TASTE-MASKED AND CONTROLLED-RELEASE FORMULATIONS OF QUININE

THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY IN THE UNIVERSITY OF LONDON

BY

AMINA I. ODIDI
B.Sc., M.Sc. Pharmacy

AUGUST, 1990

Department of Pharmaceutics,
School of Pharmacy,
Brunswick Square,
London WC1X 1AX
ABSTRACT

Due to the widespread emergence of chloroquine-resistant P. falciparum quinine became important again in malaria chemotherapy. Because of possible toxicity with parenteral routes it is often administered orally, frequently causing gastric irritation. Although malaria affects children mainly, there is no known paediatric formulation of quinine, the bitter taste of which is not masked by sweetening. From clinical experience, sustained-release of quinine would decrease the occurrence of treatment failure.

In this work, taste-masked and sustained-release quinine was formulated by a modified multiple emulsion technique of microencapsulation. It involves ionic interaction between drug and polymer in the inner aqueous phase of multiple emulsions. Two formulations were prepared using the anionic polyelectrolytes iota-carrageenan, a sulphated polysaccharide and Eudragit L, an acrylic resin.

In principle, transfer of quinine from an oil to an aqueous phase, and the binding of quinine to hydrocolloid electrolytes were demonstrated.

In vitro release of quinine was faster at lower pH values implying controlled release in the mouth, and the release kinetics were complex. Bioavailability studies and taste evaluation were carried out using healthy human volunteers. Plasma levels were more sustained in the microcapsules than the control. The release mechanism, in vitro-in vivo correlation, the different release rates from the two microcapsules and the relevance of in vivo results to malaria chemotherapy were discussed. It appears that dose forms could be designed from these, to provide sustained action of quinine.

The bitter taste was shown to be substantially reduced. Thus these microcapsules could be used for taste coverage, administered as extemporaneous suspensions.

The modified process was shown to load more drug and provide better control of release. Two other cationic drugs, acetaminophen and cimetidine were encapsulated and characterised. The drug content depended on the number of acidic groups on a polymer and the basicity of the drug. Drug loss was further minimised when the drug was in the oil phase initially. In vitro release rates depended on the strength of drug-polymer interaction. Hence this procedure could be applied for the controlled release of suitable drugs.

Some progress was also made in the development of a buccal quinine dosage form using a cross-linked alginate network. Buccal delivery would eliminate gastric irritation.
This work is dedicated to the memory of my brother, Ahmed (1966-1990)
May Allah grant him eternal rest
I would like to express my sincere gratitude to Dr Brian Warburton for his interest and encouraging supervision throughout the course of this work. Many thanks to Dr R. H. Behrens and his assistant, Mrs Doff Pryce, both of the Hospital for Tropical Diseases (HTD) for conducting the bioavailability studies and also Dr. A. Voller of the Applied Immunology Unit, Institute of Zoology, London for assay of blood samples. I would like to express my gratitude to Mr D. McCarthy for help with scanning electron microscopy and other photographic work; to the staff of the Computer Unit, Mr J McAndrew, Mr K. Barnes, Mr D. Hunt, Mrs M. Fielder and Mr D. Marley for all the assistance they gave me during the course of this work. Many thanks to Professor J. M. Newton for his interest. Thanks also to my colleagues in the department for companionship, notably Anna Ng and Ahilan Nithiananthan.

I am very grateful to the government of Kano State of Nigeria for financing this course, and also for giving me the opportunity to undertake it.

I am greatly indebted to my husband, Dr. Isa Odidi, for encouragement and support; and my young children, Nabila, Auwal and Layusa for understanding and tolerance.

I acknowledge concern, interest and goodwill from my parents, brothers, and sisters.
## CONTENTS

<table>
<thead>
<tr>
<th>TITLE</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>2</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>3</td>
</tr>
<tr>
<td>ACKNOWLEDGEMENTS</td>
<td>4</td>
</tr>
<tr>
<td>CONTENTS</td>
<td>5</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>14</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>18</td>
</tr>
<tr>
<td>LIST OF PLATES</td>
<td>20</td>
</tr>
<tr>
<td><strong>CHAPTER 1. INTRODUCTION</strong></td>
<td>22</td>
</tr>
</tbody>
</table>

1.1. Malaria
1.1.1. History
1.1.2. Prevalence
1.1.3. Malaria infection
1.1.3.1. Life cycle of malaria parasite
1.1.4. Clinical manifestations of malaria
1.1.4.1. Clinical features of severe falciparum malaria in children
1.1.5. Chemotherapy of malaria
1.1.5.1. History
1.1.5.2. Biological classification of antimalarial agents
1.1.5.3. Chloroquine
1.1.6. Drug resistance in malaria
1.1.6.1. Mechanism of resistance development
1.1.6.2. Treatment of chloroquine-resistant falciparum malaria

5
<table>
<thead>
<tr>
<th>CONTENT</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1.6.3. Prospects for new drugs</td>
<td>35</td>
</tr>
<tr>
<td>1.1.7. Potential for a malaria vaccine and the status of research</td>
<td>35</td>
</tr>
<tr>
<td><strong>1.2. Novel methods of taste-masking</strong></td>
<td>36</td>
</tr>
<tr>
<td>1.2.1. Ion-exchange technology</td>
<td>36</td>
</tr>
<tr>
<td>1.2.2. Multiple emulsions</td>
<td>38</td>
</tr>
<tr>
<td>1.2.3. Cyclodextrin (CD) inclusion complexes</td>
<td>38</td>
</tr>
<tr>
<td>1.2.4. Microencapsulation</td>
<td>40</td>
</tr>
<tr>
<td><strong>1.3. Methods of microencapsulation</strong></td>
<td>40</td>
</tr>
<tr>
<td>1.3.1. Classical methods</td>
<td>40</td>
</tr>
<tr>
<td>1.3.2. Spheronisation</td>
<td>41</td>
</tr>
<tr>
<td>1.3.3. Emulsion-solvent evaporation (ESE) method</td>
<td>41</td>
</tr>
<tr>
<td>1.3.4. Albumin microspheres</td>
<td>41</td>
</tr>
<tr>
<td>1.3.5. Multiple emulsion technique</td>
<td>42</td>
</tr>
<tr>
<td>1.3.6. Modified methods</td>
<td>42</td>
</tr>
<tr>
<td><strong>1.4. Characteristics of multiple emulsions</strong></td>
<td>44</td>
</tr>
<tr>
<td>1.4.1. Water-in-oil-in-water (w/o/w) multiple emulsion types</td>
<td>44</td>
</tr>
<tr>
<td>1.4.1. Mechanisms of instability in w/o/w multiple emulsions</td>
<td>45</td>
</tr>
<tr>
<td>1.4.3. Factors controlling the stability of multiple emulsions</td>
<td>46</td>
</tr>
<tr>
<td>1.4.3.1. Effect of electrolytes</td>
<td>47</td>
</tr>
<tr>
<td>1.4.3.2. Viscosity of the organic solvent</td>
<td>47</td>
</tr>
<tr>
<td>1.4.3.3. Type of surfactant (emulsifier)</td>
<td>48</td>
</tr>
<tr>
<td>1.4.3.4. Concentration of surfactant (emulsifier)</td>
<td>48</td>
</tr>
<tr>
<td>1.4.3.5. Properties of the interfacial films</td>
<td>48</td>
</tr>
<tr>
<td><strong>1.5. Transfer of material in primary emulsions</strong></td>
<td>48</td>
</tr>
<tr>
<td>1.5.1. Mechanisms of transfer of material across emulsion phases</td>
<td>49</td>
</tr>
</tbody>
</table>
1.5.1.1. Diffusion

1.5.2. Factors affecting rate of transport across emulsion phases

1.5.2.1. Partition coefficient

1.5.2.2. Donor phase concentration

1.5.2.3. Phase volume ratio

1.5.2.4. Viscosity

1.5.2.5. Droplet size

1.5.2.6. Nature of the organic solvent

1.6. Sustained- and controlled-release formulations

1.6.1. Introduction

1.6.2. Advantages of sustained-release dose forms

1.6.3. Evaluating drugs for sustained-release formulation

1.6.4. Design of sustained-release formulations

1.6.4.1. Multiple dosing

1.6.5. Methods of achieving sustained release

1.6.5.1. Approaches based on dosage form modification

1.6.5.1a. Embedded matrix concept

1.6.5.1b. Barrier concept

1.6.5.2. Approaches based on drug modification

1.6.5.2a. Complexes

1.6.5.2b. Drug adsorbates

1.6.5.2c. Prodrugs

1.7. Release of drugs from microcapsules

1.7.1. Introduction

1.7.2. Permeation considerations
1.7.3. Kinetics of drug release from microcapsules

1.8. Cross-linking reactions of water soluble polymers
   1.8.1. Introduction
   1.8.2. Gels
   1.8.2.1. Types of gels

1.9. Buccal dose forms
   1.9.1. Introduction
   1.9.2. Advantages of the buccal route
   1.9.3. Absorption of drugs through the buccal mucosa
   1.9.3.1. Mechanism of drug absorption through the buccal mucosa

1.10. Objectives

CHAPTER 2. MATERIALS

2.1. Wall forming materials
   2.1.1. Anionic polyelectrolytes
   2.1.1.1. iota-Carrageenan
   2.1.1.2. Eudragit L
   2.1.1.3. Agar
   2.1.2. Eudragit E
   2.1.3. Ethylcellulose
   2.1.4. Acacia (Gum Arabic)

2.2. Solvents
   2.2.1. Ethyl acetate
<table>
<thead>
<tr>
<th>CONTENT</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.2.2. Methylene Chloride</td>
<td>81</td>
</tr>
<tr>
<td>2.2.3. Water</td>
<td>81</td>
</tr>
<tr>
<td>2.3. Core materials</td>
<td>82</td>
</tr>
<tr>
<td>2.3.1. Quinine</td>
<td>82</td>
</tr>
<tr>
<td>2.3.2. Acetominophen (Paracetamol)</td>
<td>83</td>
</tr>
<tr>
<td>2.3.3. Cimetidine</td>
<td>84</td>
</tr>
<tr>
<td>2.4. Materials forming crosslinked network for buccal formulation</td>
<td>84</td>
</tr>
<tr>
<td>2.4.1. Sodium alginate</td>
<td>84</td>
</tr>
<tr>
<td>2.4.2. Calcium salts</td>
<td>85</td>
</tr>
<tr>
<td>2.5. Other materials</td>
<td>85</td>
</tr>
<tr>
<td>CHAPTER 3. PREPARATION OF MICROCAPSULES</td>
<td>86</td>
</tr>
<tr>
<td>3.1. Background</td>
<td>87</td>
</tr>
<tr>
<td>3.2. Preformulation studies</td>
<td>87</td>
</tr>
<tr>
<td>3.2.1. Choice of polymer systems</td>
<td>87</td>
</tr>
<tr>
<td>3.2.1.1. Results and discussion</td>
<td>87</td>
</tr>
<tr>
<td>3.2.1.1a. Eudragit L and Eudragit E</td>
<td>87</td>
</tr>
<tr>
<td>3.2.1.2b. Eudragit L combined with acacia and Eudragit E</td>
<td>90</td>
</tr>
<tr>
<td>3.2.1.2c. Eudragit L and ethylcellulose</td>
<td>91</td>
</tr>
<tr>
<td>3.2.1.2d. Acacia and Eudragit E</td>
<td>91</td>
</tr>
<tr>
<td>3.2.1.2e. Agar and ethylcellulose</td>
<td>91</td>
</tr>
<tr>
<td>3.2.1.2f. Carrageenan and ethylcellulose</td>
<td>92</td>
</tr>
<tr>
<td>3.2.1.3. Summary of results</td>
<td>92</td>
</tr>
<tr>
<td>3.2.2. Diffusion studies</td>
<td>93</td>
</tr>
<tr>
<td>3.2.2.1. Method</td>
<td>93</td>
</tr>
<tr>
<td>3.2.2.2. Results and discussion</td>
<td>94</td>
</tr>
<tr>
<td>3.2.2.2a. Transfer of drug from the oil phase to the aqueous phase</td>
<td>94</td>
</tr>
</tbody>
</table>
3.2.2.2b. Transfer of drug to the aqueous phase involving interaction with polyelectrolytes 96
3.2.2.3. Summary of results 99

3.3. Method of microencapsulation 102

3.4. Characteristics of microcapsules 104
3.4.1. Drug content 104
3.4.1.1. Methods of measuring drug content 104
3.4.1.1a. Method of elemental analysis (EA) 104
3.4.1.1b. Fluorescence spectroscopy (FS) 104
3.4.1.1c. Ultraviolet spectroscopy (UVS) 105
3.4.1.2. Results and discussion
3.4.2. Effect of the method of microencapsulation on drug content 107
3.4.2.1. Method 107
3.4.2.2. Results and discussion 107
3.4.3. Surface morphology 109
3.4.3.1. Method 109
3.4.3.2. Results and discussion 109
3.4.4. Particle size analysis 115
3.4.4.1. Operating principles of the Malvern 2600c particle analyser 115
3.4.4.2. Method 115
3.4.4.3. Results and discussion 115

3.5. Other drugs encapsulated 120
3.5.1. Results and discussion 120
3.5.1.1. Drug content 120
3.5.1.2. Surface morphology 123
3.5.1.3. In vitro release profiles 128
3.5.1.4. Particle size analysis 133
3.6. Conclusions 133
### CONTENT

<table>
<thead>
<tr>
<th>CHAPTER 4. RELEASE OF QUININE FROM MICROCAPSULES</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>4.1. Introduction</td>
<td>136</td>
</tr>
<tr>
<td>4.2. In vitro release</td>
<td>136</td>
</tr>
<tr>
<td>4.2.1. Method</td>
<td>136</td>
</tr>
<tr>
<td>4.2.2. Results</td>
<td>137</td>
</tr>
<tr>
<td>4.3. In vivo release</td>
<td>142</td>
</tr>
<tr>
<td>4.3.1. Protocol for in vivo studies</td>
<td>142</td>
</tr>
<tr>
<td>4.3.2. Results and discussion</td>
<td>142</td>
</tr>
<tr>
<td>4.4. General discussion</td>
<td>143</td>
</tr>
<tr>
<td>4.4.1. Mechanism of release from drug-polymer complexes</td>
<td>143</td>
</tr>
<tr>
<td>4.4.2. In vitro release kinetics</td>
<td>148</td>
</tr>
<tr>
<td>4.4.3. Comparison of release from the two microcapsule formulations</td>
<td>149</td>
</tr>
<tr>
<td>4.4.4. In vitro-in vivo correlation</td>
<td>151</td>
</tr>
<tr>
<td>4.5. Bioavailability of quinine from microcapsules</td>
<td>153</td>
</tr>
<tr>
<td>4.6. Clinical analysis of in vivo data</td>
<td>156</td>
</tr>
<tr>
<td>4.6.1. Pharmacokinetics of quinine</td>
<td>156</td>
</tr>
<tr>
<td>4.6.1.1. Adverse effects of quinine</td>
<td>157</td>
</tr>
<tr>
<td>4.6.2. Plasma levels of quinine from microcapsules</td>
<td>158</td>
</tr>
<tr>
<td>4.6.3. Incidence of adverse effects</td>
<td>159</td>
</tr>
<tr>
<td>4.6.4. Relevance of in vivo results to malaria chemotherapy</td>
<td>161</td>
</tr>
<tr>
<td>4.7. Taste evaluation</td>
<td>161</td>
</tr>
<tr>
<td>4.7.1. Method</td>
<td>162</td>
</tr>
<tr>
<td>4.7.2. Results and discussion</td>
<td>162</td>
</tr>
<tr>
<td>CONTENT</td>
<td>PAGE</td>
</tr>
<tr>
<td>---------</td>
<td>------</td>
</tr>
<tr>
<td>4.8. Conclusions</td>
<td>165</td>
</tr>
<tr>
<td><strong>CHAPTER 5. BUCCAL FORMULATION OF QUININE</strong></td>
<td>167</td>
</tr>
<tr>
<td>5.1. Introduction</td>
<td>168</td>
</tr>
<tr>
<td>5.2. Mechanism of cross-linking of alginate with calcium ions</td>
<td>168</td>
</tr>
<tr>
<td>5.3. Progress in the development of a buccal quinine dose form</td>
<td>170</td>
</tr>
<tr>
<td>5.3.1. Incorporating calcium salts in the inner aqueous phase of multiple emulsions</td>
<td>170</td>
</tr>
<tr>
<td>5.3.2. Incorporating calcium salts in the outer aqueous phase of multiple emulsions</td>
<td>170</td>
</tr>
<tr>
<td>5.3.3. Incorporating calcium salts in the inner aqueous phase with acacia instead of a polyelectrolyte</td>
<td>171</td>
</tr>
<tr>
<td>5.3.4. Attempts to solve dispersion problems using wetting agents</td>
<td>172</td>
</tr>
<tr>
<td>5.3.5. Cross-linking using a calcium salt of lower water solubility</td>
<td>172</td>
</tr>
<tr>
<td>5.4. Conclusions and suggestion for further work</td>
<td>175</td>
</tr>
<tr>
<td><strong>CHAPTER 6. GENERAL CONCLUSIONS AND SUGGESTIONS FOR FURTHER WORK</strong></td>
<td>176</td>
</tr>
<tr>
<td>6.1. General conclusions</td>
<td>177</td>
</tr>
<tr>
<td>6.2. Suggestions for further work</td>
<td>180</td>
</tr>
<tr>
<td>CONTENT</td>
<td>PAGE</td>
</tr>
<tr>
<td>---------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>APPENDICES</td>
<td>181</td>
</tr>
<tr>
<td>Appendix 1. Theory of fluorescence spectroscopy</td>
<td>182</td>
</tr>
<tr>
<td>Appendix 2. Calibration curves for spectroscopic methods of drug analysis</td>
<td>183</td>
</tr>
<tr>
<td>Appendix 3. Particle size distributions of other drugs encapsulated</td>
<td>190</td>
</tr>
<tr>
<td>Appendix 4. Relevant statistics of subjects used for bioavailability studies</td>
<td>195</td>
</tr>
<tr>
<td>Appendix 5. Plasma levels of quinine from bioavailability studies</td>
<td>196</td>
</tr>
<tr>
<td>5a. Carrageenan microcapsules</td>
<td>197</td>
</tr>
<tr>
<td>5b. Eudragit L microcapsules</td>
<td>198</td>
</tr>
<tr>
<td>5c. Quinine Bisulphate tablets</td>
<td>199</td>
</tr>
<tr>
<td>Appendix 6. MAXPOINT pharmacokinetic program</td>
<td>200</td>
</tr>
<tr>
<td>REFERENCES</td>
<td>215</td>
</tr>
<tr>
<td>Figure</td>
<td>Title</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>1.1</td>
<td>Schematic representation of the life cycle of malaria plasmodium.</td>
</tr>
<tr>
<td>1.2</td>
<td>Possible breakdown pathways in w/o/w multiple emulsions.</td>
</tr>
<tr>
<td>1.3</td>
<td>A blood level-time profile for an ideal peroral sustained release dose form.</td>
</tr>
<tr>
<td>1.4</td>
<td>Embedded matrix concept as a mechanism of controlled release in sustained release dosage form design.</td>
</tr>
<tr>
<td>1.5</td>
<td>Barrier mediated models of sustained release dosage form design.</td>
</tr>
<tr>
<td>1.6</td>
<td>Drug release profiles characteristic of barrier-mediated models. A, B, membrane-controlled diffusion.</td>
</tr>
<tr>
<td>1.7</td>
<td>Mechanism of sustained release based on drug modification.</td>
</tr>
<tr>
<td>1.8</td>
<td>Schematic representation of drug passage through an idealised homogenous coating of a microcapsule.</td>
</tr>
<tr>
<td>1.9</td>
<td>Concentration gradient across an ideal isotropic polymeric membrane.</td>
</tr>
<tr>
<td>1.10</td>
<td>Diagrammatic representation of the formation of a typical gel from a water soluble polymer (carrageenan).</td>
</tr>
<tr>
<td>2.1</td>
<td>Repeating structure of iota-carrageenan.</td>
</tr>
<tr>
<td>Figure</td>
<td>Title</td>
</tr>
<tr>
<td>--------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>2.2.</td>
<td>Repeating structure of Eudragit L.</td>
</tr>
<tr>
<td>2.3.</td>
<td>Repeating structure of Eudragit E.</td>
</tr>
<tr>
<td>2.4</td>
<td>Molecular structure of quinine.</td>
</tr>
<tr>
<td>2.5.</td>
<td>Molecular structure of acetaminophen (paracetamol).</td>
</tr>
<tr>
<td>2.6.</td>
<td>Molecular structure of cimetidine.</td>
</tr>
<tr>
<td>3.1.</td>
<td>Amount of quinine transferred with time from an oil phase (ethylcellulose in ethyl acetate) to an aqueous phase (distilled water).</td>
</tr>
<tr>
<td>3.2.</td>
<td>Effect of the concentration of Eudragit L on the amount of quinine transferred to the aqueous phase in 24 hours.</td>
</tr>
<tr>
<td>3.3.</td>
<td>Amount of quinine bound to varying concentrations of Eudragit L in 24 hours (inferred from the amounts of drug in the polymer precipitates).</td>
</tr>
<tr>
<td>3.4.</td>
<td>Amount of quinine bound to varying concentrations of carrageenan in 24 hours (inferred from the amounts of drug in the polymer precipitates).</td>
</tr>
<tr>
<td>3.5.</td>
<td>Particle size distribution of Eudragit L/quinine microcapsules (formulation A in table 3.2.).</td>
</tr>
<tr>
<td>3.6.</td>
<td>Particle size distribution of carrageenan/quinine microcapsules (formulation B in table 3.2.).</td>
</tr>
<tr>
<td>Figure</td>
<td>Title</td>
</tr>
<tr>
<td>--------</td>
<td>-------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>3.7.</td>
<td>Particle size distribution of carrageenan/quinine microcapsules (formulation C in table 3.2.).</td>
</tr>
<tr>
<td>3.8.</td>
<td>Particle size distribution of carrageenan/quinine microcapsules (formulation D in table 3.2.).</td>
</tr>
<tr>
<td>3.9.</td>
<td>In vitro release profiles of acetaminophen from Eudragit L microcapsules.</td>
</tr>
<tr>
<td>3.10.</td>
<td>In vitro release of acetaminophen from carrageenan microcapsules.</td>
</tr>
<tr>
<td>3.11.</td>
<td>In vitro release of cimetidine from Eudragit L microcapsules.</td>
</tr>
<tr>
<td>3.12.</td>
<td>In vitro release of cimetidine from carrageenan microcapsules.</td>
</tr>
<tr>
<td>4.1.</td>
<td>In vitro release of quinine from Eudragit L microcapsules (formulation A in table 3.2.).</td>
</tr>
<tr>
<td>4.2.</td>
<td>In vitro release of quinine from carrageenan microcapsules (formulation B in table 3.2.).</td>
</tr>
<tr>
<td>4.3.</td>
<td>In vitro release of quinine from carrageenan microcapsules (formulation C in table 3.2.).</td>
</tr>
<tr>
<td>4.4.</td>
<td>In vitro release of quinine from carrageenan microcapsules (formulation D in table 3.2.).</td>
</tr>
<tr>
<td>4.5a.</td>
<td>Plasma levels of quinine for individual subjects up to eight hours post dose.</td>
</tr>
</tbody>
</table>
4.5b. Plasma levels of quinine for individual subjects up to eight hours post dose. 145

4.6a. Plasma levels of quinine for individual subjects up to fifty six hours post dose. 146

4.6b. Plasma levels of quinine for individual subjects up to fifty six hours post dose. 147

4.7. Average plasma levels of quinine over fifty six hours. 154

4.8. Average plasma levels of quinine over eight hours. 155

5.1. Possible mechanism of cross-linking of alginate by calcium ions. 169
<table>
<thead>
<tr>
<th>Table</th>
<th>Title</th>
<th>PAGE</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.1</td>
<td>Dosage of antimalarial drugs for oral treatment of moderately severe malaria in non-immune children according to age.</td>
<td>34</td>
</tr>
<tr>
<td>1.2</td>
<td>Characteristics of drugs unsuitable for peroral sustained-release forms.</td>
<td>53</td>
</tr>
<tr>
<td>3.1</td>
<td>Formulations investigated for stable water-in-oil emulsion systems.</td>
<td>88</td>
</tr>
<tr>
<td>3.2</td>
<td>Compositions of quinine microcapsule formulations.</td>
<td>88</td>
</tr>
<tr>
<td>3.3</td>
<td>Amounts of quinine in microcapsules as determined by various methods of analysis.</td>
<td>104</td>
</tr>
<tr>
<td>3.4</td>
<td>Amounts of quinine in microcapsules formed by the two multiple emulsion processes.</td>
<td>106</td>
</tr>
<tr>
<td>3.5</td>
<td>Drug content of microcapsules of other drugs compared with that of quinine, as determined by elemental analysis.</td>
<td>121</td>
</tr>
<tr>
<td>4.1</td>
<td>In vitro release parameters of quinine from the microcapsules.</td>
<td>152</td>
</tr>
<tr>
<td>4.2</td>
<td>Average pharmacokinetic parameters of quinine from the tablet (control) and the two microcapsules.</td>
<td>152</td>
</tr>
<tr>
<td>Table</td>
<td>Title</td>
<td>Page</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>4.3</td>
<td>Incidences of side effects of quinine from the microcapsule formulations and the tablets.</td>
<td>160</td>
</tr>
<tr>
<td>4.4</td>
<td>Taste evaluation of suspensions of quinine microcapsules.</td>
<td>163</td>
</tr>
<tr>
<td>Plate</td>
<td>Title</td>
<td>PAGE</td>
</tr>
<tr>
<td>-------</td>
<td>----------------------------------------------------------------------</td>
<td>------</td>
</tr>
<tr>
<td>3.1.</td>
<td>Scanning electron micrograph of Eudragit L microcapsules containing quinine. Magnification : X 1250</td>
<td>110</td>
</tr>
<tr>
<td>3.2.</td>
<td>Scanning electron micrograph of carrageenan microcapsules containing quinine. Magnification : X 1250</td>
<td>111</td>
</tr>
<tr>
<td>3.3.</td>
<td>Scanning electron micrograph of empty Eudragit L microcapsules. Magnification : X 1250</td>
<td>113</td>
</tr>
<tr>
<td>3.4.</td>
<td>Scanning electron micrograph of empty carrageenan microcapsules. Magnification : X 1250</td>
<td>114</td>
</tr>
<tr>
<td>3.5.</td>
<td>Scanning electron micrograph of Eudragit L microcapsules containing paracetamol. Magnification : X 1250</td>
<td>124</td>
</tr>
<tr>
<td>3.6.</td>
<td>Scanning electron micrograph of carrageenan microcapsules containing paracetamol. Magnification : X 1250</td>
<td>125</td>
</tr>
<tr>
<td>3.7.</td>
<td>Scanning electron micrograph of Eudragit L microcapsules containing cimetidine. Magnification : X 1250</td>
<td>126</td>
</tr>
<tr>
<td>3.8.</td>
<td>Scanning electron micrograph of carrageenan microcapsules containing cimetidine. Magnification : X 1250</td>
<td>127</td>
</tr>
</tbody>
</table>
5.1. A gel network formed by dispersing microcapsules containing calcium chloride in sodium alginate solution, showing aggregation of microcapsules. 174

5.2. A gel network formed by dispersing microcapsules containing calcium lactate in sodium alginate solution. Microcapsules here are more uniformly dispersed. 174
CHAPTER ONE
INTRODUCTION
1.1. Malaria

1.1.1 History
Malaria is the most common of all protozoal diseases. It is caused by the
plasmodium protozoa and transmitted to humans by the female anopheles
mosquito. The story of malaria dates back to prehistoric times. Evidence from
fossil remains suggest that mosquitoes have been in existence and have
presumably plagued man and his ancestors for more than 100 million years
(Donno, 1986). It is therefore one of the oldest recorded human diseases.
Although its clinical symptoms were fully described by Hippocrates (460-377
B.C.) (Eleuze et al, 1989), the malaria parasite was only described in 1880 by
Alphonse Laveran, a French Army surgeon stationed in Algeria (Spencer and
Strickland, 1984). Being one of the commonest potentially fatal infections in
the world, malaria remains one of the most serious disease problems,
especially in the tropics. Before the second world war, about two thirds of the
world population was at risk. Due to a global eradication program initiated by
the World Health Organisation (WHO) in 1956, based mainly on spraying of
insecticides, there was a fall in prevalence mainly in the temperate regions.
The situation in the tropics remained the same or even worsened due to socio-
biological reasons (Noguer et al, 1978). It therefore continues to be a major
public health problem in many countries.

1.1.2 Prevalence
About half the world population is still faced with the risk of malaria (WHO,
1986) with an estimated global incidence of three hundred million cases
(Wyler, 1983). There are more than one hundred million cases yearly in
tropical Africa alone, with an estimated one million deaths mainly in infants
and young children (Bruce-Chwatt, 1979). It is found mainly in regions lying
roughly between 60°N and 40°S. It is endemic in Southeast Asia, the Indian
subcontinent, China, Oceania, the Malay subcontinent, tropical Africa, Central
and South America and parts of the middle east (Brown, 1986). Disease
transmission is a result of the interaction of four main factors; the human host,
the anopheline vector, the malaria parasite and the environment i.e. physical,
biological and socio-economical. Two epidemiological extreme situations exist;
stable and unstable malaria (Bruce-Chwatt, 1979). In unstable malaria areas
transmission is low and variable. Epidemics are likely here but eradication
programs are feasible and the impact of the disease is on all age groups.
Conversely, in stable areas transmission is high and regular, the disease is
endemic here with its impact mainly on young children. According to Bruce-Chwatt (1979), the malaria situation in most of the African continent is characterised by stability.

### 1.1.3. Malaria infection

#### 1.1.3.1. Life cycle of malaria parasites

Human malaria is caused by four plasmodia species: P. malariae, P. vivax, P. falciparum and P. ovale. Malaria parasites are in the subphylum apicomplexa, class sporoasida and sub-class coccidiasina. P. falciparum and P. malariae are found in most malarious areas, the former being predominant in Africa. P. vivax predominates in Latin America. P. falciparum and P. vivax are regarded as highly infectious strains while the others are much less so (WHO, 1987). P. falciparum poses the greatest threat. It is responsible for the high mortality in children and non-immune adults in endemic areas. The life cycle of the parasites, diagrammatically represented in figure 1.1., is characterised by one cycle of asexual division in parenchymal cells (exoerythrocytic schizogony), another cycle of pigment producing asexual division in the erythrocytes of the host (erythrocytic schizogony) and a sexual phase in the stomach of the mosquito (sporogony).

1) Exoerythrocytic stage

When an infected female anopheles mosquito feeds, sporozoites are inoculated into the blood stream of the host. After a brief period, less than an hour, these are localised in the parenchymal liver cells to develop into schizonts, which on maturation release thousands of uninucleate merozoites into the blood stream. No symptoms appear during the exoerythrocytic stage. Its duration, being 13-16 days usually or months in some cases, is shortest for falciparum and longest for malariae. The number of merozoites released into the blood also varies with species. P. falciparum releases 40,000 per schizont; P. ovale, 15,000; P. vivax, 10,000 while P. malariae releases only 2,000 per schizont (Black and Craig, 1986). The large number of merozoites in P. falciparum coupled with the short incubation period explain the high morbidity seen in this infection.

2) Erythrocytic stage

The intraerythrocytic parasites ingest 25-75% of the host red cell and undergo a series of structural changes to develop into mature trophozoites. These undergo schizogony to produce schizonts giving rise to many more merozoites. The cell ruptures and each schizont releases 6-24 merozoites which invade
Figure 1.1. Schematic representation of plasmodial life cycle. (_____ all malarias; ----, relapsing malarias only). After Rollo, (1980).
more red cells and the cycle continues. Some merozoites, other than those of falciparum, infect more parenchymal liver cells and may result in relapse for several years. This is the secondary exoerythrocytic stage. The release of merozoites coincides with the characteristic chills of malaria attacks. After one or more cycles of erythrocytic maturation a sub-population of merozoites differentiate into sexual forms or gametocytes providing the conditions for sexual reproduction in the vector. Gametogenesis is probably induced by environmental factors such as the immune status of the host, sub-curative drug pressure and metabolic stress (Carter and Miller, 1979). The gametocytes are taken into the stomach of the female anopheles when it ingests blood from an infected subject.

3) Vector (asexual) stage
In the gut of the mosquito the gametocytes are released from the parasitised red cells. These undergo structural changes and develop into sporozoites. The process takes 1-4 weeks depending on the external temperature and the species of the parasite. After a period of time a sporozoite passes through cells to lie within the duct of the secretory gland, where it is available for injection.

1.1.4. Clinical manifestations of malaria
The level of parasitaemia at which symptoms occur depends on the level of immunity of the host. Generally semi-immune individuals develop higher levels than non-immune ones before symptoms appear. The first signs are headache, malaise, anorexia, nausea, muscular pains, fatigue and dizziness. The commonest symptom is fever which results from schizont rupture, its periodicity determined by the length of the asexual cycle of the parasite. It is forty eight hours for vivax and ovale (tertian fever) and seventy two hours for falciparum (quartan fever). A typical paroxysm comprises of a sequence of a cold stage (chill, rigor), a hot stage (high fever) and a sweating stage. Other features include dry cough, abdominal pain, vomiting, and convulsion. Chronic malaria infection has been associated with tropical splenomegaly syndrome (TSS), and nephrotic syndrome. It often results in failure to survive in children and anaemia in all age groups. If not properly treated there may be recrudescence i.e. relapse due to delayed tissue schizont maturation. If properly treated, the parasitaemia may clear within three to four days. In the event that the infection is not treated at all, it could be fatal or last for years in semi-immune individuals; one year for falciparum, three-four years for vivax and ovale and thirty-forty years for malariae.

P. falciparum produces the most severe infection. If treatment is
delayed an irreversible state of shock may arise even after the peripheral blood has been cleared of parasites (Ritschel et al, 1978). The complications of severe acute malaria include renal failure, severe anaemia with heart failure, hypoglycaemia, pulmonary oedema and neuropsychiatric disturbances. Many of the pathological events can be explained by the sequestration of infected erythrocytes in the vascular beds of the internal organs.

1.1.4.1. Clinical features of severe falciparum malaria in children

Most deaths from malaria occur in young children living in areas of intense transmission of P. falciparum, the commonest fatal syndrome being cerebral malaria (Molyneux et al, 1989). Neonates are protected by the presence of HbF in their red cells, and also maternal protective IgG immunoglobulins transferred across placental tissue (Edozien et al, 1962). The disease affects children of one to five years most severely, with those under three years being at greater risk of both fatal and complicated illness. After this age immunity, acquired gradually, provides increasing protection. Onset could be gradual in cases of frequent infections or rapid in cases with hyperparasitaemia.

