Florence and Attwood, 2006).

1.3.3 Stability of o/w and w/o emulsions

Both the DLVO and steric stabilisation concepts can be used to explain at least semi-quantitatively the stability of o/w emulsions. Ionic surfactants protect droplet coalescence by increase/decrease zeta potential leading to an increase in $V_R$ whereas a hydrating layer which is created by adsorbed non-ionic surfactants on oil droplets help to maintain stability by increasing $V_S$. In the case of w/o emulsions, only steric repulsive forces play a dominant role for their stabilisation due to the low dielectric constant of the oil phase. This is supported by several papers, for example Albers and Overbeek (1959) who stated that there was no correlation between the zeta potential and coalescence of w/o emulsions but that good stabilisation occurred due to the formation of a thick film of stabilizing agent at the interface. Thompson et al. (1985) found that the presence of waxes and associated solids in crude oil could enhance the stability of w/o emulsions and by removing these solids, stability was decreased considerably. Similar results were reported by Mousa and van de Ven (1991) that the emulsions were more stable in the presence of solids (e.g. gold particles) at the water/oil interface. Thus the strength of the stabilizing layer/film adsorbed on the water droplet plays an important role in the stabilisation against coalescence of w/o emulsions. The strength of this layer/film has also been reported to be more important than the effect of lowering the interfacial tension to promote long term stability (Ford and Furmidge, 1966; Førde dal et al., 1996).
Several studies have been published on the film thickness and the surface rheological properties of the adsorption layer and have showed that these factors can be correlated with emulsion stability against coalescence (Sonntag et al., 1982; Fang and Dalgleish, 1993; Dickinson, 1999). In the case of a film, when the adsorbed layers of the two opposing surfaces come to overlap, this give rise to an osmotic pressure which induces a repulsive force to prevent the two particles from coalescing. On the other hand, if the separation distance between the two particles is smaller than the size of stabilizing agent, an attractive force overcomes a repulsive force results in the coalescence (Fig 1.4). In another way, the coalescence phenomenon can be considered to occur from two main steps, namely film drainage and film rupture. The latter is regarded to be almost immediately. Thus, the coalescence is mainly preceded by the formation of thin liquid film which the behaviour of this film plays a crucial role in determining the stability of emulsions. Much effort has been focussed on understanding the film drainage process (Ivanov, 1988).

**Figure 1.4** A schematic representing the interaction between two emulsion droplets in the presence of a stabilizing agent (represented as small particles and these can be micelles, or macromolecules). These stabilizing molecules adsorb on the interface consequently the barrier or film is formed surrounding the droplets. When the separation distance (i.e. gap) between the two adjacent particles is of the order of several sizes of small particles, the two droplets are still intact. If the two droplets approach until the adsorbing layer/film overlaps each other reaching a point that no small particles can fit in the space between then the coalescence of the two droplets takes place. This process can be considered as the film drainage. Adapted from Wasan et al., 2004.
Stenkamp and Berg (1997) stated that the efficiency of a steric stabiliser mainly depends on three factors, i) it must be well adsorbed to the surface of the emulsion droplets ii) its un-adsorbed portion must have good solvent compatibility and iii) provide sufficient thickness to prevent the approach of two droplets within a range of attractive van der Waals’ forces. These factors are in turn determined by the molecular properties of the stabilizing agent such as molecular weight and its solubility in the continuous phase. Perrin and Lafuma (1998) have reported that the stability of an emulsion was found to increase with increasing molecular weight and degree of grafting of hydrophobic branches of polymeric surfactant which is soluble in the continuous phase. However, for a polymeric surfactant which is soluble only in the disperse phase, a change in its molecular weight by modifying the backbone chain length had no noticeable effect on the emulsion stability unless an increase in the grafting branch to the backbone chain length increased the stability (Cárdenas-Valera and Bailey, 1995). Similar observations were also made by Lee (1999) who found that the emulsifying agent with a solubility higher in the oil phase than in the aqueous phase is the suitable stabiliser and hence can provide a stable w/o emulsion. These findings remind us of the general theory for formulating an emulsion which pointed out by Bancroft in 1913. The Bancroft’s rule states that the continuous phase of an emulsion will be the phase in which the surfactant is preferentially soluble. When the surfactants are more soluble in the water phase, it tends to generate o/w emulsions and the surfactants more soluble in the oil phase tend to generate w/o emulsions. Thus it was long known that the surfactant molecule greatly influences the emulsion type. This concept was later introduced by Griffin in 1949 as a quantitative way to select a suitable surfactant or to predict the emulsion type known as HLB (hydrophilic-lipophilic balance) number. A low HLB number indicates a more lipophilic surfactant which favours w/o emulsions whereas a high HLB number indicates a more hydrophilic surfactant which favours o/w emulsion formation.

However, the HLB number concept is only based on the molecular structure of the surfactant. In practical terms, the surfactant molecule will be aligned at the oil-water interface (i.e. in the case of aqueous emulsions). Consequently, the HLB number also depends on the nature of the oil phase and on the additives in both aqueous and oil
phases. Temperature must also be considered as it influences the interaction of surfactant at the interface. For example, a shift in the optimal HLB value to formulate o/w emulsions occurred in the presence of added salts (NaCl or NaI) in the water phase (Florence et al., 1975). Moreover, it has been pointed out that the mixtures of surfactants with high and low HLB number give more stable emulsions than using single surfactants. The shift in the optimum HLB value for the emulsion may occur using mixed surfactants due to the change in the interactions in the interfacial film (Florence and Attwood, 2006).

1.3.4 Stabilisation of non-aqueous systems
To date, published investigations of non-aqueous emulsions are few in number. It is still not clear what theory can be considered to allow understanding of these systems. Petersen et al. (1964) showed that the low molecular-weight non-ionic surfactants with HLB numbers ranging from 1.8 to 16.7 could produce stable olive oil-in-glycerine emulsions and those having HLB values of 4 and 11 formed glycerine-in-olive oil emulsions. They concluded that there is no correlation between the HLB value of surfactants and their capability to stabilise these non-aqueous emulsions which is reasonable in such relatively non-polar systems. These can be no hydrophilicity in non-aqueous formulations. However, HLB dependency was observed for the oil and formamide systems with an optimum HLB number ranging from 18 to 20 (Imhof and Pine, 1997b). They also studied other polar solvents such as acetonitrile, dimethylsulfoxide (DMSO), N,N-dimethylformamide (DMF) and N-methylformamide (NMF) and found that in general optimal stability could be obtained with formamide systems. The formamides are polar and hence could behave similar to water, thus their stabilisation occurs with surfactant possessing an equivalent range of HLB number as in the conventional oil-in-water emulsions shifted by the change in polarity. The similarity between formamide and water resides in their polarities, H-bonding properties and interactions with the surfactant molecules. Hence conventional non-ionic surfactants have a likelihood of being successful stabiliser in these systems. Oil-in-formamide emulsions have also been reported by Cameron and Sherrington (1996) and in our laboratory by Sakthivel et al. (2001) using stabilisers with low molecular-weight non-ionic surfactants with HLB values of 13.5 and 16.7. Both the groups suggested that hydrogen bonding of formamide appeared to play an essential role in determining the emulsion stability. Nevertheless,
Imhof and Pine (1997b) also reported that the polarities or H-bonds may not be the only factor responsible for the stabilisation of emulsions because DMSO can also form stable emulsions. Sakthivel et al. (2001) investigated a series of linear alkanes from C₈ to C₁₆ as the disperse phase of hydrocarbon-in-formamide emulsions and found that a minimum droplet size could be obtained with the C₁₀ to C₁₂ alkanes. As a result it may be indicated that the chemical nature of the two immiscible phases and their interaction with the surfactant used is important, but it is still unclear which molecular properties exactly determine the stabilisation.

The use of high molecular-weight polymeric surfactants to stabilise non-aqueous emulsions of alkanes and organic solvent was reported by Periard et al. (1970). They indicated the use of block or graft copolymers to stabilise non-aqueous emulsions of cyclohexane-in-acetonitrile and DMF-in-hexane. Cameron and Sherrington (1996) stated that PEO-PPO-PEO block copolymers could stabilise non-aqueous high internal phase emulsions of petroleum ether-in-DMSO or formamide. They also showed the higher performance of high-molecular weight polymeric surfactants as stabilizing agents over low-molecular weight surfactants. Similar findings were obtained by Imhof and Pine (1997b). Other polymeric surfactants, poly (dimethylsiloxane) based compounds, which were specially synthesized were found to be efficient stabiliser for emulsions of liquid crystals and silicone oils (Loudet et al., 2000). Such surfactant molecules must comprise of two segments, each having a preferential selectivity for the silicone oil or the liquid crystal phase.

These authors have also showed that the liquid crystal-in-silicone emulsions can be stabilised against Ostwald ripening by using an additional component in the liquid crystal phase. Briefly, Ostwald ripening is one of the important destabilisation mechanisms occurring when molecules of the disperse phase diffuse from small to large droplets due to the solubility difference between droplets of different sizes. The solubility of droplet material (or dispersed phase) in a continuous phase increases as the interfacial curvature increases i.e. droplet radius decreases. Consequently, smaller droplets shrink and finally disappear whereas the larger droplets grow with time resulting in an overall increase in the average diameter of the emulsion droplets. This mechanism may be delayed or
stopped by the addition to the disperse phase of a component which is insoluble in the continuous phase (Taylor, 1998). Even though Ostwald ripening is mainly found in aqueous w/o or o/w emulsions, this concept is adopted by some authors for destabilisation of non-aqueous emulsions (Imhof and Pine, 1997b; Loudet et al., 2000).

1.4 Emulsion formation
For non-aqueous emulsions, the choice of two immiscible oil phases and appropriate surfactants are clearly crucial to achieve stabilisation as well as the choice of suitable surfactants. In the case of an aqueous emulsion system the selection of surfactant used is based on the HLB concept discussed above. It is obvious that the absence of water molecules as a source of H-bonding forces may create a more complicated situation. There are two basic strategies which can be considered to achieve stable non-aqueous emulsions. One is to find suitable oil-immiscible polar liquids that can substantially replace water using existing non-ionic surfactants which can be selected based on the HLB concept. The other is to design surfactants having two incompatible blocks, each of which is selectively soluble in one of the immiscible liquids. Each approach has been reported as mentioned above, but still there is not enough information to guide the formulation of non-aqueous emulsions, or to understand fully the mechanism of stabilisation.

1.5 Emulsion applications
In this section various applications of emulsion technology are discussed.

1.5.1 Emulsions as drug delivery system
The development of drug delivery systems has increasingly received attention over the past decades. Much research has been carried out to find ideal systems for drug delivery which aim at optimal treatment including increase in the therapeutic index of the drug and reduction of side effects together with improving patient compliance. To achieve that, a delivery system has to provide site-specific action, for example for the delivery of toxic drugs to tumors, targeting to pathogen-infected cells and crossing the blood-brain barrier (Eniola and Hammer, 2003; Pardridge, 2002) and must localize at the targeted tissues to sustain the release of the drugs.
Colloidal carriers such as liposomes (Gregoriadis, 1979; Alving, 1983; Dass and Choong, 2006), a wide range of microspheres (DeSouza and DeSouza 1995) and nanoparticles (Soppimath et al., 2001; Müller and Keck, 2004), polymeric micelles (Jones and Leroux, 1999) and emulsions provide the possibility for the formulation of controlled and targeted drug delivery systems. The effectiveness of these systems is based on their physicochemical properties e.g. size affecting the fate of in vivo distribution (Senior, 1987; Harashima et al., 1994; Moghimi and Hunter, 2001) and the release of the drug. The modification of the surface properties can be beneficial to avoid macrophage uptake (i.e. by coating with polyethylene glycol (PEG) (Torchilin and Trubetskoy, 1995; Mosqueira et al., 2001).

1.5.2.1 Conventional emulsions

Emulsions for pharmaceutical purposes have been well recognized since the earliest times mainly for topical administration of oils and drugs. To date, there is less use of emulsion dosage forms for drug delivery compared to other forms such as tablets, capsules and solutions. The emerging variety of emulsion type, new classes of surfactant such as polymeric emulsifier and novel technologies for manufacturing may allow further investment in emulsion systems.

The resurgent interest in emulsions as drug delivery systems is due to its potential to act as a vehicle for lipophilic drugs, for drug targeting to specific sites as well as for sustained or controlled release. The more recent use of an emulsion as a drug delivery system originates from the successful use of Intralipid™ as nutritional fat emulsions and their biocompatibility and reduction of toxic effects. The emulsions as drug carriers then have been developed later to deliver lipophilic drugs or some prodrugs via parenteral route. For example, a delay of absorption of local anesthetics was observed from the emulsions as compared to solutions (Jeppson, 1975). Toxic reactions such as tissue irritation and thrombophlebitis of diazepam solution could be considerably reduced when diazepam is given in the form of o/w emulsions (Jeppson and Ljunberg, 1975; von Dardel et al., 1976; Kronevi and Ljunberg, 1983). Amphotericin B has also been incorporated
into emulsions which reduced toxicity and improved the drug's therapeutic index (Drutz, 1983). Table 1.1 shows some examples of commercial emulsions as drug delivery.

Table 1.1 Commercial formulations of emulsions based on Intralipids™.

<table>
<thead>
<tr>
<th>Preparation</th>
<th>Drug</th>
<th>Company</th>
<th>Indication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diprivan</td>
<td>Propofol</td>
<td>Zeneca Pharmaceuticals, UK</td>
<td>Anesthesia</td>
</tr>
<tr>
<td>Limethason</td>
<td>Dexamethasone palmitate</td>
<td>Green Cross, Japan</td>
<td>Rheumatoid arthritis</td>
</tr>
<tr>
<td>Lipo-NSAID</td>
<td>Flurbiprofen axetil</td>
<td>Kaken Pharmaceutical, Japan</td>
<td>Postoperative and cancer pain</td>
</tr>
<tr>
<td>Vitalipid</td>
<td>Vitamins A D E K</td>
<td>Kabi-Pharmacia, Sweden</td>
<td>Parenteral nutrition</td>
</tr>
</tbody>
</table>

Adopted from Buszello and Müller, 2000.

Emulsions may also be used as a local-specific delivery system to phagocytic cells of the reticulo-endothelial system (i.e. regional lymph nodes) or to a specific tumour tissue for releasing anticancer agent (Takahashi et al., 1976; Hashida et al., 1977a; Tanigawa et al., 1980; Yoshioka et al., 1982). Mizushima et al. (1982a, 1982b) found higher plasma concentrations and higher uptake by reticuloendothelial system (RES) of corticosteroids administered as emulsions compared to aqueous solutions.

Another advantage of emulsion system can be that of sustained/controlled release of drug incorporated into the disperse phase (Prankerd et al., 1988; Benita and Levy, 1993). With these benefits, the development of emulsions for drug delivery system has recently received a lot of attention e.g. for vaccine (Traquina et al., 1996; Martin, 1997) and gene delivery (Hara et al., 1997; Chung et al., 2001).
The type of emulsion employed is usually based on the physicochemical properties of the incorporated material (into the disperse phase) as well as the route of administration. In general, o/w emulsion is used as a carrier for hydrophobic drugs, whereas a w/o emulsion is used as a carrier for hydrophilic drugs. O/w emulsions are administered via intravenous route whereas w/o emulsions are designed for intramuscular or subcutaneous administration (where sustained release is required). A large number of papers have reported on the potential use of an aqueous o/w or w/o emulsion as drug carriers administered for transdermal (Friedman et al., 1995; Peltola et al., 2003), oral (Carrigan and Bates, 1973), parenteral (Singh and Ravin, 1986) routes. Several studies have investigated emulsion systems for ophthalmic application, (Muchtar et al., 1992; Naveh et al., 1994) and recently a newly formulated cyclosporine 0.05% ophthalmic emulsion is currently under clinical trial for dry-eye disease patients (Salib et al., 2006).

1.5.2.2 Multiple emulsions

Both w/o/w and o/w/o emulsions are also of interest as drug delivery systems. They provide advantages over w/o and o/w emulsions, for example o/w/o emulsions can be delivered intramuscularly for hydrophobic compounds whereas it can not be achieved by o/w emulsions. One problem of w/o emulsions is the high viscosity resulting from the determining viscosity of the continuous (oil) phase resulting in difficulties in injection.

The development of multiple emulsions as drug delivery systems has been studied especially using w/o/w systems. These systems have been used to prolong the drug release (Brodin et al., 1978; Omotosho et al., 1986; Davis and Walker, 1987). For example the use of w/o/w emulsions are reported to enhance therapeutics of anticancer drugs (Benoy et al., 1974; Higashi et al., 1999), to improve intestinal absorption of insulin (Engel et al., 1968) and lymphatic absorption of drugs (Lin et al., 1992). Some authors have also studied o/w/o emulsions (Brodin and Frank, 1978). However, the issues related to the stability, formation and entrapment efficiency of multiple emulsions has limited the application of these systems. Such problems may be overcome by using new emulsification technique which will be discussed in section 1.6.
1.5.2.3 Microemulsions
Microemulsions have attracted much attention in drug delivery due to their thermodynamic stability and ability to be formed spontaneously. Both o/w and w/o systems, have been used as oral preparations to improve the bioavailability of drugs and therapeutic peptides (Humberstone and Charman, 1997; Pouton, 2000). The application of microemulsions for transdermal drug delivery has also been reviewed (Kreilgaard, 2002; Peltola et al., 2003).

1.5.2.4 Magnetic emulsions
It is one of the modern technologies for drug delivery developed using nanosized magnetic particles in magnetic drug targeting (Torchilin, 2000). Nanomagnetic particles have the property to allocate and fix to the desired target area using an external magnetic field. These magnetic particles can be incorporated into the disperse phase of emulsions forming ‘magnetic emulsions’ which show a degree of magnetic response (Morimoto et al., 1983). For example, magnetic o/w emulsions have been used as carriers to deliver a lipid soluble chemotherapeutic agent and showed high drug distribution in the lungs under the influence of applied magnetic field (Akimoto et al., 1985). This topic will be discussed further in chapter 6.

1.5.2.5 Non-aqueous emulsions
Non-aqueous emulsions are less common and not widely discussed. Besides the numerous advantages of the aqueous emulsions, we believe non-aqueous emulsions can offer additional versatility. One advantage of these systems is that the properties of the phase can often be manipulated, for example by varying the molecular weight of oligomeric or polymeric liquids in one of the component phases. In aqueous systems, only the oil phase can be varied, although additives to the aqueous phase modifying the resultant properties of such systems.

Generally, non-aqueous emulsions may be used where components are sensitive to water or water is deleterious (e.g. used in engine or mechanical parts to prevent the rust formation, protection from strong electrical field or chemical reaction). Most of the
papers published have shown applications in non-pharmaceutical fields or in chemical processing such as the production of highly concentrated fuel-in-formamide emulsions for use as safety fuels (Beerbower et al., 1968), the creation of electrooptical displays made of liquid crystal droplets (Loudet et al., 2000) and formation of an elastomer film containing the liquid disinfecting phase (Riess et al., 2004). Some authors have used non-aqueous systems in the preparation of nanoparticles or as templates in the formation of silicate microstructures (Imhof and Pine, 1997a; 1998; Yi and Yang, 1999).

Bauer et al. (1987) have used non-aqueous emulsions as capsule filling substances because the presence of water even in small amount could dissolve the gelatin. Work in our laboratory has reported the slow release of drugs from these systems (Sakthivel et al., 2001; Jaitely et al., 2004). Therefore, they may be of pharmaceutical value for delivery of the water labile drugs (i.e. hydrolysable drugs) or as a potential carrier for lipophilic drug to provide sustained effects due to the phase barrier between two oil phases.

### 1.5.2 Non-pharmaceutical applications

Apart from the pharmaceutical point of view, emulsions are involved in several other applications. A basic example is food emulsions which have been primitively used prior to the definition of emulsion e.g. milk, milk products, ice cream, and mayonnaise. The choice of their component especially the emulsifying agent has to be an important issue for consideration. Many authors have extensively reviewed this subject (Dickinson, 1987). Cosmetics such as lotions, creams and other toiletry products are another familiar application of emulsions. All the components not only have to be safe, but they also have to be formulated with the proper combination to provide a pleasant sensation to the skin. Emulsion viscosity is thus one of the crucial aspects influencing their sensory properties (Barry and Grace, 1972). In the metal processing industry, emulsions could be employed as lubricants and rolling oil to dissipate the heat (Binks, 1998).

Additionally, emulsions are used in petroleum production and recovery operations at almost every step such as oil recovery process, production rate and pipeline transportation (Pal, 1994). Besides that, water-in-diesel emulsions have also been investigated as one of alternative fuels apart from bioethanol or biodiesel. These
emulsion fuels can provide an environmental benefit due to reductions in the emissions of nitrogen oxides and in the fuel consumption resulting from improved burning efficiency (Lif and Holmberg, 2006). Other industries where emulsions are of considerable importance also include herbicide or pesticidal agents in agriculture formulations (van Valkenberg, 1973), anti-static and wetting agents employed in textile industries (Mathis, 1993), bitumen emulsions for the construction of roads (Rodríguez-Valverde et al., 2003) and paints industries (Derksen, 1996). Furthermore, many hazardous materials such as alkali metals and explosives are frequently handled in the emulsion form (Jones et al., 1999).

1.6 Emulsification techniques
The fact that the emulsions can be applied in various fields such as food, pharmaceuticals and chemical industries, the production of emulsions is of great importance. Emulsions can be produced either by manual shaking or with the mechanical shakers which are suitable for laboratory or small-scale preparation. For the larger scale, they are conventionally produced using colloid mills, high-pressure homogenizers and ultrasonic homogenizers (Becher, 2001). All these emulsification devices require a certain amount of energy to break the disperse phase into smaller droplets as a result the products are subject to considerable heat which is not suitable for some active pharmaceutical ingredients. The second disadvantage of the conventional method is the uncontrolled droplet size and size distribution of emulsion resulting from inhomogeneous extensional and shear flows, generated from the devices. However, the potential uses of emulsions mostly need a precise control over droplet size and size distribution. For example, emulsions with low polydispersity have been used as templates for sol-gel chemistry to produce new materials with uniform spherical pores (Park et al., 2003) or to encapsulate electro-optical materials such as liquid crystals (Rudhardt et al., 2003). Drug-release properties from the emulsions depend on their globule size and most of the models anticipate release profiles based on the assumption that they are monodispersity. One reason concerned with the emulsion stability is often polydispersity and the polydispersity can change with time leading to coalescence. Monodisperse emulsions are also expected to be useful for fundamental studies because the interpretation of experimental results is much simpler than that of polydisperse emulsions (Mason et al.,
Additionally, the effect of Ostwald ripening will be reduced if the droplet distribution is narrow. To overcome this limitation, several techniques have been recently developed to control the droplet size as well as to produce monodisperse emulsions.

### 1.6.1 Membrane Emulsification

Over the last decade, membrane emulsification which is a relatively new method for the production of emulsions has received much attention. This technique uses microporous membranes to produce emulsion droplet in quite a simple way. The disperse phase is pressed through the membrane whilst the continuous phase flows along the membrane surface. In most cases a suitable surfactant is dissolved in dispersed or in the continuous phase. The resulting droplets are gradually developed at the pore outlets of the membrane until reaching a certain size, finally being detached by the flow of the continuous phase (Figure 1.5). This process is determined by the balance between several parameters such as the drag force on the droplet from the flowing continuous phase, the buoyancy of the droplet, the interfacial tension forces and the driving pressure (Schröder et al., 1998).

![Figure 1.5](image.png)

**Figure 1.5** Schematic representing of the membrane emulsification method. The disperse phase is pressed through a microporous membrane while the continuous phase flows along the membrane surface. Droplets grow at pores and detach at a certain size, which is determined by the balance between the forces acting on the droplet. Adapted from Nakashima et al., 2000 and Lambrich and Vladisavljevic, 2004.

A unique characteristic of membrane emulsification over the conventional emulsification technique is that the resulting droplet size is governed primarily by the choice of membrane rather than the generation of turbulent droplet break-up. Therefore, the type of
membrane surface and its porosity are the crucial factors controlling the droplet size and size distribution. For the production of o/w and w/o emulsions, hydrophilic and hydrophobic membranes are respectively required to allow droplet formation and prevent wetting of the membrane surface (Nakashima, 1991). However, the final droplet size and size distribution are not only determined by the pore size and size distribution of the membrane but also by the emulsifier type and concentration, dispersed phase flux, velocity of the continuous phase and transmembrane pressure (Joscelyne and Trägårdh, 2000).

The major advantages of membrane emulsification are the possibility to produce droplets of a defined size with a narrow droplet size distribution, low shear stress and the potential to reduce energy consumption (Okochi and Nakano, 1997; Dowding et al., 2001). Using this method both single w/o, o/w emulsions and multiple o/w/o, w/o/w emulsions can be produced (Kawashima et al., 1991; Mine et al., 1996). Mild processing conditions make membrane emulsification very useful especially for protection of the large droplets of multiple emulsions during the second emulsification step. It is also possible to generate small and monodisperse multiple emulsion droplets without using high shear stress which might cause the escape of the internal droplets (i.e. in the case of conventional emulsification method). Figure 1.6 shows the schematic diagram for the production of w/o/w emulsions.

![Figure 1.6](image_url) A schematic diagram showing the preparation of the multiple w/o/w emulsions by the membrane emulsification method. The arrows represent the direction of the fluid flow. Adopted from Vladisavljević and Schubert, 2003.
Membrane emulsification has been used to produce monodispersed emulsions for controlled delivery (Higashi et al., 1995; 1996; 1999), food industry for microencapsulation of flavours (Katoh et al., 1996; Scherze et al., 1999) and the synthesis of polymeric microspheres to be used as stationary phases in HPLC (Hosoya et al., 1996). The w/o/w emulsions have shown clinical efficacy in patients with hepatocellular carcinoma causing retention of epirubicin in the tissue for more than 3 weeks (Higashi and Setoguchi, 2000). Figure 1.7 shows examples of emulsions and microspheres some of which have been developed for industrial applications, produced from the membrane emulsification method.

Figure 1.7 Examples of products produced by membrane emulsification. (A): Photomicrograph of the w/o/w emulsions for drug delivery systems. The median value of the diameter is $30.1 \pm 0.71 \mu m$. (B): Photomicrograph of “super low fat spread” made by Moringa Milk Industry Co. Ltd. (C) scanning electron micrograph of polydivinylbenzene microspheres that were developed by Sekisui Chemical Industries Co. Ltd. as a spacer material for liquid crystal display and (D) scanning electron micrograph of monodispersed silica powder made by Suzuki Yushi Industries Co. Ltd. used for an HPLC packing material and as a cosmetic foundation. Adopted from Higashi et al., 1995; Nakashima et al., 2000.

A limiting factor of membrane emulsification is the low flux for dispersed phase through the membrane in particular when small droplets are desired. However, the flux could be
enhanced either by using a new membrane developed to have low resistance level or by modifi
ifying the process in which a coarse emulsions (prepared by conventional method) are passed through the membrane (instead of disperse phase as such) (Vladisavljevic et al., 2004). This technique is known as pre-mix membrane emulsification and is reported to produce w/o/w emulsions (Shima et al., 2004).

1.6.2 Microchannel emulsification

This is another novel method for the production of monodisperse emulsions. The principle of microchannel method is similar to that of membrane emulsification although the microchannel method provides more monodisperse droplet sizes. Instead of a membrane, a silicon microchannel plate which is manufactured by micromachining technology is used to produce the emulsions. The droplets are generated by forcing the disperse phase into the continuous phase through the silicon microchannels (Fig 1.8). A mechanism of droplet formation was suggested by Sugiura et al. (2001b). They suggested that this emulsification technique utilizes the interfacial tension to facilitate spontaneously cut off process of the distorted disperse phase into droplets.

Figure 1.8 A schematic diagram of microchannel emulsification and a photomicrograph of microchannels. Adopted from Sugiura et al., 2001b.
Microchannel emulsification is considered to be promising technique not only for preparing emulsions (Sugiura et al., 2000; Kawakatsu et al., 2001) but also lipid microparticles (Sugiura et al., 2001a), polymeric microparticles (Sugiura et al., 2001c) and microcapsules. Liu et al. (2001) employed this technique to prepare monodisperse o/w emulsions and studied the effect of surfactant concentration on stability. With monodisperse emulsions the interpretation is expected to be simpler than for polydisperse emulsions. The quality of the resulting emulsion droplet could be controlled by microchannel size (width and height), emulsifier type and concentration along with the flow rate of the dispersed phase and the continuous phase. These factors are similar to those of the membrane emulsification technique. However, the geometry and structure of the microchannel plate can be modified that make the technique more versatile. Kawakatsu et al. (1997) studied the effect of the continuous phase flow rate and the structure of the microchannel plate on the droplet size and found that using the plate with a terrace can enlarge the size of produced droplet (Fig 1.8)

As mentioned before the microchannel plate is fabricated by micromachining technology. This technology which is used for the production of such miniaturized parts and components has received a lot of attention and developed rapidly in over a decade. The microstructures have been employed in various microsystems such as micro-optical, electronic and mechanical industries. Microfluidics' has thus emerged together with the idea of microtechnology, including new methods of fabrication.

1.7 Microfluidics
Microfluidics is discussed here briefly as in this work we have studied emulsion flow in microfluidic channels.

1.7.1 Background
Whitesides (2006) stated that ‘Microfluidics is the science and technology of systems that process or manipulate small (10⁻⁹ to 10⁻¹⁸ litres) amounts of fluids, using channels with dimensions of tens to hundreds of micrometers’. The small sized plate or chips composed of one or more microchannels connecting to or separating are called as microfluidic devices. The main driving force for the development of microfluidic system originates
from the micro-scale analytical methods used in molecular analysis such as gas-phase chromatography (GPC), high-pressure liquid chromatography (HPLC) and capillary electrophoresis (CE). The revolution of genomics and proteomics is another motivation to make use of microfluidics in the area of microanalysis related to molecular biology such as high-throughput DNA sequencing. These microanalytical methods obviously require much greater throughput, and higher sensitivity and resolution than previously used in chemistry, biology and biochemistry. Therefore, microfluidics can offer new approaches to overcome these limitations.

