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DEVELOPMENT OF LIQUID FORMULATIONS FOR TARGETED DRUG DELIVERY TO THE OESOPHAGUS

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Doctor of Philosophy

ASTON UNIVERSITY

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Aston University

Development of liquid formulations for targeted drug delivery to the oesophagus

Danielle Geneviève Rebecca Russell

Doctor of Philosophy

2006

The diseases of the oesophagus range from the common (gastro-oesophageal reflux disease) to the less common but nevertheless severe (achalasia, carcinoma). Current treatments for these diseases predominantly entail surgical intervention or systemic pharmacological therapies. The novel therapeutic strategy proposed is an oral liquid with retentive properties. This drug delivery system would be a bioadhesive vehicle that maintains prolonged contact with the oesophageal epithelium, withstanding peristalsis. During prolonged retention time, drug is released from the vehicle and absorbed into the site of action. Alternatively a retentive liquid may be useful as a protective 'bandage' on the oesophagus. Few studies have investigated the oesophagus as a site of bioadhesion. Advantages of oesophageal targeting include treatment of local conditions, avoidance of first pass metabolism and inactivation by lower gastrointestinal pH, and the convenient oral delivery route.

This study investigated the retention of diverse liquids on *ex vivo* porcine oesophagus, and the rate of drug release from the carriers. Biphasic clearance from oesophageal tissue was identified, in agreement with previous studies. Drug release from most formulations was linear over the relevant time period, and of immediate onset, with no lag phase. Promising formulations were tested *in vivo* for their retention, assessed by γ-scintigraphy, and drug effects on oesophageal motor function, assessed manometrically. A temperature-sensitive polymer exhibited exceptional retention *in vivo*. Other promising formulations were oil-in-water emulsions, which exhibited prolonged retention *in vitro* and *in vivo*, and had rapid linear drug release. Polycarbophil was poorly retained in the *in vitro* model, a result confirmed by other literature methods. Some formulations were shown to have a pharmacological effect on oesophageal motility in humans, indicating their potential benefits in treating oesophageal motor disorders. The *in vivo* studies in healthy humans confirmed the validity of a targeted drug delivery strategy in affecting oesophageal motility.

Keywords: oesophagus, bioadhesion, targeting, γ-scintigraphy, gastro-oesophageal reflux, oesophageal motility disorders

Dedication

To my parents, for giving me so many opportunities.

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The *in vivo* studies were designed and carried out at St. Mark's Hospital, Harrow, Middlesex, by Dr. Chris Rayner, Dr. Chris Jordan, Prof. Michael Kamm, Dr. Anton Emmanuel and Brian Leaker. *In vivo* γ- scintigraphy studies were performed by radiographers Neil Barron and Greg Morris, and research fellow Dr. Chris Rayner and *in vivo* manometry was performed by Dr. Chris Rayner at the Physiology Unit, St. Mark's Hospital, Harrow, Middlesex.

The *in vivo* work also involved the input of Dr Chris Jordan (Pharmacologist), Dr Anton Emmanuel (Senior Lecturer), Ms Deepa Solanki (Clinical Scientist), Mr Greg Morris (Senior Scientific Officer in Nuclear Medicine), Mr Uday Bhonsle (Consultant Physicist), Prof Steve Mather (Department of Nuclear Medicine, St. Bartholemew's Hospital), Prof Keith Britton (Department of Nuclear Medicine, St. Bartholemew's Hospital), and Prof Michael Kamm (Directro, Physiology group).

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List of Contents

DEDICATION 3

ACKNOWLEDGMENTS 4

LIST OF CONTENTS 5

LIST OF FIGURES 11

LIST OF TABLES 14

LIST OF ABBREVIATIONS USED IN THE TEXT 15

CHAPTER 1 - INTRODUCTION 18

THE OESOPHAGUS 18

1.1 THE HEALTHY OESOPHAGUS 18

1.1.1 Oesophageal physiology and histology 18

1.1.2 Neurophysiology 21

1.1.3 Control of swallowing and peristalsis 22

1.1.4 Normal Swallowing and Peristalsis 22

1.1.5 Lower Oesophageal Sphincter 22

1.2. DISEASE STATES OF THE OESOPHAGUS - NON-MOTILITY DISORDERS 24

1.2.1 Gastro-oesophageal Reflux Disease 24

1.2.2 Laryngoopharyngeal reflux 29

1.2.3 Barrett's Oesophagus 29

1.2.4 Summary of current therapies for GORD and Barrett's Oesophagus 30

1.2.5 Oesophageal Cancer 32

1.3 DISEASE STATES OF THE OESOPHAGUS - MOTILITY DISORDERS 34

1.3.1 Achalasia 34

1.3.2 Diffuse Oesophageal Spasm 39

1.3.3 Nutcracker Oesophagus 40

1.3.4 Isolated hypertensive LOS 40

1.3.5 Treatment of spastic disorders 40

1.4 LIMITATIONS OF CURRENT THERAPIES FOR OESOPHAGEAL DISORDERS 42

1.5 DRUGS OF INTEREST FOR THE PROPOSED DELIVERY SYSTEM 43

1.5.1 Isosorbide dinitrate 43

1.5.2 Nifedipine 43

1.5.3 Sildenafil 44

1.5.4 Pharmacokinetics of candidate drugs 45

1.6 BIOADHESION 46

1.6.1 Theories of Bioadhesion 46

1.6.1.1 Interpenetration Theory 46
1.6.1.2 Electronic Theory 47
1.6.1.3 Wetting Theory 48
1.6.1.4 Adsorption Theory 48
1.6.2 Adverse bioadhesion within the oesophagus 48
1.6.3 Beneficial bioadhesion within the oesophagus 49
1.6.4 Bioadhesives 50
1.6.5 Measurement of bioadhesion in vitro 52
1.6.6 Measurement of oesophageal transit in vivo 52
1.7 RATIONALE FOR A BIOADHESIVE LIQUID DELIVERY SYSTEM TARGETED TO THE OESOPHAGUS . 53
1.8 THE AIM OF THIS STUDY 54

CHAPTER 2 - MATERIALS AND METHODS **57**

MATERIALS 57
2.1 MATERIAL SOURCES 57
METHODS 58
2.2. RHEOMETRY 58
2.2.1 Elastic and Viscous Moduli 59
2.2.2 Shear Methods 59
2.2.3 Oscillatory Methods 60
2.3 SURFACE TENSION 60
2.4 HPLC ANALYSIS 61
2.4.1 HPLC Methods 61
2.5 METHODS FOR QUANTIFYING RADIOACTIVITY 62
2.5.1 Gamma (γ) counting technique 62
2.5.2 Scintillation (β-counting) techniques 63
2.6 EXTRACTION OF SILDENAFIL FROM VIAGRA® TABLETS 63
2.7 PREPARATION OF OIL IN WATER EMULSIONS 64
2.8 PREPARATION OF THE SILICONE-BASED EMULSION 65
2.9 METHOD OF PREPARATION OF POLYMER SOLUTIONS 65
2.10 PREPARATION OF BLENDS OF BIOADHESIVE POLYMERS 66
2.11 PREPARATION OF DRUG-LOADED FORMULATIONS FOR FRANZ CELL EXPERIMENTS 66
2.11.1 Preparation of emulsions containing dextran, mannitol and propranolol 66
2.11.2 Preparation of emulsions containing isosorbide dinitrate, nifedipine and sildenafil 67
2.11.3 Preparation of polymer formulations containing dextran, mannitol and propranolol .. 68
2.11.4. Preparation of polymer formulations containing isosorbide dinitrate, nifedipine and sildenafil 68
2.12 PREPARATION OF CONTROLS FOR FRANZ CELL EXPERIMENTS 69
2.12.1. Preparation of controls containing dextran, mannitol and propranolol 69

2.12.2. Preparation of controls containing isosorbide dinitrate, nifedipine and sildenafil 69
2.13 PREPARATION OF DRUG-LOADED FORMULATIONS FOR RETENTION AND CHARACTERISATION STUDIES 70
2.13.1 Preparation of emulsions containing dextran, mannitol and propranolol 70
2.13.2 Preparation of emulsions containing isosorbide dinitrate, nifedipine and sildenafil 70
2.13.3 Preparation of polymer formulations containing dextran, mannitol and propranolol .. 70
2.13.4. Preparation of polymer formulations containing isosorbide dinitrate, nifedipine and sildenafil 70
2.14 STATISTICAL ANALYSIS OF RESULTS 71

CHAPTER 3 - PREFORMULATION STUDIES **73**

3.1 EMULSION- BASED FORMULATIONS 73
3.1.1 Selection of oil and water emulsions 73
3.1.2 Emulsion preparation at varying speed 74
3.1.3 Characterisation of the oil and water emulsions 75
3.1.4 Stability of silicone-based emulsion 75
3.2 POLYMER- BASED FORMULATIONS 76
3.2.1 Polycarbophil (Noveon AA1®) solutions 76
3.2.2 Alginate solution 76
3.2.3 Poloxamer 407 (Pluronic Lutrol 127NF) 77
3.2.4 Smart Hydrogel® 77
3.3. RHEOLOGY 78
3.3.1 Smart Hydrogel® rheology – effects of shear 78
3.3.2 Smart Hydrogel® rheology – effects of temperature 80
3.3.3 Smart Hydrogel® rheology – effects of drug incorporation 82
3.3.4 Poloxamer rheology 83
3.3.5 Noveon AA1® rheology 84
3.3.6 Blend A rheology 86
3.3.7 Blend B rheology 87
3.3.8 HMO emulsion rheology 88
3.3.9 LMO Emulsion rheology 90
3.3.10 Glycerol rheology 92
3.3.11 Sucralfate rheology 93
3.3.12 Summary of rheological findings 94
3.4 EFFECT OF EMULSIFICATION SPEED ON VISCOSITY OF O/W EMULSIONS 94
3.5 SURFACE TENSION 95
3.6 PROPERTIES OF CANDIDATE DRUGS 96
3.6.1 Isosorbide dinitrate 96
3.6.2 Nifedipine 97

3.6.3 Sildenafil *97*
3.6.4 Summary tables of candidate drugs and model drugs *97*

CHAPTER 4 - THE DURATION OF LIQUID RETENTION ON OESOPHAGEAL TISSUE – *IN VITRO* ASSESSMENT **100**

INTRODUCTION 100
4.1 MODELS OF LIQUID RETENTION 100
4.1.1 Previous models for assessing retention *100*
4.1.2 Strengths of the current porcine in vitro model *102*
4.2 METHOD OF ASSESSMENT OF LIQUID RETENTION *IN VITRO* 103
4.3 VALIDATION OF TC99M AS A RADIOLABEL 105
4.3.1 Benefits over fluorescent labelling *105*
4.3.2. Homogeneity of radiolabel *106*
4.3.3. Stability of radiolabel *106*
4.4 RESULTS AND DISCUSSION OF *IN VITRO* RETENTION MODEL 107
4.4.1 Shape of retention profiles *107*
4.4.2 Retention profiles of formulations - percent retention and AUC data *108*
4.4.3 Fit of retention data to models *118*
4.4.4 Importance of viscoelastic properties in retention *119*
4.4.5 Importance of surface tension in retention *122*
4.4.6 Effect of Drug incorporation into vehicles *127*
4.5 CONCLUSIONS 130

CHAPTER 5 - DRUG RELEASE FROM LIQUID FORMULATIONS *IN VITRO* **133**

INTRODUCTION 133
5.1 FRANZ CELL ASSESSMENT OF DRUG RELEASE FROM A FORMULATION 133
5.2. FRANZ CELL PROCEDURE 134
5.3 DETERMINATION OF DRUG CONCENTRATION IN RECEIVER 137
5.3.1 ISDN/NIF/SIL analysis *137*
5.3.2 DEXT/MANN/PROP analysis *137*
5.4 DATA MANIPULATION: CALCULATING DRUG RELEASE FROM FRANZ CELL SAMPLES 137
5.4.1. Assessing drug release by HPLC *137*
5.4.2. Assessing drug release by measurement of radioactive counts *139*
5.5 RESULTS AND DISCUSSION 141
5.5.1 Shape of drug release profiles *141*
5.5.2 Effect of vehicle on drug release *143*
5.5.3 Steady state flux analysis *145*
5.5.4 Effects of pH on release from polymer solutions *146*

5.5.5 Release from emulsions *147*
5.5.7 Modelling drug release kinetics *148*
5.5.8 Relevance of amounts of drug released *150*
5.6. CONCLUSIONS 151

CHAPTER 6 - THE DURATION OF LIQUID RETENTION ON OESOPHAGEAL TISSUE –*IN VIVO* ASSESSMENT **153**

INTRODUCTION 153
6.1 METHODS FOR ASSESSING LIQUID RETENTION IN HEALTHY HUMAN SUBJECTS 153
6.1.1 Gamma Scintigraphy *153*
6.1.2 Study approval and volunteer recruitment *154*
6.1.3. Selection and preparation of formulations *154*
6.1.4 Assessment of liquid transit in vivo *155*
6.1.5 Rheometry *157*
6.2 RESULTS AND DISCUSSION 157
6.2.1. Tissue attenuation of radioactive counts *157*
6.2.2 Dynamic imaging data and comparison of formulations *158*
6.2.3. Regional differences in retention *161*
6.2.4. Correlation between in vitro and in vivo data *162*
6.2.5 Importance of viscosity for in vivo retention *164*
6.2.6. Adverse events *166*
6.3 DISCUSSION 166
6.4 CONCLUSIONS 168

CHAPTER 7 - THE EFFECTS OF POLYMER FORMULATIONS CONTAINING ISOSORBIDE DINITRATE OR SILDENAFIL ON OESOPHAGEAL MOTILITY IN HEALTHY VOLUNTEERS 170

7.1. INTRODUCTION 170
7.2 STUDY DESIGN 172
7.2.1 Subjects *172*
7.2.3 Ethical and safety considerations *172*
7.2.4 Preparation of test formulations *173*
7.2.5. Study order *173*
7.3 METHODS 174
7.3.1 Oesophageal manometry catheter *174*
7.3.2 Positioning of oesophageal manometry catheter *175*
7.3.3. Swallow sequences and formulation administration *175*
7.4 RESULTS 178

7.4.1 Cardioavascular effects and adverse events *178*
7.4.2 Effect of formulations on amplitude of peristaltic pressure waves *179*
7.4.3. Impact of administration route on the effect of sildenafil on wet swallows *182*
7.4.4. Effect of formulations on lower oesophageal sphincter pressure *184*
7.5 CONCLUSIONS 186

CHAPTER 8 - CONCLUSIONS 190

REFERENCES 195

APPENDICES

APPENDIX 2A – HPLC CALIBRATION CURVES 218

APPENDIX 3A - MEASURED SURFACE TENSIONS OF FORMULATIONS (MN/M) 218

APPENDIX 4A - VALIDATION OF USE OF TC99M - EXAMPLES OF HOMOGENEITY OF RADIOLABEL IN FORMULATIONS FOLLOWING 15 SECONDS VORTEXING 219

APPENDIX 4B - TABLE SHOWING MEAN PERCENT VEHICLE RETAINED *IN VITRO* ON PORCINE OESOPHAGEAL TISSUE OVER TIME – VEHICLES ONLY (NO DRUG) 220

APPENDIX 4C - TABLE SHOWING MEAN PERCENT FORMULATION RETAINED *IN VITRO* ON PORCINE OESOPHAGEAL TISSUE OVER TIME – DRUG-LOADED VEHICLES ONLY 222

APPENDIX 4D - TABLE SHOWING MEAN AUC OF RETENTION IN VITRO ON PORCINE OESOPHAGEAL TISSUE OVER TIME. DRUG-LOADED VEHICLES ONLY 224

APPENDIX 4E - USE OF SODIUM FLUORESCEIN AS A MARKER FOR BIOADHESIVE STUDIES 228

APPENDIX 5A - TABLES SHOWING CUMULATIVE DRUG RELEASE PER UNIT AREA OVER TIME FROM DIFFERENT FORMULATIONS (FRANZ CELL STUDIES) 229

APPENDIX 5B - TABLES SHOWING Y-INTERCEPTS FOR ALL LINEAR STEADY STATE FLUX GRAPHS BASED ON FIRST 60 MINUTES. 233

APPENDIX 5C - TABLES SHOWING CUMULATIVE PERCENT DRUG RELEASE OVER TIME FROM DIFFERENT FORMULATIONS (FRANZ CELL STUDIES) 235

APPENDIX 5D - STEADY STATE FLUX (MG CM^2 MIN^{-1}) FOR DRUG RELEASE FROM DIFFERENT FORMULATIONS, BASED ON THE FIRST 60 MIN OF EACH EXPERIMENT. 239

APPENDIX 5E – CORRELATION COEFFICIENTS FOR DRUG RELEASE MODELS 241

APPENDIX 5F - TABLE SHOWING PREDICTED TOTAL DRUG RELEASE (MG) OVER THE OESOPHAGEAL SURFACE AREA 244

APPPENDIX 6A – VOLUNTEER INFORMATION FOR IN VIVO RETENTION STUDY 246

List of Figures

Figure 1.1. Diagrammatic representation of a cross-section of human oesophagus, showing the layers present. 19
Figure 1.2 Histological transverse section of human oesophageal wall 21
Figure 1.3. The dilated oesophagus in achalasia, tapering to a narrow LOS, shown by X-ray of a barium swallow 36
Figure 1.4. Corkscrew appearance of oesophagus in Diffuse Oesophageal Spasm shown by X-ray of a barium swallow. 39
Figure 1.5. Illustration of the effect of SIL on decreasing smooth muscle excitation, via the inhibition of PDE type 5. 44
Figure 3.1. Apparent shear thinning of Smart Hydrogel® observed during continuous ramp measurements, at three different pH values. 71
Figure 3.2. Photographs of Smart Hydrogel® exhibiting gelling during peak hold measurements. 79
Figure 3.3. Photograph showing solid Smart Hydrogel® gel formations remaining following peak hold measurements, after removal of the shear forces.. 80
Figure 3.4. Smart Hydrogel® 2% w/v pH 3.7, temperature ramp. 81
Figure 3.5. Smart Hydrogel® 2% w/v pH 4.7, temperature ramp 81
Figure 3.6. Smart Hydrogel® 2% w/v pH 6.0, temperature ramp 82
Figure 3.7 Effects of incorporation of ISDN or SIL on viscosity of Smart Hydrogel® 2% w/v solutions 82
Figure 3.8. Temperature ramp showing temperature dependence of the viscous and elastic moduli, and the tan δ of a 20% w/v poloxamer 407 solution. 84
Figure 3.9. pH-dependence of viscosity of Noveon AA-1 0.1% w/v aqueous solution 85
Figure 3.10. Continuous ramp for 0.1% w/v Noveon® solution. 85
Figure 3.11. Continuous ramp for Blend A. 86
Figure 3.12. Blend A temperature ramp. 86
Figure 3.13. Continuous ramp viscosity measurement for Blend B. 87
Figure 3.14. Blend B temperature ramp. 88
Figure 3.15. Continuous ramp for HMO emulsion 88
Figure 3.16. Peak hold data for HMO emulsion 89
Figure 3.17. Stress sweep for HMO emulsion 89
Figure 3.18. Temperature ramp between 5 and 40°C for HMO emulsion. 90
Figure 3.19. Continuous ramp for LMO emulsion. 90
Figure 3.20. Peak hold data for LMO emulsion 91
Figure 3.21. Stress sweep for LMO emulsion 91
Figure 3.22. Temperature ramp for LMO emulsion 92
Figure 3.23. Continuous ramp for glycerol 92
Figure 3.24. Continuous ramp for Antepsin 93
Figure 3.25. The effect of emulsification speed (rpm) on viscosity (Pa.s) of o/w emulsions. 95
Figure 3.26. The effect of emulsification speed (rpm) on surface tension (mN/m) of o/w emulsions. 96

Figure 4.1. Diagrammatic representation of apparatus used to measure liquid retention on porcine oesophagus *in vitro*.....104
Figure 4.2. Examples of stability Tc99m incorporation assessments.....107
Figure 4.3. Mean retention profile of Blend A on porcine oesophageal tissue *in vitro*, demonstrating two-phase elimination. 107
Figure 4.4. Rank order (1-20) of retention of the formulations using the different analysis parameters, percent retention or AUC at different time periods.....110
Figure 4. 5 Bar graph showing AUC 1-10 for all formulations.....112
Figure 4.6. Relationship between pH and mean in vitro percent retention of Noveon AA1® 0.1% w/v 115
Figure 4.7. Relationship between viscosity and mean *in vitro* percent retention of Noveon AA1® 0.1% w/v.....115
Figure 4.8. pH dependence of viscosity and retention of 2% w/v Smart Hydrogel® solution.....116
Figure 4.9. Scattergraph showing loss tangent tan δ (G": G') vs. percent retained on porcine tissue at 5, 10 and 30 minutes.....120
Figure 4.10. Relationship between viscosity and mean initial half-life of loss of formulation from porcine oesophageal tissue.....122
Figure 4.11. Diagrammatic representation of a liquid drop resting in equilibrium on a solid surface, showing contact angle θ.....123
Figure 4.12. Scatter diagram showing relationship between loss tangent and surface tension in polymer formulations.....124
Figure 4.13. Scatter diagram showing relationship between surface tension and percent retention of polymer formulations after 10 min.....124
Figure 4.14. Scatter diagram showing relationship between tan δ and AUC 1-10 for polymer formulations.....125
Figure 4.15. Scatter diagram showing relationship between surface tension and tan δ of emulsions.....125
Figure 4.16. Scatter diagram showing relationship between tan δ and AUC 1-10 min of emulsions.....126
Figure 4.17. Scatter diagram showing relationship between surface tension and AUC 1-10 of the emulsions. 126
Fig 4.18. Effect of drug incorporation on viscosity of 2% w/v Smart Hydrogel® at pH 4.7.....127
Figure 4.19. Effect of drug incorporation on retention of 2% w/v Smart Hydrogel® at pH 4.7.....128
Figure 4.20. Effect of drug incorporation on viscosity of LMO and HMO emulsions. 129
Figure 4.21. Effects of ISDN and NIF incorporation on the retention of LMO and HMO emulsions.....129
Figure 5.1. Diagrammatic representation of two- chamber diffusion cell used for assessment of drug release.....135
Figure 5.2.Graph showing mean cumulative μmol released per unit area over time. A selection of drugs and formulations are represented (n=3).....142
Figure 5.3. Graph showing percent drug release from polymer formulations.....144
Figure 5.4. Graph showing percent drug release from emulsions 144
Figure 5.5. Graph showing effect of pH on drug flux (μg cm^{-2} min^{-1}) from 0.01% Noveon® and 2% Smart Hydrogel® solutions 146
Figure 5.6. Graph showing drug flux (μg cm^{-2} min^{-1}) from HMO and LMO emulsions 147

Figure 6.1. Designation of three regions of interest in the oesophagus 156
Figure 6.2. Images from one subject showing transit of HMO emulsion over time 158
Figure 6.3. Retention profiles of formulations, showing % retention in the total oesophagus over time.....159
Figure 6.4. AUC 30 of formulation retention, for the total oesophagus.....160
Figure 6.5. AUC 30 for the upper, mid and lower regions of the oesophagus. 161
Figure 6.6. Comparison of AUC 30 for *in vitro* model and human total oesophagus data.....162
Figure 6.7. Graph showing relationship between the mean AUC 30 of retention curves for *in vitro* and in total oesophageal *in vivo* data, for six formulations, not including Smart Hydrogel®.....164
Figure 6.8. Scatter diagram showing relationship between viscosity (Pa.s) and retention AUC 30 for the total oesophagus, for all formulations excluding Smart Hydrogel®.....165
Figure 7.1. Diagram of the silicone rubber manometry catheter used.....175
Figure 7.2. Diagram showing timings of the swallow sequences A to E, administration of high and lose dose formulations, and the removal and insertion of the manometry catheter tube 176
Figure 7.3. The effects of oral ISDN and SIL at both low and high doses, on subject heart rate and mean arterial pressure 179
Figure 7.4. Mean amplitude of 10 dry and wet swallows, in upper, mid-, and lower oesophagus during swallow sequences A to E, after ISDN or control formulations.....180
Figure 7.5. Mean amplitude of 10 dry and wet swallows, in upper, mid-, and lower oesophagus during swallow sequences A to E, after oral SIL or control formulations.....181
Figure 7.6. Manometric recording of four wet swallows following low dose (10 mg) sildenafil in Smart Hydrogel® formulation or control Smart Hydrogel® vehicle only 182
Figure 7.7. Basal LOS pressure measured between dry and wet swallows during swallow sequences A to E, after oral ISDN in Smart Hydrogel® or control (Smart Hydrogel® only).....184
Figure 7.8. Basal lower oesophageal sphincter pressure measured between dry and wet swallows during swallow sequences A to E, after oral SIL in Smart Hydrogel® or control (Smart Hydrogel® only).. 185
Figure 7.9. Basal lower oesophageal sphincter pressure measured between dry and wet swallows during swallow sequences A to E, after oral or gastric SIL in Smart Hydrogel® or control (Smart Hydrogel® only).....186

List of Tables

Table 1.1. Summary of some previous studies, illustrating the effects of different drug on oesophageal motor function 41
Table 1.2. Summary of pharmacokinetics of candidate drugs 46

Table 2.1. Composition of the oil and water emulsions 64
Table 2.2. Composition of silicone-based emulsion 65
Table 2.3. Composition of polymer blends A and B 66

Table 3.1. Molecular weights of candidate and model drugs. 97
Table 3.2. Physicochemical data of candidate and model drugs. 98

Table 4.1. Rank order (1 to 20) of retention of the formulations using the different analysis parameters, either percent retention or AUC at different time periods 109
Table 4.2. Area under retention curve (AUC) data over a selection of time points 111
Table 4.3. Linear correlation coefficients for the percentage of vehicle retained and its transformations against time. 119
Table 4.4. R^2 correlation coefficients showing strength of relationship between rheological and retention data. 119

Table 5.1. HPLC calibration for ISDN 138
Table 5.2. HPLC peak areas for ISDN release from LMO experiments A, B and C. 138
Table 5.3. Mean HPLC peak areas for Franz cell samples containing ISDN 139
Table 5.4. Calculation of CPM per mg drug for the reference sample 140
Table 5.5. Calculation of the starting CPM in each Franz cell donor formulation. 140
Table 5.6. Calculation of the percentage of the starting radioactive counts found in receiver samples at different time points. 141
Table 5.7. Table showing formulations from which percent release after 30 min was greatest for each drug. 143
Table 5.8. The R^2 linear correlation coefficients for mean μg drug release and its transformations against time, for the different drugs 148
Table 5.9. The R^2 linear correlation coefficients for μg drug release and its transformations against time, for the different formulations. 149
Table 5.10. Mean estimated total release after 10 min for different drugs, based on high concentration formulations, excluding controls. 150

Table 6.1. Correlation coefficients obtained from comparison of mean retention data for the different oesophageal regions in humans, with mean retention data from *in vitro* experiments at equivalent time points. 163
Table 6.2. Correlation coefficients obtained from comparison of mean retention data for the different oesophageal regions in humans, with mean retention data from *in vitro* experiments at equivalent time points 163

List of abbreviations used in the text

°C	degrees Celsius
Ach	acetylcholine
ACN	acetonitrile
ANOVA	analysis of variance
AUC	area under the curve
BO	Barrett's oesophagus
bpm	beats per minute
Ca^{2+}	calcium ions
CCK	cholecystokinin
cGMP	guanosine 3',5'-cyclic monophosphate
COX-2	cyclooxygenase-2
CYP	cytochrome p450 enzyme
DBP	diastolic blood pressure
DEXT	dextran
DOS	diffuse oesophageal spasm
EGF	epithelial growth factor
FDA	Food and Drug Administration
G'	storage/elastic modulus
G"	loss/ viscous modulus
GI	gastrointestinal
GORD	gastro-oesophageal reflux disease
HCl	hydrochloric acid
HCO_3^-	hydrogen carbonate
HLB	hydrophile-lipophile balance
HMO	heavy mineral oil emulsion
HPLC	high performance (pressure) liquid chromatography
HPMC	hydroxypropyl methylcellulose
HPV	human papilloma virus
HR	heart rate
Hz	hertz
ISDN	isosorbide dinitrate
kBq	kilo bequerel
kDa	kilo Daltons
keV	kilo electron-volt
LMO	light mineral oil emulsion
LOS	lower oesophageal sphincter
LOSp	lower oesophageal sphincter pressure
LOSR	lower oesophageal sphincter relaxation
MANN	mannitol
MAP	mean arterial pressure
MeOH	methanol
mg	milligram
mL	millilitre
mmHg	millimetres of mercury (pressure)
NaOH	sodium hydroxide
NIF	nifedipine
NO	nitric oxide
NOes	nuctracker oesophagus

NOS	nitric oxide synthase
NSAID	non-steroidal anti-imflammatory drug
OeAC	oesophageal adenocarcinoma
OeSCC	oesophageal squamous cell carcinoma
Pa	Pascals
Pa.s	Pascal seconds
PDE	phosphodiesterase
PDT	photodynamic therapy
PEG	polyethylene glycol
PGOS	pressure gradient between oesophagus and stomach
PPI	proton pump inhibitor
PROP	propranolol
ROI	Region of interest
rpm	revolutions per minute
S.D.	standard deviation
S.H.	Smart Hydrogel®
SBP	systolic blood pressure
SIL	sildenafil
Tc99m	technetium 99m isotope
TLOSR	transient lower oesophageal sphincter relaxation
UV	ultra-violet
w/v	weight per volume
µg	microgram
µL	microlitre
µNm	micro Newton metres (torque)

Chapter 1: Introduction

Chapter 1

Introduction

The aim of this project is to develop a liquid oral drug delivery system to target the oesophagus for the purpose of treating oesophageal disorders.

Most current medical therapies for the oesophageal diseases discussed in this chapter are formulated for systemic administration. On absorption, systemic formulations become diluted in the body fluids, and may undergo inactivation by gastrointestinal enzymes or pH before they reach the site of action. They therefore require a high dose to allow sufficient bioavailability, but a high drug dose often increases the risk of side effects. These limitations are reasons why there is a current need for a more effective, efficient drug delivery system for targeting the oesophagus to treat these diseases. A targeted delivery system producing a local effect could avoid first pass metabolism and dilution, reducing the required drug dose.

To be successful, such a delivery system must exhibit prolonged retention within the oesophageal lumen where it allows drug diffusion out of the formulation, so that the active ingredient may be absorbed by the target tissue (e.g. smooth muscle). As the oesophagus alone is the target of this delivery system, it is important that majority of the active drug acts at this site. Formulations are to be tested for their bioadhesive potential *in vitro* and *in vivo*, and the formulation with best retention and drug release properties will be identified. The effect of formulations containing active ingredient on oesophageal motor function in healthy humans will be assessed.

The Oesophagus

1.1 The healthy oesophagus

1.1.1 Oesophageal physiology and histology

The oesophagus is a tubular structure approximately 25 mm in diameter (Boyce, 1999) and 23 cm in length (Li et al, 1994) providing the pathway via which ingested food and drink are carried to the stomach. The oesophagus begins at the pharynx at the level of the 6^{th} vertebra and enters the stomach at the cardia or gastro-oesophageal junction (Stevens and Lowe, 1997). The oesophagus is lined by non-

keratinised squamous epithelium, lying over a lamina propria and the muscularis mucosa (Stevens and Lowe, 1997; Bouchier et al, 2005). A simple representation of the layers of the oesophageal wall is shown in Figure 1.1.

The human oesophageal epithelium has an average thickness of 500-800μm. The basal epithelium may be several cell layers thick, comprising rectangular cells with dark nuclei and no glycogen in the cytoplasm (Stevens and Lowe, 1997). Some melanocytes and neuroendocrine cells also exist in the basal zone. The basal zone forms up to 15% of the normal epithelial thickness and is the site of mitosis. Above this basal layer, epithelial cells are paler-staining, glycogen rich and larger, gradually becoming flatter in shape towards the lumen. The stratified squamous epithelium is a tough lining to resist distress caused by bolus abrasion.

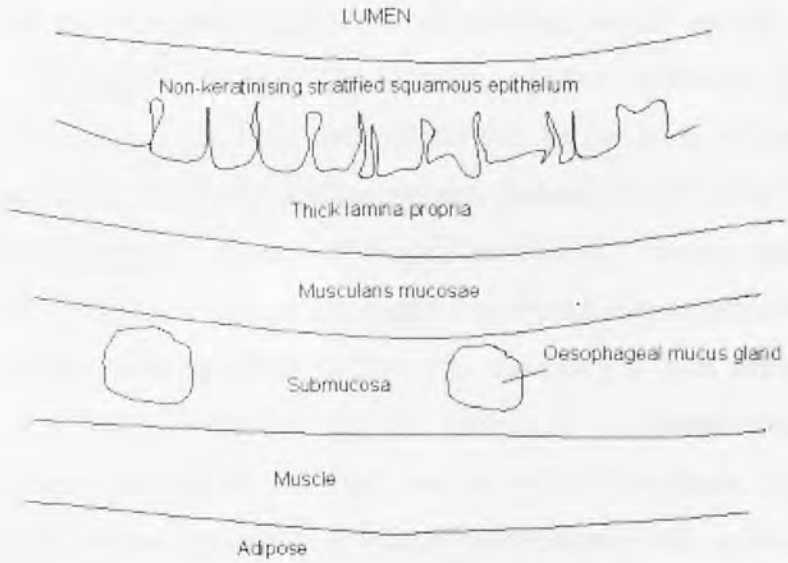

Figure 1.1. Diagrammatic representation of a cross-section of human oesophagus, showing the layers present.