The essential pathological feature of severe falciparum malaria is sequestration of erythrocytes containing highly metabolically active parasites in deep vascular beds (WHO, 1986). The sequestration is greatest in the brain (MacPherson et al, 1985), this usually causing the mortality. There is fever and listlessness followed within a few hours or more usually a day or two by altered consciousness which may progress rapidly to profound coma. Deep coma has been associated with mortality (Maguire, 1983), but many patients make a full neurological recovery despite profound and sometimes prolonged coma. Convulsions, not associated with pyrexia, are common. Motor dysfunctions (like hypertonicity, abnormal posturing and opisthonotus) suggesting decortication or decerebration, present commonly and have been associated with depth of coma and mortality (Dumas et al, 1986). The parasites rob brain cells of vital elements like glucose resulting in hypoglycaemia, acidosis and lactic anaemia. Hypoglycaemia is strongly associated with a poor prognosis, and patients with severe or recurrent hypoglycaemia are at particular risk of death and neurological sequelae (e.g. White et al, 1987). Patients with very high densities of parasitaemia are at increased risk. Field and Niven (1937) found greatly increased mortality in Malaysian patients with falciparum malaria in whom the parasite count exceeded 500 000/μl. Retinal haemorrhages
have been described in children with severe malaria (Kayembe et al, 1980). Profound anaemia may develop rapidly, the degree of this sometimes being life-threatening. The anaemia is caused by the destruction of red cells by parasites as well as dyserythropoeisis. Heart failure and altered consciousness occur in conjunction with profound anaemia. However complications in other organs like pulmonary oedema, acute renal failure and jaundice are less common than in adults. There is a prompt response to antimalarial chemotherapy and haematinic drugs, but the transfusion of packed red cells is urgently required sometimes.

Even with prompt and appropriate treatment, the mortality rate of cerebral malaria is 20%. In those who survive, consciousness is regained within one to two days of starting treatment. In a study of 131 Malawian children with falciparum malaria, Molyneux et al (1989) reported neurological sequelae in 12 (11%) of the 111 survivors. Three of 64 children who survived in Papua New Guinea had neurological deficits (Stace et al, 1982) and eleven of 66 followed for six months in Tanzania (Schmutzhard and Garstenbrand 1984). The neurological defects resolve slowly during convalescence. Associated infections could cause complications. Mabuy et al (1987) found that salmonella infection, following an acute attack of falciparum malaria in Gambian children, contributed considerably to mortality and morbidity rate.

1.1.5. Chemotherapy of malaria

1.1.5.1. History
The first potent remedy against malaria was the cinchona bark from Peru. Probably the secret of the indigenous remedy was discovered by Spanish missionaries observing the practices of local Andean herbalists (Bruce-Chwatt, 1988). The now legendary story of the cure of Lady Chincon, wife of the Viceroy of Peru, who was later supposed to have brought it back to Spain, has been disproved (Jaramillo-Arango, 1950). Cardinal Juan de Lugo promoted its use in seventeenth century Spain and the powdered bark became widely used in Europe (Guerra, 1977). It was used for the treatment of the ague, the term then used to describe a bout of malaria. The bark contains a mixture of more than twenty alkaloids, the most important of which are quinine and quinidine which are d and l isomers of the same compound. Quinine was isolated in 1820 and it remained the principal antimalarial drug until 1930, when Mepacrine, a dye derivative was introduced. Since then numerous antimalarial agents have been synthesized.
1.1.5.2. Biological classification of antimalarial agents

Antimalarial agents can be classified biologically into five main groups based on their point of action in the life cycle of the plasmodium. These are:

1) Tissue schizontocides (causal prophylactics)
   These act mainly on the primary exoerythrocytic stages. Invasion of the blood cells and further transmission of malaria is thereby prevented. The group comprise the biguanides (e.g. proguanil), the diaminopyridines (pyrimethamine, trimethoprim) and the sulphonamides (sulphalene, sulphadoxine).

2) Tissue schizontocides (anti-relapse agents)
   The 8-aminoquinolines (primaquine, pamaquine, quinocide) are tissue schizontocides which produce radical cure. The drugs act on the persistent exoerythrocytic tissue forms of P. vivax and P. ovale thus preventing relapse. Therefore in combination with an appropriate blood schizontocide, radical cure is achieved. Primaquine was employed in therapy, but its apparent usefulness has been marred by the risk of its very untoward side effects on certain peoples. In patients with glucose-6-phosphate (G-6PD) dehydrogenase deficiency it can cause marked haemolysis. Those with nicotinamide adenine dinucleotide (NAD) methaemoglobin reductase deficiency are at risk of cyanosis due to methaemoglobinaemia. The risk from the use of this agent far out-weighs its benefits since vivax and ovale malaria are not life threatening infections and clinical cure can be achieved with other agents. So it is no longer used.

3) Blood schizontocides
   These act on the erythrocytic stages. Since these forms are responsible for the clinical phase of the disease, such agents produce clinical cure in ovale and vivax infections or radical cure in falciparum and malariae infections as the later do not have secondary exoerythrocytic stages. At worst suppression to sub-clinical levels is achieved in all cases, provided the organism is susceptible. The agents include the following sub-groups: cinchona alkaloids e.g. quinine; 4-aminooquinolines e.g. chloroquine, amodiaquine; 4-quinoline methanols e.g. mefloquine; sesquiterpene lactones (qinghaosu and derivatives) and others that are still on clinical trials.

4) Gametocytocides
   These act by destroying the sexual forms of the parasite in the host blood, hence preventing transmission to the vector. The 8-aminooquinolines are active against gametocytes of all species at all stages of their maturation while the
sulphonamides are active against the gametocytes of species other than P.falciparum. The drugs in this group are used as anti-relapse agents.

5) Sporontocides
These inhibit the sexual cycle in the vector by preventing formation of oocytes and sporozoites from gametocytes derived from a patient treated with the drug. The gametocytes are not killed but rendered infertile. Antimalarial agents with such activity are the biguanides and aminopyridines. They are used for prophylaxis.

1.1.5.3. Chloroquine
Chloroquine was first synthesized in 1934, its outstanding antimalarial activity becoming obvious after an extensive clinical trial in the USA in 1944 (Elueze et al, 1989). It is still the most widely used antimalarial drug. It is the most active of the 4-aminoquinoline group of antimalarial agents, which are highly effective against the asexual blood forms (of susceptible strains) of all species of plasmodia causing human malaria. They are also effective against gametocytes of P.ovale, P. vivax and P. malariae. Chloroquine is the drug of choice in the treatment of malaria in most parts of the world, especially Africa. This has largely been due to its effectiveness on susceptible strains and freedom from serious side effects. It produces clinical cure in ovale and vivax infections, and radical cure in infections with malariae and susceptible strains of falciparum. It is also an excellent suppressive agent against all species.

1.1.6. Drug resistance in malaria
Drug resistance in malaria is defined as the ability of the parasite strain to survive and/or multiply despite the administration and absorption of the drug given in doses equal to or higher than those usually recommended but within limits of tolerance of the subject (WHO, 1973). It is commonly related to the effect of blood schizontocides particularly chloroquine on P. falciparum. However the differences in susceptibilities of species, strains and stages of malaria parasites to different antimalarial drugs do not constitute resistance.

Chloroquine resistance was first reported in Thailand in 1959 and shortly after in Columbia (Moore and Lenier, 1961). Resistant strains have now been encountered virtually everywhere that falciparum is transmitted except some parts of W. Africa (Harinasuta et al, 1965; Fogh et al, 1979; Menon et al, 1987) and it is spreading fast (WHO, 1989). Once resistant to chloroquine, the organism has a marked ability of developing resistance to other synthetic antimalarial agents. Pyrimethamine in a potentiating
combination with a sulphonamide has been known to exert a rapid and potent action, particularly useful in falciparum malaria. One such combination, co-trimoxazole (Fansidar <Roche>), was found to be effective on strains of falciparum that are resistant to chloroquine and to pyrimethamine alone. However Fansidar is now losing its effectiveness for the treatment of falciparum malaria (Chongsuphajaisiddhi et al, 1979; Reacher et al, 1981). Hurwitz et al, 1981 reported 100% failure in 9 patients with uncomplicated falciparum malaria treated with a single dose. However the degree of resistance in other parts of the world is not as severe as in Thailand. Resistance to proguanil is also widespread in some endemic areas especially where mass prophylaxis with it has been practiced. Cross resistance between it and pyrimethamine is often, but not always present. Salako and Aderounmu (1987) have reported in vitro resistance of falciparum to the new blood schizontocide, Mefloquine, in Nigeria even though the drug has not been introduced to that country. In vitro tests done on quinine in the same study showed full sensitivity. A case of mefloquine-resistant malaria in an adult acquired in Tanzania has been reported (Bygbjerg et al, 1983). Gay et al (1990) also reported prophylactic and therapeutic failure of mefloquine in a child despite an adequate plasma drug level more than two weeks after the last therapeutic dose. The child was subsequently cured with quinine, the parasite count being negative by the fourth day of therapy.

Thus multiple-drug-resistant falciparum malaria is now a serious problem and it has been increasing recently, both geographically and in intensity at an astounding rate. Although diminished sensitivity of falciparum to quinine has been confirmed in Thailand (Chongsuphajaisiddhi et al, 1981), this is not yet a widespread problem. Nguyen et al (1989) reported combined resistance to chloroquine, quinine and Fansidar in a non-immune subject who acquired the disease in a chloroquine-resistant area. He was cured with a combination of quinine and tetracycline.

1.1.6.1. **Mechanism of resistance development**

Resistance to chloroquine appears to be caused by a spontaneous mutation and is genetically expressed in multiple loci (Wernsdorfer, 1981). Malaria parasites digest haemoglobin to obtain essential amino acids, in the process a substance, ferriprotoporphyrin IX (FP) is produced. Chloroquine acts by forming a complex with FP which impairs the ability to maintain cation gradients in the parasite and the host erythrocyte by damaging cell membranes. Susceptible strains accumulate more chloroquine with a high affinity for this
complex formation. For some reason not clear now, chloroquine-resistant parasites form little or none of the FP-chloroquine complex. Free FP is removed by sequestration into haemazoin (malaria pigment) as it is lethal to the parasites. Salganic et al (1987) reported the faster inaction of chloroquine by P. berghei mutants resistant to it as a result of increased activity of plasmodium microsomal oxygenase, so this might be another mechanism of resistance development. The selection of resistant mutants is said to be affected under drug pressure. Chloroquine enhances the infectivity of resistant parasites to vector anophelines, conferring a biological advantage on resistant strains (eg. WHO, 1987). Resistance is maintained by the parasite throughout its life cycle and is transferred to the progeny, hence active malaria transmission promotes the spread of resistance. The rate of spontaneous mutations differ from strain to strain and immunity can delay the appearance of resistance in a population. The level of immunity in indigenous Africans to P. falciparum infections is higher than that of people living in other malarious areas because 95% of malaria here is caused by P. falciparum. This is likely to have contributed to the late appearance of resistance in Africa and also to the detection of the resistance in non-immune subjects in East and West Africa initially (Spencer, 1985; Olatunde, 1977).

Resistance to proguanil and pyrimethamine is due to the development of mutants that can survive through the utilisation of alternative metabolic pathways to that catalysed by dihydrofolate reductase enzyme, the blockage of which is the mechanism of action of these drugs. Resistance to sulphonamides is due to P. falciparum mutants that can circumvent and bypass the use of p-amino benzoic acid.

1.1.6.2. Treatment of chloroquine-resistant falciparum malaria

Amodiaquine, another 4-aminoquinoline is more effective than chloroquine against certain strains of P. falciparum (e.g. Ekweozor et al, 1987). It is now used in the treatment of susceptible chloroquine-resistant falciparum malaria in East Africa (Watkins et al, 1988). In other areas of the world, the first alternative is Fansidar provided the organism is sensitive to it. Where Fansidar is no longer curative and in all complicated cases of falciparum malaria, quinine is the only reliable therapeutic agent at present widely available. Even in Thailand where it is gradually losing its ability to affect radical cure, it remains the mainstay of chemotherapy. This is because of its ability to produce rapid initial reduction of parasitaemia. Fansidar and similar
combinations are commonly given with quinine in infections sensitive to these. They are given on the first day of treatment or at the end of the quinine course. Tetracyclines are effective against some multi-drug-resistant infections if given for seven or more consecutive days (Colwell et al, 1972; WHO, 1984). However the tetracyclines are slow-acting so quinine, which is more rapidly acting is given concurrently to bring down the level of parasitaemia. Paediatric doses of antimalarial drugs in current use, for the oral treatment of moderately severe malaria in non-immune subjects are given in Table 1.1.

In severe falciparum malaria; where malaria was acquired in a chloroquine-resistant area, where it has broken through chloroquine prophylaxis or where the geographical origin of the disease is not known or the species are uncertain, quinine is the treatment of choice (WHO, 1984). In ideal situations, i.e. when reliable hospital services are available, paracitidal concentrations can be rapidly and safely achieved with quinine dihydrochloride in an optimal adult dose of 10-20mg/Kg body weight diluted and infused at a constant rate over four hours (WHO, 1986). The subsequent maintenance dose is 10mg salt/Kg given eight-hourly until the patient can swallow tablets. Quinidine, which is 2-3 times as potent as quinine as a blood schizontocide can be given if the later is not available. Cardiac function must however be monitored. Up to date therefore, quinine remains invaluable in the treatment of falciparum malaria worldwide. Qinghaosu and two of its derivatives, artemether and artesunate, have been shown to have high efficacy against chloroquine- and multi-drug resistant P. falciparum (Li et al, 1984) and it has been reported to show potentiation when used with mefloquine, primaquine and tetracycline (Chawira and Warhurst, 1987). It belongs to a novel group of antimalarial agents, the sesquiterpene lactones. It is a highly active, rapidly-acting blood schizontocide. However it is not available outside China. Mefloquine has proven to be highly effective against P. falciparum infections including some strains resistant to chloroquine, but it is not available in parenteral form.
Table 1.1. Dosage of antimalarial drugs for oral treatment of moderately severe malaria in non-immune children according to age (WHO, 1983)

<table>
<thead>
<tr>
<th>Drug</th>
<th>Up to 1yr</th>
<th>1-3 yrs.</th>
<th>4-6 yrs.</th>
<th>7-11 yrs.</th>
<th>12-15 yrs.</th>
<th>Regimen</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine</td>
<td>100-200mg</td>
<td>200-300mg</td>
<td>300-500mg</td>
<td>500-1000mg</td>
<td>1-2 grn</td>
<td>Daily dose divided into 2-3 parts</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>75mg</td>
<td>150mg</td>
<td>300mg</td>
<td>300mg</td>
<td>450-600mg</td>
<td>Initial dose</td>
</tr>
<tr>
<td></td>
<td>(1/2 tab.)</td>
<td>(1 tab.)</td>
<td>(2 tabs.)</td>
<td>(2 tabs.)</td>
<td>(3-4 tabs.)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>75mg</td>
<td>113mg</td>
<td>150mg</td>
<td>150mg</td>
<td>225-300mg</td>
<td>2nd dose, after 6-24 hours</td>
</tr>
<tr>
<td></td>
<td>(1/2 tab.)</td>
<td>(3/4 tab.)</td>
<td>(1 tab.)</td>
<td>(1 tab.)</td>
<td>(1.5-2 tabs)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>37mg</td>
<td>75mg</td>
<td>75mg</td>
<td>150mg</td>
<td>150-300mg</td>
<td>Daily dose for next two days</td>
</tr>
<tr>
<td></td>
<td>(1/4 tab.)</td>
<td>(1/2 tab.)</td>
<td>(1/2 tab.)</td>
<td>(1 tab.)</td>
<td>(1-2 tabs.)</td>
<td></td>
</tr>
<tr>
<td>Amodiaquine</td>
<td>50mg</td>
<td>100mg</td>
<td>150mg</td>
<td>200-300mg</td>
<td>400-600mg</td>
<td>Dose for 1st day</td>
</tr>
<tr>
<td>Sulphadoxine +</td>
<td>50mg</td>
<td>50mg</td>
<td>100mg</td>
<td>150-200mg</td>
<td>250-400mg</td>
<td>Daily dose for next two days</td>
</tr>
<tr>
<td>Pyrimethamine</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulphalene + Pyrimethamine</td>
<td>Same as for Sulphadoxine + Pyrimethamine</td>
<td></td>
<td></td>
<td></td>
<td>Single dose</td>
<td></td>
</tr>
</tbody>
</table>
1.1.6.3. **Prospects for new drugs**

The development of resistance to chloroquine intensified research into the development of new antimalarial agents. Most of the research is carried out by the US government through the Walter Reed Army Institute for Research. The priorities for these efforts are:

1) development of blood schizontocides against P.falciparum,
2) development of a replacement for primaquine and
3) development of causal prophylactics for P. falciparum.

A number of major series are at various stages of development and marketing. Unfortunately most of these have similar structures to chloroquine and quinine so these are probably in danger of resistance development as well.

1.1.7. **Potential for a malaria vaccine and status of research**

Global malaria control eradication programs carried out over the past 50 years have been hampered by the development of drug resistance by the parasite and insecticide resistance by the mosquito vector. There is therefore a lot of interest in the development of vaccines. Three types of vaccines are being developed currently, against: sporozoites, intraerythrocytic stages and gametes. The sporozoite is the stage injected into the blood by the vector. A lot of interest has been focused on it because it is exposed to serum antibodies for a brief period before invading hepatocytes. Previous studies have shown that immunisation with x-irradiated sporozoites in humans and animals is a possibility (Nussensweig et al, 1969; Rieckmann et al, 1979). It was shown that immunised individuals develop antibodies that are almost exclusively directed against a circumsporozoite (CS) protein which covers the parasite surface (Vanderbreg et al, 1969). Dame et al (1984) determined the structure of the CS gene of falciparum and Ballou et al (1987) carried out a study on humans with a falciparum sporozoite vaccine. Their formulation proved an unsuitable candidate for field studies but the work demonstrated that human beings can be protected with a subunit CS protein vaccine. The asexual blood stages of parasites are other possible targets. Synthetic peptides corresponding to fragments of P. falciparum merozoite-specific proteins were shown to induce protection in animals experimentally infected (Patarrayo et al, 1987). A vaccine based on these peptides was shown to delay or suppress the development of parasitaemia in human volunteers (Patarrayo et al, 1988). A major problem with this clinically important stage of the infection is the high degree of
antigen polymorphism. Apparently a strong protective immunity is achieved only after exposure to a large number of variant forms.

The parasitological problems hampering vaccine development are strain diversity, stage specificity and antigenic variation. Also the actual mechanism of acquiring natural immunity is unknown and there is the difficulty of immunising 'protected' or previously infected hosts since here the body had already been challenged with the parasites before.

1.2. Novel methods of taste-masking

As well as the conventional processes like sweetening/flavoring, use of hard gelatin capsules and macrocoating, taste-masking may also be achieved by complex formation or encapsulating the drug with a suitable agent which acts as a carrier. In order for the unpleasant taste of a drug to be noticed, the drug molecules have to diffuse into the lipid bilayer of the gustatory membrane. Any procedure that slows this diffusion process would mask the taste. In the case of complex formation the protecting effect increases with increasing complex stability. Such novel methods of taste-masking include microencapsulation, multiple emulsions, cyclodextrin inclusion complexes and ion exchange technology. Although some controlled release is essential for taste coverage, the drug must however be released in the gastrointestinal tract to afford its absorption.

1.2.1. Ion-exchange technology

Ion-exchangers are synthetic, solid and slightly solubilised, cross-linked, high molecular weight electrolytes that can exchange their mobile ions of equal charge with the surrounding medium. The most frequently employed polymeric network is a copolymer of styrene and divinylbenzene that may be produced by a suspension polymerisation technique in a spherical bead form. Cation-exchangers contain acidic ionisable groups e.g. sulphonic, carboxylic or phenolic, while in anion exchangers the ionisable group is basic e.g. amine or quaternary ammonium. The later are usually presented as the chloride while the former are presented as sodium, potassium or ammonium salts. The corresponding hydrogen or hydroxyl form may be reacted with suitable anionic or cationic drug for the attainment of sustained-release, taste-masking or other effects. Weak cation exchangers have pKa values of about 6.0, so their exchange capacity tends to increase at pH 4.0 or above, while strong cation-exchangers have pKa values 1.0-2.0 so are normally highly dissociated at all
pH values encountered in the gastrointestinal tract. As a result, their exchange capacities tend to be independent of pH. Carboxylic acid type cation exchangers tend to have approximately twice the exchange capacity of the sulphonic acid type. Charged drugs are loaded onto ion-exchange resins by one of two methods. In the column procedure, a highly concentrated drug solution, buffered if necessary to enhance dissociation, is passed through a column of resin particles. In the batch procedure the drug solution is agitated with a quantity of resin particles until equilibrium is established. Upon ingestion, drugs are most likely eluted from cation exchange resins by hydrogen, sodium or potassium ions as these are most plentiful in gastrointestinal secretions. The drug-depleted resin is not absorbed, but excreted in the faeces.

The use of ion-exchange technology to achieve taste coverage has been in practice for some time. Brudney (1959) effectively masked the taste of vitamin B2 using ion-exchange resin complexes. The method was eventually patented (Brudney, 1961) and other patents exist (e.g. Keating, 1964; Siegel, 1962). Borodkin and Sundberg (1971) showed that coating the resin adsorbates further improved the taste of bitter amine drugs. Koff (1964) had earlier used castor wax to increase the palatability of cation-exchange resins loaded with amprotropine. However very few pharmaceutical grade ion-exchange resins are available. They are also not biodegradable so their use in parenteral dosage forms is not likely to be acceptable. Hinsvark et al (1973) found that the rates and variability of absorption of amphetamine and phentermine were decreased when formulated as resinates. This could be due to incomplete release from the resin and its subsequent excretion in the faeces. The non-selective properties of ion-exchange resins indicate that they could interact with any co-administered drug or food with the appropriate charge, provided the pH conditions favor ionisation of both species. Unwanted or undesirable electrolyte disturbances could also result. In a study with carbinoxamine and various cation exchangers, Schtichling (1962) discovered that increase in cross-linkage of the polymer network decreased both drug uptake and its release. Burke et al (1986) also reported a decrease in exchange rate with increase in cross-linking. This is due to a decrease in accessibility of exchanging ions.
1.2.2. **Multiple emulsions**

Emulsions are heterogenous systems of one immiscible liquid dispersed in another in the form of droplets which usually have a diameter greater than 1 μm (Becher, 1965). The two liquids are chemically unreactive and form systems characterised by a low thermodynamic stability. Therefore there is a tendency towards coalescence of the droplets to reduce interfacial free energy. Multiple emulsions are more complex systems where the drops of the dispersed phase themselves contain even smaller dispersed droplets, which normally consist of a liquid miscible with and in most cases identical with, the continuous phase. They are even more unstable than the primary emulsions. However systems with some degree of stability may be prepared by using pairs of surfactants, one stabilising the water-in-oil (w/o) emulsion and the other stabilising the oil-in-water (o/w) emulsion. There are two major types; water-in-oil-in-water (w/o/w) and oil-in-water-in-oil (o/w/o) emulsions. They can be prepared by the re-emulsification of a primary emulsion. Florence and Whitehill (1982) have reviewed the pharmaceutical applications of multiple emulsions as well as considerations in their formulations. The ability of multiple emulsions to entrap material is their most useful characteristic. The entrapped material is transferred from the internal to the external phase, across the middle phase, which is termed the membrane phase.

Multiple emulsions have been used as controlled-release delivery systems (Brodin, 1978). Dispersal of a phase containing the dissolved drug, inside the droplets of another would effectively isolate the drug in the core of the emulsion. Provided the system is stable enough to keep the drug from diffusing out for a reasonable shelf-life, the formulation could cover any taste and/or control the release of the drug. Garti et al (1983) used multiple emulsions to overcome the taste of chlorpromazine hydrochloride which is a bitter water soluble drug. These workers explored many systems of emulsifiers and were able to achieve some stability and up to 94% release in simulated gastric juice after twenty four hours. However emulsions are inherently unstable systems so it is difficult to guarantee a reasonably long shelf life. They are also bulky and difficult to transport.

1.2.3. **Cyclodextrin (CD) inclusion complexes**

Cyclodextrins were first isolated from the degradation products of starch. They are water soluble, nonreducing cyclic oligosaccharides, comprised of α-1,4-linked D-glucopyranose units (Freudenberg and Meyer-Delius, 1938).
The most common are the α-, β- and γ-CDs formed by six, seven or eight glucose units respectively. Cyclodextrins form mononuclear inclusion compounds with mostly aromatic compounds, both in the solid state and in solution, particularly aqueous. Many CD inclusion compounds owe their existence to van der Waals interactions between ‘guest’ and ‘host’ (CD) as well as the ability of the ‘guest’ molecule of suitable size to be physically accommodated in the CD cavity. The interior of the CD molecule is hydrophobic and binds a hydrophobic portion of the ‘guest’ molecule, usually forming a 1:1 complex. In solution, CDs form complexes with internal diameters of 0.6-1.0 nm. The inclusion compounds formed with apolar molecules are only slightly soluble in water whereas those formed with polar molecules are moderately soluble. The smaller the guest molecule, the greater its complexing ability. The potential pharmaceutical applications of CD complexes which include masking of unpleasant taste or odour of a compound have been reviewed by Jones et al (1984). Inclusion complexes have been prepared to utilise some of these potentials. Andersen et al (1984) suppressed the bitter taste of the anti-depressant femoxetine by formulating the solid β-CD complex of the drug as an aqueous suspension.

However CD complexes have some limitations (Fromming, 1987) viz:

1) Size and property of the CD cavity; for any successful interaction, the cavity of the cyclodextrin has to be large enough to contain the drug or at least part of it, also the drug or at least a part of it has to be lipophilic.

2) Dose of drug; there is some limit to the weight of material in a single tablet, capsule or dose of a suspension, so the amount of ‘guest’ compound in the complex is seldom higher than 20-25%. Since only a single unit of the drug is taken by a host molecule, drugs with a single dose of more than 50 mg will normally be no candidates for inclusion.

3) Toxicological problems; CDs are not metabolised as rapidly as starch and intact CDs are not absorbed. Though no signs of toxicity were observed with the oral administration of β-CD (Jones et al, 1984) and γ-CD, the danger of accumulation of higher chronic doses (especially parenteral) of β-CD in the kidney resulting in renal toxicities is there. Most CDs are surface active, so can change biological membranes, probably by complexing with membrane components. They can induce haemolysis, especially the methylated CDs.

4) The price; at present only β-CD is available at low cost.

Another short coming of this procedure is that the altered environment of the drug molecules lead to changes in stability. The cyclodextrins have been shown to catalyse a number of chemical reactions such as hydrolysis, oxidation
and decarboxylation (Tutt and Schwartz, 1971).

1.2.4. Microencapsulation
Microcapsules are an example of drug delivery systems with a large surface area. The active agent is contained within a network of polymer material or dispersed in the network of polymer, when they are termed microspheres. The most common structure is mononuclear spherical and the particle size range is between 1 and 2000 μm. Calanchi (1976) had listed the potential applications of microencapsulation in pharmaceutical dosage forms and gave examples of various products in the market in which the process has been applied to achieve some of these potentials. An important application is the development of controlled-release formulations (Harris, 1981; Bajeva et al, 1986). Taste coverage is another application of microencapsulation. Several drugs have been encapsulated to achieve this, although in most cases the taste coverage has been a subsidiary consideration to other properties. Eurand Microencapsulation S.p.A. (Italy) manufactures a range of products microencapsulated ostensibly for taste coverage (Calanchi, 1976). Examples are Diffugran, a granular preparation swallowed dry; Diffugel which is reconstituted to a pleasantly flavoured jelly and chewable acetaminophen tablets (Tylenol, marketed by McNeil in the USA). The first two are paediatric preparations. Microencapsulation is a versatile process in that both liquids and solids can be processed. The product can be presented in an appropriate selection of dose forms. There is a wide selection of coating materials to suit the various methods and the nature of the core material while still attaining the effect desired. The conditions of release may be made to depend on pH or other factors and the release mechanism may be associated with leaching, erosion, rupture or other actions depending on the wall construction (Luzzi, 1970).

1.3. Methods of microencapsulation

1.3.1. Classical methods
Merkle (1984) reviewed the classic microencapsulation processes, classified into chemical, physicochemical and mechanical. The chemical processes comprised of interfacial polymerisation and in situ polymerisation. Physicochemical microencapsulation is composed of coacervation, complex emulsion process and molten dispersion process. More recently, Li et al (1988) reviewed the basic processes too and advances in the technology. These
authors gave an analysis of the mechanical processes in particular, with a detailed account of equipment for the various methods. The newest of these mechanical processes is spheronisation. The following are the ‘more recently’ developed methods of microcapsules and microsphere formation.

1.3.2. Spheronisation
This is a form of pelletisation which refers to the formation of spherical particles from wet granulations (King, 1980). Rowe (1985) gave the main four processing steps as; blending, extrusion, spheronisation, and drying. The dry spheres can be barrier coated for sustained-release or other applications. The major advantage of the process is said to be its suitability for use with a wide range of drug concentrations (up to 75%).

1.3.3. Emulsion-solvent evaporation (ESE) method
The basic emulsification and organic solvent evaporation method of microencapsulation was patented in Britain in 1938 and then in the United States in 1939 (Suzuki and Price, 1985). Beck et al (1979) developed the technique employing poly(lactic acid) (PLA) as encapsulating agent. Yolles et al (1971) had reported the use of this biodegradable polymer for long-acting drug delivery. An aqueous solution of emulsifier e.g. gelatin is emulsified with a solution of the PLA and drug in a water-miscible organic solvent by pouring the oil into the aqueous phase rapidly with stirring. The oil is removed by either agitating the emulsion continuously, or agitation is stopped before the evaporation is complete. The microspheres are then allowed to settle, resuspended in emulsifier free water and agitated again to complete the evaporation. This later procedure is said to minimise the formation of free drug crystals in the aqueous phase or on the surface of the microspheres (Bissery et al, 1983). The procedure requires both the drug and polymer to be soluble in water, while the polymer should be soluble in the water-miscible organic solvent.

1.3.4. Albumin microspheres
Albumin microspheres are prepared by denaturation of the protein. The colloidal particles are known to sustain the release of entrapped therapeutic agent. Most methods involve the application of emulsion or suspension technology. Routine synthesis utilises an aqueous solution of a protein, a therapeutic agent at its saturation solubility and a suitable mineral oil to form
a w/o emulsion. Ultrasonication may be required in the emulsification step. Stabilisation is done by heat denaturisation (Yapel, 1979) or chemically using a suitable cross-linking agent (Senyei and Widder, 1982). Torrado et al (1989) found that the nature of the oil affects microsphere size while the size distribution is dependent on the design of the mixing cell, agitation speed and protein concentration. Albumin microspheres are used to target drugs to specific organs of the body according to their size. Gupta and Hung (1989) have reviewed their applications in drug delivery.

1.3.5. Multiple emulsion technique

This method has been reported by various workers with some modifications. The method of Nozawa et al (1979) and Yoshida et al (1980) gave three-walled microcapsules while Nozawa and Fox (1981) prepared two-walled capsules. Warburton (1982) described a process in which three-ply-walled microcapsules were formed by the loss of a volatile and slightly water soluble organic solvent from the hydrophobic layer of multiple emulsion droplets. It was further developed using more pharmaceutically acceptable materials (Morris and Warburton, 1982). The three-ply walls allow for more formulation variables than the conventional one layer walled microcapsules. Both unicored and multicored were produced and a bimodal particle size distribution was reported (Morris and Warburton, 1984). Duquemin (1987) used a wide range of wall forming materials to test the process and also encapsulated some drugs.

1.3.6. Modified methods

Over the years researchers have modified the classic microencapsulation processes to develop novel methods or improve on existing ones.

The major drawback of the emulsion solvent-evaporation technique is that water soluble and moderately water soluble drugs are poorly encapsulated because these partition into the aqueous continuous phase. Leelarasamee et al (1988) reported a solvent partitioning technique to circumvent this problem. Hydrocortisone suspended in a solution of PLA in methylene chloride was slowly injected into a stream of mineral oil. The methylene chloride partitioned into the mineral oil and the polymer precipitated around the drug particles. Tsai et al (1986) had used a system with mineral oil as continuous phase and an acetonitrile solution of PLA as the dispersed phase. Huang and Ghebre-Slassie (1989) proposed an adaptation of the emulsion-solvent evaporation procedure for making microspheres of water soluble pharmaceuticals using ethylcellulose in an alcohol-in-oil emulsion. An alcoholic solution of
ethylcellulose and diphenhydramine HCl as a model drug was emulsified in light mineral oil to form a water-in-oil emulsion. Sayed and Price (1986) developed a modified emulsion-solvent evaporation (MESE) technique. Here a fixed volume of a miscible non-solvent (for the polymer) is added to the polymer solution prior to emulsification. This resulted in a faster deposition of the polymer. Sanders et al (1984) prepared microspheres of nafarelin acetate, a potent analogue of luteinising hormone, in poly(d,L-lactide-co-glycolide) using a combined emulsification and phase separation technique. This is termed an emulsion non-solvent addition (ENSA) method. An aqueous solution of the drug and a solution of the polymer in oil is emulsified to a w/o emulsion. A non-solvent for the polymer is added to precipitate it out around the aqueous droplets, after which a large volume of non-solvent is added to complete the extraction of the solvent and harden the microspheres. The volume of non-solvent and the rate of addition are controlled. A similar technique was used by Vidmar et al (1984) to encapsulate water soluble oxytetracycline HCl. The drug was suspended in a solution of PLA in dichloromethane and phase separation was induced by non-solvent addition after emulsification. Sprockel and Prapaitrakul (1990) recently encapsulated paracetamol using the ESE, MESE and ENSE methods and compared the microsphere characteristics. The drug content of microspheres prepared by the ESE method was found to be significantly lower than those from the other methods.

Suryakusuma and Jun (1984) prepared coated hydrophillic microspheres by combining bead polymerisation and phase separation into a single step procedure. A drug dispersed in a polymer matrix was encapsulated in a polymer membrane of lower permeability. D’Onofrio et al (1979) developed a procedure which allows an oil slurry of microcapsules to be filled into soft gelatin capsules by adapting phase separation. A solution of ethylcellulose in ethylacetate was desolvated by the addition of light liquid paraffin so that a coat is deposited on the core material. The capsules are filled with the resultant slurry directly.

Ion-exchange resin beads were coated by several microencapsulation techniques to get control of drug release (Motycka and Nairn, 1979). Other workers have reported on sustained-release drug delivery systems made of coated ion-exchange resin complexes (Sprockel and Price, 1984).

Ishizaka et al (1981) prepared egg albumin microcapsules of phenacetin by a modification of the method of microspheres formation of Farhadieh (1975). A thin film of albumin, formed by denaturation, was applied onto the
surface of solid particles by adsorption.