Certain fundamental differences between the physical properties of fluids moving in large channels (macro-scale) and those in micro-scale channels have been applied in microfluidics (Stone et al., 2004; Squires and Quake, 2005). For example, the characteristics of the flow in microchannel become laminar rather than turbulent, and make fluid behaviour such as mixing become more predictable (Reynolds, 1883). Other advantages in using microfluidics in biotechnology and bioanalysis include high separation efficiency, short analysis times and enhanced detection sensitivities (Manz et al., 1992). Figure 1.9 shows a diagram representing size dependent diffusion time. Moreover, microfluidics can also be integrated with other devices to make the most use of pumps, valves, separation systems and detectors as follow the concept of micrometer-scale total (chemical) analysis systems (μTAS) or the so-called ‘lab-on-a-chip’ (Jakeway et al., 2000). Many processes of analysis include sampling, sample pre-treatment, (bio-) chemical reactions, separation and finally analytical identification. This concept was investigated for a nanolitre-DNA-analysis system with the purpose of amplification, digestion, separation, and identification of DNA sequence (Burns et al., 1998).
Figure 1.9 A comparison of different scales representing size dependent diffusion time. In the absence of turbulence in microfluidics, the mixing process for example two reactants is mainly dependent on the diffusion coefficient (D) which is assumed to be $D = 10^{-9}$ m$^2$s$^{-1}$. Thus the time for a molecule to travel a distance $d$ decreases as $1/d^2$. Short diffusion times in the millisecond range indicate that an efficient mixing of two solutions will be obtained. Adapted from Janasek et al., 2006.

### 1.7.2 Applications

Many authors have demonstrated the use of microfluidics for separations coupled to mass spectroscopy (Ramsey and Ramsey, 1997), high-throughput screening in drug development (Pihl et al., 2005; Dittrich and Manz, 2006), bioanalyses (Sia and Whitesides, 2003), examination and manipulation of samples consisting of a single cell (Wheeler et al., 2003; Werdich et al., 2004) or a single molecule (Dittrich and Manz, 2006; Stavis et al., 2005). One example which has been highly developed is their use in protein crystallization. This procedure offers the potential to screen various conditions (i.e. pH, ionic strength and composition, cosolvents and concentration), to separate nucleation and growth of crystals, and to minimize the damage to crystals by handling once they have been formed (Zheng et al., 2004). Some of this technology is now commercially available (Fig 1.10)
Figure 1.10 a) Schematic representing the process of forming droplets for screening purposes in protein crystallization. b) Photograph of a PDMS/glass capillary composite microfluidic device. c) Photomicrograph of crystals grown inside the droplet in a glass capillary. d) The crystals (top) can be directly subjected to X-ray diffraction. A diffraction pattern (bottom) of crystal produced from microfluidic device that shows the reflections on the edge of the image at a resolution of 2.0 Å. Adopted from Zheng et al., 2004.

Another area of research in which microfluidic systems show their capability is in cell biology. Eukaryotic cells have linear dimensions of 10–100 μm that are well suited for current microfluidic devices used to grow and observe cells (Taylor et al., 2003; Hung et al., 2005; Chung et al., 2005; Walker et al., 2005). Figure 1.11 shows a schematic representation of an integrated cell analysis system.

Figure 1.11 Schematic representing tissue organization, culture and analysis in microsystems: As indicated by the yellow arrows, the different microfluidic components can be connected with each other to form an integrated system, realizing multiple functionalities on a single chip. Adopted from El-Ali et al., 2006.
Microfluidic systems can also be used to generate and manipulate monodisperse droplets (Nissako et al., 2002; Dreyfus et al., 2003; Tan et al., 2004) or bubbles (Ganan-Calvo and Gordillo, 2001; Garstecki et al., 2004) of a dispersed gas or liquid phase in a continuous liquid stream, these offer new techniques to the production of emulsions and foams (Xu et al., 2005b). Nissako et al. (2005) have prepared multiple emulsions using microfluidics, a modification of microchannel emulsification discussed in section 1.6.2. They found that using this technique could control the size and number of the internal droplet size and the external droplet size. Another idea of using microfluidics for chemical reactions has also been reviewed by de Mello (2006).

**Figure 1.12** a) Schematic diagram of the formation of w/o/w emulsions in a microfluidic cell. b) to e) Photomicrographs of w/o/w emulsions with a controlled size and number of internal droplets (n). (b) n = 1 (c) n = 2 (d) n = 4 and (d) n = 8 prepared by this technique. The scale bars are 100 µm. Adopted from Nissako et al. (2005).
Although to develop these applications, request much effort and innovation in microfluidics, biomedicine and bioanalysis, the potential uses of microfluidic technologies are very promising. One is the development of new types of bioassay for diagnostic purpose or monitoring patient response to therapy that would be the next stage in the evolution of healthcare.

In general non-aqueous systems could be of great potential as seen from the various applications described in section 1.5. The various issues linked to the development of a non aqueous emulsion are discussed in the thesis.
1.8 Aims and outline of the work in thesis

The broad aim of this thesis was to formulate and investigate non-aqueous emulsions using pharmaceutically acceptable material. The applications of this system were expected not only for the sustained drug delivery but also for non-pharmaceutical purpose. It could be divided into separate parts as follows,

1. To formulate non-aqueous emulsions, starting with finding two immiscible oil phases and emulsify such systems with suitable surfactants.

2. To study the physicochemical properties of non-aqueous emulsions.

3. To assess the potential of non-aqueous emulsions and multiple (o/o/w) emulsions as lipophilic carriers for sustained drug delivery.

4. To assess the in-vivo performance of non-aqueous emulsions

5. To design magnetic non-aqueous emulsions and to study their movement under a magnetic field.

6. To study the flow behaviour of emulsion droplets in microfluidic devices.
Chapter II

FORMULATION OF NON-AQUEOUS EMULSIONS

2.1 Introduction
Non-aqueous emulsions have been studied since the mid-1960s, as mentioned in the previous chapter. The critical stage in the formulation of non-aqueous emulsions is the selection of suitable surfactant. This issue could be tackled by two approaches. One way is to synthesize a suitable surfactant which would have two different parts, each of which has a preferential affinity with either of the selected immiscible phases. Imhof and Pine (1997b) exploited this route to stabilise DMF-in-hexane emulsions using diblock copolymers of polystyrene and polyisoprene. Loudet et al. (2000) also synthesized new surfactant molecules to stabilise liquid crystal-in-silicone or silicone-in-liquid crystal emulsions. The disadvantage of this approach is clear, as there is the necessity to design and characterise a new surfactant for each combination of liquids. The second approach is to seek a suitable oil-immiscible polar liquid which can substantially replace the water phase, therefore allowing the use of existing surfactants such as non-ionic surfactants to stabilise the resultant emulsions. Cameron and Sherrington (1996) have reported that nonionic surfactants with HLB values of 12 could stabilise petroleum ether in formamide, DMF and DMSO emulsions.

51
We have been exploring non-aqueous emulsions for their pharmaceutical potential as drug delivery systems. We began to formulate non-aqueous emulsions by searching for two immiscible non-aqueous phases and emulsifying the selected phases with existing surfactants. For example, emulsions of dodecane-in-polyethylene glycol stabilised by sorbitan trioleate (Sakthivel et al., 1999) and emulsions of alkanes-in-formamide stabilised by polysorbate 20 were formulated (Sakthivel et al., 2001). Hydrocarbons and formamide are, of course, pharmaceutically unsuitable materials. We therefore moved to formulate non-aqueous systems using pharmaceutical solvents and oils, a list of which is given by Spiegel and Noseworthy (1963). However, the major difficulties in searching for stable non-aqueous emulsions arise from a lack of data relating to the formulation such as the selection for any two immiscible phases of the appropriate surfactants. In this chapter we aimed at formulating stable non-aqueous emulsions using pharmaceutical excipients. The work is in the form of a scoping exercise in order to determine, if possible, some general principles of formulation by exploring a range of oils and surfactants with different characteristics.

2.2 Materials and methods

2.2.1 Materials

2.2.1.1 Pharmaceutical solvents

Benzyl benzoate, tetracyglycol, propylene glycol, polyethylene glycol 400 (PEG 400) were obtained from Sigma (Dorset, UK). Dibutyl sebacate, ethyl oleate, isopropyl myristate, propylene carbonate, castor oil, cottonseed oil, olive oil, oleic acid, ricinoleic acid, safflower oil, sesame oil, soybean oil were obtained from Fluka (Dorset, UK). Cyclopentasiloxane, polydimethylsiloxane polymer (20 cSt, Dow Corning grade 200 silicone fluid) were supplied by S. Black (Hertfordshire, UK).

2.2.1.2 Surfactants

All surfactants used in these experiments divide roughly in three types. Firstly, conventional surfactants which are hydrocarbon-based materials (obtained from Fluka, UK) as listed in Table 2.1. Secondly, block copolymer surfactants of the polyethyleneoxide-polypropyleneoxide-polyethyleneoxide type obtained from Univar
(UK) as listed in Table 2.2. Thirdly, silicone or siloxane surfactants, silicone-based materials consisting of a methylated siloxane group (polydimethylsiloxane, PDMS) together with one or more polar groups. The most common polar groups are non-ionic based on polyethyleneoxide (PEO) and polypropyleneoxide (PPO) used in these experiments. All silicone surfactants were obtained from Dow Corning (Thailand), these are DC 190, DC 193, DC 3225C, DC 5200, DC 5225C, DC 5330 and DC 9011 as listed in Table 2.3.
Table 2.1 A list of conventional surfactants (hydrocarbon-based) explored in this work

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>INCI Name</th>
<th>HLB value</th>
<th>Chemical Structure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Span 85</td>
<td>Sorbitan trioleate</td>
<td>1.8</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Arlacel 83</td>
<td>Sorbitan sesquioleate</td>
<td>3.7</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Span 60</td>
<td>Sorbitan monostearate</td>
<td>4.7</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Tween 85</td>
<td>Polyoxyethylene sorbitan trioleate</td>
<td>11.0</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Tween 60</td>
<td>Polyoxyethylene sorbitan monostearate</td>
<td>14.9</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Tween 20</td>
<td>Polyoxyethylene sorbitan monolaurate</td>
<td>17.6</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
<tr>
<td>Triton X</td>
<td>Octylphenol ethylene oxide (n)</td>
<td>4.9, 7.8, 9.8, 12.3, 13.4, 17.6</td>
<td><img src="image" alt="Chemical Structure" /></td>
</tr>
</tbody>
</table>
Table 2.2 List of block copolymer surfactants investigated in this work

<table>
<thead>
<tr>
<th>Surfactants</th>
<th>INCI name</th>
<th>Chemical structure</th>
<th>a</th>
<th>b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synperonic PE/L 101</td>
<td>Polyethyleneoxide-polypropyleneoxide-polyethyleneoxide</td>
<td>H-(OCH(CH3)-CH2)b-(OCH2CH2)a-OH</td>
<td>4</td>
<td>56</td>
</tr>
<tr>
<td>Synperonic PE/L 121</td>
<td></td>
<td>PEOa-PPOb-PEOa</td>
<td>5</td>
<td>68</td>
</tr>
<tr>
<td>Synperonic PE/F 108</td>
<td>(Ethyleneoxide-propylene oxide block copolymer)</td>
<td></td>
<td>127</td>
<td>48</td>
</tr>
<tr>
<td>Synperonic PE/F 127</td>
<td></td>
<td></td>
<td>95</td>
<td>62</td>
</tr>
</tbody>
</table>

Table 2.3 List of silicone surfactants used in this work

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>INCI Name</th>
<th>HLB value</th>
<th>Chemical Structure</th>
<th>n</th>
<th>m</th>
<th>x</th>
<th>y</th>
<th>R</th>
<th>R₂</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC 190</td>
<td>PEG/PPG-18/18 Dimethicone</td>
<td>5.6</td>
<td></td>
<td>103</td>
<td>9.5</td>
<td>18</td>
<td>18</td>
<td>C₃H₆O</td>
<td>H</td>
</tr>
<tr>
<td>DC 193</td>
<td>PEG-12 Dimethicone</td>
<td>12.4</td>
<td></td>
<td>8.6</td>
<td>3.6</td>
<td>12</td>
<td>0</td>
<td>C₃H₆O</td>
<td>H</td>
</tr>
<tr>
<td>DC 5200</td>
<td>Lauryl PEG/PPG-18/18 Methicone</td>
<td>2.2</td>
<td></td>
<td>0</td>
<td>65</td>
<td>18</td>
<td>18</td>
<td>N/A</td>
<td>H</td>
</tr>
<tr>
<td>DC 5330</td>
<td>PEG/PPG-15/15 Dimethicone</td>
<td>N/A</td>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td>15</td>
<td>15</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>DC 3225C</td>
<td>Cyclomethicone and PEG/PPG-18/18 Dimethicone</td>
<td>N/A</td>
<td></td>
<td>394</td>
<td>4</td>
<td>18</td>
<td>18</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>DC 5225C</td>
<td>Cyclopentasiloxane and PEG/PPG-18/18 Dimethicone</td>
<td>1.7</td>
<td></td>
<td>394</td>
<td>4</td>
<td>18</td>
<td>18</td>
<td>N/A</td>
<td>N/A</td>
</tr>
<tr>
<td>DC 9011</td>
<td>Cyclopentasiloxane and PEG-12 Dimethicone Crosspolymer</td>
<td>N/A</td>
<td></td>
<td>N/A</td>
<td>N/A</td>
<td>12</td>
<td>N/A</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

\[
\begin{align*}
\text{DC 190} & : \quad \text{PEG/PPG-18/18 Dimethicone} \\
\text{DC 193} & : \quad \text{PEG-12 Dimethicone} \\
\text{DC 5200} & : \quad \text{Lauryl PEG/PPG-18/18 Methicone} \\
\text{DC 5330} & : \quad \text{PEG/PPG-15/15 Dimethicone} \\
\text{DC 3225C} & : \quad \text{Cyclomethicone and PEG/PPG-18/18 Dimethicone} \\
\text{DC 5225C} & : \quad \text{Cyclopentasiloxane and PEG/PPG-18/18 Dimethicone} \\
\text{DC 9011} & : \quad \text{Cyclopentasiloxane and PEG-12 Dimethicone Crosspolymer}
\end{align*}
\]
2.2.2 Determination of miscibility between liquid phases

The term miscible refers to the property of liquids that allows them to be mixed together and form a single homogenous phase (e.g. ethanol and water), two liquids are said to be immiscible if they cannot be mixed together e.g. oil and water. The appearance of the two distinct liquid phases after equilibration indicates that the two liquids are saturated with each another and hence indicates their immiscibility, or at least partial immiscibility.

The apparent miscibility of two non-aqueous liquids was examined by adding 1 ml of a solvent selected into 1 ml of another solvent, and mixing the two in a glass vial. The mixture was then stirred by using Rotamixer (Hook & Tucker) and the miscibility of the two liquids was observed visually after mixing for 30 min.

2.2.3 Preparation of emulsions

The emulsions were prepared by dispersing each surfactant (5% w/v) in the non-aqueous phase (continuous phase) and then adding the other non-aqueous phase (as the disperse phase) at a phase volume ratio of 0.25. The emulsification was carried out for 1 min using a Rotamixer. Benzyl benzoate-in-water or in some cases benzyl benzoate in water: propylene glycol emulsions were prepared by sonication for 1 min with a probe sonicator (Soniprep 150, Sanyo). The longer sonication time were not used as 1 min of sonication yielded sufficient dispersion.

2.2.4 Assessment of emulsion stability

There are different methods to assess the stability of a series of emulsions. One is based on the physical appearance of emulsions and simply estimates the degree of separation or creaming. Separation of the emulsion into distinct layers (i.e., cracking) may indicate gross instability. Creaming is a type of instability but a creamed emulsion may be defined as a stable system as it can be restored to its original state by gentle shaking. However, emulsion instability results from any process which causes a progressive increase in particle size until coalescence finally occurs. Thus, a more precise method for assessing emulsion stability is to study particle size and size distribution with time. An emulsion approaching the unstable state is characterised by a broadening of the width of particle
size distribution (Aulton, 2002). Both the physical appearance and droplet size measurements were used to assess the stability of the emulsions.

### 2.2.4.1 Physical appearance studies to estimate the emulsion stability

Various non-aqueous (o/o) emulsions were prepared by a Rotamixer as above and were allowed to equilibrate at room temperature. After 24 h the change in stability of the emulsion was noted by visual observation. Two destabilisation processes which could be seen in non-aqueous systems were creaming/sedimentation and cracking. Emulsions which can be restored to their original state by gently shaking are noted as the stable systems. On the other hand, with cracking the process is irreversible and this represents unstable emulsions.

### 2.2.4.2 Determination of particle size

A series of emulsions were prepared in the usual manner using the probe sonication method. Samples of the emulsions were stored at room temperature (25 °C). The mean particle size of emulsion was measured using a Mastersizer S (Malvern Instruments, Malvern, UK) at various time intervals.

### 2.2.5 Cloud point measurement

The clouding phenomenon is generally observed when the temperature of aqueous solutions of non-ionic surfactants is raised to a particular value known as the cloud point, which is a lower consolute temperature (LCT). Above their cloud points, non-ionic surfactant solutions become turbid because of the growth of their micelles and eventually insolubility as hydration levels change. The system finally separates into two phases. Cloud points are useful for measurement of the interaction of surfactants with components in the aqueous solvent.

Surfactant solutions were prepared by dissolving surfactants in the water phase or in mixtures of propylene glycol and water, the latter to determine the influence of the glycol on the behaviour of the surfactants. The cloud points were then determined by slowly
heating the surfactant solutions (0.31, 0.62, 1.25, 2.50, 5.00 and 10.00 % w/v) in sealed glass tubes. The temperature at which the surfactant solution became hazy was recorded by visual observation as the cloud point. Each measurement was made in triplicate.

2.3 Results and discussion

2.3.1 Miscibility of two liquid phases
To formulate non-aqueous emulsions, the two non-aqueous liquids have to be investigated. The non-aqueous solvents selected in this work could be categorised into three different groups. The first group consisted of polar, water, miscible liquids such as glycerol, propylene glycol, propylene carbonate, PEG 400 and tetruglycol. The second group consisted of non-polar, water immiscible liquids such as benzyl benzoate, ethyl oleate, dibutyl sebacate, isopropyl myristate, triacetin and fixed oils. The third group comprised silicone oils (polydimethylsiloxane, PDMS) which are partially inorganic polymers composed of a repeating silicon-oxygen backbone (-O-Si-O-Si-). The results of the apparent miscibility study between two non-aqueous phases are shown in Table 2.4.

Although the apparent miscibility/immiscibility of two liquids could be deduced by directly mixing them there are several properties which could be used to explain such behaviour. The molecular properties such as dielectric constant or polarity index, carbon chain length hydrogen bonding capacity could be used to explain the miscibility behaviour of two liquids. The bulk properties of liquids such as density/specific gravity, boiling point and heat of vaporization could also be of relevance to understanding their miscibility behaviour.
Table 2.4 Apparent miscibility between two non-aqueous phases

<table>
<thead>
<tr>
<th>Non-polar liquids (density, dielectric constant)</th>
<th>Polar liquids (density, dielectric constant)</th>
<th>Miscibility</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Benzyl benzoate (BB)</strong> (1.12, 4.80)</td>
<td>Glycerol (1.26, 42.5)</td>
<td>Immiscible</td>
</tr>
<tr>
<td>Propylene glycol, PG (1.04, 32.1)</td>
<td>Immiscible</td>
<td></td>
</tr>
<tr>
<td>Propylene carbonate, PC (1.20, 66.14)</td>
<td>Miscible</td>
<td></td>
</tr>
<tr>
<td>PEG 400 (1.12, 12.4)</td>
<td>Miscible</td>
<td></td>
</tr>
<tr>
<td>Tetraglycol (1.08, 15.7)</td>
<td>Miscible</td>
<td></td>
</tr>
<tr>
<td>Triacetin (1.16, 6.0)</td>
<td>Miscible</td>
<td></td>
</tr>
<tr>
<td><strong>Ethyl oleate (EO)</strong> (0.87, 3.17)</td>
<td>Glycerol (1.26, 42.5)</td>
<td>Immiscible</td>
</tr>
<tr>
<td>Propylene glycol (1.04, 32.1)</td>
<td>Immiscible</td>
<td></td>
</tr>
<tr>
<td>Propylene carbonate (1.20, 66.14)</td>
<td>Immiscible</td>
<td></td>
</tr>
<tr>
<td>PEG 400 (1.12, 12.4)</td>
<td>Immiscible</td>
<td></td>
</tr>
<tr>
<td>Tetraglycol (1.08, 15.7)</td>
<td>Immiscible*</td>
<td></td>
</tr>
<tr>
<td>Triacetin (1.16, 6.0)</td>
<td>Immiscible</td>
<td></td>
</tr>
<tr>
<td><strong>Dibutyl sebacate (DS)</strong> (0.94, 4.54)</td>
<td>Glycerol (1.26, 42.5)</td>
<td>Immiscible</td>
</tr>
<tr>
<td>Propylene glycol (1.04, 32.1)</td>
<td>Immiscible</td>
<td></td>
</tr>
<tr>
<td>Propylene carbonate (1.20, 66.14)</td>
<td>Miscible</td>
<td></td>
</tr>
<tr>
<td>PEG 400 (1.12, 12.4)</td>
<td>Immiscible</td>
<td></td>
</tr>
<tr>
<td>Tetraglycol (1.08, 15.7)</td>
<td>Miscible</td>
<td></td>
</tr>
<tr>
<td>Triacetin (1.16, 6.0)</td>
<td>Miscible</td>
<td></td>
</tr>
<tr>
<td><strong>Isopropyl myristate (IPM)</strong> (0.853, 19.92)</td>
<td>Glycerol (1.26, 42.5)</td>
<td>Immiscible</td>
</tr>
<tr>
<td>Propylene glycol (1.04, 32.1)</td>
<td>Immiscible</td>
<td></td>
</tr>
<tr>
<td>Propylene carbonate (1.20, 66.14)</td>
<td>Miscible</td>
<td></td>
</tr>
<tr>
<td>PEG 400 (1.12, 12.4)</td>
<td>Immiscible*</td>
<td></td>
</tr>
<tr>
<td>Tetraglycol (1.08, 15.7)</td>
<td>Immiscible*</td>
<td></td>
</tr>
<tr>
<td>Triacetin (1.16, 6.0)</td>
<td>Immiscible*</td>
<td></td>
</tr>
<tr>
<td><strong>Triacetin</strong> (1.16, 6.0)</td>
<td>Glycerol (1.26, 42.5)</td>
<td>Immiscible</td>
</tr>
<tr>
<td>Propylene glycol (1.04, 32.1)</td>
<td>Miscible</td>
<td></td>
</tr>
<tr>
<td>Propylene carbonate (1.20, 66.14)</td>
<td>Miscible</td>
<td></td>
</tr>
<tr>
<td>PEG 400 (1.12, 12.4)</td>
<td>Miscible</td>
<td></td>
</tr>
<tr>
<td>Tetraglycol (1.08, 15.7)</td>
<td>Miscible</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non-polar liquids (oils)</th>
<th>Miscibility</th>
</tr>
</thead>
<tbody>
<tr>
<td>Silicone oil (include Castor oil (0.96, 2.6)</td>
<td>Immiscible</td>
</tr>
<tr>
<td>Dimethicone and Cottonseed oil (0.92, 3.1)</td>
<td>Immiscible</td>
</tr>
<tr>
<td>Cyclopentasiloxane) (0.95, 2.2) Olive oil (0.92, 3.1)</td>
<td>Immiscible</td>
</tr>
<tr>
<td>Safflower oil (0.92, 2.8)</td>
<td>Immiscible</td>
</tr>
<tr>
<td>Sesame oil (0.92, 3.0)</td>
<td>Immiscible</td>
</tr>
<tr>
<td>Soybean oil (0.92, 2.8)</td>
<td>Immiscible</td>
</tr>
</tbody>
</table>

Miscible: The system was clear (transparent) after mixing

Immiscible: The system was turbid and rapidly showed a boundary between two phases

Immiscible*: The system showed a boundary between two phases after 24 h

Values in the bracket indicate the density (gm/cm^3) and dielectric constant at approximately 25°C
The miscibility of two liquids is also governed by the thermodynamics of mixing two liquid phases. Many theories have been proposed to predict the miscibility of two liquids from a knowledge of the individual component properties. These theories are based on the estimation of solubility parameter which also termed as cohesive parameters (Barton, 1983).

The solubility parameter is expressed as energy density or energy per unit volume as shown

$$\delta = \sqrt[3]{-\frac{U}{V}}$$

(2.1)

where $\delta$ is the solubility parameter and $U$ is the cohesive energy term for volume $V$. Two commonly used solubility parameters are the Hilderbrand and Hansen solubility parameter. The derivation of the equation for the estimation of these parameters is based on equation 2.1. The Hilderbrand solubility parameter can be defined as

$$\delta = \left[\frac{\Delta_f^H - RT}{V}\right]^{1/2}$$

(2.2)

where $\Delta_f^H$ is the molar enthalpy of vaporization at temperature $T$ and $R$ is the universal gas constant.

A material with high $\delta$ value requires more energy for dispersal than is gained by mixing it with material of low $\delta$ value, so this results in immiscibility, whereas two materials with similar $\delta$ values gain sufficient energy on mutual dispersion to permit mixing. The Hilderbrand solubility parameter provides a broad, qualitative indication of behaviour for most systems and good results for very small number of hydrocarbons.

Another useful parameter is the Hansen parameter which is an extension of the Hilderbrand parameter to polar and hydrogen bonding systems. Estimation of the Hansen parameter is achieved using equation 2.3 and 2.4 and assumes that dispersion, polar and hydrogen bonding parameters are valid simultaneously. Hansen parameters provide an approximate quantitative measure of the extent of interactions for all systems and good results for a limited number of systems without significant specific chemical interactions.
\[ -U = -U_d - U_p - U_h \quad (2.3) \]

\[ \delta^2 = \delta_d^2 + \delta_p^2 + \delta_h^2 \quad (2.4) \]

where \( \delta_i \) is the total Hansen parameter comprised of \( \delta_d \) [dispersive], \( \delta_p \) [polar] and \( \delta_h \) [hydrogen bonding] parameters.

It could be seen from this study that for the proportions used for these studies the miscibility of the two liquids was primarily dependent upon the dielectric constant/polarity of the two liquids. A non-polar liquid like benzyl benzoate was immiscible with polar liquids such as glycerol and propylene glycol, whereas it was miscible with non-polar liquids such as tetraglycol and triacetin.

In the case of ethyl oleate, polarity was not only the parameter relevant to the miscibility since it was immiscible with polar liquids such as glycerol and propylene carbonate as well as with less polar liquids such as triacetin. The miscibility of dibutyl sebacate a non-polar liquid with a polar propylene carbonate also indicates the presence of some parameter other than polarity coming into play.

The relevance of solubility parameters such as the Hilderband and Hansen parameters to predict the solubility of two liquids has been already discussed. The estimation of such parameters involves functionalities such as heat of vaporisation, hydrogen bonding potential etc. The estimation of such parameters could be a part of future work to predict the solubility of the two liquids.

### 2.3.2 Selection of suitable surfactants

From the results of the miscibility study two immiscible non-aqueous phases were selected, one of which was used as disperse phase and another as a continuous phase in order to prepare emulsions such as benzyl benzoate in propylene glycol, ethyl oleate in triacetin, dibutyl sebacate in propylene glycol and IPM in PEG 400 emulsions and vice versa. The surfactants listed in Table 2.1, 2.2 and 2.3 were used to emulsify a series of systems. Silicone surfactants were used only in the systems which included silicone oil.
The appearance of each emulsion was observed visually and also under microscope 1, 2, 4 and 7 days after preparation. None of the conventional and block copolymer surfactants could provide stable emulsions after 7 days. Only silicone surfactants could stabilise the oil-in-silicone oil or silicone oil-in-oil emulsions. The physicochemical properties of these systems are described in Chapter 3.

Since none of the surfactant could stabilise the non-aqueous emulsion with both the oils as continuous and dispersed phases, one of the phases was replaced with an aqueous phase. A suitable surfactant system that would stabilise o/w emulsions was then identified. The aqueous phase was then mixed with water miscible solvent to constitute the continuous phase. The proportion of the non-aqueous solvent was then increased so as to replace the aqueous phase entirely in order to identify the suitable surfactant. This approach is described in the next section.

2.3.3 The influence of non-aqueous solvent on oil-in-water emulsions
Castor oil-in-silicone oil emulsions stabilised by silicone surfactants were successfully formulated. These silicone surfactants are complex materials each of which is a dispersion of a high molecular weight silicone surfactant in cyclomethicone. The study of the stabilisation of non-aqueous emulsions using less complex materials would be advantageous. Unfortunately, no non-aqueous emulsions of the type that we wished to study for later animal use could be produced. We therefore studied the alternative system of oil-in-water emulsion adding non-aqueous solvent (propylene glycol) into the continuous phase (water) of emulsion to determine the influence of additives on the emulsion stability and the behaviour of the surfactant. Although the mixture of aqueous phase with propylene glycol was used as an external phase, the idea was to extrapolate the results to 100% propylene glycol in order to obtain a surfactant system that would suit a true non-aqueous emulsion i.e. benzyl benzoate in propylene glycol.

The stability of benzyl benzoate-in-water (BB/W) emulsions was assessed as a function of the percentage of propylene glycol in aqueous phase. In addition, the cloud point of the surfactant used to stabilise these systems were also measured in the presence of
propylene glycol, better to understand the interactions of the surfactant with water in the presence of a glycol.

The BB/W emulsions were prepared using octylphenol ethyleneoxide series (Triton X) surfactants. Triton X-100 and 405 with high HLB numbers (greater more than 12) were found to stabilise BB/W emulsions whereas Triton X-15, 35 and 45 were found to yield unstable emulsions. This result concurred with the basic rule of HLB in which states that a stable o/w emulsions could be obtained using hydrophilic surfactants with HLB number greater than 9 whereas a stable w/o emulsion could be obtained with surfactants having low HLB values (Sjöblom, 2001).

However, the above results could be deduced from the gross stability of BB/W emulsions. A more precise method used to search for the optimal stability of emulsions was to measure the mean particle size of emulsions as a function of time after preparation. Triton X-series and their combinations were used as emulsifier to stabilise the emulsion (Table 2.5) in order to get the optimal HLB for the system.

Table 2.5 The range of HLB values obtained by mixing surfactants in different proportions. These combinations were then used as emulsifiers in stabilizing BB/W systems.