The lamina propria contains collagen fibres and fibroblasts in an acellular matrix. There are some lymphocytes, eosinophils, mast cells and plasma cells present also. The muscularis mucosa is of varying thickness, thickest near the cardia where the oesophagus meets the stomach. In the upper oesophagus, striated muscle fibres have a haphazard arrangement, but in the lower third the muscle fibres are arranged uniformly in continuous muscle sheets (Stevens and Lowe, 1997). A gradual transition from striated to smooth muscle occurs in the middle third of the oesophagus, where both muscle types are found. The most distal third of the

oesophagus is almost entirely smooth muscle which is continuous with the smooth muscle of the stomach.

The submucosa contains blood vessels, mucous glands surrounded by lymphocytic infiltrate, and the Meissner's plexus (one of the two nerve plexi innervating the alimentary canal). The submucosa contains no more than three hundred mucous glands, mostly located in the proximal half (Washington et al, 2001). Submucosal mucous glands are organised in longitudinal rows with their ducts arranged obliquely, in the direction of the lumen (Sarosiek, 1995). Each gland has two to five lobes draining into a short duct lined by columnar epithelium. This duct penetrates all the above layers to open out into the lumen. Submucosal glands are similar to the mucus-secreting inner salivary glands, and in the lower 5cm of the oesophagus, the glands are identical to the cardiac glands of the stomach which secrete mucous rather than acid (Hopwood, 1986). The content of the secretion from the oesophageal glands has not been fully ascertained due to the lack of appropriate methodology; however the glands do contain mucins. Sarosiek et al (1994b) studied for the first time the secretory potential of these glands in the human oesophagus, under physiological conditions, using a six channel oesophageal perfusion catheter. Catheter channels were used to either perfuse the oesophagus with saline, or to collect (aspirate) the oesophageal and gastric secretions. Perfusion with saline resulted in continuous release of different mucin types throughout the entire procedure. When the saline was replaced with a hydrochloric acid or bicarbonate solution, the proportion of the different mucins secreted was altered by the change in perfusing solution. As the type of secretion differs with the environment, the secretions of these submucosal glands probably play a role in protecting the oesophagus under varying conditions. The amount of secretions produced within the oesophagus is still under debate. The oesophagus does not have a well-defined mucous layer like the stomach (Dixon et al, 2001). The thin layer of mucous present on the oesophageal surface is likely to have originated predominantly from saliva rather than being locally secreted despite the presence of the submucosal glands.

The muscular coat has an inner circular layer and an outer longitudinal layer. These layers are striated in the upper oesophagus but in the lower third they are smooth and continuous with the stomach muscle layers. The muscle regions are affected

differently by disease and their pharmacology differs (Storr and Allescher, 1999). Between the muscle layers of the oesophagus lies the sympathetic myenteric or Auerbach plexus (the second alimentary nerve plexus) containing mostly cholinesterase positive neurons; as such these are likely to have motor function. The other neurones (20-40 percent) are argyrophil and may be involved in the co-ordination of swallowing. The lower oesophagus has more autonomic nerves than any other area of the alimentary canal (Bouchier et al, 2005). The wall of the oesophageal body is flaccid except when a peristaltic wave is passing (see Section 1.1.3). In the relaxed state, the oesophageal lumen is highly folded.

Figure 1.2 Histological transverse section of human oesophageal wall, showing epithelium, mucosa, muscle layers and a submucosal gland. (source: http://www.lab.anhb.uwa.edu.au/mb140/CorePages/Oral/Oral.htm#Oesophagus)

1.1.2 Neurophysiology

The oesophagus has parasympathetic and sympathetic innervation. Extrinsic innervation is by vagus nerves and sympathetic fibres derived from the cervical and thoracic ganglia. Intrinsic supply is derived from Auerbach and Meissner plexi (Washington et al, 2001). It is interesting to note that the afferents from the lower oesophageal sphincter (see Section 1.1.5) have their cell bodies in the dorsal root

ganglia at the same level of the spinal cord as those from the heart. This overlapping distribution may account for the similar pain described by cardiac and oesophageal chest pain patients (Hornby and Abrahams, 2000).

1.1.3 Control of swallowing and peristalsis

As mentioned previously the oesophagus has two sections of striated (proximal) and smooth (distal) muscle, each with distinct pharmacology and disease susceptibilities. The striated region has a similar pharmacology to skeletal muscle, innervated by extrinsic vagal neurones, releasing acetylcholine, which has an excitatory effect on nicotinic receptors. Striated muscle relaxants will have an effect on the proximal oesophagus. Extrinisic innervation of the smooth muscle is not directly to the muscle, but to the myenteric plexus.

1.1.4 Normal Swallowing and Peristalsis

Initiation of a swallow of a bolus is controlled by the medullary swallowing centre. Primary peristalsis follows the initiation of a swallow and produces waves strong enough to close the lumen, although not always strong enough to propel a solid or even semi-solid bolus. Secondary peristalsis is initiated involuntarily in the oesophagus by a reflex within the myenteric plexus, activated by stretch. There is a sequential contraction of smooth muscle starting at the point of distension. It may also be initiated in response to irritation caused by gross reflux from the stomach (Washington et al, 2001; Bouchier et al, 2005). Peristalsis plays an important role not only in the progression of a food bolus but also in clearance of refluxed matter from the oesophagus. Swallowing is coordinated by the interplay between extrinsic and intrinsic nerves, and a disruption of this control results in clinical symptoms such as chest pain and difficulty swallowing (dysphagia).

1.1.5 Lower Oesophageal Sphincter

In the late 1970s, Liebermann-Meffert et al (1979) described the area where the oesophagus joins the cardia as the lower oesophageal sphincter (LOS). The LOS may be defined in several ways as there is no definite anatomical sphincter:

a) The junction of squamous and columnar epithelia
b) The region where the oesophagus enters the stomach

c) The junction between oesophageal inner muscle and the inner layer of stomach muscle.

The LOS is a smooth muscle region with residual tonic pressure and it can also be manometrically defined by a high pressure zone 15 to 40 mmHg above intra-gastric pressure (Washington et al, 2001). When a swallow is initiated, the tone of the LOS falls from basal level quickly, reaching zero in 4 to 5 seconds. Tone is recovered in 3 to 4 seconds and then a contraction occurs to about twice basal level in 3 to 4 seconds, followed by a fall to normal basal tone in 3 to 4 seconds (Washington et al, 2001). The LOS is extremely important in the prevention of acid reflux from the stomach. Control of the LOS tone is achieved by the medulla via the myenteric plexus and is still seen after transection of the upper oesophagus in man. The LOS must be compliant enough to allow passage of boluses.

Swallow-induced LOS relaxation is mediated through the central nervous system. Efferent fibres travel to the myenteric plexus of the LOS via the vagus nerve. The synapse between vagal fibres and myenteric neurons has a cholinergic mechanism (Mittal and Balaban, 1997). However, the post-synaptic neurotransmitters of the LOS are non-cholinergic and non-adrenergic. Nitric oxide (NO) and vasoactive intestinal polypeptide (VIP) are the major neurotransmitters of the LOS (Mittal and Balaban, 1997; Boeckxstaens, 2005).

Transient lower oesophageal sphincter relaxations (TLOSRs) were described by Dent et al. (1988). They are involved in the belch reflex and reflux in health and disease. A TLOSR is a spontaneous, prolonged decrease in LOS baseline pressure to the level of intragastric pressure, not triggered by a swallow (Mittal and Kassab, 2003). Generally, a TLOSR lasts longer than a swallow-induced relaxation. Gastric distension is a potent stimulus of TLOSRs which allow gas to be vented from the stomach. The gastric distension-trigger of TLOSRs can be inhibited by CCK-A receptor and NO antagonists. TLOSRs are a highly desirable target for therapeutic strategies. GABA antagonists decrease TLOSR frequency (Lehmann et al, 1999) and may therefore be useful in preventing excessive reflux. NOS inhibitors also decrease frequency of TLOSRs (Hirsch et al, 2000).

1.2. Disease States of the Oesophagus - Non-motility disorders

1.2.1 Gastro-oesophageal Reflux Disease

1.2.1.1 Epidemiology and symptoms

Gastro-oesophageal reflux disease (GORD) is caused by excessive reflux of acid and bile material from the stomach back up into the oesophagus despite the physiological mechanisms to prevent this occurring. GORD is a common affliction in the Western world, and treatment costs are substantial because of its prevalence. Gastric reflux is a physiological event occurring frequently even in healthy individuals. However, pathological reflux can lead to oesophageal damage (Orlando, 2000) and symptoms of GORD. Oesophageal reflux disease is diverse in its clinical presentations. The classic symptom is heartburn, which is commonly found even in healthy individuals. One survey suggested that 36% of individuals experience heartburn at least once a month, with 7% afflicted daily (Nebel et al, 1976). Another publication states that in the UK, up to 40% of adults may suffer from dyspepsia in a year, and 25% of those may be categorised as having GORD (NICE, 2000). Other Scandinavian surveys have suggested a lower frequency, although it appears that reflux is generally less prevalent in other areas of the globe, in particular the Far East. Often, heartburn is mild and many individuals with frequent symptoms find self-treatment with antacid preparations is sufficient (Graham et al, 1983). Acid regurgitation and belching are among the other symptoms of oesophageal reflux disease. Epigastric pain, gastrointestinal bleeding and dysphagia may also be present. More atypical presentations of reflux disease include a chest pain resembling myocardial ischaemia and respiratory symptoms such as coughing. It is often reported that severity of symptoms is exacerbated by a recumbent position and alleviated when upright. Potentially serious complications of GORD include strictures, erosive oesophagitis and development of Barrett's Oesophagus, leading to an increased risk of oesophageal adenocarcinoma (Kinnear et al, 1999; Solaymani-Dodaran et al, 2004; Savarino and Dulbecco, 2004).

1.2.1.2 Physiological causes of acid reflux from the stomach

A pH electrode can demonstrate that normal healthy subjects have small amounts of acid reflux regularly after meals; this is known as physiological reflux. The normal LOS contracts at a pressure of ~15mmHg. Intermittently, and more frequently after

eating, intragastric pressure rises transiently (Hopwood et al, 1984). If intragastric pressure rises above the LOS pressure, reflux may occur. The pressure gradient between the oesophagus and the stomach (PGOS) is a driving force in reflux (Mittal, 2003). Normally the LOS pressure continuously adapts to PGOS changes. PGOS changes are related to muscular contractions of the oesophagus and stomach, or to thoracic/abdominal cavity pressure changes. Contraction of the LOS protects against reflux. Stomach contractions lead to increased pressure in favour of reflux. Therefore, during migrating motor complex activity of the stomach, gastric contraction is coupled with LOS contraction, as a preventative mechanism against reflux. During inspiration, contraction of inspiratory muscles leads to negative intrathoracic pressure and negative intraoesophageal pressure. Therefore, the PGOS rises. Also contraction of the diaphragm and abdominal wall increases PGOS and increases stomach pressure. Therefore, all these activities are accompanied by contraction of the crural diaphragm, causing a protective increase in LOS pressure.

Heartburn may be experienced in the immediately postprandial period, despite food helping to buffer the gastric contents. Reflux in this situation may be explained by the discovery of a highly acidic pocket high in the fundus, just below the LOS (Fletcher et al, 2001). The acid pocket has the potential to reflux even at times when the stomach contains food and has an increased pH.

Pepsin is an acid-activated protease secreted by the stomach. It is found in gastric refluxate, and enhances the damaging effect of the refluxate to the oesophageal epithelium when compared to acid alone (Gotley et al, 1991; Tobey et al, 2001). Rabbit oesophageal epithelium exposed to hydrochloric acid (pH 1.7) for one hour exhibited no damage by light microscopy; however, when exposed to acid with pepsin, necrosis and cell swelling was evident (Tobey et al, 2001). Both treatments caused disruption of the epithelial barrier (as shown by a decline in electrical resistance). The acid-only damage was reversible, whereas acidic-pepsin damage was irreversible (Tobey et al, 2001).

1.2.1.3 Physiological mechanisms to resist acid damage

Acid clearance is first mechanical (bolus clearance), then chemical (acid neutralisation by saliva and oesophageal secretions). Mechanical clearance is

achieved by peristaltic waves which clear acid back into the stomach. Mucosal protection of the oesophagus operates at three overlapping levels involving pre-epithelial, epithelial and post-epithelial defences. Pre-epithelial defence involves the complementary action of salivary and oesophageal secretions (Orlando, 2000) and consists of an unstirred water layer (Heatly, 1959; Attwood, 1994) and a bicarbonate barrier. These reduce H^+ ion concentration before the epithelial surface, however this can only support a pH gradient of approximately one pH unit (Orlando, 2000). Limitations of pre-epithelial defence in the oesophagus may include a lack of well-defined resident mucous layer, scarcity of bicarbonate-secreting epithelial cells (Mertz-Nielsen et al, 1997) and poor permeability to bicarbonate diffusion from the blood. The lowest 2cm of the oesophagus is normally lined with gastric mucosa and covered by peritoneum (Washington et al, 2001) which may increase efficiency of the defences in this region.

Human oesophageal mucosa contains submucosal mucous glands arranged in longitudinal rows (Sarosiek et al, 2005) with their ducts flowing downward towards the lumen. From these glands various protective components are secreted including mucin, non-mucin proteins, EGF and prostaglandins (Sarosiek et al, 1994a; Namiot et al, 1994b). The rate of secretion is modified by luminal acid or acid/pepsin exposure. Bicarbonate ions in saliva help to neutralise refluxed acid, and the pH of the oesophagus increases during swallows. However, the pH also increases gradually between swallows, suggesting a non-salivary component of acid neutralisation. It has been demonstrated by a double-balloon technique, isolating and perfusing the distal oesophagus, that the healthy human oesophagus also secretes sufficient bicarbonate to assist in the buffering of luminal acid (Hamilton and Orlando, 1989; Meyers and Orlando, 1992). Nocturnal reflux causes the greatest damage. During sleep, salivation and peristalsis are reduced, this connection indicates how important the initial mechanical and salivary protective mechanisms are (Tobey et al, 1989).

The stratified squamous cells serve as the epithelial defence. There are intercellular junctional complexes consisting of tight junctions and glycoconjugate material (Hamilton and Orlando, 1989; Orlando et al, 1992). These limit H^+ diffusion into and between cells. The cells also have a buffering capacity by intercellular HCO_3^-

and ion exchange membrane proteins aid the control of $[H^+]$. In the stomach and duodenum, the epithelial cells can heal damaged lesions by migration over the area, although this is not seen in the oesophagus. In the oesophagus, hyperplasia at the basal cell level in some patients with GORD suggests that the oesophagus self-heals by cell replication. Post-epithelial defences include blood flow in the oesophagus, which increases during acid exposure (Feldman et al, 1996; Tanaka et al, 2003); this helps to increase delivery of HCO_3^- ions to the cells for buffering action. The blood supply also removes CO_2 produced by acid neutralisation and supplies O_2 and nutrients for cell repair.

1.2.1.4 Risk factors in GORD

The risk of GORD may be increased by a variety of factors, some of which are discussed below.

LOS function may be impaired by drugs, diet and nervous system factors. Some medications decrease the LOS pressure (e.g. sildenafil) (Eherer et al, 2002) and this can exacerbate or even precipitate GORD.

- Impairment of gastric motility may lead to delayed stomach emptying, and this build-up will increase the risk of reflux of material through the LOS.
- Impaired oesophageal motility leads to poorer clearance of acid into the stomach.
- A decline in salivary production, e.g. with advanced age or in certain disease states results in a decreased clearance of physiological reflux from the stomach, and there is a reduction of the natural pre-epithelial defence against oesophageal damage (see Section 1.2.1.3).
- The risk of reflux increases during pregnancy, with GORD occurring in an estimated 30- 50% of pregnancies (Richter, 2003). During the first trimester of pregnancy LOS pressure is less responsive to physiological stimuli. In the later two trimesters, LOS pressure gradually falls by up to 50% of basal values. Animal and human studies have found that the changes in circulating hormone levels (progesterone and oestrogen) during pregnancy affect LOS relaxation (Fisher et al, 1978; Richter, 2005). Additionally, intra-abdominal pressure increases with pregnancy. It is possible that the normal compensatory response of the LOS to these changes is impaired during pregnancy (Richter, 2003).

- The risk of GORD increases if a hiatus hernia occurs and impairs LOS function. A hiatus hernia results when the upper part of the stomach moves up into the chest through a small opening in the diaphragm, which is the muscle that separates the chest from the abdomen. This causes the LOS to become completely intra-thoracic in location. The presence of a hiatus hernia results in acidic stomach contents being retained above this opening, and within the lower part of the oesophagus. After a reflux episode, oesophageal peristalsis will clear the acid bolus from the oesophagus into the hiatal hernia (Mittal, 2003). LOS relaxation triggered by a swallow or a TLOSR will then allow reflux of the acid in the hiatal sac up into the distal oesophagus. This sequence of events results in poor, incomplete and prolonged acid clearance, thus causing GORD (Mittal and Kassab, 2003).
- There are several reports of families where multiple members have GORD, Barrett's oesophagus (BO) and oesophageal adenocarcinoma (Jochem et al, 1992). This familial trend may be due to a genetic predisposition or it could be related to the shared environmental influences experienced by a family. Two studies (Cameron et al, 2002; Mohammed et al, 2003) comparing GORD symptoms in pairs of monozygotic and dizygotic twins, have shown significantly higher concordance for GORD among monozygotic twins than dizygotic twins. Such studies suggest that genetic factors do contribute to GORD. Additionally, there may be a genetic involvement concerning inherited muscular or structural problems in the oesophagus, stomach or LOS, which result in GORD.
- Smoking causes relaxation of the LOS (Chattopadhyay et al, 1977), and therefore may exacerbate existing GORD, however no definite link with smoking has been identified (Locke et al, 1999).
- GORD mainly occurs postprandially, due to gastric distension (Holloway et al, 1985), food or cholecystokinin (CCK) (Boulant et al, 1994). CCK is released when fat enters the duodenum, therefore eating habits may affect epidemiology. The prevalence of reflux oesophagitis is increasing in developed countries. This may be explained by increased calorific intake, accompanied by increased gastric distension and frequency of TLOSRs.

1.2.2 Laryngoopharyngeal reflux

A recently described condition is laryngopharyngeal reflux (LPR), a disorder more of volume clearance and dysmotility than of acid/pepsin injury. In LPR, contents are regurgitated from the proximal oesophagus into the laryngopharynx, causing cough, dysphagia, dysphonia, and chronic throat clearing (Belafsky and Rodriguez, 2004). This clinical manifestation is not yet well investigated but pharmacological therapy includes the use of proton pump inhibitors, as with GORD (see Section 1.2.4).

1.2.3 Barrett's Oesophagus

1.2.3.1.Epidemiology and progression

Barrett's Oesophagus is an endoscopically detectable metaplastic change of the oesophageal lining so that a region is lined with columnar epithelium instead of the normal squamous epithelium. In this disease, oesophageal epithelial cells undergo a metaplastic change from the normal squamous epithelium to a columnar intestinal-type of lining (Sampliner, 1998). The new epithelium is called Barrett's epithelium. The change is only caused after destruction of the squamous epithelium, which is followed by re-epithelialisation of the oesophagus. The initial destruction responsible for the initiation of these events may be the result of acid and bile exposure in GORD. Controversy surrounds the origin of the new columnar cells, as they differ histologically from the gastric cardia lining, making simple upward migration an unlikely explanation (Shaheen and Ransohoff, 2002a). There may instead be a pluripotent cell type with features of columnar and squamous tissue (Sawhney et al, 1996). Experiments on dogs with induced oesophageal damage show that the oesophagus regenerates columnar epithelium when exposed to acid, and squamous epithelium if exposed to a neutral pH (Gillen et al, 1988). The environment in which damaged epithelium heals is therefore important. Barrett's is very rare in children, with prevalence increasing to 1% of the population older than 60. It is at least twice as common in males compared with females (Cameron, 1990). Barrett's Oesophagus is especially prevalent in Caucasian males over the age of fifty (Blot et al, 1991). Barrett's occurs in approximately 10% of patients with GORD and is associated with a 30 to 125 times increased risk of oesophageal cancer (Arguedas, 2001). If cell abnormalities occur as a result of epithelial change (dysplasia) then adenocarcinoma may develop. The risk of developing adenocarcinoma is especially high when an advanced degree of dysplasia (high-

grade dysplasia) or long segment Barrett's (more than 3 cm length of epithelium affected) is present. Some consider the cancer risk in Barrett's to be overestimated due to publication bias (Shaheen, 2000) and some small studies report higher risks than larger studies.

Epithelial growth factor (EGF) and transforming growth factor alpha (TGF-α) are peptides produced extracellularly on the cell membrane, and activated by proteolysis. Both peptides are found in many gastrointestinal tissues including the oesophagus and stomach (Hopwood, 1995). These two peptides are potential causative factors of Barrett's, dysplasia and carcinogenesis of the oesophagus, because of their proliferative effects, and because activation of the peptides is mitogenic (Souza et al, 2001). Physiological actions of EGF include cell proliferation, wound healing, differentiation, and possibly oncogenesis (Jankowski et al, 1992). In the oesophagus EGF has greatest density of distribution in the basal layer.

1.2.3.2 Treatment of Barrett's Oesophagus

Treatments designed to endoscopically destroy metaplastic areas in Barrett's Oesophagus include lasers, argon plasma coagulation and photodynamic therapy (PDT) (Haringsma, 2002). Vigorous acid suppression is usually combined with such procedures to encourage epithelial healing into the squamous type. However, an incomplete ablative therapy may leave islands of Barrett's epithelium in the treated area (Shaheen and Ransohoff, 2002b). Other surgical and pharmacological strategies have attempted to decrease the cancer risk associated with Barrett's Oesophagus. However pharmacological therapies have so far shown little success in causing regression of Barrett's Oesophagus and, so far, do not decrease the cancer risk. Prevention of Barrett's Oesophagus as a complication of GORD is therefore currently an important therapeutic area.

1.2.4 Summary of current therapies for GORD and Barrett's Oesophagus

- Antacids e.g. magnesium, aluminium and calcium salts are widely used to treat mild symptoms of GORD by neutralising acid in the stomach. Long-term use can increase the risk of kidney stone formation.

- Alginates are naturally occurring polysaccharides derived from brown seaweeds. Alginates are used for treatment of GORD symptoms and are a component of many common remedies e.g. Gaviscon®. Alginates adhere to the oesophageal mucosa and act as a protective barrier to acid damage (Batchelor et al, 2002; Tang et al, 2005). Alginate based therapies are believed to form a raft that floats above the gastric contents, which serves as a physical barrier against reflux (Tytgat, 2003). The lack of systemic absorption makes these suitable for use during pregnancy and avoids drug-drug interactions.
- Sucralfate (carafate) is the salt of aluminium hydroxide and sucrose octasulphate. It protects the mucous lining in GORD by sticking to damaged epithelium and acting as a barrier to further damage by acid and pepsin. Retention in the oesophagus and other gastrointestinal sites is though to be better at ulcerated regions than healthy mucosa (Goff, 1986; Dobrozsi, 1999).
- Histamine receptor antagonists prevent acid production and increase pH in the stomach (Tytgat 1989) hence increasing pH of the refluxate. They include famotidine, cimetidine and ranitidine.
- Proton pump inhibitors (PPIs) inhibit an ion (H^+) pump in the stomach to reduce acid production and therefore increase pH of refluxate. PPIs include omeprazole, lansoprazole and esomeprazole. These are more powerful than the H_2 blockers. They are widely used in the treatment of GORD and laryngopharyngeal reflux, and account for ~65% of the total medication cost in those with Barrett's Oesophagus (Eloubeidi 1999). Initial concerns that they would cause serum gastrin levels to rise and hence cause gastrinomas appear to have been unfounded (Klinkenberg 2000). PPIs are safe for long-term use and well tolerated. They make total symptom relief attainable in most patients.
- Cyclooxygenase-2 (COX-2) inhibitors help to reduce inflammation and pain associated with GORD, Barrett's Oesophagus and oesophagitis, and they pose less of a risk than aspirin and ibuprofen for peptic ulcers and bleeding. Although COX-2 inhibitors may protect against cancerous changes in Barrett's Oesophagus, their long-term use can result in serious side-effects such as myocardial infarction (Emery et al, 2005).

- Surgical procedures used to treat GORD are based on the principles of tightening the gastro-oesophageal junction using sutures or clips, causing collagenisation at the gastro-oesophageal junction by application of heat energy, of augmentation of the gastro-oesophageal junction by injection of substances to bulk the LOS. Fundoplication creates folds with the stomach to increase the LOS bulk. The Stretta procedure involves delivering radiofrequency energy directly into LOS tissue via endoscopically guided needle electrodes. This causes well-defined thermal lesions which upon healing cause tissue contraction, narrowing the lumen at the LOS. Microspheres may be implanted into the LOS to increase its bulk. Bulking agents may also be injected into the LOS, e.g. Enteryx polymer. Fundoplication and Stretta procedures are both FDA approved. They are outpatient procedures which are usually efficacious in the short term, and do not impede future surgical treatments. However patients with complicated GORD, Barrett's, stricture, or large hiatal hernia are not generally considered suitable candidates for the surgery (Karlowicz, 2003; Go et al, 2004).

1.2.5 Oesophageal Cancer

1.2.5.1 Types of cancer, development and epidemiology

Cancer of the oesophagus is the ninth most common malignancy worldwide (McCabe and Dlamini, 2005), and its incidence has been increasing over the past three decades (Caldas, 2000). High incidence areas include China, South Africa, Western Europe and the former Soviet Union (Pickens and Orringer, 2003). Oesophageal cancer is eight times as likely to occur in whites as other ethnic races. Over 7,000 new cases are diagnosed each year compared with 10,000 gastric carcinomas (Cancer Research Campaign, 1998). Oesophageal cancer is more prevalent in males, in whom the incidence is four times that of women (Blot et al, 1991). It is the second most common cancer among males in South Africa (McCabe and Dlamini, 2005). Most cases occur in people over 45 years old. The two main categories of oesophageal cancer are squamous carcinoma (OeSCC) and adenocarcimona (OeAC). As discussed earlier, the oesophagus is normally lined with squamous epithelium. Thirty years ago, most oesophageal cancers were squamous carcinomas. The incidence of OeAC has been increasing more rapidly

than any other cancer over the last 3 decades. Oesophageal adenocarcinoma is no longer a rare neoplasm and is now at least as common as squamous carcinoma (Cameron, 1997). Symptoms of oesophageal cancer include difficulty in swallowing, pain in the oesophagus, chronic cough, vomiting, hiccups and weight loss.

Tumorigenesis is a multi-phased process associated with accumulated genetic changes in the somatic cells that give rise to a cancer (Caldas, 1998). Cancer cells acquire a selective growth advantage because of mutations in their genes that control cellular proliferation and death (Nowell, 1976). Squamous cell carcinoma starts in the squamous epithelial cells lining the oesophagus and usually affects the upper and middle thirds of the oesophagus. Adenocarcinoma starts in the mucus-producing glands of the oesophagus and mainly affects the lower third of the oesophagus. This type of cancer is most associated with GORD and Barrett's Oesophagus and the absolute risk of OeAC in those with uncomplicated reflux is low (Blot 1991).

1.2.5.2 Risk Factors

White men are the highest risk group for contracting OeAC, and black women the lowest risk group (El Serag et al, 2002). Other risk factors for OeAC include smoking, obesity, and history of chronic GORD. It is estimated that 40% of oesophageal cancers are related to smoking (Cameron, 1997) and alcohol has a strong aetiological link with oesophageal cancer (McCabe and Dlamini, 2005). Ethanol has been found to decrease LOS pressure and oesophageal body contraction amplitude in humans (Storr and Allescher, 1999), and smoking causes relaxation of the LOS (Chattopadhyay et al, 1977). This could partly explain why drinking alcohol and smoking are implicated in OeAC, as the mechanical defence mechanisms against acid reflux are diminished. Diets high in salty, preserved and pickled foods, and low in vitamins and minerals, are additional risk factors. Smoked food, which contains high levels of nitrites and nitrosamines, are likely inducers of oesophageal carcinoma (McCabe and Dlamini, 2005). Approximately 60% of people with the motility disorder achalasia will develop OeSCC and 86% of cases of primary OeAC originate from Barrett's (McCabe and Dlamini, 2005) Much research is in progress to elucidate the precise mechanisms causing the metaplasia

to dysplasia sequence of oesophageal carcinoma at a molecular level (Yuan et al, 2002). It is interesting to note that the risk for OeAC in patients with short segment Barrett's Oesophagus is not substantially lower than in patients with long segment Barrett's (Rudolph et al, 2000). The level of risk of OeAC with Barrett's is uncertain. Quoted figures vary widely from 30 to 125 times increased risk (Mandal et al, 2003). However it is generally accepted that Barrett's metaplasia pre-disposes the patient to subsequent OeAC development (Cameron, 1997). Other risk factors for oesophageal cancer include extended exposure to soot, metal, dust and diesel exhausts. Certain serotypes of the human papilloma virus (HPV) are also associated with development of OeSCC (Ashworth et al, 1993). Tylosis is a rare inherited skin condition that is also associated with a very high risk of oesophageal carcinoma (Ashworth et al, 1993; Ellis et al, 1994).

1.2.5.3 Treatment of Oesophageal Cancer

Management of oesophageal cancer remains an unsolved health problem (McCabe and Dlamini, 2005). Surgery is the most common treatment and a partial or total oesophagectomy may be carried out (Haringsma, 2002). Oesophagectomy is curative in patients with high-grade dysplasia or early OeAC, and removal of the whole oesophagus means that the risk of progression to cancer is removed (NICE, 2004). If the cancer has metastasised to the stomach, part of the stomach may also be removed in an oesophagastrectomy. Lymphadenectomy is also carried out around the oesophagus to prevent the survival of any cancer cells inside the lymph nodes. It is thought that pre-operative chemoradiotherapy improves survival rate compared with surgery alone (Sheehan and Meade, 2000).

1.3 Disease States of the Oesophagus - Motility disorders

The disorders of oesophageal motor function, also known as motility disorders, include achalasia, diffuse oesophageal spasm and nutcracker oesophagus (Adler and Romero, 2001). These disorders of motor function are described in more detail below.

1.3.1 Achalasia

1.3.1.1 Disease presentation

Achalasia is the best understood oesophageal motor disorder, most common in middle age, but occurring in all age groups and both sexes (Mittal, 1997). Achalasia is a motor disease of the oesophageal body smooth muscle and the LOS, causing failure of the hypercontractile LOS to relax when swallowing and aperistalsis of the oesophageal body (Triadafilopoulos et al, 1991; Richter, 2001). What causes motor dysfunction in achalasia is still uncertain (Boeckxstaens, 2005), but smooth muscle denervation may be responsible, as achalasia sufferers lack inhibitory ganglion cells in the myenteric plexus (Hirano, 1999). Immunohistochemistry staining shows that nitric oxide synthase (NOS) is absent from the LOS of achalasia patients. Nitric oxide is an inhibitory neurotransmitter which normally controls swallow-induced LOS relaxation and oesophageal peristalsis (Preikisatis et al, 1994; Hirsch et al, 1998; Sivaro et al, 2001). Through the production of cGMP, nitric oxide usually causes relaxation of the LOS and (Du et al, 1992; Konturek et al, 1997). The denervated LOS is therefore hypertensive and cannot relax normally in response to swallows. Furthermore, the normal swallow-induced wave-like peristaltic contractions are absent. Instead, low amplitude simultaneous contractions may occur. Some patients may be diagnosed with vigorous achalasia, when these simultaneous contractions are high amplitude (50 mmHg) (Mittal, 1997; Adler and Romero, 2001).

The result of achalasia is delayed oesophageal emptying through the LOS, resulting in residual food and fluids remaining in the oesophagus, which can cause irritation. Symptoms include chest pain which may be felt widely from the chest to the ears, teeth and fingers (Hopwood et al, 1984). The pain may occur at any time and can last minutes or hours. The lack of peristalsis causes dysphagia (difficulty swallowing). Later complications may include food regurgitation, weight loss and aspiration pneumonia (Boeckxstaens, 2005). Some infections or malignancies may cause pseudoachalasia, with similar symptoms. Achalasia is associated with an increased risk of developing oesophageal carcinoma, in particular OeSCC (Sandler, 1995).

Diagnosis is carried out manometrically and it is identified by a lack of primary peristalsis and increased LOS tone. A wet swallow results in failure of the LOS to open completely, or for sufficient duration, to allow passage of the bolus. All these

manometric findings in achalasia are highly reproducible. Radiography of a barium swallow will show a dilated oesophagus containing residual matter tapering distally to a narrow hypertensive LOS (see Figure 1.3).

Figure 1.3. The dilated oesophagus in achalasia, tapering to a narrow LOS, shown by X-ray of a barium swallow (Source: http://www.achalasia.info/images.html)

1.3.1.2 Treatment of achalasia

The target of achalasia treatment is predominantly the hypertensive LOS. There are pharmacological and non-pharmacological methods of treatment.