Levy et al (1988) developed a microencapsulation procedure involving interfacial cross-linking of proteins and/or polysaccharides by means of bifunctional reagents. The types of walls obtained by this process include cross-linked haemoglobin (for red blood cells substitutes), cross-linked enzymes (for immobilised enzymes) and cross-linked serum albumin or gelatin which are biodegradable drug carriers.

Yeung and Nixon (1988) incorporated liposomes into nylon-walled microcapsules. The liposomes were coated by the polyamide formed by interfacial polymerisation. The release of drug from such a formulation was found to be slower when compared with that from simple nylon-walled microcapsules. However the final product could only exist as a suspension or slurry, thus suffering from many problems associated with conventional liposomal suspensions like drug leakage, microbial growth and oxidative degradation both of the drug and phospholipid content. To take care of these problems, Nixon and Yeung (1989) prepared microencapsulated liposomes with a more complex matrix containing gelatin and/or acacia associated with the nylon. In each case the product was recovered as a free-flowing powder.

1.4. Characteristics of multiple emulsions

The definition and nature of multiple emulsions, including the major types, have been covered in section 1.2.2.

1.4.1. W/O/W multiple emulsion types

Depending on the efficiency of the secondary emulsification step, and probably other factors including the nature of the secondary surfactants, a multiple emulsion drop could contain one, two, many or even numerous internal droplets. Indeed empty drops are not uncommon, although these would not be expected to predominate.

Florence and Whitehill (1981) identified three types of w/o/w emulsion systems distinguished by the nature of the predominating multiple emulsion droplets. The workers designated these types A, B and C respectively. The type A w/o/w emulsion was composed of relatively small drops containing one and occasionally more, relatively large internal aqueous droplets while the type B emulsion consisted of larger multiple drops which had smaller but more numerous internal droplets. Type C systems on the other hand comprised very large multiple drops, which contained very large numbers of internal droplets.
that were difficult to resolve. It was shown that the type C system retards the release of entrapped material to a much greater extent than the other types and hence thought to be more promising as a drug delivery system.

1.4.2. Mechanisms of instability in w/o/w multiple emulsions

Considering their composition and structure, w/o/w emulsions may be expected to deteriorate by one or more of the following mechanisms:
1) coalescence of the oil drops
2) coalescence of the internal aqueous droplets
3) rupture of the oil film separating the two aqueous phases and
4) passage of water to and from the internal droplets through the oil layer.

Florence and Whitehill (1981) investigated and analysed the mechanisms of instability in the three different w/o/w multiple emulsion types from section 1.4.1. Figure 1.2. represents some of the breakdown pathways put

![Figure 1.2. Possible breakdown pathways in w/o/w multiple emulsions](image)

Figure 1.2. Possible breakdown pathways in w/o/w multiple emulsions
forward by these workers. The internal oil drop may coalesce with other oil drops (which may contain internal aqueous droplets) as in [a], the internal aqueous drops may be expelled individually: [b], [c], [d], [e]; more than one may be expelled [f] or they may be expelled in one stage [g], less frequently. The internal aqueous drops may coalesce before expulsion: [h], [i], [j], [k]. Another possible mechanism is the gradual diffusion of water through the oil phase resulting in shrinkage of the internal droplets: [l], [m], [n].

Although a number of factors will determine the breakdown mechanism in any particular system, a tendency towards a reduction in the interfacial area and subsequent reduction in the free energy of the system will be a major driving force in the "choice" of a pathway. Thus coalescence of the oil drops would result in a large change in free energy and this was shown to occur in all three different emulsions. On the other hand coalescence of the internal droplets would not be expected to be a major breakdown pathway since it would not result in large decreases in free energy. It was reported to have been favoured by type A rather than type B emulsion systems. Although the internal droplets of type C systems are small, these are numerous so the overall change in free energy would be higher than in type B systems, as such the former may break via this mechanism. Expulsion of the internal aqueous droplets through rupture of the oil phase is a major breakdown mechanism. Complete rupture, with the release of internal phase in type C and shrinkage of internal droplets in type B, in both cases said to be due to differences in osmotic pressure, were observed under an osmotic gradient.

1.4.3. Factors controlling the stability of multiple emulsions

The stability of any multiple emulsion system will depend on the stability of the corresponding primary emulsion. If the primary emulsion is unstable, the internal droplets within the membrane phase of the multiple emulsion will coalesce resulting in an increased break-up of the multiple drop due to rupture. Therefore the factors controlling the stability and properties of simple emulsions (e.g. composition, pH, viscosity) will also influence the stability of the more complex systems. The internal aqueous phase volume may influence rate of transfer of material from this phase and hence stability (Collings, 1971). The stability of the multiple systems will however be determined by factors specific to multiple systems as well. Florence and Whitehill (1982) reviewed these factors.

Any factor which affects either the ability of droplets to coalesce or the
rate of transfer of material through the membrane phase will affect the stability of the multiple emulsion system.

1.4.3.1. Effect of electrolytes
The presence of electrolytes appears to be one of the most important factors in determining the stability of multiple emulsions, the effect being two-fold: osmotic and interfacial.

a) Osmotic effects
Water droplets may pass from one aqueous phase to the other. With a high osmotic pressure in the internal aqueous phase, water may pass to this phase leading to the swelling and eventual bursting of the droplets. When the osmotic pressure in the external aqueous phase is higher, water is transferred to this phase and the internal droplets shrink. If the osmotic pressure difference across the oil phase is so large, as occurs when the oil layer is so thin, water passes very rapidly causing immediate rupture of the oil drops with subsequent expulsion of the internal droplets. The inner aqueous phase then mixes with the external aqueous medium leaving a simple oil drop. The presence of material other than simple electrolytes e.g. drugs, protein or sugar in either aqueous phase may exert this effect.

b) Interfacial effects
Brodin et al (1978) reported an initial decrease in the diffusion coefficient of naltrexone hydrochloride from the internal aqueous phase of a w/o/w emulsion with increasing sodium chloride concentration. They suggested that the electrolyte competes with surfactant for water molecules at the inner water/oil interface. This would result in a rigid interfacial layer which will be a more effective barrier to transfer of material across the membranes. Such an effect will confer stability on the system by retarding transfer of material.

1.4.3.2. Viscosity of the organic solvent
Transfer of material occurs across the organic phase, so any property of this phase that will affect the rate of this transfer will determine stability. In a study with different w/o/w systems using a mixture of two oils, a high viscosity oil and a normal paraffinic oil, Frankenfeld et al (1976) found that the stability of the membrane towards rupture and leakage of entrapped material decreased with decreasing viscosity. This is as a result of a higher rate of transfer of material with the decrease in viscosity.
1.4.3.3. Type of surfactant (emulsifier)

Li (1971) found that ionic surfactants gave a much higher transport rate of toluene through an aqueous membrane. The rate of diffusion in systems with non-ionic surfactants also varied with hydrophilic chain lengths of the polymers. Apparently then, the nature of the surface active agent could affect stability.

1.4.3.4. Concentration of surfactant (emulsifier)

This is critical in determining stability especially in the primary emulsification step. Collings (1971) reported differences in percentages of sodium chloride released per unit time with varying concentrations of primary surfactant.

1.4.3.5. Properties of the interfacial films

A multiple emulsion has two liquid-liquid interfaces. The nature of each film will depend on the nature of the primary and secondary emulsifiers used in the preparation. It will also depend on the presence of materials in the internal and continuous phases. The viscosity and elasticity of the interfacial films are vital in determining the stability by hindering the close approach of drops (by surfactant chain effect) and preventing coalescence by acting as a mechanical barrier. The nature of the films will also determine the rate of transfer of material. Since the two interfaces are in close proximity, diffusion of emulsifier between the interfacial films may occur and this would change the composition and thickness of the adsorbed multi-layer, probably till an equilibrium is attained. Using microscopy with cross polarisers, Kavaliunas and Frank (1978) detected liquid crystal phases at both inner (w-o) and outer (o-w) interfaces in a w/o/w system. Liquid crystalline phases were also detected in both interfaces in o/w/o emulsions. The presence of these structures was found to improve the stability of the emulsions a lot.

1.5. Transfer of material in primary emulsions

1.5.1. Mechanisms of transfer of material across emulsion phases

The most obvious method of passage of material across a membrane is by diffusion for unionised material. Other possible transfer mechanisms put forward are micellar transport, carrier-mediated transfer and, a much less
important mechanism, which is the solubilisation of minute amounts of one phase in the other.

1.5.1.1. Diffusion
Diffusion is the spontaneous penetration of one substance into another under the potential of concentration gradient. In solutions, it affords the distribution of material between various phases. Hence the material will tend to move from a region of higher concentration to one of lower concentration. Diffusion rate of drugs across a membrane can be described according to Fick's first law as

\[ J = - D \frac{dC_m}{dx} \tag{1.1} \]

where \( J \) = flux per exposed surface area in mg/s.cm²; \( D \) = diffusion co-efficient (cm²/s); \( C_m \) = concentration in the membrane (mg/cm³); \( x \) = distance into the membrane i.e., membrane thickness (cm) and \( \frac{dC_m}{dx} \) = concentration gradient within the membrane. The negative sign indicates that positive flow occurs down a negative concentration gradient. The rate of diffusion of a substance in solution is a function of molecular size and shape, and degree of interaction with the solvent. The diffusion co-efficient is approximately proportional to the reciprocal of the cube root of the molecular weight for solutes the molecules of which are large compared with those of solvent. An analysis of diffusion of solute from one phase of an emulsion to another requires a knowledge of the distribution of the drug in the phases of the emulsion. Partitioning is the movement of material from one phase to another. For a water-in-oil emulsion containing a lipophilic drug, some of the drug partitions into the aqueous phase. As drug concentration in the aqueous phase depletes into a sink, which could be a distribution phase or adsorption site, it is compensated by partitioning of the drug from the oil phase. This concentration gradient creates a driving force for diffusion. The rate of transfer of material will depend on the concentration gradient and the diffusion rate.

1.5.2. Factors affecting rate of transport across emulsion phases
The factors affecting the rate of diffusion will by inference influence the rate of transfer of material across the phases of emulsions.

1.5.2.1. Partition co-efficient
A drug in one phase of an emulsion will partition between the aqueous and
oily phases. The partition co-efficient is defined as:

\[ P = \frac{C_o}{C_w} \tag{1.2} \]

where \( C_o \) is the concentration in the organic phase and \( C_w \) the concentration in the aqueous phase. It is therefore a measure of the relative affinities of the solute for an aqueous and a non-aqueous phase. However it would only be constant if a single molecular species is involved and ionisation or any other association will disrupt it. Many drugs are weak electrolytes and will ionise in at least one phase, usually the aqueous phase. If ionisation and its consequences are neglected, an apparent partition co-efficient can be got. It is related to the partition co-efficient by the following equations:

for acids,

\[ \log P = \log P_{app} - \log \left[ \frac{1}{1+10^{pH-pK_a}} \right] \tag{1.3} \]

for bases,

\[ \log P = \log P_{app} - \log \left[ \frac{1}{1+10^{pK_a-pH}} \right] \tag{1.4} \]

where \( P_{app} \) is the apparent partition co-efficient.

If the aqueous phase contains a micellar phase, i.e. oily dispersed phase, at equilibrium drug could be distributed among the three phases. Another equation defining the partition coefficient is:

\[ P = \frac{J}{C} \tag{1.5} \]

in which \( C \) is the concentration difference across the membrane, which is taken to be equal to the donor phase concentration (mg). The partition co-efficient is therefore directly proportional to the flux, \( J \), which is in turn directly proportional to the diffusion co-efficient according to equation 1.1. The extent of partitioning of the drug between the various phases and the interdependence of this and other parameters will influence mass transfer. Any procedure that will concentrate the solute in the aqueous phase e.g. formation of a water soluble protonated salt or complex formation in the aqueous phase will lower the apparent partition co-efficient and hence diffusion rate.
1.5.2.2. Donour phase concentration
From equation 1.5., the value of the partition co-efficient and hence the diffusion rate will depend on the concentration in the donor phase. Ayres and Lasker (1974) reported an increase in diffusion rate with increasing amount of drug originally in w/o and o/w emulsions, up to saturation point. This is because rate of diffusion for drugs actually depends on the amount rather than the concentration. The release pattern from emulsions has however been said to be the same whether diffusion co-efficient is concentration dependent or not (Koizumi and Higuchi, 1968).

1.5.2.3. Phase volume ratio
Increasing the volume of the phase the drug is in originally decreases the rate of transfer. Friedman and Benita (1987) observed this and attributed it to the retention capacity of the dispersed oily droplets, the larger number of which was able to sustain the drug release over longer periods of time.

1.5.2.4. Viscosity
Several workers have noted an inverse relationship between viscosity and diffusion rate (e.g. Garrett and Chemburker, 1968).

1.5.2.5. Droplet size
Omotosho et al (1986) and Friedman and Benita (1987) both noted a decrease in diffusion rate with increasing mean droplet size. This is a direct result of decreased contact area between the phases of the emulsions.

1.5.2.6. Nature of the organic solvent
If the oil is slightly water soluble diffusion of the lipophilic drug from this vehicle is enhanced (Plakogiannis and Yaacob, 1977). The solubilisation of the vehicle can hence render more drug in the aqueous phase than would be present if the vehicle was completely water insoluble.

1.6. Sustained* and controlled-release formulations

1.6.1. Introduction
Sustained-release, prolonged-release, timed-release and controlled-release dosage forms are terms used to identify drug delivery systems that are designed to achieve a prolonged therapeutic effect by continuously releasing
medication over an extended period of time after administration of a single dose (Lordi, 1986). In the case of orally administered forms, this period is measured in hours and critically depends on the residence time of the dosage form in the gastrointestinal (GI) tract. Basically they are comprised of a biologically active agent and a suitable excipient, commonly a polymeric material. The term 'controlled-release' has become associated with systems that deliver therapeutic agents automatically. The concept implies a quantitative understanding of the physicochemical mechanism of drug availability to the extent that the dosage form release rate can be specified. Such predictability and reproducibility of release kinetics is achieved using therapeutic systems, which are dosage forms that precisely control rate and duration of drug delivery (Chandrasekaran, 1978). They comprise a drug component, a drug delivery module, a platform and a therapeutic program. Examples of controlled-release delivery systems are implants, inserts, osmotic pumps, transdermal devices etc. The hydrodynamic pressure controlled systems, intragastric floating tablets, transmucosal tablets, and microsporous membrane coated tablets (Chien, 1983) are some of the potential developments and new approaches to oral controlled-release drug delivery technology. Microencapsulation is a means of attaining control of drug release.

1.6.2. Advantages of sustained-release dose forms

The administration of sustained-release dose forms enables increased reliability of therapy (Urquhart, 1981). The obvious desirable therapeutic advantages that can be achieved from prescribing such dose forms are given below.

1) The frequency of drug administration is reduced, thereby increasing patient compliance and convenience. 2) The blood level oscillation characteristic of multiple dosing in conventional dose forms is reduced as a more even blood level is maintained.

3) The total amount of drug administered can be reduced, hence maximising availability with a minimum dose.

4) The safety margin of high potency drugs can be increased, while the incidences of local and systemic adverse side effects can be reduced.

1.6.3. Evaluating drugs as candidates for sustained-release formulation

Table 1.2. is a list of characteristics of drugs unsuitable for peroral sustained-release forms and some examples. It indicates that the drug should be
Table 1.2. Characteristics of drugs unsuitable for peroral sustained release dose forms.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Drugs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Not effectively absorbed in the lower intestine.</td>
<td>Riboflavine, Ferrous salts</td>
</tr>
<tr>
<td>Absorbed and excreted rapidly, short biological half-lives (&lt;1 hr).</td>
<td>Penicillin G, Furosemide</td>
</tr>
<tr>
<td>Large doses required (&gt;1 gm).</td>
<td>Sulphonamides</td>
</tr>
<tr>
<td>Long biological half-lives (&gt;12 hr).</td>
<td>Phenytoin, Diazepam</td>
</tr>
<tr>
<td>Cumulative action and undesirable effects; low therapeutic indices.</td>
<td>Digitoxin, Phenobarbital</td>
</tr>
<tr>
<td>Precise dosage titrated to individual required.</td>
<td>Cardiac glycosides, Anticoagulants</td>
</tr>
<tr>
<td>No clear advantage for sustained-release.</td>
<td>Griseofulvin</td>
</tr>
</tbody>
</table>
amenable to at least one of the advantages of controlling release given in section 1.6.2. to justify formulation. Another aspect that must be considered in evaluating drugs for sustained delivery is some disadvantages of such formulations. These include the following:

1) Dosage regimens are fixed by the dosage form design, so there is not much flexibility here.
2) There is no possibility of prompt termination of therapy in cases of immediate changes in drug need during therapy such as significant adverse drug effects.
3) Sustained-release forms are designed on the basis of average drug biological half-lives, therefore disease states that alter drug disposition, significant patient variation including specific needs of individuals, etc. are not accommodated.
4) Economic factors should be assessed since the equipment and processes used for manufacturing many sustained-release forms are more costly than those for conventional dose forms.

1.6.4. Design of sustained-release formulations

To formulate a sustained release dosage form, there must be a correlation between the blood levels of the drug and its pharmacological effect. It is necessary to have a clear understanding of the pharmacokinetics of the candidate and the therapeutic range, including the minimum effective and maximum safe doses before proceeding with design. There are also some specific parameters that must be taken into account in optimising sustained release dosage form designs. These are: Di, the loading or immediately available portion of the dose; Dm, the maintenance or slowly available portion of the dose; Tm, the time at which release of maintenance dose begins and kr, the specific rate of release of the maintenance dose.

One property of blood-level-time profiles characteristic of multiple dosing therapy in conventional dose forms is the oscillations (peaks and troughs). As the dosing interval is reduced, the number of doses required to attain a steady state drug level increases, the amplitude of the oscillations diminishes, and the steady state average blood level is increased. The drug level oscillations can be eliminated by a constant rate intravenous infusion. The ultimate objective in formulating an oral sustained release dose form is to provide such a constant blood level for up to 12 hours. Figure 1.3. depicts the sort of drug-level time profile that characterises an ideal peroral sustained release dose form after a single dose. It approximates to a design consisting of
a loading dose and a zero-order release maintenance dose, as described by Robinson and Eriksen (1966).

In the figure (1.3.), $T_p$ is the peak time, $h$ is the total time after administration in which the drug is effectively absorbed. $C_p$ is the average drug level to be maintained constantly for a period of time equal to $(h-T_p)$ hours, it is also the peak blood level observed after administration of a loading dose. The portion of the area under the curve contributed by the loading and maintenance doses is indicated. To obtain a constant drug level, the rate of drug absorption must be made equal to its rate of elimination.

Figure 1.3. A blood-level time profile for an ideal peroral sustained release dosage form
Thus the drug must be provided by the dosage form at a rate such that the drug concentration becomes constant at the absorption site. If this zero order release characteristic is implemented, the release process becomes independent of the maintenance dose and does not change during the effective maintenance period. Lordi (1986) has given the equations that can be used to estimate design parameters for an optimised zero order model, for both simultaneous and delayed release of maintenance dose.

1.6.4.1. Multiple dosing

Even with the zero order model, multiple dosing can result in non ideal blood level profiles. Only the dosing interval is adjustable in sustained-release therapy. If the dosing interval is equal to or lower than the total anticipated drug release time, accumulation results from formulations designed with loading doses. On the other hand for such designs, if two units are administered followed by single-unit subsequent doses, after several doses the overall drug level falls slightly lower than the average level determined by the dosage form design. Blood level peaks may be observed with the zero order release model, but minimal fluctuation could be obtained if the dosing interval is set equal to \( (h + t_{up}) \). Increasing the dosing interval further diminishes the peak and deepens the trough in the blood level profile, thus defeating one of the objectives of the dosage form design. Welling and Dobrinska (1978) have described some strategies for attaining approximate ideal profiles in multiple dosing of different sustained release designs based on both cumulative and noncumulative approaches. However, administration of formulations without loading doses can result in minimal fluctuations during long term therapy.

For a zero order based design consisting only of a slow release maintenance dose administered at intervals of \( h \) hours, if absorption is consistent, drug profiles equivalent to those from constant rate infusion could be obtained. In such a case, only one dosing interval is required to attain near steady state drug levels and accumulation will only take place if the dosing interval is less than the effective maintenance time.

1.6.5. Methods of achieving sustained release

The two general sets of methods developed for implementation of practical sustained release dosage form designs are:
1) methods based on modification of physical and/or chemical properties of the drug and
2) methods based on modification of the drug release rate characteristics of the dosage form that affect bioavailability.

1.6.5.1. **Approaches based on dosage form modification**

Formulations based on the modification of the physicochemical properties of the dosage form can be classified into three product types: encapsulated slow release beads (or granules), tabletted mixed or slow release granulations and slow release tablets. Two general principles are used in retarding drug release from most sustained release formulations involving dosage form modification: the embedded matrix and the barrier principles (Lordi, 1986). Both are based on membrane-moderated controlled-release devices.

1.6.5.1a. **Embedded matrix concept**

The embedded matrix concept as a mechanism of controlling release in sustained release dosage form design is schematically represented in figure 1.4. (Lordi, 1986). The drug is dispersed in a matrix of retardant material (i.e. a ‘monolithic’ device), which can be encapsulated as particles or compressed into tablets. The release is controlled by a number of physical processes: permeation of the matrix by water, leaching of drug from the matrix and erosion of the matrix material. Alternately, drug may dissolve in the matrix material and be released by diffusion through the matrix material or partitioned between the matrix and extracting fluid.

The kinetics of release is based on the Higuchi (1963) equation for defining release from inert matrices:

\[ Q = \left[ \frac{D \epsilon C_s}{t} \left( 2A - \epsilon C_s \right) t \right]^{1/2} \]  \hspace{1cm} (1.6)

where \( Q \) is the amount of drug released per unit surface after time \( t \), \( D \) is the diffusion coefficient of the drug in the elution medium, \( T \) is the tortuosity of the matrix, \( C_s \) is the solubility of the drug in the elution medium, \( \epsilon \) is the porosity of the matrix, and \( A \) is the initial loading dose of the drug. Linear diffusion is assumed. Drug release is triggered by penetration of eluting media into the matrix, dissolving the drug and thereby creating channels through which diffusion occurs (figure 1.4). The porosity and tortuosity are functions of the amount of dispersed drug, the physicochemical properties of the matrix, and the dispersion characteristics of the drug in the matrix.
Figure 1.4. Embedded matrix concept as a mechanism of controlled release in sustained release dosage form design. Network model (a): drug is insoluble in the retardant material. Dispersion model (b): drug is soluble in the retardant material. Diffusion profile (c) characterises drug release from a matrix.
If the drug is freely soluble in the elution medium i.e., $C_s \gg A$, such that the dissolution rate is rapid, the following equation which describes the release of drug from a solution entrapped in an insoluble matrix, then applies:

$$Q = 2A(Dt/\pi T)^{1/2}$$  \hspace{1cm} (1.7)

Here the rate of release is directly proportional to the amount of dispersed drug, $A$; it is proportional to $A^{1/2}$ for insoluble drugs if $2A = C_s$, and plots of $Q$ versus $t^{1/2}$ will be linear. Chien and Lambert (1974) has described the application of a general expression for the case of a drug dispersed in a matrix in which the drug dissolves, where both matrix and partition control are possible:

$$Q = KDCst/h$$  \hspace{1cm} (1.8)

where $K$ is the partition coefficient ($K = C_s/C_p$), $C_p$ is the solubility in the matrix phase, and $h$ is the thickness of the hydrodynamic diffusion layer. Here the drug has a low solubility, partition control dominates, and the release is zero-order. The zero order model is described by the following equation:

$$X_t = X_0 - k_0t$$  \hspace{1cm} (1.9)

where $X_0$ is the initial concentration, $X_t$ is the concentration at time $t$ and $k_0$ is the zero order rate constant. The equation implies that a plot of amount of drug released versus time is linear. The variables affecting drug release, which have been studied using these models, included the nature of retardant, drug solubility, effect of added diluents, drug loading, drug mixtures and drug-matrix interaction (Desai et al, 1966).

1.6.5.1b. Barrier concept

The barrier concept of controlled release implies that a layer of retardant material is imposed between the drug and the elution medium, resulting into a 'reservoir' device (Baker and Londsdale, 1974). The release mechanism involves diffusion of drug through the barrier, permeation of the barrier by moisture, and/or erosion of the barrier. The release rate is dependent on barrier permeability which is governed by its composition, physicochemical properties, thickness and integrity, and also the device configuration. Figure 1.5. is a
schematic representation of the more significant models of barrier-mediated release. For case B, the barrier is permeable to the elution medium; a lag time is involved since drug is not released until moisture has penetrated the barrier, dissolving the drug in the reservoir. Case C involves timed erosion of the barrier, while for case D the release mechanism is rupture of the barrier after sufficient moisture has penetrated the membrane.

Figure 1.5. Barrier-mediated models of sustained release dosage form designs. A, drug diffusion through the barrier. B, Permeation of barrier by elution media followed by drug diffusion. C, Erosion of barrier, releasing drug. D, Rupture of barrier as a result of permeation of elution media.
Figure 1.6. shows the form of the drug release profiles characteristic of these models. For case A, drug is present in the reservoir as a solution or suspension; the barrier is impermeable to the elution medium. At steady state the rate of release into a sink is:

\[
\text{DEPLETION PHASE} < \text{BURST EFFECT} < \text{STEADY STATE PHASE}
\]

Figure 1.6 Drug-release profiles characteristic of barrier-mediated models. A, B, Membrane-controlled diffusion.
\[ R = \frac{SDmCs_m}{I} \]

where \( S \) is the surface area, \( D_m \) is the diffusion coefficient of drug in the membrane, \( I \) is the thickness of the membrane barrier, and \( Cs_m \) concentration of drug in the membrane, assuming constant activity of drug in the reservoir (Lordi, 1986). For the membrane-encapsulated solution, release is first order, the kinetics described by the following equation:

\[ \log W = -kt/2.303 + \log W_0 \]

where \( W \) = amount of drug left; \( W_0 \) = initial amount of drug; \( k \) = first order rate constant and \( t \) = time. Plots of logarithms of amount of drug released versus time will be linear. For membrane-encapsulated suspensions, release is zero order if membrane diffusion is slower than dissolution. A burst effect results if the membrane is saturated with drug (curve A in fig. 1.6.), and a lag time if drug has not penetrated the membrane (curve B in fig. 1.6.). Drug release approximates first order during the depletion phase.

Dosage form designs may be applied to all drug types, the selection of a particular dosage form being limited by the specific drug properties (e.g. solubility, dissociation constant etc.), the manufacturing technology available and the methodology needed to establish the validity of the design.

1.6.5.2. Approaches based on drug modification
The physicochemical properties of a drug can be altered by complex formation, drug-adsorbate preparation, or prodrug synthesis. These techniques are only possible with drug moieties containing appropriate functional groups e.g. acidic or basic. CD inclusion complexes and ion-exchange resin-drug adsorbates have been discussed in sections 1.2.3. and 1.2.1. respectively. The main advantage of this approach is that it is independent of the dosage form design. Drugs thus modified may be formulated as liquid suspensions, capsules or tablets. Loading doses of unmodified drug could be included.

Figure 1.7 identifies the mechanisms involved in controlling drug release from complexes, adsorbates and prodrugs.

1.6.5.2a. Complexes
Complexes can be formed from drugs and appropriate polyelectrolytes or cyclodextrins. Here the effective release rate is a function of two processes: the rate of dissolution of the solid complex into the biological fluids and the
rate of dissociation or breakdown of the complex in solution. The dissolution step may be described by the following expression:
Dissolution rate = ks(solubility)(surface area)

where ks is the dissolution rate constant, a function of the hydrodynamic state as well as factors influencing the diffusion process. The surface area could be altered by control of particle size and/or solubility of the drug complex via selection of the complexing agent. The two processes are both dependent on pH and composition of the gastric and intestinal fluids, but the dissociation step is critically so.

Figure 1.7. Mechanisms of sustained release based on drug modification
This is because its rate may be pH-dependent, may be determined by the ionic composition of the fluid, and may be affected by the natural enzymatic processes. Thus the specific in vivo processes involved have to be known and this should inform the selection of the complex. For example drug release could depend on the availability of a specific ion and its concentration in gastro-intestinal fluids, and although some salts of weak bases resist the action of gastric fluid, natural digestive processes in the intestine act to dissociate the complexes.

If the rate of dissolution is greater than the rate of dissociation, a zero order release profile may be realised. This is because if the solubility of the complex is sufficiently low, its concentration is maintained at the saturation point, such that excess solid complex is present during most of the effective maintenance time. Further control of release may be achieved by preparing high specific surface material to promote dissolution. On the other hand, if dissociation rate is greater than dissolution rate, dissolution of the complex becomes rate-determining.

1.6.5.2b. Drug adsorbates
This is a special case of complex formation where the product is essentially insoluble e.g. ion-exchange resin-drug complexes. Availability of drug is determined only by the rate of dissociation (desorption) of the complex, and therefore, access of the adsorbent surface to water as well as the effective surface area of the adsorbate. Ion-exchange resin-drug adsorbates for sustained-release have been prepared by many workers (see section 1.2.1.).

1.6.5.2c. Prodrugs
Prodrugs are therapeutically inactive drug derivatives that regenerate the parent drug by enzymatic or nonenzymatic hydrolysis. For sustained release, the solubility, specific absorption rate, and/or elimination rate constant of an effective prodrug should be significantly lower than that of the parent compound (Lordi, 1986). Kwan (1978) has described the pharmacokinetics of a prodrug in which the sustained blood level is determined by the metabolic rate, i.e., by formation of the active moiety after absorption. Elslager (1969) designed several sustained release forms of the sulphonamide, dapsone (DDS) by modifying its free amino acids to get compounds of very low solubility. Sinkula (1978) has reported the synthesis of prodrugs of isoproterenol, isoniazid, and penicillin designed for prolonged-release. In the event that the solubility of a drug has been significantly reduced by prodrug formation, and
where breakdown of the prodrug takes place at the absorption site, availability will be limited by dissolution rate.

1.7. **Release of drugs from microcapsules**

1.7.1. **Introduction**
Release of drug from microcapsules and microparticles is a mass transport phenomenon involving diffusion of drug molecules from a region of high concentration in the dosage form to a region of low concentration in the surrounding environment (Deasy, 1984). Due to the great diversity in the physical form of microcapsules and also differences in the physicochemical properties of core and coating materials, modelling of drug release from such systems is difficult. Thus several workers have reported such attempts to be less than satisfactory (Nixon and Walker, 1971; Madan, 1981).

1.7.2. **Permeation considerations**
Diffusion as a means of transfer of material was discussed in section 1.5. In microcapsules the coating normally acts as the first barrier to diffusion and the second barrier would be the unstirred diffusion layer between the coating and the receiving phase. Figure 1.8. depicts a microcapsule arrangement whereby a homogenous coating is interposed between a core composed of solid or liquid drug and an aqueous receiving phase containing a negligible bulk drug concentration. The thickness of the stagnant diffusion layer is a function of stirring rate in the bulk aqueous phase, its composition and viscosity, and the molecular volume of the diffusant at a particular temperature.

Diffusion in microcapsules may involve transport not only through an isotropic medium such as drug in solution, but also through a homogenous polymeric membrane. Transport here involves dissolution of the permeating drug in the polymer at the high concentration side of the membrane interface and diffusion across the membrane in the direction of the concentration gradient as shown in figure 1.9. The drug concentrations just inside the membrane interfaces are:

\[ C_{m(h)} = KC_h \]  \hspace{1cm} (1.12)

at the high concentration side and
Core solid or liquid drug

Coating

Aqueous receiving phase which contains a negligible bulk drug concentration

Stagnant diffusion layer

direction of diffusant flux

Figure 1.8. Schematic representation of drug passage through an idealised homogenous coating of a microcapsule (After Deasy, 1984).

C_h

C_{m(h)}

C_{m(l)}

C_l

High concentration solution side \( (C_h) \)  

Membrane thickness \( (t_m) \)  

Low concentration solution side \( (C_l) \)

Figure 1.9. Concentration gradient across an ideal isotropic polymeric membrane \( (K < 1) \)  
(After Deasy, 1984).
\[ C_{m(0)} = KC_i \]  

(1.13)

at the low concentration side, where \( K \) is the partition or distribution coefficient of the drug
toward the polymer and is assumed to be constant.
Assuming a steady state, Fick's first law equation (Eq.1.1) may be integrated
from \( x = 0 \) to \( x = 1 \) and from \( C = C_{m(0)} \) to \( C = C_{m(0)} \) to give

\[ J = D_m A/l_m \left( C_{m(0)} - C_{m(0)} \right) \]  

(1.14)

where \( dC_m \) is the difference in concentration across the two faces of the
membrane, \( l_m \) is the membrane thickness, \( D_m \) is the diffusion coefficient in
polymeric membrane and \( A \) is the surface area. As the concentration of drug
dissolved in the membrane is usually unknown, Eq. (1.14) is written as

\[ J = D_m KA dC/l_m \]  

(1.15)

where \( dC \) is the difference in concentration of drug in solution on either side
of the membrane. The overall permeation of a solute through a polymer is
described by the term permeability coefficient \( P \), where

\[ P = D_m K \]  

(1.16)

Where the membrane thickness is known, \( D_m K/l_m \) is used to denote the
permeability. Takamura et al (1971) have described how this value for
electrolytes may be determined with polyphthalamide microcapsules. From this
value the apparent diffusion coefficient can be calculated (Takamura et al,
1973).

1.7.3. Kinetics of drug release from
microcapsules
Mathematical models used to describe the kinetics of drug release from
microcapsules and microparticles are usually based on drug release from
macrocapsular or macromatrix devices, respectively. Due to the size difference
however, drug release from the microdosage forms tends to attain a steady
state that is of shorter duration faster. Also, the sometimes irregular shape of
particles in this dosage forms often show a poor fit for release data from conventional models based on spherical, cylindrical, or other regular geometries (Deasy, 1984). Many microcapsules are multinuclear or aggregates of smaller particles so their release kinetics do not follow that expected of a reservoir-type device i.e. zero order but rather that of a monolithic devise i.e. $t^{1/2}$ dependent first, followed by first order in some cases. The mechanisms and rate theories of the release models have been treated in section 1.6.5.