<table>
<thead>
<tr>
<th>Surfactants (% wt)</th>
<th>Triton X 15</th>
<th>Triton X 35</th>
<th>Triton X 45</th>
<th>Triton X 100</th>
<th>Triton X 405</th>
</tr>
</thead>
<tbody>
<tr>
<td>HLB</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7.45</td>
<td>70</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>10.60</td>
<td>-</td>
<td>50</td>
<td>-</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>10.88</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>30</td>
<td>-</td>
</tr>
<tr>
<td>11.60</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>50</td>
<td>-</td>
</tr>
<tr>
<td>11.96</td>
<td>-</td>
<td>-</td>
<td>40</td>
<td>60</td>
<td>-</td>
</tr>
<tr>
<td>12.32</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>70</td>
<td>-</td>
</tr>
<tr>
<td>12.68</td>
<td>-</td>
<td>-</td>
<td>20</td>
<td>80</td>
<td>-</td>
</tr>
<tr>
<td>13.04</td>
<td>-</td>
<td>-</td>
<td>10</td>
<td>90</td>
<td>-</td>
</tr>
<tr>
<td>13.40</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
<td>-</td>
</tr>
<tr>
<td>14.66</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>70</td>
<td>30</td>
</tr>
<tr>
<td>15.50</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>50</td>
<td>50</td>
</tr>
<tr>
<td>16.34</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>30</td>
<td>70</td>
</tr>
<tr>
<td>17.60</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>100</td>
</tr>
</tbody>
</table>

The coloured area in the Table represents the circled region in the Figure 2.1.
Figure 2.1 The effect of the HLB value of the surfactant mixture (using Triton X-series and their combinations) on the stability of BB/W emulsions, represented as the mean particle size of globule (μm) after storage for different time intervals (2-168 h). The circled region shows the HLB number required for optimal stability.

Figure 2.1 shows that the HLB range from 11.60 to 13.40 provided the most stable BB/W emulsions. The combination of Triton X-45 and Triton X-100 with the HLB number 11.60 and 11.96 offered a better emulsion stability compared to an individual Triton X surfactant. The synergistic effect of mixed surfactant on the emulsion stability has been known for a long time and is discussed by several authors including Velev et al. (1994) and Kan et al. (1999).

Although the combination of surfactants provides better emulsion stability, Triton X-100 was selected as a surfactant for BB/W because it provides comparable emulsion stability compared to mixed surfactants. This system was then used to study the influence of propylene glycol on the stability of o/w emulsions and the cloud point of Triton X-100 solutions.
2.3.3.1 The influence of propylene glycol on the stability of o/w emulsions

The stability of BB/W emulsions decreased as the amount of propylene glycol in the water (continuous phase) increased. It could be seen for BB/water: propylene glycol (50:50) (Figure 2.2 F and Figure 2.3 F) that the mean particle size of the dispersed phase significantly increased within 3 days. Further increase in the proportion of propylene glycol in the aqueous phase (≥ 60 %) resulted in unstable emulsions. Extrapolated to 100% propylene glycol, it is clear that the surfactants used in these systems could not stabilise a BB/PG system. The addition of propylene glycol therefore reduces the ability of Triton X-100 to act as an emulsifier protecting benzyl benzoate droplets against coalescence. To clarify aspects of the instability of the emulsion system, the cloud points of surfactant solutions were measured as a function of surfactant concentration. Initially the studies were performed using pure aqueous phase and then mixtures of water and propylene glycol in different proportions were used.

Stabilisation of o/w emulsions by non-ionic surfactants (such as ethylene oxide based surfactants) is mainly due to enthalpic forces. The ether oxygen group of PEO chain contributes to the hydrogen bonding with the surrounding water molecules. Consequently a hydrated layer of PEO chains is formed around the emulsion droplets, this layer provides the repulsive force preventing the emulsion droplet from coming close to each other, hence preventing the coalescence. As the temperature is increased the hydration of the ethylene oxide groups reduces resulting in the decrease in area per head group as well as the thickness of non-ionic surfactant layers increases on reduction of the extended hydration (Claesson and Kjellander, 1986 and Florence and Attwood, 2006). The effect of increasing the temperature on the hydration of ethylene oxide side chains might be similar to that of increasing the amount of propylene glycol in the aqueous phase, ultimately leading to instability of the systems. Higher cloud points indicate a stronger interaction between the surfactant molecule and solvent molecules and hence more stable emulsions. Therefore, there is a correlation between the emulsion stability and the cloud point of non-ionic surfactant solution. Florence et al. (1975) found that the cloud point of
non-ionic surfactant solutions was directly proportional to their HLB value and thereby influencing the emulsion stability.

Figure 2.2 Photomicrographs of benzyl benzoate-in-water/propylene glycol emulsions taken 2h after preparation. The ratio of the water: propylene glycol is (A) 100:0, (B) 90:10, (C) 80:20, (D) 70:30, (E) 60:40 and (F) 50:50. The scale bar is 10 μm.
Figure 2.3 Photomicrographs of benzyl benzoate-in-water/propylene glycol emulsions taken 3 days after preparation. The ratio of the water: propylene glycol is (A) 100:0, (B) 90:10, (C) 80:20, (D) 70:30, (E) 60:40 and (F) 50:50. The scale bar is 10 μm.
2.3.3.2 The cloud point of surfactant solutions

The most commonly used non-ionic surfactants have the polyethyleneoxide (PEO) chain as their hydrophilic group. One property of these non-ionic surfactants in solution is that they become turbid after heating due to the separation of the solution into two phases, namely a dilute and a concentrated surfactant phase. The temperature at which this phase separation occurs is known as the cloud point (lower consolute temperature). The presence of the cloud point is generally considered to be due to growth of the micelles (Brown et al., 1983) or the aggregation of micelles (Gu et al., 1989; Huang and Gu, 1990) resulting in the reduction in the critical micellar concentration (cmc).

The strong attractive interaction between micelles must arise due to the dehydration of the PEO chains when the surfactant solution is heated (Hiemenz and Rajagopalan, 1997). The cloud point is very sensitive to the presence of additives in the system even at very small amounts. Many authors have investigated the effect of additives on the cloud point, additive ranging from ionic surfactants (Maclay, 1956; Marszall, 1989; Gu and Galera-Gómez, 1995), inorganic electrolytes (Schott and Han, 1977; Schott and Royce, 1984) to organic compounds (Marszall, 1977; Kumar et al., 2000). The additives change the surfactant-solvent interactions within the surfactant solution system, and hence affect the cloud point, reflecting changes in the cmc, the HLB value, micelle size and phase behaviour. These alterations may affect the stability of the emulsions. Therefore we studied the cloud points of Triton X-100 solutions as a function of additive (propylene glycol) in attempt to correlate the results with the stability of BB/W emulsions, ultimately to better understand the instability of BB/PG emulsions.

In the absence of propylene glycol the cloud point of 1 %w/w of Triton X-100 solutions is 67 °C, in agreement with the result in the literature (Valaulikar and Manohar, 1985; Marszall, 1988; Gu and Galera-Gómez, 1999). Above 1.25 % w/v of propylene glycol, the cloud point remained nearly constant or decreased very slowly as surfactant concentration increased (Fig 2.4, 0). For Triton X-114 the cloud point of 1% w/v solution is 23.5 °C. In the range of surfactant concentration studied the cloud point of Triton X-114 solutions remain nearly constant or gradually increased as surfactant concentration
increased (Fig 2.4 ♦). This result compares well with the data in literature (Gu and Galera-Gómez, 1995; Koshy et al., 1996).

![Graph showing the effect of propylene glycol on the cloud point of Triton X-100 and Triton X-114 solutions. The cloud points (°C) are expressed as a function of surfactant concentration (% w/w). The closed symbols represent Triton X-114 and the open symbols represent Triton X-100.](image)

**Figure 2.4** The effect of propylene glycol on the cloud point of Triton X-100 and Triton X-114 solutions. The cloud points (°C) are expressed as a function of surfactant concentration (% w/w). The closed symbols represent Triton X-114 and the open symbols represent Triton X-100. This graph represents a series of concentrations of propylene glycol in water (% v/v): (♦ O) 0%, (■ □) 5%, (▲ △) 10%, (○) 15% w/v of propylene glycol.

It is well known that the cloud point increases with the number of ethylene oxide units as seen here the cloud point of Triton X-100 is higher than Triton X-114. On addition of propylene glycol, the cloud point of both Triton X-100 and Triton X-114 were found to be increased (Fig 2.4). A similar effect on the cloud point in the presence of propylene glycol was also observed in the surfactant system of oleic acid conjugated with ethoxylated (10) stearylamine (Liang et al., 2005).

Previous published works have investigated the effect of polar organic additives on the cloud point of Triton X-100 and Triton X-114. Koshy et al. (1996) reported the effect of polyethylene glycols (PEGs) on Triton X-100 and Triton X-114 solutions. The cloud point increased when polyethylene glycols (PEGs) were added to the Triton X-100 solutions. This effect was more pronounced as the concentration as well as the molecular
weights of PEGs increased. For example at the same concentration of PEGs added, the cloud point followed the trend: PEG 400 > PEG 300 > PEG 200. The effect of the addition of PEGs was similar to the effect of an increase in the number of the ethylene oxide groups in the Triton molecules resulting in the increase in the cloud point. This was also suggested earlier by Lin and Metzer (1971). However, the contrary effect was found after adding PEGs (400, 300 and 200) to the Triton X-114 solutions. Similar results were observed by Sharma et al. (2003) who studied the cloud point behaviour of C_{12}E_{n} on the addition of PEG 4000. They suggested that the effect may be due to the additive (PEGs) being solubilised in the core of the micelles. Marszall (1977) also reported that the increase or decrease in the cloud point depended on the ethylene oxide chain length of the non-ionic surfactant.

Gu and Galera-Gómez (1999) investigated the effect of various alcohols and other organic liquids such as acids, esters, ketones, ether, formamide and dioxane on the cloud point of Triton X-100. The presence of alcohols with a carbon chain length < 4 increased the cloud point of Triton X-100, but alcohols with a carbon chain length ≥ 4 (except tert-butanol) decreased the cloud point. The longer the chain length of the added alcohol, the higher the effect on the cloud point depression or increase. This result is in agreement with the data obtained using other non-ionic surfactants (Sobisch and Wustneck, 1992) and certain uncharged polymers (Karlström et al., 1990).

The addition of polar organic liquids such as formic acid, acetic acid, acetone, dioxin and N,N-dimethyl formamide leads to a significant increase in the cloud point, whereas the addition of less polar organic liquids such as methyl acetate, ethyl acetate, ethyl ether and methyl ethyl ketone lead to a decrease in cloud points Gu and Galera-Gómez (1999). It has been suggested that the adsorption of the additives at the micelle/water interface was the key factor determining the change in the cloud point.

We also found that the cloud point temperature was increased in a linear fashion as the concentration of propylene glycol was increased. A comparable relationship could be observed for various concentrations of the surfactant Triton X-100 (from 0.31 to 10 %w/v) (Fig 2.5). Similar results were also observed by Liang et al. (2005).
As propylene glycol is a polar organic liquid, the addition of propylene glycol was expected to affect the cloud point of Triton X-100 solutions. Cloud point behaviour is determined by the balance between attractive and repulsive forces involving with hydrophilic and hydrophobic portions of the surfactant (Kjellander and Florin, 1981 and Claesson and Kjellander, 1986). The rise in the cloud point after adding propylene glycol implies that the solubility of Triton X-100 in the propylene glycol-water mixture was improved. This will reduce the concentration of surfactant molecules at the interface of the emulsion droplet resulting in a decrease in emulsion stability.

Extrapolating the relationship between propylene glycol concentration and the cloud point of Triton X-100 aqueous solutions are shown in Figure 2.6. Such extrapolations would yield a cloud point of Triton X-100 in 100% propylene glycol (Fig 2.6).
**Figure 2.6** The effect of propylene glycol concentration on the cloud point of Triton X-100 aqueous solutions. The extrapolation of this relation is used to estimate the cloud point of the surfactant (Triton X-100) in 100% propylene glycol.

**Table 2.6** The equation of the line obtained by plotting the relation ship between concentrations of propylene glycol on the cloud point of various concentrations of Triton X-100 solutions. The equation was then used to estimate the cloud point of the surfactant in 100% propylene glycol by extrapolation.

<table>
<thead>
<tr>
<th>Triton X-100 concentration</th>
<th>Equation</th>
<th>Cloud point extrapolated to 100% Propylene glycol</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.31</td>
<td>( y = 1.77x + 70.60 )</td>
<td>247.6</td>
</tr>
<tr>
<td>0.62</td>
<td>( y = 1.70x + 69.00 )</td>
<td>239.0</td>
</tr>
<tr>
<td>1.25</td>
<td>( y = 1.77x + 66.35 )</td>
<td>243.4</td>
</tr>
<tr>
<td>2.5</td>
<td>( y = 1.74x + 63.70 )</td>
<td>237.6</td>
</tr>
<tr>
<td>5</td>
<td>( y = 1.74x + 63.45 )</td>
<td>237.7</td>
</tr>
<tr>
<td>10</td>
<td>( y = 1.77x + 70.6 )</td>
<td>237.5</td>
</tr>
</tbody>
</table>
This approach indicated an extremely high cloud point (> 235°C) for Triton X-100 in 100% propylene glycol, hence very strong interaction. The extremely strong interaction between the surfactant and the solvent would imply that the Triton X-100 could not be used as a stabiliser with systems of propylene glycol. This in fact supported the observation that Triton X-100 could not stabilise an emulsion containing benzyl benzoate in propylene glycol as the surfactant molecules were predominantly associated with the bulk of the propylene glycol rather than the interface (propylene glycol and benzyl benzoate).

A hydrated layer of surfactant molecules adsorbed on droplets provide stability of emulsions via steric interaction can be explained by the free energy of mixing of surfactant molecule with the continuous phase (solvent) as (Florence and Attwood, 2006)

$$\frac{\Delta G_m}{kT} = \frac{4\pi}{3} B N_A c^2 \left( \frac{\delta}{2} \right)^2 \left( 3a + 2\delta + \frac{H}{2} \right)$$ (2.5)

where \( k \) the Boltzmann constant, \( T \) is the temperature, \( N_A \) is the Avogadro constant, \( c \) is the concentration of surfactant in the interfacial layer, \( H \) is the distance between two droplets, \( \delta \) is the thickness of surfactant adsorbed layer, \( a \) is the radius of droplet.

This equation can describe the effect of temperature and additives on the emulsion stability as \( B \) is proportional to \((1-\theta/T)\), where \( \theta \) ‘theta temperature’ is the temperature at which the surfactant molecule and solvent have no affinity for each other. When the temperature is raised reaching the point \( T \approx \theta \), results in a zero value of \( B \) and the lost of hydration. Consequently, the hydrated surfactant layer is disappeared (no steric stabilisation) and thus emulsions will be easily coalesced. In this case heating reduces \( \Delta G_m \), a positive value of \( \Delta G_m \) is thus required for emulsion stability.

The cloud point is similar to theta temperature (\( \theta \)), the more addition of propylene glycol the higher cloud point and this cause the negative value of \( \Delta G_m \). Hence, the instability of emulsion occurs.
2.4 Summary

This chapter describes the approach to select the two immiscible phases required in the formation of non-aqueous emulsions. It was also observed that the physicochemical parameters of the liquids as such (e.g. dielectric constant, bulk density etc.) could not be used to predict the immiscibility. A comprehensive estimation of solubility parameters (e.g. Hilderbrand or Hansen solubility parameter) could rather be required to predict the immiscibility of two liquids.

The selection of a suitable surfactant was understood to be the key step in stabilising the o/o emulsions. Since none of the surfactant used in this study could stabilise the o/o emulsion, an approach was adapted to partially replace the aqueous phase (of o/w system) with a water miscible solvent (propylene glycol). A surfactant that will stabilise such systems was then identified. The results of an effect on the propylene glycol concentration on the emulsion stability and the cloud point of surfactant solution systems were studies and could then be used to extrapolate the results to the water free emulsions if the proportion of the non-aqueous solvent is increased.
3.1 Introduction
This chapter mainly discusses non-aqueous emulsions composed of castor oil and silicone oils (dimethicone and cyclopentasiloxane). These were selected because optimal stability could be achieved from these systems compared to other systems studied, as described in the previous chapter. Emulsions with a polar continuous phase such as DMSO, DMF and formamide have a greater similarity with aqueous systems compared with systems comprising two non-polar liquids, which present, therefore a greater challenges. There are relatively few publications on non-aqueous emulsions and even less if systems are composed of both non-polar liquids. Emulsions of castor oil-in-silicone oils of varying viscosity stabilised by octylphenol ethyleneoxide (10) (Triton X-100) have previously been reported by our group. The optimization of the viscosity of silicone oils to obtain maximal stability was examined (Jaitely et al., 2004). Without addition of a stabilizing agent, emulsions comprised of castor oil in silicone oil of different viscosities have also been used as models to study rheological behaviour in an electric field (Ha and Yang, 2000).
In this chapter, we are aimed at defining the physicochemical parameters which contribute to the stabilisation of these systems, the parameters taken into account include the apparent miscibility of surfactants in the oil phases, interfacial tension, resultant globule size and size distribution as well as the rheological properties of the non-aqueous emulsions. Multiple \( \text{o}_1/\text{o}_2/\text{w} \) and \( \text{o}_2/\text{o}_1/\text{o}_2 \) emulsions were also prepared and are described here.

### 3.2 Materials and methods

#### 3.2.1 Materials

Castor oil (Fluka) and silicone oil; cyclopentasiloxane, demethicone (20 cSt, Dow Corning grade 200 silicone fluid) were used as the oil phases. The non-ionic surfactants varying with HLB number in the range from 1.8 to 17.6 that of Sorbitan sesquioleate (Arlacel 83), Sorbitan monostearate (Span 60), Sorbitan tri-oleate (Span 85), PEO 20 sorbitan mono-stearate (Twee 60), PEO 20 sorbitan tri-oleate (Twee 85) were obtained from Fluka (UK) and octylphenol ethylene oxide (Triton X-series) where \( X \) is number of ethylene oxide residues were obtained from Dow Chemical (Belgium), block copolymer surfactants (Univar, UK) and silicone surfactants (Dow Corning, Thailand) all the chemical structure of these surfactants are list in Table 2.1, 2.2 and 2.3 (in Chapter 2).

#### 3.2.2 Determination surfactant miscibility in liquid phases

Miscibility was gauged by adding 10 mg surfactant (1% w/v) in 1 ml to both castor oil and silicone oil; dimethicone and cyclopentasiloxane in 50 x 12 mm a vial. The mixtures were stirred using a Rotamixer (Hook & Tucker) and the apparent miscibility of surfactants in each phase was observed.

#### 3.2.3 Preparation of emulsions

The emulsions were prepared by dispersing each surfactant in the oil phase in which they were most soluble and then adding another oil phase (as disperse phase) at a phase volume ratio of 0.25 or 0.50. Emulsification was carried out for 1 min using a Rotamixer. Similar composition emulsions were also prepared by sonication for 1 min with a probe sonicator (Soniprep 150, Sanyo) at room temperature.
3.2.4 Preparation of multiple emulsions

A two-step process was used to formulate the multiple emulsions. First, the primary emulsions were prepared using a probe sonicator for 1 min. The silicone surfactants DC 3225C (cyclomethicone/PEG/PPG-18/18 dimethicone) and DC190 (PEG/PPG-18/18 dimethicone) were used to emulsify castor oil-in-dimethicone and dimethicone-in-castor oil systems respectively. In the second step the non-aqueous emulsions were dispersed in i) an aqueous outer phase containing Tergitol NP-7 (nonylphenol ethoxylate) to form castor oil-in-dimethicone-in-water \(\text{o}_1/\text{o}_2/\text{w}\) emulsions and ii) the dimethicone phase containing DC 3225C to form dimethicone-in-castor oil-in-dimethicone \(\text{o}_2/\text{o}_1/\text{o}_2\) emulsions. The final emulsification stage was carried out for 1 min using a Rotamixer.

3.2.5 Assessment of emulsion stability

The classical method of particle sizing by direct visual observation of the emulsion was chosen to determine the particle size of castor oil in silicone oil emulsions. Each droplet was measured from enlarged photomicrographs. The statistical validity of particle counting of 300 droplets results in a cumulative distribution in which the error at any value will be less than 8% within a 95 % confidence limit, whereas an error of less than 5 % can be obtained with 2960 particles in the same level of confidence (Becher, 2001). In this experiment, a population of 300 to 500 droplets in each system were sized. In general, in order to carry out the count, the concentrated emulsions were diluted immediately prior to the experiments as dilution can cause changes in properties. Both the physical appearance and determination of droplet size were used to assess the stability of the emulsions.

3.2.5.1 Physical stability of coarse emulsions

Various non-aqueous \((\text{o}/\text{o})\) emulsions were prepared by placing 1% w/v of surfactant in one oil phase; another oil phase at phase volume ratio of 25% was added in a tall 3-ml vial. The vial was subsequently shaken for 1 min by Rotamixer and was left to settle at 25° C. After 24 h the stability of emulsion was observed.
3.2.5.2 Determination of particle size and size distribution

With the three selected surfactants: DC 3225C (Cyclomethicone and PEG/PPG - 18/18 Dimethicone), DC 5225C (Cyclopentasiloxane and PEG/PPG - 18/18 Dimethicone), and DC 9011 (Cyclopentasiloxane and PEG-12 Dimethicone Crosspolymer). A series of o/o emulsions were prepared in usual manner by varying the surfactant concentrations (1%, 3% and 5%) and sonicating for 1 min. After certain storage times, random samples of each emulsion were pipetted onto a slide. The slide was covered carefully with a cover slip avoiding pressure thus preventing the flattening of the particles. Particle sizes were observed by optical microscope (Nikon Microphot-FXA) with x10 and x40 magnification. Random pictures of the slide were taken and used for measuring particle size. Populations of 300-500 globules were sized from each system.

3.2.6 Determination of interfacial tension

The pendent drop method was used to determine the interfacial tension between castor oil and silicone oil. To provide equilibrium measurements, the experiments were carried out at a constant temperature without disturbing the system. The pendant drop method allows a change in interfacial tension to be followed during the drop’s ageing process. A drop hanging from a capillary tip elongates as it grows larger. The shape of the drop may be related to its surface tension using the theory of Bashforth and Adams (Adamson, 1990). Interfacial tension was calculated from the shape of drop (Fig 3.1) where $d_e$ is the equatorial diameter and $d_s$ is the diameter measured at a distance $d_e$ from the apex of the pendent drop. A shape-dependant quantity, $S = d_s/d_e$ can therefore be defined. The $S$ value of corresponding to the $H$ value is obtained from tables (for example, Adamson, 1990 page 31 to 33) and the interfacial tension ($\gamma$) calculated by the equation 3.1.

$$\gamma = \frac{\Delta \rho g d_e^2}{H} \quad (3.1)$$

where $\Delta \rho$ is the density difference of the two liquid phases and $g$ is the gravitational constant.
Solutions of surfactant in silicone fluid were prepared in the concentration range 0.001-10% w/v. The pendant drop method was used to determine the interfacial tension of castor oil in silicone oils. A hanging drop of castor oil was formed by gently releasing castor oil through a 1-ml pipette tip into silicone fluid at each surfactant concentration. All elements of the set-up were located on an antivibration table. The image of the drop was digitally photographed (Nikon ‘Coolpix’ 4500 digital camera) with a fine image quality of 2272 x 1704 pixels. The experimental method was validated by measuring the interfacial tension between \( n \)-hexane and water which was found to be 51.48 ± 1.23 mN/m. This value is comparable with the reported value, 50.38 mN/m (Zeppieri et al., 2001). All measurements were performed at 25 ± 1 °C.

### 3.2.7 Determination of viscosity

The rheological characteristics of the emulsions were examined with a CSL^2500 Carri-Med Rheometer (TA Instruments, West Sussex, UK) using cone-plate geometry. Cone radius and angle were 4 cm and 2 degrees, respectively. The rheometer was run in stepped flow mode at 25°C. Equal volumes of emulsion samples were carefully placed in the middle of the gap. Each sample was measured in triplicate at the volume of 0.3 ml.
3.3 Results and discussion

3.3.1 Determination of surfactant miscibility in liquid phases

To prepare the emulsion for this study, each surfactant was dissolved in one phase before mixing two immiscible liquids together. Consequently, miscibility tests were carried out to assess which dispersed or continuous phase could dissolve the surfactants. Various surfactants were used and their effects on both phases are showed in Table 3.1.

It shows that only three silicone surfactants: DC 3225C, DC 5225C, and DC 9011 were miscible in the two possible continuous phases, dimethicone and cyclopentasiloxane. Other non-ionic surfactants such as Triton X-series, Span 85 and Tween 85 as well as block copolymers seem to be immiscible in the silicone fluid, but most of them were readily miscible in the castor oil. The miscibility between the surfactant and the castor oil may be explained by the presence of the hydroxyl groups of ricinoleic acid which is the major component in castor oil (87%) contributing to hydrogen bonding between the surfactant and the castor oil. There can also be hydrophobic interactions between the hydrophobic portions of the oil and that of the surfactants.

In the case of silicone surfactants such as DC 190, DC 193, and DC 5330 even though they contain silicone backbones, their long polyethyleneoxide (PEO) chains play a dominant role in determining miscibility in castor oil. On the other hand, the silicone backbone in the hydrophobic part of DC 3225C, DC 5225C and DC 9011 is the crucial factor in making these surfactants miscible in the silicone oil.
Table 3.1 Miscibility of the surfactants in each oil phase

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Castor oil</th>
<th>Dimethicone</th>
<th>Cyclopentasiloxane</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conventional surfactants</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Span 85</td>
<td>miscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>Arlacel 83</td>
<td>miscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>Span 60</td>
<td>miscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>Tween 85</td>
<td>miscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>Tween 60</td>
<td>miscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>Triton-X 15</td>
<td>miscible</td>
<td>miscible*</td>
<td>miscible*</td>
</tr>
<tr>
<td>Triton-X 100</td>
<td>miscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>Triton-X 207</td>
<td>miscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>Triton-X 405</td>
<td>immiscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td><strong>Block copolymer surfactants</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synperonic PE/L101</td>
<td>miscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>Synperonic PE/L121</td>
<td>miscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>Synperonic PE/F108</td>
<td>immiscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>Synperonic PE/F127</td>
<td>immiscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td><strong>Silicone surfactants</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DC 190</td>
<td>miscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>DC 193</td>
<td>miscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>DC 5200</td>
<td>immiscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>DC 5330</td>
<td>miscible</td>
<td>immiscible</td>
<td>immiscible</td>
</tr>
<tr>
<td>DC 3225C</td>
<td>immiscible</td>
<td>miscible</td>
<td>miscible</td>
</tr>
<tr>
<td>DC 5225C</td>
<td>immiscible</td>
<td>miscible</td>
<td>miscible</td>
</tr>
<tr>
<td>DC 9011</td>
<td>immiscible</td>
<td>miscible</td>
<td>miscible</td>
</tr>
</tbody>
</table>

Miscible: The solution was clear and transparent after mixing
Miscible*: The solution became clear after leaving it for a period (cloudy in the beginning)
Immiscible: The solution became turbid after mixing or could be seen to form two phases
Molecular structure of the surfactants are shown in the previous chapter (section 2.2.1.2)
3.3.2 Stability assessment for selection of suitable surfactants

It has been pointed out in Chapter 1 that there has been limited research published on non-aqueous emulsions. This makes the choice of suitable surfactant extremely difficult. In aqueous systems, conventional theory for selection of surfactants is based on the concept of the hydrophile-lipophile balance (HLB). The HLB number reflects the relative proportions of the hydrophilic and lipophilic regions of the surfactant molecule. One criterion to select the surfactants used in this experiment was based on the range of HLB number from 1.8 to 17.6. The purpose of this study was to determine the suitable surfactant characteristic to generate stable castor oil-in-silicone oil emulsions and to observe whether the HLB concept in a modified form could be applied to non-aqueous emulsions.

Two destabilisation processes which could be seen in these systems were sedimentation (rather than creaming) and cracking (Fig 3.2). Emulsions which could be restored to their original state by gentle shaking are relatively stable systems. On the other hand, with cracking the process is irreversible and represents unstable emulsions. Various o/o emulsions were left to equilibrate at 25° C and the appearance of emulsion was observed after 24 h (Table 3.2).

Figure 3.2 Physical appearance of emulsions. (A): Sedimentation of an o/o emulsion (castor oil-in-dimethicone) emulsified with silicone surfactant DC 3225 (Cyclomethicone and PEG/PPG-18/18 Dimethicone) after 24 h. (B): Cracking of an o/o emulsion (castor oil-in-dimethicone) emulsified with silicone surfactant DC 193 (PEG-12 dimethicone) after 24 h.
Table 3.2 Stability of castor oil in silicone oil emulsions in the presence of a range of conventional and silicone surfactants

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>INCI Name</th>
<th>HLB value</th>
<th>Stability after 24 h</th>
<th>Castor oil in dimethicone emulsions</th>
<th>Castor oil in cyclopentasiloxane emulsions</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Conventional surfactants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Span 85</td>
<td>Sorbitan trioleate</td>
<td>1.8</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Arlacel 83</td>
<td>Sorbitan sesquioleate</td>
<td>3.7</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Span 60</td>
<td>Sorbitan monostearate</td>
<td>4.7</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Tween 85</td>
<td>Polyoxyethylene sorbitan trioleate</td>
<td>11.0</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Tween 60</td>
<td>Polyoxyethylene sorbitan monostearate</td>
<td>14.9</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Triton-X 15</td>
<td>Octylphenoxypoly (1) ethoxylethanol</td>
<td>4.9</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Triton-X 100</td>
<td>Octylphenoxypoly (9.5) ethoxylethanol</td>
<td>13.5</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Triton-X 207</td>
<td>Octylphenoxypoly (20) ethoxylethanol</td>
<td>16.2</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Triton-X 405</td>
<td>Octylphenoxypoly (40) ethoxylethanol</td>
<td>17.9</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><strong>Block copolymer surfactants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synperonic PE/L 101</td>
<td>PEO₄-PPO₅₆-PEO₄</td>
<td>N/A</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Synperonic PE/L 121</td>
<td>PEO₅-PPO₆₈-PEO₅</td>
<td>N/A</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Synperonic PE/F 108</td>
<td>PEO₁₀₇-PPO₄₈-PEO₁₀₇</td>
<td>N/A</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>Synperonic PE/F 127</td>
<td>PEO₉₅-PPO₆₂-PEO₉₅</td>
<td>N/A</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td><strong>Silicone surfactants</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>DC 190°</td>
<td>PEG/PPG-18/18 Dimethicone</td>
<td>5.6</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>DC 193°</td>
<td>PEG-12 Dimethicone</td>
<td>12.4</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>DC 5200°</td>
<td>Lauryl PEG/PPG-18/18 Methicone</td>
<td>2.2</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>DC 5330°</td>
<td>PEG/PPG-15/15 Dimethicone</td>
<td>N/A</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>DC 3225C</td>
<td>Cyclomethicone and PEG/PPG-18/18 Dimethicone</td>
<td>N/A</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>DC 5225C</td>
<td>Cyclopentasiloxane and PEG/PPG-18/18 Dimethicone</td>
<td>1.7</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
<tr>
<td>DC 9011</td>
<td>Cyclopentasiloxane and PEG-12 Dimethicone Crosspolymer</td>
<td>N/A</td>
<td>√</td>
<td>√</td>
<td></td>
</tr>
</tbody>
</table>

X unstable emulsions, √ stable emulsions, N/A No data available
The results show that only the silicone surfactants could stabilise silicone based emulsions and of those determined only DC 3225C, DC 5225C, and DC 9011 could produce appreciable stability of the castor oil-in-silicone oil emulsions. DC 190, DC 193, DC 5220, and DC 5330 surfactants did not provide stability. None of the conventional non-ionic surfactants or block copolymer surfactants could form stable non-aqueous emulsions. Clearly, the HLB concept could not be applied to these non-aqueous emulsions. Some authors have studied the correlation between emulsion stability and HLB value for example; Niraula et al. (2004) stated that the stability of o/w emulsions decreased with a decrease in HLB values. Zaki et al., 1996 also reported that the coalescence percentage of w/o emulsions was in accordance with the increase in HLB of PPO-PEO block copolymers.