Pharmacological

a) Calcium channel blockers

Intracellular calcium is required by LOS smooth muscle for maintenance of tone. The oesophagus, and in particular the LOS region, also requires extracellular calcium for contraction. Ca^{2+} channel antagonists reduce entry of extracellular calcium into the smooth muscle cells, thus reducing LOS tone and decreasing amplitude and duration of oesophageal contraction (Mittal, 1997). The type 1 Ca^{2+} channel antagonists have mostly cardiac effects whereas type 2 antagonists (e.g. nifedipine (NIF)) are more potent to gastrointestinal smooth muscle (Mittal, 1997). The role of Ca channel blockers in achalasia treatment is not well defined but studies have shown some benefit. NIF administered sublingually has been found to decrease LOS pressure in a dose dependent fashion (Hongo et al, 1984); 10, 20, 30 and 40 mg doses reduced LOSp by 13%, 30%, 34% and 35% respectively in normal subjects.

Bortolotti and Labo (1981) found that NIF decreased LOS pressure and alleviated most of the dysphagia and chest pain in 20 achalasia patients. However, Gelfond et al. (1982) found that NIF only relieved symptoms in 8 out of 15 patients and that isosorbide dinitrate therapy was more effective. More recent studies by Tridafilopoulos (1991) showed that NIF can significantly lower the LOS pressure baseline in achalasia patients, but this decrease varied widely from 4% to 43% decrease, and failed to induce a symptomatic benefit as assessed by diary cards. NIF significantly reduced the amplitude of non-peristaltic contractions in the smooth muscle (distal) portion of the oesophagus but this effect was to varying degrees in different subjects. NIF does not affect the amplitude of contractions in the proximal striated region. In these studies, despite the manometric effect reported, no significant improvement of clinical symptoms was obtained. 20mg NIF in fact halved dysphagia and chest pain scores in only two patients. Although Traube et al. (1984) found that 20mg sublingual NIF decreased LOSp by 32% in achalasia patients, they observed poor clinical efficacy of sublingual NIF in treating achalasia (Traube and McCallum, 1985). During all the aforementioned studies, the NIF therapy was administered for systemic action.

b) Nitrates

It has been shown that oral and sublingual administration of nitrates relaxes the LOS smooth muscle. Nitrates release nitric oxide, one of the neurotransmitters responsible for relaxation of gastro-intestinal smooth muscle (Kummerle, 1994). An important nitrate in the treatment of oesophageal motor disorders is isosorbide dinitrate (ISDN). Sublingual ISDN significantly reduced LOS pressure in healthy subjects (Kikendall and Mellow, 1980) and in achalasia patients (Gelfond et al, 1982). In the latter study, there was a 47% decrease in LOSp after 30mg sublingual ISDN and a significant improvement in oesophageal emptying was observed.

c) Phosphodiesterase type 5 inhibitors

As mentioned in section 1.2.1.4, the phosphodiesterase type 5 inhibitor sildenafil (SIL) can precipitate GORD as a side effect in individuals without oesophageal motility disorders, by decreasing LOS pressure (Eherer et al, 2002). By the same mechanism, SIL has shown promise in the treatment of achalasia. In patients with

achalasia or a hypertensive LOS, a 50 mg dose of SIL infused directly into the stomach has been found to decrease LOS pressure (Bortolotti et al, 2000; 2002b). Lee et al. (2003) also demonstrated that a similar quantity of sildenafil (0.8 mg kg^{-1}) decreased LOS tone and prolonged swallow-induced LOS relaxation in healthy subjects. Additionally Zhang et al. (2001) found that sildenafil (1 mg kg^{-1}) significantly decreased basal LOS pressure in cats. The effects of sildenafil on oesophageal motility have been shown to persist for an hour or more after acute dosing (Rhee et al, 2001; Bortolotti et al, 2001; Eherer et al, 2002; Lee et al, 2003), and the reduction in LOS pressure suggests that sildenafil might be useful as a pre-mealtime treatment for achalasia patients, to enable passage of food into the stomach.

Non-pharmacological

Pneumatic Dilatation

Balloon dilatation may be used to open up the tight LOS. Dilatation of the LOS using solid or pneumatic dilators (e.g. a balloon) under endoscopic/fluoroscopic guidance may be used with varying degrees of long-term success. Approximately 60-80% of patients experience improvements after their first session (Koshy and Nostrant, 1997). Dilatation is a well-established technique but carries the risk of tissue perforation (5% risk) and bleeding (Mittal, 1997).

Surgery

Botulinum toxin is a highly potent natural toxin. It effectively blocks acetylcholine release from motor neurone synapses, causing muscle paralysis. When injected into porcine LOS smooth muscle, it can dramatically reduce LOS pressure (Pasricha et al, 1994). Endoscopically-guided injections of botulinum toxin directly into the LOS are used in treating achalasia, resulting in significant decrease in LOS pressure. Improved oesophageal emptying and symptomatic relief has been observed (Annese et al, 1995). The treatment involves four injections, each of 20 units of toxin. It is thought that numerous repeated injections over time can scar the LOS and make later surgery more complicated. There is some uncertainty regarding the duration of relief offered (Mittal, 1997; Vaezi et al, 1999). A surgical myotomy involves making incisions at the gastro-oesophageal junction to sever the muscle fibres causing oesophageal blockage. The procedure can result in scarring or be

incomplete and may have associated complications, particularly in elderly or otherwise debilitated patients (Triadafilopoulos et al, 1991).

1.3.2 Diffuse Oesophageal Spasm

Diffuse oesophageal spasm (DOS) is a spastic oesophageal disorder in which manometry findings include abnormal, intermittent, prolonged (>6 sec), swallow-induced, simultaneous contractions of the oesophageal body (Adler and Romero, 2001; Richter, 2001). More specifically, the disease is defined by the presence of simultaneous contractions after 20 percent or more of wet swallows (Clouse and Hallett, 1992; Richter, 2001). Sperandio at al. (2002) reported that the simultaneous contractions (spasms) in DOS patients are exclusively found in the distal smooth muscle segment. The symptoms of DOS are dysphagia and chest pain. The pain is highly variable in severity and duration (Richter, 2001) and is not only associated with swallowing or eating. Unlike achalasia, symptoms are typically non-progressive. Many patients also have some LOS dysfunction, this can be raised LOS pressure or impaired LOS relaxation, sometimes both (Mittal, 1997). Radiography of a barium swallow classically shows a corkscrew-like appearance during a simultaneous contraction of the oesophageal body (Fig. 1.4).

Figure 1.4. Corkscrew appearance of oesophagus in Diffuse Oesophageal Spasm shown by X-ray of a barium swallow.
(http://www.gastro-pro.org/motility/esophageal/clinicalapp/clinapp-07.html)

1.3.3 Nutcracker Oesophagus

In Nutcracker Oesophagus (NOes), also known as hypercontractile oesophagus, the patient has normal peristalsis but the swallow-induced pressure waves are of high amplitude (>180 mmHg) and duration (>6 sec) (Castell, 1995; Adler and Romero, 2001; Lee et al, 2003). This results in chest pain and dysphagia which are non-progressive and not associated with eating. Manometric diagnosis requires a mean distal peristaltic amplitude of >180mmHg. The LOS has normal relaxation but baseline pressure is elevated to above 40mmHg. Although the pathogenesis of NOes is unknown, it may result from an imbalance between the excitatory and inhibitory elements of oesophageal innervation (Lee et al, 2003).

1.3.4 Isolated hypertensive LOS

This is an uncommon manometric abnormality involving a raised LOS pressure (>35-40 mmHg) (Tamhankar et al, 2003), but otherwise there is normal oesophageal contraction amplitude, duration and peristalsis. This can cause mild to moderate dysphagia in the individual.

1.3.5 Treatment of spastic disorders

The three disorders, DOS, NOes and hypertensive LOS are also known as spastic disorders of the oesophagus (Mittal, 1997). Pharmacotherapy of these three disorders may be considered together as the symptoms and goals of treatment are similar. There is hyper-contractility of the LOS muscle, oesophageal body, or both, which require correction. The treatment options for these disorders are based on smooth muscle relaxant therapy. Many drugs used in achalasia are also used to treat these disorders. Table 1.1 summarises a few studies that have investigated the effects of drugs on oesophageal function.

In the study by Richter and Bozymski (1985), blood levels of NIF were twice as high after oral administration than after sublingual administration. This suggests that in normal subjects, sublingual absorption is apparently not as effective as absorption through the GI tract.

Dose (mg)	Effects found	Subject	Investigator
Verapamil 80 μg/kg	↓ distal oesophageal contraction amplitude, ↓ duration of contraction	Baboon	(Richter et al, 1982)
Diltiazem 90– 150 mg	↓ amplitude & duration of contraction	Healthy human	(Catteau Jr et al, 1991)
Diltiazem 90 mg	↓ amplitude (from 190 to 154 mmHg)	Nutcracker patients	(Catteau Jr et al, 1991)
Nifedipine 10/20/30/40 mg sublingual	13/ 30/ 34/ 35 % ↓ amplitude	Healthy human	(Hongo et al, 1984)
Nifedipine 10/20/30 mg oral	17/ 38/ 40 % ↓ amplitude	Healthy human	(Richter et al, 1985)
Nifedipine 20 mg oral	↓ LOS pressure baseline, ↓ amplitude	Healthy human	(Blackwell et al, 1981)
Nifedipine 20 mg oral	↓ amplitude & contraction frequency	DOS patients	(Blackwell et al, 1981)
Isosorbide dinitrate 10 – 20 m oral	Long term symptomatic improvement	DOS patients	(Orlando and Boyzmski, 1973)
Sildenafil 0.8 mg/kg	Prolongs swallow-induced LOS relaxation, ↓ amplitude	Healthy human & NOes patients	(Lee et al, 2003)

Table 1.1. Summary of some previous studies, illustrating the effects of different drugs on oesophageal motor function

Hydralazine relaxes arteriolar smooth muscle, and at 25mg taken four times a day, it is more effective than placebo in relieving symptoms of DOS (Mellow, 1982). Finally, work carried out by Miller et al. (1995) suggests that botulinum toxin, already used in achalasia treatment, could be useful in treating DOS, as it relieves dysphagia and chest pain. Richter (1982) had disappointing results using verapamil in treating non-cardiac chest pain in nutcracker oesophagus.

Nifedipine is used to treat these disorders in doses of 10 – 20 mg three times a day. Many patients have symptomatic relief with sublingual nitroglycerine (Mittal, 1997), and this also has been seen to correct manometric abnormalities in DOS (Orlando and Boyzmski, 1973).

1.4 Limitations of current therapies for oesophageal disorders

All current therapies used in GORD management either provide symptomatic relief (alginates) or reduce acidity of the refluxate (antacids, proton pump inhibitors, H_2 antagonists). No formulations are available which address the inherent cause of gastric reflux, i.e. the transient lower oesophageal relaxations. Neutralisation of stomach acid may make refluxate less acidic, and reduce discomfort caused by reflux, however pepsin and bile acids are still present, and the oesophageal epithelium is still under threat. Acid suppression may improve quality of life in the short-term but could allow disease progression to go unnoticed as the number of reflux events reported by the patient decreases but pepsin is still entering the oesophagus to cause damage.

For treatment of achalasia, botulinum toxin therapy is effective. However, it is inconvenient as the procedure needs repeating every few months (Storr and Allescher, 1999). Botulinum toxin injections into the LOS are invasive procedures and repeated injections may eventually result in scar tissue formation and fibrosation at the LOS (Bhutani, 1997). For treatment of achalasia, surgical procedures are often effective but dangerous in frail patients, with an associated risk of oesophageal perforation, and stricture. A non-invasive treatment would be safer and preferable. The chronic use of orally administered nitric oxide donors or sildenafil in patients with oesophageal motility disorders has been limited by variable efficacy and adverse effects (Gelfond et al, 1981; Traube and McCallum, 1985; Thomas et al, 1986; Bortolotti, 1999; Richter, 2001; Storr et al, 2001; Eherer et al, 2002). For example, side effects associated with high doses of nitrates include headache (Stacher et al, 1997). At 20- 30 mg doses nifedipine also has side effects including headache and hypotension in many patients (Mittal, 1997).

With the exception of alginate preparations and antacids, current drug therapies for the aforementioned diseases are systemic formulations. As mentioned at the start of this chapter, systemic doses may need to be higher than if the drug was targeted directly to the site of action. High doses of drugs are prone to causing the side effects that limit clinical use of a formulation, and a local effect may reduce this possibility.

1.5 Drugs of interest for the proposed delivery system

Three drugs were of interest during this project for their potential use in treating oesophageal motor disorders. These were isosorbide dinitrate, nifedipine and sildenafil.

1.5.1 Isosorbide dinitrate

ISDN is an organic nitrate, and a vasodilator with an action on both veins and arteries. Organic nitrates are converted to nitric oxide, one of the neurotransmitters responsible for relaxation of gastro-intestinal smooth muscle (Kummerle, 1994). It has been shown that oral and sublingual nitrates relax the LOS smooth muscle (Kikendall and Mellow, 1980; Gelfond et al, 1981,1982).

1.5.2 Nifedipine

Nifedipine (NIF) is used widely in the management of angina pectoris and hypertension (Kostewicz et al, 1996), Raynaud's Syndrome, congestive heart failure and cardiomyopathy. It is a lipophilic molecule, light sensitive, practically insoluble in water but soluble in ethanol. The drug is rapidly absorbed from the gastrointestinal tract (Kostewicz et al, 1996) and undergoes extensive first pass metabolism (Varshosaz and Dehghan, 2002). NIF is therefore a good candidate for a targeted drug delivery system which can bypass this first pass effect and improve bioavailability.

NIF inhibits transmembrane influx of Ca^{2+} into both cardiac muscle and smooth muscle, and affects oesophageal smooth muscle function in healthy individuals and patients with oesophageal motility disorders (Blackwell et al, 1981; Becker and Burakoff, 1983). However, there have been some disappointing results in clinical symptom reduction despite these manometric effects of nifedipine (Richter et al, 1982; Traube et al, 1989; Triadafilopoulos et al, 1991).

NIF is rapidly absorbed following oral administration, with detectable levels in serum 10 minutes after administration (Pfizer Inc., 2000). The bioavailability is proportional to dose and peak blood levels are reached within 30 minutes and its half life in plasma is approximately 2 hours. In formulations prepared during this

study, for example some polymer solutions and emulsions, NIF was often in suspension rather than fully dissolved, due to its very low aqueous solubility. Absorption of a drug through an epithelium occurs when the particle size is no larger than 2 μm diameter (Thanos et al, 2003). It may be helpful in further development of these formulations for drug particle size to be reduced. Such particle size reduction would also be expected to allow faster drug diffusion and release out of the vehicle, and hence a more effective formulation.

1.5.3 Sildenafil

Nitric oxide (NO) is an inhibitory neurotransmitter that controls relaxation of the lower oesophageal sphincter (LOS) and oesophageal peristalsis. It raises intracellular concentrations of guanosine 3',5'-cyclic monophosphate (cGMP) by activating soluble guanylyl cyclase (Shahin et al, 2000). Cyclic GMP then phosphorylates other intracellular signalling molecules that make smooth muscle cells less excitable. Cyclic nucleotide phosphodiesterases (PDEs) degrade and terminate the effects of cyclic nucleotides by the hydrolytic cleavage of their 3'-ribose-phosphate bond. PDE type 5 inactivates nitric oxide -stimulated guanosine cGMP (Moreland et al, 1998), making it an important modulator of the NO/cGMP signalling system (Lee et al, 2003).

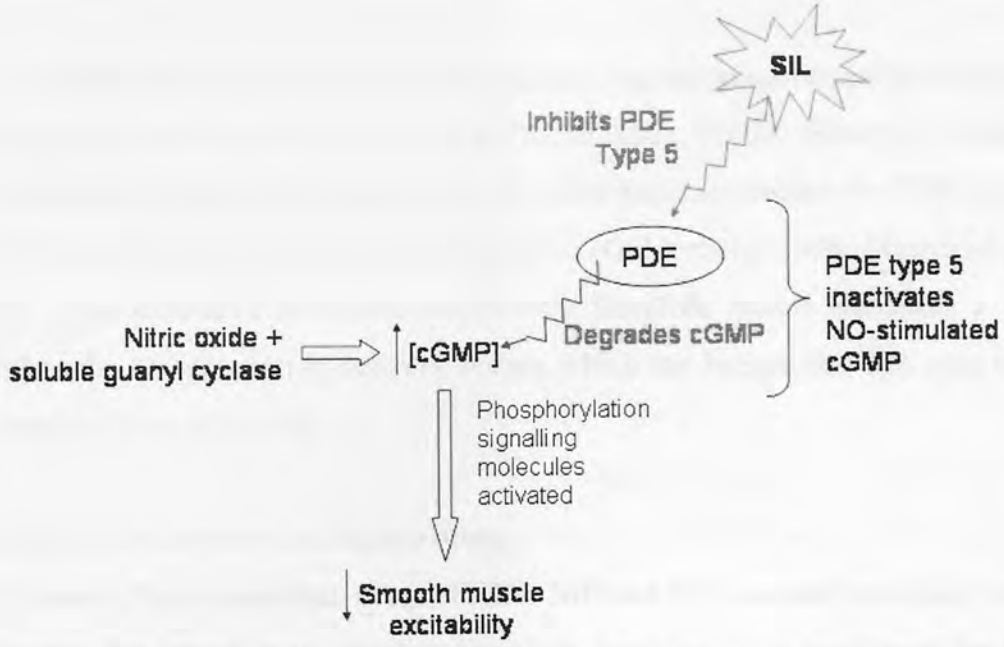

Figure 1.5. Illustration of the effect of SIL on decreasing smooth muscle excitation, via the inhibition of PDE type 5.

SIL selectively inhibits PDE type 5, thereby promoting cGMP accumulation, leading to smooth muscle relaxation and decreased smooth muscle excitability. This process is shown in the diagram in Figure 1.5.

Lee et al. (2003) showed that sildenafil prolongs swallow-induced LOSRs and decreases peristaltic wave amplitude in both healthy subjects and nutcracker oesophagus patients. Moreover, SIL can bring wave amplitudes of nutcracker patients into the normal range. As with the effects of nifedipine, SIL did not affect motor function of the striated proximal oesophagus in either group. The proximal striated muscle is controlled by a different mechanism to the NO-mediated cGMP signalling which occurs in smooth muscle. SIL may prolong swallow-induced LOSRs because cGMP generated by neuronal NO during swallowing is degraded more slowly when SIL is present (as SIL decreases the effects of PDE type 5).

The duration of the effect of SIL on oesophageal motor function has been investigated previously. One study found the onset of effects occurred within 10 minutes, being maximal around 15 to 20 minutes, with a duration of up to one hour following administration as a tablet (Bortolotti et al, 2001). A similar time course of the effects of SIL has been reported in achalasia patients (Lee et al, 2003). However, one study by Eherer (2002) found than the effects may endure for up to 8 hours.

SIL is rapidly absorbed, with maximum plasma concentration occurring around 30 minutes to 2 hours after oral dosing (Pfizer Inc., 2002). However, absolute bioavailability is about 40% due to extensive first pass metabolism by CYP3A4 and CYP2C9, and not due to incomplete absorption (Goldenberg, 1998; Muirhead et al, 2002). This extensive first pass metabolism therefore makes sildenafil a good candidate for a targeted drug delivery system which can bypass this first pass effect and improve bioavailability.

1.5.4 Pharmacokinetics of candidate drugs

As discussed, three candidate drugs, ISDN, NIF and SIL, are all promising agents for treating the oesophageal motility disorders; however, their usefulness has been limited in the past by side effects. Their high first pass clearance means that a large dose is required for the desired oesophageal effects if a systemic formulation is

used. The extent and rate of hepatic metabolism, that tends to limit their bioavailability is summarised in table 1.2.

Drug	% absorption	Metabolism	$t_{½}$ (hours)
isorsorbide dinitrate	Variable, 10 –90%. Average bioavailability ~ 25%	hepatic	1
nifedipine	Rapid & full. Bioavailability limited by first pass metabolism	hepatic	2
sildenafil	Approx. 40%	hepatic	4

Table 1.2. Summary of pharmacokinetics of candidate drugs

It is proposed that a targeted approach will require a smaller dose, as the first pass metabolism is avoided. It is predicted that this strategy will reduce side effects and could help to optimise the usefulness of these drugs in treating oesophageal motor disorders.

1.6 Bioadhesion

Bioadhesion is the attachment of a synthetic or natural material to a biological substrate for a prolonged period of time. Where the substrate is a mucous membrane the more specific term mucoadhesion may be used. A bioadhesive material must come into intimate contact with the tissue for bioadhesion to be possible. Several theories have been developed in an attempt to fully explain the phenomenon of bioadhesion. These theories are not mutually exclusive and bioadhesion/retention is likely to be due to the synergy of several mechanisms and cannot be explained by any single theory.

1.6.1 Theories of Bioadhesion

1.6.1.1 Interpenetration Theory

In 1963 Voyutskii first proposed this mechanism that describes molecular chain interpenetration in the interfacial region. This is a popular theory for describing bioadhesion of polymers onto a mucous membrane. The concentration gradient across the interface causes diffusion of bioadhesive chains into the mucous layer, and glycoprotein chains of mucous also diffuse into the bioadhesive. Chains from the mucous and the bioadhesive must interpenetrate to a sufficient depth to allow

formation of semi-permanent bonds. The diffused chains temporarily 'anchor' the mucoadhesive in place. Such chain interpenetration may be used as evidence of mucoadhesion (Jabbari et al, 1993). The rate of diffusion is dependent on a concentration gradient, chemical potential gradient, and polymer chain size. Small molecular weight chains and the chain ends take part in the interdiffusion process. If the polymer chains are too long, diffusion is slower and may even be too slow for the rate of mucous turnover. It is important that the adhesive and substrate are compatible and this is most likely to be achieved when the two have similar chemical structures. An uneven tissue surface assists bioadhesion by allowing penetration of the bioadhesive into tissue crevices. This theory is of questionable importance in un-crosslinked, non-polymer materials that demonstrate bioadhesive properties.

Factors affecting bioadhesion include polymer concentration. If a polymer is too concentrated in solution, the adhesive force can be reduced. Some polymers swell in contact with water and this can disentangle polymer chains leaving more of them free to interpenetrate. Swelling is water dependent and affected by polymer concentration. pH is also important as most polymers have an optimal pH for adhesion. Molecular 'adhesion promoters' (e.g. PEG chains grafted onto cross-linked polymers) are potential contributors to strong bioadhesion. Adhesion promoters may also be linear chains which are free for diffusion (De Ascentiis et al, 1995).

1.6.1.2 Electronic Theory

This theory describes the electrostatic forces between the adhesive and substrate. These are due to the chemical nature of cell surfaces and mucin, making them negatively charged at physiological pH (Woodley, 2001). Positively charged molecules are therefore likely to interact with the cell surface. The interface will have a double layer of electrical charge resulting from electron transfer upon contact of the adhesive and the substrate (Mikos and Peppas, 1990). This electron transfer is due to structural differences between mucous glycoprotein chains and the bioadhesive material. Bioadhesion is caused by attraction across the double layer of charge (Huang et al, 2000). This theory would suggest that charged materials should make the best bioadhesives, in particular cations. However the good bioadhesion of

some anions e.g. carbopols shows that other interactions are present. These may include hydrogen bonding, Van de Waals forces and hydrophobic interactions. Hydrophobic bonding occurs when non-polar regions associate with each other in aqueous solution due to the tendency of water molecules to exclude non-polar molecules.

1.6.1.3 Wetting Theory

The wetting theory of bioadhesion states that adhesion is dependent on the ability of a liquid to spread over a tissue surface (Mikos and Peppas, 1989; Chickering and Mathiowitz, 1999; Bruinsma and Sackmann, 2001). This theory would suggest that materials of low viscosity should be the most bioadhesive. This is an important theory in the description of adhesive materials without polymer chains capable of entanglement.

1.6.1.4 Adsorption Theory

This theory describes how within the interface between adhesive and substrate, bonds are formed (Mikos and Peppas, 1990; Chickering and Mathiowitz, 1999). The bonds may be either primary or secondary. Primary bonds are high strength, covalent and permanent and therefore not suitable for a drug delivery device. Secondary bonds include electrostatic forces, Van der Waals forces, hydrogen bonding and hydrophobic bonding. Hydrophobic forces occur when non-polar groups associate with each other in an aqueous solution, and are considered to be of great significance in bioadhesion (Mortazavi, 1995).

1.6.2 Adverse bioadhesion within the oesophagus

Tablets and capsules sometimes adhere and remain within the oesophagus for longer than the usual transit time, particularly if taken whilst recumbent or with insufficient liquid (Marvola et al, 1982; Swisher et al, 1984; Wilson et al, 1986; Higuchi et al, 2001; Perkins et al, 2001). Adhesion of tablets is especially common at anatomical narrowings of the oesophagus e.g. at the aortic arch level. Some coatings such as gelatin, PEG and HPMC are particularly prone to adhesion (Perkins et al, 1999; Mathias et al, 2002; Honkanen et al, 2002), and adherence is proportional to tablet size (Swisher et al, 1984; Perkins et al, 2001). Adhesion of a

tablet to the oesophagus can result in delivery of a high concentration of drug to a small mucosal area; this may result in damage causing dysphagia or stricture formation. Such injury was first described by Pemberton (1970). Drugs can cause local damage by caustic or acidic effects, heat production, hyperosmosis, impaired oesophageal acid clearance and other effects including the particular drug's toxic effects (Marvola et al, 1982). Commonly used medications such as tetracyclines, doxycycline and NSAIDs may cause such injury. Swallowed corrosives may destroy or temporarily damage the inner circular muscle, Auerbach's plexus and longitudinal muscle. Superficial lesions may stop peristalsis temporarily or even permanently. This adverse bioadhesion in the oesophagus has been well documented and concerns solid dosage forms, especially in the elderly (Perkins et al, 1994).

1.6.3 Beneficial bioadhesion within the oesophagus

Although retention of tablets in the oesophagus may be potentially harmful, adhesion of a drug delivery system could be extremely valuable for localised treatment of disease. Conditions which might be treated locally include all the aforementioned diseases. If an orally delivered formulation were able to adhere to the oesophagus then this would increase the available time for absorption and interaction of a drug with the epithelium. A sufficiently bioadhesive system might be able to withstand oesophageal peristalsis which usually is effective in displacing luminal boluses. A mucoadhesive drug delivery device can help maintain the drug between effective and toxic levels, prevent dilution of drug in the body fluids and allow targeting of the drug to a desired site (Huang et al, 2000). Another advantage is that drugs absorbed through mucosal surfaces can directly enter the bloodstream and avoid inactivation by enzymes in the gastrointestinal tract (Park and Robinson, 1984). Localised targeting by bioadhesion may allow a lower drug dose to be administered, increase treatment efficacy and cost effectiveness (Ch'ng et al, 1985). However there is likely to be variability in oesophageal tissue between individuals and especially so in patients with reflux or motility disorders. Therefore, it may be difficult to predict treatment outcome.

1.6.4 Bioadhesives

A mucoadhesive must be soluble in mucous for good adhesion to be possible. A material with similar structure to the mucous on the substrate surface can achieve this solubility, and it is therefore hypothesised that bioadhesive polymeric devices might benefit from containing hydroxyl, carboxyl, amines and sulphate groups (Huang et al, 2000). It is thought beneficial for a bioadhesive system to bind to surface structures of the absorptive epithelial cells themselves (e.g. glycoproteins and glycolipids on the apical surface), but preferably not to mucous (Lehr, 1992). The oesophageal lumen is coated with an unstirred water layer, which contains mucus, but this mucous is not a well-defined adherent layer as found in the stomach. The mucous present is mostly derived from swallowed saliva, and therefore has a rapid turnover (see Section 1.1.1.). For this reason, it is bioadhesion/ retention on the tissue surface itself, and not mucoadhesion to a mucous layer, that is of interest in this project. However, this interaction is likely to follow the theories of bioadhesion.

Bioadhesives tend to hydrate and swell in contact with water. Bioadhesive materials so far identified tend to be high molecular weight polymers e.g. poly(acrylic acid) amd HPMC (Nagai, 1985; Gu et al, 1988; Lehr et al, 1990; Potts et al, 1997b). Polycations are often associated with high toxicity due to local regions of high cationic charge density and non-biodegradability (Fischer et al, 2003). Polyanions are considered be better than polycations for toxicity reasons (Duncan, 2003). However toxicity of high molecular weight polymers in an oesophageal drug delivery system is not a concern as they are not absorbed (see below, within Section 1.6.4). Poly(acrylic acid) is strongly bioadhesive and is a polyanion. Gelatine is adhesive and a neutral polymer. However although neutral polymers may have good bioadhesive potential, they are often easily washed off the adhesion site. Hydrogels, polycarbophils and carboxymethylcellulose are all reported to be bioadhesive (Smart, 1984; Mathiowitz, 1997; Bernkop-Schnurch, 1999; Huang et al, 2000).

Over a decade ago, Ito et al. (1990) used ferrite and a dye with polymer as an adhesive/releasable delivery system to deliver drugs directly to the oesophagus for cancer chemotherapy. A similar approach was investigated by Nagano et al. (1997). Other groups have used polymers to target the oesophagus, some of which have

inherent bioadhesive properties (Batchelor et al, 2004; Richardson et al, 2005). Others gel in response to stimuli e.g. pH or temperature changes, to delay transit once they reach the oesophagus (Potts et al, 1997a; Potts et al, 1997b; Bromberg and Ron, 1998; Soppimath et al, 2002).

Carbopol polymers and Noveon polycarbophils are well documented as being bioadhesive (Ch'ng et al, 1985; Gu et al, 1988; Lehr et al, 1990; Solomonidou et al, 2001; Kockisch et al, 2004). Carbopol polymers, Pemulen polymeric emulsifiers and Noveon polycarbophils are very high molecular weight polymers of acrylic acid, crosslinked with polyalkenyl ethers or divinyl glycol (Noveon Inc, 2002) Acrylic-based hydrogels form highly adhesive bonds (Park and Robinson, 1985; 1987). The polymers swell up to 1000 times their original volume in water to form a gel and this swelling provides a large adhesive surface area for maximum contact with the mucous membrane. The exact mechanism by which poly (acrylic acid) polymers interact with mucin is not yet understood. Noveon, Inc. pharmaceutical polymers are safe for oral human use and approved by many international pharmacopoeias. They might also protect protein and peptide drugs from degradation in order to increase bioavailability (LueBen et al, 1995; 1996).

Another material which has previously shown bioadhesive strength is Smart Hydrogel®. In aqueous solution, Smart Hydrogel® is fluid at room temperature. It is thought to be bioadhesive and forms a gel at body temperature (see Section 3.3.2), (Potts et al, 1997a), and therefore is an interesting potential candidate for targeting the oesophagus (Potts et al, 1997a; Potts et al, 1997b). Alginates are natural polymers that are also well documented bioadhesives (Smart, 1984; Batchelor et al, 2000; 2002).

The polymers used to create drug delivery vehicles for this study were alginates, poloxamer, polycarbophil, and Smart Hydrogel®. These polymers all have high molecular weights. Because of their large molecular weights, the polymers themselves are not likely to be absorbed and to exert undesired systemic effects (Lehr, 1994a). The literature states that polycarbophil is not absorbed (Singla et al, 2000), and alginates are not absorbed (Del Buono et al, 2000). Toxicity of high

molecular weight polymers in an oesophageal drug delivery system is not a concern due to the lack of absorption.

1.6.5 Measurement of bioadhesion *in vitro*

Bioadhesion is difficult to measure. Tests vary widely depending on the factors considered when designing the test, and it is difficult to extrapolate from *in vitro* testing to the *in vivo* performance (Mortazavi and Smart, 1995). *In vivo* situations have constantly varying conditions whereas *in vitro* tests are usually performed in a controlled environment. For example, *in vitro* work does not take into account the rate of mucous turnover, and so *in vitro/ in vivo* correlations may be difficult. There are often significant discrepancies between studies giving information on the relative adhesive properties of materials (Mortazavi and Smart, 1995).

Extent of adhesion may be studied using Fourier transform infrared (FTIR) spectroscopy and microscopy (Edsman and Hagerstrom, 2005). Tensiometers may also be used to measure the strength of adhesion for solid mucoadhesive dosage forms, measuring work of adhesion or maximum detachment force; the work of adhesion has been considered the best measurement for assessing bioadhesion (Ponchel, 1987). Most existing oesophageal *in vitro* models are still lacking in peristaltic simulation and in most *in vitro* models, the question is whether adhesion to the substrate, or, cohesion of the material to itself, is actually being measured.

1.6.6 Measurement of oesophageal transit *in vivo*

In a clinical situation, oesophageal transit may be assessed by an X-ray study of a swallowed suspension of barium sulphate. However, barium is dense and may have a faster transit than more physiological substances. An additional problem with this method is the high radiation dose carried by an X-ray study. Gamma (γ) scintigraphy is now the method of choice for monitoring transit of a suitably radiolabelled pharmaceutical dosage forms in the gastro-intestinal tract (Wilding et al, 2001; Newman et al, 2003). Gamma scintigraphy can provide information about the movement and fate of a material after administration. The radiation dose is smaller and more easily quantifiable than X-ray studies. Some of the formulations produced in this study were tested *in vivo* in humans using γ-scintigraphy to measure their bioadhesion in the oesophagus (Potts et al, 1997a) (see Chapter 6).