Sometimes more stringent tests than the conventional ones have to be applied to characterise the release. Studying the release of salicylic and benzoic acids from inert wax matrices, Schwartz et al (1968) found that the data fitted both the square root of time dependent and first order release kinetics, giving linear plots. They derived rate equations corresponding to the Higuchi (matrix) and first-order equations. For the matrix mechanism, the release rate will be inversely proportional to the total amount of drug released $Q'$ according to the equation:

$$\frac{dQ'}{dt} = k_H^2/2Q' \quad (1.16)$$

where $Q' = QS$ and $S$ is the surface area of the microcapsules; $Q$ is the amount released per unit surface area; $k_H$ is the Higuchi rate constant. Here a plot of rate of dissolution versus the reciprocal of amount released is linear. The rate predicted by the first order kinetics is given by the following equation:

$$\frac{dQ'}{dt} = k_1w_0 - k_1Q' \quad (1.17)$$

Here $w = w_0 - Q'$; where $w$ = amount of drug left, $w_0$ = initial amount of drug, $k_1$ = first order rate constant and $t$ = time. Here the rate is directly proportional to $Q'$, so a plot of release rate versus $Q'$ will be linear. This provided a basis for differentiating the two mechanisms. The release profile was found to follow the matrix model. Benita et al (1984) used the same procedure to ascertain that the release of paracetamol from methacrylate copolymers microcapsule followed first order rather than both this and the matrix model. Satyanarayana Gupta and Sparks (1980) presented a mathematical model for progesterone release from spherical injectable polylactic acid microcapsules. The model was based on a matrix device where a boundary layer and a time-variant drug concentration on the outside of the particles were taken into account. A ‘boundary layer’ on the surface of
microcapsules is made up of an appreciable amount of free drug in contact with the surface. This layer is stagnant and hinders release of drug by diffusion. The effect is more marked with low solubility drugs, where their concentration in the unstirred layer can tend toward the solubility of the drug with a resultant loss in driving force for diffusion.

1.8. Cross-linking reactions of water soluble polymers

1.8.1. Introduction
Cross-linking is the combination of reactive groups in a polymer with other groups, polymeric or otherwise, to form a network under conditions which do not significantly degrade the polymer. Water solubility implies the presence of a substantial number of polar groups in a molecule, so the potential for chemical and other types of reactions is considerable. Gel formation may be regarded as a form of cross-linking which is possibly reversible by physical change. There are two types of cross-linking reactions by chemical modification (Finch, 1983):
1) Cross-linking with functional groups
Polymers with pendant or backbone functional groups may be cross-linked by typical organic chemical reactions involving functional groups. Linking could be between groups on the polymer and those of an added compound or between groups on two reactive polymers. The mechanism of bond formation may be by addition, condensation or ionic interaction. The reaction could be inter or intramolecular and added agents could be mono or bifunctional. An example is the cross-linking reactions involving metal ions which occur in many polymers with hydroxyl side groups.
2) Cross-linking by hydrogen bonding
This could be either inter or intramolecular bonding. The reversible gels produced on cooling a water soluble polymer solution can be regarded as chemically cross-linked networks, in which the cross-linking function is due to hydrogen bonding (Bochard, 1983).

1.8.2. Gels
Hermans (1949) defined a gel as a coherent system of at least two compounds, which exhibits mechanical properties characteristic of a solid, where both the dispersed component and the dispersion medium extend themselves continuously throughout the whole system. According to Flory (1963), the most striking property of gels is their rigidity, and on a molecular level, the
presence of an 'infinite' network of macroscopic dimensions is said to explain the stability of their shape towards external force (Flory, 1963). The gel point of a polymerising system is considered to be the point at which a 3-dimensional network, infinite in extent, first appears (Flory, 1963). The equilibrium transition is a cooperative intrachain reaction which may be caused by thermally induced glass transition, a partial crystallisation or an intermolecular ionic bonding of chains of one of the blocks. The latter can be induced by the addition of a third component to the polymer solution such as divalent ions e.g. Ca\textsuperscript{2+} in the form of salts in disaccharides (e.g. Vrij, 1964). The gel structure is lost if monovalent alkali metal ions such as Na\textsuperscript{+} are added. This type of transition is not necessarily thermoreversible. The common feature of the secondary and tertiary structure of gelling copolymers like polysaccharides seems to be their capability to form single and double helices, where two or more chains are involved (Bochard, 1983). This coil to helix transition, which occurs even in extremely dilute solutions, is an intramolecular transformation in homogeneous phase.

1.8.2.1. Types of gels

Flory (1974) categorised gels into four types as follows:

1) Well-ordered lamellar structures, the forces between these being electrostatic or dipolar. These include gel mesophases such as soap gels and clays.

2) Covalent polymer networks that are completely amorphous. Examples are vinyl-divinyl copolymers, alkyd and phenolic resins and silica gel.

3) Polymer networks formed by physical aggregation. These are predominantly disordered with regions of local order. The structure of the primary molecules, by means of which the network is built, is usually linear and of finite size. The network junctions are multiple stranded helices, bundles of chains or crystalline regions. These include gel systems formed by polysaccharides and polypeptides.

4) Particulate disordered structures, such as flocculant precipitates, which usually consist of particles of large geometric anisotropy, e.g. disordered network-like accumulations of fibrils as in gels formed by aggregation of proteins.

Most water soluble polymers form type 3 gels, i.e. gels which are mainly disordered, with regions of local order. For thermoreversible gels, at the gel melting temperature $T_m$, this order is lost, and a viscous solution containing the polymer in a random spatial distribution is formed. If the temperature is lowered below $T_m$, the gelation begins again. This thermally induced process
may be repeated several times under isothermal conditions. However strictly, gel formation is an irreversible process (Bochard, 1983). Figure 1.10. represents the formation of a typical gel from a water soluble polymer, that of carrageenan, showing the junction zones.

Fig. 1.10. Junction zones of carrageenan gel. The arrows indicate galactose sulphate or disulphate residues replacing anhydrogalactose units; these residues break up the regular helical structure. (Finch, 1983).
1.9. Buccal dose forms

1.9.1. Introduction
Absorption from the gastrointestinal tract is frequently a problem for many drugs. This may be due to unfavourable physicochemical properties (Atkinson et al, 1962) or degradation of the drug (Nelson, 1962) which may be enzymatic or non-enzymatic. The degradation may take place in the gastric lumen (Renwick, 1982), the brush border membrane (Adibi and Kim, 1981) or during the first-pass through the liver after absorption (Benet, 1979). Several alternative routes of delivery have been employed to circumvent these problems. Gibaldi and Kanig (1965) used the buccal route for this purpose. Anders et al (1983) reported on the buccal absorption of protirelin. Bogaert and Rosseel (1972) measured the plasma levels of nitroglycerin after buccal administration in human subjects. Assay of the saliva and rinsing fluid at 3.5 minutes revealed that 25-40% of the dose of the drug was not absorbed. These results indicate the possibility of prolonging the release of drugs administered via this route. Davidson et al (1971) presented data of plasma levels of pentaerythritol absorbed through the buccal mucosa. Bardget et al (1984) reported on the plasma concentration and bioavailability of a buccal preparation of morphine sulphate.

1.9.2. Advantages of the buccal route
1) Potential for controlled drug delivery with a suitable device.
2) Reduction of drug degradation possible with gastrointestinal administration.
3) Ease of administration and removal of drug dosage.
4) Elimination of direct gastric irritation.
5) Ability to alter site of drug application to avoid mucosal irritation.
6) Avoidance of variable gastrointestinal transit time and chemical environment as factors in absorption.

1.9.3. Absorption of drugs through the buccal mucosa
One of the unexpected functions of the mouth is its capability to serve as a site for absorption of drugs. It contains a highly vascular mucous membrane, so any drug diffusing in has ready and direct access to the general circulation via numerous arteries and capillaries. The mucous membrane contain many minute buccal glands which pour their secretions into the mouth. The bulk of the secretion is supplied by the three pairs of salivary glands.
(parotid, submaxillary and sublingual). Each parotid gland is located just under and in front of the ear and its ducts open upon the inner surface of the cheek which is the buccal cavity. Blood is richly supplied to the salivary glands and their ducts by branches of the external carotid artery and is returned after travelling through many arteries and capillaries via the jugular veins.

The main disadvantages of using this route are 1) its narrow area of application compared with say the dermal one and 2) its higher barrier property for permeation of foreign materials compared with other parts of the alimentary canal such as the small intestine. However there are regional variations in the epithelial thickness and the degree of keratinisation in relation to the mucosal function (Squier and Hall, 1985a; Squier and Hall, 1985b) and these differences may affect permeability to drugs. Kurosaki et al (1989) have reported increased permeability of keratinised oral mucosa to salicylic acid. Keratinised oral mucosa is found in regions which are particularly susceptible to the stresses and strains of masticatory activity (Berkovitz et al, 1978).

1.9.3.1. Mechanism of drug absorption through the buccal mucosa

Work on the amphetamines (Beckett and Triggs, 1967) indicated a relation between the buccal absorption and the concentration of unionised drug in the mouth. As the buffer solution was made more alkaline, the amount of amphetamines absorbed with time increased. When more than one drug was put in the mouth at the same time the absorption occurred as if the drugs are placed there singly. Also optical isomers absorbed to the same extent. From this evidence the workers concluded that buccal absorption occur by passive diffusion of the unionised form of the drug. Bickel and Weder (1969) also reported such a dependence on the buccal absorption of imipramine and its metabolites. Beckett and Moffat (1970) measured the rate constants of buccal absorption of ten carboxylic acids from solutions at pH 4.0 using a single subject. They found a positive correlation between these and the logarithms of previously determined partition coefficients. This further supports passive diffusion as a mechanism of drug transfer as the absorption is seen to obey the pH-partition theory. Schurmann and Turner (1977) found agreement of the buccal absorption of atenolol and propranolol with the pH-partition theory. However Beckett and Moffat (1971) found that the relation between lipid solubility and absorption into the buccal mucosa is not clear for barbiturates, though the mucosa was clearly selectively permeable to the unionised forms. These authors proposed that this step is probably preceded by interaction or
complex formation between the drug molecule and the protein of the mucosa, and that the release of the barbiturate into the lipid is the rate controlling step in the absorption process.

1.10. Objectives

The main objective of this work was the taste-masking of quinine for a paediatric dose form using a multiple emulsion technique of microencapsulation.

Quinine is essential in the treatment of chloroquine-resistant falciparum malaria infections (WHO, 1984). This is due to the fast rate at which these resistant strains are being developed worldwide (WHO, 1989). The inherent high mortality rate of the disease and its severity further underline the importance of correct therapy. Intravenous quinine injections are dangerous (White et al, 1983b) and clinical experience has created doubts about its safety given intramuscularly (Thuriaux, 1983). It is therefore administered orally in uncomplicated cases or as maintenance in more severe cases. Although malaria affects children mainly and more severely, there is no known paediatric formulation of quinine. The drug has a very bitter taste which cannot be masked by sweetening or flavoring. Therefore taste coverage of a paediatric formulation would be beneficial.

Duquemin and Warburton (1986) have reported controlled-release in three-ply-walled microcapsules as a result of polymer-drug binding. It was decided to employ such interactions with polyanions as a mechanism of controlled release of quinine in the present work. The two polyelectrolytes chosen are iota-carrageenan, a naturally occurring biodegradable sulphated polysaccharide and Eudragit L which is a synthetic acrylic resin. In vitro release studies of quinine from the microcapsules and bioavailability studies on human subjects, as well as other investigations, will be carried out. The taste coverage will also be evaluated. Two other drugs will be encapsulated to further evaluate the modified process. Paracetamol and cimetidine, both being cationic and bitter, have been selected. The characteristics of the microcapsules such as drug content, release profiles, particle size and surface morphology will be investigated. The results of these will be discussed in chapter three. The results of in vitro and in vivo release of quinine, and those of taste evaluation will be presented in chapter four.

Oral quinine causes gastric irritation manifested as pain, nausea, diarrhoea and vomiting at therapeutic doses (Powell and McNamara, 1972).
Rectal instillation had been found to be too irritant (Fletcher, 1923) as well. It was thought that a buccal formulation of this drug could be beneficial. Another objective of this work was to formulate a buccal dosage form of quinine from the microcapsules. This will be reported in chapter five.
CHAPTER TWO
MATERIALS
2.1. Wall forming materials

2.1.1. Anionic polyelectrolytes

2.1.1.1. iota-Carrageenan

Carrageenan is a structural polysaccharide found in sea plants. The most widely utilised source is Irish moss (Chondrus crispus and Gigartina stellata). They are salts of sulphate esters having a ratio of sulphate to hexose units of almost unity. There are two fractions separated by selective separation of potassium salts (Smith and Cook, 1953). The potassium-sensitive fraction is designated kappa (k) and the other one lambda. A third, iota-(i)carrageenan, which is essentially calcium sensitive is extracted from certain red sea weeds such as Eucheuma spinosum. i-Carrageenan is composed primarily of 1,3-linked galactose 4-sulphate with 1,4-linked 3,6-anhydro-D-galactose 2-sulphate (figure 2.1). A certain percentage of 6-sulphate exists in the 1,4-linked unit and may be removed by alkaline modification to form 3,6-anhydro groups.

\[ \text{Figure 2.1. Repeating structure of iota-carrageenan} \]
Carrageenan is a strongly charged anionic polyelectrolyte of large size. The molecular weight range is 100,000 - 800,000 (Smith and Cook, 1953). The theoretical approximate sulphate content of iota-carrageenan is 32% (Glicksman, 1969). A value of 28.83% was got in the present work by elemental analysis. In water carrageenan exhibits typical hydrocolloid properties of thickening and gelling. Iota- and kappa-carrageenan require heat for solution while lambda-carrageenan is fully soluble in cold water. Organic solvents and salts retard the hydration and solution of carrageenans. They are stable above pH 7.0, but below this pH stability decreases especially with increase in temperature. Degradation at pH 5-7 is mild, but quite rapid at lower levels. The order of stability at low pH is iota > lambda > kappa. iota-Carrageenan gels most strongly with calcium ions to develop very elastic gels characterised by absence of syneresis. Carrageenan has proved a safe food and pharmaceutical ingredient at all consumable quantities. Life time studies with rats and mice, involving up to 25% of carrageenan in the dry diet weight, showed no deleterious effects except for expected inefficiency of food utilisation at the 25% level (Stoloff, 1959).

iota-Carrageenan, lot 115-0643 was supplied by Sigma Chemical Company, St. Louis, USA.

Eudragit L

Eudragit L (acrylic resin) is a copolymer, anionic in character, based on methacrylic acid and methacrylic acid methyl ester. It is soluble in a neutral to weakly alkaline milieu. 1 gm dissolves in 7 gm of methanol, ethanol, isopropyl alcohol, acetone containing small amounts of water or IN sodium hydroxide to give clear or slightly opaque solutions. It is practically insoluble in ethylacetate, methylene chloride, petroleum ether and water. It is a white, very fine free-flowing powder with at least 95% of the particles less than 0.25 mm. It has a weakly sour odour. Figure 2.2. shows the repeating structure of the Eudragit L molecule. The ratio of the free carboxyl groups to the ester groups is approximately 1:1 and the mean molecular weight is 135,000 (Rohm Pharma). It is used as a coating material.

Eudragit L powder, lot number 03-8005 was supplied by Rohm Pharma, GMBH.
2.1.1.3. Agar
Agar is a sea weed extract, said to be a mixture of at least two polysaccharides: a neutral polymer (agarose) and agropectin which is a sulphated polymer (Araki and Arai, 1957). The ratios of these two vary widely, the percentage of agarose ranging from 50-90%. It forms gels with a hysteresis lag, e.g. a 1.5% solution of a good quality agar forms a gel upon cooling to about 30°C that does not melt below 85°C. It forms tough gels at concentrations as low as 0.5%. 'Lab M' agar, batch number 91004, was supplied by the Microbiological Supply Company, Toddington, England.

2.1.2. Eudragit E
This is an acrylic resin like Eudragit L. It is a cationic copolymer based on dimethylaminoethyl methacrylate and neutral methacrylic acid esters (figure 2.3.). The mean molecular weight is 150,000. It is a light yellow granule with
a characteristic amine-like odour. 1 gm dissolves in 7 gm of methanol, ethanol, isopropyl alcohol, acetone, ethylacetate, methylene chloride or 1N HCl to give clear to slightly opaque solutions. It is practically insoluble in water, becoming water soluble by forming salts with acids thus affording film coatings which are soluble in gastric juice. One of its possible applications is said to be masking of unpleasant taste or odour (Rohm Pharma). Eudragit E granules, lot number 01-80203 was supplied by Rohm Pharma, GMBH.

\[
\begin{array}{c}
\text{CH}_3 & \text{CH}_3 \\
\text{\ldots - CH}_2 - \text{C - CH}_2 - \text{C - \ldots} \\
\text{C = O} & \text{C = O} \\
\text{\ldots} & \text{OR} \\
\text{CH}_2 & \\
\text{CH}_2 - \text{N - CH}_3 \\
\text{CH}_3
\end{array}
\]

\[R = \text{CH}_3, \text{C}_4\text{H}_9\]

Figure 2.3. Repeating structure of Eudragit E

2.1.3. Ethylcellulose (EC)
Ethylcellulose is an ethyl ester prepared by the reaction of ethyl chloride with alkali cellulose. It contains 44 -51% of ethoxy \((\text{-OC}_2\text{H}_5)\) groups, calculated on the dried basis. EC N10 NF was used for preparing microcapsules for in vivo studies, while EC T10 was used for the rest of the work. The NF grade of ethylcellulose meets National Formulary requirements (Hercules, Ltd). This
grade has ethoxyl substitution range 48-49.5% and a viscosity value of 8-11 cps. EC T10 has a minimum ethoxyl substitution of 49.6% and viscosity range 8-11 cps as well.

EC T10 batch number 68552 and EC N10 NF batch number 63739 were supplied by Hercules Ltd., London.

2.1.4. Acacia (Gum Arabic)
This is the dried gummy exudate obtained from various species of Acacia trees of the Leguminosae family. It is a heterogenous material and may be composed of several slightly different molecular species. It comprises a main chain of β-galactopyranose units linked through positions 1-3, with side chains of 1,6-linked galactopyranose units terminating in glucuronic acid or 4-O-methylglucuronic acid residues. Additional groups are attached to the C-3 positions on the galactose side chains. Molecular weight ranges from 250,000 to 1000,000. It is extremely soluble in water, yielding solutions up to 50% w/v. It forms solutions of low viscosity at concentrations up to 5%.

Acacia powder product number 33214 was supplied by BDH Ltd., Poole, England.

2.2. Solvents

2.2.1. Ethyl acetate
This is a colourless, inflammable liquid, with molecular weight 88.11. Its density is 0.90 gm per millilitre at 20°C. It is soluble 1 in 10 of water at 25°C, more soluble at lower and less soluble at higher temperatures. Analar grade was used for preparing microcapsules used for in vivo studies.

Ethyl acetate Analar (Prod 10108, lot 8981430K) and Ethyl acetate GPR (Prod 28311) were supplied by BDH Ltd, Poole, England.

2.2.2. Methylene Chloride
This is a clear, colourless, volatile liquid of molecular weight 84.93 and boiling point 39-41°C. It is soluble 1 in 50 of water, miscible with alcohol and ether.

Methylene chloride, UN 1593, product number 28096, lot number 9540910K was supplied by BDH Chemicals, Poole, England.

2.2.3. Water
The water used was double distilled, pH about 5.9.
2.3. Core materials (drugs encapsulated)

2.3.1. Quinine
Quinine is a cinchona alkaloid. These substances occur most notably in plants of the genus cinchona and the related genera Remijia and Ladenbergia of the family Rubiaceae, where they are almost completely located in the bark. The molecular structure of quinine (figure 2.4.) consists of two moieties, a quinoline and a quinuclidine ring linked by a hydroxymethylene bridge. It is laevorotatory and has an erythro configuration at C-8 and C-9. It is a tertiary amine and has two nitrogen groups so two sets of salts can be obtained with either one or both nitrogens protonated.

Figure 2.4. Molecular structure of quinine
It is used as an antimalarial agent, the therapeutic effect derived mainly from its capacity to check the multiplication of the erythrocytic stages of the parasite.

Quinine base GPR (Prod. 30006) was supplied by BDH Chemicals, Poole, England. The quinine base encapsulated for in vivo studies was prepared by precipitation of Quinine HCl EP with ammonia solution. This is because no pharmacopoieal grade base was available. Quinine hydrochloride EP (Batch numbers 259543 387, 240932 1284 and 290414 689) was supplied by Fluka Chemie AG Buchs, Switzerland. Quinine sulphate (Prod 30010) and Quinine hydrochloride (Prod 30008) were also supplied by BDH Chemicals.

2.3.2. Acetaminophen (Paracetamol)
Paracetamol is a white, crystalline, odourless powder, with a slightly bitter taste. The molecular structure is shown in figure 2.5., it has a secondary amine group and a molecular weight of 151.16. The pH of a saturated solution is 5.3 - 6.5 and its pKa range is 9.0 - 9.5. It is soluble 1 gm in 70 ml water, 20 ml boiling water and 40 ml glycerin, solubility in ether is slight. It was found to be very soluble in ethylacetate in the present work. It is used as an analgesic/antipyretic.
Paracetamol GPR (Prod 27007) was supplied by BDH Chemicals Ltd., Poole, England.

\[
\text{HO-} - \text{NHCOCH}_3
\]

Figure 2.5. Molecular structure of acetaminophen (paracetamol)
2.3.3. Cimetidine

Figure 2.6. is the molecular structure of cimetidine, the molecular weight is 252.34. In water pKa of the imidazole ring N (-NH-) is 6.8. It is a white to off-white, crystalline powder with an unpleasant odour. 1 gm is soluble in 200 ml water, 18 ml alcohol, 100 ml chloroform, it is insoluble in ether. Cimetidine is a H2-receptor antagonist used in the treatment of confirmed duodenal ulcers and pathological hypersecretory conditions.

Cimetidine for experimental use, batch number 38/103 with particle size less than or equal to 160μm, from SK & F Ltd., was used.

![Molecular structure of cimetidine](image)

Figure 2.6. Molecular structure of cimetidine

2.4. Materials forming cross-linked network

for buccal formulation

2.4.1. Sodium alginate

Alginate is a sea weed extract. It is a copolymer of anhydro-1, 4-β-D-mannuronic acid and L-glucoronic acid. Sodium alginate is readily soluble in hot and cold water. Molecular weight ranges from 32,000 to 200,000 and correspond to a degree of polymerisation of 180-930. Alginate gels by reacting
with calcium, the reaction thought to be both intermolecular and intramolecular. Complete reaction of soluble sodium alginate forms insoluble calcium alginate. Varying consistencies are got by using smaller amounts of calcium.

Sodium alginate from Laminaria hyperboria, product number 30105, was supplied by BDH Ltd, Poole, England.

2.4.2. Calcium salts
Calcium sulphate dihydrate, batch number 6464430, was supplied by BDH Chemicals Ltd., England.
Calcium chloride (fused lump) was supplied by McCarthys Medical Ltd., Romford, Essex, England.
Calcium acetate GPR, product number 27576, was supplied by BDH Chemicals Ltd., Poole, England.
Calcium lactate GPR was also supplied by BDH Ltd.

2.5. Other materials
1) Ammonia solution AnalaR (specific gravity 0.91, ammonia content about 25%); product number 10011, UN number 2672 was supplied by BDH Chemicals, Poole, England.
2) Tragacanth powder was supplied by BDH Chemicals, Ltd.
3) Sodium lauryl sulphate was supplied by Marchon Products Ltd.

Unless specified, information was got from Merck Index or Martindale.
CHAPTER THREE
PREPARATION OF MICRO CAPSULES
Duquemin and Warburton (1986) have reported control of drug release in three-ply-walled microcapsules through binding of the drug to the polymer in the inner aqueous phase of multiple emulsions during manufacture. They showed that binding of quinine in particular does occur, the extent of which controls the release of the drug. The initial rapid release from these microcapsules and the low drug loading inherent in the method was also reported (Duquemin, 1987). The main aim of the present work was to formulate taste-masked and controlled-release formulations of quinine. For taste masking, control of release in the buccal cavity is imperative at least for the brief period the formulation resides there. It was decided to explore complex-formation, using polyanions because quinine is cationic. This would then be a way of controlling drug release. The complex-formation would also be expected to improve drug loading by decreasing the amount of drug available for transfer to the outer aqueous phase of the multiple emulsion, which would minimise drug loss.

3.2. Preformulation studies

3.2.1. Choice of polymer systems
From chapter one, section 1.4.3., a stable primary emulsion is necessary for the formulation of a corresponding multiple emulsion with any reasonable degree of stability. Primary emulsions (w/o) were prepared using various combinations of polymers, with or without the drug, to determine which of these will give stable w/o and hence w/o/w multiple emulsions. Aqueous solutions of the water-soluble polymer are emulsified into equal volumes of solutions of the oil-soluble polymer in ethyl acetate.

3.2.1.1. Results and discussion
Table 3.1. is a list of the composition of the w/o emulsion systems investigated with a summary of the result obtained in each case.

3.2.1.1a. Eudragit L and Eudragit E
These are both acrylic resin copolymers. Eudragit E is cationic, based on dimethylaminoethyl methacrylate and neutral methacrylic esters. On the other hand, Eudragit L is anionic, based on methacrylic acid and methacrylic acid methyl ester. The substances are therefore oppositely charged.
Table 3.1. Formulations investigated for stable water-in-oil emulsion systems

<table>
<thead>
<tr>
<th>System</th>
<th>Formulation</th>
<th>Aqueous Phase</th>
<th>Non-aqueous Phase</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Eudragit L  (2-4% w/v)</td>
<td>Eudragit E (2-4% w/v)</td>
<td>Precipitation</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Eudragit L  (0.1% w/v)</td>
<td>Eudragit E (0.1% w/v)</td>
<td>Gelation, then separation</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Eudragit L  (0.1-0.9% w/v) + 0.5% w/v Quinine SO₄</td>
<td>Eudragit E (0.1-0.9% w/v)</td>
<td>Emulsions break down almost immediately</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Eudragit L  (0.25-1%)+ 4% w/v Acacia</td>
<td>Eudragit E (1-2% w/v)</td>
<td>Separation with interfacial film</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Eudragit L  (0.75-4% w/v)</td>
<td>Ethylcellulose (4-8% w/v)</td>
<td>Phase inversion</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Eudragit L  (0.5% w/v)</td>
<td>Ethylcellulose (8%, 6%, w/v)</td>
<td>Stable w/o emulsions formed</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Acacia (4% w/v)</td>
<td>Eudragit E (0.25-1% w/v)</td>
<td>Separation, and phase inversion</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>Agar (0.25% w/v)</td>
<td>Ethylcellulose (8% w/v)</td>
<td>Homogenous, w/o stable emulsion</td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>Agar 0.5% w/v</td>
<td>Ethylcellulose (8% w/v)</td>
<td>Stable w/o emulsion with dispersible gel</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Carrageenan (0.25%, 0.5%, w/v)</td>
<td>Ethylcellulose (8% w/v)</td>
<td>Stable, w/o emulsions formed</td>
<td></td>
</tr>
</tbody>
</table>
polyelectrolytes. A polyelectrolyte is a long chain polymer molecule whose repeating building block contains an ionisable group. They are either polyacids, polybases or polyampholytes depending upon the nature of the ionisable centers. (Silberberg, 1974).

Concentrations of 2-4% w/v of Eudragit L and Eudragit E, i.e. system 1 in table 3.1., precipitate out immediately with no formation of emulsions. Unfortunately changing the organic solvent from ethyl acetate to dichloromethane did not make any improvement. With a concentration of 0.1% w/v of each polymer (system 2, table 3.1.), the emulsion started gelling during preparation. It separated completely soon after, with the gelation in the aqueous phase. However there was an interfacial film between the two phases. After forty eight hours the aqueous phase became cloudy. Thus although at low concentrations of the two polymers there was evidence of strong interfacial interaction, the interaction in the bulk did not favor emulsion stability. It appears that these two substances are incompatible. Katchalsky and Spitnik (1955) have studied the interaction between polyacids and polybases in solution. They found that, independent of molecular size, the molecules pair to form essentially neutral aggregates with a strong tendency to precipitate. When dissolved in a suitable solvent the groups on polyelectrolytes ionise producing charged groups. Under well defined conditions the macromolecules will bear a considerable charge distributed along the chain (the ‘fixed’ charges) accompanied by an equivalent number of small ions of opposite sign (the ‘counter’ ions) in solution. Electrostatic interactions arise between the fixed charges and the counter charges. If the polyelectrolyte solution is dilute, especially in the absence of low molecular weight salt, the counter ions to the charged groups tend to move away from the polymer leaving the charged sites on the polymer to interact more strongly with each other (Silberberg, 1974). Thus where oppositely charged polyions are present together in solution in the same environment, interactions would occur forming neutral aggregates.

At low concentrations of the polyelectrolytes some gelation was observed. The necessary condition for gel formation is a transition of one of the blocks of monomer units to domains, where the macro-Brownian motion of polymer chains is not free i.e., where the chains cannot move along each other (Bochard, 1983). In the present work the gel was seen in the aqueous phase or at the interface. Probably blocks of monomer units on the Eudragit E molecule were pulled into the aqueous phase through the ionic bonding between molecules of the two polyelectrolytes, resulting in gel formation.

The electrostatic repulsion between like fixed charges along the spine
of a polyelectrolyte chain may be partially screened off by the presence of small ions in the solution and by the interaction between the polyions themselves (Mandel, 1983). The ‘fixed’ charges, once they are on the same chain, can only move through the solution in a highly cooperative way, with the severe restriction that the maximum separation between any two of these charges is that corresponding to a maximum extension of the chain between them. Small ions in the solution would not be hampered by this restriction. So using this analogy, it was thought that introducing the drug at this stage would neutralise some of the charge on the polyaicd and possibly stabilise the system. When a range of concentrations (0.1-0.9% w/v) of each polymer were used, in each case incorporating 0.5% w/v quinine sulphate in the aqueous phase, (system 3, table 3.1.), none of the emulsions was stable for more than twenty minutes. As the concentration of the polymers was increased, the stability of the emulsion decreased. The emulsion always breaks, giving a solid gel at the interface and some precipitate in the aqueous phase, the degree of which depended on the concentration of the polymer. Apparently the small cations did not screen off the charges on the Eudragit L molecule effectively.

It became increasingly impossible to incorporate the drug in the aqueous phase with increasing polymer concentration as precipitation occurred. Being an alkaloid, quinine base is precipitated out of its salt in the presence of electrolytes in solution. The higher the concentration of the polyelectrolyte relative to the amount of quinine salt, the greater the tendency to precipitate. This is because the number of polyanions in solution would increase with increasing polyelectrolyte concentration. For a fixed polyelectrolyte concentration, the degree of precipitation will obviously increase as the amount of salt is increased.

3.2.1.1b. Eudragit L combined with acacia and Eudragit E

4% w/v acacia was incorporated in the aqueous phase in addition to Eudragit L (system 4 in table 3.1.). Concentrations of Eudragit L of 0.25-1.0% w/v and 1-2% w/v of Eudragit E were used. None of these formulations formed an emulsion, separation into two distinct layers occurring with a film in between. Probably the acidic groups on acacia increased the anionic charge density in the system, thereby further promoting interaction with the polybase. Thus the effects of the interaction discussed in the preceding section are magnified even more.
3.2.1.1c. Eudragit L and ethylcellulose

A series of experiments were carried out, attempting to prepare stable w/o emulsions using Eudragit L. The formulations (system 5 in table 3.1.) comprised of Eudragit L in a concentration range of 0.75-4% w/v in the aqueous phase, and ethylcellulose in the oil phase (concentration range of 4-8% w/v). In all the formulations there was phase inversion, thus o/w emulsions were formed instead. The phase inversion here is probably indicative of an imbalance in the emulsifier property of the polymer combination. Thick, homogenous w/o emulsions which were stable for days were however prepared from 0.5% Eudragit L and 8% or 6% ethylcellulose in ethyl acetate (system 6, table 3.1.). Stable multiple emulsions were subsequently produced from these.

3.2.1.1d. Acacia and Eudragit E

An attempt was made to prepare a stable emulsion with an aqueous solution of acacia and an organic solution of Eudragit E. In system 7 (table 3.1.) a 4% w/v acacia solution was combined in each case with concentrations of Eudragit E ranging from 0.25-1% w/v. The phases of the emulsions separated a few minutes after preparation, on further homogenisation the phases inverted. One system was not homogenised further. Here there was some precipitation at first and later gelation of the aqueous phase while the oil phase became cloudy, clearing after twenty four hours.

Eudragit E is positively charged and there are residual acidic groups in the acacia molecule. Thus there was the potential for ionic interaction as well as hydrogen bonding and van der Waals interactions. The opposing charges in the two polymers clearly interact so much at the concentrations used that phase separation occurred instantly. The nitrogen atom in the Eudragit E repeating unit is tertiary, making it a strong electrolyte. The same argument used to explain the interaction between the two oppositely charged acrylic resins (section 3.2.2.1a.) can be applied here. The result from the system that was not emulsified further does indicate some modification in the structure of acacia which could have been brought about by such an association.

3.2.1.1e. Agar and ethylcellulose

An aqueous solution of 0.25% w/v agar was emulsified into 8% w/v ethylcellulose in ethyl acetate (system 8, table 3.1.). A thick homogenous w/o emulsion was formed which was stable after twenty four hours. When the
concentration of agar was increased to 0.5% w/v (system 9, table 3.1.), a thick w/o emulsion was formed which contained some dispersible gel that was however soluble in the oil. There was slight creaming after ninety six hours but it was redispersible. Thus this formulation gave a stable primary emulsion as well. However agar forms gels with a hysteresis lag, i.e., it gels at temperatures far below the gel melting temperature. Since gel formation is essentially a modification of the molecular structure of the polymer, this might affect drug-polymer interaction. Also, the sulphate content here appears to be lower than that of carrageenan since agar is composed of a sulphated as well as a neutral polysaccharide with the percentage of the former usually being much lower (Araki, 1937).

3.2.1.1f. Carrageenan and ethylcellulose
In system 10 (table 3.1.), aqueous solutions of 0.25% w/v and 0.5% w/v carrageenan were emulsified in each case into 8% w/v ethylcellulose solution in ethyl acetate. Stable, thick, homogenous w/o emulsions were formed. Stable w/o/w emulsions were subsequently produced from these with 4% w/v acacia in the outer aqueous phases. The formulation with 0.25% w/v carrageenan was stable with up to 0.25% w/v quinine sulphate in the aqueous phase, but higher concentrations of the drug could not be incorporated as it was precipitated out. Solutes hinder the solution of carrageenan, the larger the amount of the solute the greater being this effect (Glicksman, 1969). Evidently the amount of drug that could be successfully solubilised with the carrageenan at the desirable concentration was too low for the purpose of this work.

3.2.1.2. Summary of results
Stable w/o/w multiple emulsions could be formulated from the following w/o primary emulsion systems:
0.5% w/v Eudragit L and 8% w/v ethylcellulose
0.5% w/v Eudragit L and 6% w/v ethylcellulose
-(system 6, table 3.1.) and
0.25% w/v carrageenan and 8% w/v ethylcellulose
0.5% w/v carrageenan and 8% w/v ethylcellulose
-(system 10, table 3.1.)
Between the formulations in system 6, the one with the higher concentration of ethylcellulose was deemed more favorable as it would be expected to give a thicker coat.

Although a stable w/o emulsion was formed from agar and
ethylcellulose, the gelation properties of the water soluble polymer and its apparent low sulphate content made this combination unsuitable for the purpose of this work.