Many publications have reported that the HLB concept was not entirely applicable for selecting suitable surfactants even for w/o and o/w emulsions (Ford and Furmidge, 1966; Boyd et al., 1972; Gullapalli and Sheth, 1999). Al-Sabagh (2002) also stated that there is no relationship between the stability of some emulsions and the HLB of the surfactants employed. It is clear that other factors relating to the physical interactions of the surfactants, for example, are important. Two surfactants can have the same HLB and yet have very different hydrophilic and hydrophobic head groups, for example.

The stability of non-aqueous emulsions could be related to the apparent miscibility of the surfactants in the continuous phase. Since the major approach for the stability of non-aqueous emulsions was to find a suitable surfactant whose two structural parts were selectively soluble in either of the immiscible phases; e.g. use of diblock copolymers of polystyrene and polyisoprene to stabilise DMF/hexane emulsions (Imhof and Pine, 1997b). Similarly, the silicone surfactants can stabilise castor oil-in-silicone emulsions because their molecules contain the bulky silicone backbone part in which found to be preferably miscible with the silicone continuous phase.

The miscibility of the used surfactants in the silicone oil is an important requirement to obtain a stable castor oil-in-silicone oil emulsion. As a general rule, the Bancroft rule, proposes that an effective surfactant for an o/w emulsion should be that it is more soluble
in the water phase and *vice versa*, i.e. a surfactant should be more soluble in the oil phase to produce a w/o system. This could explain why castor oil-in-silicone oil emulsions could be emulsified by the surfactants more soluble in the silicone oil. Among the silicone surfactants used DC 190, DC 193, DC 5200, and DC 5330 could not stabilise the castor oil-in-silicone oil emulsions as they were immiscible with the silicone oil phase. However we could use such surfactants to produce stable silicone oil-in-castor oil emulsions, which emphasises the point of solubility in the continuous phase. Ivanov and Kralchevsky (1997) described the emulsion stability based on equilibrium and dynamic condition of the interfacial film between the droplets. They stated that the films drain much quicker if the surfactant is dissolved in the disperse phase more than in the continuous phase. This concept also helps to recognize the process of chemical demulsification which is one way to separate two immiscible liquid by adding additives such as surfactants that are more soluble in the disperse droplets than in the continuous phase.

Adsorption of a surfactant molecule at the phase boundary can prevent attractive forces between two neighbouring particles by forming an interfacial film, inhibiting close proximity of the droplets. This monomolecular film adjusts itself in that its more polar moiety is oriented towards the aqueous phase and non-polar hydrocarbon moiety towards the oil (Florence and Attwood, 2006) in o/w systems. In the same way, at the castor oil/silicone oil interface the arrangement of silicone surfactant molecule most likely appears in a way that the hydrophobic groups\(^1\) (siloxane backbone) protrude into the silicone phase and the polar groups (PEO chains) embed in the castor oil (Hill, 1999; Nagatani *et al.*, 2001). This adsorbed surfactant layer offers the added steric barrier to the castor oil droplets and hence protects them to coalesce as shown in schematic diagram (Fig 3.3). This steric stabilisation effect which plays a dominant role in the case of non-ionic macromolecule-stabilised systems can also be attributed to the siloxane surfactant

\(^{1}\) *It is difficult to avoid the terms hydrophilic and hydrophobic, although strictly we should employ the terms solvophobic and solvophilic when there is no aqueous component. However as there are two oil phases it can be confusing to use the terms solvophobic and solvophilic without defining solvophbic and solvophbic.*
protecting the castor oil in silicone emulsions by virtue of its molecular size and flexibility (Hill, 2002).

Figure 3.3 A schematic illustration of the assumed orientation of a silicone surfactant copolymer (DC 3225C, DC 5225C) at the castor oil and silicone oil interface (not to scale).

Steric stabilisation is related to the possible conformational changes in the surfactant molecule adsorbed at interface and can be explained in two principal ways. First the ‘osmotic pressure effect’ (enthalpic stabilisation) is caused by the increase in the osmotic pressure between the macromolecular chains on neighbouring particles. To reduce the osmotic pressure the particles move apart. Second, the ‘volume restriction effect’ (entropic stabilisation) is caused by the loss of configurational entropy in a polymeric surfactant molecule, leading to a repulsive force between opposing particles. In non-aqueous systems, the latter concept is more likely to be applicable to describe stabilisation. March and Napper (1977) reported the steric stabilisation of both w/o and o/w emulsions and found that for o/w emulsions the repulsive force could be based on either enthalpic or entropic origins, whereas in the case of w/o only entropic stabilisation was observed.
3.3.3 Determination of interfacial tension

For spontaneous emulsification of two immiscible liquids to occur, a surfactant is required to reduce the interfacial tension at the liquid/liquid interface to close to zero. Adsorption of surfactant aids stabilisation of dispersed emulsion phases by lowering the interfacial free energy at the interface thus aiding initial dispersion and subsequent stability. In addition, the presence of adsorbed surfactant layers at the droplet surface reduces the possibility of droplet coalescence. Therefore, it was important to investigate the interfacial tension between castor oil and silicone oil and attempt to correlate this with the stability of the castor oil in silicone oil emulsions. The selected surfactants were DC 3225C, DC 5225C, DC 9011 and DC 190, the last representing a silicone surfactant immiscible in silicone fluids. The first three surfactants were dissolved in the two silicone fluids, dimethicone and cyclopentasiloxane, and the castor oil drop formed in the silicone at varying surfactant concentrations. The interfacial tensions were measured and plotted against log (surfactant concentration) (Fig 3.4, A and B).

Typical plots of interfacial tension versus log concentration were found. The first portion represented high, more or less invariant interfacial tensions due to the small effect of low surfactant concentrations. In the second and third regions, the interfacial tension value decreases almost linearly with increasing (log) concentration, and shows a break point, and again remain almost constant. The transition point is generally considered to correspond to the critical micelle concentration (cmc).

The interfacial tension of castor oil against silicone oils decreased more obviously in the presence of DC 3225C and DC 5225C than with DC 9011 (Fig 3.4). The interfacial tensions of castor oil-cyclopentasiloxane systems, for example, at 1% levels of DC 3225C, DC 5225C, and DC 9011 are 6.78, 4.92, and 26.16 mN/m, respectively. Of the three surfactants used DC 9011 could not lower the interfacial tension sufficiently and hence could not produce a stable emulsion (as seen with the growth in droplet size in section 3.3.4). The results showed both DC 3225C and DC 5225C, as might be expected from their similar structures had similar properties as far as interfacial tension was concerned. They showed more or less the same in the two silicone fluids.
Figure 3.4  The interfacial tension of castor oil in two silicone oils with three silicone surfactants soluble in the silicones plotted as a function of the logarithm of the surfactant concentration; ♦ DC 3225C, ■ DC 5225C and ▲ DC 9011. The upper graph (A) shows the interfacial tension of castor oil-dimethicone systems as a function of surfactant concentration, the lower graph (B) shows the interfacial tension of castor oil-cyclopentasiloxane systems as a function of surfactant concentration for the same three surfactants.
Similar experiments were carried out for DC 190. Since DC 190 was soluble in castor oil, it was dissolved in this instead of silicone oils. DC 190 decreased the interfacial tension more than DC 3225C or DC 5225C. For example, at a concentration 5% the interfacial tension (castor oil-cyclopentasiloxane) of DC 190 value 0.57 mN/m whereas DC 3225C and DC 5225C values were 5.14 and 4.05 mN/m respectively (Fig 3.5).

**Figure 3.5** The interfacial tension of castor oil-silicone oil systems; • castor oil-dimethicone and • castor oil-cyclopentasiloxane plotted as a function of the logarithm of the silicone surfactant concentration (DC 190: PEG/PPG-18/18 Dimethicone), which is soluble in the castor oil phase rather than the silicone oil phase.

Although DC 190 provided the lowest limiting interfacial tension when compared with DC 3225C, DC 5225C and DC 9011, it did not function as good stabiliser of castor oil-in-silicone oil emulsions. An effective interfacial activity is not always an indicator of an efficient emulsifying agent. On the contrary, for example some natural polymeric emulsifiers, such as gums have been used to stabilise emulsions without substantial lowering of the interfacial tension at interface (Becher, 2001). Ford and Furmidge (1966) stated that a sufficiently strong interfacial film is more important for emulsion stabilisation than a low interfacial tension between the two phases. The mobility of the surfactants at the interface is also an issue, and possibly replenishment of the surfactant
layer from the bulk phase leading to elasticity of the surface film is required, hence the need for there to be a sufficient reservoir of stabiliser in the continuous phase.

The break points of the interfacial curve represent typically the cmc value of the surfactants. The interfacial tension curves (Fig 3.4) showed that the cmc values for the silicone surfactants in this system are approximately at a concentration of 5% (w/v). Table 3.3 and Table 3.4 show the effect of different silicone surfactants as well as their concentration on the interfacial tension at the castor oil/silicone oil interface.

Table 3.3 The interfacial tension of different silicone surfactants at the castor oil/dimethicone interface.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Interfacial tension at 1% (mN/m)</th>
<th>Interfacial tension at 5% (mN/m)</th>
<th>Interfacial tension at 10% (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC 3225C*</td>
<td>9.97 ± 1.43</td>
<td>3.68 ± 0.30</td>
<td>3.51 ± 0.22</td>
</tr>
<tr>
<td>DC 5225C*</td>
<td>11.04 ± 0.94</td>
<td>5.43 ± 0.27</td>
<td>5.31 ± 0.49</td>
</tr>
<tr>
<td>DC 9011*</td>
<td>16.18 ± 1.79</td>
<td>11.48 ± 1.37</td>
<td>11.67 ± 0.51</td>
</tr>
<tr>
<td>DC 190**</td>
<td>4.87 ± 0.35</td>
<td>2.00 ± 0.12</td>
<td>-</td>
</tr>
</tbody>
</table>

Table 3.4 The interfacial tension of different silicone surfactants at the castor oil/cyclopentasiloxane interface.

<table>
<thead>
<tr>
<th>Surfactant</th>
<th>Interfacial tension at 1% (mN/m)</th>
<th>Interfacial tension at 5% (mN/m)</th>
<th>Interfacial tension at 10% (mN/m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DC 3225C*</td>
<td>6.78 ± 0.62</td>
<td>5.14 ± 0.66</td>
<td>4.65 ± 0.54</td>
</tr>
<tr>
<td>DC 5225C*</td>
<td>4.92 ± 0.68</td>
<td>4.05 ± 0.58</td>
<td>4.05 ± 0.49</td>
</tr>
<tr>
<td>DC 9011*</td>
<td>26.16 ± 0.58</td>
<td>25.46 ± 1.01</td>
<td>21.17 ± 2.29</td>
</tr>
<tr>
<td>DC 190**</td>
<td>1.65 ± 0.26</td>
<td>0.57 ± 0.08</td>
<td>-</td>
</tr>
</tbody>
</table>

* Each of DC 3225C, DC 5225C, DC 9011 was dissolved in the silicone oil and the pendant drop of castor oil formed in the silicone oil.

** DC 190 was dissolved in the castor oil pendant drop.
3.3.4 Determination of particle size and size distribution

3.3.4.1 Particle size
As in the previous experiment, the surfactants DC 3225C, DC 5225C, and DC 9011 were found to form stable non-aqueous emulsions. So, only these three silicone surfactants were further evaluated to differentiate more precisely difference in stability. The particle size distribution was determined as a function of surfactant concentration (1%, 3%, and 5% w/v).

Two different silicone oil phases were used to prepare the castor oil in silicone oil emulsions in order to compare the influence of continuous phase on the stability of the observed system. Table 3.5 to 3.7 show the particle size of the castor oil-in-dimethicone emulsions and castor oil-in-cyclopentasiloxane emulsions, respectively at various concentrations with time. All the systems showed increases in droplet size over time.

**Table 3.5** Mean particle size (µm) of castor oil-in-dimethicone emulsions and castor oil-in-cyclopentasiloxane emulsions with DC 3225C as a function of surfactant concentration.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Castor oil/dimethicone emulsions (stabilising with DC 3225C)</th>
<th>Castor oil/cyclopentasiloxane emulsions (stabilising with DC 3225C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1%</td>
<td>3%</td>
</tr>
<tr>
<td>2</td>
<td>4.88 ± 0.95</td>
<td>2.35 ± 0.46</td>
</tr>
<tr>
<td>24</td>
<td>5.26 ± 1.15</td>
<td>3.29 ± 0.46</td>
</tr>
<tr>
<td>96</td>
<td>5.57 ± 1.19</td>
<td>3.52 ± 0.57</td>
</tr>
<tr>
<td>168</td>
<td>5.56 ± 1.28</td>
<td>3.65 ± 0.70</td>
</tr>
</tbody>
</table>
Table 3.6 Mean particle size (μm) of castor oil-in-dimethicone emulsions and castor oil-in-cyclopentasiloxane emulsions with DC 5225C as a function of surfactant concentration.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Castor oil/dimethicone emulsions (stabilising with DC 5225C)</th>
<th>Castor oil/cyclopentasiloxane emulsions (stabilising with DC 5225C)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1%</td>
<td>3%</td>
</tr>
<tr>
<td>2</td>
<td>5.61 ± 1.08</td>
<td>2.36 ± 0.44</td>
</tr>
<tr>
<td>24</td>
<td>5.65 ± 1.34</td>
<td>3.08 ± 0.69</td>
</tr>
<tr>
<td>96</td>
<td>5.86 ± 1.24</td>
<td>3.37 ± 0.74</td>
</tr>
<tr>
<td>168</td>
<td>6.29 ± 1.33</td>
<td>3.59 ± 0.74</td>
</tr>
</tbody>
</table>

Table 3.7 Mean particle size (μm) of castor oil-in-dimethicone emulsions and castor oil-in-cyclopentasiloxane emulsions with DC 9011 as a function of surfactant concentration.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Castor oil/dimethicone emulsions (stabilising with DC9011)</th>
<th>Castor oil/cyclopentasiloxane emulsions (stabilising with DC9011)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1%</td>
<td>3%</td>
</tr>
<tr>
<td>2</td>
<td>6.14 ± 1.18</td>
<td>2.42 ± 0.50</td>
</tr>
<tr>
<td>24</td>
<td>6.13 ± 1.52</td>
<td>3.13 ± 0.76</td>
</tr>
<tr>
<td>96</td>
<td>7.44 ± 1.23</td>
<td>4.11 ± 0.87</td>
</tr>
<tr>
<td>168</td>
<td>7.61 ± 1.74</td>
<td>5.02 ± 0.89</td>
</tr>
</tbody>
</table>

When DC 3225C, DC 5225C, or DC 9011 were used as the surfactants at a concentration of 5%, the lowest mean particle size was observed in both the continuous phases. Among these three, DC9011 provided the least stable emulsion. DC 9011 also showed poorer effectiveness in lowering the interfacial tension when compared at the same concentration. Solubility in the continuous phase and efficient interfacial activity is thus required for the emulsion stability.

As the mean particle size is a key parameter indicating emulsion stability, castor oil-in-dimethicone emulsions appeared more stable than castor oil-in-cyclopentasiloxane emulsions. This could perhaps be explained on the basis of Stoke's law (Chapter 1); an
increase in the viscosity of the continuous phase increases the stability of emulsion. Dimethicone has higher viscosity, 20 cSt compared to 4.2 cSt for cyclopentasiloxane, therefore emulsions composed of dimethicone as continuous phase are more stable.

Clarification of the role of molecular structure (e.g. area per molecule of surfactant at the interface) which could influence to the stability is limited by the lack of exact structures of these surfactants. Manufactures either did not know or were unwilling to pass on the information, and we were unable to source simpler silicone based surfactants.

3.3.4.2 Particle size distributions

Figure 3.6A shows the difference in particle size distribution with the three surfactants for both dimethicone and cyclopentasiloxane. Distribution curve of emulsions formed with DC 9011 is broad and consists of more than one peak. Clearly, emulsions which were prepared with DC 9011 proved to least stable. The system which consists of castor oil/dimethicone with 5% DC 3225C seems to be the best system in this study. The same three surfactants form emulsions of castor oil/cyclopentasiloxane which have broader size distribution (Fig 3.6 B) with larger mean sizes.

![Graphs showing particle size distributions of castor oil-in-silicone oil emulsions stabilised by three silicone surfactants: DC 3225C, DC 5225C and DC 9011.](image)

**Figure 3.6** Particle size distributions of castor oil-in-silicone oil emulsions stabilised by three silicone surfactants; • DC 3225C, * DC 5225C and – DC 9011. (A) Particle size distribution of castor oil/dimethicone emulsions (B) Particle size distribution of castor oil/ cyclopentasiloxane emulsions. (Particle size of emulsions measured 2h after preparation)
In the castor oil-in-dimethicone system with a high surfactant concentration (5%), the shape distribution curve was found to be a narrow single peak. Broader shapes and less distinct peaks were obtained at lower concentrations (Fig 3.7). A similar result was observed in emulsions of cyclopentasiloxane.

Figure 3.7 Particle size distributions of castor oil-in-dimethicone emulsions varying in the concentration (% w/v); ♦ 1, ■ 3 and ▲ 5, of three silicones surfactants A) emulsions with DC 3225C, (B) emulsions with DC 5225C and (C) emulsions with DC 9011. (Particle size of emulsions measured 2h after preparation).
The interfacial tension data could correlate with the mean particle size of castor oil-in-dimethicone emulsions at concentrations approaching cmc. It is found, as expected, that the mean particle size of emulsion decreases with the increase in the surfactant concentration up to the cmc. At concentrations above the cmc, the particle size of emulsions in this system does not decrease significantly with increasing concentration (Fig 3.8). Moreover, the size distribution curves show the following from these results that their profiles become narrower as the concentration approaches the cmc (Fig 3.7). Similar findings were also observed (Goloub and Pugh, 2003). The results also appear similarly in the case oil-in-cyclopentasiloxane emulsion systems.

![Graph](image)

**Figure 3.8** Mean particle size of castor oil-in-dimethicone emulsions as a function of silicone surfactant concentration as well as different type of silicone surfactant; ♦ DC 3225C and ■ DC 5225C. The mean particle size of emulsions was measured using Mastersizer.

### 3.3.5 Rheological behaviour of emulsions

An attempt to understand some of the factors contributing to the stabilisation of castor oil-in-silicone oil was investigated by studying their rheological behaviour. Many studies have been carried out to relate the rheological properties of emulsions to their stability (Campanella *et al.*, 1995; Perrin and Lafuma, 1998; Kontogiorgos *et al.*, 2004). Several factors that affect the emulsion rheology are the particle concentration (phase volume), the viscosity of the continuous phase, the particle size distribution and the interaction between the particles (Barnes, 1994).
A log plot of the viscosity versus shear rate for castor oil-in-dimethicone emulsions stabilised with three different silicone surfactants at the same phase volume ($\phi = 25$) is shown in Fig 3.9 A. Emulsions stabilised with DC 9011 exhibit pronounced shear-thinning behaviour whereas emulsions stabilised with DC 3225C or DC 5225C shows less shear-thinning behaviour over shear rate range tested (fairly Newtonian or near Newtonian behaviour). A shear-thinning or pseudoplastic system is a non-Newtonian fluid characterised by a decrease in apparent viscosity with increasing shear rate or shear stress (Barnes et al., 1989).

A great deal of research has been reported on the shear-thinning behaviour of oil-in-water emulsions (Dickinson et al., 1995; Manoj et al., 1998; Floury et al., 2000; Radford et al., 2004) or water-in-oil emulsions (Pal, 1993; 1997 and Lee et al., 1997) and verified to be due to the flocculation of emulsion droplets. The results may imply that DC 9011 may somehow produce attractive interactions between droplets leading to flocculation or aggregation of castor oil-in-dimethicone emulsions. The flocculated droplets may enhance the sedimentation and lead to coalescence of the droplets. Earlier experiments on the particle size distribution of these emulsions showed that DC 9011 provided less stable emulsion than either DC 3225C or DC 5225C.

The rheograms of castor oil-in-dimethicone emulsions stabilised with DC 3225C, DC 5225C and DC 9011 were also studied as a function of aging time (32 months) and results are shown in Figure 3.9 B, C and D respectively. An emulsion with DC 9011 was affected by ageing as considerable change in the rheological pattern was found (Fig 3.9 D). At the same shear rate, the apparent viscosity of the stored emulsions was significantly decreased compared to the freshly prepared emulsions, especially in the low shear rate range. This may be due to the significant increase in average particle size of emulsions, as shown in Fig 3.10, over the duration of the storage period. Similar results were found by Pal (2000) who concluded that the viscosity of shear-thinning emulsions is strongly influenced by the droplet size; an increase in droplet size resulting in a decrease in the viscosity. On the other hand, the effect of the droplet size does not show any influence on the viscosity of emulsions exhibiting Newtonian behaviour.
Figure 3.9 A plot of log [apparent viscosity] versus shear rate for castor oil-in-dimethicone emulsions using three silicone surfactants (5% w/v): • DC 3225C ▼ DC 5225C and ■ DC 9011. The filled symbols represent the freshly prepared emulsions at (2h) while the open symbols represent the emulsions after storage for 32 months.

In the case of the emulsions stabilised with DC 3225C and DC 5225C, the rheological patterns of emulsions after long storage of time illustrated as slightly shear-thinning behaviour which the viscosity was slightly lower than that from freshly prepared emulsions (Fig 3.10 B, C). Moreover, the rheological behaviour was not affected by ageing as the shape of the flow curve did not change significantly upon storage, compared to emulsions formed with DC 9011. This may be due to the particle size of emulsions formulated with DC 3225C and DC 5225C which did not show pronounced changes (Fig 3.10) to be able to detect by the rheological test. The rheological data and photomicrograph support the stability of emulsions over a long period of time when DC 3225C or DC 5225C are employed as emulsifiers.
Figure 3.10 Photomicrographs of castor oil-in-dimethicone emulsions formulated with three silicone surfactants: DC 3225C, DC 5225C and DC 9011. Here are compared freshly prepared (2h) systems as shown in A, C and E and 32 months after preparation as shown in B, D and F. The scale bar is 20 μm.
3.3.6 Multiple emulsions

3.3.6.1 Oil-in-oil-in-water emulsions

Our previous studies indicated that stable non-aqueous emulsions of castor oil and silicone oil could be formulated using silicone surfactants. Castor oil in silicone oil emulsions with a mean particle size 1.08 ± 0.02 μm were used as the internal phase for preparing castor oil-in-silicone oil-in-water (o₁/o₂/w) multiple emulsions. Different concentrations of hydrophilic surfactant (Tergitol NP-7) from 1 up to 7 % w/v were used to emulsify the primary non-aqueous emulsions into the aqueous outer phase. Photomicrographs of the o₁/o₂/w emulsions demonstrated that the mean particle size of dimethicone droplets (containing castor oil dispersion) decreased with increase in surfactant concentration (Fig 3.11). This may affect the stability of multiple emulsions via the external drop coalescence pathway (Garti and Aserin, 1996).

Figure 3.11 Photomicrographs of o₁/o₂/w emulsions formulated with Tergitol NP-7 as the secondary emulsifier (A) 1 % (B) 3 % (C) 5 % (D) 7 % w/v. The scale bar is 10 μm.
3.3.6.2 oil-in-oil-in-oil anhydrous multiple emulsions

Anhydrous multiple \((o_2/o_1/o_2)\) emulsions could also be formulated by dispersing dimethicone-in-castor oil emulsions in the dimethicone phase. These demonstrated the possibility to formulate either \(o_1/o_2/w\) or \(o_2/o_1/o_2\) emulsions from non-aqueous emulsions (Fig.3.12) and expand the portfolio of emulsion systems.

![Photomicrograph of dimethicone-in-castor oil-in-dimethicone \(o_2/o_1/o_2\) emulsions. The scale bar is 50 \(\mu\)m.](image)

**Figure 3.12** Photomicrograph of dimethicone-in-castor oil-in-dimethicone \(o_2/o_1/o_2\) emulsions. The scale bar is 50 \(\mu\)m.
3.4 Summary

Stable non-aqueous emulsions of castor oil-in-silicone oil could be obtained using silicone surfactants. Such emulsions can show stability over long periods. Among the various surfactants studied only silicone surfactants (DC 3225C, DC 5225C, and DC 9011) were found to be miscible with the silicone oil continuous phase thus stabilising the systems. Interfacial tension studies with these surfactants (DC 3225C, DC 5225C, and DC 9011) showed their effectiveness at lowering the interfacial tension of the castor oil/silicone oil interface. Moreover, DC 3225C and DC 5225C were found to be more effective in lowering the interfacial tension than DC 9011.

Emulsions of castor oil-in-silicone oil with DC 3225C and DC 5225C were found to be more stable than those with DC 9011, as shown by the mean particle size and size distribution studies and are in accordance with the interfacial tension studies. Emulsions stabilised with DC 9011 exhibits pronounced shear-thinning behaviour whereas DC 3225C and DC 5225C shows less shear-thinning behaviour.

Possibly the most vital factor contributing to the stability of these systems is the solubility of the surfactants in the continuous phase, coupled with the bulky side chains of the surfactants which provide steric stabilisation. With this understanding it is possible to tailor suitable surfactant/s to successfully stabilise the castor oil/silicone oil systems. A low interfacial tension between the two oil phases is desirable, but is not sufficient.

Comparing the external phase dimethicone to cyclopentasiloxane, the former showed a slower increase in particle size versus time, most likely due to the fact that dimethicone has a higher viscosity than DC 245 which may lead to a slower rate of particle sedimentation and slower film thinning, reducing the chance for particle surfaces to collide.
Chapter IV

IN VITRO STUDIES: RELEASE OF TWO MODEL DRUGS FROM NON-AQUEOUS EMULSIONS

4.1 Introduction

Conventional emulsions (o/w or w/o), multiple emulsions (o/w/o or w/o/w) as well as emulsions containing dispersed microspheres (Hashida et al., 1977c), or vesicles (Yoshioka et al., 1995) have for a long time been considered as prolonged release carriers. Many papers have stated that the \textit{in vitro} release rate can be modified by varying the physicochemical properties of the emulsion system. Windheuser et al. (1970) demonstrated that the release rate of benzocaine from w/o emulsions could be controlled by several parameters such as the phase volume ratio, the viscosity of continuous phase and the partition coefficient of drug. Other parameters which also have significant influence on drug release are the droplet size of emulsions (Cole and Whateley, 1997), surfactant concentration (Garti \textit{et al}., 1994; Vasiljevic \textit{et al}., 2006), the nature of the surfactant (Jager-Lezer \textit{et al}., 1997), osmotic imbalance in w/o/w systems (Hino \textit{et al}., 1995 and Geiger \textit{et al}., 1998) and the viscosity of the emulsion (Adeyeye and Price, 1990).

Oil-in-oil or non-aqueous emulsions were formulated in the form of castor oil-in-silicone oil systems and showed no phase separation over long storage times (at least 32 months). Such emulsions could be proposed as prolonged delivery systems for hydrophobic
compounds. In this chapter in vitro studies were carried out to explore the potential use of o/o emulsions as anhydrous drug carriers. $^3$H-Dehydroepiandrosterone and $^3$H-dexamethasone were chosen as the lipophilic model drugs and incorporated in the disperse phase (castor oil). From among silicone surfactants, DC 3225C (5%) was selected as the most suitable to emulsify the castor oil-in-dimethicone emulsions for the reason that it provided the most stable system (see Chapter 3).

Two techniques have been used to evaluate the in vitro release behaviour of drug from emulsions - the cell diffusion technique (Friedman and Benita, 1987; Lostritto et al., 1987) and the dialysis bag diffusion technique (Sasaki et al., 1984; Washington, 1989; Levy and Benita, 1990). The cell diffusion technique, which comprises of two chambers separated by dialysis membrane, was designed mainly for transdermal preparations. The dialysis technique therefore was selected to study drug release from all the emulsion systems in this chapter. The aim of this chapter was to examine the feasibility of using non-aqueous systems as vehicles for drug delivery.

4.2 Materials and methods

4.2 Materials

$^3$H-Dehydroepiandrosterone (Dupent/NEN, USA), $^3$H-Dexamethasone (Amersham, UK), dialysis tubing (SnakeSkin®, MWCO 3500, Pierce Chemical Company, U.S.A.) and double-distilled water were used in the release studies. Optiphase 'safe', a radioactive reagent for aqueous and non-aqueous samples, was purchased from Perkin Elmer.

4.2.2 The assessment of the particle size in the presence of water

The castor oil-in-dimethicone emulsions stabilised with DC 3225C was prepared as usual and divided in two equal parts. A 2.0 ml sample was placed in dialysis tubing and this was then placed in 200 ml distilled water. This system was stirred at 25 °C and left for 24 h. After 24 h, the particle size of the emulsion was determined by the photomicrographic method mentioned earlier (see Section 3.2.5.2). A sample (2 ml) of emulsion was kept at the same temperature and particle size was determined after 24 h.
4.2.3 In vitro release

$^3$H-dehydroepiandrosterone and $^3$H-dexamethasone were first dissolved in the castor oil phase at a level of 0.5 mg per 0.5 ml. The drug solution was then added to the dimethicone phase containing 5 % w/v of DC 3225C (silicone surfactant) at phase volume ratio ($\phi$) 0.25 and 0.50. The emulsification was carried out by probe sonication for 1 min. The o/o/w multiple emulsions were prepared by a two-stage emulsification procedure. In the first step the o/o primary emulsion with incorporated drug was formed as described ($\phi_1 = 0.25$). In the second step the o/o primary emulsion was dispersed in an aqueous phase containing 5% w/v of the nonylphenol ethoxylate (Tergitol NP-7) at a secondary phase volume of 0.25 and 0.50 ($\phi_2 = 0.25, 0.50$). The castor oil-in-water (o/w) emulsions were prepared as for castor oil-in-silicone oil (co/so, silicone oil mentioned from this point until Chapter 7 is referred to dimethicone) emulsions but a mixture of polysorbate 20 and sorbitan monooleate (75:25) was used instead of the single silicone surfactant.