1.7 Rationale for a bioadhesive liquid delivery system targeted to the oesophagus

As mentioned previously, there are no existing GORD treatments that address the underlying cause of reflux. A simple approach to preventing the oesophageal damage that causes metaplasia, dysplasia and carcinoma is to prevent reflux occurring through the LOS. This could be achieved by a targeted drug delivery system which inhibits the transient lower oesophageal sphincter relaxations. Motility disorders of the oesophagus are treated with varying degrees of efficacy and risk using surgical intervention and systemic drugs. A novel strategy in treatment of achalasia, nutcracker oesophagus and diffuse oesophageal spasm would be an oral liquid drug delivery system that adheres to the oesophageal surface. No previous literature examines the use of targeted non-invasive therapies in treatment of these diseases. For treatment of oesophageal diseases, the novel drug delivery strategy proposed is the use of an oral liquid, which undergoes prolonged retention within the oesophagus and delivers drug at this site. After swallowing, the liquid formulation will be retained in the oesophageal lumen, to maximise the intimacy of contact with the mucosa. Although targeted drug delivery to the oesophagus is a challenge, an ideal formulation will resist normal rapid clearance from the oesophageal lumen by swallowing, peristalsis and gravity. The transit time of a swallowed bolus is of the order of 7 to 8 seconds (Kjellen et al, 1984; Chadhuris et al, 2000; Tutuian et al, 2003). However, with disordered peristalsis, gravity still propels a bolus from mouth to stomach in under 1 minute (Orlando, 1991). Strongly bioadhesive liquids would be expected to overcome the peristaltic and gravitational forces, allowing the drug delivery system prolonged residence time within the oesophagus. The formulation will then have more opportunity for intimate contact with the epithelium, during which time drug could diffuse out of the formulation and be absorbed into the target tissue. The resultant effect therefore could be, for example, a direct action on smooth muscle. Prolonged contact of swallowed, adhered liquid with the oesophageal mucosa must not cause any caustic damage, as observed when tablets adhere in the oesophagus (see Section 1.6.2). The vehicle should release the drug quickly and to a sufficient extent whilst retained at the target site.

Bioadhesive or retentive drug delivery systems have been proposed with a variety of applications in the eye, nasal, buccal and vaginal cavities, and GI tract. However delivery specifically to the oesophagus is an area which has received little coverage in the past. Most other attempts in this area have addressed the need for a protective coating or 'bandage' on the oesophagus (Dettmar et al, 1986; Tang et al, 2005) rather than being systems to deliver drugs into the oesophageal smooth muscle directly. Potts et al (2000) described the concept of oesophageal 'bandaging' for symptomatic treatment and improved healing in GORD. Solutions of sodium alginate can adhere to the oesophageal epithelium *in vitro* for up to 60 min (Batchelor et al, 2002). Tang et al. (2005) reported promising results with such a system, showing that an alginate layer coating the oesophagus can significantly decrease porcine gastric pepsin and hydrochloric acid diffusion to oesophageal epithelium *in vitro*. Such a protective coating could limit the contact of refluxate with the oesophageal epithelial surface.

In summary, the use of a bioadhesive liquid drug delivery system targeted to the oesophagus may allow a lower dose to be used in treating oesophageal motility disorders and hence reduce the potential side effects. The strategy may also be used to form a protective coating over the oesophageal epithelium.

1.8 The aim of this study

The aim of this work was to develop a liquid oral drug delivery system to target the oesophagus for the purpose of treating oesophageal disorders. The delivery system must undergo prolonged retention within the oesophageal lumen where allows drug diffusion out of the formulation, so that it may be absorbed by the target tissue. A variety of vehicles were assessed, abandoning the common assumption that all liquid bioadhesives are polymer solutions. Formulations were tested for their bioadhesive potential *in vitro* and *in vivo*, and the formulations with the best retention and drug release properties were examined. The effect of formulations containing active ingredient on oesophageal motor function in healthy humans was also assessed.

Bioadhesion was measured *in vitro* by the duration of liquid retention on porcine oesophageal mucosa during continuous washing, using an adaptation of the method described by Batchelor et al (2002). Bioadhesion *in vivo* was tested by imaging the transit of liquids through the human oesophagus using gamma scintigraphy in healthy volunteers. Candidate drugs for the delivery system included isosorbide dinitrate, nifedipine and sildenafil. Release of model drug from the various formulations was tested *in vitro* using Franz-style diffusion cells. The effects of promising formulations on healthy oesophageal motor function were assessed *in vivo* by manometric methods, to investigate the feasibility of the system for treating oesophageal motor disorders.

Chapter 2: Materials and Methods

Chapter 2

Materials and Methods

Materials

2.1 Material sources

The following chemicals were all purchased from Sigma, Dorset, UK: Ammonium acetate solid, calcium carbonate, calcium chloride, calcium phosphate, glycerol, heavy mineral oil, lemon oil, light mineral oil, magnesium stearate, methylparaben, olive oil, potassium dihydrogen phosphate, phosphoric acid, proplyparaben, sodium chloride, sodium dihydrogen phosphate, sodium hydroxide pellets, sorbitol 70, sucrose, triethylamine, Tween® 80, Tween 85®, xanthan gum, isosorbide dinitrate laboratory grade (40% with lactose), nifedipine, propranolol, D-mannitol, dextran (average mol wt 10,000), ^{14}C-labelled mannitol, ^{3}H-labelled propranolol, ^{14}C-labelled dextran (10,000 mw).

Scintillation fluid used was Optiphase HiSafe, purchased from PerkinElmer. Technetium 99m tin colloid Hepatate was supplied by the Radiopharmacy Dept., Birmingham City Hospital NHS Trust, UK. Holdings, use and disposal of all radioactive materials were recorded on Isostock® software (Gillett Partnership).

Antepsin® sucralfate suspension (Wyeth, Taplow, UK) was purchased from a local pharmacy. Poloxamer 407 (Pluronic Lutrol 127NF) was a gift from BASF, Germany. FMC Biopolymer (Philadelphia, USA) supplied sodium alginates LF120M and LF200S as a gift. Smart Hydrogel® powder was supplied as a gift from Advanced Medical Solutions, Cheshire, UK. Noveon AA1® was a gift from Noveon Inc. (Ohio, USA). Dimethicone 300,000 mPa.s viscosity was supplied by GE Bayer Silicones (Leverkusen, Germany). Viagra® 100mg tablets (Pfizer) were purchased from AAH Pharmaceuticals Ltd (Coventry, UK). HPLC grade solvents (acetonitrile and methanol) were supplied by Fisher Scientific, Loughborough, UK.

2.2. Rheometry

Viscosity is a measure of the internal friction of a fluid that occurs when a layer of fluid is made to move in relation to another layer. The amount of force required to produce movement is called shear, and highly viscous fluids require more force to move than lower viscosity fluids (Marriot, 1988). Isaac Newton assumed that the viscosity of all materials, at a given temperature, is independent of the shear rate, a behaviour called "Newtonian", however not all fluids behave in this way. Water is one example of a Newtonian fluid. Non-Newtonian fluids are more common, and they include the shear-thinning materials. These display a decreasing viscosity with increasing shear rate, for example paints or toothpaste. Dilatant, or shear-thickening materials are less common, and demonstrate an increasing viscosity with increasing shear rate e.g. a paste of cornflour in water. Other effects that may be seen upon viscosity measurements are thixotropy, when the material undergoes a decrease in viscosity with time, whilst subjected to constant shear. Rheopexy is the opposite, more rare behaviour, where viscosity increases with time upon application of constant shear.

A TA Instruments AR1000 rheometer with a 60mm diameter metal cone (hard anodised aluminium) with 1° angle and 31μm truncation was used to measure the viscoelastic properties of some of the materials that demonstrated a good bioadhesive profile. Acquisition of rheological data enabled an investigation of the relationship between viscoelastic properties and retention of liquids {Tamburic, 1995 69 /id}. Overall viscosity of each formulation was measured by controlled stress measurements at a constant shear rate of 10 s^{-1}. This shear rate was selected as it is comparable to the physiological forces of swallowing and draining under gravity (Barnes et al, 1989). All solutions were measured at a temperature of 25° C. Evaporation and drying at the edge of the sample may lead to large errors in the measured torque, as the effect is maximal at the greatest distance from the centre of rotation, therefore a solvent trap was employed to prevent sample evaporation over time.

In small angle cone and plate geometry, the velocity at any point on the rotating surface is equal to $r\omega$, where r= the distance from the centre of rotation and ω= the rate of rotation. If the angle is <4° as used here, the distance between the cone and plate becomes $r\phi$ where ϕ= the angle between the cone and plate in radians.
Due to the shape of the cone geometry, the shear rate (s^{-1}) is the same at any point on the plate, and may be expressed as in equation 2.1.

$\frac{r\omega}{r\phi}$ or $\frac{\omega}{\phi}$ Equation 2.1. Expression for shear rate.

The shear stress σ (dynes.cm^{-2}) is then calculated as in Equation 2.2.

$\frac{3T}{2\pi a^3}$ where T= torque and a= radius of plate. Equation 2.2. Expression for shear stress.

The viscosity of the sample, η, in Pa.s, is calculated by dividing shear stress by shear rate.

2.2.1 Elastic and Viscous Moduli

The elastic modulus, also known as the storage modulus, G', is a measure of the energy stored and recovered per cycle of oscillatory deformation, and it reflects the solid-like component of the visco-elasticity of the material. The viscous modulus, also called the loss modulus, G", reflects the liquid-like properties of the material. The ratio of viscous to elastic moduli is a useful description of the overall elasticity of the sample. It may also be called the loss tangent (tan δ), where tan δ = G"/ G', and a higher numerical value of tan δ indicates a lower degree of elasticity.

2.2.2 Shear Methods

Shear measurements involve a rotational motion of the cone above the plate, with the sample in between. During peak hold measurements, the temperature was constant at 25°C. The applied shear rate was held at $10s^{-1}$ for 60 s. This type of experiment was used to assess the overall viscosity of the formulations. For continuous ramp measurements, temperature was constant at 25°C; shear rate was ramped from 0.01 to 100 s^{-1} over 1 minute, and data were plotted logarithmically.

This data revealed any shear dependent properties of the formulations and enabled identification of the linear region within this shear range.

2.2.3 Oscillatory Methods

Oscillatory measurements involve very small movements of the cone over the sample on the plate, rather than rotating in full circles as in shear measurements. This non-destructive measurement allows the internal structure of the material to be investigated. Two important parameters that can be measured using oscillatory techniques are the elastic modulus and viscous moduli. During stress sweep measurements, temperature was kept constant at 25°C and the frequency held at 1 Hz. Torque was gradually increased from 1 – 100 μNm and data recorded logarithmically. For temperature ramp measurements, the temperature was increased from 5 – 40°C at a rate of 10°C/min. A single frequency of 1Hz was used and torque was controlled at 10 μNm.

2.3 Surface tension

The surface tension of the formulations is potentially an important factor in the spreading and subsequent bioadhesion of liquids. This is discussed further in Section 4.4.5. Surface tension of the liquid formulations was measured using a Camtel Ltd QCT-100 machine. Data was generated using the CIT 100S (Mk2) software. The surface tension measurements were performed using a Wilhelmy plate system. The measurements are made on the principle that if the lower end of the plate, micro-roughened to improve its wettability, is brought into contact with the surface of a liquid, the effect will be to pull the plate into the liquid. This pulling force is measured and represents the surface forces of that liquid. The plate approached the liquid at 0.2 mm/s and accepted a 25 mg change as indication of plate contact with the surface. Once contact was identified, the plate was immersed to 2 mm depth within the liquid, and then raised again to the surface point at 0.2 mm/s. The plate then was held at the surface for 100 seconds to measure the surface tension.

The known density values of air (1.300 g cm^{-3}) and water (0.9986 g cm^{-3}) were used as reference values for the light phase and heavy phase respectively, to allow the

software data analysis. Surface tension measurements were recorded and had the units mN/m.

2.4 HPLC analysis

High performance (pressure) liquid chromatography (HPLC) is a useful tool to investigate identity, quantity and resolution of a compound. HPLC was used during this project for measuring drug concentrations within formulations, and to analyse samples collected during Franz cell drug release studies. Detection settings were optimised until a clean peak of the sample was observed on the chromatograph. The variables that were altered systematically during optimisation of each protocol were the mobile phase, flow rate and UV detection wavelength of the system. Once the protocol had been optimised, a series of known compound concentrations were injected into the HPLC, giving a series of peaks correlating to the concentration of the injected compound. The area under each peak was calculated and this data was used to generate a calibration curve (concentration of sample in solution against peak area). The best-fit line of the calibration curve should be a straight line $y = mx + c$ with a good fit. The calibration curve was then used to calculate concentrations of unknown samples from their peak area on the chromatogram. For all optimised HPLC methods, the linear correlation coefficient was greater than $R^2 = 0.95$ each time. Some examples of calibration curves for each drug are displayed in Appendix 2A. A calibration was performed prior to sample analysis on each study day to minimise the effects of inter-day variation. The calibration data was used to assess drug concentrations within unknown samples from the Franz cell receiver fluid (Chapter 5).

2.4.1 HPLC Methods

HPLC analysis was performed using an Agilent HP1100 series quaternary pump in conjunction with an HP 1100 series UV detector and autosampler. The column used was a Hypersil ODS-2, 150mm × 4.7mm (Thermo Electron Corporation). The mobile phases for all methods were filtered through a scintered glass funnel, de-gassed by sonication and flushed through the HPLC for 30 minutes to equilibrate the system.

2.4.1.1 Isosorbide dinitrate HPLC method

HPLC analysis of isosorbide dinitrate was performed using a method developed following experimentation with methods by Baaske et al. (1983) and Olsena and Scroggins (1984). Mobile phase was acetonitrile:water (60:40), and the flow rate was 1 mL/min. Injection volume was 25µl. UV detection wavelength was 215 nm and temperature was controlled at 20°C. The retention time for isosorbide dinitrate was a little over three minutes.

2.4.1.2 Nifedipine HPLC method

Due to the photosensitivity of NIF, all vials were covered in aluminium foil to reduce the risk of drug degradation. A method for nifedipine HPLC analysis was optimised by adaptation of the methods described by Zarghi et al. (2000) and Shahriyar and Lau-Cam (2000). For HPLC analysis, the mobile phase consisted of methanol: ammonium acetate (0.04M): acetonitrile: triethylamine (400:400:200:8), and the flow rate was 1mL/min. The injection volume was 25µl and a UV detection wavelength of 235nm was used. The temperature was controlled at 20°C. The retention time for nifedipine was approximately 3.5 minutes.

2.4.1.3 Sildenafil HPLC method

The method used for sildenafil was optimised following variations on the method described by Dinesh et al. (2002). HPLC mobile phase was acetonitrile:water (95:5), and the flow rate was 1.5 mL/min. Temperature was maintained at 20°C, the injection volume was 10µL, and detection wavelength was set at 245 nm. The retention time for sildenafil was approximately eight minutes.

2.5 Methods for quantifying radioactivity

2.5.1 Gamma (γ) counting technique

The retention studies performed in this work required the liquids to be labelled. The radioactive label chosen was Tc99m. Washings from the retention apparatus were collected over designated time periods, directly into scintillation tubes. At the end of each experiment, all samples were analysed together in one batch in order that they were counted within a few minutes of each other. This minimised the effects of

radioactive decay over time, as Tc99m has a short half- life (approximately 6 ½ hours). The gamma counter used was a Packard Instruments Cobra Auto-Gamma machine, and data were generated in terms of counts per minute within each sample.

2.5.2 Scintillation (β-counting) techniques

Some of the formulations used in the Franz cell drug release studies contained a radiolabelled drug, which could be identified in samples from the receiver chamber by counting the radioactivity. Both radionuclides involved (tritium and ^{14}C) are β-emitters. The 1 mL samples from receiver medium were collected at designated time points directly into scintillation tubes. 4mL of scintillation cocktail Optiphase HiSafe® was added to the tube, sealed, and mixed thoroughly by vortexing. The samples were counted using a Packard Instruments model 1600 TR machine, and counts per minute generated. These data were compared to a reference sample of the donor material, to assess drug release over time.

2.6 Extraction of sildenafil from Viagra® tablets

Sildenafil (SIL) was extracted from Viagra® tablets for use in the studies. The blue film coating was carefully removed from a Viagra® tablet (100mg active ingredient) using a scalpel. The uncoated tablet was then dissolved in 40 mL distilled water. SIL has an aqueous solubility of approximately 3.5 mg/mL. The 100 mg SIL dissolved in 40mL therefore achieved a SIL concentration of 2.5 mg/mL. The remaining excipients of Viagra tablet (microcrystalline cellulose, calcium hydrogen phosphate, croscarmellose sodium, magnesium stearate) are relatively water insoluble and remained in suspension. The mixture was centrifuged and the supernatant was filtered through filter paper (Grade 1 type, 11 μm pore, Whatman, UK) to remove these insoluble excipients. The resulting aqueous solution was assigned a nominal concentration of 2.5 mg/mL sildenafil and was used either diluted or undiluted in the subsequent preparation of formulations for retention and drug release studies.

2.7 Preparation of oil in water emulsions

A wide range of emulsions prepared were variations on those described in US patent 5,670,163 (Cuca et al, 1999), which states that emulsions may be bioadhesive for up to 8 hours. A variety of oils and surfactants were used in the manufacture of these emulsions. The surfactants used were Span 80, and Tween® 20,65, 80 and 85. The oils used were liquid paraffin, castor oil, heavy mineral oil, light mineral oil, olive oil, soybean oil, sunflower seed oil, safflower seed oil and lemon oil. Nut oils were avoided due to their potential allergenic properties.

In addition to the oil, water and surfactant, each emulsion also contained sorbitol 70 as sweetener and methylparaben and propylparaben as anti-microbial preservatives. The composition of the emulsions is shown in Table 2.1.

	% w/v
Water	72.8
Oil	10.0
Surfactant	9.0
Sorbitol 70	8.0
Methylparaben	0.13
Propylparaben	0.07

Table 2.1. Composition of the oil and water emulsions

The water was stirred and heated to 60– 70°C in a 100 mL beaker on a magnetic stirring plate, and then the Sorbitol 70 was added while continuously stirring until dissolved. The parabens esters were added to this aqueous phase, and dissolved whilst stirring and maintaining the temperature for approximately 15 minutes. The aqueous phase was then cooled to 35°C or below before use. The oil and surfactant were mixed at room temperature in a separate beaker using an overhead propeller mixer for about 15 minutes to ensure satisfactory mixing. The cooled aqueous phase was slowly added to the oil phase, stirring constantly using the overhead propeller mixer set at 800 rpm. Following the addition of the aqueous phase, mixing was continued for 15 minutes to allow complete homogenisation of the emulsion.

2.8 Preparation of the silicone-based emulsion

A silicone-based emulsion was prepared based on an example described in US patent 5,711,936 (Hill et al, 1998). In brief, poloxamer 407 (89% w/v) was heated at 70-80°C until molten, before lemon oil (1% w/v) and dimethicone (10% w/v) were added. This was stirred for 90 min whilst maintaining the temperature. The resulting silicone phase was dispersed in an aqueous solution that contained sweeteners (sorbitol, sucrose and glycerol) and thickening agents (xanthan gum) (Table 2.2).

Constituent	% w/w
Dimethicone/ poloxamer mix	2.0
Distilled water	84.5
Sorbitol 70	5.0
Sucrose	5.0
Glycerol	3.0
Xanthan gum	0.5

Table 2.2. Composition of silicone-based emulsion

2.9 Method of preparation of polymer solutions

Noveon AA1® powder (0.1% w/v) was dispersed in double distilled water and mixed until fully dissolved using a propeller mixer. The pH was accurately measured using a calibrated Sartorius pH meter and adjusted to pH 6.0 or 7.0 as required by drop-wise addition and thorough mixing of concentrated sodium hydroxide solution. A 2% w/v solution of the alginate LF120M was prepared by gradual addition of the powdered solid to distilled water under constant stirring at 800 rpm by an overhead propeller mixer. Smart Hydrogel® (2% w/v) was dispersed in distilled water and thoroughly mixed using a propeller mixer. The pH was measured and adjusted as before to 4.7 or 6.0, or left unadjusted at a pH of 3.6. Solutions of poloxamer 407 were made by dispersing the solid in cold double distilled water to a concentration of 20% w/v and stirring until dissolved. All the polymer solutions were prepared at a mixing speed of 800 rpm.

2.10 Preparation of blends of bioadhesive polymers

In addition to the individual polymer solutions previously mentioned, two solutions were prepared that incorporated a physical mixture of bioadhesive polymers (alginates and polycarbophil) with a thermally responsive component (poloxamer 407). These formulations were prepared in 100 mL beakers using an overhead rotary mixer, with 800 rpm mixing speed. The solid materials were added to water whilst stirring continuously. The resulting vehicles were called blend A and blend B and their respective compositions are detailed in Table 2.3.

	% w/v				
	Alginate molecular weight		Noveon AA1®	Poloxamer 407	dH_2O (mL)
	Medium	High			
Blend A	2.35	-	0.10	10.00	87.55
Blend B	-	2.00	0.10	10.00	87.90

Table 2.3. Composition of polymer blends A and B

2.11 Preparation of drug-loaded formulations for Franz cell experiments

In addition to the manufacture of vehicles alone, formulations were prepared that contained model drugs. These were used to assess drug release from the liquids *in vitro*. Two methods of formulation preparation were used, depending on the method of drug detection to be used in sample analysis (radioactivity counts or HPLC).

2.11.1 Preparation of emulsions containing dextran, mannitol and propranolol

For drug release from emulsions containing dextran (DEXT), mannitol (MANN) and propranolol (PROP), analysis was performed by measurement of a radioactive label. The three drugs could be purchased pre-labelled. DEXT was purchased with ^{14}C radionuclide incorporated. MANN and PROP were tritiated (^{3}H- labelled). Both ^{14}C and ^{3}H are low energy β- emitters (emax = 0.156 MeV and 18 keV respectively), with long half lives (5730 and 12.26 years respectively). This made them relatively safe labels for handling outside of the body, in a designated working environment, with standard protective clothing.

The aqueous phase and the oil phase of each emulsion were prepared as before (see section 2.7). Standard non-radioactive drug was incorporated into the aqueous phase (for MANN and DEXT) or the oil phase (PROP) in amounts necessary to produce a concentration in the final emulsion of 2.5 mg mL^{-1}. Small quantities of the radioactive drugs were then used to 'spike' each formulation; these materials were mixed directly into the emulsions, in sufficient quantities to allow a total starting activity in each Franz cell of 5kBq. This level of radioactivity would be equivalent to 300,000 disintegrations per minute (dpm) in the donor of each Franz cell at the start of each experiment. This amount was sufficient to allow detection of very small (less than one percent) amounts of the added drug in samples from the receiver compartment. Hydrophilic DEXT or MANN was incorporated into the aqueous phase of the emulsions, whereas liphophilic PROP was incorporated into the lipid phase. The two phases were then combined and the emulsion prepared as before (section 2.6). The quantities of radiolabelled drug added were small enough to avoid significant alteration of the final drug concentration in the emulsion, but large enough to be detected if small amounts diffused in the Franz cell receiver fluid (at least 5kBq in each starting Franz cell). The radiolabelled drug was stirred thoroughly into the relevant phase, before the two phases were combined and the emulsion prepared. The emulsification process was the same as for preparation of emulsions for retention studies.

2.11.2 Preparation of emulsions containing isosorbide dinitrate, nifedipine and sildenafil

For formulations containing isosorbide dinitrate (ISDN), nifedipine (NIF) and sildenafil (SIL), analysis of Franz cell samples was carried out using HPLC analysis to determine the drug concentration in the receiver. HPLC methods are detailed in section 2.4.2. The emulsions were prepared as described in section 2.7, but the drugs were incorporated before the aqueous and lipid phases were combined. Hydrophobic NIF powder was incorporated into the oil phase, and the more hydrophilic ISDN powder was incorporated into the aqueous phase. NIF and ISDN were added in the amounts necessary to achieve emulsions with drug concentrations of either 1.0 mg mL^{-1} or 2.5 mg mL^{-1}. The emulsions containing SIL were prepared by using the extracted SIL stock solution (see section 2.6), diluted with distilled water as necessary, for the aqueous phase, instead of water alone. The low

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concentration emulsion was prepared with a final SIL concentration of 1.0 mg mL^{-1}. The high SIL concentration was only 2.0 mg mL^{-1}. This was the highest concentration that could be achieved using the 2.5 mg mL^{-1} SIL stock solution, as the aqueous phase became diluted with the oily phase during the emulsification process.

2.11.3 Preparation of polymer formulations containing dextran, mannitol and propranolol

For Franz cell drug release experiments, drugs were also incorporated into polymer formulations. The polymer vehicles used were Noveon AA1® polycarbophil and Smart Hydrogel®. As with the emulsions (section 2.11.1) the drugs DEXT, MANN and PROP in Franz cell receiver fluid were analysed by measuring the radioactivity. The same radiolabelled drug materials were used. Noveon AA1® powder (0.1% w/v) or Smart Hydrogel® (2% w/v) powder was dispersed in distilled water under constant stirring with an overhead propeller mixer. Non-radioactive drug was added to these solutions to produce a final concentration of 2.5 mg mL^{-1}. The pH of the solutions was then adjusted by addition of concentrated NaOH. Polymer solutions were adjusted to achieve a pH or either 4.7 or 6.0. The volumes of NaOH added were negligible compared to polymer solution and the formulation was assigned a drug concentration of 2.5 mg mL^{-1}. The small amount of radiolabelled drug 'spike' was then mixed directly into the solution using an overhead propeller mixer, in sufficient quantity to allow a total starting activity in each Franz cell of 5kBq.

2.11.4. Preparation of polymer formulations containing isosorbide dinitrate, nifedipine and sildenafil

As with the emulsions (Section 2.11.2) the drugs ISDN, NIF, and SIL in Franz cell receiver fluid were analysed directly by HPLC. In the case of ISDN and NIF formulations, Noveon AA1® powder (0.1% w/v) or Smart Hydrogel® (2% w/v) powder was dispersed in distilled water under constant stirring with an overhead propeller mixer, then drug powder was added to these solutions to produce a final concentration of either 1.0 mg mL^{-1} or 2.5 mg mL^{-1}. In the case of the SIL formulation, the polymer powders were dispersed in the extracted SIL stock solution (see section 2.6), diluted with distilled water as necessary, to create a final SIL concentration of 1.0 mg mL^{-1} or 2.5 mg mL^{-1}. The pH of the solutions was then

adjusted by addition of concentrated NaOH solution as before. Polymer solutions were adjusted to achieve a pH or either 4.7 or 6.0. The small amount of radiolabelled drug 'spike' was then mixed directly into the solution using an overhead propeller mixer, in sufficient quantity to allow a total starting activity in each Franz cell of 5 kBq.

2.12 Preparation of controls for Franz cell experiments

The controls used for the Franz cell experiments were solutions or suspensions of drugs, in either distilled water only, or an acetonitrile and water mixture (ACN:H_2O 60:40). Propranolol and nifedipine formed suspensions whereas the other drug controls were solutions.

2.12.1. Preparation of controls containing dextran, mannitol and propranolol

The non-radiolabelled drugs were dispersed in either water or ACN:H_2O at a concentration of 2.5 mg mL^{-1} by mixing with an overhead propeller mixer. These drug-containing controls were then 'spiked' with the relevant quantity of radiolabelled drug, to allow a total starting activity in each Franc cell of 5 kBq. The radiolabelled drug was mixed into the control preparation using an overhead propeller mixer.

2.12.2. Preparation of controls containing isosorbide dinitrate, nifedipine and sildenafil

For ISDN and NIF controls, the drug was dispersed in either water or ACN:H_2O (95:5) at a concentration of 2.5 mg mL^{-1} by mixing with an overhead propeller mixer. For the aqueous SIL control, the stock solution of 2.5 mg mL^{-1} SIL was used without any dilutions. To prepare the ACN:H_2O control, the SIL extraction was performed as described in section 2.6, however a mixture of ACN:H_2O (60:40) was used to dissolve the Viagra® tablet, instead of water only. The resulting solution contained 2.5 mg ml^{-1} SIL dissolved in the acetonitrile and water mixture.

2.13 Preparation of drug-loaded formulations for retention and characterisation studies

Other formulations containing model drugs were prepared that were used to assess the effects of drugs on bioadhesion, formulation viscosity and surface tension. The drugs were not radiolabelled as analysis of drug concentration was not required for these studies.

2.13.1 Preparation of emulsions containing dextran, mannitol and propranolol

The aqueous phase and the oil phase of each emulsion were prepared as before. The drug in powder form was incorporated into the aqueous phase (for MANN and DEXT) or the oil phase (PROP) in amounts necessary to produce a concentration in the final emulsion of 2.5 mg mL^{-1}. The two phases were then combined and the emulsion prepared as before (section 2.7).

2.13.2 Preparation of emulsions containing isosorbide dinitrate, nifedipine and sildenafil

The emulsions were prepared as described in section 2.11.2

2.13.3 Preparation of polymer formulations containing dextran, mannitol and propranolol

The polymer vehicles used were Noveon AA1® polycarbophil and Smart Hydrogel®. Noveon AA1® powder (0.1% w/v) or Smart Hydrogel® (2% w/v) powder was dispersed in distilled water under constant stirring with an overhead propeller mixer. Drug in powder form was added to these solutions to produce a final concentration of 2.5 mg mL^{-1}. The pH of the solutions was then adjusted by addition of aqueous 1M NaOH solution. Polymer solutions were adjusted to achieve a pH or either 4.7 or 6.0.

2.13.4. Preparation of polymer formulations containing isosorbide dinitrate, nifedipine and sildenafil

In the case of ISDN and NIF formulations, Noveon AA1® powder (0.1% w/v) or Smart Hydrogel® (2% w/v) powder was dispersed in distilled water under constant stirring with an overhead propeller mixer, then drug powder was added to these

solutions to produce a final concentration of either 1.0 mg mL^{-1} or 2.5 mg mL^{-1}. In the case of the SIL formulation, the polymer powders were dispersed in the extracted SIL stock solution (see section 2.6), diluted with distilled water as necessary, to create a final SIL concentration of 1.0 mg mL^{-1} or 2.5 mg mL^{-1}. The pH of the solutions was then adjusted by addition of aqueous 1M NaOH solution. Polymer solutions were adjusted to achieve a pH or either 4.7 or 6.0.

2.14 Statistical analysis of results

Analysis of variance tests (ANOVA) were performed to determine differences between data, with the significance level set at $p<0.05$.

Chapter 3: Preformulation Studies

Chapter 3
Preformulation Studies

Introduction

This chapter details the characterisation of the formulations used during the project, selection of candidate vehicles for further studies, and some of the tests used to characterise their properties, including viscosity and surface tension. The effects of different variables on the resulting formulations produced were examined. The physicochemical properties of the model drugs used during the project were also identified for later reference. Additionally, the effect of model drug incorporation into some of the vehicles was examined and the data generated are discussed.

A number of studies have reported previously that viscosity is an important factor in retention of liquids on biological surfaces (Sahlin and Peppas, 1997b; Young and Smart, 2000; Young et al, 2002; Russell et al, 2003). Additionally, the wetting theory of bioadhesion suggests that a small contact angle allows a liquid to spread more easily over a substrate surface and the liquid has a greater opportunity for intimate contact with the substrate. This contact is necessary for bioadhesion to occur (Peppas and Buri, 1985; Mikos and Peppas, 1989; Chickering and Mathiowitz, 1999). The contact angle is related to the surface tension of a liquid, as liquids with a low surface tension will be able to spread more easily and achieve a small contact angle. The surface tensions and viscosities of the liquid formulations were therefore tested during this project in order to examine the impact of these parameters on liquid adhesion to oesophageal tissue.

3.1 Emulsion- based formulations

Two different types of emulsion were formulated in order to test claims in patent literature that they exhibit prolonged retention in the oesophagus (Hill et al, 1998; Cuca et al, 1999).

3.1.1 Selection of oil and water emulsions

A wide range of emulsions prepared were variations on those described in US patent 5,670,163 (Cuca et al, 1999), which states that emulsions may be bioadhesive

for up to 8 hours. A variety of oils and surfactants were used in the manufacture of these emulsions. The formulation technique and the materials used are detailed in Chapter 2.

Two emulsions were selected for further development; these were selected on the basis of their superior stability and relatively high viscosity. The two emulsions selected had been prepared using either light mineral oil (LMO) or heavy mineral oil (HMO), both using Tween® 85 as a surfactant. These emulsions were stable for over seven days as assessed by the consistency, lack of microbial growth and homogeneity (absence of visible phase separation) of the emulsion. There was no phase separation observed after storage for a week at room temperature or 4°C. Another factor used for selecting suitable candidate emulsions was a high viscosity as this was initially considered to be an important factor in prolonged retention (Tamburic and Craig, 1995). The viscosity in Pa.s of each emulsion was assessed using a TA Instruments AR1000 rheometer. The LMO and HMO emulsions selected both had a higher viscosity (0.24 and 0.04 Pa.s respectively) compared with the other emulsions prepared. The less stable emulsions and those with low viscosities were discarded.