It was not possible to incorporate quinine salts by solubilisation in the aqueous phase of any of systems 6 and 10 due to incompatibility problems.

3.2.2. Diffusion studies
It was not possible to incorporate substantial amounts of quinine salt in the aqueous phase with the polyelectrolytes (section 3.2.1. of this chapter). Hence it was decided to experiment adding the drug base in the oil phase for subsequent transfer to the aqueous phase before the preparation of the multiple emulsion. It was also considered necessary to find some evidence of the ionic interaction between quinine and the polyelectrolytes, if any, before proceeding with the microencapsulation process. The aims of the experiments here therefore were:
1) to demonstrate the transfer of quinine from the oil phase to the aqueous phase of w/o primary emulsions and,
2) to demonstrate ionic interaction of quinine to the polyelectrolyte in the aqueous phase.

3.2.2.1. Method
A solution of ethylcellulose (8% w/v) and quinine (0.5% w/v) in ethyl acetate was layered over an equal volume (50 ml) of double distilled water in a covered glass beaker, both solvents having been mutually saturated. The aqueous phase was stirred with a magnetic flea at speed 1 of a Griffin magnetic stirrer and hot plate (about 30-35 rpm). Samples were taken from the aqueous phase at time intervals, diluted appropriately and analysed for quinine by fluorescence spectroscopy. The volume difference due to sampling was taken into account while calculating the amount transferred at each time interval.

To demonstrate transfer of drug with adsorption into polyelectrolyte solution, an 8% w/v ethylcellulose and 2% w/v quinine solution in ethyl acetate was layered on an equal volume of an aqueous solution of the polyelectrolyte in a stoppered separating funnel, the solvents mutually saturated. The concentration of polyelectrolyte was varied to give a series. No stirring was employed as this would result in emulsification. The aqueous
phase was sampled at twenty four hours. Part of the sample was analysed directly for drug while some was treated with a large volume of a non-solvent for the polymer to precipitate the later out. After washing the precipitate was treated with sulphuric acid (0.1M) and the resultant extract analysed for drug. The non-solvents used were ethyl acetate for Eudragit L and methanol for carrageenan.

The experiments were carried out at room temperature. Quinine analysis was done by fluorescence spectroscopy using the Perkin Elmer (PE) 3000 uv-visible spectrophotometer at excitation and emission wave lengths of 343 and 450 nm respectively. The theory of fluorescence spectroscopy is given in appendix 1. and the calibration curve from which concentrations were extrapolated is given in appendix 2.

3.2.2.2. Results and discussion

3.2.2.2a. Transfer of drug from the oil phase to the aqueous phase

Figure 3.1. is a plot of the amount of drug transferred with time from the oil phase to the aqueous phase. At twenty four hours 47.8 mg or 19.1 % w/w of the drug has been transferred. Although the profile indicates that the process is nowhere near equilibrium, it does show that the transfer does occur, albeit slowly. Duquemin (1987) had demonstrated transfer of drug to the outer aqueous phase of multiple emulsions using a similar procedure. She layered the primary emulsion on a solution of the polymer in the outer aqueous phase. Here diffusion of drug was taking place across the emulsion phases in the first stage, and from emulsion droplets to a solution in the second stage of the process. However equilibrium was not reached over a period of 60 hours. In the present experiments the surface area and hence interfacial area available for diffusion would be many orders of magnitude lower than in the second stage of the former case (Duquemin, 1987). It would definitely be expected to be even lower compared with the case of water droplets dispersed in an oil phase as in a w/o emulsion. This small interfacial area would result in a slow rate of diffusion, while the transfer should be much faster across the phases of a primary emulsion.

Walkow and McGinity (1987) investigated the diffusion rate of methylsalicylate in solutions and emulsions across membranes and found it higher in one emulsion system where the vehicle is slightly water soluble than in the solutions. Diffusion across the two phases was found to be the slowest, i.e. rate determining step. The kinetics of diffusion from emulsions was found
Fig. 3.1. Amount of quinine transferred with time from an oil phase (ethylcellulose in ethyl acetate) to an aqueous phase (distilled water).
to fit better to the square root of time relationship. Bottari et al (1974), working on the release rates of salicylic acid from ointment bases, also found that release was faster from emulsions than the corresponding anhydrous bases. They found no clear trend to faster release from w/o compared to o/w type emulsions. Cajkovac (1984) on the other hand reported faster release of chloramphenicol from o/w than w/o emulsions. In most of the cases though, the drug is more soluble in the aqueous phase and the release was followed into an aqueous medium so this could explain why release was faster from an emulsion with an aqueous continuous phase. Friedman and Benita (1987) studied the in vitro release of morphine base from stable o/w emulsions. Their release kinetic analysis indicated that the transfer of drug from the oily to the aqueous phase was the rate limiting step in the overall process, provided the main portion of the drug was localised in the oil phase. This was confirmed by the dependence of release on the oily phase volume ratio and the mean size of emulsion droplets. Although non-ionic surfactants were used, preliminary studies had shown that the presence of the drug in micelles did not affect its rate of transport. Looking at the release of solutes from multiple emulsions, Omotosho et al (1986) found that diffusion of unionised species of 5-fluoro uracil across the oil phase is the most probable mechanism of transfer of this compound. Overall the authors concluded that in such stable systems, diffusion across the oil layer or through localised thin oil lamellae is the primary transport mechanism. Brodin and Frank (1978) had measured the diffusion coefficients of thymol in an o/w/o emulsion and also the primary (o/w) emulsion and found the two values to be similar. Their results indicate that diffusion in the oil is the rate-limiting step in both systems.

3.2.2.2b. Transfer of drug to the aqueous phase
with interaction with polyelectrolyte

The amounts of drug in the aqueous phase at different concentrations of Eudragit L at twenty four hours are shown in figure 3.2. The trend is an increase in the total amount of drug in the aqueous phase as the concentration of polymer is increased.

However even with the highest concentration of Eudragit L, the amount of drug transferred here after twenty four hours is not very much higher than that in the case where the aqueous phase contained no polymer (section 3.2.2.2a.). This is despite the fact that the concentration of drug in the donor phase is four times higher here (with polymer in the aqueous phase). Any association between quinine and polyelectrolyte in the aqueous phase will
Fig. 3.2. Effect of the concentration of Eudragit L on the amount of quinine transferred to the aqueous phase in 24 hours.
lower the apparent partition coefficient of the drug between the two phases. On the other hand, the rate of diffusion of material and hence its transfer across the phases of a primary emulsion has been seen to depend on its partition coefficient between the two phases (chapter one, section 1.5.2.1.). The lower partition coefficient would thus result in a lower diffusion coefficient and smaller amount of drug transferred. Crank (1967) had found diffusion to be slower when the process involved adsorption onto internal sites. According to this worker, in such a case a lag time may result in the kinetics due to non perfect sink conditions. It is probable therefore that the interaction between quinine and the polyelectrolyte slowed the diffusion process. A contributing reason could be the absence of the very gentle agitation that was applied to the aqueous phase in the experiment with no polyelectrolyte.

Again due to a difference in interfacial area, the amounts of drug transferred here will be many orders of magnitude smaller than what will be expected to be transferred between the two phases in a w/o emulsion.

Viscosity effects produced some inconsistency in the determination of total drug transferred with carrageenan in the aqueous phase. The Eudragit L solutions were not viscous in the range studied so there was no such complication. In dilute solutions and in the absence of low molecular weight salt, the repulsion between like charges on polyelectrolyte chains tend to favor the open conformation of the macromolecule accompanied by an increase in average dimensions and a corresponding increase in the viscosity of the system (the polyelectrolyte effect) (Silberberg, 1974). Because of the greater physical dimension of the carrageenan molecule, the polyelectrolyte effect and hence the hindrance to diffusion is greater here than with Eudragit L. The higher the polymer concentration, the higher the viscosity of the solution and the lower the diffusion rate of drug into it. This viscosity effect was even more pronounced because samples were taken from the bottom of the aqueous phase. At 0.5% w/v the carrageenan solution was so thick that it was almost a gel. Here the molecules are so closely packed that movement of even small molecules in the system will tend to be slow, the result of which would be a relative decrease in the amount of material transferred. Sampling under these conditions is therefore unlikely to give a complete picture of the process. A realistic estimation of total drug transferred in carrageenan can hence only be achieved if the experiments are carried out at a higher temperature. However the application of heat over the duration of the experiment might affect the structure of the polyelectrolyte.
Figure 3.3. shows that the amount of quinine in the precipitate of Eudragit L was directly proportional to the concentration of the polyelectrolyte solution. Both Eudragit L and carrageenan form polyanions in solution which could interact with co-existing cations. Quinine has a pKa of about 8.8, so it would be expected to be protonated in the polyelectrolyte solutions. Ionic interactions could therefore occur between the polyanions of each polyacid and the quinine cations. Ion-binding with polyelectrolytes is said to be ionic not covalent (Nagasawa, 1974). That is, it is not due to complex formation or other non-ionic forces but due to electrostatic attractions. The small counter ions accumulate around the polyion skeleton due to strong electrostatic interactions, the phenomenon corresponding to ion-pair formation in simple electrolytes. Thus the amounts of quinine in the polymer precipitates are by inference the amounts bound to the polymer in each case. Despite the constraints in sampling due to viscosity, the amount of drug in the precipitate of carrageenan solution was found to increase with increasing concentration of the later as well (figure 3.4.) up to a point.

From figures 3.3. and 3.4., the amounts of drug bound weight for weight were higher with Eudragit L in the aqueous phase. This would indicate that Eudragit L has a higher capacity for interacting with quinine. For such interaction, at a constant concentration of the small ion, the degree of binding would depend on the number of binding sites available per polymer molecule. The more polyions available, the greater the number of binding sites and the higher the amount of drug bound. Indeed for each polymer, the amount of precipitate from the same volume of solution was seen to increase with increasing polymer concentration. And this resulted in corresponding larger amounts of drug in the precipitate as stated earlier. Although these results do not provide any quantitative characterisation of binding, they would indicate that ionic interaction did occur in the systems studied. It should be emphasized that the values of drug bound are just inferred from the results under the conditions the experiments were carried out. Errors may also have arisen from inadequate washing of precipitate resulting in overestimation but the results do give a trend.

3.2.2.3. Summary of results
Quinine was transferred from the oil phases to the aqueous phases of the w/o primary emulsion systems under study. It appears that diffusion of the drug was followed by ionic interaction between it and the polyelectrolyte in the aqueous phase. Thus it is possible to formulate a w/o/w multiple emulsion with
Fig. 3.3. Amount of quinine bound to varying concentrations of Eudragit L in 24 hours (inferred from the amounts of drug in the polymer precipitates).
Fig. 3.4. Amount of quinine bound to varying concentrations of carrageenan in 24 hours (inferred from the amounts of drug in the polymer precipitates).
the drug in the inner aqueous phase by incorporating it in the oil phase initially, and leaving the primary emulsion for some time before further emulsification.

3.3. Method of microencapsulation

It became necessary to modify the original multiple emulsion technique of forming three-ply-walled microcapsules (Morris and Warburton, 1982) because:
1) There was a need for more control of drug release and also improvement on drug loading, and
2) There was incompatibility between quinine and the polyelectrolytes as seen in section 3.2.1.

In this modified method, the drug is incorporated in the oil phase and gets into the inner aqueous phase by diffusion, to interact with the polyanion. Transfer of drug from the oil phase to the aqueous phase and subsequent interaction between it and the polyelectrolyte in the aqueous phase have been shown to occur (section 3.2.2.).

Each water-in-oil emulsion was prepared from equal volumes of an aqueous solution of the polyelectrolyte in double distilled water and a solution of ethylcellulose and the drug in ethyl acetate, the solvents having been mutually saturated. The emulsification was done by using a Silverson mixer/emulsifier made by Machines Ltd., Waterside, Bucks, England. The primary emulsion was transferred into a stoppered jar and left for twenty four hours, after which it was further emulsified into an equal volume of a solution of acacia in double distilled water to form a w/o/w multiple emulsion. This second emulsification was carried out at a lower mixing speed than the first one to prevent breakage of already formed multiple emulsion droplets. Table 3.2. shows the composition of the formulations prepared. The oil was removed from the emulsion by evaporation using a Buchi rotary evaporator (Rotavapor) model R 110, supplied by Orme Scientific Ltd, Manchester; fitted with a vacuum pump. Evaporation was carried out at 30°C and a rotation speed of 1. The suspension of microcapsules was left to settle, the supernatant decanted and the slurry air-dried.

Duquemin (1987) had reported an optimum phase volume ratio of the primary emulsion of one for minimal aggregation and ease of extraction.
Table 3.2. Compositions of quinine microcapsule formulations

<table>
<thead>
<tr>
<th>Code</th>
<th>Inner Aqueous Phase: Distilled Water</th>
<th>Oil Phase: Ethyl acetate</th>
<th>Outer Aqueous Phase: Distilled Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>0.5% w/v Eudragit L</td>
<td>8% w/v Ethylcellulose</td>
<td>4% w/v Acacia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2% w/v Quinine</td>
<td></td>
</tr>
<tr>
<td>B</td>
<td>0.25% w/v Carrageenan</td>
<td>8% w/v Ethylcellulose</td>
<td>4% w/v Acacia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2% w/v Quinine</td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>0.25% w/v Carrageenan</td>
<td>8% w/v Ethylcellulose</td>
<td>4% w/v Acacia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3% w/v Quinine</td>
<td></td>
</tr>
<tr>
<td>D</td>
<td>0.5% w/v Carrageenan</td>
<td>8% w/v Ethylcellulose</td>
<td>4% w/v Acacia</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1% w/v Quinine</td>
<td></td>
</tr>
</tbody>
</table>
3.4. **Characterisation of microcapsules**

3.4.1. **Drug content**

Elemental analysis was used initially to determine drug content in the microcapsules. This is because it was thought the total pyrolysis involved in the process could separate the drug from the polymer in a short while to make the former available for estimation. However it became evident that although a rank order was always maintained in any one series, the method is not reproducible at the concentrations of the drug being encountered. Therefore fluorescence and ultraviolet spectroscopy were both used as well to analyse drug content during the course of this work.

3.4.1.1. **Methods of measuring drug content**

3.4.1.1a. **Method of elemental analysis (EN)**

The general method involves combustion in an oxidation furnace at 120°C, elution and introduction of gases into a reduction furnace at 60°C and then separation using a chromatographic column. Peaks corresponding to the elements are seen on a detector. A known weight of microcapsules was used to afford calculation of amount of each element. The instrument used was the Carlo Erba Elemental Analyser model MOD 1106 attached to a Carlo Erba DP 110 PRC recorder. Quinine was estimated by nitrogen analysis, it being the only compound in the formulation containing this element.

3.4.1.1b. **Fluorescence spectroscopy (FS)**

The instrument used was a Perkin Elmer (PE) 3000 fluorescence spectrometer attached to an RE 541 Servoscribe potentiometric recorder. Quinine emits radiation at 450 nm when excited at 350 nm. An experimentally determined excitation wavelength of 343 nm and emission wavelength of 450 nm and a slit width of 5 nm were used. The drug was extracted into dilute sulphuric acid (0.1M) by triturating the microcapsules and acid in a glass mortar. A weighed amount of microcapsules was triturated, a sample removed and the same volume of acid replaced to keep the volume constant. The sample was diluted appropriately and the fluorescence was measured. The optimum extraction time for each polymer was first determined by analysing samples at time intervals until a constant concentration value was attained at which stage it was assumed that all the quinine had been extracted. Extractions were then carried out over the appropriate periods.
3.4.1.1c. Ultraviolet spectroscopy (UVS)

A PE 554 ultraviolet - visible spectrophotometer made by Perkin Elmer Beaconsfield, Bucks, England was used. Quinine was analysed at 330 nm. The drug was extracted by triturating a known weight of microcapsules in dilute (0.1M) hydrochloric acid. It was filtered, the extract made up to 50 ml and analysed.

3.4.1.2. Results and discussion

For spectroscopic methods concentrations of drug were extrapolated from suitable calibration curves, which are given in appendix 2. The fluorescence spectra of pure quinine and the microcapsule extract were found to be similar. Duquemin (1987) had used uv spectroscopy for analysis of drug content. The optimum extraction times were found to be seventy and one hundred minutes for the Eudragit L and carrageenan microcapsules respectively. Drug contents of the four formulations of the microcapsules arrived at using the three methods of analysis are given in table 3.3.

The composition of the formulations A, B, C and D were given in table 3.2.

There was more agreement between results from the two spectroscopic methods. The Eudragit L formulation (A) took up almost 74% of the drug incorporated while the formulations with 0.25% carrageenan (B, C) took up 37% and 41% respectively (FS method). Assuming that the total of the amount of drug left in the oil phase and that which might have been lost to the outer aqueous phase in the two types of formulations was equivalent, the results indicate that Eudragit L has about twice the capacity of iota-carrageenan for interacting with quinine. An indication of this difference in binding capacity of the two polymers was seen in section 3.2.2.2b. It could be explained by the difference in the number of reactive groups (binding sites) per molecule for the two polyelectrolytes. Apart from charge, size, polarisability etc., other features determining the degree of short-range ionic interactions involving polyatomic ions are the charge density distribution and in some instances the presence of hydrophobic groups (Kauzmann, 1959). Eudragit L has an ester to carboxylic acid ratio of 1:1, this means that about 50% of the molecule is available for ionic interaction. Determination of the sulphate content of the carrageenan used by elemental analysis gave a value of 28.8%, the approximate figure for iota-carrageenan is said to be 32% (Stoloff, 1959). From the foregoing it can be deduced that the binding sites on the carrageenan molecule are just 50-60% of those on the Eudragit L molecule which roughly agrees with uptake of drug by the microcapsules. Formulation D was prepared using 0.5% w/v
Table 3.3. Drug content of microcapsules as determined by various methods of analysis

<table>
<thead>
<tr>
<th>Code</th>
<th>Amount of drug in emulsion (% w/w)</th>
<th>Amount of drug in microcapsules (% total of amount in emulsion)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>EA</td>
<td>UVS</td>
</tr>
<tr>
<td>A</td>
<td>10.81</td>
<td>98.61</td>
</tr>
<tr>
<td>B</td>
<td>10.95</td>
<td>49.77</td>
</tr>
<tr>
<td>C</td>
<td>15.58</td>
<td>50.58</td>
</tr>
<tr>
<td>D</td>
<td>5.79</td>
<td>97.93</td>
</tr>
</tbody>
</table>

EA = elemental analysis
UVS = ultraviolet spectroscopy
FS = fluorescence spectroscopy
carrageenan i.e., twice the concentration in A and B. From table 3.3., the amount of drug taken up by these microcapsules, i.e. formulation D is 80% (FS method), while about half of this was taken up by B an C. Although it would be wrong to make an absolute direct quantitative comparison of the two sets of data, the higher uptake of drug in formulation D could probably be due to an increase in the number of binding sites as a result of increase in polymer concentration.

3.4.2. Effect of the method of microencapsulation on drug content

3.4.2.1. Method

1) Microcapsules were prepared using the original method with 4% w/v acacia and 2% w/v quinine hydrochloride (1.72% w/v base) in the inner aqueous phase, 8% w/v ethylcellulose in the oil phase and 4% w/v acacia in the outer aqueous phase. 50 mg of the resulting microcapsules was extracted into 0.1M HCl and drug content was determined by ultraviolet spectroscopy.

2) Microcapsules were prepared by the modified method but with acacia (4% w/v) in the inner aqueous phase instead of a polyelectrolyte. 2% w/v quinine base was incorporated in the oil phase. Drug analysis was carried out as above.

3.4.2.2. Results and discussion

Table 3.4. depicts the content of drug in microcapsules prepared by the two multiple emulsion techniques. It shows that the modified method is a more efficient process in terms of drug loading. While only 19.77% of the total amount of drug incorporated initially was found in the microcapsules prepared by the original method, up to 86.94% of total drug was found in those prepared by the modified method.

Duquemin (1987) investigated the amount of quinine lost to the aqueous continuous phase during manufacture and extraction of microcapsules prepared by the original method using different extraction methods. This occurred despite the mutual saturation of the solvents. She found that although the microcapsules extracted by rotary evaporation had the highest drug content, the amount of drug in the aqueous continuous phase was a lot more (up to four times) than in the microcapsules. This was said to be due to the equilibration of drug in the two aqueous phases of the multiple emulsion according to their respective volumes, thus theoretically twice more drug is expected to be in the outer aqueous phase. The transfer was promoted by the high water solubility.
Table 3.4. Drug content of microcapsules formed by the two multiple emulsion processes

<table>
<thead>
<tr>
<th>Method</th>
<th>Amount of drug in emulsion (% w/w)</th>
<th>Amount of drug in microcapsules (% w/w)</th>
<th>% of total in emulsion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Original</td>
<td>7.818</td>
<td>1.546</td>
<td>19.77</td>
</tr>
<tr>
<td>Modified</td>
<td>9.090</td>
<td>7.903</td>
<td>86.94</td>
</tr>
</tbody>
</table>
The problem of low drug loading with water-soluble and slightly water-soluble drugs is also encountered with the emulsion-solvent evaporation (ESE) technique of microencapsulation (Bodmeir and McGinity, 1987). Jalil and Nixon (1989) explained the low phenobarbitone load in microcapsules formed from an o/w emulsion system using the ESE method in the light of drug crystal migration into the aqueous continuous phase as a result of crystal development at the droplet interface. Benita et al (1984) had reported the same phenomenon in the even more poorly water soluble drugs, lomoustine and progesterone. Both Duquemin (1987), and Jalil and Nixon (1989) improved drug loading by the previous addition of core material to the aqueous continuous phase. However the later workers showed that despite the presaturation, the aqueous phase contained a large proportion (15.5%) of the original drug. Thus even under this condition, spontaneous crystal growth occur; according to these authors, via a stage of supersaturation and precipitation of crystals whilst the polymer was still in solution. In the modified method, once drug diffuses into the aqueous phase of the primary emulsion the proportion of it that interact with polyelectrolyte molecules cannot diffuse out because the interfacial membrane will not be permeable to the large complex. Diffusion of any unbound drug and that remaining in the oil phase would occur, but its rate will be greatly reduced by the low water solubility of quinine base and the absence of a high concentration gradient. The greater loss of drug to the aqueous continuous phase of multiple emulsions in the original method is therefore due to partitioning of the free water soluble drug between this and the inner aqueous phase.

3.4.3. Surface morphology

3.4.3.1. Method
Each specimen was gold coated in an argon atmosphere using an Edwards sputter coater, prior to viewing. The specimens were viewed and photographed under a Jeol (model JSM 36) scanning electron microscope at an accelerating voltage of 10KV.

3.4.3.2. Results and discussion
Plates 3.1. and 3.2. are the scanning electron micrographs of Eudragit L and carrageenan microcapsules respectively, each containing quinine. Although the walls do not appear to be porous, the surfaces are not smooth, but rather seem
Plate 3.2. Scanning electron micrograph of carrageenan microcapsules containing quinine.
Magnification: X 1250
Plate 3.1. Scanning electron micrograph of Eudragit L microcapsules containing quinine.
Magnification: X 1250
to have the so-called ‘orange peel’ texture reported by other workers (Nixon and Matthews, 1976; Duquemin, 1987). The effect seem more pronounced in the carrageenan microcapsules where some surfaces are even convoluted. Florence et al (1979) encountered such a morphology in microcapsules formed from butyl 2-cyanoacrylate by in situ polymerisation and attributed it to the influence of the organic phase on the morphology of interfacial films. In the process, the monomer was dissolved in the oil phase of a w/o emulsion with the reaction occurring at the interface of the disperse phase. Duquemin (1987) also explained the ‘orange peel’ appearance and wall porosity of microcapsules formed by a multiple emulsion technique in terms of the influence of the organic phase component (ethylcellulose). Goto et al (1988) found a similar effect in ketoprofen microcapsules prepared with Eudragit S (an anionic acrylic resin) by a solvent evaporation process in oil phase. The surfaces of these were coarse, the higher the drug load the more pronounced the effect. Microcapsules prepared from the neutral acrylic resins were smooth, becoming relatively less so with higher drug contents. The difference was said to be due to a possible reduction of interfacial tension between the microcapsules (dispersed phase) and the dispersion medium by an additive which could have prevented the electrification and flocculation of the microcapsules (Goto et al, 1986).

Plates 3.3. and 3.4. are micrographs of empty microcapsules of Eudragit L and carrageenan respectively. The particles are either sickle-shaped or indented in several points on the surface. Thus it seems that when the particles are empty and hence hollow, the walls collapse onto themselves. The sickle-shaped structures can also be seen in some of the micrographs of microcapsules containing drugs where a microcapsule is not loaded. These collapsed structures have been reported elsewhere (Duquemin, 1987).
Plate 3.3. Scanning electron micrograph of empty
*Eudragit L* microcapsules
Magnification: X 1250
Plate 3.4. Scanning electron micrograph of empty carrageenan microcapsules
Magnification: X 1250
3.4.4. Particle size analysis
This was carried out using the Malvern 2600c droplet and particle analyser.

3.4.4.1. Operating principles of the Malvern 2600c particle analyser
The instrument is based on the principle of laser diffraction, i.e. light scattering. Sizing is accomplished without forming an image of the particle onto a detector. The light from a low power Helium-Neon laser is used to form a collimated and monochromatic beam of light, the analyser beam. Any particles present within this laser light will scatter it. The scattered light is gathered by a detector in the form of a series of concentric annular rings, over a range of solid angles of scatter. Each particle scatters light at a favored scattering angle which is related to its diameter; large particles scatter light over small angles and vice versa. At 1 µm and above, the scattering with angle is independent of the optical properties of the material or the medium of suspension. The result of the measurement analysis is a volume distribution projected from surface area, characterised over the size limits of the range lens used. By making many measurements of the detector readings and averaging, the instrument builds up an average light scattering characteristic based on millions of individual particles over a very short time (seconds).

3.4.4.2. Method
Microcapsules were suspended in double distilled water for measurement.

3.4.4.3. Results and discussion
The particle size distributions of formulations A, B, C and D (as in table 3.2.) are fairly wide. These are given in figures 3.5., 3.6., 3.7. and 3.8. respectively. The mean diameters derived from volume distributions were found to be 55.8 µm, 47.7 µm, 47.8 µm and 52.5 µm respectively. These are larger than acacia/ethylcellulose microcapsules manufactured using the original method which had a mean diameter of 10 µm and 15.68 µm respectively for unicore and multicored microcapsules (Morris and Warburton, 1984). This is probably due to a difference in the nature of the multiple emulsion droplets. Florence and Whitehill (1981) had categorised w/o/w multiple emulsion systems according to the predominant multiple droplet types and their sizes. Probably the larger microcapsules obtained in the present work resulted from large, multicored multiple emulsion droplets. Another reason could be a difference in mixing speeds. The possibility of aggregation of some of the
Fig. 3.5. Particle size distribution of Eudragit L/quinine microcapsules
(formulation A in table 3.2.).
Fig. 3.6. Particle size distribution of carrageenan/quinine microcapsules (formulation B in table 3.2.).
Fig. 3.7. Particle size distribution of carrageenan/quinine microcapsules
(formulation C in table 3.2.).
Fig. 3.8. Particle size distribution of carrageenan/quinine microcapsules (formulation D in table 3.2.).
microcapsules during extraction cannot be miscounted too. As the organic solvent is removed, the particles tend to stick together probably due to the tackiness of the acacia solution in the outer aqueous phase.

Nixon and Jalil (1989) found that short evaporation times of solvent from w/o as well as o/w systems resulted in tacky microcapsules which aggregated, this indicating the presence of residual solvent. This could be the case here as the solvent is removed over a short period of time, usually less than thirty minutes. Increasing the concentration of carrageenan (formulation D) gave larger microcapsules, which could be due to a viscosity effect. The difference in the amount of drug in microcapsules does not seem to have any effect on particle diameter. Thus the two carrageenan formulations (B and C, table 3.2.) with varying drug load have basically the same mean diameter and a similar size distribution. Duquemin (1987) observed this non-dependence of drug concentration on particle size in acacia microcapsules containing quinine hydrochloride over a drug concentration range of 0.5 -4% w/w.

### 3.5. Other drugs encapsulated

Two other drugs, acetaminophen (paracetamol) and cimetidine, were encapsulated and characterised to test the modified method. Paracetamol was incorporated in the oil phase as in the case of quinine while cimetidine was encapsulated by incorporating it in the inner aqueous phase directly. Analysis of drug content was done by nitrogen analysis. Particle size analysis, scanning electron microscopy and dissolution studies were all carried out as for quinine (sections 3.4.4., 3.4.3. and 4.2.1. respectively).

#### 3.5.1. Results and discussion

#### 3.5.1.1. Drug content

The results of analysis of drug content are given in table 3.5., that of quinine from elemental analysis is included for comparison.

For paracetamol the results are similar to those of quinine in the sense that the loading in the Eudragit L formulation is about twice that of the carrageenan formulation. This has been explained in the light of a difference in binding sites of the two polyelectrolytes (section 3.4.1. in this chapter). Microcapsules of paracetamol were prepared using the same method as for quinine. The larger amount of drug in the quinine microcapsules probably has to do with the nature of the amine groups in the two compounds, secondary
Table 3.5. Drug content of quinine microcapsules and microcapsules of other drugs, determined by elemental analysis

<table>
<thead>
<tr>
<th>Drug</th>
<th>Amount of drug in microcapsules</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(% w/w)</td>
<td>(% of total in emulsion)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Eudragit L</td>
<td>Carrageenan</td>
<td>Eudragit L</td>
</tr>
<tr>
<td>Quinine</td>
<td>10.66</td>
<td>5.45</td>
<td>98.7</td>
</tr>
<tr>
<td>Paracetamol</td>
<td>5.08</td>
<td>3.02</td>
<td>44.5</td>
</tr>
<tr>
<td>Cimetidine</td>
<td>9.40</td>
<td>6.58</td>
<td>35.23</td>
</tr>
</tbody>
</table>
in paracetamol and tertiary in quinine. Ammonia and the amines are truly weak bases as long as there is at least one hydrogen atom attached to the nitrogen; but as soon as this is replaced, the basicity increases ten thousand fold and they become strong electrolytes (Sidgwick, 1950). Quinine will therefore have more affinity for interacting with the polyelectrolytes than paracetamol. Due to this, the proportion of free drug will be higher in the paracetamol microcapsules resulting in greater loss. Studying the interaction between a polycarboxylic acid ion-exchange resin with amine drugs, Borodkin and Yunker (1970) also found that tertiary amines exhibited a much greater affinity for the resin than other type of amines. The existence of empty (collapsed) microcapsules in micrographs (plates 3.5. and 3.6.), underlines the low drug load in the paracetamol microcapsules compared to those of quinine (plates 3.3. and 3.4.). The predominance of these in the carrageenan/paracetamol microcapsules also shows the difference in affinity for the drug between the polyelectrolytes.

The amount of drug in the two formulations of cimetidine does not differ much though it is still slightly higher in the Eudragit L formulation. The amounts are lower than those of quinine in both cases. Compared with paracetamol, it is lower in the Eudragit L formulation and higher in the carrageenan formulation. Cimetidine is a cyanoguanidine (see structure in figure 2.6.), the basicity of the guanidine having been markedly reduced by a strong electron-withdrawing substituent, the cyano group. Such compounds are weakly amphoteric, and hence unionised in a broad pH range (2 - 12) (Durant, 1977). Cimetidine has a pKa of 0.4 (Hirt et al, 1961). This weak basicity would not be expected to result in a high affinity between the drug and any of the two polyelectrolytes. It would indicate that the bulk of the drug in these microcapsules is probably not ionic bonded. Cimetidine is very polar and hydrophilic. Thus apart from the very low degree of protonation, this water solubility (1gm in 88) would have been expected to result in a much lower drug loading than for paracetamol since loss to the outer aqueous phase was enhanced. On the other hand, larger amounts of drug were incorporated in the multiple emulsions in the case of cimetidine. Although it is percentages that are being compared here, the lower initial amounts for paracetamol could have been responsible for the relatively lower drug loading here, especially since paracetamol will partition into the aqueous phase as well. It is not possible to analyse these results precisely because the drugs are not located entirely in the microcapsules as ionic complexes.
3.5.1.2. Surface morphology

Plates 3.5. and 3.6. are the scanning electron micrographs of paracetamol containing microcapsules made from Eudragit L and carrageenan respectively. The ‘orange peel’ morphology, pronounced here in the Eudragit L microcapsules, has been discussed in section 3.4.3.2. (surface characteristics of quinine microcapsules) based on reports from other workers. Microcapsules of cimetidine made with Eudragit L and carrageenan (plates 3.7. and 3.8. respectively) are both essentially spherical and have smooth surfaces. However the walls appear to be porous with numerous holes. While quinine and paracetamol microcapsules were prepared with drug initially in the oil phase, with cimetidine the drug was dissolved in the inner aqueous phase. This might account for the differences observed. Duquemin (1987) reported porous walls in microcapsules formed by the original method, said to be due to the interaction of ethylcellulose films with water.
Plate 3.5. Scanning electron micrograph of Eudragit L microcapsules containing paracetamol.
Magnification: X 1250
Plate 3.6. Scanning electron micrograph of carrageenan microcapsules containing paracetamol.
Magnification: X 1250
Plate 3.7. Scanning electron micrograph of Eudragit L microcapsules containing cimetidine.
Magnification: X 1250
1.3.1.3. In-vitro Release Profile

The release profiles of piroxicam from Rodograph I, and carrageenan are, given in figures 3.8 and 3.9 (supplementary). When the carrageenan microcapsules 40-52% were released after quickly a small initial burst followed by slow release, the same obtained in profile was even higher whereas of piroxicam showed the release after 7 days. The release mechanism can be explained by Fick's law which states that the rate of diffusion is directly proportional to the concentration difference. However, the difference in release profiles might be due to the differences in the structure and properties of the microcapsules.

Plate 3.8. Scanning electron micrograph of carrageenan microcapsules containing cimetidine.
Magnification: X 1250
In vitro release profiles

The release profiles of paracetamol from Eudragit L and carrageenan are given in figures 3.9. and 3.10. respectively. From the carrageenan microcapsules 30-32% was released fast initially in 0.1M HCl followed by slow release, the same obtained in distilled water with an initial release of about 20% of drug. The profiles from the Eudragit L microcapsules were similar, the difference being that the distinction between the two release phases was more pronounced with the Eudragit L microcapsules (in 0.1M HCl). A higher proportion of drug was also released from the carrageenan microcapsules in 0.1M HCl in one hour (over 80%), compared with 60% released from the Eudragit L microcapsules. This could be because most of the drug in the carrageenan microcapsules was on the surfaces, probably due to an interplay of lower binding capacity of the polymer and weaker basicity of the drug compared to quinine. Indeed the micrograph of carrageenan/paracetamol microcapsules (plate 3.6.) show most of these to be empty. Certainly the release profiles do not reflect release from a strong complex with drug crystals on the surface of the microcapsules. Overall the release was fast here for the two formulations compared to quinine despite the lower drug load in the paracetamol microcapsules. (About 80% and 45% of quinine were released from the Eudragit L and carrageenan microcapsules in 0.1M HCl in one hour). This is because of the stronger association between quinine and the polyelectrolytes, it being a tertiary amine while paracetamol is a secondary amine. A weaker association between drug and polymer would result in faster dissociation and hence faster drug release. Borodkin and Sundberg (1971) studied the release of amine drugs from cation ion-exchange resin adsorbates in simulated intestinal fluid. They found that greater than 90% of drug was eluted from the secondary amine adsorbates and less than 50% from the tertiary amine adsorbates during the same period.