A 2.0 ml sample of each emulsion was placed in dialysis tubing and this was then placed in 200 ml of a continually stirred (100 rpm) dialysing medium (PBS, phosphate buffer saline at pH 7.4) maintained at 37 ± 0.5°C. A sample of the dialyzing medium (1 ml) was withdrawn periodically and replaced with an equivalent volume of medium after each sampling. A 1 ml sample containing drug was diluted with 10 ml OptiPhase 'Safe' cocktail. The count rate was recorded by a multi-purpose scintillation counter (Beckman LS 6500) with a 5 min measurement time for each sample. All systems were tested three times followed the same protocol.

4.2.4 Determination of the partition coefficient of drug

The partition coefficient (K) of the following systems was studied: octanol/water, castor oil/dimethicone, dimethicone/PBS and castor oil/PBS; by the shake-flask method. All these systems were conducted by the same procedure. The octanol/water system is mentioned here as an example. Prior to the start of the experiment, both phases (octanol and water) have to be presaturated with each other (Wong and McKeown, 1988). Octanol was equilibrated with an excess of water and left overnight at room temperature and vice
versa. Stock solutions of drug were used; $^{3}$H-dehydroepiandrosterone ($^{3}$H-DHEA) and $^{3}$H-dexamethasone ($^{3}$H-DEXA), prepared in the phase which provided better solubility. For example, in the case of the octanol-water partition coefficient, octanol was used as a solvent to prepare the drug stock solutions. The drug stock solution (1 ml) was added to 80 ml of another phase (water) in the centrifuge tube and left shaking at least 24 h at 37° C to ensure that the equilibrium was reached. The tube was then centrifuged at 2500 rpm for 30 min to separate the two phases. Both phases were then analyzed for radioactivity using liquid scintillation counting. Finally, the octanol/water partition coefficient ($P_{oct/w}$) was calculated using following equation,

$$P_{oct/w} = \frac{C_{oct}}{C_{water}}$$  (4.1)

where $C_{oct}$ and $C_{water}$ are the the drug concentrations found respectively at equilibrium in the octanol and water phase. To obtain reliable values, partitioning experiments were performed at least three concentration levels of drug stock solution covering at least 10 fold ranges (Hansch and Leo, 1995).

4.3 Results and discussion

4.3.1 The assessment of particle size in the presence of aqueous phase

The release of drug from multiple emulsions may occur by mainly two mechanisms (Florence and Attwood, 2006). One is due to the coalescence of internal emulsion droplets resulting in the mass transfer of drug molecules. This mechanism depends upon the physical stability of emulsion and may cause uncontrolled or unpredicted release rate of drug (Chung et al., 2001). The other occurs in the normal or expected manner by diffusion or partition of the solute across the surfactant film barriers. In either in vitro or in vivo studies non-aqueous emulsions come in contact with an aqueous phase. Therefore, the stability of emulsions and the influence of the presence of water or dialysing medium were investigated. To estimate the stability of the emulsion, the mean particle size was measured after contact with water and in the absence of water (Fig 4.1).
These results suggested that there was no significant difference in the stability of castor oil/dimethicone emulsions after contact with water. Without this change in morphology and the size of emulsion particles, it can be assumed that the release of the drug molecule is not the result of differences in the stability of the emulsions. Similar results were observed in that there was no significant change in the mean particle size measured before and after in vitro release under the experimental conditions, using the Mastersizer. The release mechanism therefore can be considered to be due to the diffusion of drug from castor oil droplets through the external oil phase to the aqueous phase.

4.3.2 Measurement of release rate of entrapped drug

4.3.2.1 The release profile of simple o/o emulsions

The release profiles of $^3$H-DHEA and $^3$H-DEXA from non-aqueous emulsions are shown in Fig 4.2. Both $^3$H-DHEA and $^3$H-DEXA release profiles from the emulsions presented similarly with a slow release up to 48 h (Fig 4.2 A and B). Earlier studies in our laboratories of dodecane-in-formamide emulsions, showed the percentage release of $^3$H-DHEA at 24 h to be in approximately 40 % (Sakthivel et al., 2001). This is most likely due to the difference in the solubility of DHEA in each system, as well as differences in
the stability and composition of the emulsions. Release of each drug dissolved in castor oil alone was also investigated as a control experiment.

It was found that the release rate of $^3$H-DHEA from the castor oil control system (Fig 4.2 An) was comparable with the data on emulsions at a phase volume 0.25 (●) but higher when compared with the emulsions of phase volume 0.50 (▼). In the case of DEXA, the release rate from the castor oil control system (Fig 4.2 Bn) was significantly higher than from both the emulsions (● 0.25 and 0.50▼). This suggested an extended release of drug from the emulsions compared to an oily solution. Moreover, these results lead us to conclude that non-aqueous emulsion show beneficial effects over the oily solution as a sustained delivery system for hydrophobic drugs, in part because the drug release can be modified by changing the physicochemical properties of emulsions.

The release rate of both lipophilic drugs from the emulsions is represented in Fig 4.2 as a function of the phase volume 0.25 and 0.50. Emulsions with phase volume 0.25 provided the faster release rate of $^3$H-DHEA and $^3$H-DEXA compared to 0.50 phase volume systems. Friedman and Benita (1987) reported that the increase in the oil phase volume of o/w emulsions reduced significantly morphine release. They inferred that the larger the oil disperse phase which was the rate-determining step in the overall kinetic process, the greater the amount of the drug was located in the oil phase.
Figure 4.2 In vitro release of two lipophilic model drugs from the disperse phase of castor oil-in-dimethicone emulsions into an aqueous dialysing medium (pH 7.4). (A) $^3$H-DHEA: ○ phase volume 0.25; ▼ phase volume 0.50; ■ castor oil alone (B) $^3$H-dexamethasone: ○ phase volume 0.25; ▼ phase volume 0.50; ■ castor oil alone; ● positive control (composed of $^3$H-dexamethasone in a mixture of ethanol and PBS to indicate that the dialysis membrane itself was not a barrier for drug to be released under the experimental conditions)
Generally, the release rate should be faster with increasing phase volume of emulsions because an increase in phase volume leads to a proportional increase in total amount of particles/droplets in the emulsion according to the following equation (Grassi et al., 2000).

\[ N = \frac{6\phi}{\pi d^3} \]  

(4.2)

where \( N \) is the number of emulsion particle/droplet, \( \phi \) is the phase volume and \( d \) is diameter of emulsion. Equation 4.2 is based on the assumption that the emulsion droplets are characterised by a monodisperse size distribution. Bjerregaard et al. (1999) found that the release rate of glucose from w/o emulsions was raised 41% by a 68% increase in the volume fraction of dispersed phase. Similar results occurred with non-aqueous emulsions. Sakthivel et al. (2001) observed the slow release of \(^3\)H-DHEA from dodecane-in-formamide emulsions, and the release rate was slower with lower dodecane volume fractions.

Nevertheless, the contrary results illustrated in Fig 4.2 may be explained due to differences in the particle size of the emulsions. The mean particle size of castor oil-in-dimethicone emulsions at disperse phase volumes of 0.25 and 0.50 were 1.20 and 3.05, respectively. As in equation 4.2, the number of emulsion droplets depends on two variable factors, phase volume (\( \phi \)) and globule diameter (\( d \)). The total particle number of castor oil/dimethicone emulsions with phase volume 0.25 was 8.21 times higher than emulsion at phase volume 0.50.

Windheuser et al. (1970) studied the physicochemical parameters influencing the rate of drug release from w/o emulsions. One of those factors was the particle size of emulsions. Reduction in particle size increases the release rate resulting from the increase in surface area exposed for diffusion. Similar behaviour was observed in this experiment if we consider the specific surface area of the emulsion particles which can be calculated from (Grassi et al., 2000)
where $S$ is the specific area of emulsion particles/droplets, $\phi$ is the phase volume and $d$ is diameter of emulsion. The total surface area of emulsion with a phase volume 0.25 is 1.28 times higher than emulsion at phase volume 0.50. Consequently, the higher release rate was detected as a result of the relative increase in the contact area of totality of emulsion droplets were surrounded by dimethicone continuous phase. Moreover, it was observed that the surface area ($S$) dependency was more pronounced for the release rate of drug from emulsions studied than on the droplet number ($N$). The explanation is that the slightly increase of surface area (1.28) produces a similar release rate, compared to the greater increase in total droplet number (8.21). Similar behaviour was observed by Friedman and Benita (1987).

Hydrophilic drugs are usually incorporated in the aqueous phase of w/o emulsions with oil phase and interfacial layers acting as a release barrier (Davis et al., 1985). Since the biological fluids are miscible with water, the water phase of o/w emulsions in which hydrophobic drugs are dissolved in the oil inner phase may not serve as an effective barrier. One advantage in using o/o emulsions as prolonged release carriers is that they may provide a lower release rate compared to o/w emulsions due to the presence of the external oil phase. This was demonstrated by comparison of the release behaviour of $^3$H-DEXA from both castor oil-in-water (o/w) and castor oil-in-dimethicone emulsions (Fig 4.3).
Figure 4.3 *In vitro* release of $^3$H-DEXA from • castor oil-in-dimethicone emulsions and ▼ castor oil-in-water emulsions into an aqueous dialysing medium (pH 7.4).

4.3.2.2 The release profile from multiple emulsions

In the previous chapter, it was seen that multiple emulsions (o/o/w) could be formulated using o/o emulsions as the internal phase. Therefore, the release of drug from castor oil-in-dimethicone-in-water (o/o/w) emulsions was followed, as shown in Figure 4.4. $^3$H-DEXA release from o/o/w was prolonged up to 48 h. Many multiple emulsions mainly formulated as w/o/w or o/w/o systems have been viewed as being a useful carrier for prolonged delivery system (Omotosho *et al.*, 1989; Sela *et al.*, 1995; Mishra and Pandit, 1990)

The *in vitro* release profile of $^3$H-DEXA from castor oil-in-dimethicone-in-water (o/o/w) multiple emulsions were also studied as a function of secondary phase volume $^2$ ($\phi_2$) between 0.25 and 0.50 (Fig 4.4). It was found that ~18 % and 37 % of drug was released in 48 h from o/o/w systems with secondary phase volumes ($\phi_2$) of 0.50 and 0.25, respectively. These different release profiles can be attributed to the different

$^2$ phase volume of castor oil/dimethicone emulsions dispersing in the water outer phase
physicochemical characteristics of the emulsion. In this case, the external droplet size of o/o/w emulsions at $\phi_2$ of 0.25 were smaller than those of emulsions at $\phi_2$ of 0.50 (Fig 4.5) resulting larger surface area from which drug diffusion could occur. Thus, the $^3$H-DEXA release from emulsion at $\phi_2$ 0.25 was faster than from emulsion at $\phi_2$ 0.50. Okoshi and Nakano (1996) stated that release from the w/o/w emulsions was slow and found to be faster with a decrease in external particle size because of the higher surface area exposed to external water phase.

Although o/o emulsions have shown potential uses as anhydrous vehicles to provide sustained delivery systems, they may have drawbacks due to their viscosity as occurs with w/o systems. For example, the tacky feeling when using this system as a topical application and the difficulty in injection if used as a parenteral. To overcome such limitations, the use of o/o emulsions may replaced with o/o/w emulsions.

![Figure 4.4 In vitro release of $^3$H-DEXA from castor oil-in-dimethicone-in-water (o/o/w) emulsions at secondary phase volume ($\phi_2$) $\triangledown$: 0.25 and $\triangleleft$: 0.50.](image)

Figure 4.4 In vitro release of $^3$H-DEXA from castor oil-in-dimethicone-in-water (o/o/w) emulsions at secondary phase volume ($\phi_2$) $\triangledown$: 0.25 and $\triangleleft$: 0.50.

$^3$ droplet size of dimethicone dispersing in the water outer phase
4.3.3 Determination of partition coefficient

As the $^3$H-DHEA or DEXA molecule has been entrapped within the dispersed droplets, it is important to visualize the diffusion of the drug molecule across the border of the two immiscible interfaces. This involves the partitioning of the drug in the particular phase that controls the release rate. Many authors have studied the release behaviour from emulsion systems and stated that the partition coefficient was an efficient parameter to predict the diffusion rate (Windheuser *et al*., 1970). Therefore, the partition coefficient ($K$) of the model drug has to be measured to determine the factors related to the release profile. Since the drug has to partition across two interfaces, castor oil/dimethicone and dimethicone/PBS, the partition coefficient of $^3$H-DHEA and $^3$H-DEXA in these systems was evaluated (Table 4.1).

Table 4.1 The partition coefficient ($K$) of $^3$H-DHEA and $^3$H-DEXA between two phases
The method used in this study was validated by measuring the octanol-water partition coefficient of \(^3\text{H}-\text{DHEA}\) and \(^3\text{H}-\text{DEXA}\). It was found that octanol-water \(\log K\) values of \(^3\text{H}-\text{DHEA}\) and \(^3\text{H}-\text{DEXA}\) were 3.08 and 1.89, respectively, close to the values obtained from the reference data; DHEA = 3.23 (Hansch et al., 1995) and DEXA = 1.83 (Sjöblom, 1967). Table 4.1 shows that the \(^3\text{H}-\text{DHEA}\) partition is in favour of castor oil rather than dimethicone phase \((K = 229.1)\). The similar result was found for \(^3\text{H}-\text{DEXA}\) partition between castor oil/dimethicone with the larger extent due to the higher \(K\) value \((20417)\) comparing with \(^3\text{H}-\text{DHEA}\). In the case of drug partition between dimethicone/PBS phase, \(^3\text{H}-\text{DEXA}\) was significantly localized in the PBS phase rather than the dimethicone \((K = 7.94 \times 10^4)\) whereas \(^3\text{H}-\text{DHEA}\) was localized in dimethicone rather than PBS phase \((K = 4.07)\).

### 4.3.4 Release profile mechanism considerations

During all the *in vitro* experimental period the emulsions were stable as described in section 4.3.1. Therefore, the release of drug was considered as due to diffusion mechanisms rather to disintegration of the emulsion. The kinetic model proposed is based on previous reports which examined release from a drug carrier enclosed in a small membrane-enveloped compartment (Friedman and Benita, 1987) as represented in Figure 4.6. The overall drug detected in an aqueous dialysing medium deal with the partition between castor oil-dimethicone phase of the emulsion and the permeation of drug through the membrane.

Figure 4.6 shows that a kinetic model can be proposed, if we consider the system to consist of three compartments. Firstly, the castor oil phase is represented as emulsion droplet dispersed throughout the dimethicone continuous phase, which is the second compartment. The last one is the aqueous dialysing medium. Each compartment is separated by the interfacial surfactant boundary and the dialysis membrane.
Figure 4.6 Schematic representation of drug release from castor oil-in-dimethicone emulsions. The drug diffuses from the castor oil droplets to the dimethicone continuous phase and then through the dialysis membrane reaching to the aqueous dialysing medium.

The rate of drug transport from the castor oil droplets to dimethicone continuous phase can be described as a process of mass transfer of drug in the castor oil phase to the dimethicone continuous phase. Consequently, the flux between two phases across the interfacial barrier based on Fick’s law can be given (Boddé and Joosten, 1985; Lian et al., 2004)

\[ J = \frac{1}{A} \left( \frac{dM}{dt} \right)_{1 \rightarrow 2} = P \left( C_1 - K_{12} C_2 \right) \]  

(4.4)

\[ J = \frac{1}{A} \left( \frac{dM}{dt} \right)_{2 \rightarrow 1} = P \left( C_2 - K_{21} C_1 \right) \]  

(4.5)

where \( A \) is the total area of castor oil-dimethicone interface; \( P \) is the permeability coefficient across the interfacial barrier; \( C_1 \) and \( C_2 \) is the drug concentration in castor oil and dimethicone phase, respectively; \( K_{12} \) is the castor oil-dimethicone partition coefficient, and \( K_{21} \) is the dimethicone-castor oil partition coefficient. Equation 4.4 and 4.5 assumes that the presence of the surfactant boundary layer does not affect the total transport and allows partition equilibrium across it. Also the flux between the dimethicone phase and aqueous dialysing medium can be stated as
\[
J = \frac{1}{B} \left( \frac{dM}{dt} \right)_{2 \rightarrow 3} = P_{23}(C_2 - K_{23}C_3) \tag{4.6}
\]

where \( B \) is the surface area of the dialysis membrane; \( P_{23} \) is the permeability coefficient across the dialysis membrane; \( C_2 \) and \( C_3 \) is the drug concentration in dimethicone phase and dialysing medium, respectively, and \( K_{23} \) is the dimethicone-dialysing medium partition coefficient.

These basic kinetic equations were proposed to determine the factors which involve the rate of drug release from co/so emulsions rather than to solve the theoretically mathematic model to fit with the experimental data. The mathematic evaluation of the release profile using kinetic approach is complicated especially in the case of release from colloidal carriers (e.g. emulsion systems). Many constraints arise from the physicochemical properties of the systems studied such as the size of particles, the presence of interfacial surfactant barrier as well as micelles, and various drug-excipient interactions (Ammoury et al., 1990). All these factors are unavoidable and difficult to keep constant.

Equation 4.4, 4.5, and 4.6 demonstrate that the overall level of drug detected in an aqueous dialysing medium is governed by not one but several parameters. However, the effect of partition coefficient is of interest because it depends on the property of drug molecule (in a given system) molecular weight and lipophilicity, whereas the others depend on the emulsion system itself such as the surface area of emulsion particle. Many papers have reported that the release rate of drug from emulsions (Windheuser et al., 1970; Mhando and Po, 1990; Laugel et al., 1996), microemulsions (Grassi et al., 2000), nanoparticles (Duclairoir et al., 2003) which demonstrate that release is strongly dependent on the partition coefficient.

It is shows that the rate of drug release in each phase is correlated to the partition coefficient in term of the higher of partition coefficient (for example) from dimethicone phase to dialysing medium \( (K_{23}) \), the lower of amount of drug release in dialysing medium per unit time (Eq 4.6). Therefore, the partition coefficient may be used as a
parameter to predict the release rate in a comparative fashion for different compounds from the same carrier system. Sasaki et al. (1984) illustrated that the in vitro release profile of mitomycin prodrugs from liposomes or o/w emulsion found to be correlated to the octanol-water partition coefficient of the derivatives in an inverse proportional manner. They evaluated the release rate using the dialysis technique which was similar to the method applied here. However, in the case of castor oil-in-silicone oil (o/o) emulsion, the release behaviour of both drugs; \(^3\)H-DHEA and \(^3\)H-DEXA, was identical (Fig 4.7) and could not be correlated with their octanol-water partition coefficient. This can be explained by the fact that the overall release of both drugs detected in an aqueous dialysing medium, is dependent on \(K_{21}\), \(K_{23}\), and \(K_{12}\) (Eq 4.4, 4.5, and 4.6) as the emulsion is composed of at least three phases (castor oil, dimethicone, and aqueous dialysing medium) which makes the system more complex than o/w emulsions.

Nevertheless, for a less complex system such as an oily solution, the total amount of drug release in aqueous phase was found to be significantly influenced by the magnitude of the partition coefficient (Schultz et al., 1997; Fredholt et al., 2000; Larsen et al., 2001). This behaviour was also investigated in the experiment of the release behaviour of \(^3\)H-DHEA and \(^3\)H-DEXA from castor oil solutions. It clearly shows that the release rate of DHEA from castor oil solution was slower than the release of DEXA from castor oil solution (Fig 4.8). This observation relates well to the castor oil-PBS partition coefficient or even its octanol-water partition coefficient; the greater of the partition coefficient value the lower of the release rate (Table 4.1).
Figure 4.7 *In vitro* release profiles of two lipophilic model drugs from castor oil-in-dimethicone emulsions at phase volume of 0.25 (●) and 0.50 (▼) into an aqueous dialysing medium (pH 7.4). ^H- DHEA and ^H-dexamethasone (DEXA) are represented by filled and open symbols, respectively.

Figure 4.8 *In vitro* release profiles comparing two lipophilic model drugs from castor oil solution alone, into an aqueous dialysing medium (pH 7.4). ^H- DHEA and ^H-dexamethasone were used as the lipophilic model drug is and represented by filled and open symbols, respectively.
4.4 Summary

In vitro release of both model drugs $^3$H-DHEA and $^3$H-DEXA was found to be slow from castor oil-in-dimethicone emulsions lasting up to 48 h. The release of $^3$H-DEXA from non-aqueous emulsions was slower than from castor oil-in-water emulsions or castor oil solutions. Emulsions with the lower phase volume of 0.25 provide faster release of the drug than systems with phase volume 0.50, due to smaller diameter, thus larger surface area of their globules. Slow release of drug was also observed from multiple o/o/w emulsions and their size also affects the release rate in a similar fashion of o/o emulsions. The mechanism of drug release from emulsion is partition between two liquid phases, thus the main factor determining the release rate is the partition coefficient. These results demonstrate the potential use of non-aqueous systems as carriers of water insoluble drug for sustained or controlled release.
5.1 Introduction

Drugs for intramuscular administration were often been formulated as aqueous or oily suspensions or solutions (Dekanski and Chapman, 1953). Other formulations have been developed later in order to prolong the release of the drug to reduce the dose frequency: these include emulsions, liposomes (Arrowsmith et al., 1984; Cabanes et al., 1998), gels (Wenzel et al., 2002) and microparticles (Garcia del Barrio et al., 2004).

The use of emulsions as depot preparations to be given by intramuscular injection was first discussed in the 1970s. Nakamoto et al. (1975) suggested that parenteral administration of mitomycin C (MMC) as an emulsion preparation (either w/o or o/w) was more effective for lymphatic transport than administration as an aqueous solution. These authors reported an increase in the lymph:plasma concentration ratio following the intramuscular injection of w/o emulsions of MMC when compared to o/w emulsions. The absorption of $^{131}$I-iodohippuric acid (a hydrophilic model compound) in the rat from muscle into lymph nodes was enhanced in the following order: aqueous solution < w/o emulsions < gelatin-containing w/o emulsions (Hashida et al., 1977c). More recent
studies on w/o emulsions suggested their possible use as vehicles for sustained release of hydrophilic drugs from intramuscular injection sites (Bjerregaard et al., 2001). Multiple (w/o/w) emulsions have also been studied to achieve prolonged release of hydrophilic compound after intramuscular administration in animals such as the beagle dogs (Florence et al., 1976), the rabbits (Davis et al., 1987) or the rats (Otomosho et al., 1989).

Most of the studies previously discussed have employed w/o or w/o/w emulsions as a vehicle for parenteral administration of hydrophilic molecules. Lipophilic drugs on the other hand, have generally been formulated as oily solutions or suspensions (Tanaka et al., 1974), but do not appear to have been formulated as emulsions for intramuscular administration. However, the use of emulsions may provide some advantages over oily solutions or suspensions because of the flexible properties of emulsions which allow drug release rate to be adjusted by varying parameters such as droplet size, phase volume and viscosity (Bjerregaard et al., 1999). Moreover, lipophilic drugs may be associated with problems of precipitation at the site of injection and this may result in pain at the injection site; however, such precipitation may be minimized if the drug is released slowly from the emulsion. Lee et al. (2002) reported that the use of an o/w microemulsion formulation for clonixic acid (a lipophilic drug) could reduce the pain at the injection site compared with a commercial formulation of lysine clonixinate.

In this chapter, non-aqueous or oil-in-oil (o/o) emulsions are investigated as a potential vehicle for poor water soluble compounds and sustained delivery. Although some in vitro experiments have reported the use of non-aqueous emulsions in sustained/prolonged release (Sakthivel et al., 2001; Jaitely et al., 2004), there is little known about the biological behaviour of these systems. An in vivo study was carried out to evaluate the possibility of non-aqueous emulsions acting as drug reservoirs and which were compared with oil-in-water (o/w) emulsions. Drug absorption from the muscle injection site and its distribution into various organs of animals was also estimated.
5.2 Materials and methods

5.2.1 Materials

$^3$H-dexamethasone (specific radioactivity 261 mCi/mg) (Amersham Biosciences, Buckinghamshire, UK), dexamethasone, hydrogen peroxide, Isoamyl alcohol (Sigma, Gillingham, Dorset, UK) were used. Biosol and Bioscint were obtained from National Diagnostics, Hull, UK. A Polytron homogeniser (PT 3000) Kinematica AG, Switzerland was utilized for tissue homogenizing.

5.2.2 Preparation of $^3$H- dexamethasone emulsions

Dexamethasone 6 mg was dissolved in 2 ml absolute ethanol in a round bottomed flask and $^3$H-dexamethasone 600 µCi were added. The solution was dried in a rotary evaporator. The $^3$H-dexamethasone was incorporated into the castor oil disperse phase to prepare emulsions. Castor oil-in-silicone oil (co/so) and castor oil-in-water (co/w) emulsions were prepared by sonication. Dimethicone was used as a silicone oil in this chapter. The silicone surfactant (DC3225C) and the mixture of polysorbate 20:sorbitan monooleate (75:25) were used as the surfactants to emulsify the co/so and co/w emulsions, respectively. A similar mean particle size for both emulsions was obtained (Mastersizer S, Malvern Instruments, Worcestershire, UK). All emulsions were freshly prepared and the uniformity of distribution of the tracers was checked prior to administration.

5.2.3 Animals

Male, Sprague-Dawley rats (Harlan, Blackthorn, Bicester, Oxon, UK), mean body weight 282 g, were caged in groups of 3 to 4 and acclimatized for at least 7 days before the start of each experiment; animals were allowed free access to diet (Extruded Global Rodent Diet; Harlan Teklad, Blackthorn, Bicester, Oxon, UK) and water. A temperature of 19 to 22 °C was maintained, with a relative humidity of 45–65%, and a light:dark cycle of 12:12h (lights on at 07.00h). All procedures followed the UK Home Office (1989) “Code of Practice for the Housing and Care of Animals used in Scientific Procedures”.

122
5.2.4 Intramuscular injection

Two experiments were carried out. In the first experiment, rats (n = 4) were lightly anesthetized with isoflurane and injected with a single dose of 100 µl $^3$H-dexamethasone in the co/so emulsions into the gastrocnemius (calf) muscle of the lower left hind limb. $^3$H-dexamethasone was given at a dose of 0.1 mg/kg and the specific activity of $^3$H-dexamethasone was 100 µCi/mg. For the injection, a 25G ½ 0.5 x 16 needle (Becton Dickinson, Oxford, UK) was used and care was taken to achieve uniformity of the injection site in all animals. After injection, animals were allowed free access to diet and water. A second (control) experiment was conducted using the same procedure as in the first study but the animals (n = 4) were injected with $^3$H-dexamethasone in the co/w emulsions. In each experiment an additional group of 4 rats served as blank controls, the animals being injected with the corresponding emulsions without drug.

In addition to the two main experiments (above) preliminary studies were performed to validate all procedures (e.g. the presence and location of the emulsion at the injection site and the amount of radioactive drug in different organs).

5.2.5 Blood and organ sampling

In each of the two experiments, four rats were autopsied at 0 (control), 0.5, 2, 4, 8, 24, and 48 h post injection following the intraperitoneal (ip) injection of pentobarbital sodium (Euthatal, Merial, Harlow, Essex, UK). Blood was withdrawn from the abdominal aorta and collected into 4.5 ml lithium-heparin tubes (Sarstedt, Beaumont Leys, Leicester, UK) which were centrifuged (2500g, 10 min, room temperature) and the plasma harvested and stored at -80 °C. All muscle tissue at the injection site and surrounding the tibiofibular was removed and weighed. Organs were removed (contralateral muscle, spleen, liver, adrenals, kidneys, heart, lungs, stomach, cervical lymph nodes, thymus and testes), weighed and stored at -80 °C. For paired organs, both organs were taken.
5.2.6 Preparation for liquid scintillation counting

Tissues were homogenized with an equal amount (w/w) of PBS. An aliquot of 200 mg of tissue homogenate was solubilised in 1.0 ml of tissue solubiliser (Biosol®) and shaken overnight at 55 °C. After incubation, 17 ml of self-acidified (Bioscint®) scintillation liquid was added to each sample and kept in darkness for 72 h before counting in a Beckman LS6500 Multi-Purpose Scintillation Counter (GMI, Ramsey, Minnesota, USA). Coloured samples were decolorized with 200-400 µl of 30% hydrogen peroxide, and isoamyl alcohol was added to reduce foaming.

5.2.7 Pharmacokinetic analysis

Pharmacokinetic parameters for ³H-dexamethasone were determined according to model-independent analysis. The maximum plasma concentration (C_max) and the corresponding time (T_max) were obtained directly from the individual plasma concentration-time profile. The elimination rate constant (k_e) was estimated from the slope of a semilogarithmic concentration-time curve in the terminal phase and the elimination half-life (t_1/2) was then obtained from 0.693/k_e. The area under the plasma concentration-time curve (AUC₀,∞) and area under the first moment curve (AUMC) were calculated by the trapezoidal rule (Gibaldi and Perrier, 1975). The other parameters such as the area under the plasma concentration-time curve from time zero to infinity (AUC₀,∞), mean residence time (MRT) and total body clearance (CL) were calculated using the following standard equations (Wagner, 1993).

\[
AUC_\infty = AUC_0,\infty + \frac{C_i}{k_e} \quad (5.1)
\]

\[
AUMC_\infty = AUMC_0,\infty + \frac{C_i T}{k_e} + \frac{C_i}{k_e^2} \quad (5.2)
\]

\[
MRT = \frac{AUMC}{AUC} \quad (5.3)
\]

\[
CL = \frac{Dose}{AUC} \quad (5.4)
\]

where C_i is the plasma concentration observed at time t (T).
5.2.8 Statistical analysis
Mean values of unpaired data were analyzed using Student’s t-test. Differences between values were considered statistically significant where \( P \) values were less than 0.05.

5.3 Results and discussion
5.3.1 The \(^3\)H-dexamethasone plasma concentration
The \(^3\)H-dexamethasone plasma concentration profiles in the rat following a single intramuscular injection of the castor oil-in-silicone oil and the castor oil-in-water emulsions are shown in Figure 5.1. Statistically significant differences \((P < 0.05)\) were found between co/so and co/w emulsions in the mean levels at each time point except at 48 h. The pharmacokinetic parameters for both formulations are summarized in Table 5.1.