3.1.2 Emulsion preparation at varying speed

The LMO and HMO emulsions were prepared at different final mixing velocities, to determine the effect of emulsification speed on viscosity and retention of the emulsions. Therefore, in addition to the 800 rpm speed, stirring velocities of 200 and 2000 rpm were used to prepare the emulsions. The viscosity and stability of emulsions made at each speed was also assessed. Finally, the LMO and HMO emulsions were additionally prepared using a Silverson® mixer for the final mixing stage at a 4000 rpm velocity using the emulsor screen. However, these emulsions prepared using the Silverson® mixer were discarded as they had a very low viscosity and phase separation was observed within a few hours of preparation, indicating poor stability. The high shear involved in homogenisation using the Silverson mixer was considered to be the cause of these problems. The emulsions prepared using an overhead propeller mixer at 200, 800 and 2000 rpm were satisfactorily stable with no phase separation or visible microbial growth within 14 days post-formulation. These emulsions were stored in a refrigerator at 4°C and used for *in vitro*

experiments within five days. Emulsions prepared for *in vivo* studies were used within two days.

3.1.3 Characterisation of the oil and water emulsions

The emulsions were tested in order to determine whether they were oil-in-water (o/w) or water-in-oil (w/o). One test involved placing a drop of the emulsion onto filter paper and observing the spreading. In theory, an o/w emulsion should spread over the filter paper, and a w/o emulsion will not spread (Hauser and Lynn, 1940). This gave inconclusive results, as both emulsions did spread. However, further investigation of the technique showed that either a drop of water, or a drop of oil, onto filter paper resulted in similar spreading. This test was therefore not considered reliable for characterisation of the emulsions. In another investigation, a water-soluble dye (Evans blue) was incorporated into the aqueous phase and an oil-soluble dye (Sudan III) into the lipid phase before combination of the two phases. Visualisation under a microscope did not clarify whether the emulsion was w/o or o/w. Ease of dilution of each emulsion with water revealed that both emulsions were o/w, as an emulsion can be diluted by its continuous phase (Mabrouk, 2004).

Parabens are esters of 4-hydroxybenzoic acid, and have been used increasingly over the last 50 years as anti-microbial preservatives (Perlovich et al, 2005). However, they may have allergic and oestrogenic activity, with possible carcinogenic potential (Harvey and Everett, 2004). If these risks are significant, an alternative means of preserving the emulsions would be required. However, parabens are acceptable for use in pharmaceuticals at the low concentrations used in this project. Concentrations of parabens up to 0.25% are deemed acceptable (Martindale, 1999).

3.1.4 Stability of silicone-based emulsion

A silicone-based emulsion (SBE) was prepared based on the examples described in US patent 5,711,936 (Hill et al, 1998). A stability assessment was carried out in a similar manner to the o/w emulsions. The SBE was found to be visually stable with no phase separation occurring after 2 weeks' storage at room temperature or 4°C. The SBE was stored in a refrigerator at 4°C and used for *in vitro* experiments within five days.

3.2 Polymer- based formulations

Traditionally bioadhesive formulations described in the literature have comprised polymer materials. A range of polymer-based formulations were therefore assessed during this project. These included polycarbophil, alginate and synthetic block co-polymer solutions, and mixtures of these polymers in solution.

3.2.1 Polycarbophil (Noveon AA1®) solutions

Noveon AA1® is a commercially available poly(acrylic acid) (PAA) synthetic polymer, also known as polycarbophil, and considered to be mucoadhesive (Smart, 1984; Duchene et al, 1988). It is a long-chain, high molecular weight, cross-linked polymer, composed of carboxyvinyl groups. When placed in water, PAA polymers swell and form weak hydrogel structures. With increasing pH, these structures become more rigid upon formation of a three dimensional viscoelastic network (Tamburic and Craig, 1996). The established bioadhesive properties of this family of polymers, and the potential for manipulation of the polymer structure and behaviour by pH alterations, made Noveon AA1® a desirable candidate for a targeted drug delivery formulation to the oesophagus.

3.2.2 Alginate solution

Alginates are natural polysaccharides derived from seaweed. They are block co-polymers of guluronic acid and mannuronic acid. In an acidic environment of below pH 3, hydrogen bonds are formed between the acid and hydroxyl groups within the alginate, to form a cross-linked gel structure. The molecular weight of the alginate affects the strength of this gel, with higher molecular weights forming a stronger gel (Draget et al, 1997). LF120M is a medium-sized alginate with an intermediate molecular weight of approximately 139 kDa (Johnson et al, 1998). LF 200S is a larger sized alginate with a molecular weight of 210 kDa (Drury et al, 2004). Solutions of sodium alginate were interesting for this study because they have previously been shown to adhere to oesophageal tissue *in vitro* (Batchelor et al, 2002). Sodium alginate solutions that contain calcium carbonate (e.g. Gaviscon®), release calcium ions and carbon dioxide in the presence of acid. These calcium ions cross-link the alginate to create a gel that is better retained on the oesophageal

surface (Tonnesen and Karlsen, 2002). Alginate solutions therefore have potential as oesophageal protectants during a reflux episode (Tang et al, 2005).

3.2.3 Poloxamer 407 (Pluronic Lutrol 127NF)

Poloxamers are synthetic tri-block copolymers of ethylene oxide and propylene oxide. Poloxamer 407 has the formula $HO(C_2H_{4O})_{141}(C_3H_6O)_{44}C_2H_4O)_{141}H$. Poloxamer 407 was of interest in this project for two reasons. Firstly it is has been shown to be adhesive to the oesophagus (Mathias et al, 2002) and adhesive to rat rectal mucous membranes at 36.5°C for 6 hours in rats (Charrueau et al, 2001). Polyethylene oxide (PEO) chains are able to penetrate into mucous (De Ascentiis et al, 1995; Peppas and Sahlin, 1996) and so may be mucoadhesive. The addition of poloxamer to a formulation might therefore enhance the adherence to the oesophageal mucosa. Another interesting property of this material is that a 20% w/v solution undergoes increased viscosity with increasing temperature (Brown et al, 1995b; Soppimath et al, 2002), and gelation of the solution occurs between 20°C and 30°C (Fig 3.8) with the gel-transition point occurring around 20°C.

This temperature-triggered gelation may be very useful in targeting the oesophagus, where a liquid is taken orally, and once swallowed, warms to body temperature. It is predicted that upon ingestion, the increase in temperature of a small volume of the solution would be fast enough to permit gelling to occur during passage through the oesophagus. This would slow transit through the oesophagus and prolong the retention time.

3.2.4 Smart Hydrogel®

Smart Hydrogel® is a synthetic co-polymer poly(oxyethylene-b-oxypropylene-b-oxyethylene)-g-poly(acrylic acid). This polymer also exhibits thermally sensitive behaviour (Brown et al, 1995b; Orkisz et al, 1997). As with poloxamer, this characteristic makes the Smart Hydrogel® an exciting candidate as a vehicle to target the oesophagus. In a previous study in human volunteers performed by Potts et al. (1997a), a 5 mL volume of Smart Hydrogel® solution labelled with Tc99m was swallowed by healthy subjects, and scintigraphic imaging used to visualise the gel transit through the oesophagus. Biphasic clearance of the hydrogel was observed, with 65 percent of the hydrogel seen to pass into the stomach in the first 5

seconds. The Smart Hydrogel® was well retained within the oesophagus, however, with 13 percent still remaining in the oesophagus 10 min after administration. The group concluded that the temperature-induced gelation upon entering the body resulted in coating of the oesophagus.

3.3. Rheology

Methods used for taking rheological measurements are detailed in Chapter 2. The data obtained from these rheological characterisation experiments helped to better understand the formulation properties. All graphs show typical plots for formulations.

3.3.1 Smart Hydrogel® rheology – effects of shear

The Smart Hydrogel® (SH) caused problems in obtaining reliable rheological measurements as it displayed unusual behaviour. A 2% w/v solution is shear-thickening, a property that can be demonstrated in practice by prolonged stirring, or syringing of the material. For this reason the continuous ramp, peak hold and stress sweep measurements were expected to reveal the same characteristics. However, in all measurements, the solution failed to display shear-thickening properties in the rheological measurements. On the contrary, shear-thinning properties were observed. This pseudo-shear thinning was observed at all pH assessed (Fig 3.1).

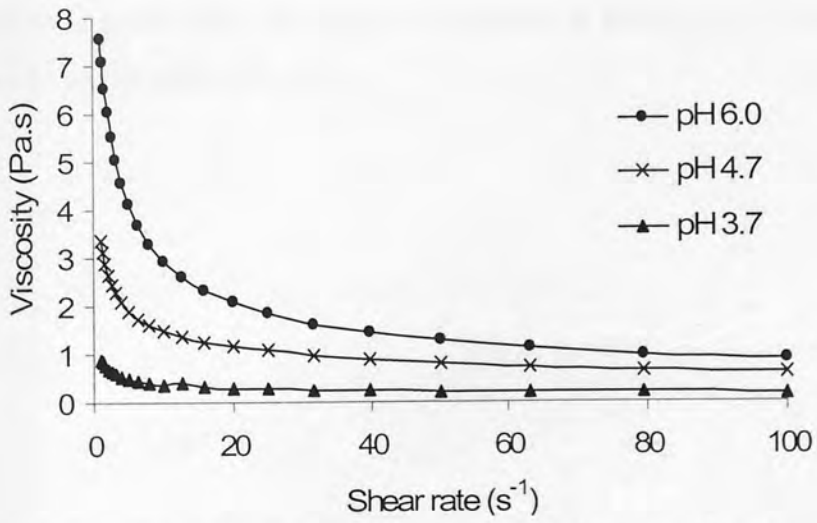


Figure 3.1. Apparent shear thinning of Smart Hydrogel® observed during continuous ramp measurements, at three different pH values.

These unexpected data are considered unreliable as it is evident that the hydrogel exhibits a gelling upon application of shear. Although this gelling is not necessarily consistent throughout the material, there is a definite change of state in areas of the material. Small gel spheres are formed under the rheometer, which move out from under the plate, seemingly because the gap does not accommodate their diameter. This phenomenon may be seen in Fig 3.2, taken during peak hold measurements at $10s^{-1}$ shear rate. The behaviour may be described as thixotropic, as the phenomenon is observed upon application of a constant shear.

Figure 3.2. Photographs of Smart Hydrogel® exhibiting gelling during peak hold measurements. Arrows indicate areas of solid gel appearance at the edge of the cone.

Once the cone is lifted from the sample the spherical beads of gel remain formed and are visible on the plate. (Fig 3.3)

Figure 3.3. Photograph showing solid Smart Hydrogel® gel formations remaining following peak hold measurements, after removal of the shear forces. Arrows indicate areas of solid gel on the plate.

3.3.2 Smart Hydrogel® rheology – effects of temperature

Another characteristic of Smart Hydrogel® is its thermogelling properties, which can be simply observed by eye after holding a container of pH-adjusted solution in the hand for a few seconds, or placing the gel in a warm oven. This property was investigated during temperature ramp measurements, increasing the temperature to 37 °C at a rate of 10 °C per minute.

For a 2% w/v Smart Hydrogel® solution where the pH is unadjusted at 3.6, there is no point in this temperature range at which the elastic modulus rises above the viscous modulus (Fig 3.4). There is therefore no gel-point in the unadjusted pH solution.

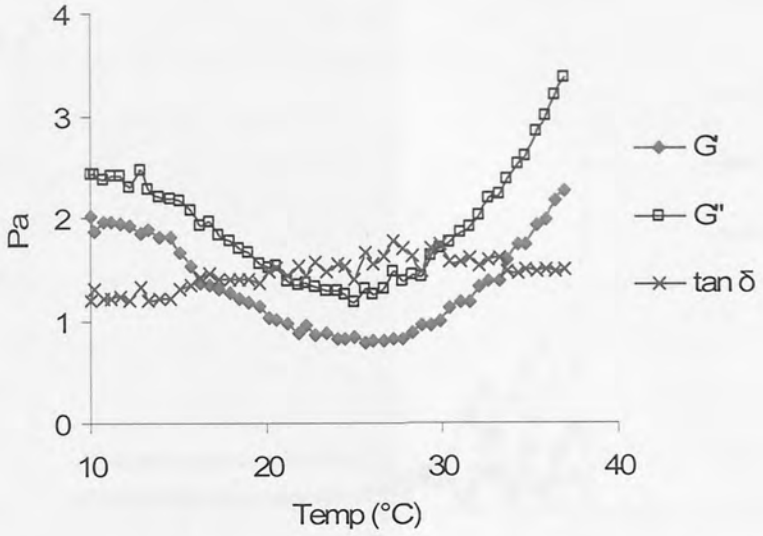


Figure 3.4. Smart Hydrogel® 2% w/v pH 3.7, temperature ramp, during which there is no clear gel point.

However, an increase in pH of the Smart Hydrogel® yields a thermogelling solution, as the pH 4.7 and pH 6.0 solutions exhibit a dramatic increase in elastic modulus above the viscous modulus at around 30°C (Figs 3.5 and 3.6). This is accompanied by a decrease in tan δ, indicating an increase in solid-like properties. This finding suggests that Smart Hydrogel® would be a valuable drug delivery vehicle in the oesophagus where the pH is approximately pH 5, and so the oesophageal environment could promote gelling.

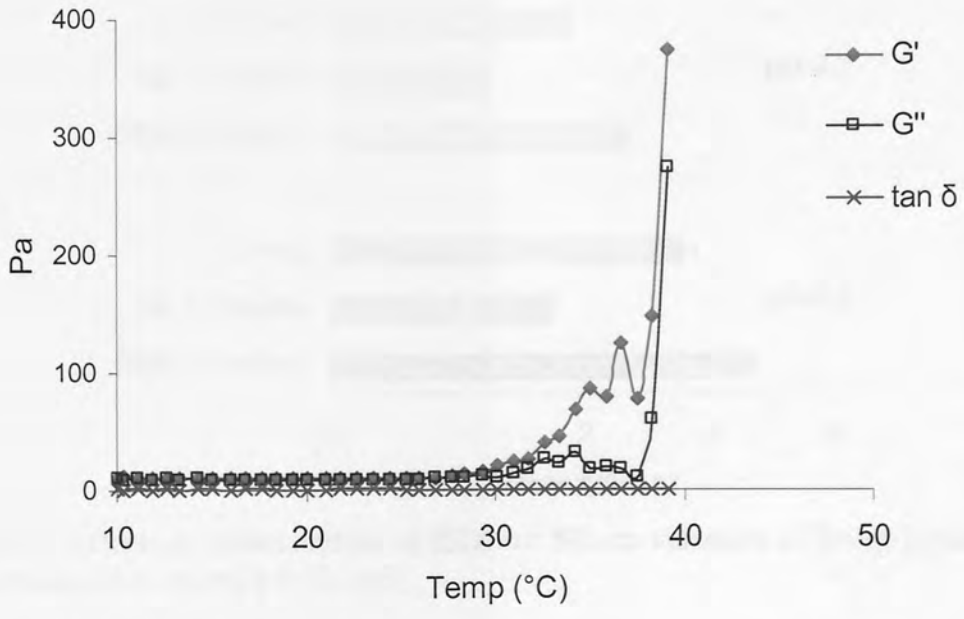


Figure 3.5. Smart Hydrogel® 2% w/v pH 4.7, temperature ramp exhibiting gelling at around 35°C.

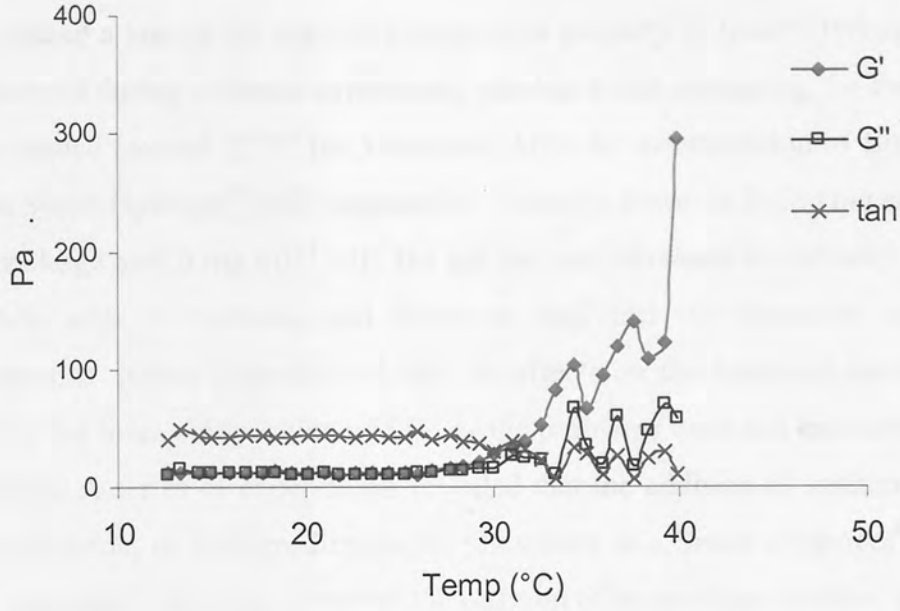


Figure 3.6. Smart Hydrogel® 2% w/v pH 6.0, temperature ramp exhibiting gelling at around 35°C.

3.3.3 Smart Hydrogel® rheology – effects of drug incorporation

It appears that at pH 4.7 and pH 6.0, the incorporation of ISDN into the Smart Hydrogel® solution increases viscosity, whereas the incorporation of SIL reduces the viscosity of the hydrogel solution (see figure 3.7)

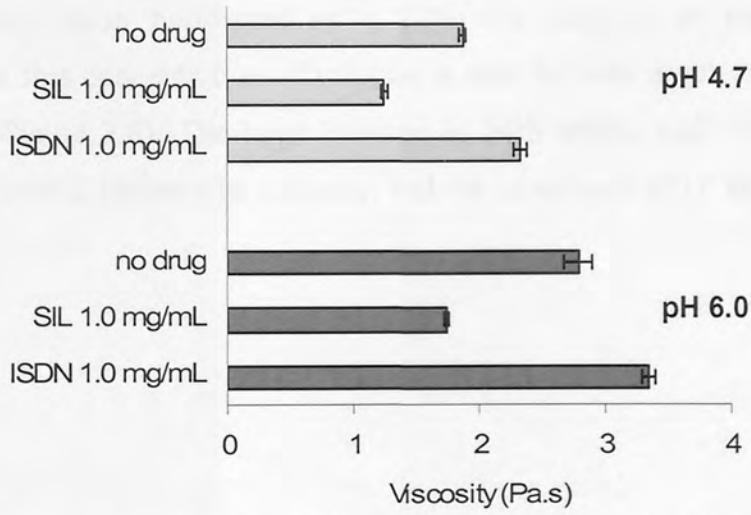


Figure 3.7 Effects of incorporation of ISDN or SIL on viscosity of Smart Hydrogel® 2% w/v solutions (mean ± S.D. n=3).

The addition of SIL into Smart Hydrogel® has a number of detrimental effects on the properties of the polymer. Syneresis of the gel was observed, causing clumps of

gel to form around a fluid of very low viscosity. Additionally, sufficient quantities of SIL caused a loss of the thermally-responsive property of Smart Hydrogel®. This was observed during a simple experiment, placing a vial containing the formulation in a pre-heated oven at 37 °C for 5 minutes. After the incorporation of 1.0 mg mL^{-1} SIL, the Smart Hydrogel® still increased in viscosity, however following addition of a dose as large as 4.0 mg mL^{-1} SIL, the gel had not increased in viscosity by visual inspection after 5 minutes, and therefore had lost its thermally responsive characteristics. It was hypothesised that the effects on the hydrogel solution were caused by the ionic composition of SIL, as the problems were not encountered with other drugs. A series of experiments revealed that the addition of sodium chloride, calcium chloride, or sodium dihydrogen phosphate to a Smart Hydrogel® solution caused decreased viscosity, However the addition of magnesium stearate, potassium dihydrogen phosphate, calcium carbonate and calcium phosphate did not affect the hydrogel viscosity. It may be concluded that there is some interaction between sodium and chloride ions and the gel that causes a weakening of the gel structure (Yong et al, 2001). Similar loss of gel structure of a hydrogel due to presence of salts has been previously reported, for example the loss of gelation in Poloxamers caused by salts (Pandit and Kisaka, 1996).

3.3.4 Poloxamer rheology

The temperature ramp performed on a 20% w/v solution of poloxamer 407 confirmed that this concentration of solution is able to form a gel at temperatures above 20°C (Figure 3.8). The large increase in both elastic and viscous moduli, indicating an overall increase in viscosity, and the cross-over of G' and G" shows a gel point.

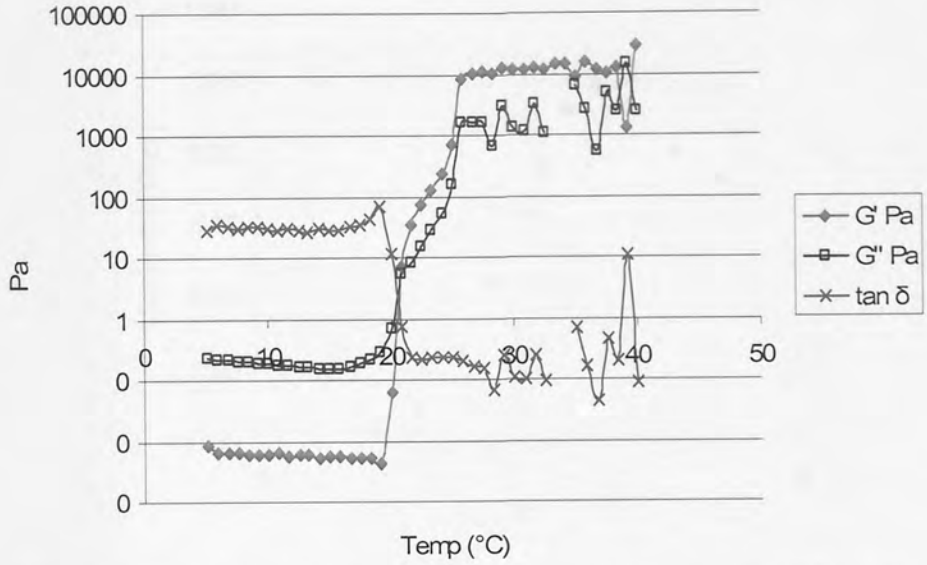


Figure 3.8. Temperature ramp showing temperature dependence of the viscous and elastic moduli, and the tan δ of a 20% w/v poloxamer 407 solution (torque = 10μNm).

Peak hold measurements were also performed on a 20% w/v aqueous solution of poloxamer 407 at a constant shear rate of 10 s^{-1}, during which there was no change in viscosity over time. The mean viscosity at poloxamer 407 measured at this shear rate was 5.60 Pa.s (± 0.34 Pa.s).

3.3.5 Noveon AA1® rheology

Studies were carried out on an aqueous solution of Noveon AA1® to assess the importance of pH on viscosity. The pH was adjusted using drops of aqueous 1M NaOH solution, and the viscosity in centipoise was measured using a Brookhaven viscometer. The viscosity of a 0.1% w/v solution of Noveon® was highly pH dependent as shown in Figure 3.9. The viscosity increased dramatically with pH above pH 5. This effect was found to be reversible upon addition of concentrated HCl to acidify the solution.

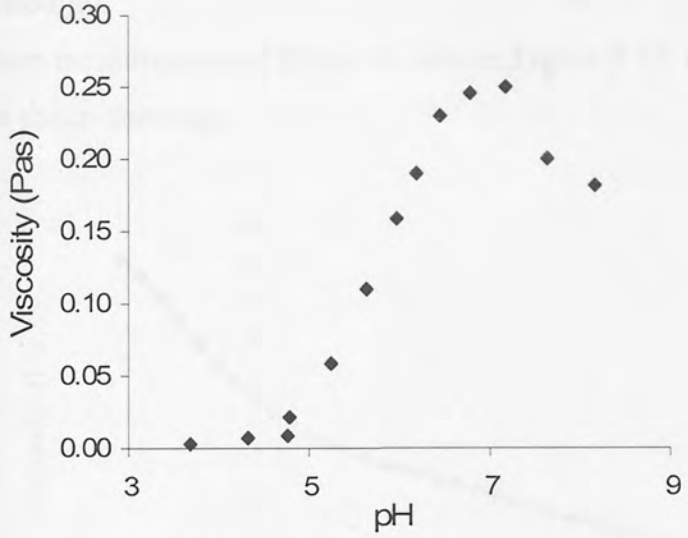


Figure 3.9. pH-dependence of viscosity of Noveon AA-1 0.1% w/v aqueous solution.

Flow rheometry continuous ramp also revealed that the viscosity of a Noveon® solution decreases with increasing applied shear, between 1 and 10 s^{-1}, however above this level of shear there is a minimal effect on viscosity (Figure 3.10).

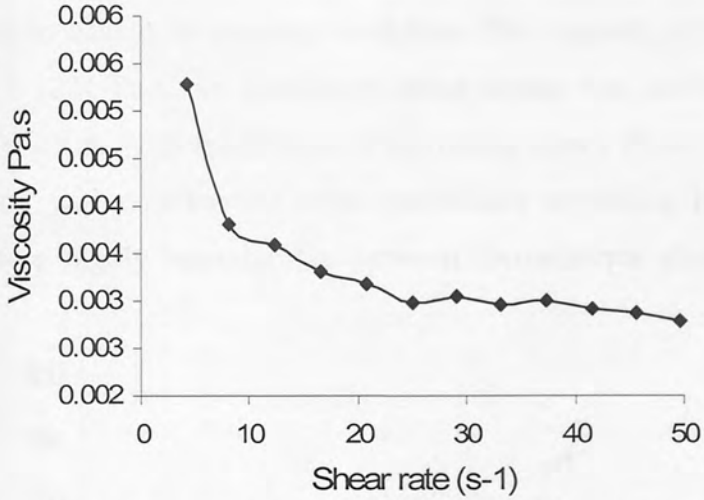


Figure 3.10. Continuous ramp for 0.1% w/v Noveon® solution, showing shear thinning over a shear rate range 0.1 to 100 s^{-1}.

Peak hold measurements performed on 0.01% Noveon solutions at pH 4.7 and 6.0 at a constant shear rate of $10s^{-1}$ did not result in variations in viscosity. The mean viscosity values measured for the pH 4.7 and pH 6.0 solutions were 0.009 and 0.158 Pa.s respectively (quoted to 3 significant figures).

3.3.6 Blend A rheology

A continuous ramp measurement of Blend A, seen in Figure 3.10, revealed that the polymer blend is shear- thinning.

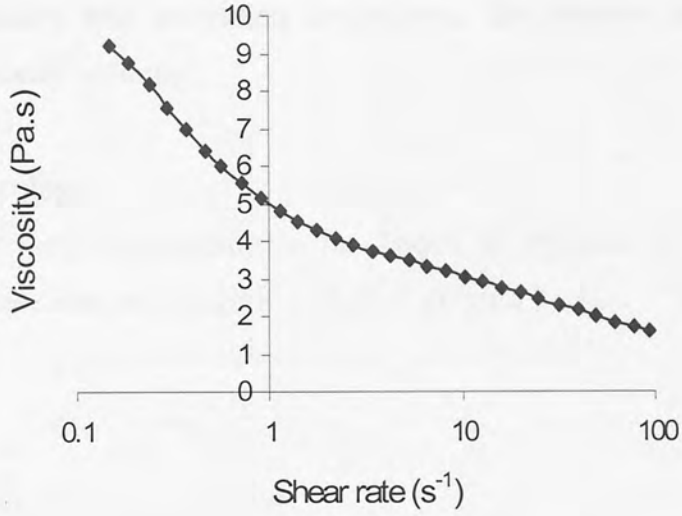


Figure 3.11. Continuous ramp for Blend A, showing shear thinning over a shear rate range 0.1 to 100 s^{-1}.

Peak hold measurements were also performed at a constant shear rate 10 s^{-1}, during which there was no change in viscosity over time. The viscosity of Blend A at this shear rate was 3.1221 Pa.s. An oscillatory stress sweep was performed, between torque 1 and 100 μNm. With application of increasing shear, Blend 1 remained at a constant viscosity, with no alteration in the viscoelastic properties. It was noted that these results were highly reproducible between formulations made on different days.

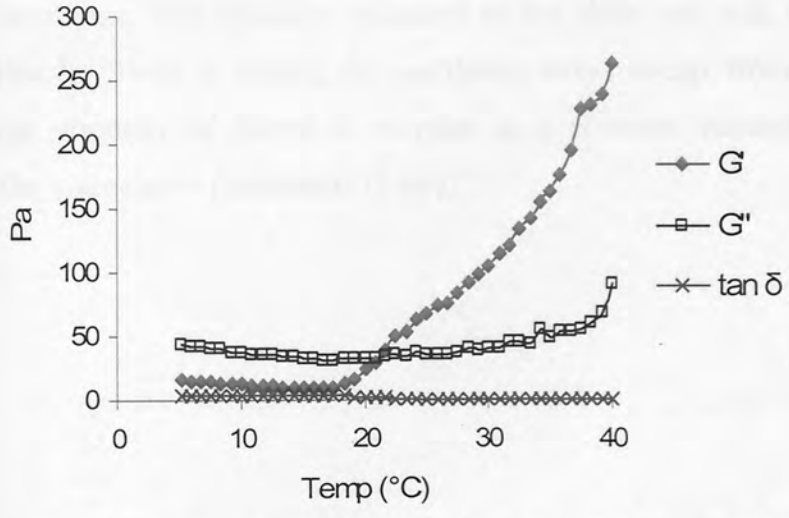


Figure 3.12. Blend A temperature ramp exhibiting gelling at around 22°C, at a low torue of 1 μNm.

A temperature ramp experiment between 5 and 40 °C was also performed on the blend A (see figure 3.12). At a low stress (torque = 1 μNm), the blend shows gelling and transition from liquid to solid material at 22°C. At a higher torque, however (torque = 10 μNm), this gel point was not observed, and there is actually a gradual decrease in viscosity with increasing temperature. The viscous modulus remains higher than the elastic modulus.

3.3.7 Blend B rheology

The continuous ramp measurements for Blend B revealed a shear thinning behaviour over the shear rate range 15 to 100 s^{-1} (Figure 3.13).

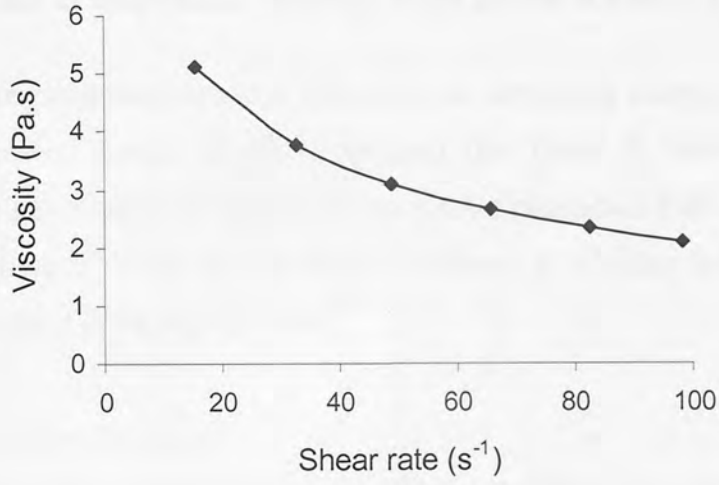


Figure 3.13. Continuous ramp viscosity measurement for Blend B, showing shear thinning over a shear rate range 15 to 100 s^{-1}.

During peak hold measurements, at a constant shear rate of $10s^{-1}$ there is no change in viscosity over time. The viscosity measured at this shear rate was 6.3054 Pa.s, higher than that for Blend A. During the oscillatory stress sweep, from torque 1 to 100 μNm, the viscosity of Blend B remains at a constant viscosity, with no alteration in the viscoelastic parameters G' or G".

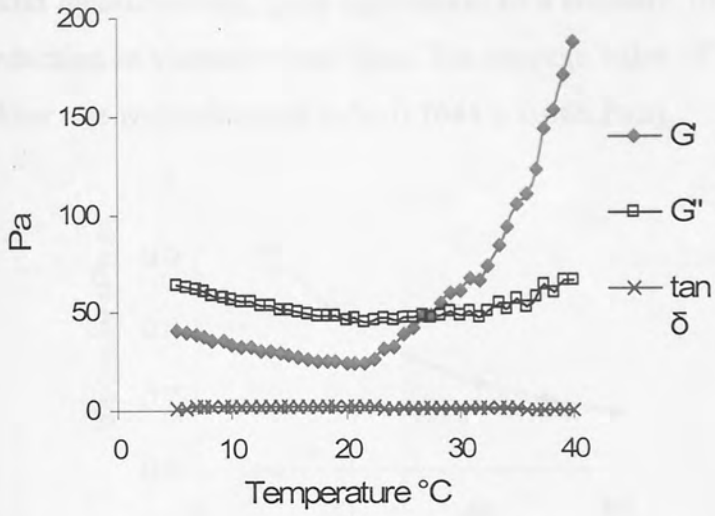


Figure 3.14. Blend B temperature ramp exhibiting gelling at around 27 °C.

The temperature ramp measurement, performed at increasing temperature from 5 to 40°C, at a constant torque of $10s^{-1}$, revealed that blend B shows gelling and transition from more liquid properties to more solid properties from approximately 27°C. The gelling of Blend B is therefore achieved at a higher temperature than Blend A, although it is equally dramatic.