Figures 3.11. and 3.12. are the release profiles of cimetidine from Eudragit L and carrageenan microcapsules respectively. The profiles are similar to those of Eudragit L/paracetamol microcapsules. This characteristic release profile, which is despite the supposed stronger association between the polyelectrolytes and paracetamol, could be due to the higher drug load in the cimetidine microcapsules. The release profiles are also similar to those of quinine microcapsules (figures 4.1.-4.4.) with the important difference that the relative amounts released are higher here in both formulations although the drug load was higher in the quinine microcapsules. This is again a reflection of the weaker basicity of cimetidine (section 3.5.1.1.). However the difference
Fig. 3.9. In vitro release profiles of acetaminophen from Eudragit L microcapsules.
Fig. 3.10. In vitro release of acetaminophen from carrageenan microcapsules.
Fig. 3.11. In vitro release of cimetidine from Eudragit L microcapsules.
Fig. 3.12. In vitro release of cimetidine from carrageenan microcapsules.
between the two cimetidine formulations in terms of ability to control drug release is still apparent. Thus while about 90% of total drug was released from the Eudragit L microcapsules in 0.1M HCl in one hour, 80% was released from the carrageenan microcapsules in the same medium over the same period. The proportion of total drug released in distilled water is also different for the formulations, 24% for carrageenan and 60% for Eudragit L microcapsules. This indicates that the weak association between Eudragit L and cimetidine allowed a substantial amount of drug to be released in distilled water with pH about 5.9. Therefore the effect of the lower basicity of cimetidine compared to quinine is more pronounced with the weaker electrolyte.

3.5.1.4. Particle size analysis
The size distributions of the two microcapsule formulations of the two drugs are given in appendix 3. The mean diameter of Eudragit L microcapsules of paracetamol was found to be 143.2 μm, and that of carrageenan 40.4 μm. In both cases the size distributions were wide (appendix 3.) implying partial aggregation. The Eudragit L formulation containing cimetidine comprised of particles with a mean diameter of 110.7 μm while those of carrageenan had a mean diameter of 110.2 μm. All round, microcapsules prepared with Eudragit L were larger in size as in the case of quinine (section 3.4.4.3.).

3.6. Conclusions

A multiple emulsion technique of forming three-ply-walled microcapsules has been modified using quinine as a model core material. Transfer of quinine from the oil phases to the aqueous phases of primary emulsions as well as ionic interaction between the drug cations and polyanions in the inner aqueous phase were demonstrated in principle. This gives credence to the modified process.

The present method was found to load more drug than the original one. This is partly due to the interaction of drug and polyelectrolyte in the former. As an oil-soluble form of the drug was used, the low water solubility minimised loss to the aqueous continuous phase, thus further increasing drug content. The particle size distributions were found to be fairly wide, indicating partial aggregation of the microcapsules. Scanning electron microscopy showed empty microcapsules to be largely sickle-shaped, rather than spherical.

Two other drugs were encapsulated and characterised. It has been shown that the magnitude of drug loading in the microcapsules reflects the
affinity for interaction between drug and polyelectrolyte. The strength and magnitude of association between the drugs and a particular polyelectrolyte depended on the basicity of the drug molecule. Thus quinine, a tertiary amine had the highest drug load. The stronger the interaction between drug and polyelectrolyte, the slower was the release of drug from the complex.
CHAPTER FOUR
RELEASE OF QUININE FROM MICROCAPSULES
4.1. Introduction

The results of in vitro release studies may not always give an insight into the performance of a dose form in vivo. Such results can however be a powerful source of information for the purposes of dosage form design. Thus, in vitro dissolution tests are now the established tool for checking oral controlled release products (Graffner and Lakamedal, 1989).

The procedures for in vitro release study are normally determined by the dosage form, the process utilised to control release and the maintenance period. The test is preferably carried out over a period of 6-12 hours. However such stringent tests are more often required during the development stage of the dosage form. For the purposes of the present work, it was thought that a simply designed in vitro test carried out over a period of one hour is sufficient to give the trend of release profile.

Validation of sustained release can only be achieved by in vivo testing (Lordi, 1986). The aim is to determine the bioequivalence of the product in relation with conventional dosage forms of the formulated drug. In the present work, it was also of interest to find out whether the bound drug would be released sufficiently in vivo to justify any claim on the process, in view of the mechanism of controlled release employed (chapter 3, section 3.1.).

The release studies were not intended to produce a comprehensive comparison between the two dosage forms of quinine i.e. microcapsules and tablets. Therefore the in vitro release of the tablet was not studied.

4.2. In vitro release

4.2.1. Method

The dissolution apparatus consisted of a 1 litre cylindrical, flat bottom dissolution flask complying with the specifications of the British Pharmacopoeia 1980, appendix X11. The dissolution medium was stirred at 100 rpm. Dissolution was monitored by a continuous flow-through technique using a polytetrafluoroethylene (PTFE) tubing with internal diameter 1 mm and a Cecil CE 292 uv-visible spectrophotometer at a wave length of 330 nm. The absorbance readings are read directly. A sintered polypropylene depth filter is fitted to the intake of the continuous flow line to prevent particulate matter entering the spectrophotometer cells. The release studies were carried out at 25°C. Duquemin (1987) found no change in release in the temperature range 25 - 37°C. 0.1M HCl and double distilled water (pH about 5.9) were the dissolution media used. 500 mg of microcapsules and 500 ml dissolution
4.2.2. Results and discussion
The calibration curves from which the results were extrapolated are given in appendix 2. The values of amounts released are means of two determinations. Figure 4.1 is the in vitro release profile of the Eudragit L microcapsules (formulation A in table 3.2.), while figures 4.2., 4.3. and 4.4. are the release profiles of the three carrageenan formulations (B, C and D in table 3.2.). The profiles followed the same pattern. In all the formulations amounts corresponding to about 25% of drug released in 0.1M HCl, were released in double distilled water. This pH-dependence indicates that the release mechanism is probably the dissociation of drug-polymer ionic complexes.

The profiles were characterised by a lag phase, followed by a burst-effect of rapid drug release over a short period of time, and finally a slow release phase which is thought to correspond to release through dissociation of the complexes. Bodmeir et al (1989) have reported a similar release profile for quinidine from microspheres of PLA prepared by an emulsification-solvent evaporation method. They attributed it to ionic interactions between the end carboxylic acid groups of PLA and the basic quinidine which could lead to the formation of an insoluble drug-carrier complex or salt.

In the present study the profiles are also thought to be as a result of the method of microencapsulation used. The cores of the microcapsules will contain drug-polymer complexes as well as some free drug dispersed in the polymer network. Some drug would have remained attached to the oil-soluble polymer, while some could be on the surface of the microcapsules. Jalil and Nixon (1989) reported results indicating crystal development at the droplet interface in an o/w emulsion system during microencapsulation of phenobarbitone by the emulsion-solvent evaporation method. Some drug had partitioned into the aqueous phase and since solvent evaporation took place at the interface between the organic and aqueous phases, crystals of the drug grew at the interface while the coating material was still liquid or semi solid as the solvent evaporated. This crystal growth on the surface of microcapsules could also occur with methods not involving emulsification. On examination of cellulose acetate butyrate/phenylpropanolamine HCl microcapsules that produced rapid release of drug, Prapaitrakul and Whitworth (1989) observed multiple drug particles within the microcapsules and protrusion of some of the particles through the surrounding membrane.
Fig. 4.1. In vitro release of quinine from Eudragit L microcapsules (formulation A in table 3.2.).
Fig. 4.2. In vitro release of quinine from carrageenan microcapsules (formulation B in table 3.2.).
Fig 4.3. In vitro release of quinine from carrageenan microcapsules (formulation C in table 3.2.).
Fig. 4.4. In vitro release of quinine from carrageenan microcapsules (formulation D in table 3.2.).
4.3. In vivo release

Having got sufficient loading of drugs in microcapsules and established in vitro release profiles of the drug in the formulations, the next step was to find out the performance of the microcapsules in vivo. This is particularly in view of the supposed mechanism of interaction of the drug with the polyelectrolytes. Taste evaluation was also required to assess the taste coverage.

4.3.1. Protocol for in vivo studies

The study was carried out at the Hospital for Tropical Diseases, St. Pancras Way, London. Six healthy volunteers, four females and two males with body weight ranging from 44-87.5 Kg were used in a cross-over design. Relevant statistics of the subjects are given in appendix 4. Each subject received two microcapsule formulations and quinine bisulphate tablets, i.e. the control, on separate occasions. Each formulation comprised of either 0.5% w/v Eudragit L or 0.25% w/v carrageenan in the inner aqueous phase of multiple emulsions (see table 3.2.). A dose of 540 mg quinine base was administered eight hourly, two times. A weekly interval was allowed between each study to allow complete elimination of quinine from the body before the next dose was given. Microcapsules were administered as extemporaneous suspensions in 0.5% w/v tragacanth with no other additive. The subjects had no breakfast and fasting was continued until three hours after drug ingestion. Blood samples were taken at time intervals for quinine analysis. The protocol was approved by the University College London (UCL) and University College Hospital committee on the ethics of clinical investigation, Faculty of Clinical Sciences, UCL. One subject dropped out of the trial after the first week due to adverse reactions, but another volunteer continued.

4.3.2. Results and discussion

Plasma quinine levels were determined using a modification of an enzyme-linked immunosorbent assay (ELISA) (Eslava et al, 1988). The assay was done at the Applied Immunology Unit, Institute of Zoology, Reagent’s Park, London. The plasma levels at time intervals for individual subjects after microcapsule formulations and the control, including the means and standard deviations, are given in appendix 5. Figures 4.5a and 4.5b., and 4.6a and 4.6b. are the plasma concentration versus time curves for each subject over eight hours and fifty six hours respectively showing individual variations.

Despite some individual variations, the profiles followed a similar
pattern with almost identical levels for the Eudragit L microcapsules and the control at first, levels becoming lower for the control during the elimination phase. On the other hand, the plasma levels from the carrageenan microcapsules were generally lower in all the subjects. The results show that the release of quinine is faster from the Eudragit L than the carrageenan microcapsules, thus being in accord with in vitro release results. Also the in vivo release profiles seem to support the proposed pH-dependent release from these microcapsules. Probably the carrageenan formulation was not resident long enough in the stomach for most of the drug to be released. The high pH values in the small intestine would not favour elution. However carrageenan is biodegradable, so it is expected that most of the drug could be released eventually. An indication of this is seen in the final release phase where quinine appeared to be released continuously, albeit at a slow rate.

4.4. General discussion

4.4.1. Mechanism of release from drug-polymer complexes

Release from drug-polymer complexes requires two processes: drug dissociation and drug diffusion. It is however governed by the kinetics of the bioerosion/biodegradation reaction (Hellar, 1980). The rate of hydrolysis and hence rate of release depends on the strength and chemical nature of the drug-polymer combination, e.g. an anhydride linkage is more susceptible to alkaline hydrolysis than an ester linkage. For a polymer-drug combination to release the drug when required, and hence be effective as a sustained or controlled release formulation, the polymer must have some hydrophillic groups to enhance degradation. The number of groups required depend on the characteristics of the medium and the nature of the linkage, e.g. an anhydride linkage would require the least percentage of hydrophillic groups. The frequency of such groups would affect the period of activity. Neogi and Allan (1974) have shown that for copolymers of 2,3,5-trichloro-4-pyridyl methacrylate and acrylic acid, the one with the higher percentage of acrylic acid offers protection for less time than that with a lower one. This is because large numbers of -COOH groups on the chain make the surrounding ester linkages more susceptible to attack and also serve as catalysts for hydrolysis of the surrounding ester linkages. The rate of hydrolysis of a linkage is also dependent on the groups surrounding it, hydrophobic groups offer protection against hydrolysis, thus a
Fig. 4.5a. Plasma levels of quinine for individual subjects up to eight hours post dose.
Fig. 4.5b. Plasma levels of quinine for individual subjects up to eight hours post dose.
Fig. 4.6a. Plasma levels of quinine for individual subjects up to fifty six hours post dose.
Fig. 4.6b. Plasma levels of quinine for individual subjects up to fifty six hours post dose.
copolymers of acrylic acid and p-nitrophenyl acrylate hydrolyses much faster than the homopolymer of the later (Morawetz and Gaetjens, 1958). Dimension, structure and stereochemistry play a role in determining the rate of release by hindering the approach to a particular bond in question. Hence a highly cross-linked polymer is much less susceptible to hydrolysis compared to an uncross-linked one. Also a stereoregular or crystalline polymer is less susceptible than an amorphous or atactic polymer.

Such complexes are quite sensitive to in vivo variables since release rate depends on the characteristic of the eluting medium. Sprockel and Prapairtrakul (1988) showed that the in vitro release of phenylpropanolamine adsorbed onto sulphonic acid cation-exchange resin particles required counterions in the eluting media. In the presence of hydrogen ions the release was rapid, but it was slow with calcium ions. At constant ionic strengths eluants with low pH gave a more rapid release, and an increase in ionic strength at fixed pH values resulted in enhanced release with the effect being more noticeable at lower pH values. Goto et al (1988) reported the release of ketoprofen microcapsules prepared by the solvent evaporation process to be pH-dependent when an anionic acrylic resin was used as coating material. They found no such dependence when the microencapsulation was achieved with neutral acrylic resins. This pH dependence of release has been encountered in in vitro release studies in the present work.

Feely and Davis (1988) reported controlled release of chlorpheniramine from hydroxypropyl methylcellulose (HPMC) by a drug-surfactant ionic interaction. The complexes of sodium alkyl sulphates, were formed in situ within the (HPMC) matrix. Several methods were patented using ion-exchange technology to form complexes (e.g. Hays, 1962). Amphetamine and antihistamine tannate complexes were once marketed in both tablet and suspension forms as sustained-release formulations (Lordi, 1986). Other complex acids that are used to prepare relatively insoluble and degradable complexes of basic drugs include polygalacturonic acid, alginic acid, and arabogalactone sulphate. Products prepared by interaction of some clays (e.g. bentonite) with cationic drugs or amine salts and certain nonionic drugs have also been investigated (McGinity and Lach, 1977).

4.4.2. In vitro release kinetics

For sustained release formulations prepared by drug modification, if the rate of dissolution is greater than the rate of dissociation, i.e. the dissociation step is rate determining, a zero order release pattern might be realised (Lordi,
This is because the concentration of complex is maintained at its saturation point if the solubility of the complex is sufficiently low so that excess solid is always present.

The profiles in the present study resulted in mixed and complex kinetics for the two formulations, which could not be characterised in a straightforward manner. This is due to the complex release mechanism arising from the method of microcapsulation. The overall release of drug from microcapsules in the present work is thought to be the result of an interplay of the following processes:

1) dissociation and subsequent diffusion of drug from ionic complexes
2) erosion/degradation of polymer matrix and diffusion of unbound drug from the cores
3) leaching through pores from the insoluble ethylcellulose matrix and
4) diffusion of drug from the surface of microcapsules.

Bodmeir et al (1989) found the kinetics of release of quinidine from PLA microspheres to be complicated due to ionic complexation between the drug and carboxylic groups on the polymer molecule. The kinetics of drug release from ion-exchange resin-drug complexes is also not easily characterised (e.g. Burke et al, 1986). Boyd et al (1947) and subsequently, Reichenberg (1955) derived equations to determine whether the exchange is controlled by a chemical, film diffusion or particle diffusion process.

4.4.3. **Comparison of release from the two microcapsule formulations**

Both in vitro and in vivo release profiles show faster release from the Eudragit L microcapsules. The amount of drug released in vitro from the Eudragit L microcapsules (A in table 3.2.) in 0.1M HCl in one hour was approximately 80% (figure 4.1.). On the other hand 33%, 46% and 41% were released from the three carrageenan formulations (B, C and D in table 3.2.) respectively in the same medium, during the same period as seen in figures 4.2., 4.3. and 4.4. From these results then, the amount of drug released in vitro from the carrageenan microcapsules is about 50-55% of that released from the Eudragit L microcapsules. The in vitro release parameters in table 4.1. show the difference in the rate of release from the two formulations. The results of in vivo studies were fitted by a least squares method into a three parameter, two compartment pharmacokinetic model using a computer programme called MAXPOINT.
The model is described by the following equation:

\[ C = B \left( e^{\beta t} - e^{-\alpha t} \right) \]  \hspace{1cm} (4.1)

where \( B \) = characteristic constant, \( t \) = time (hr), \( C \) = plasma concentration (mg/l) at time \( t \), \( \alpha \) = absorption rate constant (hr\(^{-1}\)) and \( \beta \) = elimination rate constant (hr\(^{-1}\)). The programme is given in appendix 6. The derived pharmacokinetic parameters are given in table 4.2. The elimination rate constant and hence the \( t_{1/2} \) for elimination of quinine from the carrageenan formulation is just two thirds that of the Eudragit L formulation. This is a reflection of the slower availability of quinine from the carrageenan microcapsules for elimination. The area under the plasma level time curves (AUC) for the Eudragit L microcapsule is larger, as would be expected from the difference in release rates of the two microcapsules. The smaller AUC in the carrageenan microcapsules emphasizes the higher level of control of drug release here.

These results can be explained by the difference in the chemical structure of the polymers. Sulphonic acid based polyelectrolytes (pKa 1-2) form stronger complexes than carboxylic acid based ones (pKa 5-6). Except for heparin, carrageenan is the most strongly charged natural anionic polyelectrolyte known (Stoloff, 1959). Since ion-binding is due to electrostatic interactions, carrageenan would form stronger ionic complexes with quinine. This resulted in a much slower rate of hydrolysis of the peptide linkage and hence slower rate of drug release. Complex formation was seen to be one of the means of achieving sustained-release by dosage form modification (chapter one, section 1.6.3.). The stronger the complex is, the more effective it is in producing sustained-action. Even at low pH values acid hydrolysis in the carrageenan molecule involves the glycosidic linkages before the sulphate groups (Stoloff, 1959), making dissociation slower still.

It was seen in section 4.4.1. that factors such as frequency of hydrophilic groups and the nature of groups surrounding these, size and stereochemistry of the polymer also influence the rate of dissociation of a drug-polymer complex. The higher percentage of hydrophilic groups in Eudragit L which has a carboxylic acid to ester ratio of 1:1, will make its complexes dissociate faster than those of carrageenan with a hydrophilic group (sulphate) content of about 28-32%. The carrageenan molecule is more bulky than Eudragit L, hence this should make the former less susceptible to degradation. The portion of drug that is not bonded will be in the polymer matrix. Daly et al (1984)

150
found that the release rate from hydrophilic matrix systems decrease as the viscosity of the polymer increases. If this holds, the more viscous carrageenan will be expected to release drug at a slower rate.

4.4.4. **In vitro-in vivo correlation**

Though in vitro-in vivo correlations have often been of little use in predicting the bioavailability of formulations not included in the study under discussion, the correlation may apply to specific products manufactured by similar methods (Smolen, 1971). Substances with good solubility in both water and lipids, and which show linear pharmacokinetics, are said to offer more favourable conditions for in vitro-in vivo correlation.

A comparison of the in vitro release parameters (table 4.1.) and the pharmacokinetic parameters (table 4.2.) respectively for the Eudragit L and carrageenan formulations gives an indication of correlation of in vitro release data with in vivo performance. A graphical representation is not possible with only two sets of data, but the maximum amounts released in vitro for the two formulations compare relatively in magnitude with the maximum plasma levels, time to peak plasma levels or the elimination rate constants. Such a correlation in controlled release delivery systems is said to lend credence to the reliability of the system (Colombo et al, 1986).

In a study, Florence and Jenkins (1976) found the \( t_{50\%} \) and \( t_{100\%} \) to be much shorter in vitro than in vivo. They concluded however, that the measurement of the in vitro rates of release could be used to compare the performance of several preparations. Raghunathan et al (1981) got a direct linear relationship between the amount of phenylpropanolamine released in three hours from suspensions of coated ion-exchange resin-drug complexes and the mean peak plasma concentration from in vivo studies. Sjovall et al (1984) studied the release of suspensions of bacampicillin/ethylcellulose microcapsules. They found an inverse linear relationship between dissolution half life and peak plasma level, urinary recovery, area under the plasma levels time curve and calculated oral bioavailability. Sedman and Wagner (1976) were able to get control of aspirin absorption from enteric coated granules through in vitro dissolution data.
Table 4.1. In vitro release parameters of quinine from microcapsules

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Eudragit L Microcapsules</th>
<th>Carrageenan Microcapsules</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maximum Amount Released (%)</td>
<td>80</td>
<td>46</td>
</tr>
<tr>
<td>$t_{50%}$ (min)</td>
<td>7.5</td>
<td>&gt; 60</td>
</tr>
</tbody>
</table>

Key

$t_{50\%} = $ time for 50% release

Table 4.2. Average pharmacokinetic parameters of quinine from the two dosage forms

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Quinine Bisulphate Tablets</th>
<th>Eudragit L Microcapsules</th>
<th>Carrageenan Microcapsules</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C_{\text{max}}$ (mg$l^{-1}$)</td>
<td>1.39</td>
<td>1.42</td>
<td>0.66</td>
</tr>
<tr>
<td>$AUC$ (mg.hr$l^{-1}$)</td>
<td>17.20</td>
<td>22.57</td>
<td>15.32</td>
</tr>
<tr>
<td>$T_{\text{max}}$ (hr)</td>
<td>1.97</td>
<td>2.02</td>
<td>3.25</td>
</tr>
<tr>
<td>$\alpha$ (hr$^{-1}$)</td>
<td>1.02</td>
<td>1.88</td>
<td>1.69</td>
</tr>
<tr>
<td>$\beta$ (hr$^{-1}$)</td>
<td>0.114</td>
<td>0.070</td>
<td>0.047</td>
</tr>
<tr>
<td>$t_{1/2}$ (hr)</td>
<td>6.08</td>
<td>9.90</td>
<td>14.75</td>
</tr>
</tbody>
</table>

Key

$C_{\text{max}} = $ maximum plasma level attained

$AUC = $ area under the plasma level/time curve

$T_{\text{max}} = $ time at which maximum plasma level was attained

$\alpha = $ absorption rate constant

$\beta = $ elimination rate constant
4.5. Bioavailability of quinine from microcapsules

Figure 4.7. is the average plasma levels time profile for all the subjects over fifty six hours, while figure 4.8. is the average plasma levels time profile for the first eight hours post dose. Thus the results of in vivo studies indicate that plasma levels were more sustained in the microcapsules than the quinine tablet.

The time to maximum plasma level for the Eudragit L microcapsules was not that much higher than that of the tablet, and the peak plasma level was even slightly higher in the microcapsules (table 4.2.). The portion of drug in the microcapsule not bound to the polymer would be released immediately. On the other hand the dissociation of the complex might have proceeded almost immediately due to the weaker binding between drug and polymer here. The net effect was a high maximum plasma level during a relatively short time (2.02 hours) when compared to the tablet which is film-coated. While the coating on the tablet has to dissolve for release of drug to commence, the drug crystals on the surface of the microcapsules are available for release immediately. Despite the faster release from this particular microcapsule formulation however, the elimination rate constant of quinine here was much lower than that from the conventional tablet. The elimination rate constant from the tablets is 0.114 hr⁻¹ while that of the microcapsules is 0.070 hr⁻¹. This is due to slower release of drug from the microcapsules at later stages. It confirms the sustained-release property of a portion of the dose. The interplay of higher initial release rate and lower elimination rate constant resulted in a larger AUC for the microcapsule formulation, implying greater bioavailability in the later.

While the plasma levels from the tablet and the Eudragit L formulation were almost identical initially, the levels from the carrageenan formulation were much lower. The Cmax was just about half, and the time to peak was over two thirds higher than that of the tablet (table 4.2.). The elimination rate of the drug was much lower here, the value being less than half that from the tablet. This is due to slower release of drug from the carrageenan microcapsules because of the stronger association between it and quinine. The AUC is larger for the tablet than the carrageenan microcapsules due to the lower rate of availability of drug for absorption in the later.

It appears then, that with the right choice of polyelectrolyte, a dose form designed from the microcapsules under study could maintain the required
Fig. 4.7. Average plasma levels of quinine over fifty six hours.
Fig. 4.8. Average plasma levels of quinine over eight hours.
blood level of a drug for a specified period of time. The potentially rapid release in the low pH of the stomach would tend to negate the sustained-release property of the dose form, especially in the Eudragit L formulation. However it would depend on the requirement of the dosage form, and the loading dose could be designed to be released in this initial 'burst'.

Suryakusuma and Jun (1984) conducted bioavailability studies on indomethacin microcapsules prepared by a combined bead polymerisation and phase separation technique, on dogs. The AUC, Cmax and Tmax were significantly different from those of an equal dose of drug powder. The duration at which serum levels from the microcapsules remained above the minimum effective concentration (MEC) of the drug was almost twice as great as that of the drug powder. It was suggested that the results confer sustained release properties on the product. Leucuta (1990) studied the pharmacokinetics of nifedipine from a single oral dose of hardened gelatin microspheres administered to human volunteers. He found statistically significant differences between values of maximum plasma levels, time to maximum levels and absorption rate constants of this preparation and the control, which is the drug granules. He concluded that cross-linking the gelatin had prolonged drug release, thus the hardened gelatin microspheres might be suitable for a sustained release formulation of the drug. Results obtained from the in vitro release, and analysis of urine samples, of five volunteers indicated that nitrofurantoin microcapsules from complex coacervation with gelatin provided prolonged release compared with that of the control formulation (Mortada et al, 1988). Guler et al (1989) also demonstrated the sustained release of dextropropoxylene hydrochloride from microcapsules in vivo.

4.6. Clinical analysis of in vivo data

4.6.1. Pharmacokinetics of quinine

Quinine is readily and almost completely absorbed when administered orally, absorption mainly occurring from the small intestine. Peak plasma concentrations are achieved 1-3 hours after absorption.

It is distributed throughout most of the body fluid. The concentration in erythrocytes is one quarter to one third that in plasma but this rises to about half in severe malaria (White et al, 1983a). Drug-sensitive malaria parasites are said to concentrate quinoline antimalarials within parasitised red cells (Fitch et al, 1979). This explains the higher red cell/plasma concentration ratios seen in patients with high parasitaemias. It is over 70% bound to plasma proteins.
Silamut et al (1985) found binding to be significantly higher in cerebral malaria than in uncomplicated malaria or following recovery. They also found the ratio of cerebrospinal fluid to free plasma quinine to be very low suggesting that quinine does not cross the blood brain barrier freely. Quinine readily crosses the placenta. Pharmacokinetic data from falciparum malaria patients in the third trimester of pregnancy show significant differences compared to that of other adults in the acute phase of malaria (Looreesuwan et al, 1984).

Metabolism largely occurs in the liver with less than 5% of drug excreted unchanged in the urine. The metabolism is mainly by hydroxylation, principally to a 2-hydroxyquinoline derivative, which shows little antimalarial activity (Trenholme et al, 1976). After termination of therapy quinine plasma levels fall rapidly, only negligible concentrations being present after 48 hours (WHO, 1986). Renal excretion is limited by the high percentage of protein binding. It is more rapid in acid urine because of its basicity. The elimination half-life of quinine is said to be shorter in children than adults but the volume of distribution is similar.

White et al (1983b) studied the pharmacokinetics of quinine after slow intravenous injection to healthy volunteers. Its disappearance from the blood was described by two exponential terms (two compartment open model) with a rapid distribution phase (\( t_{1/2a} \) 1.9 min) and a slower terminal elimination phase (\( t_{1/2b} \) 11.1 hr). Garnham et al (1976) have reported similar half-lives. The apparent volume of the central compartment (0.57 lKg\(^{-1}\)) was approximately one third of the total apparent volume of distribution.

4.6.1.1. Adverse effects of quinine

A characteristic symptom complex may occur during the administration of quinine. It is commonly referred to as cinchonism and consists of tinnitus, nausea, dysphoria and blurring of vision. These symptoms are usually mild and unless they persist, do not warrant termination of therapy. Toxic doses produce more severe adverse reactions including hypotension, myocardial conduction disturbances, blindness, deafness and central nervous system abnormalities. Hypersensitivity reactions occur in some individuals and are manifest as pruritis, rashes, fever and asthma. Quinine is a potent stimulant of insulin production and may cause hypoglycaemia, particularly in pregnant or seriously ill patients (e.g. Misagena, 1983).
4.6.2. Plasma levels of quinine from microcapsules

The mean peak plasma levels of quinine obtained from the microcapsules are promising (see table 4.2.), this representing absorption after only one dose of 540 mg quinine base in healthy individuals. In malaria the pharmacokinetics of quinine is significantly altered, with a decrease in the apparent volume of distribution, prolongation of elimination $t_{1/2}$ and a reduction in systemic clearance that is proportional to the severity of infection.

Hall et al (1973) studied the bioavailability of quinine from enteric-coated tablets and gelatin capsules in healthy volunteers. A dose of 540 mg base was given 8 hourly for three days in each case. Plasma levels were determined by a benzene extraction method which measures unmetabolised quinine. At two hours post dose the mean plasma levels were 1.82 mg/l for the tablet and 2.79 mg/l for the capsule. These levels actually correspond to peak levels after a single dose. The value for the tablet is comparable to the mean Cmax values for the tablet and Eudragit L microcapsules in this study, giving allowance for the different analytical methods. Plasma levels from their study was maximal at 72 hours with a mean value of 4.08 mg/l for the tablet, and at 64 hours with a mean value of 3.91 mg/l for the gelatin capsule. The results from the present work indicate that levels higher than these could probably be realised with continuous dosing of the microcapsules in view of the sustained-release properties of the formulations.

Sabchareon et al (1982) assessed the serum quinine concentrations after different durations of i/v infusion and after oral administration in 51 children with uncomplicated falciparum malaria. 22 convalescent children were used as control. For the group treated with oral quinine at a dose of 10 mg salt/Kg body weight, at four hours a mean peak serum level of 22.59 nmol/ml (7.33 mg/l) was got in the patients while that of the controls was 10.17 nmol/ml (3.30 mg/l). The mean elimination half life for the patients was almost four times that of the control, and the mean volume of distribution was lower in the patients. This shows that higher levels are realised in malaria and the drug is also cleared slower in disease. In another study, Shann et al (1985) obtained the lowest elimination $t_{1/2}$ in children with no malaria, followed by that in conscious patients while it was highest in those children with severe malaria. White et al (1982) found plasma quinine concentrations to be higher in uncomplicated malaria compared with convalescence, and higher still in cerebral malaria. The high levels were accompanied by reduced clearance and a decrease in volume of distribution. Therefore the higher levels in malaria...
appear to be due in part to a contraction of volume of distribution. Trenholme et al (1976) got similar results when they studied quinine disposition in subjects before and during an experimentally induced infection of chloroquine-resistant falciparum. An artificially induced fever produced the same effect as malaria. Analysis of their results indicate that the longer elimination half life in malaria is due to impaired metabolism.

4.6.3. Incidence of side effects

The side effects encountered and the number of subjects affected by each, for the three forms of quinine studied are given in table 4.3. The most common were neurological, manifested as tinnitus and deafness, and also gastrointestinal in the form of abdominal pain and nausea. Tinnitus was usually associated with a sensation of deafness (fullness or stuffiness). One person was so much affected by nervous related symptoms during the first course of quinine (carrageenan microcapsules) that this warranted withdrawal from the trial. Another subject was particularly vulnerable to gastrointestinal effects, getting these more severely and more frequently than all the other subjects. One subject experienced itching and hot flushes with the Eudragit L microcapsules. More subjects were affected after taking the Eudragit L microcapsules and the tablets, which produced higher plasma levels, especially the former, and the severity of effects also followed this order. Generally the occurrence and duration of effects tended to correspond with periods during which quinine levels were high. This indicates that incidences of side effects are related to blood levels.

Powell and McNamara (1972) analysed the side effects of quinine in experimentally-induced infection with chloroquine-resistant P. falciparum. They noted a dependence of incidences of side effects on high plasma levels. 1% of the volunteers were reported to have complained of unusual nervousness and 5% had pruritus. The treatment was discontinued in one case due to severe gastrointestinal symptoms and three others due to cinchonism with marked tinnitus and vertigo. Trenholme et al (1976) also noted higher incidences of quinine side effects during induced fever compared with afebrile condition. This is said to be due to the higher blood levels attained in febrile conditions and with malaria infection.
Table 4.3. Incidences of side effects of quinine from the microcapsules formulations and the tablets

<table>
<thead>
<tr>
<th>Side effect</th>
<th>Number of subjects affected</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eudragit L Microcapsules</td>
<td>Carrageenan Microcapsules</td>
<td>Quinine Bisulphate Tablets</td>
<td></td>
</tr>
<tr>
<td>Tinnitus</td>
<td>4</td>
<td>2</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Deafness</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Visual disturbances</td>
<td>2</td>
<td>1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Depression</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Headache</td>
<td>2</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Dizziness</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Distraction</td>
<td>3</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Confusion</td>
<td>3</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Anxiety</td>
<td>1</td>
<td>1</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Feeling shaky</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Tiredness</td>
<td>5</td>
<td>3</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Retching</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Diarrhoea</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Nausea</td>
<td>3</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Itching</td>
<td>1</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Other skin reactions</td>
<td>2</td>
<td>-</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>
4.6.4. Relevance of in vivo results to malaria chemotherapy

Chongsuphajaisiddhi et al (1981) assessed the efficacy of quinine in 50 children with falciparum malaria in relation to in vitro sensitivity (measured by the minimum inhibitory concentration, MIC) and to trough serum levels of the drug during the course of treatment. Quinine was administered orally eight hourly for 14 days. A dose of either 10 mg/Kg body weight or one adjusted to body surface area calculated from an adult dose were given. The results of their work revealed a relationship between the outcome of treatment and the persistence of trough serum quinine levels above the MIC. There were no treatment failures in the 26 cases in which trough serum levels of the drug remained above the MIC for longer than six days. Resistance developed in one case where this level was maintained above the MIC for only two days and another where it was maintained for six days. Thus treatment failures may occur in cases infected by parasites of high MIC, where the trough serum levels cannot be maintained above the MIC for longer than six days during the course of treatment.