![Figure 5.1](image)

Figure 5.1 A semilog plot of \(^3\)H-dexamethasone plasma concentration as a function of time following the intramuscular injection of single dose of (●) castor oil-in-silicone oil emulsions; (▼) castor oil-in-water emulsions; each datum point is the mean value ± SD of 4 rats; statistically significant differences (\( P < 0.05 \)) were found between two emulsions at each time point except at 48 h.
Table 5.1 Plasma pharmacokinetic parameters following the intramuscular injection of rats with $^3$H-dexamethasone in castor oil-in-silicone oil (co/so) emulsions and castor oil-in-water (co/w) emulsions.

<table>
<thead>
<tr>
<th>Parameter (Units)</th>
<th>co/so emulsions</th>
<th>co/w emulsions</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_e$ (h$^{-1}$)</td>
<td>0.033</td>
<td>0.046</td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>$C_{max}$ (µg/ml)</td>
<td>0.048</td>
<td>0.078</td>
</tr>
<tr>
<td>$t_{1/2}$ (h)</td>
<td>20.87</td>
<td>15.04</td>
</tr>
<tr>
<td>$AUC_{\infty}$ (µg h/ml)</td>
<td>1.23</td>
<td>1.26</td>
</tr>
<tr>
<td>$MRT$ (h)</td>
<td>28.19</td>
<td>19.85</td>
</tr>
<tr>
<td>$CL$ (l/h/kg)</td>
<td>0.08</td>
<td>0.08</td>
</tr>
</tbody>
</table>

*Abbreviations: $k_e$, elimination rate constant; $T_{max}$, time to reach maximum plasma concentration; $C_{max}$, maximum plasma concentration; $t_{1/2}$, elimination half-life; $AUC_{\infty}$, plasma concentration-time curve from time zero to infinity; $MRT$, mean residence time; $CL$, total body clearance.

Following the intramuscular injection of the co/w emulsion a peak plasma concentration ($T_{max}$) of the drug appeared at 2.0 h post dosing (Fig. 5.1). However, following the administration of the non-aqueous emulsions, $T_{max}$ occurred at 4.0 h post dosing. A lower $C_{max}$ was detected after the administration of the non-aqueous emulsions (0.048 µg/ml) compared to the oil-in-water system (0.078 µg/ml). No significant difference was found between the areas under the plasma concentration-time curve ($AUC_{\infty}$), indicating a comparable extents of absorption for both formulations. However, a lower peak concentration with a longer $T_{max}$ confirmed that $^3$H-dexamethasone was absorbed somewhat slower from co/so emulsions than from co/w emulsions.

The mean residence time ($MRT$) is the average time a drug molecule spends in the body, introduced by Yamaoka et al. (1978). Such a parameter was found to be useful for predicting the sustained-release behaviour of the drug after intramuscular administration. The MRT value is 1.42 times greater for co/so emulsions than for the co/w emulsions.
Prolonged release of drug from co/so emulsions was also reflected in the elimination half life ($t_{1/2}$) which was found to be 1.39 times longer.

Although co/so emulsions could provide slower absorption of $^3$H-dexamethasone than from co/w emulsions, both formulations demonstrated modestly prolonged drug absorption when compared to the dexamethasone aqueous solution (Fig 5.2). These results were confirmed with the studies from other laboratories (Hansen et al., 1999; Varma and Mulay, 1980). For example, Samtani and Jusko (2005) were found that dexamethasone was absorbed rapidly reaching the maximum concentration within 45 min after intramuscular dosing of dexamethasone sodium phosphate (aqueous solution). They concluded that the pharmacokinetic profile for dexamethasone given by intramuscular route was not significantly different from the intravenous route. Mager et al. (2003) also reported that a short time of both $MRT$ (7.8 h) and $t_{1/2}$ (5.55 h) were obtained following intravenous injection of dexamethasone sodium phosphate.

![Figure 5.2](image)

Figure 5.2 A plot of dexamethasone plasma concentration as a function of time following (▼) intramuscular (IM) injection of $^3$H-dexamethasone-castor oil-in-silicone oil emulsions; (▲) intramuscular (IM) injection of $^3$H-dexamethasone-castor oil-in-water emulsions; (●) intravenous (IV) injection of dexamethasone sodium phosphate* at a dose of 0.1 mg/kg; (●) intramuscular (IM) injection of dexamethasone phosphate** at a dose of 1 mg/kg. Plasma concentration profiles of dexamethasone sodium phosphate (aqueous solution) were obtained from Mager et al.* (2003) and Samtani and Jusko** (2005).
Dreyfuss et al. (1976) studied the absorption of $^{14}$C-fluphenazine base, $^{14}$C-fluphenazine enanthate and $^{14}$C-fluphenazine decanoate when each compound was administered intramuscularly in sesame oil to dogs. They found that the administration of $^{14}$C-fluphenazine base did not illustrate slow-release characteristics and a large amount was released from the site of injection within 12 h of dosing. The result was confirmed by Luo et al. (1997) who reported that the half-life of fluphenazine following intramuscular fluphenazine decanoate in sesame oil was 30 times longer than the value obtained after dosing of fluphenazine base in sesame oil. These findings indicate that the ester form of fluphenazine is important to produce the slow-release system. The need for using the ester form may be replaced by using co/so non-aqueous emulsions as a reservoir vehicles without modification of such drug molecule.

5.3.2 The absorption of $^3$H-dexamethasone from the muscle injection site

The clearance of $^3$H-dexamethasone from the site of the intramuscular injection of the two types of emulsions is shown in Figure 5.3.

![Figure 5.3](image_url)

**Figure 5.3** Clearance of $^3$H-dexamethasone from the injection site after intramuscular injection of emulsions (●) castor oil-in-silicone oil emulsions; (▽) castor oil-in-water emulsions. The results are expressed as percentage of initial dose remaining in the muscle after injection into rats for up to 48 h. Each datum point is the mean value ± SD of 4 rats.
The drug was cleared more slowly from the non-aqueous formulation than from co/w emulsions, as might be expected, 49% of the drug remaining at the injection site 2h after administration of the co/so formulation compare to 19% for the co/w emulsion. At 4 h post dosing, the percentages were 33% and 4% and at 8 h, 18% and 1%, respectively. The decrease in the clearance from the site of injection was related to the increase of plasma level in both formulations (Fig 5.4).

**Figure 5.4** Relationship between clearance and plasma level of $^3$H-dexamethasone. A) castor oil-in-silicone oil emulsions and B) castor oil-in-water emulsions. The results are expressed as the average amount ($\mu$g) remaining per gram of muscle (injection site) for clearance and as the mean value of plasma concentration ($\mu$g/ml) for the plasma level, as a function of time.
Assuming that amount of drug released from the two emulsions was totally absorbed into the blood, the rate of drug release from the emulsions \( (k_r) \) is equivalent to the rate of drug absorption from the injection site \( (k_a) \). A semilog plot of \(^3\text{H}-\text{dexamethasone} \) remaining at the injection site \textit{versus} time is non-linear, and appears to comprise at least an initial phase of rapid absorption followed by a slower absorption phase (Fig 5.5). The absorption half life \( (t_{1/2}) \) of drug in the immediate post dosing period from the co/so and co/w emulsions was found to be \( \sim 200 \) and \( 50 \) min, respectively \( (t_{1/2} = 0.693/k_a) \).

![Figure 5.5](image)

**Figure 5.5** A semilog plot of \(^3\text{H}-\text{dexamethasone} \) remaining at the injection site \textit{versus} time. \(^3\text{H}-\text{dexamethasone} \) was intramuscularly injected to the animals as emulsions (\( \bullet \)) castor oil-in silicone oil-emulsion and (\( \triangledown \)) castor oil-in-water emulsion. Each data point is the mean value ± SD of 4 rats.

Nevertheless, the absorption half life \( (t_{1/2}) \) at the immediate post dosing was found to be shorter than that calculated at later times. This may be explained by a number of reasons such as varying diffusion coefficient, a longer diffusion path for molecules at the centre of injection site, the impairment of blood or lymph flow from the local site (Sund and Schou, 1964; Ballard, 1968).
Tanaka et al. (1974) and Hirano et al. (1981) studied the absorption mechanism of drug from oily solutions after intramuscular injection and suggested that there were two different absorption routes to be considered. Firstly, drug molecules were taken up together with direct absorption of small oil droplets and secondly drug was absorbed after being transferred from the oily depot into the aqueous phase surrounding the injection site. The latter route appeared to be the major one for absorption, consequently the transport process of drug from the oil phase into the aqueous phase might be the rate-limiting step. However, in the emulsion delivery system the presence of two interfaces; the disperse/continuous phase as well as the continuous phase/aqueous body fluids, has to be considered in estimating the absorption rate of drug after intramuscular injection. The drug concentration in the aqueous body fluids depends on the partition of drug molecules through both interfaces in which the slower transport process is considered as the rate limiting step. This could be explained by Hashida et al. (1977b) who revealed that the clearance from the muscle injection site of $^3$H-5-fluorouracil encapsulated in gelatin microsphere-in-oil (s/o) emulsions was slower than those from water-in-oil (w/o) emulsions.

The extent to which emulsions retain their integrity following intramuscular injection is not known. When water is the continuous phase, this must be lost rapidly leaving the castor oil droplets to coalesce or disperse throughout the muscle tissue. However, drug molecules were released slowly from the co/so emulsion droplets due to their partition through the castor oil/silicone oil interface, as well as the silicone oil/aqueous body fluid phase. Schematic diagram in figure 5.6 describe the presence of interfaces in non-aqueous emulsions.
Figure 5.6 Schematic diagram representing the sequence of drug absorption from emulsion system: A) Castor oil-in-silicone oil emulsions illustrate that First, the drug molecules are transported from emulsion droplets (castor oil) to the continuous phase (silicone oil) (1) where they are then transported further to the aqueous body fluid phase (2). Subsequently, the drug molecules in the aqueous phase diffuse into the blood or lymph capillary (3). B) Castor oil-in-water emulsions illustrate that the drug molecules are transported from emulsion droplets (castor oil) to the aqueous body fluid phase (1) where they are then diffuse further into the blood or lymph capillary (2).

A similar finding was also suggested from the gross pathological findings at the injection site at autopsy. At all time points up to 48 h after intramuscular injection, at autopsy the $^3$H-dexamethasone-co/so emulsion deposits were still clearly visible between the muscle bundles of the hind leg (Fig. 5.7). Conversely, there was no evidence of the $^3$H-dexamethasone-co/w emulsion deposits at the autopsied muscle injection site after administration for 2 h.
Figure 5.7 The thigh of a rat autopsied at 48 h after a single injection of \(^3\)H-dexamethasone-co/so emulsion. The superficial muscle has been dissected away to reveal the emulsion (yellow arrowheads) which appears to be enveloped by a transparent membrane. There is an area of fat below the injected emulsion (black arrows).

5.3.3 The tissue distribution of \(^3\)H-dexamethasone from emulsions

The biodistribution of \(^3\)H-dexamethasone after intramuscular injection in the form of co/so emulsion and co/w emulsion is shown in Table 5.2 and 5.3, respectively. The percentages of the administered dose of the drug (per g of wet tissue) in various organs after the dosing of co/w emulsions were generally higher than the percentages for the co/so emulsion in many of the tissues (Fig. 5.8 and 5.9).
Table 5.2 Tissue distribution of $^3$H-dexamethasone administered as castor oil-in-silicone oil emulsions to rats (0.1 mg/kg). The amount of $^3$H-dexamethasone in each organ is shown at 0.5, 2, 4, 8, 24, and 48 h after single intramuscular injection into the gastrocnemius muscle of the lower left hind limb. The results are expressed as the mean (± SD) percentage of the initial injected dose per organ. (n = 4).

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>Time post injection (h)</th>
<th>0.5</th>
<th>2</th>
<th>4</th>
<th>8</th>
<th>24</th>
<th>48</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
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<td>±0.052</td>
<td>±0.053</td>
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<tr>
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<td>0.063</td>
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</tr>
<tr>
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<tr>
<td></td>
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<td>±0.009</td>
<td>±0.009</td>
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</tr>
</tbody>
</table>

$^a$ Contralateral gastrocnemius muscle

$^b$ Cervical lymph nodes
Table 5.3 Tissue distribution of $^3$H-dexamethasone administered as castor oil-in-water emulsions to rats (0.1 mg/kg). The amount of $^3$H-dexamethasone in each organ at 0.5, 2, 4, 8, 24, and 48 h after single intramuscular injection into the gastrocnemius muscle of the lower left hind limb. The results are expressed as the mean (± SD) percentage of the initial injected dose per organ. (n = 4).

<table>
<thead>
<tr>
<th>ORGAN</th>
<th>Time post injection (h)</th>
<th>0.5</th>
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<th>4</th>
<th>8</th>
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<td>0.028</td>
<td>0.219</td>
<td>0.342</td>
<td>0.256</td>
<td>0.066</td>
<td>0.079</td>
</tr>
<tr>
<td></td>
<td>±0.007</td>
<td>±0.052</td>
<td>±0.063</td>
<td>±0.019</td>
<td>±0.010</td>
<td>±0.006</td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Contralateral gastrocnemius muscle

$^b$ Cervical lymph nodes
Figure 5.8 Tissue distribution of $^3$H-dexamethasone (0.1 mg/kg) administered intramuscularly as castor oil-in-silicone oil (co/so) emulsion to rats. The amount of $^3$H-dexamethasone distributed in each organ is shown at 0.5, 2, 4, 8, 24, and 48 h post dosing. The results are expressed as the percentage of the injected dose per g of wet tissue ± SD (n=4). The inset shows the $^3$H-dexamethasone plasma concentration profile after injection of the emulsion. Muscle is the contralateral gastrocnemius muscle.
Figure 5.9 Tissue distribution of $^3$H-dexamethasone (0.1 mg/kg) administered intramuscularly as castor oil-in-water (co/w) emulsion to rats. The amount of $^3$H-dexamethasone distributed in each organ is shown at 0.5, 2, 4, 8, 24, and 48 h post dosing. The results are expressed the percentage of the injected dose per g of wet tissue ± S.D (n=4). The inset shows the $^3$H-dexamethasone plasma concentration profile after injection of the emulsion. Muscle is the contralateral gastrocnemius muscle.
For both emulsions, the distribution patterns of $^3$H-dexamethasone from 0.5 h to 48 h in each organ reflect the plasma concentration profiles. For example in the case of co/so emulsions, the concentration of drug in all tissues rose from 0.5 h, reaching maximum concentration at 4 h, and then reduced thereafter to 48 h; this pattern of response was similar to that of was concomitant with the plasma concentration profile (see the inset in Fig 5.8). This pattern of response was also observed for the co/w emulsion but have the maximum concentration of $^3$H-dexamethasone in the plasma and tissues occurred at 2 h, post dosing (see the inset in Fig 5.9).

The liver contained the highest concentration of drug at 4 h, followed by the kidneys in the case of non-aqueous emulsion (Fig 5.8). Little radioactivity (a 10 times lower level than in the liver) was detected in the contralateral gastrocnemius muscle, in the thymus gland and in the testes. The uptake of $^3$H-dexamethasone into the tissues at the 4 h time point was in the following order: liver > kidneys > spleen > lung > stomach > adrenal glands > heart > lymph nodes > thymus gland > contralateral gastrocnemius muscle > testes.

The results of tissue distribution obtained from co/w emulsion were generally similar to that of co/so emulsion (Fig 5.9). The highest level of radioactivity was detected at 2 h in liver and the next highest was in the kidneys; the lowest radioactivity was detected in the contralateral gastrocnemius muscle, thymus gland and testes. The ranked order of $^3$H-dexamethasone tissue distribution at the 2 h time point was liver > kidneys > adrenal glands > lung > heart > stomach > lymph nodes > spleen > thymus gland > contralateral muscle > testes.

In the case of both formulations therefore, only minimal amounts of $^3$H-dexamethasone were found outside the muscle injection site apart from in the liver and kidneys; in both these organs a high uptake of radioactivity was detected. This may be due to the fact that the metabolism and excretion of dexamethasone mainly take places in the liver and kidney. Farshi et al. (1996) noted that high level of dexamethasone sodium phosphate was detected in the kidney, spleen and liver after the intramucosal injection of the dose in solution. It has also been reported that there is a high uptake of dexamethasone by the liver, kidney and adrenal glands;
dexamethasone metabolism in the liver is slow and excretion is mainly in the urine (Dumasia et al., 1986; Dollery, 1998; Agnew et al., 2003).

From the biodistribution results (Fig 5.8 and 5.9), the amount of $^3$H-dexamethasone in the major organs, especially in the liver and kidneys, after dosing with the co/so emulsion was relatively lower than with the co/w emulsion. This finding may indicate that the administration of $^3$H-dexamethasone as a non-aqueous emulsion could reduce the systemic drug concentration in various tissues, and this is an important factor if the adverse effects of the drug are being considered. Dexamethasone sodium phosphate encapsulated in liposomes has a similar sustained effect at the site of administration (Farshi et al., 1996).

The amount of $^3$H-dexamethasone in the liver and kidneys at 2 h after injection of the oil-in-water emulsions was approximately 1.80 and 1.95 times higher than for the non-aqueous emulsions (Fig 5.10), result reflected in the plasma level, at 2 h post dosing. This observation, together with the plasma concentration profile may indicate a modest advantage for co/so non-aqueous emulsions, which would therefore have the potential to reduce toxicity to the liver and kidneys.
Figure 5.10 The percentage of $^3$H-dexamethasone distributed in A: the liver and B: the kidneys at 0.5, 2, 4, 8, 24, and 48 h post intramuscular injection. The results compare between (■ ■) castor oil-in-water (co/w) emulsions and (■ ■) castor oil-in-silicone oil (co/so) emulsions. Each data point is the mean value (± SD) of 4 rats.
5.4 Summary

Administration of $^3$H-dexamethasone in a co/so non-aqueous emulsion showed the anticipated slower absorption following intramuscular injection when compared to release from co/w emulsions. This was due to the co/so formulation having a longer $T_{\text{max}}$ together with an increase in the mean residence time ($\text{MRT}$) and a longer elimination half life ($t_{1/2}$). The peak plasma concentration ($C_{\text{max}}$) was 1.6 times lower following intramuscular injection of co/so compared to co/w emulsions. At the muscle injection site, the clearance of $^3$H-dexamethasone from the co/so emulsion was slower than that from the co/w emulsion. After the dosing of the co/w emulsion, drug was distributed throughout the major organs, but especially in the liver and kidneys where levels were higher than with the co/so emulsion. Therefore, co/so non-aqueous emulsions could be useful as drug carriers to avoid high plasma peak levels and related drug toxicity, particularly in cases of a narrow therapeutic index. In addition, such emulsions could be considered as drug carriers for sustained release systems however, further work on the choice of the external oil phase would be necessary, to obtain more significant effects.
Chapter VI

MAGNETIC NON-AQUEOUS EMULSIONS

6.1 Introduction
Magnetic nanoparticles have increasingly received attention for mechanical or electronic applications (e.g. data storage devices) due to the progress in modern technology and miniaturization. Magnetic particles, particularly those containing the iron oxides maghemite ($\gamma$-Fe$_2$O$_3$) or magnetite (Fe$_3$O$_4$) can also be applied in a wide range of in vitro and in vivo biomedical and bioengineering fields.

The magnetic properties of magnetic particles are strongly dependent on their size especially if in the nanometer size range, therefore narrow size distributions or monodispersity is also important. In the nanosized diameter range (5-20 nm) magnetic particles show dramatic changes in their physicochemical properties when compared to their atomic or bulk counterparts (Murray et al., 2001). For example, they exhibit unusual magnetic properties, a superparamagnetic phenomenon, which is demonstrated in a plot of magnetization versus field as having no hysteresis loop since each particle is considered as a single magnetic domain (Goya et al., 2003).

A hysteresis loop is generated when there is a different response on magnetization and demagnetization of magnetic material under an applied field (Fig 6.1). When the applied field is increased, the magnetic material is magnetized and a magnetization curve is produced which is different from the curve obtained during a decrease in the applied field.
Magnetization of material

Figure 6.1 A typical hysteresis loop for a magnetic material showing the magnetic parameters which are obtained from the hysteresis loop - that of saturation magnetization ($M_s$), remanent magnetization ($M_r$) and coercivity ($H_c$). At point ‘a’ and ‘d’ the magnetization approach a maximum value when increase an applied field. After an applied field decrease reaching to zero ($H = 0$) at point ‘b’ and ‘c’, the magnetic material still have a residual magnetization. This magnetization reduce to zero ($M = 0$) at ‘c’ and ‘f’ when an applied field is reverse. Adapted from Leslie-Pelecky and Rieke, 1996).

As the applied field is increased, the magnetization of the material is raised to the maximum value at point ‘a’ which is called the saturation magnetization ($M_s$). When the applied field is reduced from point ‘a’ to point ‘b’ at zero magnetic field ($H = 0$), the magnetic flux remains in the material as residual magnetization. This is called the remanent magnetization ($M_r$). After point ‘b’ the applied field is reversed, the magnetization is reduced to zero at point ‘c’, which is the coercivity ($H_c$).

As the applied field is increased in the opposite direction, the magnetic material again becomes saturated as shown in point ‘d’. The curve is shifted from point ‘d’ to ‘e’ when the applied field is reduced to zero. Finally, the curve is shifted from point ‘e’ to ‘f’ as the applied field increase in the positive direction. Therefore, the hysteresis measurement can be used to characterise magnetic materials by means of several
parameters: coercivity, remanent magnetization, and saturation magnetization (Talapin et al., 2004). These magnetic parameters depend on the size, chemical composition and crystalline structure of the material (Sun and Murray, 1999). The different preparation methods provide magnetic particles with different size, shape and crystalline structure as well as the synthetic procedure (Kim et al., 2001). One of the common methods used is solution phase chemical synthesis (see section 6.2.2). Several approaches have also been used for controlling size and shape during chemical synthesis, such as the use of a water-in-oil microemulsion technique (Pillai and Shah, 1996) and a flow injection technique (Salazar-Alvarez et al., 2006). The latter approach is based on the microfluidic technology in order to obtain magnetic particle with a narrow size distribution.

Magnetic particles are sometimes in the form of colloidal dispersions which is electrostatically or sterically stabilised in water or organic solvents. These are also known as ferrofluids. Various stabilisers have been used to protect the agglomeration between particles. These include oleic acid, cyclodextrin (Bocanegra-Diaz et al., 2004), polymers such as polyacrylamide (Sun et al., 2006). Another advantage of coated with polymer is that magnetic particles can easily bind with biomaterials offering the potential use as carriers in biomedicine and biochemistry fields (Fig 6.2 A).

Many applications of magnetic nanoparticles arise from their possibility to be manipulated by an application of an external magnetic field. These include the use for cell separation and analysis, detection of pathogenic bacteria and viruses, isolation and analysis of nucleic acid as well as for protein purification (Häfeli et al., 1997). Ligand-coupled magnetic beads have been commercially developed as Dynabeads® (Bosnes et al., 1997) (Fig 6.2 B). Superparamagnetic iron oxide particles can be used as contrast agents in magnetic resonance imaging (MRI) (Tiefenauer et al., 1996; Shieh et al., 2005) for diagnostic purposes. Functionalized magnetic nanoparticles can be specially attached to a cancer tumor and heated up by an a.c. magnetic field, resulting in inactivation in the tumor cell namely magnetic fluid hyperthermia (Jordan et al., 1999; Brusentsov et al., 2005).
In the past few decades, iron oxide nanoparticles have also been investigated as a magnetic drug delivery to allow the localization of the drug and site-specific direction to targeted cells as well as minimal interaction and toxicity to normal cells. The magnetic carriers have often been studied to deliver anti-cancer drugs such as adriamycin (Gallo et al., 1989) or doxorubicin (Widder et al., 1981) entrapped in albumin microspheres and paclitaxel binding with an MTC™ (Magnetic Delivered Therapeutics Inc. SanDiego) composed of an alloy of iron and activated carbon (Allen et al., 1997). Magnetic erythrocytes (Vyas and Jain, 1994) and magnetic nanoparticles (coated with polyvinyl alcohol) (Schulze et al., 2005) have also been used for local delivery anti-inflammatory agents to treat painful inflamed joints.

Akimoto and Morimoto (1983) investigated magnetic o/w emulsions to localize chemotherapeutic agents using magnetic guidance to specific sites. The emulsions consisted of methyl-CCNU entrapped into an ethyl oleate based magnetic fluid as a disperse phase and casein solution as a continuous phase. A high concentration of the drug was found higher in the lungs where the magnets were directly applied at the thorax to the both side of rats after intravenous administration. A similar finding was also obtained by Akimoto et al. (1985).
This chapter describes the preparation of magnetic nanoparticles of iron oxide (magnetite, Fe$_3$O$_4$) and their incorporation into the castor oil disperse droplet with dimethicone as the continuous phase in order to formulate magnetic non-aqueous emulsions. The movement and behaviour of these emulsions was investigated under the influence of an applied magnetic field.

6.2 Materials and methods

6.2.1 Materials
Ferric chloride hexahydrate FeCl$_3$·6H$_2$O, ferrous chloride tetrahydrate FeCl$_2$·4H$_2$O, oleic acid were obtained from Sigma (Dorset, UK). Sodium hydroxide, nitric acid, ammonium hydroxide, dichloromethane were obtained from BDH (Leicestershire, UK).

6.2.2 Synthesis of magnetic particles
Two methods, based on co-precipitation of iron II (Fe$^{2+}$) and iron III (Fe$^{3+}$) chloride salt in the presence of strong base, were used to prepare iron oxide nanoparticles (Fe$_3$O$_4$). In the first method, the nanoparticles were prepared using the technique of Massart (1982) as described below.

The preparation of iron oxide nanoparticles begins with the separate preparation of an aqueous solution of 1M FeCl$_3$ 40 ml and 2M FeCl$_2$ 10 ml reagent. A 400 ml of NaOH solution was also prepared, heated and maintained at 100°C with vigorous stirring. An aqueous solution of FeCl$_3$ and FeCl$_2$ was slowly added into NaOH solution. The mixture gradually turned black and was maintained at 100°C for 10 min and then left to cool to room temperature. The precipitated magnetite was collected from the mixture and then agitated with 200 ml 1M nitric acid for 10 min. This step was repeated twice. The recovered precipitate was stirred with 200 ml water for 5 min, a step repeated three times. The magnetic dispersion was centrifuged at 15000 rpm for 1 h and the supernatant was removed. Finally, the magnetite was redispersed in water and brought to a final volume of 10 ml. Figure 6.3 shows the schematic of magnetite preparation (method I).
Figure 6.3 A Schematic showing the synthesis of magnetic iron oxide (magnetite, \( \text{Fe}_3\text{O}_4 \)) using method 1 as follows (1) add of an aqueous solution of \( \text{FeCl}_3 \) and \( \text{FeCl}_2 \) into \( \text{NaOH} \) solution, (2) leave the mixture for 10 min, (3) collect the precipitate, (4) wash with \( \text{HNO}_3 \) and decant, (5) wash with water, (6) centrifuge at 15000 rpm for 1h and (7) lyophilize and carry out powder analysis (X-ray diffraction).
The magnetic nanoparticles were then coated with oleic acid to allow their ready dispersal in the castor oil. The coating method began with dilution of the magnetic dispersion 2 ml by adding 50 ml water. Flocculation appeared after addition of a few drops of 25% NH₃ and the precipitate sedimented using a permanent magnet. The precipitate was washed with 50 ml. Another, 100 ml water was added to the precipitate. After adding 2 ml of oleic acid, all magnetic nanoparticles were transferred to the oil phase within a few minutes. Finally, the black oil dispersion was easily separated from the colourless water phase. This magnetic dispersion was incorporated into castor oil to prepare the castor oil-in-dimethicone emulsions.

The second method for magnetite preparation followed the technique of Wilson et al. (2002). All solutions used in this method were prepared separately and purged with N₂ prior to use for a minimum of 30 min to avoid unwanted oxidation. The first step of the synthesis started with forming magnetite nanoparticles in anaerobic conditions at room temperature. Aqueous solutions of FeCl₃ (0.389M) and FeCl₂ 4H₂O (0.195M) reagents were syringed into a 3-necked, 250 ml, round bottomed flask equipped with a mechanical stirrer and attached with vacuum-tight adapters to maintain an inert nitrogen environment. 10 ml of NH₄OH (50% v/v aqueous) was quickly syringed into the flask with rapid stirring immediately after the addition of aqueous iron salts. The solution quickly turned black indicating formation of the magnetite. The magnetic dispersion was stirred for 30 min under a N₂ atmosphere. After this, the magnetic nanoparticles were grafted with block copolymer (Synperonic L101). This polymer 2 g was dissolved in dichloromethane (CH₂Cl₂) 25 ml and the solution was syringed into the flask and allowed to react with the magnetite for 30 min with stirring. The CH₂Cl₂ was later removed with a nitrogen flow for 2 h and the prepared polymer-magnetite nanoparticle aqueous suspension was neutralized with dilute HCl (25 % v/v aqueous) to a pH 6.5-7. This magnetic dispersion was then transferred to a dialysis membrane and dialyzed against water for three days, refreshing the dialysis water twice daily. Finally, the magnetite nanoparticles were centrifuged 15000 rpm for 1 h. Figure 6.4 shows the scheme of this method (II) of magnetite preparation.
Figure 6.4 Schematic showing the synthesis of magnetic iron oxide (magnetite, Fe₃O₄) using method II as follows (1) add an aqueous solution of FeCl₃ and FeCl₂, (2) add NH₄OH solution, (3) leave the mixture for 30 min, (4) add polymer, leave for 30 min and remove CH₂Cl₂, (5) neutralize the mixture, dialyse and centrifuge at 15000 rpm for 1h and (6) lyophilize and conduct powder analysis (X-ray diffraction).
6.2.3 Characterization of magnetic particles

6.2.3.1 Particle size determination
The average diameter of the magnetic nanoparticles was measured using photon correlation spectroscopy (PCS) using a Malvern 4700C sub-micron multi-angle particle analyzer (Malvern Instruments, Malvern, UK). The diameter determined by PCS is the hydrodynamic diameter which is calculated using Stokes-Einstein equation base on the Brownian movement of particle within dispersing fluid. Measurements were carried out in triplicate to provide an average hydrodynamic diameter (nm) ± SD.

6.2.3.2 Transmission Electron Microscopy
The magnetic nanoparticles were characterised by transmission electron microscopy (TEM). A drop of sample was placed on to a copper grid (Gilder, Grantham, UK) and stained with 1% uranyl acetate. The grid was air dried and photomicrographs were viewed by Philips CM 120 (201L) electron microscope (Eindhoven, The Netherlands) at magnifications ranging from 52,000 to 105,000 at an operating voltage of 120 kV.