3.3.8 HMO emulsion rheology

The continuous ramp assessment of the HMO emulsion demonstrated a shear thinning effect with increasing rate of shear (Fig 3.15).

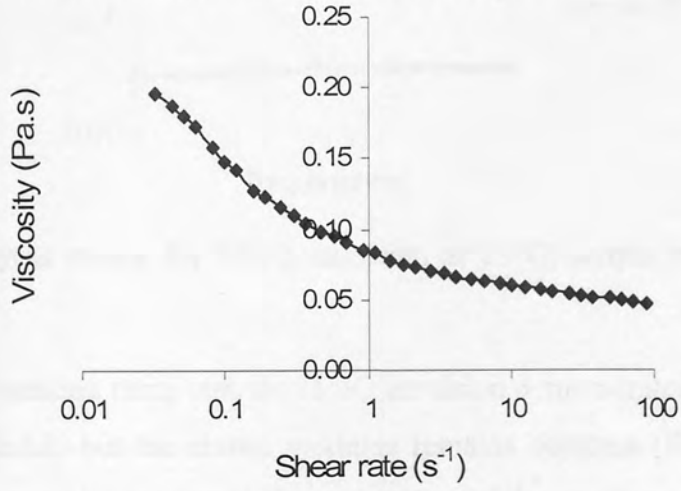


Figure 3.15. Continuous ramp for HMO emulsion at 25 °C, between shear rates 0.01 and 100 s^{-1}.

During peak hold measurements, upon application of a constant 10 s^{-1} shear, there was a slight reduction in viscosity over time. The average value of the viscosity of HMO at this shear rate was calculated to be 0.7641 ± 0.086 Pa.s).

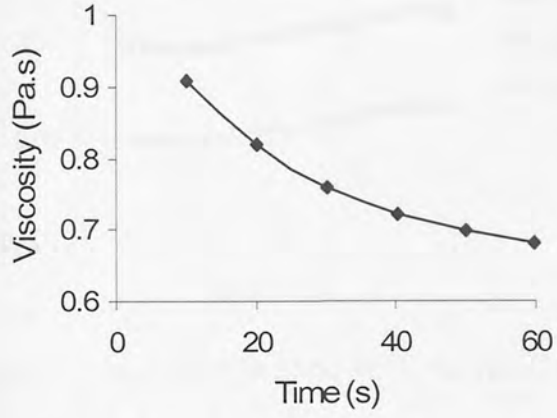


Figure 3.16. Peak hold data for HMO emulsion at 25°C, at a constant shear of $10s^{-1}$.

A stress sweep study, however, shows that with application of an increasing strain, the structure of the emulsions is not broken down, as the viscous and elastic moduli remain constant. The maintenance of the internal structure is seen also in the constant values for tan δ over the course of the experiment (Figure 3.17).

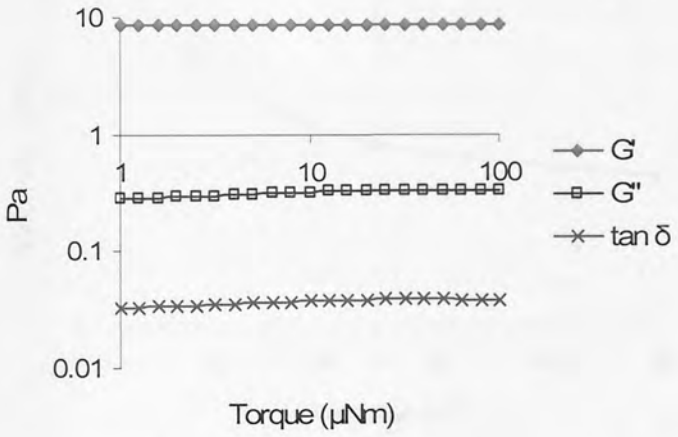


Figure 3.17. Stress sweep for HMO emulsion at 25°C, torque range = 1 to 100 μNm.

During the temperature ramp test, the HMO emulsion demonstrates a slight increase in viscous modulus, but the elastic modulus remains constant (Figure 3.18). This indicates an increase in the liquid-like properties of the emulsion compared to the solid-like properties; this is also reflected in the slight increase in loss tangent.

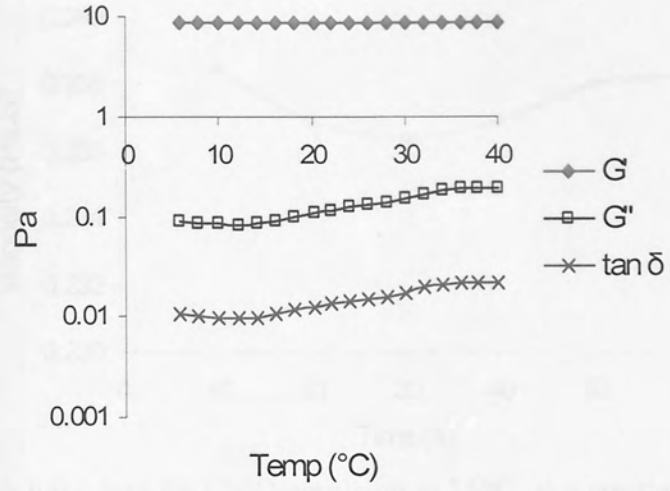


Figure 3.18. Temperature ramp between 5 and 40°C for HMO emulsion.

3.3.9 LMO Emulsion rheology

As with the HMO emulsion, during continuous ramp measurements, there was a slight reduction in viscosity of the LMO emulsion with increasing shear (Figure 3.19).

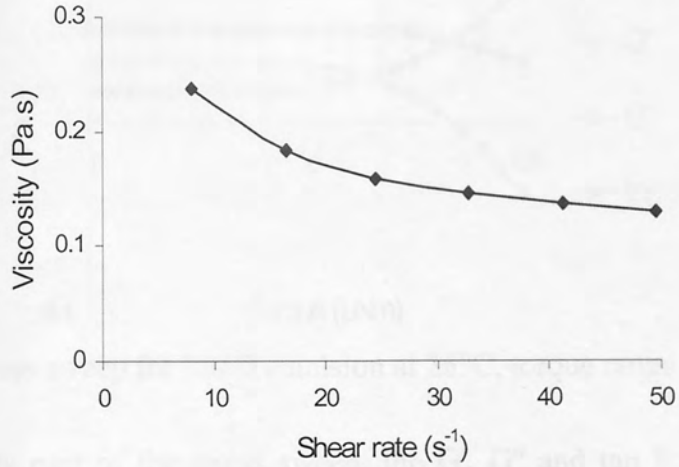


Figure 3.19. Continuous ramp for LMO emulsion at 25°C, between shear rates 10 and 50 s^{-1}.

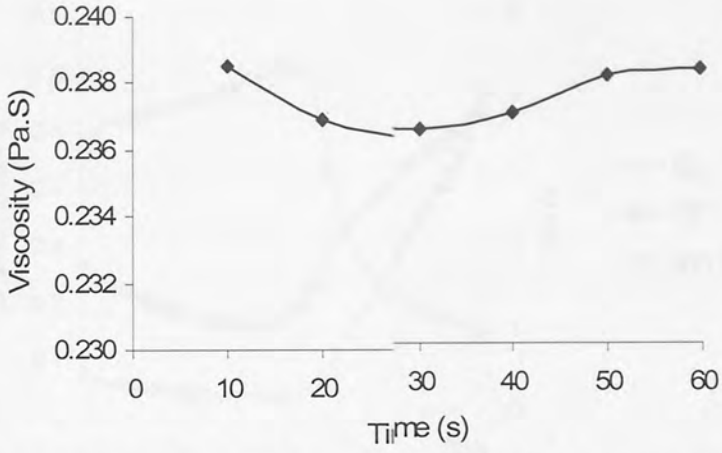


Figure 3.20. Peak hold data for LMO emulsion at 25°C, at a constant shear of $10s^{-1}$.

The peak hold measurement for LMO showed a constant viscosity, with only minor fluctuations over time (Figure 3.20). The average value for the viscosity of LMO at this shear rate was 0.2375 Pa.s (± 0.004), significantly lower than that of the HMO emulsion ($P<0.05$).

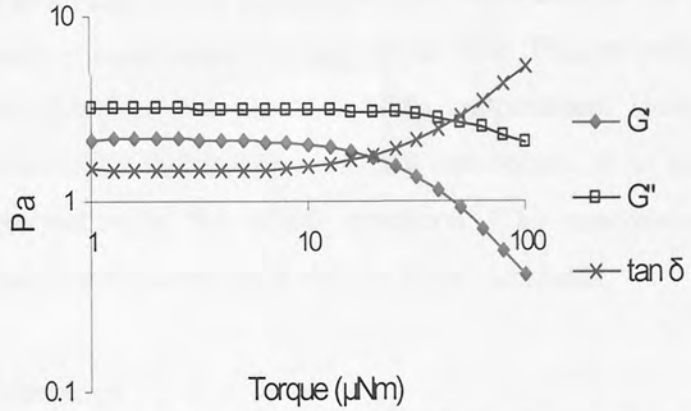


Figure 3.21. Stress sweep for LMO emulsion at 25°C, torque range = 1 to 100 µNm.

During the early part of the stress sweep, the G', G" and tan δ remain constant. When the torque is raised above 10 µNm, the elastic modulus decreases, indicating an increase in the liquid-like properties of the emulsion compared to the solid-like properties, this is also reflected in the slight increase in loss tangent (see Figure 3.21). This is similar behaviour to that observed in the HMO emulsion previously.

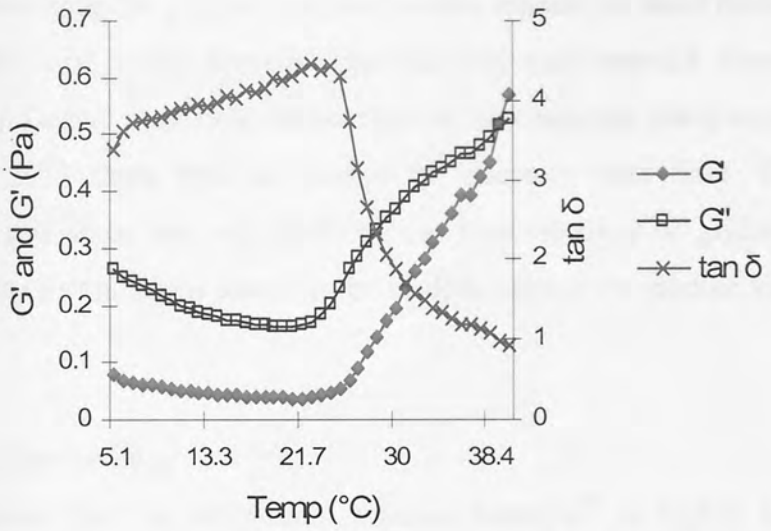


Figure 3.22. Temperature ramp for LMO emulsion, exhibiting gelling at around 39°C.

The temperature ramp data for LMO are shown in Figure 3.22. As the temperature is increased above approximately 25°C, the G' and G" begin to increase, until a gel point is reached at around 39°C, when there is a cross-over of the two moduli, and the emulsion becomes more solid-like than liquid-like. This gel point is only seen at 39°C and would therefore not occur at body temperature. However, there is a surprising and interesting increase in elasticity that occurs up to body temperature, that is not observed with the HMO emulsion. The mechanisms behind this temperature-dependent viscosity have not yet been elucidated.

3.3.10 Glycerol rheology

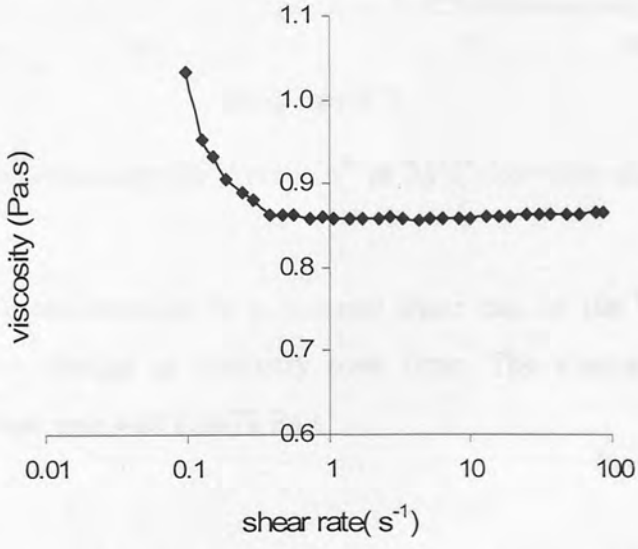


Figure 3.23. Continuous ramp for glycerol at 25°C, between shear rates 0.1 and $100s^{-1}$.

The continuous ramp for glycerol showed a small amount of shear thinning between shear rates 0.1 and 1 s-1, however the viscosity was constant above this shear (Figure 3.23). During peak hold measurements, at a constant shear rate of $10s^{-1}$ and temperature 25°C there was no change in viscosity over time. The viscosity measured at this shear rate was 0.8417 Pa.s. This viscosity of glycerol was mid-range of all the formulations tested in the studies, having the median viscosity of all the vehicles.

3.3.11 Sucralfate rheology

Fig. 3.24 shows that the sucralfate solution Antespin® is highly susceptible to destructive shear forces, with a decreasing viscosity observed with increasing shear rate. This could be due to the reduction in size of the particles in the suspension with an increasing applied shear. The polymeric nature of Antepsin® means that it could align during the shear measurement, which would result in reduced viscosity.

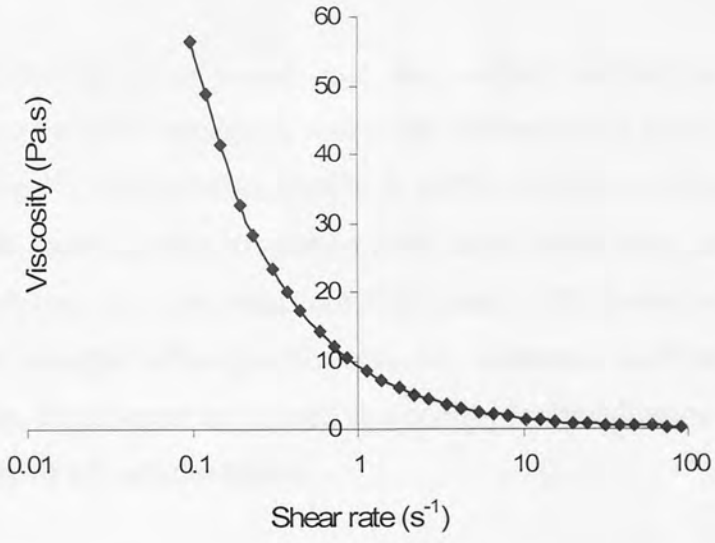


Figure 3.24. Continuous ramp for Antepsin® at 25°C, between shear rates 0.1 and $100s^{-1}$.

During peak hold measurements, at a constant shear rate of $10s^{-1}$ and temperature 25°C there was no change in viscosity over time. The viscosity of Antepsin® measured at this shear rate was 1.0072 Pa.s.

3.3.12 Summary of rheological findings

Shear thinning effects might be important *in vivo* in this project as the formulation will be subject to swallowing and peristalsis. Changes in viscosity might affect bioadhesion of the vehicle. Temperature effects were also considered very important for this application as the formulation will increase to body temperature once administered. Both of these factors might therefore affect the duration and pattern of adhesion within the oesophagus. It was found that Smart Hydrogel® formed areas of solid gel under shear forces. This might also occur *in vivo*, and gel areas may adhere to the folds and crevices in the oesophageal surface. The 2% w/v solution of Smart Hydrogel® had thermogelling properties with a dramatic increase in viscosity and elasticity observed above approximately 30 °C. This feature may impart prolonged retention in the oesophagus at body temperature (Potts et al, 1997a; Potts et al, 1997b). However, although the vehicle has exciting potential characteristics for a potential oesophageal drug delivery vehicle, incorporation of drugs might affect the viscosity and thermogelling capability.

The data confirmed previous reports that the viscosity of Noveon AA-1 is pH-dependent. Poloxamer 407 was confirmed to be thermogelling above 20 °C, similar to Smart Hydrogel®. The polymer Blends A and B, which incorporate Poloxamer 407, show some thermogelling property which could allow them to adhere well in the warm oesophagus. The o/w emulsions HMO and LMO showed a slight response to temperature changes although this was not dramatic, and both were shear thinning. Finally, the glycerol to be used as a control for bioadhesion studies had the median viscosity of all vehicles tested.

3.4 Effect of emulsification speed on viscosity of o/w emulsions

The viscosities of the o/w emulsions prepared using an overhead propeller mixer at different speeds were measured. The viscosity of each emulsion was reproducible throughout the formulation.

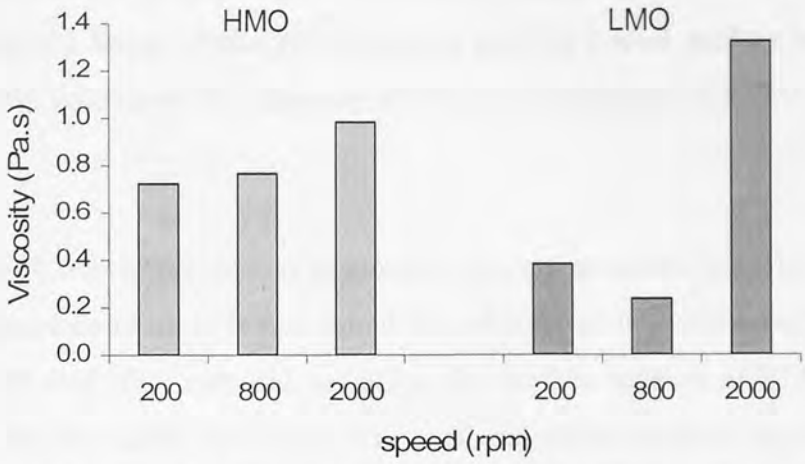


Figure 3.25. The effect of emulsification speed (rpm) on viscosity (Pa.s) of o/w emulsions. Graph shows one representative viscosity measurement.

As can be seen in Figure 3.25, there was a modest increase in viscosity with increasing speed of emulsification for HMO. However the LMO emulsion did not follow the same trend, as the viscosity of the emulsion prepared at 800 rpm was lower than that prepared at 200 rpm, and the high speed (2000 rpm) formulation had a markedly increased viscosity.

3.5 Surface tension

The wetting theory of bioadhesion describes the importance of spreading in adhesion of a liquid. The better a liquid spreads, the more opportunity it has to achieve intimate contact with the target biological substrate, and this contact is vital for bioadhesion (Peppas and Buri, 1985; Mikos and Peppas, 1989; Chickering and Mathiowitz, 1999). A low surface tension would allow a liquid to spontaneously spread over the substrate surface, with a small contact angle. It was therefore assumed that a low surface tension would be conducive to bioadhesion. The surface tensions of the liquid vehicles were therefore tested during this project.

The method used to measure surface tension is detailed in Chapter 2. Additionally, correlations between surface tensions and viscosity were investigated. No linear relationship was found between the surface tensions of all vehicles and their viscosity (R^2 = 0.07). When examining all the formulations collectively, 0.01% Noveon® solutions, water, Antepsin®, glycerol and 2% alginate LF120M were

found to have the highest surface tensions. Mid-range surface tensions were measured in 2% Smart Hydrogel® solutions, and the lowest surface tensions were found in 20% Poloxamer 407 solution and the o/w emulsions (full data in Appendix 3A).

The speed at which the two o/w emulsions were prepared was investigated in relation to surface tension. It was found that alterations in the emulsification speed between 200 and 2000 rpm did not affect the surface tension of HMO emulsion. However, for the LMO emulsion, the speed did affect surface tension, with the highest surface tension produced by the mid-range 800 rpm speed (Figure 3.26)

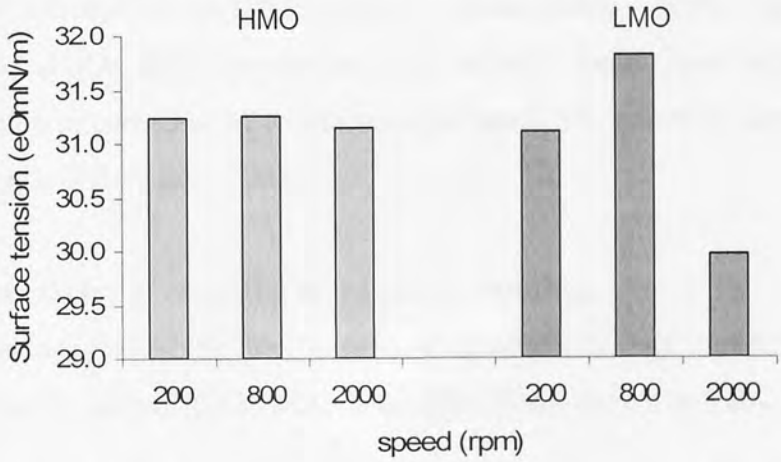


Figure 3.26. The effect of emulsification speed (rpm) on surface tension (mN/m) of o/w emulsions.

3.6 Properties of candidate drugs

Some of the physicochemical properties of the three drugs of interest, isosorbide dinitrate, nifedipine and sildenafil, are detailed in this section.

3.6.1 Isosorbide dinitrate

Isosorbide dinitrate (ISDN) is the compound 1,4:3,6-dianhydro-D-glucitol 2,5-dinitrate. It is a white, crystalline solid, stable in both air and solution. ISDN is sparingly soluble in water (1.09 mg/mL) but freely soluble in organic solvents, and its melting point is 70°C (Rahway, 1983).

3.6.2 Nifedipine

Nifedipine (NIF) is the compound 3,5-pyridinedicarboxylic acid,1,1-dihydro=2,6=dimethyl-4=(2=nitrophenyl I)-,dimethyl ester, with the formula $C_{17}H_{18}N_2O_6$. It is a yellow crystalline solid, and it is unstable, and sensitive to light, which causes photodegradation of the chemical. It is practically insoluble in water (< 1 mg/mL) (Liu et al, 1999; Chowdary and Kamalakara, 2003).

3.6.3 Sildenafil

Sildenafil (SIL) (purchased as Viagra® tablets, as a citrate salt) is the compound 1-[[3-(6,7-Dihydro-1-methyl-7-oxo-3-propyl-1H-pyrazolo[4,3-d]pyrimidin-5-yl)-4-ethoxyphenyl]sulfonyl]-4-methylpiperazine monocitrate, with the formula $C_{22}H_{30}N_6O_4S.C_6H_8O_7$. Both the neutral and cationic forms have relatively high lipophilicity due in part to an internal hydrogen bond. SIL citrate is soluble in water up to 3.5 mg/mL (Pfizer Inc., 2002).

3.6.4 Summary tables of candidate drugs and model drugs

The molecular weights of the three candidate drugs ISDN, NIF and SIL, as well as those of the model drugs DEXT, MANN and PROP are shown in Table 3.1.

Drug	Mw
PROP	259.34
NIF	346.34
ISDN	236.14
SIL	474.58
MANN	187.17
DEXT	approx. 10,000

Table 3.1. Molecular weights of candidate and model drugs.

Other information by which the drug properties might be compared is displayed in Table 3.2. These parameters are the aqueous solubilities, log P and pKa values where known. These drug properties might affect release rates from the vehicles during the study.

Drug	Aqueous solubility (mg/mL)	Log P		pKa	
PROP	0.07	2.53	(Shao and Kaushal, 2004)	9.15	(Palmgren et al, 2004)
NIF	< 1	2.50	(van der Lee et al 2001)		
ISDN	<100	1.31	(Feldstein et al, 1998)		
SIL	3.5	Log D = 2.7	(Liaw and Chang 2001)	6.5	(Liaw and Chang, 2001)
MANN	220	- 2.5	(Shao and Kaushal, 2004)		

Table 3.2. Physicochemical data of candidate and model drugs.

Chapter 4: The duration of liquid retention on oesophageal tissue – *in vitro* assessment

Chapter 4

The duration of liquid retention on oesophageal tissue – *in vitro* assessment

Introduction

The aim of this work is to develop a liquid oral drug delivery formulation to target the oesophagus for the purpose of treating oesophageal disorders. This is to be achieved by using a liquid that exhibits prolonged contact with the epithelium. There are no reported studies that screen a wide range of liquid formulations with diverse, different properties for their retention on the oesophagus. This study aimed to achieve this, with a diverse collection of liquids being tested on the same model, to achieve a screening/ranking process that would aid selection of candidate vehicles for further studies. Investigation of the rheological and surface tension properties of formulations was used to understand the mechanisms of retention in the model.

4.1 Models of liquid retention

4.1.1 Previous models for assessing retention

In order to develop a liquid formulation with the ability to adhere to the oesophageal mucosa, an assessment of bioadhesive capability was required. A range of promising formulations (mineral oil emulsions, silicone based emulsions, polycarbophil, poloxamer 407, Smart Hydrogel® and polymer blends) and a commercially available product Antepsin® were assessed in comparison to two controls, glycerol and water. The emulsions were chosen due to patents stating that similar formulations had good adhesion in the oesophagus (Hill et al, 1998; Cuca et al, 1999). Polycarbophil was chosen due to its reported bioadhesive properties (Smart, 1984; Duchene et al, 1988). Poloxamer 407 (Soppimath et al, 2002; Ricci et al, 2002) and Smart Hydrogel® (Potts et al, 1997a; 1997b) were selected for their thermal-induced gelation and reported bioadhesive properties. Also the polymer blends were created in order to combine the beneficial properties of a range of interesting polymers. Other oesophageal bioadhesion models have also examined retention of Antepsin®, as it adheres to damaged and ulcerated oesophageal mucosa (Dobrozsi, 1999). Glycerol was chosen as a control as it is a relatively viscous non-polymeric liquid (Dobrozsi, 1999), with no known bioadhesive properties.

A range of *in vitro* models has been reported in the literature that assess bioadhesion in different ways. For solid dosage forms, force of detachment studies have been employed to assess adherence of films and tablets *in vitro* on dialysis membrane and pig cornea (Henriksen et al, 1996), on porcine oesophageal tubes (Honkanen et al, 2002), and filter paper hydrated with a mucin dispersion (Bonferoni et al, 2004). Llabot et al (2004) measured force of detachment of a tablet from the surface of a 30% mucin gel Varshosaz and Dehghan (2002) coated thin glass plates with a polymer solution, before drying them. The coated plates were attached to a tensiometer and dipped into 1% mucous or alginate solutions, and the force of detachment measured. Many previous bioadhesion measurements made under a controlled environment are difficult to extrapolate to the performance *in vivo,* as the conditions are so different from the *in vivo* situation. The considerable discrepancies between studies are probably related to the different experimental conditions. Mortazavi (1995) therefore produced a tensiometer test in an attempt to standardise bioadhesion measurements, however this was for solid materials and there is no similar standard model for liquid retention. The adhesive properties of polymers are somewhat altered by hydration; Gurny (1984) and Mortazavi (1995) stressed that over hydration can cause the formation of a slippery mucilage and loss of adhesive properties. The application of liquid bioadhesion, although desirable, is therefore a challenge in pharmaceutical development, particularly where conventional bioadhesive polymers are concerned, and a means for assessment of bioadhesive performance of a liquid is required.

Models for assessing liquid bioadhesion have previously included an agar-coated glass tube, washed to mimic saliva flow (Ito et al, 1990). The polymers applied to the tube had been dyed, and the eluate was assayed colorimetrically. Park and Robinson (1984) performed a novel study of polymer binding to cultured cells. A fluorescent probe pyrene, localised in the lipid membrane produced a fluorescent spectra. The degree of change in fluorescence was proportional to binding of polymer to the cell membrane. Their study concluded that carboxylated polyanions were favoured polymers in terms of both bioadhesion and toxicity. Dobroszi and colleagues (1999) measured retention of commercially available sucralfate suspensions on rat oesophagus everted over a plastic rod. The dipping technique used had the benefits of physiological temperature control and fluid movement over

the tissue surface, but lacked peristaltic simulation and the amount of fluid available for contact was far greater than the saliva volume available in the oesophageal lumen *in vivo*. Richardson et al (2005) more recently adapted the tube-based model to measure alginate retention in the lumen of an oesophageal tube, but added the simulation of peristalsis by running a roller down the oesophageal tube accompanied by a volume of artificial saliva. Proportions of alginate remaining on the top middle and bottom of the oesophagus were assessed, and photographs were taken to record the appearance of the remaining tissue sections. This method was used to determine whether an alginate suspension composition could be altered to manipulate adhesion specifically to the distal oesophagus.

Despite all these different studies using a variety models to measure liquid retention, there are no reported studies that screen a wide range of liquids with different properties for their retention on the oesophagus, rather than e.g. a polymer series. This study aimed to achieve this, with a diverse collection of liquids being tested on the same model, to achieve a screening process that would aid selection of candidate vehicles to carry through to further studies.

4.1.2 Strengths of the current porcine *in vitro* model

The structure and function of organs such as the oesophagus differ between animal species. Hamilton and Orlando (1989) discovered that the opossum, but not the rabbit, oesophagus, secretes bicarbonate ions into the oesophageal lumen, this enables more rapid acid clearance in the opossum oesophagus. Submucosal glands are present in the opossum, but not rabbit oesophagus (Abdulnour-Nakhoul et al, 2000). As these glands are present (albeit in small numbers) in the human oesophagus, it is possible that human oesophagus is also capable of secreting bicarbonate acid (Hamilton and Orlando, 1989; Meyers and Orlando, 1992). Porcine tissue was used in the *in vitro* model as the biological substrate. It is considered to be one of the most comparable histologically with human oesophagus, as the amount of mucous and mucous glands present are of a similar order (Clemente et al 1990; Christie et al, 1995). Rat oesophagus contains a large amount of salivary mucin, but is devoid of oesophageal submucosal glands, and may be less representative of the human environment (Kinoshita et al 1999). The porcine gastrointestinal tract bears similarities to that of the human, in many aspects of

anatomy and physiological functions, probably due to the non-ruminant omnivorous diet that they both consume, unlike that of carnivores, ruminants, rabbits and rodents (Swindle et al, 2000).

4.2 Method of assessment of liquid retention *in vitro*

Bioadhesion was represented by the duration of liquid retention on porcine oesophageal mucosa during continuous washing, using an adaptation of the method described by Batchelor et al (2002). Porcine oesophagi were obtained fresh from a local abattoir on the day of slaughter. The oesophagi were gently rinsed with water to remove foodstuff and the outer muscular layer stripped away. The resulting oesophageal tubes consisting of epithelium and some external residual connective tissue were frozen at -70°C until required. Freezing the tissue prior to use has been discussed previously by Young et al (2002) and is considered suitable for storing animal tissue with minimal damage. Furthermore, Smart et al (2003) reported that freezing porcine oesophageal tissue was necessary to inactivate the muscle tissue and provide the flat surface needed for the retention study. Freezing also reduces the detrimental effects of bacterial and enzymatic tissue degradation.

Prior to each experiment, an oesophageal tube was allowed to equilibrate to room temperature. Longitudinal sections were cut, each approximately 6cm long. One section was mounted on a Perspex® platform with the mucosal surface upwards, at an angle of approximately 30°, in a humidity chamber. During the retention experiment the oesophageal strip was maintained at 37°C in high humidity (>90% relative humidity). Figure 4.1 shows a diagram of the apparatus used.

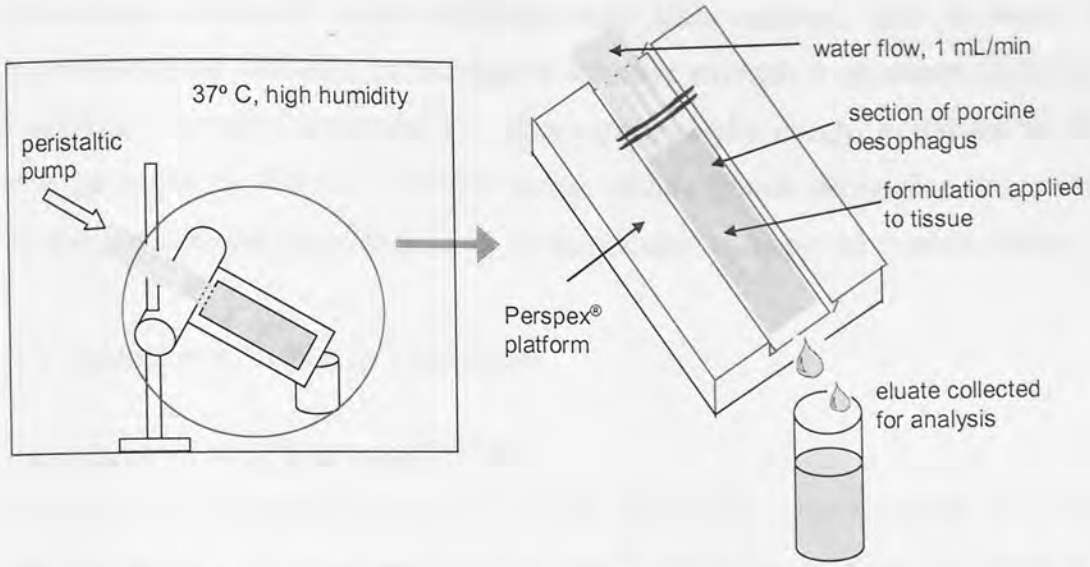


Figure 4.1. Diagrammatic representation of apparatus used to measure liquid retention on porcine oesophagus *in vitro*.