These findings provide an indication for the development of a sustained-release dosage form of quinine for the treatment of chloroquine-resistant falciparum malaria. This will be particularly relevant in areas where the parasites have high MIC values e.g. Southeast Asia, particularly Thailand where MIC values are now rather high (Chongsuphajaisiddhi et al, 1983). A dosage form of the drug, designed to deliver a certain concentration in the blood and maintain this level for the desired period, would be very beneficial. The differences in MIC values of the parasite according to the locality of the isolate could be accommodated by varying dosing intervals to attain the most suitable therapeutic response in each circumstance. The sustained-release property of microcapsules prepared in the present study indicate that they could be suitable for such a formulation. The difference in the release rate of the two formulations is an indication of the amount of room for manoeuvre in dosage form design. With the right choice of polyelectrolyte, it should be possible to attain a desirable release characteristics and hence dose response.

4.7. Taste evaluation

Taste evaluation of the microcapsule suspensions was carried out simultaneously with bioavailability studies.
4.7.1. Method
The method was adapted from that used by Sjovall et al (1984). Each subject assessed the taste of the formulation from the dose given. The evaluation was done on two levels: the overall palatability and the bitter aftertaste. Overall palatability was based on a scale of 0-4, with the end points extremely bad (0) and excellent (4). The bitter aftertaste evaluation was based on a scale of 0-3 with the end points threshold (0) and strong (3).

4.7.2. Results and discussion
The sensory evaluation by individual subjects is given in table 4.4. On average the overall palatability of the carrageenan formulation was rated fair, while the bitter aftertaste was rated moderate. The Eudragit L formulation got an average rating of good for overall palatability and slight for the bitter aftertaste.

Thus it appears that the Eudragit L formulation was more acceptable, despite the faster release of drug from this formulation (section 4.1.). Some of the subjects were of the opinion that the bitter taste of quinine was masked in the carrageenan microcapsules, but there was a residual taste of something else. It was not figured out what the source is as none of the other constituents has any unpleasant taste. It should be emphasized that evaluation was carried out on the raw microcapsules in a bland vehicle with no sweetening or flavouring agent added which is the practice when evaluating such formulations. The taste was substantially improved later, by incorporating peppermint oil in the oil phase during preparation of microcapsules, showing that flavouring will make some difference to the palatability of the formulations. The taste of quinine from these microcapsules cannot be masked completely by the process alone due to the burst in release even at moderate pH values.
Table 4.4. Sensory evaluation of microcapsules suspensions

<table>
<thead>
<tr>
<th>Subject</th>
<th>Overall palatability</th>
<th></th>
<th>Bitter aftertaste</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Carrageenan</td>
<td>Eudragit L</td>
<td>Carrageenan</td>
<td>Eudragit L</td>
</tr>
<tr>
<td>1</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>2</td>
<td>3</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>3</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
</tbody>
</table>

**Key**

Overall palatability scale:
0 - extremely bad; 1 - fair; 2 - good; 3 - very good; 4 - excellent

Bitter aftertaste scale:
0 - threshold; 1 - slight; 2 - moderate; 3 - strong
Borodkin and Yunker (1970) did some studies on the interaction of amine drugs with polycarboxylic acid ion-exchange resins. Their results indicated that saliva, with an average pH of 6.7 would only elute a limited amount of drug from the drug-resin adsorbates. However rapid and quantitative elution occurred as soon as the drug was exposed to the low pH of the stomach. The results of in vitro release studies in the present work followed the same pattern with only a proportion of the amount of drug released in 0.1M HCl (pH 2.0) being released in double distilled water (pH about 5.9). This has been explained in the light of the mechanism of drug release from the ionic complexes. Drug release in the saliva should be even slower due to its higher pH.

The findings from in vitro release studies of quinine and other drugs in the present work indicate that taste coverage in complexes of amine-containing drug molecules would be more effective with tertiary amines. This is because of the stronger ionic bonding between these and the polyanions which slows release. Borodkin and Sundberg (1971) found that adsorption onto ion-exchange resins reduced the bitter taste of amine drugs. Their results also indicated that in such formulations, taste coverage should be most efficient with tertiary amine drugs. Evaluating the taste of microcapsule suspensions of bacampicillin hydrochloride using volunteers, Sjovall et al (1984) found a significant direct linear relationship between the dissolution half-times and overall taste and bitterness.

Coating the microcapsules of quinine would mask the taste further as this would control the fast initial release. Borodkin and Sundberg (1971) improved the taste of their resin adsorbates by coating with a mixture of ethylcellulose-hydroxypropyl methylcellulose using an air suspension coater. The coating procedure however has to be selected such that minimal damage is done to the microcapsules. The present results do indicate that these formulations have potential for taste coverage when suitably formulated and presented as extemporaneous suspensions in a flavoured vehicle.

Alpar and Walters (1981) masked the bitter taste of phenithicillin potassium by microencapsulation with ethylcellulose. Baichwall and Abraham (1980) effectively masked the taste of metronidazole without the process affecting drug release significantly. Polli and Shoop (1976) developed a method for preparing a palatable formulation of cholestyramine. The formulation comprises coacervates of the drug and a modified gum colloid or cellulosive (e.g. ethylcellulose) and a charged anionic gum (e.g. sodium alginate or carrageenan), reconstituted in an aqueous flavoured vehicle like
fruit juice or water.

4.8. Conclusions

The in vitro release of quinine from the microcapsules was faster at low pH values, indicating that the mechanism of dissociation of the polyelectrolyte-drug ionic complex is acid hydrolysis. This release pattern also implies controlled release in the saliva pH (about 6.7) and availability in the lower pH of the stomach. The release from the microcapsules appeared to be biphasic after a lag phase, due to the initial rapid release of drug crystals from the surface of the microcapsules. The final, slow release phase is believed to correspond to dissociation of complexes. The kinetics of release appeared to be complex. The release from the carrageenan microcapsules was slower, in line with a stronger interaction with quinine here. The faster release from the Eudragit L microcapsules could also have been partly due to the higher proportion of hydrophilic groups here. This would increase the rate of hydrolysis and hence the rate of dissociation of the complexes. The size of the carrageenan molecule could also have hindered the hydrolysis, thus contributing to the slower release.

The Cmax, Tmax and β values for quinine from the tablet are higher than those from carrageenan microcapsules. There was not much difference between the Cmax and Tmax values of the tablet and Eudragit L microcapsules but the elimination rate constant (β) was lower in the microcapsules. The microcapsules thus seem promising for sustained-release of quinine and other suitable drugs. In vitro release parameters and pattern correlate reasonably with plasma level time profiles and bioavailability which further underlines the reliability of the dosage forms for controlled release. The results of in vivo studies are consistent with previous work done on oral bioavailability of quinine in healthy volunteers.

The microencapsulation process employed reduced the bitter taste of quinine appreciably. It was not possible to cover the taste completely due to the nature of the release characteristics of the microcapsules. However, the taste was improved when peppermint oil was incorporated during the preparation of the microcapsules. This was done after taste evaluation had been carried out. This shows that the taste would improve further with flavouring. Therefore these microcapsule formulations could have potential for taste coverage of quinine and other suitable drugs with bitter or other unpleasant
For the successful treatment of chloroquine-resistant falciparum malaria in cases infected by parasites with high minimum inhibitory concentrations (MIC), plasma levels of quinine have to be maintained over a critical part of the treatment period. This is not easily achieved with conventional quinine dose forms at the recommended regimens. The results of the bioavailability studies conducted indicate that sustained-release forms of quinine could reduce the occurrence of treatment failure in falciparum malaria chemotherapy.
CHAPTER FIVE
BUCCAL FORMULATION OF QUININE
5.1. Introduction

Quinine is a marked local irritant; thus when taken orally it often causes abdominal pain, diarrhoea, nausea, and vomiting (e.g. Powell and McNamara, 1972). Rectal instillation did not produce any significant reduction of gastric irritation (Fletcher, 1923). The majority of the populace in many parts of the areas where malaria is endemic do not have access to the attention of skilled medical personnel always. This further compounds the hazards of parenteral administration (White, 1983; Thuriaux, 1982). In vivo studies in the present work have shown that although the gastric irritation was lessened by microencapsulation, it was not eliminated in a greater proportion of the subjects used. Another mode of administration which could circumvent this problem of local irritation would therefore have advantages.

The mechanism of absorption of drug administered by the buccal route, its advantages and short comings were discussed in chapter one, section 1.10. One of the advantages is the elimination of local irritation to the gastric mucosa. Indeed although there does not seem to be any documentation of this, apparently quinine solution dipped in cotton wool used to be applied into the buccal cavity of unconscious malaria patients (mainly children). Therefore the possibility of developing a buccal formulation of quinine using microcapsules successfully prepared in the course of this work was explored. The basic idea is to disperse the microcapsules in a cross-linked network of alginate. The resulting gel could then be moulded into dosage forms for administration.

5.2. Mechanism of cross-linking of alginate by calcium ions

Sodium alginate solutions form gels by reacting with calcium ions, the reaction thought to be both intermolecular and intramolecular (Glicksman, 1969). Crosslinking takes place through carboxyl groups by primary valences and through hydroxyl groups by secondary valences (figure 5.1.). Calcium links with carboxyl groups by means of its primary valences, probably onto adjacent molecular units. Coordinate bonds then extend to two nearby hydroxyl groups of a third unit that may be in the same molecular chain, to retain the coiled shape of the macromolecule. If this third unit is in another chain, this results in the formation of a huge molecule with a three dimensional, netlike structure. Complete reaction of soluble sodium alginate forms insoluble calcium alginate, varying consistencies can be attained by using a range of calcium concentrations.
Figure 5.1. Possible mechanism of cross-linking of alginate by calcium ions
Some preparatory work was carried out to investigate the feasibility of producing a buccal quinine dosage form. The general method involved ‘dispersion’ of microcapsules containing calcium salts in aqueous sodium alginate solutions. The alginate molecules could then be crosslinked through bonding with the calcium ions to produce gel networks. Calcium salts were encapsulated by incorporating these in the inner or outer aqueous phase of multiple emulsions as the case may be. The findings from these experiments are briefly reported in the following sections.

**5.3.1. Incorporating calcium salts in the inner aqueous phase of multiple emulsions**

Small concentrations (0.1 - 0.2% w/v) of calcium chloride or calcium acetate were incorporated in the inner aqueous phases of multiple emulsions, with either Eudragit L or carrageenan. An amount (up to 750mg) of dried microcapsules was dispersed in 25 ml 2% w/v sodium alginate solution by means of a magnetic stirrer at moderate speed.

Although some thickening of the alginate solution occurred, none of the systems investigated formed a gel. Apparently the amount of calcium in the microcapsules was not sufficient to crosslink the polymer to produce a gel network. The amount of salt that could be incorporated in the inner aqueous phase was limited by the incompatibility of solutes with solutions of the polyelectrolytes, so it was not possible to increase the amount of calcium salt.

**5.3.2. Incorporating calcium salts in the outer aqueous phase of multiple emulsions**

The idea of incorporating calcium salts in the outer aqueous phase for subsequent transfer to the inner aqueous phase was based on the thinking that a lot more salt could be added since these would not prevent the solution of acacia. 5% w/v calcium chloride was added to the outer aqueous phase. The multiple emulsion was left for 24 hours for the calcium to diffuse in before the microcapsules were extracted.

It was not possible to disperse the dried microcapsules in 25 ml of 2-4% w/v sodium alginate solution, because the microcapsules lumped together with a hardened surface immediately on getting in contact with the alginate. Also there appeared to be a considerable amount of charge on the surface of the microcapsules as these were sticking to dry surfaces. Probably some
interaction took place between the cationic calcium ions and acacia, which has some residual acidic groups. This would reduce the amount of calcium ions available for transport. Another reason could be that the acacia-calcium complexes that might have been formed became a barrier for diffusion of the remaining free calcium ions out of the outer aqueous phase. So some of the calcium might have been trapped in these complexes in the outer aqueous phase, and after drying left on the surface of microcapsules. When the microcapsules were left in the alginate solution as such, no gelling occurred after 24 hours. Therefore the microcapsules only gave a ‘burst’ of calcium. Washing the microcapsules briefly in distilled water seemed to remove all the calcium ions, as dispersing these in sodium alginate solution produced neither the initial lumping nor any thickening when left for 24 hours. This confirms that most of the calcium salt had remained in the outer aqueous phase and hence the surface of the microcapsules. Some gelation occurred after several days but the microcapsules were lumped together. This might have been due to the dissociation of the proposed calcium-acacia complexes or slow diffusion of free calcium out of the matrix, over the period.

5.3.3. Incorporating calcium salt in the inner aqueous phase with acacia instead of a polyelectrolyte

Here the polyelectrolytes were substituted with acacia, 4% w/v, with quinine still in the oil phase. Another batch was made without the drug at all. In both cases 4% w/v calcium chloride was added. 500 mg of the dried microcapsules were stirred into 25 ml of either 2 or 4% w/v alginate solution.

These microcapsules were more dispersible in the 4% w/v alginate solution while lumping was more pronounced in the 2% w/v series. This could be because there were more calcium ions per alginate molecule for crosslinking in the 2% w/v solution. For the microcapsules without drug, the mixture for both concentrations of alginate gelled into an immovable mass within an hour. That dispersed in the 2% w/v alginate solution was more solid to the touch, this also being because there were more calcium ions for reaction here per polymer molecule. After 24 hours a firm gel had resulted in each case but the microcapsules were all lumped together. Plate 5.1. is a picture of a gel formed by dispersing the microcapsules in 25ml of a 2% w/v sodium alginate solution showing the microcapsules lumped together in the network. After 24 hours no gelation had occurred in the microcapsules containing drug even with the 2% w/v alginate solution.

Obviously there were more calcium ions in the microcapsules with no
quinine incorporated. A plausible explanation could be that quinine and the calcium competed for ionic binding sites on the acacia molecule, so a lot more ‘free’ calcium was lost to the outer aqueous phase during manufacture and extraction.

Incorporation of 4% w/v calcium acetate (a less soluble calcium salt) in the microcapsules without drug gave the same result. Here also no uniform dispersion of microcapsules was formed in the gel.

5.3.4 Attempts to solve dispersion problem using wetting agents

It was believed that wetting the microcapsules prior to mixing or by adding wetting agent in the alginate might make these more dispersible, so a series of experiments were performed with wetting agents.

Wetting the microcapsules containing calcium chloride in a small amount of 0.1% w/v sodium lauryl sulphate prior to mixing produced gelation immediately contact was made with the alginate solution. The microcapsules were all lumped together non-uniformly. It appears that the calcium salt is so soluble that it was all solubilised during wetting. When the microcapsules were wetted with drops of glycerol triacetate prior to mixing, the same result was obtained with the only difference being that gel formation was less instantaneous. Mixing with a mixer/emulsifier instead of a magnetic stirrer was tried, but lumping was even worse here. 2 ml of a 0.1% w/v solution of sodium lauryl sulphate was added to alginate solution and the microcapsules added with mixing. The dispersibility of the microcapsules was not improved at all.

Reducing the amount of calcium chloride in the microcapsules did not reduce the ‘burst’ effect. The problem therefore seem to be that of high water solubility of the calcium salts (chloride and acetate).

5.3.5 Cross-linking using a calcium salt with low water solubility

Microcapsules were prepared with acacia and 3% w/v calcium lactate in the inner aqueous phase. The microcapsules here were finer and flow more freely.

When 500 mg of microcapsules was mixed with 25 ml of 2% w/v sodium alginate, over 24 hours a uniform gel was formed which was not as firm to the touch as the one with the chloride salt (section 3.6.3.3.). There was no lumping of microcapsules but these were not uniformly dispersed in the matrix, some sections being a clear gel. When a larger amount of microcapsules (750mg) were stirred into 20ml of 2% w/v sodium alginate
solution, a soft gel matrix with the microcapsules fairly well dispersed (plate 5.2.), was formed over a period of three days.

Calcium lactate has a solubility of 3.1 gm/100 ml in cold water, almost 25 times less than that of calcium chloride and about 12 times less than that of calcium acetate. The low water solubility improved dispersion of microcapsules ostensibly by reducing the amount of calcium lost to the outer aqueous phase. This in turn minimised the amount on the surface of microcapsules. However there appeared to be insufficient calcium for interaction. This is because there is three times more calcium weight for weight in the chloride salt than the lactate. Heat afforded the incorporation of more calcium lactate (7.6% w/v), increasing its solubility by about half. The results of gelation experiments showed that this did increase the amount of calcium in the microcapsules. The alginate solution indicated that some interaction did occur. On the other hand lumping of the microcapsules increased. This is probably because the more calcium lactate is added to the inner aqueous phase, the more is transferred to the outer aqueous phase and hence to the surface of microcapsules after equilibration.

With 3 ml of 1% w/v sodium lauryl sulphate in 2% w/v sodium alginate and mixing with a mixer/emulsifier, a white soft gel was formed which hardened a bit overnight. Dispersion seemed to be complete here but the high concentration of wetting agent is probably unacceptable as it might irritate the buccal mucosa. Another thing is that the gel was very soft, it is possible that the shear from the emulsifier might have destroyed the gel structure.
Plate 5.1. A gel network formed by dispersing microcapsules containing calcium chloride in sodium alginate solution, showing aggregation of microcapsules.

Plate 5.2. A gel network formed by dispersing microcapsules containing calcium lactate in sodium alginate solution. Microcapsules here are more uniformly dispersed.
5.4. Conclusion and suggestions for further work

From the results of the work done so far it appears that the concept of dispersing microcapsules in a network of gel for a buccal quinine dosage is probably plausible. It could also be extended to other drugs with similar physicochemical properties. The main problem seem to be getting the right source of calcium ions in the right concentration such that:

1) Sufficient calcium ions are available for crosslinking with no much loss to the outer aqueous phase to prevent aggregation of the microcapsules.
2) The drug and calcium ions coexist in the microcapsules with minimal competition for possible ionic binding with polymer molecules and indeed any other type of interaction that might compromise drug loading or the crosslinking process.

Though the untoward effect of poorly dispersed microcapsules is likely to be only a lack of cosmetic appeal, it is a short coming worth looking at nevertheless.

Incorporating an organic (oil-soluble) calcium salt in the oil phase of the multiple emulsion might take care of these problems. Thus organic calcium salts with the general structure:

$$\text{RCOO}^- \leftrightarrow \text{RCOO}^- \text{Ca}^{++} \leftrightarrow \text{Ca}^{++} \text{COOR}$$

like stearate or oleate, with pKa values of about 4-6 rather than 2-3 can be used to control the production of calcium ions. Transfer of calcium to the outer aqueous phase would certainly be minimised, thus taking care of its resultant release in a burst. These being weaker, interaction with polymer molecules is likely to be to a much lesser extent than in the case of Ca from stronger ones. The effect of this would be less competition for binding sites between drug and the calcium ions. If this holds it will then be possible to disperse microcapsules of quinine prepared by the modified multiple emulsion technique in this work (with calcium salt in the oil phase) in the crosslinked alginate network.
CHAPTER SIX
GENERAL CONCLUSIONS
6.1. Conclusions

Taste-masked and controlled-release quinine formulations were prepared using a modified multiple emulsion technique of microencapsulation. The process involves ionic interaction between cationic drug and anionic polyelectrolyte in the inner aqueous phase of the multiple emulsion. Two formulations were prepared using carrageenan and Eudragit L. Acetaminophen and cimetidine were also encapsulated and the characteristics (particle size, drug content, in vitro release rates and morphology) of microcapsules of the three drugs compared.

Transfer of quinine from the oil phase to the aqueous phase of primary emulsions was demonstrated. It was also shown, in principle, that the drug interacts with the polyanion in the inner aqueous phase. More drug was shown to be transferred with Eudragit L than carrageenan. Although no quantitative analysis of ionic-binding was made, the results indicated that higher amounts of drug were bound to Eudragit L. This was explained by the difference in the number of binding sites on the polymer molecules. While the carboxylic acid content of Eudragit L is 50%, the sulphate content of iota-carrageenan is not more than 32%. The diffusion process was generally slow, partly because the rate of diffusion was decreased by a lowering of the apparent partition coefficient of the drug as a result of the complex formation in the inner aqueous phase. The other reason is the small surface area presented for diffusion between the phases. That transfer of drug between the phases does occur gives credence to the method of incorporation of quinine used in this work.

The process was seen to be superior in drug loading to the original method. Drug content was higher here because of the ionic interaction involved. Thus the drug already bound to the polyanion in the inner aqueous phase was not available for partitioning to the outer aqueous phase during manufacture and extraction. The drug loss inherent in the method was hence minimised. Where an oil soluble form of a drug is used, the loss was further reduced due to a lower water solubility. More drug was loaded in the microcapsules prepared with Eudragit L than those with carrageenan, thus confirming the findings from diffusion and interaction studies. The magnitude of loading in microcapsules of the other drugs encapsulated, i.e. acetaminophen and cimetidine followed the same pattern. For a particular polyelectrolyte, drug loading was found to be a function of the basicity of the drug molecule. The loading for quinine,
which is a tertiary amine, was therefore found to be over twice that for acetaminophen which has a secondary amine group. These two could be compared as the drug was initially incorporated in the oil phase in both cases. Drug content was also higher in quinine than in cimetidine. The distinction between paracetamol and cimetidine was not very apparent as the differences between the amounts loaded were neither appreciable nor consistent. Cimetidine was initially incorporated in the aqueous phase, this and its higher water solubility would have resulted in a substantial amount being lost to the aqueous phase. Also from the chemistry of its molecule no extensive ionic-binding would be expected to occur between it and the acidic groups on the polyelectrolytes. On the other hand the higher percentage of drug started with here compared to paracetamol might have resulted in a relatively higher amount of free drug than would have been expected. Thus the two results are not easily comparable.

The in vitro release of quinine was much faster at a lower pH, implying that hydrolysis is the mechanism of dissociation of the complexes. It also shows that drug release will be fast in the low stomach pH (1-2) but slow in the mouth since the pH of saliva (about 6.7) is higher. The profiles gave an indication of sustained release, the rate of release depending on the strength of drug-polymer interaction. It was faster from the Eudragit L microcapsules because carrageenan being a stronger polyelectrolyte, with a lower pKa value, form stronger complexes with the drug. Apart from this, other differences in the chemistry of the two polymers favour faster hydrolysis in Eudragit L. The kinetics of drug release were complex but consistent with what is expected from the method of microencapsulation.

In terms of the plasma levels attained and the adverse effects encountered, the results of bioavailability studies conducted on healthy human volunteers were consistent with those reported from previous studies. Plasma levels were more sustained in the microcapsules than the control tablet, confirming the sustained release property of the formulations. The correlation between in vitro and in vivo release profiles and parameters underline the ability of the formulations for controlled release. It also supports the proposed mechanism of controlled release in the formulations, and confirms the distinction in drug release rate between ionic complexes formed from the two polyelectrolytes as a result of their different chemical structures. There was no much difference between the Eudragit L microcapsules and the tablet in terms of peak plasma level and the
time the peak was reached. This was attributed to fast initial release in the microcapsules due to drug crystals on the surface followed by high rate of hydrolysis in line with a weak association here. The elimination rate was however lower in the microcapsules, this resulting in the blood levels being sustained longer. The in vivo release was found to be faster from the Eudragit L than the carrageenan microcapsules with a higher peak plasma level, shorter time to peak and higher elimination rate constant.

From the assessment of clinical data on falciparum malaria chemotherapy, it appears that a sustained release quinine dose form could reduce the occurrence of treatment failure. This could also prevent incidences of adverse effects, the mild forms of which were encountered in the present study, by preventing the build-up of unnecessarily high quinine plasma levels. The dependence of the MIC for a particular parasite on its geographical origin could be accommodated by adjustment of dosage intervals to produce the desired blood levels. Alternatively, different dosage form designs could be used, some with loading doses. The microcapsules could be used as basis for both adult and paediatric dosage forms.

Taste evaluation of the microcapsules suspended in a bland vehicle with no added excipient showed that the process reduced the bitter taste substantially. The taste coverage was found to be more effective subsequently when peppermint oil was incorporated in the oil phase during the preparation of microcapsules. This shows that the suspensions would be even more palatable with flavouring. Therefore these microcapsules could have potential for taste coverage of not only quinine but other unpleasant tasting drugs if suitably presented as extemporaneous preparations for paediatric use. A taste-masked paediatric quinine preparation would improve patient compliance.

The rate of drug release from microcapsules of other drugs encapsulated was found to depend on the strength of association between the drug and the polyelectrolyte, as in the case of quinine. It appears that tertiary amine drugs would be better candidates for sustained-release and taste coverage using the microcapsule formulations evaluated in this work. The principles involved imply that the process could also be applied to suitable anionic drugs and cationic polyelectrolytes for the control of drug release.
Some progress was made in the development of a buccal quinine formulation. Microcapsules containing calcium salts were dispersed in alginate solutions to form cross-links of the alginate resulting in gel structures. The main constraints encountered were encapsulating adequate calcium salt, and aggregation of the microcapsules which is thought to be due to immediate reaction of alginate with the calcium ions on the surface of microcapsules. In addition to bypassing the oral mucosa, such a formulation would control drug release more effectively.

6.2. Suggestions for further work

The main shortcoming of these microcapsules as potential sustained release formulations seem to be the fast initial release, which could be undesirable. The procedure of the microencapsulation is such that formation of drug crystals on the surface, which is responsible for the initial rapid release, will be difficult to eliminate. Coating the dried microcapsules using a process which would not rupture the microcapsules might realise better control of initial release. This would also increase the taste coverage since release in the mouth will be slowed. Using another oil soluble polymer which does not form porous films like ethylcellulose could improve the integrity of the wall formed from that layer.

A polyelectrolyte with a strength somewhere between that of Eudragit L and carrageenan will probably serve most purposes although the two extremes may well serve particular purposes. Thus another important suggestion for further work, and probably the most obvious, is the choice of polyelectrolyte to suit the release properties desired. This should ensure the attainment of the desirable in vitro release profiles of quinine prior to sustained-release dosage form design.

For the buccal quinine dose form, retention of calcium in the microcapsules can be improved by using an oil soluble, organic calcium salt. This would control the production of calcium ions for crosslinking, and thereby also reduce the interaction between the ions and the polymer in the inner aqueous phase of multiple emulsions. In addition it would minimise the loss of calcium salt to the outer aqueous phase and hence its crystallisation on the surface of microcapsules.
Appendix 1. Theory of fluorescence spectroscopy

Molecular fluorescence is the almost immediate emission (in the order of $10^{-8}$ seconds) of light from a molecule following the absorption of radiation. Molecules in a higher level of excited singlet state quickly return to the lowest vibrational level of the excited state by transferring their excess energy to other molecules through collisions, as well as partitioning to other possible modes of vibration or rotation within the excited molecule. Fluorescence therefore arises from a singlet-singlet transition. The process is characterised by two spectra, an excitation spectrum corresponding to the absorption of energy and a fluorescence spectrum corresponding to the emission of energy. The fluorescence intensity is expressed by the following equation:

$$F = I_0 \Theta e l CK$$  \hspace{1cm} (3.1)

where $I_0$ is the intensity of exciting radiation, $e$ is the absorption coefficient, $l$ is path length, $C$ is the concentration, $K$ is a constant depending upon instrumental design and $\Theta$ is the quantum efficiency; a constant which takes account of the fact that not all the molecules which absorb radiation will fluoresce. Equation 3.1. indicates that the fluorescence signal is proportional to the concentration of the compound.

The fluorescence of a substance is generally more affected by the environment than is the absorption. When the fluorescence is decreased by the presence of another substance the phenomenon is termed quenching. There are many types of quenching processes, however, a common one is collisional quenching in which the excited fluorescent species loses its energy of excitation by collision with another substance termed the quencher. The degree of quenching is related to the concentration of the quencher by the following equation:

$$F'/F = 1 + kC$$  \hspace{1cm} (3.2)

where $F'$ = fluorescence intensity in absence of quencher, $F$ = fluorescence intensity when quencher is present, $C$ = concentration of quencher and $k$ = constant.
Appendix 2. Calibration curves for spectroscopic methods of drugs analysis
Fluorescence calibration curve for quinine

(diffusion studies)

Fluorescence Intensity

Concentration (ug/ml)

1000  900  800  700  600  500  400  300  200  0.2

0.3  0.4  0.5  0.6  0.7  0.8  0.9  1
Fluorescence calibration curve for quinine

(drug content of microcapsules)
Beer–Lambert calibration curve for quinine

(drug content of microcapsules)

Absorbance

Concentration (mg/100ml)
Beer-Lambert calibration curve for quinine

Concentration (mg% w/v)

Absorbance

0.1M HCl
Distilled water

187
Beer–Lambert calibration curve for paracetamol

Concentration (mg/l)

Absorbance

0.8  0.7  0.6  0.5  0.4  0.3  0.2  0.1

Double distilled water

0.1 M HCl

0  1  2  3  4  5  6  7  8  9  10
Beer–Lambert calibration curve for cimetidine

Absorbance

Concentration (mg/l)

0.1M HCl
Distilled water

1 8 9
Appendix 3. Particle size distributions of microcapsules of otherdrugs encapsulated
Particle size distribution of Eudragit L/paracetamol microcapsules

% undersize

Particle size (um)
Particle size distribution of carrageenan/paracetamol microcapsules

% Undersize vs. Particle size (μm)
Particle size distribution of Eudragit L/cimetidine microcapsules
Particle size distribution of carrageenan/cimetidine microcapsules
Appendix 4. Relevant statistics of subjects used for bioavailability studies

<table>
<thead>
<tr>
<th>Subject</th>
<th>Weight (Kg)</th>
<th>Height (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>87.5</td>
<td>1.83</td>
</tr>
<tr>
<td>2</td>
<td>44.0</td>
<td>1.58</td>
</tr>
<tr>
<td>3</td>
<td>55.0</td>
<td>1.60</td>
</tr>
<tr>
<td>4</td>
<td>58.0</td>
<td>1.60</td>
</tr>
<tr>
<td>5</td>
<td>74.5</td>
<td>1.73</td>
</tr>
<tr>
<td>6</td>
<td>60.3</td>
<td>1.52</td>
</tr>
<tr>
<td>7</td>
<td>59.5</td>
<td>1.65</td>
</tr>
</tbody>
</table>

N.B. Subject 7 dropped out after the first week, i.e., after one formulation
Appendix 5. Plasma levels of quinine from bioavailability studies
### 5a. Carrageenan microcapsules

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Plasma levels (mg/l)</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subject 1</td>
<td>Subject 2</td>
<td>Subject 3</td>
</tr>
<tr>
<td>0</td>
<td>0.00</td>
<td>0.01</td>
<td>0.00</td>
</tr>
<tr>
<td>1</td>
<td>0.57</td>
<td>0.41</td>
<td>0.84</td>
</tr>
<tr>
<td>2</td>
<td>0.58</td>
<td>0.41</td>
<td>0.63</td>
</tr>
<tr>
<td>3</td>
<td>0.65</td>
<td>0.41</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>0.63</td>
<td></td>
<td>0.62</td>
</tr>
<tr>
<td>5</td>
<td>0.59</td>
<td></td>
<td>0.62</td>
</tr>
<tr>
<td>6</td>
<td>0.69</td>
<td>0.48</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>0.55</td>
<td>0.42</td>
<td>0.62</td>
</tr>
<tr>
<td>8</td>
<td>0.52</td>
<td>0.39</td>
<td>0.51</td>
</tr>
<tr>
<td>24</td>
<td>0.75</td>
<td>0.59</td>
<td>0.79</td>
</tr>
<tr>
<td>32</td>
<td>0.50</td>
<td>0.46</td>
<td>0.60</td>
</tr>
<tr>
<td>48</td>
<td>0.38</td>
<td>0.34</td>
<td>0.13</td>
</tr>
<tr>
<td>56</td>
<td>0.27</td>
<td>0.29</td>
<td>0.10</td>
</tr>
</tbody>
</table>
5b. Eudragit L microcapsules

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Plasma levels (mg/l)</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Subject 1</td>
<td>Subject 2</td>
<td>Subject 3</td>
</tr>
<tr>
<td>0</td>
<td>0.08</td>
<td>0.03</td>
<td>0.03</td>
</tr>
<tr>
<td>1</td>
<td>1.41</td>
<td>0.61</td>
<td>2.26</td>
</tr>
<tr>
<td>2</td>
<td>1.41</td>
<td>1.28</td>
<td>1.82</td>
</tr>
<tr>
<td>3</td>
<td>1.62</td>
<td>1.16</td>
<td>1.23</td>
</tr>
<tr>
<td>4</td>
<td>1.40</td>
<td>1.25</td>
<td>1.43</td>
</tr>
<tr>
<td>5</td>
<td>0.98</td>
<td>1.03</td>
<td>1.15</td>
</tr>
<tr>
<td>6</td>
<td>1.09</td>
<td>1.26</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>0.91</td>
<td>0.97</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>0.98</td>
<td>1.13</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>2.13</td>
<td>1.54</td>
<td>-</td>
</tr>
<tr>
<td>24</td>
<td>1.49</td>
<td>0.78</td>
<td>0.60</td>
</tr>
<tr>
<td>32</td>
<td>0.74</td>
<td>0.66</td>
<td>0.55</td>
</tr>
<tr>
<td>48</td>
<td>0.52</td>
<td>0.44</td>
<td>0.24</td>
</tr>
<tr>
<td>56</td>
<td>0.38</td>
<td>0.16</td>
<td>0.12</td>
</tr>
</tbody>
</table>
5c. Quinine Bisulphate tablets