6.2.3.3 Powder X-ray diffraction analysis
The crystalline nature of materials can be investigated using X-ray diffraction analysis which is very useful for the initial identification of differences in morphology of crystalline compounds. The spacing in a crystal lattice can be described using Bragg’s equation (Willard et al., 1988)

$$n\lambda = 2d \sin \theta$$  \hspace{1cm} (6.1)

where $n$ is the order of the diffraction, $\lambda$ is the wavelength of the X-ray beam, $d$ is the spacing between atomic planes in the lattice, and $\theta$ is the angle of diffraction. A scanning X-ray powder diffractometer (PW3830 Philips, Netherlands) was used to identify the magnetic nanoparticles synthesized. The radiation used was generated by a Cu, $K\alpha$ monochromatic filter, with a wavelength of 1.5418 Å at 45 kV and 30 mA. Samples were scanned over a range of $2\theta$ values from 5° to 70°.
6.2.3.4 Vibrating sample magnetometer

Basic magnetic properties of materials can be characterised using a Vibrating sample magnetometer (VSM, Lake Shore Model 7304). The results of VSM in this chapter were provided by courtesy of Prof Chao-Ming Fu (National Taiwan University, Taiwan). The magnetization of the magnetic particles (powder) was measured over a range of applied fields between -10,000 and 10,000 oersted (Oe). In the VSM system, the sample is mounted at the end of a rod for sample holder, to be placed between an electromagnet which generates a uniform magnetic field gradient. This applied field induces the magnetic moments of particles to line up with the field. The other end of the sample rod is attached to a vibration unit which generates vertical oscillation at a specific frequency (Fig 6.5). During the measurement, the magnetic sample is oscillated by the vibrating rod and causes a time-varying of magnetic flux which consequently produces induced voltage being sensed by the detection coils. Before measurement, a calibration with standard sample was carefully performed in order to obtain correct value of the magnetization of samples.

The results obtained from the VSM are demonstrated as a hysteresis loop: the magnetization (M) curve of the magnetic material under an applied magnetic field (H) in the positive and negative directions, as discussed in section 6.1.
Figure 6.5 This schematic diagram represents the vibrating sample magnetometer showing the vibration unit and the sample chamber surrounded by a set of sensing coils.

6.2.4 Formulation of magnetic non-aqueous emulsions

A stock magnetic dispersion was prepared by adding magnetite particles (40 mg) to castor oil (2 ml). The emulsions were prepared by adding this castor oil dispersion into the mixture of 5% silicone surfactant DC 3225C in dimethicone (continuous phase) at a phase volume of 0.25. Emulsification was carried out for 1 min using a Rota Mixer. A similar method was used to prepare water-in-dimethicone emulsions to compare the response under an applied field with magnetic non-aqueous emulsions.

6.2.5 Preparation of micropipettes

Borosilicate glass capillaries (inner diameter of 1.17 mm; outer diameter of 1.5 mm) were obtained from Harvard Instruments, UK. The glass capillaries were pulled into micron sized exit diameter tips using a Narishige pipette puller (model PC-10). The micropipettes were placed on a microscope slide and observed the movement of emulsion droplet under an applied magnetic field using an inverted light microscope. A micromanipulator (Narishige, Japan, Model Mo-203) was used to control the movement of the pipettes in the x, y, and z directions.
6.2.6 Image capture

Two types of images were captured. They may be called the centimeter sized image and the micron sized image. The micron sized sample was viewed under a light microscope (Nikon-Microphot-FXA) utilizing a distant lens with Nikon UV light (model HB-10101 AF). The experimental microscope observations were recorded using a video camera, as shown in Figure 6.6. The results of the experiment were captured as MPEG video clips on a computer using Hauppage capturing software (Win TV model No. 404). From each video clip, a sequence of pictures was captured using Imagegrab 30-EN software, edited using Adobe Photo Shop. The centimeter sized images were captured using a similar procedure but a normal camera lens (Nikon) was attached to the video camera.

*Figure 6.6* The experimental imaging set up, showing an inverted microscope to which is attached a micromanipulator, placed on an anti-vibration table. The camera is mounted on the microscope and connected to TV-video which captures the images as a video clip. The clips are subsequently converted to a sequence of images. (This experimental instrument was set up by Dr. Behrooz Nasseri)
6.2.7 Construction of electromagnet

The electromagnet was constructed by Mr Christopher Courtice (Experimental Officer, Department of Pharmacology). An insulated copper wire was coiled around a soft iron which was used as an iron core. Once the electrical current flows in the copper wire, it generates heat, consequently the copper wire may melt inside the coil. The coil thus was attached between the copper wire in which the water can pass through to cool the system as shown in Figure 6.7. Gaussmeter (Hirst Magnetic Intrument Ltd, UK) was used to measure generated magnetic field strength.

![Side perspective image](image)

![Front perspective image](image)

**Figure 6.7** The electromagnet used to generate the magnetic field in the experiment.

6.2.8 Experimental set up

Two main experiment procedures were carried out and are described in this chapter. First, the movement of emulsion samples using an applied magnetic field from an electromagnet was observed (Fig 6.8 A). A small drop of sample was placed on a Petri dish half filled with water allowing free movement of the sample. A series of centimeter sized of images were capture from a video clip. Second, the movement of emulsion samples in which individual droplets were observed using an applied magnetic field from a bar magnet (Fig 6.8 B). Emulsion samples were put in either micropipette or the microchannel of a microfluidic chip, experiments which will be discussed in Chapter 7. The emulsion droplets were viewed under an inverted microscope and captured as a video clip.
Figure 6.8 Schematic diagram representing the experimental set up to obtain the sequence of images. A) The movement of an emulsion drop under an applied field using an electromagnet. B) The movement of emulsion droplets under an applied field using a bar magnet.

6.3 Results and discussion

6.3.1 Particle size of magnetic particles

The morphology of magnetite nanoparticles synthesized by both methods is illustrated in Figure 6.9. TEM photograph of both the samples showed the cubic crystalline shape of magnetite particles with a diameter ~10 nm.
Figure 6.9 TEM images of magnetic nanoparticles synthesized by: method I (A) photo taken before coating the particle with oleic acid and method II (B) photo taken after coating the particle with block copolymer (Synperonic L101). The scale bar is 20 nm.

The mean particle size of the magnetic nanoparticles was also observed using PCS (Table 6.1 and Figure 6.10). There are three categories of mean particle size generated using PCS: intensity, volume, and number mean particle size. These three mean values for each magnetic nanoparticle were found to be different (Table 6.1). For example for uncoated magnetic particles, the mean particle size was 38.30, 15.53, and 9.90 when interpreted by intensity, volume, and number value, respectively.

Table 6.1 The mean particle sizes (nm) of magnetic nanoparticles.

<table>
<thead>
<tr>
<th>Size by</th>
<th>Mean particle size (nm) of magnetic nanoparticles</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Uncoated</td>
<td>Coated with Synperonic L101</td>
</tr>
<tr>
<td>Intensity</td>
<td>38.30 ± 2.75</td>
<td>38.27 ± 3.34</td>
</tr>
<tr>
<td>Volume</td>
<td>15.53 ± 0.83</td>
<td>18.60 ± 1.33</td>
</tr>
<tr>
<td>Number</td>
<td>9.90 ± 0.75</td>
<td>12.87 ± 1.26</td>
</tr>
</tbody>
</table>

*aMagnetic particles synthesized by method I
*bMagnetic particles synthesized by method II
*cThis value obtained from the higher peak in Figure 6.10 B

The difference in the mean value of particle size was due to the aggregation of magnetic nanoparticles as shown in TEM image (Fig 6.10). The mean number value from the PCS is used as this value matches the result obtained from TEM. Although,
the coated particles were found to be more aggregated than the uncoated particles, without this coating magnetic particles could not be dispersed in the castor oil phase. The coated particles were thus used to formulate magnetic non-aqueous emulsions.

**Figure 6.10** The mean particle size distribution of the magnetic nanoparticles uncoated (A) and coated with poloxamer (B). The results expressed as the % in class and the diameter (nm) which is interpreted in three values; intensity (○), volume (▼), and number (■) value.
6.3.2 Powder X-ray diffraction

The powder X-ray diffraction pattern of magnetic nanoparticles is presented in Figure 6.11. A similar pattern was also obtained by others (Pillai and Shah, 1996; Montagne et al., 2002; Xu et al., 2004; Pich et al., 2005).

**A** Magnetic nanoparticles (uncoated)

**B** Magnetic nanoparticles (coated with poloxamer)

*Figure 6.11* The X-ray diffraction patterns of synthesized magnetic nanoparticles uncoated (A) and coated with poloxamer (B). The diffraction peak positions (2θ) were at 30.20, 35.56, 43.26, 53.67, 57.14 and 62.77 as shown in black arrows.
From the X-ray diffraction pattern, the d-spacing value (Å) at the diffraction peak position (2θ) can be calculated from Bragg’s Law. The experimental d-spacing value of both magnetite nanoparticles was found to be similar to the d-spacing value of iron oxide (Fe₃O₄) obtained by Deng et al. (2003). Based on the X-ray diffraction data it can be stated that the iron oxide particles synthesized by both methods are composed of magnetite (Fe₃O₄).

Table 6.2 X-ray diffraction data expressed as the position of peak (2θ) and d-spacing (Å) for magnetic nanoparticles Fe₃O₄.

<table>
<thead>
<tr>
<th>position (2θ)</th>
<th>d-spacing (Å)(experimental value)</th>
<th>d-spacing (Å)ᵃ</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>uncoated</td>
<td>coated SynL101</td>
</tr>
<tr>
<td>18.3362</td>
<td>-</td>
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</tr>
<tr>
<td>30.2025</td>
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<td>2.9592</td>
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<td>2.5298</td>
<td>2.5243</td>
</tr>
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<td>2.0885</td>
<td>2.0914</td>
</tr>
<tr>
<td>53.6702</td>
<td>1.7094</td>
<td>1.7078</td>
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<td>1.6088</td>
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<tr>
<td>62.7741</td>
<td>1.4806</td>
<td>1.4803</td>
</tr>
</tbody>
</table>

ᵃDeng et al., (2003)
ᵇThe peak position was shown in the X-ray diffraction pattern but no value stated

6.3.3 Magnetic properties

The magnetic properties of these magnetic nanoparticles investigated by vibrating sample magnetometer (VSM) are illustrated as the magnetization (M) of the samples versus the applied magnetic field (H) (Fig 6.12). The magnetization of the synthesized particles decreases from the saturation state and reaches zero (H = 0) when the applied field is decreased. This indicates there was no residual magnetization in the synthesized magnetic particles even though the applied field is zero (no remanence effect). With zero remanence and coercivity, these magnetic nanoparticles can be considered as superparamagnetic materials.
Figure 6.12 The plot of magnetization (M) and applied field (H) obtained from the different samples of Fe₃O₄ magnetic nanoparticles. ♦ uncoated (prepared by method I) ■ coated with poloxamer (prepared by method II).

The saturation magnetization which was determined from the plateau region of the magnetization curve (Fig 6.12) was found to be ~ 40 emu/g and 50 emu/g for uncoated magnetic particles and poloxamer coated magnetic particles, respectively. This however may be due to the different method used to prepare the magnetic nanoparticles rather than the presence of the adsorbed layer. The magnetite coated with poloxamer was synthesized under a nitrogen atmosphere (method II) whereas uncoated magnetite was synthesized in open air. The similar results were observed from others who stated that higher saturation magnetization was obtained from magnetic particles prepared under a non-oxidizing environment (Kim et al., 2001).

6.3.4 Magnetic emulsions
The magnetic nanoparticles incorporated in the castor oil were used to form castor oil-in-dimethicone (non-aqueous) emulsions (Fig 6.13).
These results show that magnetic non-aqueous emulsions can be formulated. These magnetic non-aqueous emulsions were used to study their movement under the magnetic field to exhibit their potential use for biomedical application and a few studies of their flow properties were conducted for intrinsic interest.

6.3.5 Effect of an applied magnetic field

A magnetic particle encapsulated in castor oil droplet is a single-domain magnet with a magnetic moment. In the absence of an applied field the magnetic moments are randomly oriented, and thus have no net magnetization (Fig 6.14 A). When an external field is applied, all the particles eventually align their moments along the direction of the field to produce a bulk magnetic moment. The force \( F_m \) acting on each magnetic particle which is induced by the applied field can be defined as (Rosensweig, 1985)

\[
F_m = (m \nabla) B
\]  

(6.2)

where \( m \) is magnetic dipole moment and \( B \) is the magnetic field gradient. This magnetic force will lead the magnetic particles to assemble, forming a chain-like aggregation in the droplet (Fig 6.14 B). If the total magnetic force of magnetic particles in the castor oil droplets is sufficiently high, it will cause droplet movement.
Figure 6.14 Photomicrograph of magnetic particles incorporated in non-aqueous emulsions. With no applied field (A) magnetic particles randomly disperse inside the droplet. An assembly of magnetic particles occurs when an external field (12 V) is applied (B). Schematic representation of the alignment of magnetic particles inside the castor oil droplet under an external magnetic field (H) inducing the magnetic force (Fm) (C).
The direction of the applied magnetic field influences the morphology of the assembly of the magnetic nanoparticles leading to the movement of droplets. When the field was applied in the plane of the emulsion sample (parallel), chain-like structure can be seen (Fig 6.15 B) whereas vertical assembly is formed when the field applied perpendicularly to the plane (Fig 6.15 C).

Figure 6.15 Photomicrograph of water-in-dimethicone emulsions representing an effect of the direction of an applied field (12 V): (A) without the field, (B) parallel direction of field and (C) perpendicular direction of field. The scale bar is 50 μm.

6.3.5.1 Movement of magnetic emulsion droplets in microchannels

The movement of magnetic non-aqueous emulsion droplet was observed under the microscope. Even though a magnetic field generated by an electromagnet can be adjusted, a magnetic bar was used instead due to a limitation of the experimental setup. Thus, the data obtained here are phenomenological observations. Most of the results obtained showed that emulsion droplets could be retained at or not surprisingly, moved directly to the bar magnet. For example in Figure 6.16 A the magnetic bar was placed near one side of the micropipette wall, and emulsion droplets were retained near that wall until magnetic bar was removed. In Figure 6.16 B the magnet bar was placed to the opposite side of the vessel. Consequently the magnetic droplets gradually moved against the flow direction due to the magnetic force ($F_m$) and finally move towards the bar magnet (Fig 6.16 F).
Figure 6.16 Photomicrographic sequences of emulsion droplets moving under an external magnetic field. After change of the position of the bar magnet from A to B, the emulsion droplets move against the fluid flow towards the magnet due to the magnetic force. The scale bar is 30 µm.

Another example of the movement of magnetic emulsion droplets was observed at the diversion region of microchannel where the flow splitting into two (Fig 6.17). After the magnetic bar was placed near one side arm channel the droplets were directed toward that channel as expected. This is a useful model of a simple fluid valve.
Figure 6.17 Photomicrographic sequence of the movement of magnetic non-aqueous directed to one side arm channel where a bar magnet is placed – a model fluid valve effect. The scale bar is 60 μm.
The velocity of emulsion droplets is dependent on the magnetic force exerted by external applied field. If this force is much more than the fluid flow, the emulsion droplets will move against the flow. However in this experiment with the limitation in the size and the magnetic field strength of a magnetic field generator, we could not obtain quantitative data from the microscopic observations. Several papers have recently been reported on the manipulation of magnetic particles on a micro-electromagnet chip (Lee and Westervelt, 2001; Mirowski et al., 2004) with higher sensitivity and more precision compared to bar magnet. The integration of micro-electromagnet and microfluidic systems has also been developed (Smistrup et al., 2005; Guo et al., 2006).

6.3.5.2 Movement of magnetic emulsions in centimetre scale
To visualize the movement of magnetic emulsions and study the factors affecting their viscosity, the experiment was carried out in a larger scale. The response of magnetic non-aqueous emulsions under an applied magnetic field was observed by placing a drop of emulsion sample (in millimeter size) on a Petri dish filled with water allowing free movement of the sample. The movement of magnetic emulsions was externally controlled by the electromagnet.

When the applied magnetic field on, the drop start moving slowly toward an iron core of electromagnet and finally reach another end of Petri dish if the field is maintained (Fig 6.18). When the field is off, the emulsion sample suddenly stops moving. The velocity of the movement depends on the magnetic field strength, which varies along the distance from the iron core of electromagnet. The magnetic property of the particles (i.e. magnetization) and the amount incorporated in the emulsions also affects the velocity.
Figure 6.18 Image sequence (centimeter sized) of the movement of magnetic non-aqueous emulsions from one side toward the electromagnet (with the applied field 10 volts). At time 00.00 s (first image) the emulsion as a drop was placed on the Petri dish filled with water and did not move. Once the field was applied, the emulsion drop started to move toward the electromagnet.

The factors (i.e. phase volume of emulsions and magnetic field strength) which influence the velocity of emulsion sample under the magnetic field were also studied (Fig 6.19). It could be seen that the velocity decreases exponentially with the increase in distance from the iron core as the magnetic field gradient is decreased. The applied field rises from 5 V to 10 V could make emulsions move faster. Similar results also obtained with an increase in phase volume of emulsions with a more pronounced effect than the increase in the applied field. Similar results were observed by Melle et al. (2005) who stated that the magnetic field required for decane droplets to move decreases with the magnetic particle concentration.
Figure 6.19 A plot of the velocity (cm/s) of emulsion samples as a function of the distance from the iron core representing the exponential decay. This also shows the relationship between the velocity and phase volume of the emulsions: 0.25 and 0.50 as well as the different in an external applied field: 5 and 10 Volts.

The correlation between the velocity of emulsions and magnetic field strength generated from electromagnet, can provide a general mean of predicting the velocity from magnetic field strength or vice versa (Fig 6.20). This experiment studied the flow of emulsions considering only magnetic force acting on the emulsion droplets. However, the flow of magnetic particles or magnetic carriers is not only related to magnetic force but also the force of the liquid flow exerted on the magnetic particles (Ally et al. 2005; Kim and Park, 2005).
Figure 6.20 A plot of the velocity (cm/s) of emulsion samples as a function of magnetic field strength generated by electromagnet. This also shows the effect of emulsion phase volume: •) 0.25 and ▲) 0.50.
6.4 Summary

Iron oxide magnetic nanoparticles in size range of ~ 10 nm were synthesized and characterised by X-ray powder diffraction and TEM. The magnetization curve with no hysteresis loop confirmed their superparamagnetic character. Magnetic nanoparticles could be incorporated into the disperse phase to form magnetic non-aqueous emulsions of castor oil-in-dimethicone systems. The movement of such emulsions could be controlled using an external magnetic field with appropriate field strength. The velocity of emulsion droplets was mainly dependent on the amount of magnetic particles incorporated as well as the applied field strength. The possibility of retaining and controlling magnetic non-aqueous emulsions could prove to be important finding for the development of new materials in biomedical technology such as drug delivery system where water is undesirable.
FLOW BEHAVIOUR OF EMULSION DROPLETS IN MICROFLUIDIC DEVICES

7.1 Introduction

This chapter describes studies on the flow behaviour of emulsion droplets in microfluidic devices. Emulsion rheology has been the subjected of many papers. Most rheological studies at low shear rate assume that the individual globules remain separate and intact. A few studies have been conducted into non-aqueous emulsion rheology to study the aging effect on viscosity exerted through droplet size distribution change (Hamill and Petersen, 1966; Reichmann and Petersen, 1973). As mentioned in Chapter 1, microfluidics are of increasing interest in the filed of biomedicine. They can also be used to obtain a variety of parameters including enzyme reaction kinetics (Hadd et al., 1997; Duffy et al., 1999), molecular diffusion coefficients (Kamholz et al., 2001) and viscosity (Lee and Tripathi, 2005; Srivastana et al., 2005; Guillot et al., 2006). Here we use microfluidic devices, not to measure viscosity but to study the flow behaviour of some emulsions as we believe that this is intrinsically interesting and may provide some fundamental properties to aid development of both emulsion and microfluidic sciences, some relevant to some processing issues.
The motivation for doing the experiments in this chapter was first to study the flow of emulsion droplets at Y- or T-junctions to mimic the flow of droplets in vessels of the biological situation after administration, although of course non-aqueous systems would not be given by the intravenous route. Emulsion globules can be considered to be models of a variety of elastic delivery vehicles in this regard. The ability to formulate systems in which the properties of both the disperse phase and the external phase can be adjusted is a technical advantage in both basic and applied work. Movement of disperse systems in porous and complex media (not only bifurcating blood vessels) is an important topic. An example for this issue is the study of the flow of blood in the circulation which is the most common topic in the subject of biofluid mechanics. Due to the complexity of biological systems the study of fluid or particle movement in vivo is complicated; therefore the simulation model of the flow is very challenging. Even though the microfluidic devices used here do not entirely represent channels of biological vessels, they provide some information which may lead to better understanding of non-aqueous systems, or biological system, or even can create novel applications, such as production of double emulsion systems (Okushima et al., 2004). Most of the data in this chapter is visual. In this sense they are phenomenological observations (videomicroscopic clips printed here a sequences of images).

In this study, there were mainly four phenomena considered in the flow of emulsion droplets inside the channels of the microfluidic chips, 1) chaining, 2) oscillatory movement of droplets, an unexpected finding, 3) the breakup and 4) the formation of new droplets, a phenomenon which has been observed by other authors (Nie et al. 2005; Xu et al., 2005a; Nguyen et al., 2006; Tan et al., 2006).

7.2 Materials and methods
7.2.1 Materials
The microfluidic devices were obtained from ThinXXS GmbH (Mainz, Germany) and Epigem (Redcar, UK). The device was connected to a multi-channel cartridge pump model 205U/CA (Watson-Marlow Breddel Pumps, Cornwall, UK).
7.2.2 Microfluidic devices

Two patterns of microfluidic devices were used. One was called the ‘Snake mixer’ slide which is made of the transparent cyclic olefin copolymer. The slide contains five channels with different diameter sizes (Fig 7.1). Each channel can be connected to the others by attaching them through the tube connector. We have employed the chip for its individual channels rather than using it as a mixer. Therefore some channels with the larger sized diameters have not been used, mostly due to the difficulty in capturing images under the microscope. The other chip is made of polymethyl methacrylate (PMMA) (Fig 7.2). It represents as one bifurcating and complex connecting channel system. This chip was used only in the experiments on droplet breakup phenomena.

Figure 7.1 (A) The illustration of the ‘Snake mixer’ slide. The channel size diameter from left to right is of (1) 100 μm (2) 640 μm (3) 320 μm (4) 320 μm (5) 320 μm (Not to scale) (detail from ThinXXS GmbH). All the slide dimensions are shown in mm. (B) Image shows the ‘Snake mixer’ slide to provide an idea of size.
7.2.3 Preparation of emulsions
The emulsions were prepared by adding the disperse phase (castor oil) into the mixture of silicone surfactant DC 3225C in dimethicone (continuous phase) at a phase volume of 0.25 as described in chapter two. Emulsification was carried out for 1 min using a Rotamixer. For many of studies it was important to have large droplets so that their properties could be captured microscopically.

7.2.4 Image capturing
The motion of droplets was viewed under an inverted microscope (Nikon-Microphot-FXA). The experimental observation from the microscope was recorded using a video camera. The images were captured as described in Chapter 6.

7.2.5 Experimental set up
There are two main experimental procedures carried out in this chapter. First, emulsion samples were directly injected to the microfluidic devices using the pressure from a syringe. Both microfluidic chips were used in this method and results were observed. Figure 7.3 shows the schematic diagram represents to the first method.

Figure 7.2 The diagram of the second microfluidic chip (Not to scale). All the slide dimensions are shown in mm.
Figure 7.3 Schematic diagram representing the experimental set up of the first method. The emulsion samples were injected into the microchannel of both microfluidic chips. A) Using the ‘Snake mixer’ slide: samples were injected mainly to three different microchannels (red encircled) and observed under the inverted microscope as discussed in section 7.3.1 (chaining association) and section 7.3.2 (oscillatory movement). B) Using the more complex chip: samples were injected to the bifurcating and complex microchannel system and the results were discussed in section 7.3.4 (droplet breakup).

In the second method, samples were place to the ‘Snake mixer slide’ using the pressure from a multi-channel pump which was connected to the microfluidic devices through Teflon microtubes (inner diameter 0.5 mm, Brendal). In this method the disperse and continuous phases were driven into the same channel through separate inlets. One inlet was filled with a mixture of silicone oil and 5% silicone surfactant (DC 3225C) and the other with the castor oil, as shown in Figure 7.4. The same flow rate was used from the pump at 3 rpm and this was kept constant during the experiment. Both phases were driven into the channels through separate inlets.
Figure 7.4 Schematic diagram showing the experimental set up of the second method. Both castor oil and dimethicone phases were pumped into the microchannel through the opposing inlet. The emulsion droplets were then formed at the Y junction (red encircled) as discussed in section 7.3.3 (droplet formation).

7.3 Results and discussion
7.3.1 Chaining linear or quasi-linear association of emulsion droplets
Aggregation of colloidal particles in chainlike formations has been examined in several situations. For example, magnetic emulsion droplets are attracted to each other by dipole moment interactions under a magnetic field, as in our experiments (Fig 7.5) the details of which are discussed in chapter six where the chaining of droplets is seen as the particles become polarized. Many authors have been used chainlike behaviour to directly measure the interaction forces between colloidal particles (Calderon et al., 1994; Dimitrova et al., 2004).
Figure 7.5 Chaining behaviour of magnetic emulsion droplets (see chapter 6) under a magnetic field. In the absence of the magnetic field (A) the droplets do not move. After applying an electromagnetic field (12 volts) for 5.71(B), 6.74 and 24.50 s magnetic emulsion droplet form chain-like association. The magnetic (w/o) emulsion composed of water droplets dispersing in the dimethicone emulsifying with silicone surfactant (DC 3225C). Under the magnetic field, the magnetic particles become polarized and each particle acquires a dipole moment parallel to the field. The attractive interactions between the dipoles induce the formation of chains of droplets. The scale bar is 20 μm.

Not only does a magnetic field induce the assembly of particles, but electric fields also can induce chain formation (Fig 7.6 A) as in electrorheological fluids (Wu and Conrad, 1998; Wang et al., 2001; Shen, 2006). Without an applied field, self chain-like assemblies of water droplets were also reported by Poulin et al. (1997) (Fig 7.6 B). They exhibited a novel class of emulsion composed of water droplets in an anisotropic liquid (a nematic liquid crystal) or a multiple emulsion of water-in-nematic liquid crystal-in-water. The presence of water droplets disturbs the nematic field ordering of the nematic liquid crystal molecule. The distortion of the nematic field causes the dipole moment of the droplet to align either parallel or antiparallel to the nematic field. This dipole-dipole interaction between the droplets accounts for the attractive force leading to the formation the chain highlighted in Figure 7.6 B.
Figure 7.6 A) Photomicrograph of glass beads (radius 10-15 μm) suspended in silicone oil under an external electric field of 1 kV/mm, in an image adopted from Shen et al. (2006). B) Photomicrograph of a nematic multiple (water-in-nematic liquid crystal-in-water) emulsion taken under crossed polarizers. A chain of water droplets forms inside a bigger droplet of the nematic liquid crystal. An image adopted from Poulin et al. (1997).

We surprisingly found that the castor oil-in-dimethicone emulsion droplets began to move as a chain especially when the droplets were flowing relatively slowly in the microchannels. This behaviour is similar to other configurations shown in other dipole systems such as magnetic emulsions, electrorheological fluid and nematic emulsions, but in our work in some systems it occurred in the absence of either applied magnetic or electric fields. The chain formation of non-aqueous emulsion droplets was often observed close to the wall of the microchannels resulting in occasionally joining between two nearby chains across the microchannel, as shown in Figure 7.7 and Figure 7.8. In Figure 7.7 shows the chaining of large and small globules approximately in the axis of the channel. The chain is not always linear but as in the example at 15.00 s (see red squared in Fig 7.7) the chain is more complex.
Figure 7.7 Photomicrographic sequence of chain formation of droplets of castor oil in the continuous phase of dimethicone containing silicone surfactant. In this case the particles are flowing near the central axis of the microfluidic vessel. The droplets move with an average flow rate of 240 μm/s. Black arrows depict joining of two separate chains. Red square box depict the complex association as quasi-linear. The scale bar is 100 μm.
Figure 7.8 Photomicrographic sequence showing chain formation of castor oil emulsion droplets in dimethicone containing silicone surfactant. F) It also illustrates the movement of the liquid in the channel (red arrows) which has the highest velocity at the centre of microchannel (black arrow). The chains follow the velocity profile and in H the chain is seen to separate. The scale bar is 100 µm.

Figure 7.8 clearly shows the anticipated velocity gradients across a microchannel with the maximum velocity at the axis and slowest movements at the vessel walls. This flow behaviour, known as ‘laminar’ flow, describes the flow of liquid/gas in a cylindrical pipe in which the flow can be predicted using the Poiseuille equation (Morrison and Ross, 2002). Turbulence which occurs where laminar flow breaks down characterises the movement of liquid in a chaotic way as illustrated in Figure 7.9. The chain formation allows the clear visualization of this well-known phenomenon. The rupture of the chain is a result of the velocity gradient at this point (Fig 7.8 H).
Figure 7.9 Diagram representing the flow behaviour of fluids in a cylindrical tube. A) shows turbulent flow: the liquid moves right to left but in a highly disorganized manner. This generally occurs in large scale channels at high velocities. B) shows laminar flow: the liquid also moves from right to left but in a highly organized manner showing linear velocity of each parallel layers. This behaviour often happens in small scale channels (i.e. microchannel). The application of microfluidics (i.e. mixing) based on this concept as is described in Chapter 1. The flow can be quantified in terms of the unitless parameter, Reynolds number $(Re) = uL/v$, where $u$ is the mean fluid velocity, $L$ is diameter of the pipe (tube) and $v$ is the kinematic viscosity of fluid. The critical Reynolds number for a pipe is $\approx 2300$ and above this value the liquid flow becomes turbulent and less predictable.

The chain-like behaviour of castor oil droplets at the channel wall in Figure 7.8 may be encouraged by the slow movement of the liquid near to the wall/surface of the microchannel. When globules associate with each other with attractive dispersion forces ($F_a$), they do not coalesce due to a repulsive steric force ($F_r$). There is however, another force caused by the fluid phase flow. As stated above this can be considered to move as parallel layers, each layer will move with a velocity directly proportional to the distance from the wall as shown in figure 7.9. This force per unit area ($A$) known as shear stress ($F_s$) generates the flow and may be defined as

$$F_s = \eta \frac{dv}{dr}$$  \hspace{1cm} (7.1)
where \( \eta \) is the viscosity of fluid, \( \frac{dv}{dr} \) is the velocity gradient. In Figure 7.8 H, consider the droplets on the point of separation where \( Fa = Fs \), the attractive force \( (Fa) \) between the globules thus can be obtained using equation (7.1) and found to be 0.034 Pa (\( \eta = 0.020 \) Pa.s obtained using rheometer in Chapter 3 and \( \frac{dv}{dr} = 13.58/8 \)). At the periphery (walls) \( Fs \) is very low and \( Fa \) predominates \( (Fa > Fs) \) resulting in chain formation but at the centre, at one stage \( Fs > Fa \) and the chain ruptures (Fig 10).