The wide range of liquids, both vehicles and drug-loaded formulations were prepared as described in Sections 2.7–2.10 and 2.13. For each experiment 0.1 mL (approx. 0.2 MBq activity) of the radionuclide Technetium 99m (Tc99m) was mixed with 1.5 mL of the formulation under investigation by vortexing for 15 seconds. 1 mL of this labelled formulation was dispensed onto the tissue surface using a syringe, and washing initiated; the surface was washed with distilled water at a rate of 1 mL/min to simulate saliva flow. Normal basal saliva flow rate in humans is 0.36 mg/min (Namiot et al, 1994a); The flow rate used in the *in vitro* model is faster than this, and therefore liquids may in reality have better opportunity for prolonged contact with the epithelium, and hence better retention *in vivo*. During the experiment, the eluate flowing off the tissue section was collected directly into 2.5 mL scintillation tubes at regular intervals up to 30 minutes. The radioactivity in each tube was measured in terms of counts per second (cps) (see section 2.5.2) to determine the percentage of the dose washed off at each time point ($n \geq 3$); from this a retention profile for each formulation was generated in terms of percent retention on the oesophageal tissue over time. For each experiment, all tubes were measured together at the end of the procedure, this resulted in all tubes being counted within 3 minutes, minimising the effects of Tc99m degradation over time.

The lack of equilibration time before the adhesive is washed off makes this model a closer representation of the oesophagus *in vivo* when a liquid medication is

swallowed. Although many adhesion tests allow contact time between the bioadhesive and substrate surface before adhesive strength is measured, this would not be a reasonable representation of oesophageal physiology, where saliva flow will be quick to follow a luminal bolus, without much delay. For this reason, washing was commenced as soon as the formulation was applied over the tissue.

4.3 Validation of Tc99m as a radiolabel

4.3.1 Benefits over fluorescent labelling

Previous studies reported using this model have used a water-soluble fluorescent dye as a marker that was associated with each applied formulation (Batchelor et al, 2002). However, in this study a radioactive label was selected in place of fluorescence as it is not affected by alterations in pH of the applied formulation, and is also unaffected by the opacity of a solution, unlike fluorescence spectrophotometer readings. This was considered important when washing biological tissue, as cellular matter can be present in the eluate, causing cloudiness. Also some of the formulations in this study were opaque (e.g. emulsions), or contained solid matter where drugs were in suspension. In addition, in tracing the adhesion of a solution *in vivo*, a radiolabel such as Tc99m is advantageous as it may be detected quickly using a gamma counter and its short half-life of approximately 6 hours is beneficial. Additionally, studies to measure oesophageal transit of the liquids in humans would be carried out using gamma scintigraphy and Tc99m would be a relatively safe material to use. The use of Tc99m *in vitro* would facilitate and enhance subsequent comparisons with *in vivo* scintigraphic studies. A dye that covalently binds to the analyte would be preferable. Tc99m tin colloid is aqueous and therefore can only be incorporated into the aqueous component of a formulation. This may have limitations e.g. when labelling an emulsion, if the oil phase is adhering to the tissue and the aqueous phase is washed away then the technetium label will not reflect this, and simply a poor retention profile will be seen as the Tc99m is washed off. An advantage of using Tc99m as a label is that at the end of each retention experiment, the tissue section may be placed inside a scintillation tube. The actual activity still remaining on the tissue may be directly measured in this way; this is interesting and helps to validate the γ-counter data.

A medical examination and training was carried out before the commencement of any experiments involving radioactive material. The ALARA (as-low-as-reasonably-achievable) principle was observed in the design and implementation of these studies. Two simple studies were performed to validate the use of this label; one to ascertain that the radiolabel is homogeneously distributed throughout the formulations, and a stability study.

4.3.2. Homogeneity of radiolabel

1.5 mL of the formulation under investigation was mixed with 0.1 mL of the tin colloid label (activity approximately 0.2 MBq), using a vortex mixer for 15 seconds. The volume was then divided into five aliquots; each volume was weighed and the radioactivity measured using a Packard Cobra Auto-Gamma Counter. The activity per gram in each portion (cps/g) was calculated (Appendix 4A). The values for cps/g were highly consistent in all samples of the vortexed material, and all within 95 percent of the mean cps/g. This was performed on a range of formulations from the lowest to highest viscosities and the results demonstrated that for the small volumes involved (2 mL) the 15-second mixing process was sufficient to distribute the Tc99m label homogenously within the sample.

4.3.3. Stability of radiolabel

When evaluating retention on mucosal surfaces by monitoring the retention of a label dispersed within a formulation, there is potential for loss of the label as it diffuses out of the formulation into the tissue or surrounding medium (Riley et al, 2002). A preliminary study was therefore performed to assess loss of radiolabel from the formulations over time (Jackson et al, 2001). 1.5 mL of labelled formulation was sealed inside dialysis tubing of 12-14 kDa MW cut-off. This tubing was placed within 100 mL of water and dialysed for at least 60 minutes. In each case the degree of associated label was maintained within the formulation at greater than 90 percent for time periods up to 60 minutes. Water was used as a control and the associated label was less than 10 percent retained after 40 minutes. This study demonstrated a satisfactory incorporation of radiolabel within the test formulations over the time course of each retention experiment.

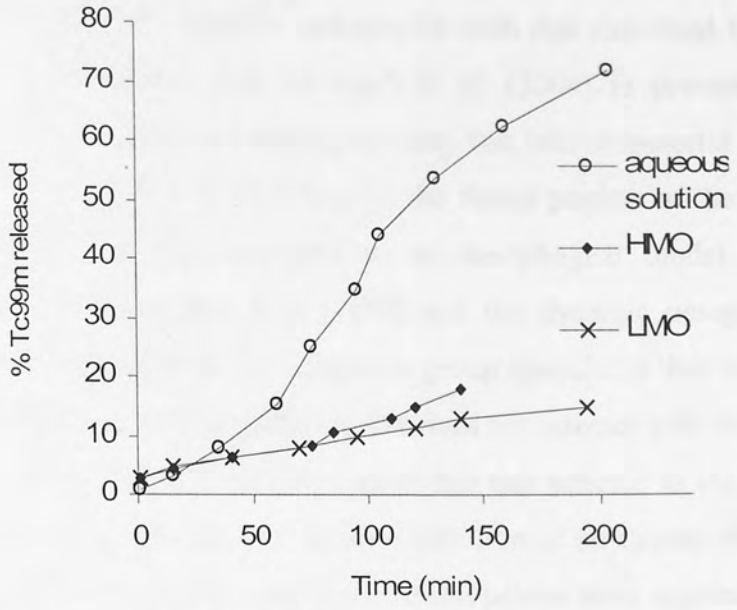


Figure 4.2. Examples of stability Tc99m incorporation assessments. Emulsions retain the label well with little released over time, but Tc99m diffuses quickly from aqueous solution.

4.4 Results and Discussion of *in vitro* retention model

4.4.1 Shape of retention profiles

Retention profiles of each formulation were plotted in terms of percentage of the applied formulation retained on the tissue against time. An example of the shape of retention profile observed is shown in Figure 4.3.

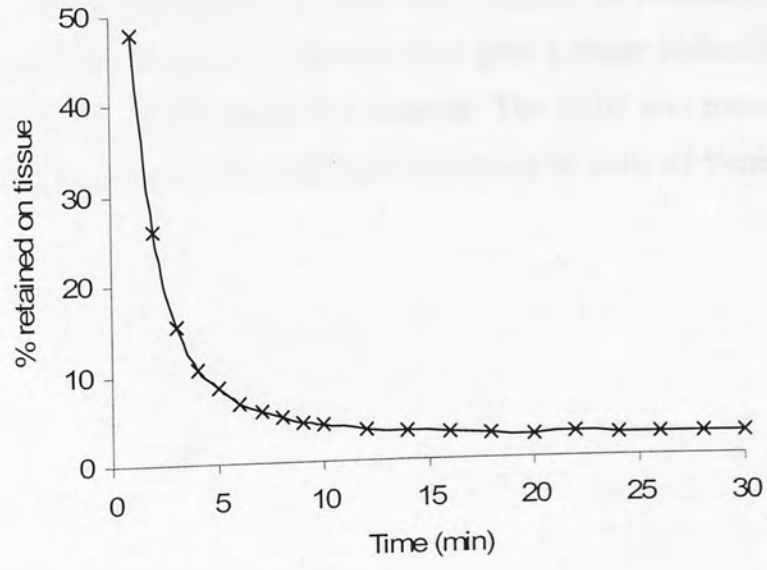


Figure 4.3. Mean retention profile of Blend A on porcine oesophageal tissue *in vitro*, demonstrating two-phase elimination.

The shape of the retention profiles, comparable with that described by Riley et al. (2002), Smart et al. (2003) and Kockisch et al. (2004) in previous work, and illustrated in Fig. 4.3, has two domains; an early fast loss of material followed by a slower loss of material that is identified by the flatter portion of the curve at later time points. This was also observed in an oesophageal model incorporating peristalsis used by Richardson et al (2005) and the dynamic oesophageal model used by Kockisch et al (2004). Richardson's group speculated that the initial stage represents the detachment of formulation that does not interact with the mucosa, and the second represents the loss of formulation that has adhered to the mucosa. This biphasic clearance may also be due to administration of an excess of material, and might be less evident if smaller volumes of formulations were applied. To facilitate comparisons between different formulations at early and later time regions, the area under the curve (AUC) was calculated for selected time periods.

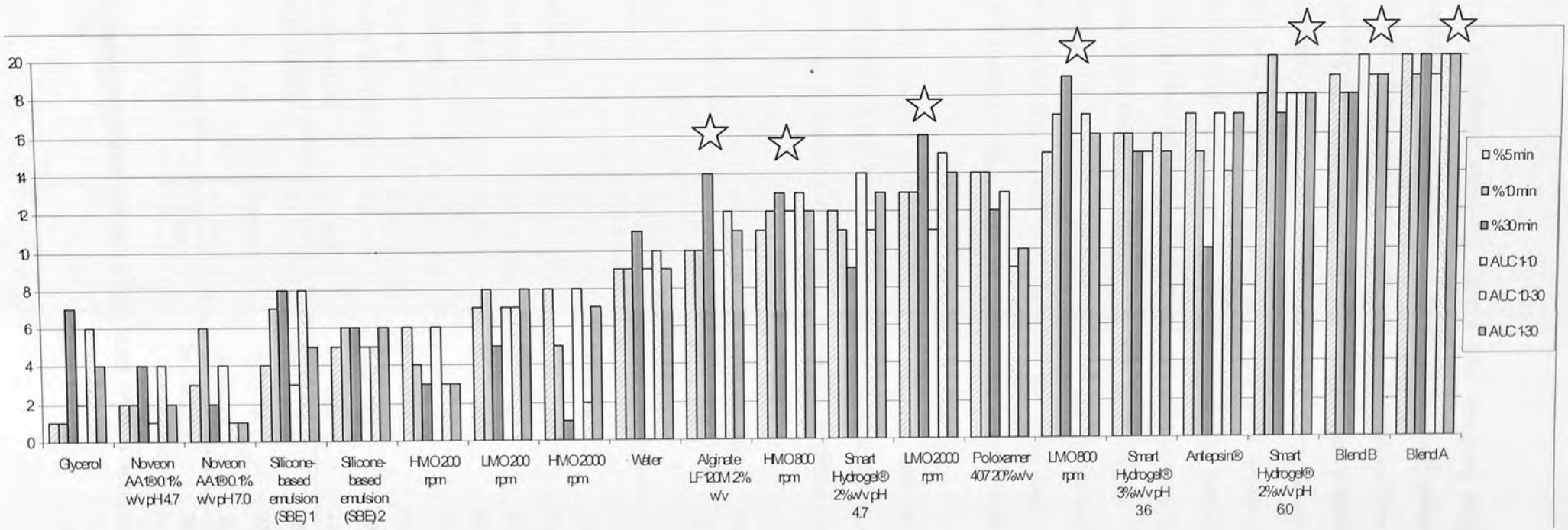
4.4.2 Retention profiles of formulations - percent retention and AUC data

Once the data had been analysed in terms of percent retained at different time points, and AUC over different time periods (see Appendix 4B and 4C), the differences in rank order of retention of the formulations using the different analysis parameters was assessed. The percentage of the dose retained at specific time points is one means of ranking the relative performance of adhesive materials over time however the specific time point selected can influence the rank observed. The AUC between two time points offers an alternative means of evaluating the overall adhesion between the two time points and may give a better indication of overall performance in terms of adhesion of a material. The AUC was measured via the trapezoidal method using Excel. AUC was expressed in units of %min throughout the study.

	Rank - % retained on tissue			Rank - area under retention curve		
	% 5 min	% 10 min	% 30 min	AUC 1-10	AUC 10-30	AUC 1-30
Glycerol	1	1	7	2	6	4
Noveon AA1® 0.1% pH 4.7	2	2	4	1	4	2
Noveon AA1® 0.1% pH 7.0	3	6	2	4	1	1
Silicone-based emulsion (SBE) 1	4	7	8	3	8	5
Silicone-based emulsion (SBE) 2	5	6	6	5	5	6
HMO 200 rpm	6	4	3	6	3	3
LMO 200 rpm	7	8	5	7	7	8
HMO 2000 rpm	8	5	1	8	2	7
Water	9	9	11	9	10	9
Alginate LF120M 2%	**10**	**10**	**14**	**10**	**12**	**11**
HMO 800 rpm	**11**	**12**	**13**	**12**	**13**	**12**
Smart Hydrogel® 2% pH 4.7	12	11	9	14	11	13
LMO 2000 rpm	**13**	**13**	**16**	**11**	**15**	**14**
Poloxamer 407 20%	14	14	12	13	9	10
LMO 800 rpm	**15**	**17**	**19**	**16**	**17**	**16**
Smart Hydrogel® 2% pH 3.6	16	16	15	15	16	15
Antepsin®	17	15	10	17	14	17
Smart Hydrogel® 2% pH 6.0	**18**	**20**	**17**	**18**	**18**	**18**
Blend B	**19**	**18**	**18**	**20**	**19**	**19**
Blend A	**20**	**19**	**20**	**19**	**20**	**20**

Table 4.1. Rank order (1 to 20) of retention of the formulations using the different analysis parameters, either percent retention or AUC at different time periods (1 = poorest retained, 20 = best retained). Bold figures indicate formulations that maintained a high ranking at both early and late time points.

Figure 4.4. Rank order (1-20) of retention of the formulations using the different analysis parameters, either percent retention or AUC at different time periods. Starred bars indicate vehicles that are well retained in the early stages, and also maintain good retention in the later stages. This was assessed by comparison of their position within the rank order at the earlier and late stages, and this may be seen in both the percent retention and the AUC data.



Glycerol used as a mid-range viscosity control (the viscosity of glycerol was the median of all formulations tested) performed poorly in the early phase of retention but compared well to other formulations at later stages. The tin colloid diluted in water alone performed averagely in the model, and any formulations performing poorer than water were considered poor candidates for retentive drug delivery systems. As the objective of these studies was to identify a liquid with prolonged retention on oesophageal tissue, it was considered that late retention was particularly important. The starred data in Fig. 4.4 indicate vehicles that are well retained in the early stages, but also maintain high retention ranking in the later stages. This is seen by comparison of their position within the rank order at the earlier and late stages, and this may be seen in both the percent retention and the AUC data. These promising formulations were amongst those chosen for subsequent *in vivo* studies.

The AUC of the early section of the profiles, 1-10 minutes, and the later section, 10-30 minutes as well as the total AUC 1-30 minutes were calculated. Table 4.2 shows how the retention of the materials tested varied according to these analyses.

	AUC 1- 10	**AUC 10 – 30**	**Total AUC 1 - 30**
Water	11.9 ± 5.0	12.5 ± 6.0	23.2 ± 10.3
Glycerol	6.5 ± 0.1	6.6 ± 0.1	12.6 ± 0.2
Blend A	108.8 ± 48.6	65.5 ± 39.8	172.4 ± 84.1
Blend B	113.2 ± 25.2	53.1 ± 25.4	162.6 ± 44.1
Smart Hydrogel® 2% pH 6.0	67.4 ± 18.0	52.0 ± 25.6	114.8 ± 35.5
Smart Hydrogel® 2% pH 4.7	46.2 ± 26.2	36.9 ± 6.4	80.4 ± 31.6
Smart Hydrogel® 2% pH 3.6	32.5 ± 9.1	18.8 ± 10.3	49.8 ± 13.5
Alginate LF120M 2 % w/v	22.1 ± 7.1	22.8 ± 17.1	43.7 ± 23.3
Poloxamer 407 2 0% w/v	33.4 ± 25.0	28.8 ± 19.2	60.2 ± 42.3
Noveon AA1® 0.1% w/v pH 7.0	9.6 ± 3.5	10.2 ± 5.5	19.1 ± 8.7
Noveon AA1® 0.1% w/v pH 6.0	7.8 ± 0.1	5.3 ± 0.0	12.8 ± 0.2
Silicone-based emulsion (SBE)	87.7 ± 84.4	68.0 ± 63.6	150.0 ± 134.8
LMO emulsion	46.6 ± 47.0	56.3 ± 72.1	99.4 ± 113.9
HMO emulsion	21.6 ± 8.3	18.6 ± 6.8	38.9 ± 11.7
Antepsin®	77.8 ± 5.7	26.7 ± 17.3	79.1 ± 44.7

Table 4.2. Area under retention curve (AUC) data over a selection of time points (mean ± S.D. n≥ 3).

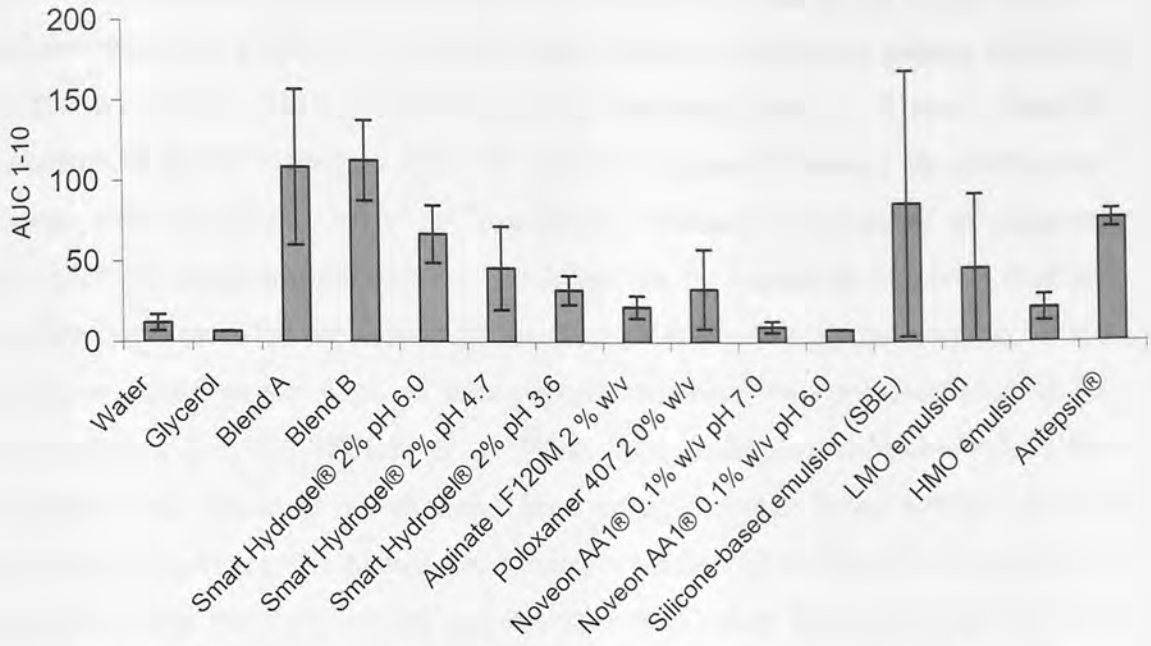


Figure 4. 5 Bar graph showing AUC 1-10 for all formulations (mean ± S.D. $n \geq 3$).

Figure 4.5 shows the data for the early AUC 1-10 minutes. It demonstrates that there was variability in the data, with large standard deviations produced as a result. Large standard deviations were observed with all time points, and during analysis of both percent retention data and AUC data. This means that many of the differences described between formulations are more descriptive rather than significant. The large errors encountered in the data may be due to a number of factors. The most obvious cause of variation is the use of animal tissue. A fresh section of porcine oesophagus was used for each experiment; therefore no two formulations were tested on the same surface. Animal tissue is variable between animals, and even within the same animal, with the proximal oesophagus having a different thickness and texture to the distal oesophagus. Other causes of variability in the results include fluctuations in the temperature and humidity inside the chamber, which could affect the properties of the formulations. The rate of syringing the formulation on to the tissue surface was kept as constant as possible, so as to keep the shear force experienced by each formulation constant, however this is subject to human error. Another source of variability is the time in between syringing the formulation onto the tissue and the onset of water flow over the tissue surface. This was done as quickly as possible, although human error may have led to a 1 to 2 second difference each time.

When analysing data for the polymer blends, it was noted that Blend A and Blend B exhibited retention profiles of a similar order. Blend A displayed greater retention over the later time period (10-30 min) and for the total AUC (1-30 min). Blend B demonstrated greater retention over the first 10 minutes. However, the differences between the two blends were not significant. Although the blends of alginate, poloxamer and polycarbophil were well retained on the oesophageal tissue, it might have been predicted that the poly(ethylene oxide), a H-bond accepting polymer within poloxamer, could in fact form an inter-polymer complex with polycarbophil, an H-bond donating polymer (Bailey et al 1964), thus producing cohesion within the formulation and reducing the potential for bonding between tissue surface and the formulation. However, the blends had greater retention AUCs than any other liquid tested over both the early period and the total AUC. Any free polyethylene oxide chains may have been available for interpenetration with mucous on the oesophageal surface (De Ascentiis et al, 1995), however, such an interaction is probably minimal due to the lack of a well-defined mucous layer on the porcine tissue used. It was noted that the physical blends had significantly higher AUC 1-10 than any of their single components, Noveon AA1®, alginate or Poloxamer ($p<0.05$). On the basis of the poloxamer concentration within the blends, thermogelling properties would not be predicted (at least 16 % w/v would be expected to impart this property (Brown et al, 1995a)), but the blends showed thermogelling behaviour when tested in our laboratory between 4 and 40°C (Chapter 3). This is unexpected, as solutions of the thermogelling component, Poloxamer 407 alone do not gel until a concentration of approximately 10% w/v is reached. This phenomenon is possibly a result of interactions between the components present and may be worthy of further investigation. Despite the excellent retention of the polymer blends in the *in vitro* study, their high viscosity may make them difficult to swallow in practice and their high poloxamer content may present toxicity concerns.

Retention of Smart Hydrogel® and Noveon AA1® displayed pH dependence in this *in vitro* model, which is in concordance with studies by Hassan and Gallo (1990) which found Smart Hydrogel® bioadhesion to be pH dependent and Ch'ng et al. (1985) and Tobey et al. (1996) who found Noveon adhesion to pig gastric tissue to be affected by the degree of neutralisation. Both of these polymers were best retained at the highest pH values tested, pH 6.0 for Smart Hydrogel® and pH 7.0 for Noveon AA1®. The

viscosity of both polymer solutions is pH dependent, with an increase in pH causing an increase in viscosity.

Noveon AA1® is the acidic form of polycarbophil, and is a well-documented bioadhesive (Smart, 1984; Duchene et al, 1988). The carboxyl groups present in the polycarbophil structure may enhance its ability to bond with mucin on a mucous membrane. Unexpectedly the two Noveon AA1® solutions did not exhibit more prolonged retention than the distilled water control over any time period. The reason for this is unclear, as Noveon AA1® is a recognised bioadhesive that would be expected to be well retained on a mucous membrane (Duchene et al, 1988). It is possible that in this model, Noveon AA1® in fact does bind strongly to mucin, which is present on the oesophagus due to salivary content, but is not an adherent layer (Dixon et al, 2001). The mucous and bound polymer would therefore be rapidly washed away. This highlights a possible disadvantage of using a specifically mucoadhesive substance to target the oesophagus, where the presence of an adherent mucous layer is disputable.

Other researchers have reported the poor adhesion of polycarbophil under experimental conditions. A solution of Noveon AA1® (0.5% w/v) was found previously to show a poor bioadhesive performance when compared to other polymer hydrogels (Young et al, 2002). In that study, the work of adhesion was proportional to the mucoadhesive polymer-surface concentration, highlighting the importance of interactions at the surface to bioadhesion. Also Vermani et al (2002) tested both the bioadhesive strength of polymers and their retention on excised sheep vaginal tissue. Although they found both sodium alginate and polycarbophil to have significant inherent bioadhesive strength, in the retention studies polycarbophil performed poorly under their experimental conditions. Henriksen et al (1996) found that neither tablets nor films of Noveon AA1® were adhesive at pH 7.2 in force of detachment studies on dialysis membrane. They also discovered Noveon AA1® gel did not adhere at neutral pH when applied to buccal mucosa in organ culture. Polycarbophil reportedly shows inconsistent mucoadhesion depending on experimental features or mucosal characteristics (Singla et al, 2000; Accili et al, 2004). However the bioadhesion of Noveon AA1® as assessed by this model did demonstrate the pH dependence that was expected (Fig 4.6).

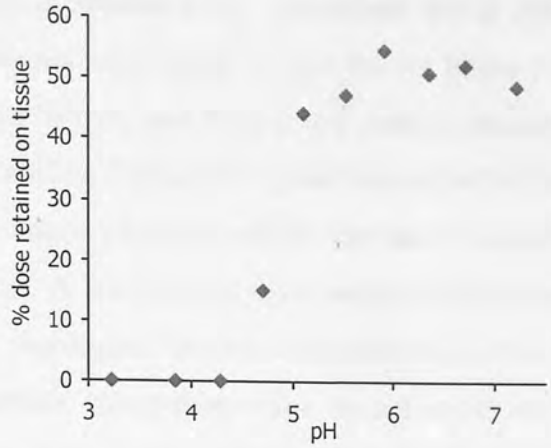


Figure 4.6. Relationship between pH and mean in vitro percent retention of Noveon AA1® 0.1% w/v. (Note: this experiment used a fluorescent label.–See Appendix 4F)

As the viscosity of Noveon AA1® is closely related to pH (Chapter 3), it follows that the retention of the polymer solution is related to its viscosity also (Fig 4.7).

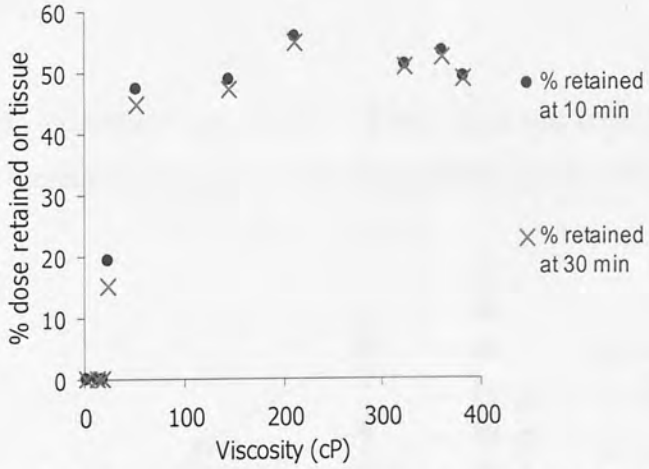


Figure 4.7. Relationship between viscosity and mean *in vitro* percent retention of Noveon AA1® 0.1% w/v. (Note: this experiment used a fluorescent label).

Poloxamer 407 is a thermally responsive non-ionic, surfactant, tri-block copolymer of ethylene oxide and propylene oxide. Such a molecule might be capable of hydrogen bonding with mucin as a result of the H-bond accepting property of ethylene oxide. The poloxamer 407 used in this study demonstrated more prolonged retention than Noveon AA1®, alginate LF120M, Smart Hydrogel® at unadjusted pH, and the HMO emulsion, although not reaching significance due to the large variability (see table 4.2). A previous study by Mathias et al. (2002) also found poloxamer 407 to be more adhesive on porcine oesophageal tissue than alginate. However that study investigated polymers as dried coatings on a glass disc, and therefore tested them in the solid form

rather than in solution. In another study, poloxamer 407 at 20% w/v was found to be adhesive to rectal mucous membranes in rats for six hours (Charrueau et al, 2001). Poloxamers are surface active, and have a low surface tension (see section 3.4) and therefore high spreadability. This allows good contact with the substrate, which may account for their enhanced adhesion when compared to more viscous liquids with higher surface tensions. A study found no substantial bioadhesion of poloxamer 407 when measured by a rheological method with porcine gastric mucin, suggesting that the interdiffusion between this polymer and mucin is not an important factor in its bioadhesion (Hassan and Gallo, 1990). The good performance observed for poloxamer 407 may be due to its low surface tension and ability to gel at body temperature (Brown et al, 1995a; Mathias et al, 2002), see Chapter 3 . It has been reported that some multidrug- resistant carcinoma cells are hypersensitive to poloxamers; therefore their bioadhesive properties could aid targeted treatment in oesophageal cancer by enhancing the activity of antineoplastic agents against tumours (Amiji and Park, 1992).

All Smart Hydrogel® solutions were retained better than the controls, and retention was pH dependent. Increased pH and viscosity both led to better retention (Fig. 4.8).

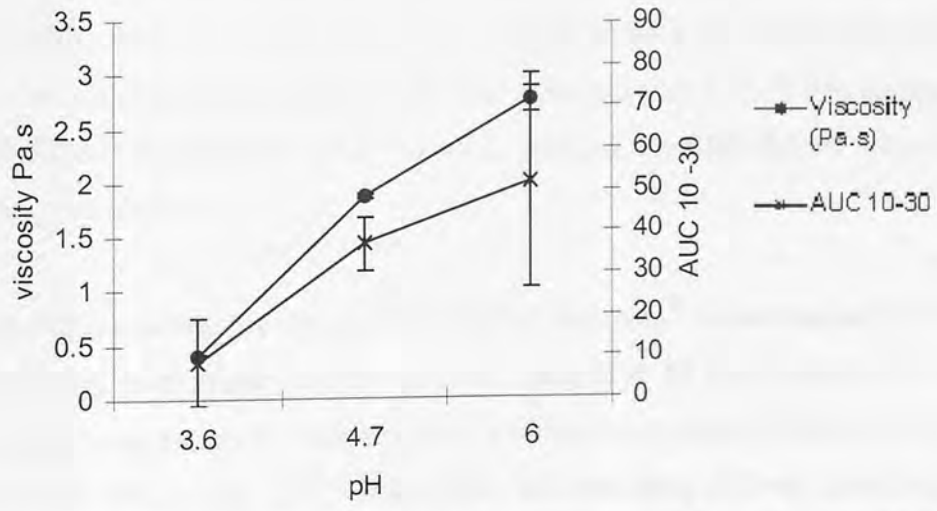


Figure 4.8. pH dependence of viscosity and retention of 2% w/v Smart Hydrogel® solution (retention data = mean AUC 10-30 ± S.D n ≥ 3).

All Smart Hydrogel® solutions adhered significantly better than Noveon AA1® solutions, for AUC and percent retention data ($p<0.05$) (Fig 4.4). Smart Hydrogel® at pH 6.0 was better retained than Noveon AA1®, alginate and HMO emulsion (for AUC

1- 10 and AUC 1-30, $p<0.05$). Its increased viscosity (1.5 times higher than the pH 4.7 viscosity and 7 times more viscous than the unadjusted pH 3.6 solution) may prolong passage time sufficiently to allow the complete bulk of the liquid to warm to 37°C and gel *in situ*. As the lower pH 3.6 solution is less viscous it may pass over the tissue before it has time to respond to the change in temperature. At all three pH values (pH 3.6, 4.7 and 6.0), Smart Hydrogel® 2% w/v was found to gel from approximately 27°C when tested using an oscillatory rheometry temperature ramp between 4 and 40°C (Chapter 3). As the high molecular weight polymers investigated are unlikely to be absorbed, there are few concerns regarding their systemic toxicity (see section 1.7.4). However, Smart Hydrogel® is likely to be a safer drug delivery system than the physical blends used here as it contains a far lower percentage concentration of polymers and has lower potential for any toxicity. Moreover, both components of Smart Hydrogel® have approved regulatory status and an animal toxicity study has demonstrated the low toxicity of the polymer (Bromberg and Ron, 1998).

In the category of emulsions, the silicone-based emulsion (SBE) consistently yielded the greatest AUC for all time periods calculated (see Table 4.2). However, the errors for SBE were larger than those observed with other formulations (see Fig 4.5). This may be due to the relatively complex formulation process for the SBE, which might result in inconsistent products produced each time (section 2.8). When comparing all the formulations, for the early AUC period (1-10 min), the SBE did not adhere as well as the polymer blends.

All three emulsions and the commercial product Antepsin® were retained on the tissue for longer than both water and the glycerol control at all time points. For all time periods, LMO was retained to approximately twice the extent of HMO (46.6 ± 4.7 vs. 21.6 ± 8.3 at AUC 1-10). LMO is therefore the best drug delivery candidate of the three emulsions, in terms of retention.