<table>
<thead>
<tr>
<th>Time (hr)</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
<th>Subject 5</th>
<th>Subject 6</th>
<th>Mean</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>0.050</td>
<td>0.02</td>
<td>0.01</td>
<td>0.04</td>
<td>0.01</td>
<td>0.01</td>
<td>0.023</td>
<td>0.0159</td>
</tr>
<tr>
<td>1</td>
<td>1.523</td>
<td>0.745</td>
<td>1.548</td>
<td>1.227</td>
<td>1.536</td>
<td>0.711</td>
<td>1.215</td>
<td>0.3617</td>
</tr>
<tr>
<td>2</td>
<td>1.553</td>
<td>1.170</td>
<td>1.553</td>
<td>1.559</td>
<td>1.238</td>
<td>0.894</td>
<td>1.328</td>
<td>0.2503</td>
</tr>
<tr>
<td>3</td>
<td>1.743</td>
<td>1.227</td>
<td>1.170</td>
<td>1.503</td>
<td>1.101</td>
<td>0.906</td>
<td>1.275</td>
<td>0.2741</td>
</tr>
<tr>
<td>4</td>
<td>1.284</td>
<td>0.906</td>
<td>1.112</td>
<td>1.032</td>
<td>1.456</td>
<td>0.814</td>
<td>1.101</td>
<td>0.2179</td>
</tr>
<tr>
<td>5</td>
<td>1.707</td>
<td>1.170</td>
<td>0.963</td>
<td>0.998</td>
<td>1.112</td>
<td>0.975</td>
<td>1.154</td>
<td>0.2587</td>
</tr>
<tr>
<td>6</td>
<td>1.800</td>
<td>1.112</td>
<td>1.204</td>
<td>0.998</td>
<td>1.215</td>
<td>0.699</td>
<td>1.171</td>
<td>0.3303</td>
</tr>
<tr>
<td>7</td>
<td>1.181</td>
<td>0.768</td>
<td>-</td>
<td>0.963</td>
<td>0.940</td>
<td>0.940</td>
<td>0.958</td>
<td>0.1315</td>
</tr>
<tr>
<td>8</td>
<td>1.098</td>
<td>0.986</td>
<td>0.871</td>
<td>0.860</td>
<td>1.055</td>
<td>0.768</td>
<td>0.940</td>
<td>0.1162</td>
</tr>
<tr>
<td>24</td>
<td>1.043</td>
<td>0.860</td>
<td>0.493</td>
<td>1.559</td>
<td>1.066</td>
<td>0.722</td>
<td>0.957</td>
<td>0.3320</td>
</tr>
<tr>
<td>32</td>
<td>0.860</td>
<td>0.550</td>
<td>0.390</td>
<td>0.447</td>
<td>0.619</td>
<td>0.470</td>
<td>0.556</td>
<td>0.1545</td>
</tr>
<tr>
<td>48</td>
<td>0.390</td>
<td>0.287</td>
<td>0.046</td>
<td>0.367</td>
<td>0.229</td>
<td>0.252</td>
<td>0.262</td>
<td>0.1124</td>
</tr>
<tr>
<td>56</td>
<td>0.355</td>
<td>0.195</td>
<td>0.023</td>
<td>0.080</td>
<td>0.275</td>
<td>0.161</td>
<td>0.182</td>
<td>0.1117</td>
</tr>
</tbody>
</table>
Appendix 6. MAXPOINT pharmacokinetic program
A FORTRAN COMPUTER PROGRAMME FOR MODELLING PHARMACOKINETIC BEHAVIOUR BASED ON THREE PARAMETERS

Prof. L. SAUNDERS, SCHOOL OF PHARMACY

C SCHOOL OF PHARMACY, PHYS CHEM LIBRARY CALL NO 770
C EVALUATION OF PARAMETERS FOR EXPONENTIAL EQUATIONS
C FROM ORAL DOSE, REVISED MAXIMUM POINT METHOD
C OCTOBER 1981
C PROGRAM EDITED BY PROFESSOR L. SAUNDERS
C SCHOOL OF PHARMACY
C UNIVERSITY OF LONDON
C
COMMON NP, XO, T(50), C(50), NPAR, TC(100)
COMMON/BLOCKD/ CM, TM, IX, AREA, VKA, ITER, KIT, TGM
COMMON/BLOCKG/ AA, BB, ALPHA, BETA, VK12, VK21, VKEL
COMMON/CONTR/ IS1, IS2, IS3, IPP, NIT, ESUM, KITC
CHARACTER*10 TITLE(8)
DIMENSION E2(20), V(20)
C*
C CALL DISCON(4)
C CALL CONNEC(5)
C CALL CONNEC(6)
WRITE(6, 900)
WRITE(4, 900)
900 FORMAT(1H, 'MAXIMUM POINT METHOD, ORAL DRUG')
WRITE(6, 901)
901 FORMAT(1H, 'ENTER NSET, NPAR')
111 READ(5,*) NSET, NPARI
IF(NPAR.EQ.0)NPAR=5
IS1=1
IS2=1
C IS1=IS2=1
IPP=2
IS3=3
NTAP=0
VKA2=9999.
2000 WRITE(6, 902)
902 FORMAT(1H, 'ENTER TITLE (INCLUDE YOUR NAME)')
90 READ(5,1) TITLE
1 FORMAT(8A10)
91 WRITE(6, 903)
903 FORMAT(1H, 'ENTER NP, KITC')
READ(5,*) NP, KITC
C STEVE MOD, 5/11/82
IF(NP.GT.50) GOTO 91
WRITE(6,905)
905 FORMAT(1H,'ENTER LIMBET, 1 RESTRICTS LSQ')
READ(5,*) LIMBET
WRITE(6,904)
904 FORMAT(1H,'ENTER T,C IN PAIRS, NO ZERO C')
READ(5,*) (T(I),C(I),I=1,NP)
ALPHA = 0.
IF(KITC.EQ.0) KITC= 5
SL1 = (C(2)-C(1))/(T(2)-T(1))
SL2 = (C(3)-C(2))/(T(3)-T(2))
IF(SL2.LT.SL1) GO TO 7300
TLAG = (C(3)*T(1)-C(1)*T(3))/(C(3)-C(1))
GO TO 7310
7300 CALL GETLAG(TLAG)
7310 TIMEL= TLAG
IF(TIMEL.LT.0.) TIMEL= 0.
IF(TIMEL.GT.T(1)) TIMEL= T(1)/2.
KIT= 0
JITER= 1
WRITE(6,3) TITLE
WRITE(4,3) TITLE
3 FORM AT(1H ' EVALUATION OF PARAMETERS FOR EXPONENTIAL EQUATION
2, FROM ORAL DOSE '/ 1H ,8A10)
WRITE(6,7) TIMEL
WRITE(4,7) TIMEL
7 FORMAT(1H ' LAG TIME =',E12.4)
DO 5 I=1,NP
T(I)= T(I)-TIMEL
IF(C(I).GT.0.) GO TO 5
WRITE(6,72)
72 FORM AT(1H 'STOP,NEGATIVE CONCENTRATION')
GO TO 622
5 CONTINUE
IF(NP.LT.5) STOP
C REGRESSION ANALYSIS FOR LAST THIRD OF POINTS
NSUM= NP/3
IF(NSUM.LT.3) NSUM=3
NST = NP-NSUM+ 1
CALL REGR(1,NSUM,NST,A,B)
BETA= -B
IF(BETA.GT.1.E-6) GO TO 620
WRITE(6,621)
WRITE(4,621)
621 FORMAT(4H BB=,E12.4,5X,BETA=,E12.4)
DO 73 I=1,NP
73 E2(I)= BB* EXP(-BETA*T(I))
C DETERMINE AREA UNDER C,T PLOT
AREA= 0.

202
NM1 = NP-1
DO 19 I = 1, NM1
19 AREA = AREA + (C(I) + C(I+1)) * (T(I+1) - T(I))/2.
AREA = AREA + C(I) * T(I)/2. + C(NP)/BETA
WRITE(6,20) AREA
WRITE(4,20) AREA
20 FORMAT(21H AREA UNDER C,T P L O T = £ 18.4)
CALL CMAX
TMAX = TM + TIMEL
WRITE(6,201) CM, TMAX, TGM
WRITE(4,201) CM, TMAX, TGM
201 FORMAT(1H 'CMAX = ', E12.4, 4X, 'TIME OF CMAX AFTER DOSE = ',
1E12.4/1H, 'AUC TO CMAX = ', E12.4)
VKA = 1.5/TM
IF(NPAR.EQ.3) GOTO 9000
WRITE(6,1000)
WRITE(4,1000)
1000 FORM AT(1H ' KIT', 5X, 'VK1', 6X, 'ALPHA', 3X, 'BETA', 4X,
9000 IF(TM.LT.T(NP-2)) GOTO 50
WRITE(6,777)
WRITE(4,777)
777 FORMAT(1H ' INSUFFICIENT DATA AT LATE TIMES')
GO TO 622
50 CALL CIV(LIMBET)
IF(ITER.EQ.20) GOTO 556
IF(ITER.EQ.99) GOTO 622
KIT = KIT+1
IF(KIT.GT.KITC) GOTO 555
VKA1 = VKA
IF(ABS(VKA2-VKA1)/VKA2 .LT. 0.01) GOTO 555
VKA2 = VKA1
GO TO 50
555 WRITE(6,15) VKA, ALPHA, BETA, AA, BB
WRITE(4,15) VKA, ALPHA, BETA, AA, BB
15 FORMAT(1H ' FINAL VALUES OF RATE CONSTANTS / BI-EXPONENTIAL /
1 ' VKA = ', E12.4, 6X, 'ALPHA = ', E12.4, 6X, 'BETA = ', E12.4/
2 ' AA = ', E12.4, 7X, 'BB = ', E12.4)
556 WRITE(6,149)
WRITE(4,149)
149 FORMAT(1H ' CALCULATED VALUES FOR ORIGINAL DATA ' /
2 14X,4HT(I), 11X, 7HCCAL(I), 14X, 4HC(I))
DIF = 0.
DO 210 I = 1, NP
IF(ITER.EQ.5.OR.JITER.EQ.40) GOTO 25
V(I) = BB * (EXP(-BETA*T(I)) - EXP(-VK1*T(I)))
GO TO 26
25 V(I) = AA * EXP(-ALPHA*T(I)) + E2(I) -(AA + BB) * EXP(-VK1*T(I))
26 DIF = DIF + (V(I) - C(I))**2
WRITE(6,153) T(I), V(I), C(I)
WRITE(4,153) T(I), V(I), C(I)
210 CONTINUE
153 FORMAT(1H , 3F18.4)
WRITE(6,155) DIF
155
WRITE(4,155) DIF
155 FORMAT(1H,'SUMSQ=',E18.4)
622 CONTINUE
   IF(NSET.EQ.0) GO TO 90
   NTAP= NTAP+1
   IF(NTAP.GE.NSET) GO TO 110
   GO TO 2000
110 STOP
   END
SUBROUTINE GETLAG(SLAG)
   COMMON NP,XO,T(50),C(50),NPAR,TC(100)
   A2=((C(1)-C(2))*(T(2)-T(3))-(C(2)-C(3))*(T(1)-T(2)))/
   ((T(1)-T(3))*(T(1)-T(2))*(T(2)-T(3)))
   A1=(C(1)-C(2))/(T(1)-T(2))-A2*(T(1)+T(2))
   AO=C(1)-A1*T(1)-A2*T(1)**2
   TLAG= 0.
   DO 11=1,50
      FT= AO+ A1*TLAG+ A2*TLAG**2
      DFT= A1+ 2.*A2*TLAG
      TNEW= TLAG-FT/DFT
      IF(ABS(TLAG-TNEW).LT. 1.E-4) GO TO 2
   1   TLAG= TNEW
   2   SLAG= TNEW
   RETURN
   END
SUBROUTINE CIV(LIMBET)
   COMMON NP,XO,T(50),C(50),NPAR,TC(100)
   COMMON/BLOCKD/CM,TM,IX,AREA,VKA,JITER,KIT,TGM
   COMMON/BLOCKG/ AA 3  B ,ALPHA,BET A, V K 12, VK21, VKEL
   COMMON/CONTR/ IS 1  ,IS2,IS3,IPP,NITJESUM,KITC
   DIMENSION P(5),PO(5),P1(5)
   IF(JITER.EQ.20) GO TO 101
   IF(NPAR.EQ.3)GOTO 101
   CALL VMIN
   IF(JITER.EQ.20) GO TO 101
   1002 CALL BIORAL
   C THIS WRITE TO CHANNEL 4 INCLUDED BY STEVE
   WRITE(4,2000)KIT,VKA,ALPHA,BETA,AA,BB,VK12,VK21,VKEL
   WRITE(6,2000)KIT,VKA,ALPHA,BETA,AA,BB,VK12,VK21,VKEL
   2000 FORMAT(I3,2X,3(2XJF6.3),2(1X,E9.3),F6.3,2(1XJ7 6.3))
   IF(JITER.NE.20) GO TO 299
   101 JITER= 20
   1001 WRITE(6,45)
   45 FORMAT(33H CONTINUED WITH MONO-EXPONENTIAL )
   26 CONTINUE
   JITER=20
   VKA= 1.5/TM
   C PREPARE ARRAYS FOR LEAST SQUARES
   P(1)= BB
   P(2)= BETA
   P(3)= VKA
   DO 30 I=1,NPAR
   P(I)= 0.0
30 P1(I) = 1.E20
   IF(LIMBET.NE.1)GOTO 50
   P0(1) = 0.95*BB
   P1(1) = 1.05*BB
   P0(2) = 0.95*BETA
   P1(2) = 1.05*BETA
   WRITE(6,102)
   WRITE(4,102)
102 FORMAT(1H,'BB AND BETA RESTRICTED TO 5 PERCENT VARIATION')
50 J=0
   DO 3020 I=1,NP
      J=J+1
      TC(J)= T(I)
      J=J+1
      TC(J)= C(I)
   3020 CONTINUE
   NPW= NP
   CALL MINV(2,2,NPW,3,'0.0 ','0.0 ',ITR,1,0,'0 ',-1,
   1P,P0,P1,TC)
   BB= P(1)
   BETA= P(2)
   VKA= P(3)
   IF(VKA.LE.0.) JITER=99
   WRITE(6,25) BB,BETA,VKA
   WRITE(4,25) BB,BETA,VKA
   AA= 0.
   ALPHA= 0.
25 FORMAT(1H,'FINAL VALUES OF RATE CONSTANTS '/
   16H MONOEXPONENTIAL/4H BB=,E12.4/ 6H BETA=,E12.4/
   15H VKA=,E12.4)
299 RETURN
END
SUBROUTINE VMIN
COMMON NP,XO,T(50),C(50),NPAR,TC(100)
COMMON/BLOCKD/CM,TM,IX,AREA,VKA,JITER,KIT,TGM
COMMON/BLOCKG/ AA,BB,ALPHA,BETA,VK12,VK21,VREL
IF(ALPHA.LE.O.) GO TO 77
IF(KIT.GT.0) GO TO 7
77 ALPHA = (VKA + BETA)/2.0
   IF((VKA-ALPHA).LT..01) ALPHA=VKA-.01
   ATM= EXP(-ALPHA*TM)
   BTM= EXP(-BETA*TM)
   AA=(CM*VKA-BB*BTM*(VKA-BETA))/(ATM*(VKA-ALPHA))
   DO 101=1,50
      D= AA+ BB
      VKTM= VKA*TM
      IF(VKTM.GT.200.) GO TO 4
      SLM= VKA*D*EXP(-VKA*TM)-ALPHA*AA*EXP(-ALPHA*TM)-BETA*BB*EXP(-BETA*TM)
   101
   DSLM=(1.-VKA*TM)*(AA+BB)*EXP(-VKA*TM)
   IF(ABS(DSLM).LT.0.1) DSLM= -0.1
   IF(DSLM.GT.0.) DSLM= -DSLMS
   VREW= VKA- SLM/DSLMS
   IF(VREW.LE.0.) VREW= 1.5/TM
7  IF((VKA-ALPHA).LT..01) ALPHAt=VKA-.01
   ATM= EXP(-ALPHA*TM)
   BTM= EXP(-BETA*TM)
   AA=(CM*VKA-BB*BTM*(VKA-BETA))/(ATM*(VKA-ALPHA))
   DO 101=1,50
      D= AA+ BB
      VKTM= VKA*TM
      IF(VKTM.GT.200.) GO TO 4
      SLM= VKA*D*EXP(-VKA*TM)-ALPHA*AA*EXP(-ALPHA*TM)-BETA*BB*EXP(-BETA*TM)
   101
   DSLM=(1.-VKA*TM)*(AA+BB)*EXP(-VKA*TM)
   IF(ABS(DSLM).LT.0.1) DSLM= -0.1
   IF(DSLM.GT.0.) DSLM= -DSLMS
   VREW= VKA- SLM/DSLMS
   IF(VREW.LE.0.) VREW= 1.5/TM
205
IF(ABS(SLM).LT.1.E-4) GO TO 3
10 VKA= VREW
3 VKA= VREW
IF((VKA-ALPHA).LT.0.01)ALPHA=VKA-0.01
RETURN
4 JITER= 20
RETURN
END
SUBROUTINE BIORAL
COMMON NP,X0,T(50),C(50),NPAR,TC(100)
COMMON/BLOCKD/ CM,TM,IX,AREA,VKA,JITER,KIT,TGM
COMMON/BLOCKG/ AA,BB,ALPHA3ETA,VK12,VK21,VKEL
DO 12 J= 1,50
ATM= EXP(-ALPHA*TM)
BTM= EXP(-BETA*TM)
VKTM= EXP(-VKA*TM)
FALF=AA*(1.-ATM)/ALPHA+BB*(1.-BTM)/BETA-(AA+BB)*(1.-VKTM)/VKA-TGM
DFAL=AA*(TM*ATM-(1.-ATM)/ALPHA)/ALPHA
ANEW=ALPHA-FALF/DFAL
IF((ABS(ALPHA-ANEW).LT.1.E-4) GO TO 15
12 VKA= ANEW
15 VKA= ANEW
IF((VKA-ALPHA).LT.0.01)ALPHA=VKA-0.01
IF(ALPHA.GT.0.) GO TO 77
WRITE(6,23)
23 FORMAT(' ALPHA FROM REGRESSION')
call VMIN
77 JITER= 5
VKEL=(AA*(VKA-ALPHA)+BB*(VKA-BETA))/(VKA*AREA)
VK21= ALPHA+ BETA- VK21- VKEL
IF(KIT.EQ.0) GOTO 21
IF(VKEL.LE.0. OR. VK21.LE.0. OR. VK12.LE.0.) JITER= 40
AR=EXP(-BETA*TM)-0.9*CM/BB
IF(AR.GT.0.)JITER=20
IF(JITER.NE.40)GOTO 21
WRITE(6,50)
WRITE(4,50)
50 FORMAT(1H ,MAXPT ABOVE REGR BUT A RATE CONSTANT -VE')
21 RETURN
END
SUBROUTINE REGR(M,N,NST,A,B)
COMMON NP,X0,T(50),C(50),NPAR,TC(100)
C NST= THE FIRST POINT IN ARRAY
KI= NST-1
SUMX=0.0
SUMY=0.0
XSQ=0.0
YSQ=0.0
TS=0.0
XN=0.0
C SUMX=SUMY=XSQ=YSQ=TS=XN=0.0
DO 1 I=1,N
KP= KI+1

X=T(KP)
Y=ALOG(C(KP))
SUMX=SUMX+X
XSQ=XSQ+X**2
SUMY=SUMY+Y
YSQ=YSQ+Y**2
TS=TS+X*Y
XN=XN+1.0
1 CONTINUE
Q=XN*TS-SUMX*SUMY
D=XN*XSQ-SUMX**2
B=Q/D
A=(SUMY-B*SUMX)/XN
RETURN
END
SUBROUTINE CMAX
COMMON NP,XO,T(50),C(50),NPAR,TC(100)
COMMON/BLOCKD/CM,TM,IX,AREA,VKAJITER,KIT,TGM
XM=-10.E10
DO 11=1,NP
IF(XM.GT.C(I)) GO TO 1
XM=C(I)
IX=I
1 CONTINUE
IF(XM.EQ.C(1)) GO TO 10
K=1
L=1
IF(IX.LE.2) GO TO 15
IF(C(IX-2).GT.C(IX-1)) K=2
15 IST=NP-IX
IF(IST.LE.2) GO TO 17
IF(C(IX+2).GT.C(IX+1)) L=2
17 P1=T(I-K)
E6=C(I-K)
GO TO 20
10 CM=C(1)
TM=T(1)
TGM=0.5*C(1)*T(1)
WRITE(6,40)
WRITE(4,40)
40 FORMAT(1H,'FIRST POINT TAKEN AS MAXIMUM')
GOTO 50
20 E7=XM
E3=C(I+L)
P2=T(I)
P3=T(I+L)
TM=(E6*(P3**2-P2**2)+E7*(P1**2-P3**2)+E3*(P2**2-P1**2))/
(2.*(E6*(P3-P2)+E7*(P1-P3)+E3*(P2-P1)))
IF(P2.NE.TM) GO TO 30
TM=P2
CM=E7
GO TO 31
30 R=((P1-TM)/(P2-TM))**2
CM=(E6-R*E7)/(1.-R)
31 TGM= 0
32 N1= IX-1
DO 3 I=1,N1
 33 TGM=TGM+(C(I)+C(I+1))*(T(I+1)-T(I))*0.5
3 CONTINUE
34 TGM= TGM+ 0.5* C(1)* T(1)
35 TGM=TGM+ 0.5* (C(IX)+ CM)* (TM-T(IX))
50 RETURN
END

SUBROUTINE FUN (F,J,D,P,X)
DIMENSION D(3),P(3),X(1)
31 TA= (-P(2)*X(1))
32 IF(TA.GT.-1.E-3 ) GO TO 15
33 IF(TA .LT.-1.E+2) GO TO 17
34 GO TO 10
15 E2= 1.
36 GO TO 20
17 E2= 0.
37 GO TO 20
10 E2= EXP(TA)
20 TB= (-P(3)*X(1))
21 IF(TB.GT.-1.E-3 ) GO TO 25
22 IF(TB.LT.-1.E+2) GO TO 27
23 GO TO 30
25 E3= 1.
26 GO TO 40
27 E3= 0.
28 GO TO 40
30 E3= EXP(TB)
40 F= P(1)*(E2-E3)
31 D (1)= E2-E3
32 D(2)= -P(1)*X(1)*E2
33 D(3)= P(1)*X(1)*E3
RETURN
END
SUBROUTINE MINV(NV,ND,NC,NP,TO,EP,MXIT,NF,IT,RE,IW,P,P0,PI,A)
COMMON/CONTR/ IS 1,IS2,IS3,IPP,NIT,ESUM,KITC
DIMENSION A(40),P(5),P0(5),P1(5)
CHARACTER*6 TO,EP,PPJ>C
CHARACTER*4 F(40)
CHARACTER*10 MT(8)
INTEGER DATE(4)
CHARACTER*6 GUESS,PROBLM,FINISH,AMON,AMOX
CHARACTER*6 YES,ONO,RE
DATA YES,ONO,GUESS,PROBLM,FINISH,AMON,AMOX/3HYES,2HN0,5HPARAM,6HPR
XOBLM,6HPHINISH,6HMINMUM,6HMAXMUM/
31 LLK=0
32 IF(IS1.EQ .l) G O T O  16
15 READ(5,1)PP,PC,NV,ND,NC,NP,TO,EP,MXIT,NF,IT,RE,IW
1 FORMAT(2A6,2I4,I6,I4,2A6,3I4,A3,I4)
16 IF(NP.LE.0.OR.NP.GT.100)GO TO 700-
17 IF(ND.LE.0.OR.ND.GT.NV)GO TO 800
33 LLK=LLK+1
34 DECODE(6,500,TO) TOL
IF(TOL.EQ.0.0) TOL = 0.00001
DECODE(6,500,EP) EPS
IF(EPS.EQ.0.0) EPS = 0.00001

500 FORMAT(E6.0)
IF (MXIT.LT.1) MXIT=100
IF(KITC.GT.MXIT)MXIT=KITC
IF(IW.LT.0.OR.IW.GT.NV)IW=0
TOL=ABS(TOL)
IF(RE.NE.YES) RE=ONO
IF(IT.EQ.0) IT=5
IF(RE.EQ.YES) REWIND IT
NF=MAX0(1,NF)
NF=MIN0(NF,9)
1070 IF(IS2.EQ.1) GO TO 11
NF = NF * 18
READ (5,2) (F(I),I=1,NF)
2 FORMAT (18A4)
WRITE (6,1002) (F a),1=1 W
1002 FORMAT(16H VARIABLE FORMAT, 16X,24A4/(32X,24A4))
11 J=1
IF(IS2.EQ.1) GO TO 1000
DO 3 L=1,NC
READ (IT,F) (P(I),I=1,NV)
DO 3 I=1,NV
A(J)=P a)
3 J=J+1
1000 NP1=NP+1
L1=1+NV*NC
L2=L1+NP1*NP1
IF(L2.GT.15000)GO TO 600
LJ=0
IF(IS3.EQ.5) GO TO 1700
IF(IS3.EQ.1) GO TO 1700
IF(IS3.EQ.3)GOTO 1700
81 READ(5,25)MT
25 FORMAT(8A10)
DECODE(6,26,MT) PP
26 FORMAT(A6)
IF(PP.EQ.AMON) DECODE (72,12,MT) (P0(I),I=1,NP)
IF(PP.EQ.AMOX) DECODE (72,12,MT) (P1(I),I=1,NP)
IF(IS3.EQ.2) GO TO 1700
IF(PP.EQ.PROBLM) GO TO 22
IF(PP.EQ.FINISH) GO TO 24
IF(PP.EQ.GUESS) GO TO 18
IF(PP.EQ.AMON.OR.PP.EQ.AMOX) GO TO 81
WRITE(6,23) MT
23 FORMAT(' NON CONTROL CARD (',8A10,') ')
GO TO 81
22 DECODE(61,1,MT) PP,PC,NV,ND,NC,NP,TO,EP,MXIT,NF,IT,RE,IW
GO TO 16
24 RETURN
18 DECODE(72,12,MT) (P(I),I=1,NP)
12 FORMAT(6X,11F6.0)
   CALL MINIZ(A,A(L1),P,NV,NC,NP1,TO,EPS,EE,ND,MXIT,LLK,P0,P1,IW)
GO TO 81
600 WRITE(6,601)
601 FORMAT(' TOTAL AMOUNT OF DATA, EXCEEDS 1500')
602 GO TO 24
700 WRITE(6,701)
701 FORMAT(' NUMBER OF PARAMETERS EXCEEDS 100')
GO TO 602
800 WRITE(6,801)
801 FORMAT(' INDEX OF DEPENDENT VARIABLE EXCEEDS NUMBER OF VARIABLES')
GO TO 602
1700 CONTINUE
   CALL MINIZ(A,A(L1),P,NV,NC,NP1,TOL,EE,ND,MXIT,LLK,P0,P1,IW)
RETURN
END
SUBROUTINE MINIZ(X,A,P,NV,NC,NP1,TOL,EE,ND,MXIT,LLK,P0,P1,IW)
COMMON/CONTR/ IS1,IS2,IS3,IPP,NIT,ESUM,KITC
DIMENSION A(NP1,NP1),X(NV,NC),P(5),FP(5),D(5),P0(5),P1(5)
1),B(10)
DIMENSION PP0(5)
IF(IW.EQ.0)C=1.0
NP=NP1-1
MNAIL = 10
NAIL = 0
DO 557 I=1,NP
557 PP0(I)=P(I)
EPS1=5.*EPS+1.
IF(IPP.EQ.1) GO TO 194
WRITE(6,195)
WRITE(4,195)
195 FORMAT(' ITERATION ERROR PARAMETERS'/14X,'MEAN'/14X,'SQUARE
1')
194 LLL=5
KLIT= 0
LIT=0
HU=NC
H=0.
LLLLL=5
E0=1.E20
DO 556 I=1,NP
556 P(I)=PP0(I)
FNC=NC-NP
29 DO 85 I=1,NP1
DO 85 J=1,1
85 A(I,J)=0.
DO 2 L=1,NC
C CALL FUN(F(L),FP,X(I,L),P,LLK)
   CALL FUN(Q,FP,P,X(I,L))
FP(NP1)=X(ND,L)-Q
IF(IW.NE.0)C=X(IW,L)
IF(C.LT.(1.E-20)) GO TO 2
DO 1 I=1,NP1
FPIC=FP(I)*C
DO 1 J=1,1
1 A(I,J)=A(I,J)+FPIC*FP(I)
240
CONTINUE
EE=A(NP1,NP1)/FNC
IF(EE.LE.E0) GO TO 871
NAIL=NAIL+1
IF(NAIL.GT.MNAIL) GO TO 871
DO 872 I=1,NP
P(I)=PP0(I)+D(I)/2.
872 D(I)=D(I)/2.
GO TO 29
IF (IPP.EQ.1) GO TO 1725
IF(LIT.LE.10) GO TO 701
KLIT= KLIT+1
IF(KLIT.LT.10) GO TO 1725
701 WRITE(6,198)LIT,NAIL,EE,(P(I),I=1,NP)
WRITE(4,198)LIT,NAIL,EE,(P(I),I=1,NP)
198 FORMAT(I6,1X,I2,1X,IP 10E12.4/(22X,1P9E12.4))
KLIT= 0
1725 NAIL=0
8191 LIT= LIT+1
CALL STEP(A,D,P0,P1,NP1,TOL)
IF(LIT.GT.MXIT) GO TO 200
IF(EE.EQ.O.) GO TO 470
PC=ABS((E0-EE)/EE)
E0=EE
IF(PC.LT.EPS) GO TO 100
LLL=LLLL
121 DO 28 I=1,NP
PP0(I)=P(I)
28 P(I)=P(I)+D(I)
GO TO 29
200 WRITE (6,201)
WRITE(4,201)
201 FORMAT(30H THE PROCESS IS NOT CONVERGING)
GO TO 470
100 LLL= LLL-1
IF(LLL.NE.0) GO TO 121
470 DO 778 I=1,NP
778 FP(I)=SQRT(ABS(A(I,I)*EE))
IF(IPP.EQ.0) GO TO 1700
IF(KLIT.EQ.0) GO TO 705
WRITE(6,198)LIT,NAIL,EE,(P(I),I=1,NP)
WRITE(4,198)LIT,NAIL,EE,(P(I),I=1,NP)
WRITE(6,198)LIT,NAIL,EE,(P(I),I=1,NP)
705 WRITE(6,1710) LIT,EE
WRITE(4,1710) LIT,EE
1710 FORMAT(1H ,NON LINEAR LEAST SQUARES'/'
1 1H ,TIR='15,5X,'SUMSQ='1E16.6)
1700 WRITE (6,779) (FP(I),I=1,NP)
WRITE(4,779) (FP(I),I=1,NP)
779 FORMAT(' ASYMPTOTIC STANDARD DEVIATIONS OF THE PARAMETERS'//
X(10X,1P10E12.4))
DO 841 I=1,NP
FP(I)=SQRT(ABS(A(I,I)))
DO 841 J=1,I
841 A(I,J)=A(I,J)
WRITE(6,774)
774 FORMAT(' ASYMPTOTIC CORRELATION MATRIX OF THE PARAMETERS')
L1=0
371 L0=L1+1
L1=MIN0(NP,L1+10)
WRITE(6,372)(L,L=L0,L1)
372 FORMAT(' ;',10I12)
DO 842 I=1,NP
K=0
DO 373 J=L0,L1
K=K+1
IF(FP(I).EQ.0.0.OR.FP(J).EQ.0.0.AND.A(I,J).EQ.0.0)GO TO 843
B(K)=A(I,J)/(FP(I)*FP(J))
GO TO 373
843 B(K)=0.0
373 CONTINUE
842 WRITE(6,374)(L,L=1,K)
374 FORMAT(I9 ,10F12.5)
IF(L1.NE.NP)GO TO 371
1515 WRITE(6,553)
553 FORMAT(' CASE F Y-F STANDARD VARIABLES
1/30X,'DEVIATION'/30X,'OF ESTIMATE')
DO 551 L=1,NC
SDEST=0.0
CALL FUN(Q,JFP,JP,X(1JL))
DO 24 I=1,JP
DO 24 J=1,NP
24 SDEST=SDEST+FP(I)*FP(J)*A(I,J)
SDEST=SQRT(SDEST*EE)
TT=X(ND,L)-Q
551 WRITE(6,552)L,Q,TT,SDEST,(X(I,L),I=1,NV)
552 FORMAT(1X,I4,10F12.5/(41X,7F12.5))
NIT=LIT
ESUM=EE
RETURN
END
SUBROUTINE STEP(A,D,P,P0,P1,NP1,TOL)
DIMENSION A(NP1,NP1),V(40),D(5),IN(5),P0(5),P1(5),P(5)
NP=NP1-1
DO 20 I=1,NP
V(I)=SQRT(A(I,I))
DO 20 J=1,NP
20 IF(V(I)*V(J).EQ.0.)GO TO 212
A(I,J)=A(I,J)/(V(I)*V(J))
GO TO 20
212 A(I,J)=0.
20 CONTINUE
DO 2 I=1,NP
IN(I)=0
2  K=0
R=0.
DO 1 I=1,NP
IF(IN(I).NE.0 .OR. A(I,J).LE.TOL)GO TO 1
Q=A(NP1,I)*A(NP1,I)/A(I,J)
IF(Q.LE.R) GO TO 1
K=I
R=Q
1 CONTINUE
IF(K.EQ.0) GO TO 3
C=-1.
4 DO 5 I=1,K
D(I)=A(K,I)
5 A(K,I)=0.
PP=D(K)
DO 6 I=K,NP1
D(I)=A(I,K)
6 A(I,K)=0.
D(K)=C
IN(K)=IN(K)+1
DO 7 J=1,NP1
IF (PP.EQ.0..OR.D(I).EQ.0.) GO TO 24
Y=D(I)/PP
GO TO 25
24 Y=0.
25 CONTINUE
DO 7 J=1,I
7 A(I,J)=A(I,J)-D(J)*Y
GO TO 8
3 R=1.
DO 9 I=1,NP
IF(IN(I).NE.1) GO TO 9
IF (V(I).EQ.0.) GO TO 15
D(I)=A(NP1,I)*V(NP1)/V(I)
16 IF (D(I).EQ.0.) GO TO 17
H=AMAX1((P0(I)-P(I))/D(I),(P1(I)-P(I))/D(I))
GO TO 18
15 D(I)=0.0
17 H=0.
18 CONTINUE
IF(H.GE.R) GO TO 9
R=H
K=I
9 CONTINUE
C=1.
IF(R.LE.TOL) GO TO 4
DO 10 I=1,NP
IF(IN(I).NE.1) GO TO 11
D(I)=D(I)*R
DO 21 J=1,I
IF ((V(I)*V(J)).EQ.0.0) GO TO 30
A(I,J)=-A(I,J)/(V(I)*V(J))
GO TO 21
30 A(I,J)=0.
21 A(I,J)=A(I,J)
GO TO 10
11 D(I)=0.
DO 22 J=1,NP
A(I,J)=0.
22 A(J,J)=0.
10 CONTINUE
   RETURN
END
REFERENCES


1481-1483


588-589


Bajeva, S. K., Ranga Roa, K. V. & Kumar, Y (1986) J. Microencapsulation,
3: 33-37

Medicine and Biology’ Vol. 47, A. C. Tanquary & R. E. Lacey (eds.). Plenum
Press, New York p15-71

Wasserman, G. F., Diggs, C. L., Hoffman, S. L., Hollingdale, M. R.,
Hockmeyer, W. T., Schneider, I., Young, J. F., Reeve, P., Chulay, J. D. (1987)
Lancet, 1: 1277-1281

Bardgett, D., Howard, C., Murray, G. R., Calvey, T. N., Williams, N. E.

New York. p2


217


218


219


Farhadieh, B (1975) U. S. Patent, 3,922,379


Flory, P. J. (1963) J. Amer. Chem. Soc. 63: 3083, 3091, 3096


221


222


223


Koff, A. (1964) U. S. Patent, 1,138,525


224


Morawitz, H. & Gaetjens, E. (1958) J. Polymer Sci. 32: 526

225


226


228
Seigel, S., Pettebone, R. H. & Hanus, E. J. (1962) U. S. Patent, 3,070,508


Yapel, A. F. (1979) U. S. Patent, 4,147,767


Yoshida, H., Uesugi, T & Noro, S (1980) Yakugaku Zasshi, 100: 1203-1214