![Diagram representing the shear stress (Fs) exerted on castor oil droplets at each layer of the liquid with different viscosities. At position near to the wall of droplets 1 and 4, Fs encourages the chain formation. At the central of the microchannel, Fs aid the separation of the chain as seen in Figure 7.8 H.](image)

**Figure 7.10** Diagram representing the shear stress \( (Fs) \) exerted on castor oil droplets at each layer of the liquid with different viscosities. At position near to the wall of droplets 1 and 4, \( Fs \) encourages the chain formation. At the central of the microchannel, \( Fs \) aid the separation of the chain as seen in Figure 7.8 H.

From these results, the study of droplet flow in the microchannel can be used as a direct technique to obtain the attractive force between two droplets by measuring the force applied by the fluid flow. Although this chain-like formation observed is not always easy to control. A similar approach using electrorheological fluids in which the chains formed in an applied electric field has been addressed by Fu and Resca (1996) and Atten et al. (1997).
7.3.2 Oscillatory movement of emulsion droplets

During experiments on emulsion flow in microchannels, another phenomenon was occasionally observed with some of emulsion droplets, that of oscillatory movement as shown in Figure 7.11.

![Figure 7.11 Photomicrographic sequence of the oscillation of an emulsion droplet in a period less than 1s. From (a) to (e) 0.04 sec, (e) to (Q) 0.44 sec, and (Q) to (U) take 0.04 sec (total 0.8 second). The scale bar is 20 μm.](image)

This is a complex phenomenon: the droplets oscillate even when the liquid flow is from right to left (see in Fig 7.12). The oscillations are rapid with the rate shown in Figure 7.12.
Figure 7.12 Diagram represent the oscillation movement of droplet in one cycle. The lower graph plotted between the velocity of droplet (from Fig 7.10) and time.

As we observed, this behaviour usually occurred near to the surface of the microchannel where the shear forces are lower. In previous microscopic scale studies, some important characteristics have been observed when droplets flow along the fibres (Mullins et al., 2004a; 2004b; 2005). Those features include droplet formation and coalescence, droplet evaporation, droplet detachment by drag forces as well as oscillation of the liquid droplets. These authors believed that the droplet oscillation was induced by the transition region where the flow transforms from a purely laminar flow to turbulent flow. They also found that there are two types of this movement, transverse and radial oscillation and that there was evidence of vertical oscillation (Fig 7.13). The study of droplet oscillation is important especially in the fibrous filter system because most droplets will remain attached to the fibres until the flow around droplets reaching the oscillation region. Such behaviour is expected to have an effect on the flow and particle capture inside a fibrous filter (Mullins et al., 2006).
However, the oscillatory motion in our experiments was different from the above mentioned as we also observed vertical oscillation in which the droplets move forward and backward in a direction perpendicular to the liquid flow, in addition to the radial and transverse oscillation shown in Figure 7.14.
Figure 7.14 Oscillation and chaining of propylene glycol-in-silicone oil emulsions captured at intervals 3 s. The transverse and vertical oscillation of droplets is seen (pink encircled droplets). The scale bar is 100 μm.

Nevertheless, it might be that this is the driver: the particle disengages from one layer of fluid flow to be propelled into the central axis layer and escapes to the low velocity region. Otherwise it appears if there are electrical forces involved, that the particle is charged and discharged. However both walls will have the same electrical and surface properties. Similar to globule chain formation, oscillation often occurs near the wall where slow fluid flow. Therefore, other factors might also be considered to have an effect on both chaining and oscillation; these include the interaction of droplet and wall, interaction between the droplets (disperse phase), interaction between the
continuous phase and also the effect of surfactant which affects the interaction between the droplets.

These experimental data have also demonstrated the existence of chain formation and oscillatory movement of droplets in other emulsion formulations using the same experimental set up as shown in the Table 7.1. The experiment was carried out in a similar way to that of the experiments with the castor oil-in-dimethicone emulsion, but the disperse phase or surfactant was varied.

**Table 7.1** The flow behaviour of emulsions in microfluidic devices

<table>
<thead>
<tr>
<th>Emulsion formulations</th>
<th>Chain association</th>
<th>Oscillatory movement</th>
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<tbody>
<tr>
<td>1. Castor oil-in-dimethicone</td>
<td>++++</td>
<td>++++</td>
</tr>
<tr>
<td>2. Propylene glycol-in-dimethicone</td>
<td>+++</td>
<td>++++</td>
</tr>
<tr>
<td>3. Water-in-dimethicone</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>4. NaCl solutions-in-dimethicone</td>
<td>4. 0.1 % NaCl</td>
<td>++</td>
</tr>
<tr>
<td>5. 1% NaCl</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>6. 10% NaCl</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>7. Benzyl benzoate-in-dimethicone</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>8. Castor oil-in-dimethicone*</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

*Formulation 8 emulsified with Triton X-100 1% w/v

*Formulation 1 to 7 emulsified with the same silicone surfactant 1 % w/v (DC 3225C)

Evidence of the behaviour with the degree observed from + to ++++ - no evidence of the behaviour

We observed that with the change of surfactant from a silicone based emulsifier to Triton X-100, no evidence either of chaining or oscillation of droplets occurred. Different disperse phase also affected the degree of these occurrences. On the other hand, the degree did not change when increasing the dielectric constant of the disperse phase (water). These findings suggested that the polymeric surfactants with high molecular weight and disperse phase may involve the change in interactions between the droplets, continuous phase, wall surface and somehow affect the behaviours of droplets inside the microchannels. Moreover, the charge on the droplets has less
influence on these interactions because the surfactant used non-ionic and dimethicone has a low dielectric constant.

The subject of gas/liquid flow behaviour has many applications in the fields of chemical engineering, biomedical engineering and fluid dynamics. Many flow types of liquid or gas have been proposed, mainly in the form of mathematical models including oscillatory flow of gas in the model of human central airways (Tanaka et al., 2001) or of dispersion in an annulus or small vessels (Sarkar and Jayaraman, 2004). Our findings provide the image evidence of at least two types of flow behaviour which might be used to develop mathematical models of these complex processes. Further work is required to determine the source of the oscillatory behaviour and also the boundary conditions in which these phenomena occur.

7.3.3 Formation of droplets

Many studies on the formation of emulsion droplets using microfluidic devices have been conducted (Sugiura et al., 2001a; Nisisako et al., 2004). Using the same microfluidic chip used in the studies of droplet flow above, we successfully formed three different sizes of monodisperse emulsion droplets at the Y-junction of a microchannel (Fig 7.4). The experimental study began by supplying 2 liquids (castor oil and dimethicone) in separate inlets of the microchannel as briefly shown in Figure 7.15. Pressure driven flow was used to push each liquid into the microchannel. Figure 7.15 shows the sequence of photomicrographs for three sized droplets were appearing orderly at the junction (from Fig 7.15 A to C) representing the first series. The second series, with three sizes of droplets was later performed (from Fig 7.15 D to F) followed by the third (from Fig 7.15 G to I) and then last by the fourth series (from Fig 7.15 J to L). Each series comprises of three different sized droplets generated with the size in length at 160, 25 and 100 μm.
Figure 7.15 Schematic representing of the formation of castor oil droplets at the Y-junction of a microchannel driven by the flow of the continuous phase (upper image). The photomicrographic sequences show the formation of droplets having three different sizes. The first droplet formed begins with the flow of castor oil phase from one side arm channel into the other side arm at a flow rate of 6 μl/min. The continuous phase flow (120 μl/min) comes from the main channel entering the side arm channel (outlet) and splits the castor oil segment into the first droplet (A). After that, the second droplet is formed suddenly (B) and followed by third droplet (C). After a series of three different sized droplets was formed, another series (D to F) of the same size was later generated and follow by series G to I and J to L. There are 4 series shown here over a period of 30 s. An average time for droplet formation in each series [i.e. from image (C) to (F)] is 9.53 ± 0.45 s. The scale bar is 100 μm.
The first droplet was formed with the movement of the castor oil phase into the junction where the continuous phase was streaming from the main channel. The castor oil was gradually extended until the whole diameter of outlet channel was filled, forming a barrier for the incoming continuous phase (Fig. 7.16). During the process, a castor oil thread gradually developed connecting the drop to the castor oil bulk phase (Fig. 7.16 C). The stretching of this thread leads to the development of thinned area at its both ends (Fig. 7.16 E). Finally, the droplet splits as the thin film ruptures. As a consequence the satellite droplet is also generated at the same time (Fig. 7.16 G).

**Figure 7.16** Photomicrographic sequence showing the serial stages of the formation of the primary and secondary (satellite) droplet. The first stage shows the detaching droplet and the thin ‘thread’ separating the droplet from the single mass of castor oil phase. This thread is unstable and it is the breakup of the thinned area that allows the formation of the primary and satellite droplets.

Many authors have reported the formation of satellite droplets in various droplet generation devices (Shi et al., 1994; Henderson et al., 2000; Cristini et al., 2003; Anna et al., 2003). Zhang (1999) studied the generation of satellite droplets under various breakup conditions and concluded that the viscous forces and interfacial tension play an important role. The volume of satellite droplet formed varied depending on the flow rate of the continuous phase, such that less than 1% of the primary drop was found in the quiescent continuous phase and almost 10% when the continuous phase flow rate was 200 times faster than disperse phase (Zhang, 1999).
Several authors have investigated theoretical and experimental data in attempt to predict this behaviour (Zhang, 1999; Henderson et al., 2000; Cristini et al. 2003; Ko and Ryou, 2005). The presence of satellite droplets can be a major drawback in the formation of monodisperse emulsions. However, and paradoxically, this interesting phenomenon may be used as one reliable technique to produce monodisperse nanoparticle droplets. With the satellite sorting technique first developed by Tan et al. (2004), by controlling the shear gradient at the microchannel junction, satellite droplets can be separated and collected into the desired reservoir (Tan and Lee, 2005). The combination of droplet formation and sorting technique could generate nanoparticles droplet with diameters less than 100 nm (Tan et al., 2006).

After the satellite droplet is formed, a third droplet is subsequently generated. This behaviour was similar to the formation of the first droplet except that no satellite droplet was observed (Fig 7.17). During the “pinching-off” of the third droplet, a thin thread was also observed but thinner than the thread generated from first droplet (Fig 7.17 F).

**Figure 7.17** Photomicrographic sequence (continued from Figure 7.16) showing the formation of the third droplet with a length of 100 µm. While the continuous phase is still streaming, another castor oil droplet is generated following the appearance of a satellite drop (at 1:28 s). During the disintegration of the droplet, the thread is developed and elongated, finally breaking off from the remaining castor oil phase.
The formation of castor droplets arise from the rupture of the thin film which forms between the phases is followed by a “dewetting” process of the castor oil phase on the microchannel surface. Dewetting is the process where a liquid film deposited on a non-wettable surface is eventually transformed into droplets (Neto and Jacobs, 2004). Both theoretical and experimental studies have been carried out in an attempt to understand the stability of thin liquid films and subsequent dewetting processes (Reiter, 1992; Sharma, 1993; Faldi et al., 1995) because thin liquid films are essential in many technological applications, such as in lubricating, coatings (paints) and insulating layers of many microelectronic devices. The stability of thin films can be described quantitatively using a ‘disjoining pressure’ which is a function of the difference in chemical potential or partial molar free energy of material in a thin liquid film and a bulk liquid (Kheshgi and Scriven, 1991). The disjoining pressure is essential for describing wetting and dewetting phenomena. A positive disjoining pressure leads to stable thin films and hence these can wet the surface, whereas a negative disjoining pressure (conjoining pressure) tends to make the liquid form into droplets and hence dewet the surface (Fig 7.18). The film break up induced by the conjoining pressure begins to play an important role when the thickness of the film less than 0.1 μm (Sharma, 1993; Sharma and Rieter, 1996).

![Disjoining Pressure Profiles](image)

**Figure 7.18** Disjoining pressure profiles of (1) the formation of a stable thin film (wetting), (2) an unstable thin layer (dewetting) and (3) the formation of a metastable film with a sessile drop on a wetted surface leading to satellite droplet formation. Adapted from Padday (1970) and Seemann et al. (2001).
Three sized droplets were generated in this experiment in a periodical and reproducible manner with the estimated time in each cycle of $9.53 \pm 0.45$ s. The size distribution was also found to be very narrow in repeated production of the droplets. These can be removed by conventional filtration techniques for larger droplets or by sorting techniques for small droplets. Even though further work needs to be done, many authors have investigated the droplet formation under various conditions (Sugiura et al., 2002; Köhler and Kirner, 2005; Van der Graaf et al., 2005). They found that the size of the forming droplet can be varied by changing the flow rate; increasing the flow rate reduces the droplet size. A disadvantage of droplet formation by microfluidic production (microchannel emulsification) is that the fluxes and the volume fractions of dispersed phase are low. Moreover, only single drops can be generated at a time by each orifice and hence high production rates cannot be reached. This drawback can be overcome using the highly parallel production offered by the development in microfluidic technology (Thorsen et al., 2001, Anna et al., 2003) and also using the breakup technique to further adjust droplet size in order to increase the volume fraction and to make the most out of microfluidic production.

### 7.3.4 Break-up of emulsion droplet

The motion and breakup of deformable drops through constant and variable cross-section capillaries is one fundamental problem which has been of interest in fluid mechanics. Such phenomena have been involved in a variety of apparent problems in many engineering and scientific applications. Instead of considering this as a problematic situation, controlled droplet breakup can be applied beneficially to form mono-disperse emulsion droplets (Link et al., 2004). After each droplet is formed using the microchannel emulsification technique, the size of the droplet can be reduced using by droplet breakup phenomena using the microfluidic device i.e. at the T-junction. This would enhance the potential use of microfluidic devices.

We observed droplet breakup when a coarse emulsion produced by a Rotamixer was passed through a microchannel system. The large emulsion droplets were broken into smaller droplets at the T-junction. This breakup was dependent on i) the continuous phase, ii) the disperse phase, and iii) on the geometry of the device, among of other factors.
7.3.4.1 Continuous phase flow induced break-up

Figure 7.19 shows the image sequence of droplet breakup by the continuous phase at a T-junction. The emulsion droplet reaches a T-junction (Fig 7.19 A), a branch in the channel creates the possibility of movement in two flow directions. Consequently, the droplet is often deformed and flows into both arms of the branch (Fig 7.19 B-H). If the droplet is large enough, the deformation caused by flow of the continuous phase will finally split the mother droplet into two daughter droplets (Fig 7.19 I).

![Image of droplet breakup](image.png)

**Figure 7.19** The breakup of a droplet by the continuous phase flow at T-junction. The droplet flows into a T-junction in (A), where the flow is split in two opposite directions. At the corner of T-junction the droplet is deformed (B-H) and splits into two daughter droplets by the pressure exerted by the flowing continuous phase (I). The scale bar is 100 μm.

It can be seen that the daughter droplets were of different size (Fig 7.19 I). Normally, the droplet would break into two smaller droplets of equal size if the flow rate in both side arms is equal, when the forces exerted on each side of the mother droplet would be equal leading to symmetric breakup. Conversely, asymmetric breakup arises from
unequal flow rate in the side arms which is easily achieved by adjusting their geometry. For example, Link et al. (2004) reported that the ratio of the flow rates \( q_i \) in each side arm was inversely proportional to the ratio of the arm lengths \( l_i \) (Fig 7.20) i.e. \( q_1/q_2 \approx l_2/l_1 \) and this value was related to the ratio of the volume \( V_i \) of both daughter droplets \( (l_2/l_1 \approx V_i/V_2) \). Tan et al. (2004) also studied asymmetric breakup by changing the ratio of the channel width \( w_1 \) and \( w_2 \) (Fig 7.20). They suggested that the daughter droplet volume was dependent on the channel resistance in both side arms as well as the volume of the mother droplet.

![Figure 7.20 Schematic of the flow of a droplet (length, \( l_0 \)) inside a channel (width, \( w_0 \)) towards a T-junction where the flow is divided into two side arms (channels) with adjustable length \( l_1 \) and \( l_2 \), width \( w_1 \) and \( w_2 \), and flow rate \( q_1 \) and \( q_2 \). This diagram adapted by Tan et al., 2004.](image)

To design the desired ratio volume of the daughter droplets, the relative flow rate can also be controlled varying the outlet pressure in the two side arms (Song et al., 2003). The breakup mechanism for the laminar flow condition was explained by Briscoe et al. (1999). Droplet breakup occurs when the droplet is extended and aligned with the disruptive strain due to the viscous stress exerted by the continuous phase, causing an imbalance of the interfacial tension. These factors are generally described by the capillary number \( (C_a) \) which determines the qualitative importance of drop deformation. Such a parameter was defined by Link et al. (2004) as

\[
C_a = \frac{\eta v}{\gamma}
\]

where \( \eta \) is the viscosity of the continuous phase, \( v \) is the velocity of droplet entering the junction, and \( \gamma \) is the interfacial tension between disperse and continuous phase.

195
Link et al. (2004) also determined the conditions in which the droplet breakup occurred by examining both the non-breaking and breaking condition for droplets at the T-junction. They obtained a factor to predict the breakup criteria, called the critical capillary number ($C_{cr}$) which can be expressed as

$$C_{cr} = \alpha \varepsilon_0 \left( \frac{1}{\varepsilon_0^{2/3}} - 1 \right)^2$$  \hspace{1cm} (7.3)

where $\alpha$ is a dimensionless constant equal to 1 for the square channel of the T-junction, and $\varepsilon_0$ is the initial extension of droplet before entering to the T-junction which is defined as

$$\varepsilon_0 = \frac{l_0}{\pi w_0}$$  \hspace{1cm} (7.4)

where $l_0$ is the initial length of droplet, and $w_0$ is the width of the channel as illustrated in the Figure 7.20. When $C_a$ is greater than $C_{cr}$, droplets will break providing the initial extension of droplet is less than one ($\varepsilon_0 < 1$). In the case where the initial extension of droplet is greater than one ($\varepsilon_0 > 1$) namely the sufficiently long droplet, will always break at the T-junction even at a very low capillary number. These parameters can be applied in our experiment data discussed in the next section.

### 7.3.4.2 Disperse phase induced droplet break-up

Breakup of droplets at the T-junction occurred due to the collision between two droplets at the bifurcating junction (Fig 7.21). In this figure two emulsion droplets move toward (Fig 7.21 A) and the first droplet is sufficiently long to deform and flow into both sidearm channels (Fig 7.21 B). The second droplet also moves towards and collides with the first one at the junction (Fig 7.21 C). This leads to the formation of two daughter droplets (Fig 7.21 D-I).
The mechanism of droplet breakup by the disperse phase would be similar to the breakup by the continuous phase but the disruptive strain on the mother droplet may occur due to the viscous stress exerted by the continuous phase as well as the disperse phase (the impacting droplet). This was demonstrated through examination of the various flow conditions of different sizes of breaking and non-breaking droplets using the plot the parameters involved with the break-up mechanism (Fig 7.22).
It can be seen that breaking droplets are sometimes located in the non-breaking region of the graph (Fig 7.22) and all those droplets have been split owing to the disperse phase or the continuous phase and the geometry of the channel. The critical capillary number with $\alpha = 1$ cannot predict the T-junction breakup of droplets due to the disperse phase. These results imply that the disperse phase can facilitate the breakup of droplet and that the critical line may shift to the left of the graph (Fig 7.22). Another droplet breakup study in a channel of different geometry also showed that using $C_{cr}$ with $\alpha = 1$ could not entirely predict the breakup of droplets (Tan et al., 2004).
Breakup of a droplet due to pressure from the continuous and the disperse phase at the T-junction was also observed in our experiment where the flow is generated from the both sidearm channels and joined together at the bifurcating junction. A mother droplet coming from the left sidearm channel reaching T-junction branching and was split by the disperse phase in Figure 7.23 and by the continuous phase in Figure 7.24.

**Figure 7.23** Breakup of a droplet by disperse phase flow at a T-junction. A mother droplet comes from the one side channel and is split by another disperse phase droplet flowing from the other side channel. When each breakup occurs from the different sizes of dispersing phase droplet (B), daughter droplets are obtained with the length of $L_1$, $L_2$, and $L_3$ (C). Similar sizes of daughter droplets are observed in I and II ($L_1 = L_2$) and these daughter droplet sizes are larger than the daughter droplet in III ($L_1, L_2 > L_3$). The scale bar is 100 μm.
It was found that when a similar size of the disperse phase (Fig 7.23 IA and IIA) was approaching the mother droplet, daughter droplets of equal size ($L_1 \approx L_2$) were obtained (Fig 7.23 IC and IIC). The daughter droplet size was further reduced ($L_3 < L_1, L_2$) (Fig 7.23 IIC) with an increasing of the size of the disperse droplet (Fig 7.23 IIB). This could be due to the greater shear force from the larger droplet size applied on the mother droplet. Moreover, when the mother droplet experienced the continuous phase flow (Fig 7.24), the larger ($L_4, L_5 > L_1, L_2, L_3$) the size of the daughter droplet was acquired compared to the breakup with disperse phase due to the less shear force. This observation was in good agreement with the above results as seen in the graph (Fig 7.22).

Using this breakup technique, the size of generated emulsion droplets can be controlled for use in various fields such as micromixers (Gunther et al., 2005; Garstecki et al., 2005) and chemical reactors (de Mello, 2006). An advantage of using microfluidic devices in those fields is that the chemical concentration and the reaction time can be managed by adjusting the geometry. Figure 7.25 shows an example of droplet break-up from one droplet into eight droplets assuming each time symmetric break-up occurs. From this diagram, the size, area, and volume of the daughter droplets can be calculated and predicted using a simple approach that is the volume of the mother droplet ($V_A$) equal to the total volume of daughter droplets ($V_B, V_C, V_D$) which is defined as

\[ V_A = V_B + V_C + V_D \]
\[ V_A = \frac{1}{6} \pi r^2 a + \frac{4}{3} \pi r^3 \]  
(7.5)

\[ V_B = 2(\pi r^2 b + \frac{4}{3} \pi r^3) \]  
(7.6)

\[ V_C = 4(\pi r^2 c + \frac{4}{3} \pi r^3) \]  
(7.7)

\[ V_D = 8(\pi r^2 d + \frac{4}{3} \pi r^3) \]  
(7.8)

\[ V_n = 2^n (\pi r^2 n + \frac{4}{3} \pi r^3) \]  
(7.9)

where \( a, b, c, d \) and \( n \) is the length of the droplets and \( r \) is the radius of the tube.

Figure 7.25 A schematic diagram representing the break-up of one large drop into 8 smaller droplets. Using simple equations, the size, surface area and volume of daughter droplets can be obtained and predicted as well as some control the number of the daughter droplets as follows: \( V_A = V_B = V_C = V_D, V_A = 1(\pi r^2 a + \frac{4}{3} \pi r^3), V_B = 2(\pi r^2 b + \frac{4}{3} \pi r^3), V_C = 4(\pi r^2 c + \frac{4}{3} \pi r^3), V_D = 8(\pi r^2 d + \frac{4}{3} \pi r^3) \) and \( V_n = 2^n (\pi r^2 n + \frac{4}{3} \pi r^3) \).

When the droplets are split, this causes an increase in surface area and hence interfacial energy. Consequently, the work of dispersion increases and more energy is required to generate smaller of droplets. The production of emulsions with small size can be achieved using microchannel emulsification with less energy input compared to conventional emulsification method. However, the interfacial tension between the droplet and continuous phase is the main factor governing droplet breakup.
Combining microchannel emulsification and the continuous phase break-up techniques can produce small emulsion droplets at a high volume fraction without altering the total disperse phase volume (Link et al., 2004). The droplet breakup technique by the disperse phase can further expand the use of the breakup technique by the continuous phase. For example, the range of minimum breaking droplet size could be extended, the volume fraction of droplet could be increased, and also the time used for breaking the droplet would be expected to be less. However, more work is needed to study further these possibilities.

The geometry of the microchannel facilitates the breakup of droplets at the same T-junction where flow direction is changed. The mother droplet moves close to a corner of the bifurcating junction (Fig 7.26 A). A branch in the channel creates two directions of streamline which are perpendicular to each other. Consequently, the droplet is deformed and flows in both arms (Fig 7.26 B-E). This deformation will finally split the mother droplet into two daughter droplets (Fig 7.26 F) by continuous phase flow, as well as through the geometry of the channels. These results were also calculated and plotted between capillary number ($C_a$, $C_a = \eta v/\gamma$) and the initial extension of the droplets ($e_0$) as shown in Figure 7.22.

**Figure 7.26** The breakup of droplet by the geometry of the microchannel at a T-junction. The mother droplet flows into one side arm of the channel in (A), where the flow is split into two directions. At the corner of the T-junction the droplet is deformed (B-E) and split into two daughter droplets (F). The scale bar is 100 μm.
These results show the flexibility of this fission technique, even if only one geometry of microfluidic device is used. By varying the geometry, the flow magnitude, flow direction and resistance of the channels can also be controlled at various junction downstream (Link et al., 2004; Tan et al., 2004).
7.4 Summary

Microfluidics have invited much attention due to the variety of applications in biomedical technology and basic sciences. Flow behaviour in microchannels is an important topic. The phenomenological results of flow behaviour of non-aqueous emulsions in microchannels may be used to further develop and understanding of the production and stability of emulsion systems. In a way also they give some insight into the behaviour of fluid carrier systems and their behaviours in complex vessels and veins from the point of view of understanding the range of forces that impinge on the droplets i.e. emulsions or flexible liposomes.
CONCLUSIONS AND FUTURE PERSPECTIVES

8.1 Conclusions

This thesis describes studies on non-aqueous emulsions both from the point of view of potential delivery vehicles and as interesting systems in their own right. With the diverse application of emulsion systems, non-aqueous emulsions may expand the use of traditional aqueous emulsions to allow use where the water is undesirable. Adapt from the $0_1/0_2$, $0_2/0_1$ systems multiple $0_1/0_2/w$ and $0_2/0_1/0_2$ emulsions were produce to expand the portfolio.

Formulation began with searching for the two immiscible non-aqueous phases selected from the pharmaceutically acceptable materials. Various non-aqueous liquids were immiscible depending on physicochemical parameters such as dielectric constant, bulk density and functional groups. The main problem of formulations was to find suitable surfactants to stabilise the two oil phases without custom synthesis of surfactants. The difficulties arise from the fact that there is no water phase the HLB concept is not applicable especially if both phases are non-polar. Because non-aqueous emulsions are so rare, few if any surfactants have been designed for the express purpose of stabilizing them.

Only castor oil-in-silicone oil emulsions were found to be stable using silicone surfactants which were miscible with the silicone phases. Therefore the most vital factor contributing to the stabilisation is the solubility of the surfactant in the
continuous phase, a concept put forward many years ago. However, if the solubility is too high that surfactant will be an insufficient stabiliser, as we found from the extrapolation results of Triton X-100 in benzyl benzoate-in-propylene glycol/water system. The adsorbed silicone surfactant film on the interface of castor oil and silicone oil offers steric stabilisation to protect coalescence of droplets through the bulky silicone backbone of the surfactant molecule.

Some physicochemical properties of castor oil-in-silicone oil systems were studied as a function of different silicone surfactants and silicone oils. DC 3225C and DC 5225C were found to be more effective in lowering the interfacial tension and offered more stable emulsions compared to DC 9011. The sign of lower emulsion stability was pronounced shear-thinning behaviour of emulsions stabilised with DC 9011. Emulsions of dimethicone as the continuous phase presented more stable behaviour compared to those of cyclopentasiloxane, most likely due to the higher viscosity of the former.

Non-aqueous emulsions may be used for drug delivery system as a depot or reservoir vehicle for lipophilic or hydrolytically unstable therapeutic materials. Slow release $^3$H-dexamethasone was observed from castor oil-in-dimethicone emulsions compared its release from castor oil-in-water emulsions or castor oil solutions. These in vitro results were confirmed by the in vivo data which showed a slower absorption of $^3$H-dexamethasone following intramuscular injection of castor oil-in-dimethicone emulsions to rats.

Magnetic non-aqueous emulsions were also formulated by loading iron oxide magnetic nanoparticles (10 nm) in the disperse phase. The response of these systems under an external magnetic field was investigated. Magnetic forces induced by an applied field could immobilize or manipulate magnetic emulsions at the desired point or in specific directions. The velocity of emulsions was determined by the magnetic force and this force was dependent on the magnetization of magnetic particle and the magnetic field strength.

Investigation of the flow behaviour of non-aqueous emulsions in the microchannels of microfluidic devices showed phenomena such as chain-like aggregation, oscillatory
droplet movement and we were able to study the breakup of large droplets. These findings may provide a better understanding in the production and stability of emulsion systems. In all the fields studied more work would be necessary to completely understand the systems, as discussed below.

8.2 Future perspectives

Further potential investigation following on the results obtained in this thesis could be:

- Study of the solubility parameters of liquid phases and also of surfactants to predict the solubility of two liquids and of surfactant in both phases. This may lead to developing a logical approach to formulation of non-aqueous emulsions.

- Investigation of the interfacial rheology and film thinning behaviour as the rupture of the surfactant film between the disperse phase on close contact leading to instability.

- Transdermal delivery could be another potential use of non-aqueous emulsions as silicone oil is widely applied topically.

- Toxicity and histological studies on castor oil-in-silicone oil emulsions.

- Magnetic particles may be coated with other polymers in order to enhance their dispersion in the disperse phase. Such magnetic emulsions possibly can be used as drug delivery system to localise effects, but the main approach would be with aqueous systems (magnetic particles in oil in water).

- Magnetic emulsions may be used for direct measurement the interaction forces (attractive and repulsive forces) between the droplets because coalescence between droplets can occur under a magnetic field (Fig 8.1). However, the magnetic force exerted on the droplet has to be well defined. This can provide the development of quantitative theory to explain stabilisation mechanisms which depend on controlling the balance between attractive and repulsive interactions.
Figure 8.1 Sequential photomicrograph of droplet coalescence of magnetic castor oil-in-dimethicone emulsions under an applied field using bar magnet.

- Designing the geometry of microfluidic devices to produce monodispersed emulsion systems of specified droplet size with high yield.
REFERENCES


229