The aqueous sucralfate suspension Antepsin® demonstrated appreciable adhesion during the early period, only surpassed by the silicone-based emulsion and the blends (see table 4.2). However for the later time period it did not continue to adhere as well.

These results were as expected; sucralfate is used to treat ulcerated areas of mucosal tissue, where it specifically adheres. Although it does adhere to non-ulcerated tissue (Dobrozsi, 1999), this is not where its bioadhesion is most effective, and not what the product is designed to do. A scintigraphic study by Taal et al (1995) found that sucralfate was ineffective in coating the oesophagus for prolonged periods. Dobrozsi's group found that other types of sucralfate suspensions, containing the gel form of sucralfate, are more adhesive to oesophageal mucosa than Antepsin® (1999).

Glycerol was used as a control (Section 4.1.1), due to its non-adhesive but viscous properties (Dobrozsi et al, 1999). Over all the time periods, AUC was consistently lowest for the glycerol control, i.e. all formulations were better retained on the tissue than glycerol. All of the formulations, with the exception of Noveon AA1®, were retained for longer than the water control also. It was concluded that these are suitable controls for this study. The poor retention of glycerol is evidence that high viscosity is not the only important factor in bioadhesion.

4.4.3 Fit of retention data to models

Graphs were drawn of time against percent retained on tissue, time against ln percent retained, and time against the reciprocal percent retained, in order to determine whether the retention profiles of the vehicles demonstrated zero, first or second order clearance. The linearity of each graph was indicated by the linear R^2 correlation coefficient value as shown in Table 4.3. The retention profiles of all vehicles were best fitted to a second order removal process, where time versus reciprocal percent retention was linear over the full 30 minutes. Correlation coefficients for this second order removal ranged from $R^2 = 0.71$ to $R^2 = 1.00$ (see Table 4.3).

	Zero order % vs. t	First order ln % vs. t	Second order 1/% vs. t
SBE	0.65	0.91	0.99
Smart Hydrogel® 2% pH 4.7	0.36	0.77	0.98
Smart Hydrogel® 2% pH 6.0	0.65	0.94	0.99
Blend A	0.36	0.68	0.89
Antepsin	0.51	0.89	0.99
Gaviscon Advance	0.28	0.65	0.94
Noveon 0.1% pH 7	0.52	0.86	0.94
Noveon 0.1% pH 6	0.47	0.78	0.97
Noveon 0.1% pH 4.7	0.52	0.79	0.95
Blend B	0.33	0.70	0.96
Alginate LF120M 2%	0.26	0.48	0.71
dH_2O	0.53	0.77	0.94
Glycerol	0.40	0.63	0.93
HMO 200 rpm	0.46	0.80	0.97
HMO 800 rpm	0.54	0.79	0.95
HMO 2000 rpm	0.52	0.90	0.98
LMO 800 rpm	0.51	0.85	1.00
LMO 200 rpm	0.60	0.83	0.96
LMO 2000 rpm	0.59	0.77	0.90

Table 4.3. Linear correlation coefficients for the percentage of vehicle retained and its transformations against time.

4.4.4 Importance of viscoelastic properties in retention

The rheological parameters of viscosity, elastic modulus (G') and viscous modulus (G") were assessed for all formulations, using the shear and oscillatory rheometry as described in Chapter 3. Additionally, from this data, the loss tangent, tan δ, was calculated by the formula G"/G'. Tan δ is a description of the overall elasticity of the material and provides a more intrinsic comparison of liquids with respect to each other than G" or G' alone (Caramella et al, 1999). A higher loss tangent indicates a lower elasticity. All rheological parameters were plotted against percent retention data and AUC data, in order to determine whether there was a relationship, and the strength of any relationship. Strength of linear correlations between rheological parameters and *in vitro* retention data were represented by the correlation coefficient (R^2).

	% 5 min	% 10 min	% 30 min	AUC 1-10	AUC 10-30	AUC 1-30
Viscosity (Pa.s)	0.3983	0.3193	0.2121	0.4568	0.2615	0.3979
G' (Pa)	0.0314	0.0175	0.0031	0.0194	0.0017	0.0058
G" (Pa)	0.1218	0.0389	0.0113	0.1155	0.1912	0.0125
G": G' (tan δ)	0.4777	0.3131	0.2284	0.157	0.2212	0.1799

Table 4.4. R^2 correlation coefficients showing strength of relationship between rheological and retention data (retention data = mean, n ≥3).

Of particular interest was the relationship between loss tangent and vehicle retention on tissue. This data is presented in Figure 4.9.

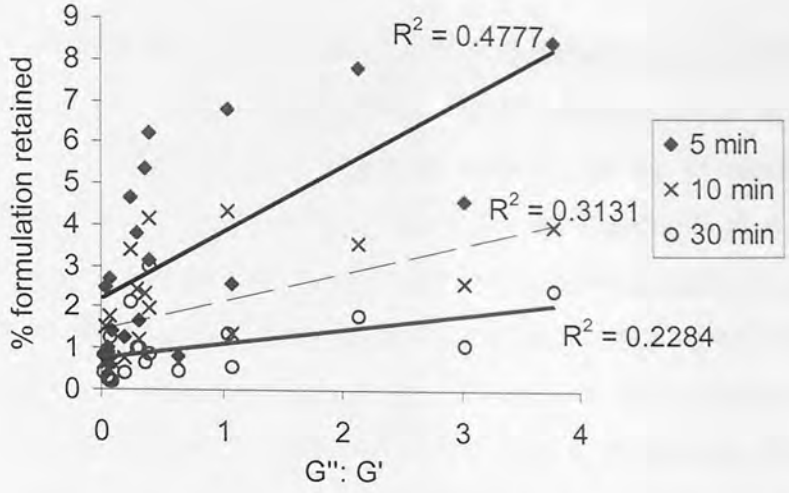


Figure 4.9. Scattergraph showing loss tangent tan δ (G'': G') vs. percent retained on porcine tissue at 5, 10 and 30 minutes. The correlation coefficient for each time point is indicated by R^2 value.

There was a weak positive correlation between tan δ (G'':G') and liquid retention. This indicates that there is a weak relationship between retention and the extent of elasticity within the vehicle. The greater the viscous: elastic ratio within the material's structure, the better retained it is on the tissue. It is interesting to note that the correlation weakens further with retention assessed at later time points (e.g. $R^2 = 0.48$ at 5 min, vs. 0.23 at 30 min). As the relationship is stronger between this rheological parameter and retention at early time points, this suggests that the fluidity of the sample is most important in early retention.

The wetting theory of bioadhesion suggests that low viscosity and a high spreading ability (represented by a small contact angle) are conducive to adhesion, as these properties allow the fluid to spread freely, and achieve a more intimate contact with the substrate surface (Sahlin and Peppas, 1997a). A high loss tangent means a liquid is more viscous rather than elastic, and therefore spreads over the tissue surface more easily, moving into crevices and folds in the target tissue. A high tan δ should mean that the liquid achieves more intimate contact with the biological surface and therefore has a better opportunity for bioadhesion.

It can be concluded that a liquid with a greater liquid than solid character is more likely to achieve bioadhesion at early time points, as a high viscous to elastic ratio is important for initial contact with the tissue.

It is important to note that none of the rheological parameters examined showed a very strong correlation with retention. The overall viscosity of a liquid therefore cannot be used as a predictor of its retention capacity, as the relationship between rheology and retention is undoubtedly not a simple one. Caramella et al. (1999) also found no correlation between rheological and tensile testing parameters, apart from the tan δ. They attributed a lack of correlation between the rheological parameters and work of adhesion to the diversity of the mature of the polymers they were considering. The liquids tested in this study were even more diverse. However some homologous series do demonstrate a trend. When a series of Noveon AA1® solutions encompassing a range of viscosities is examined using this model, the retention and viscosity are closely correlated (Russell et al, 2003). It is also interesting to note that for the three emulsions, there is in fact a tentative relationship between viscosity and retention, i.e., SBE> LMO> HMO for both retention and viscosity.

Young et al (2002) found a correlation between the viscous modulus G' and mucoadhesion of polymer solutions, within a concentration series. As can be seen in Table 4.4, no relationship is observed between the viscous modulus G' and any of the retention time points examined, in terms of percentage retention of AUC of retention curve. The R^2 values shown in Table 4.4 indicate a lack of linear correlation, ranging from 0.03 to 0.31 only. A study by Young and Smart (2000) also discovered that the viscosity (in Pa.s) of semi-solid hydrogels was proportional to the half-life of the initial first order removal from biological surfaces, and their experiments were performed similarly using porcine oesophagus. The same assessment was performed on the data gathered using this model, using the first six minutes of each retention profile to obtain a half-life value for initial oesophageal clearance. Figure 4.10 demonstrates that such a relationship was not found when examining the wide variety of liquids tested in this model. The discrepancies may be due to the similarity of formulations tested by Young et al. in both studies (all polymer dispersions) compared with the diversity of the formulations tested in this study.

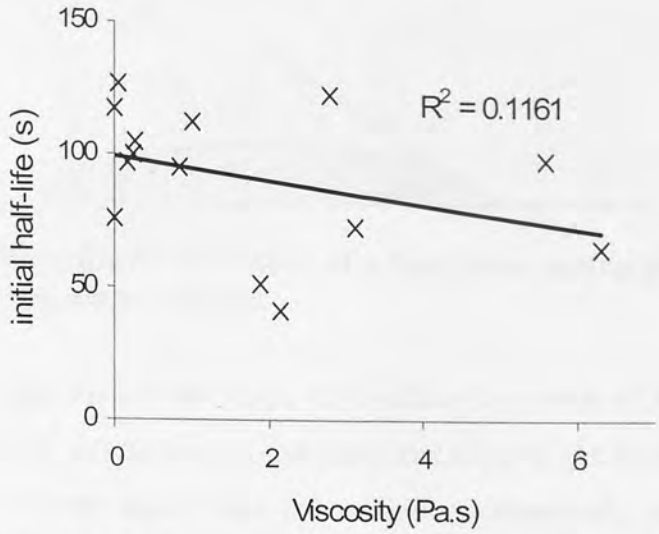


Figure 4.10. Relationship between viscosity and mean initial half-life of loss of formulation from porcine oesophageal tissue

4.4.5 Importance of surface tension in retention

Kinloch (1980) stated that the factors important for bioadhesion were a zero or near-zero contact angle, a relatively low viscosity, and contact at the interface that prevents air entrapment. These factors are collectively known as the Sharpe-Schonhorn criteria (Peppas and Buri, 1985). The wetting theory of bioadhesion suggests that the ability of a liquid to spread over the biological substrate surface is an important factor in liquid bioadhesion (Peppas and Buri, 1985; Mikos and Peppas, 1989; Chickering and Mathiowitz, 1999).

Tension in surface layers is the result of the attraction of bulk material for the surface layer. This attraction tends to decrease the number of molecules in the surface region, resulting in an increase in intermolecular distance. This increase requires work to be done and returns work to the system upon a return to a normal configuration. This can be explained by the Dupré equation $W_A = \gamma_b + \gamma_t - \gamma_{bt}$. The equation states that the work of adhesion W_A is equal to the sum of the surface tension of the bioadhesive γ_b and the surface tension of the tissue γ_t , less the interfacial tension γ_{bt}. It can be seen from this that the lower the surface tension of the bioadhesive, the lower the work of adhesion.

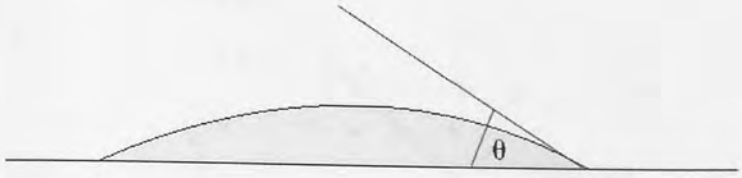


Figure 4.11. Diagrammatic representation of a liquid drop resting in equilibrium on a solid surface, showing contact angle θ.

Figure 4.11 represents the contact angle experienced by a drop of liquid resting on a solid surface. When $\theta > 0$, there is incomplete spreading of the liquid over the tissue surface. When $\theta = 0$, the liquid wets the surface spontaneously and spreads freely. The lower the surface tension, the more spreading occurs, and the more likely intimate contact is to be achieved between the adhesive and the substrate. Thermogelling polymers may be particularly successful in targeting the oesophagus, as they start as a flowable, pourable liquid that is able to spread over the tissue, but quickly warms up to body temperature and gels on the tissue surface. The spreading coefficient and surface tension are likely to be important factors in determining retention.

Rather than investigating the analysis of loss tangent and surface tension for all formulations, polymer-based and emulsion-based formulations were considered separately.

When analysing the polymers alone, a linear trend ($R^2 = 0.78$) was found between tan δ and surface tension (Fig 4.12). Therefore, for polymer solutions, a high viscous: elastic ratio correlates with a low surface tension, and therefore such a formulation is likely to have a high spreading ability.

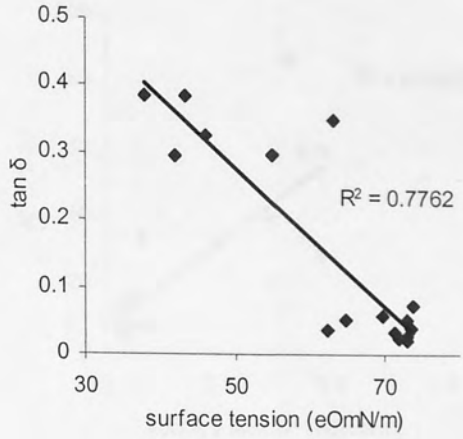


Figure 4.12. Scatter diagram showing relationship between loss tangent and surface tension in polymer formulations.

It was found that for polymers, there were moderate correlations between surface tension and percent retained on tissue ($R^2 = 0.48$) (Fig. 4.13) and between tan δ and early AUC 1-10 ($R^2 = 0.61$) (Fig. 4.14). Both of these correlations become weaker as later retention time points are examined, which implies that these relationships are more significant in the early stages of adhesion.

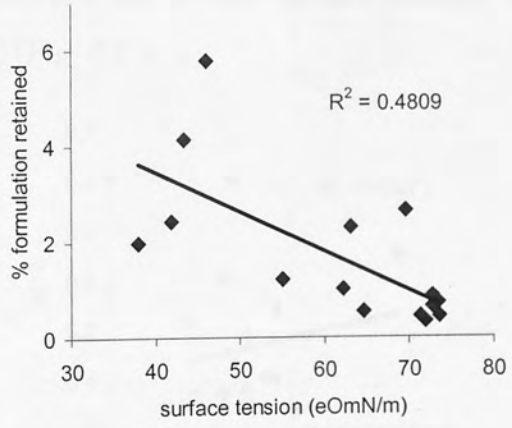


Figure 4.13. Scatter diagram showing relationship between surface tension and percent retention of polymer formulations after 10 min.

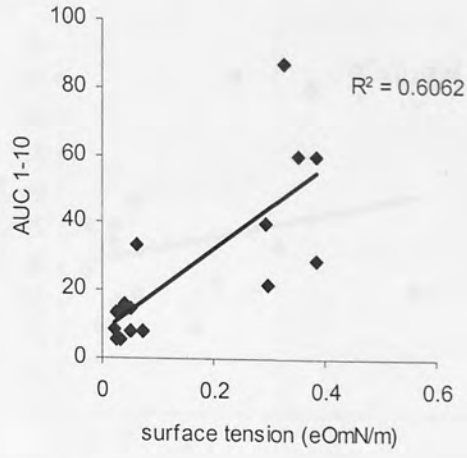


Figure 4.14. Scatter diagram showing relationship between tan δ and AUC 1-10 for polymer formulations.

It can be concluded that a low surface tension or a high viscous: elastic ratio (loss tangent) within the material are beneficial for retention on oesophageal tissue *in vitro*, particularly at the early time points.

A different situation was observed when examining the emulsions in isolation. There was no relationship between tan δ and surface tension of the emulsion-based formulations ($R^2 = 0.03$) (Fig. 4.15).

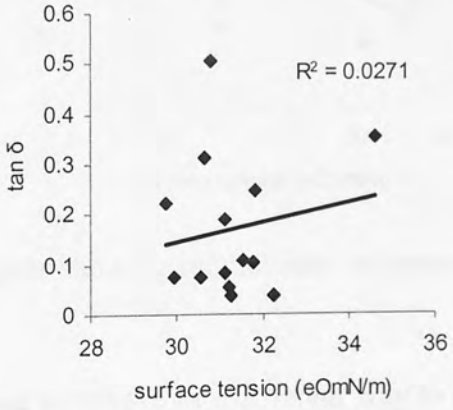


Figure 4.15. Scatter diagram showing relationship between surface tension and tan δ of emulsions.

The lack of linear relationship suggests that the loss tangent would not be expected to affect the surface tension of emulsions, or their wetting ability. This was confirmed as loss tangent is not related to retention of the emulsions (Fig 4.16.)

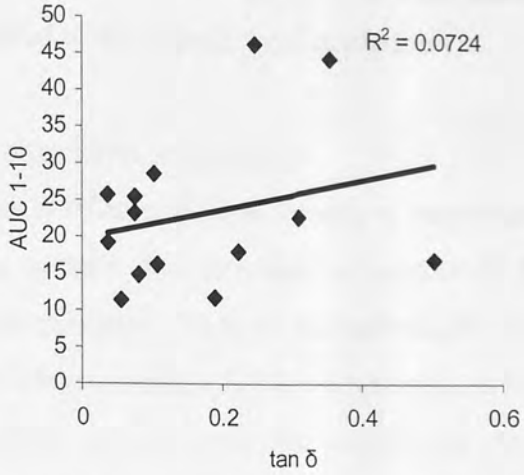


Figure 4.16. Scatter diagram showing relationship between tan δ and AUC 1-10 min of emulsions.

In fact, retention of the emulsion-based formulations is not related to the surface tension either ($R^2 = 0.08$) (Fig. 4.17).

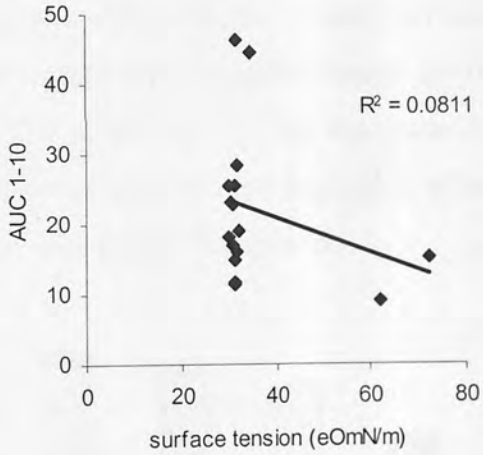


Figure 4.17. Scatter diagram showing relationship between surface tension and AUC 1-10 of the emulsions.

Emulsions therefore appear to behave in a different way to polymer solutions, both in terms of their loss tangent not relating to surface tension, and in the factors influencing their retention on porcine oesophageal tissue *in vitro*. The mechanisms by which emulsions adhere to the oesophagus and are able to exhibit prolonged retention are unclear, and need further investigation. Bioadhesive drug delivery systems involving liquids have usually focussed on polymer solutions, and emulsions are a less-explored strategy. The adhesion of emulsions to the surface may be the result of hydrophobic or electrostatic interactions. This might be investigated by lipophilicity

and surface charge studies, and it would be helpful to determine which phase(s) of the emulsion remain(s) adhered to the oesophageal epithelium.

4.4.6 Effect of Drug incorporation into vehicles

Although the identification of bioadhesive liquids is important in the design of the proposed drug delivery system, the retention properties of the system with drug incorporated must also be assessed. These investigations are crucial because a vehicle demonstrating excellent adhesion might fail to be retained on the tissue once a drug is incorporated, and another vehicle may be unaffected by drug incorporation, simplifying prediction of its behaviour. This effect would depend on the mechanism(s) of adhesion to the oesophagus.

4.4.6.1 Drug incorporation into Smart Hydrogel®

When the candidate drugs ISDN and SIL were incorporated into 2% w/v Smart Hydrogel®, there were notable effects on the viscosity of the system (Fig. 4.18). Both drugs caused a significant reduction in overall viscosity ($p<0.05$), with SIL having the more prominent effect. The effect on viscosity was dose-dependent for both drugs, and the incorporation of drugs also had a comparable effect for the pH 6 hydrogel solution as the effect seen with the pH 4.7 solution.

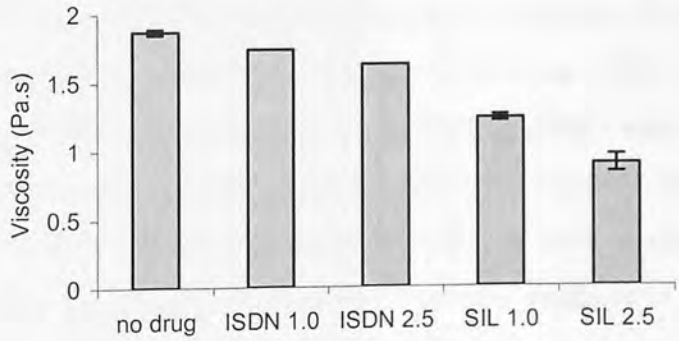


Fig 4.18. Effect of drug incorporation on viscosity of 2% w/v Smart Hydrogel® at pH 4.7. Drug concentrations = mg/mL (viscosity measurements are mean ± S.D. n= 3).

This reduction in viscosity did not result in the expected detrimental effect on retention, however. Both ISDN and SIL enhanced retention of the hydrogel, with best retention achieved with the lower drug concentrations (1 mg/mL) (Figure 4.19).

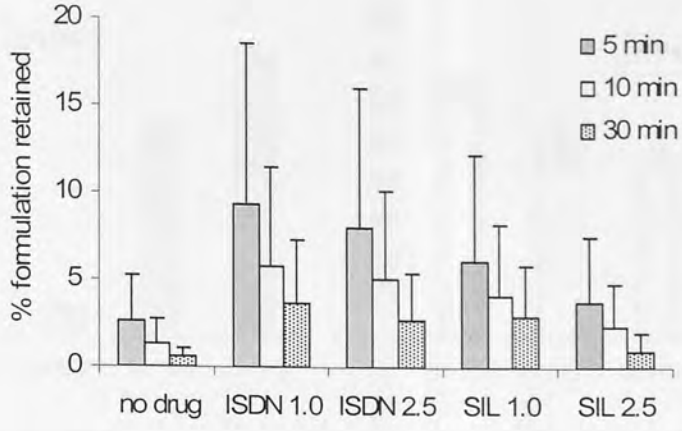


Figure 4.19. Effect of drug incorporation on retention of 2% w/v Smart Hydrogel® at pH 4.7. Drug concentrations = mg/mL. Means ± S.D, n≥ 3.

The impact of drug incorporation on formulation retention must be considered in the selection of a suitable drug delivery system. It would be of interest to test other drugs in the Smart Hydrogel® vehicle to investigate further how the drug affects the properties of the hydrogel and if particular characteristics of the drugs are responsible. There may be an ionic interaction between the drug and the hydrogel polymer that affects the polymer structure and prevents it from normal interdiffusion or attractive electrostatic interactions with the oesophageal surface.

4.4.6.2 Drug incorporation into emulsions

The large variability in formulation and hence unreliability of the SBE made it unsuitable for further investigation. The o/w emulsions LMO and HMO were investigated further. When the candidate drugs ISDN and NIF were incorporated into o/w emulsions, there were notable effects on viscosity (Figure 4.20). ISDN caused a significant reduction in overall viscosity ($p<0.05$) of both emulsions, with a 28 percent decrease in viscosity of HMO and a 77 percent decrease in viscosity of LMO caused by ISDN 2.5 mg/mL. Incorporation of NIF affected the emulsions differently. The viscosity of LMO was increased by 62 percent with a 1.0 mg/mL NIF concentration, and more than doubled by a 2.5 mg/mL concentration.

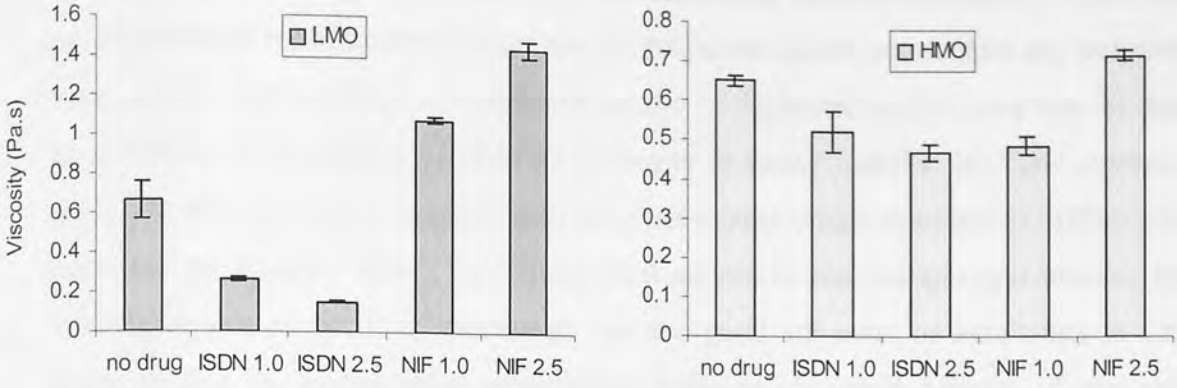


Figure 4.20. Effect of drug incorporation on viscosity of LMO and HMO emulsions. Drug concentrations = mg/mL. Mean ± S.D, n=6.

NIF caused a 25 percent decrease in HMO viscosity at low concentration and a 10 percent increase at high concentration. The reasons for these changes in viscosity have not yet been elucidated.

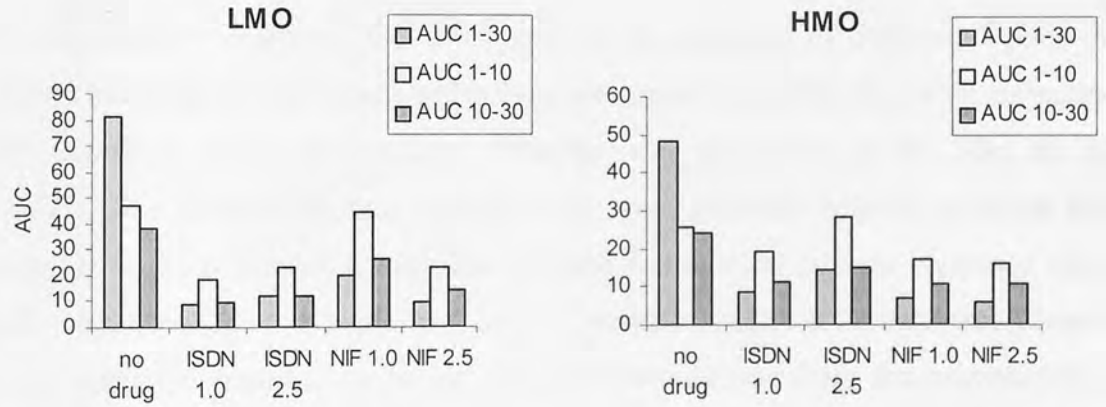


Figure 4.21. Effects of ISDN and NIF incorporation on the retention (of LMO and HMO emulsions. Mean, n≥ 6.

Both emulsions demonstrated reduced retention caused by the incorporation of drugs (Fig. 4.21). Although both ISDN and NIF appeared detrimental to the retention of LMO, due to the large standard deviations of the data collected, this effect was not statistically significant. The negative effects of ISDN on HMO retention also failed to reach statistical significance. However, retention of the HMO emulsion was significantly impaired by NIF incorporation. These experiments indicate that LMO might be the stronger candidate for further development, as it is less likely to be negatively affected by the incorporation of drugs. Similar studies could be carried out using different model drugs to verify this.

The prolonged retention exhibited by emulsions in this study is interesting. These are unconventional bioadhesives as they are not polymers and do not contain any polymer constituents. The retention of emulsions cannot be explained in the same way as that of polymers. Bioadhesion of cationic polymers is easily justified by their opposite charge to the cell membrane and their polymer chains which are able to diffuse and penetrate the mucous layer. Emulsions must adhere to the oesophageal mucosa by different mechanisms. It is speculated that the good adhesion of emulsions in this study is due to hydrophobic interactions between the lipid bilayer of the cell membrane and the lipid component of the emulsion. Further work must be carried out to elucidate which phase(s) of the emulsion remain adhered to the epithelium over the course of the experiments.

4.5 Conclusions

In vitro studies considered the differences in the retention of different liquids on porcine oesophageal epithelium under dynamic conditions, when placed on an incline and washed to mimic saliva flow. Retention was monitored in this way for 30 minutes. Two distinct clearance phases were seen, probably related to excess free formulation (high clearance rate) and adhered formulation (slower clearance rate). Both the porcine *in vitro* model and *in vivo* retention studies in humans (see Chapter 6) demonstrated biphasic clearance of liquid formulations from the oesophagus, a phenomenon that has been observed previously in the literature. Large standard deviations were consistently found in the data gathered; this may be due to the variable properties of the animal tissue used.

This study found that viscosity is not closely correlated with retention performance. Therefore some of the emulsions which were rejected during preformulation on the basis of their low viscosity may be worthy of further attention, provided their stability is satisfactory (see Section 3.1.1). Noveon AA-1 solutions were not well retained in this model, and this poor adhesion confirms work carried out previously by other researchers (Henriksen et al, 1996; Young et al, 2002; Vermani, 2002). However it was observed that both the viscosity and retention of Noveon and Smart Hydrogel® solutions were pH –dependent.

Investigation of the rheological properties revealed that for polymers, loss tangent was related to surface tension, and both parameters were related to retention *in vitro*. Emulsions, however, showed no link between loss tangent and surface tensions, and neither parameter was related to retention. Finally it was noted that drug incorporation into the vehicles can affect their physical and rheological properties, and could have detrimental effects on retention. The most robust vehicles, unaffected by drugs, will be the best candidates for this drug delivery system.

This *in vitro* retention study was used to select candidates for further drug release work. The Smart Hydrogel® was of interest as both its viscosity and retention are affected by pH. This pH-dependence was worthy of further investigation in drug release tests. LMO and HMO emulsions were both retained better than controls *in vitro*. Emulsions were of particular interest as they are a novel, less explored strategy for bioadhesion that polymer formulations. Noveon AA1® was also considered an interesting candidate, despite its failure to show good retention *in vitro*. It is a much studied polymer, which could serve as a known control by which the more novel formulations would be compared. Additionally both the viscosity and retention of Noveon were pH-dependent.

In vitro tests using this model may be valuable in determining formulations with good retention in the oesophagus at late time points. The formulations which excel at later time points, as well as in the early phase of the experiment, will be the most valuable for the proposed drug delivery system.

Chapter 5: Drug release from liquid formulations *in vitro*

Chapter 5

Drug release from liquid formulations *in vitro*

Introduction

The aim of the project is to develop a liquid drug delivery formulation to target the oesophagus for the purpose of treating oesophageal disorders. Where a pharmacological action is required, rather than a protective bandaging role, the strategy requires the formulation to have a direct pharmacological action on oesophageal tissue. This is to be achieved by using a liquid with prolonged contact with the epithelium. However, in the development of a drug delivery system targeted to the oesophagus, it is not sufficient to prepare a carrier which is merely well retained in the oesophagus. Once the drug delivery system is retained within the oesophagus, resulting in prolonged intimate contact with the epithelium, the active ingredient must then be released from the carrier system, so that it may permeate the target tissue and have its effect, for example contracting or relaxing the smooth muscle. The amount and rate of release of the drug from the vehicles will be an important factor in determining the concentration of drug available to the target tissue. It has been suggested that *in vitro* release studies can help to predict the best vehicle in further experiments (Ozsoy et al, 2004). The rate of release of candidate drugs ISDN, NIF, and SIL, and model drugs dextran (DEXT), mannitol (MANN), and propranolol (PROP) from both formulations and controls was assessed. The influences of vehicle composition, pH, viscosity, and the physicochemical properties of the drug on rate and extent of drug release were examined.

5.1 Franz cell assessment of drug release from a formulation

Release of drugs from formulations was assessed using ISDN, NIF, SIL, dextran (DEXT), mannitol (MANN) and propranolol (PROP). PROP, MANN and DEXT were used as model drugs in the release studies. These drugs are not of interest for their action on the oesophagus, and are not intended candidates for this drug delivery system. They are, however, an interesting selection of model drugs due to their range of physicochemical properties (see section 3.6). PROP is a lipophilic molecule, available in solution in ethanol and water. MANN is a small, hydrophilic molecule. DEXT is a larger MW hydrophilic molecule. The dextran used in this study was

10,000 Daltons MW. All three drugs were also available commercially in radio-labelled form.

The blends were not included in these experiments due to concerns regarding their highly variable retention observed during *in vitro* retention studies (Chapter 4). The formulations chosen for this stage of testing were the most promising and interesting carriers from the *in vitro* retention studies. These formulations were Smart Hydrogel® 2% w/v solutions at pH 3.7, 4.7 and 6.0, Noveon AA1® 0.1% w/v solution at pH 4.7 and 6.0, and the LMO and HMO oil-in-water emulsions (see section 4.5).

5.2. Franz cell procedure

Drug release was measured *in vitro* using glass Franz-type diffusion cells (Siewert et al, 2003), with a receptor compartment volume of 43 mL and an effective diffusion area of 1.86 cm^2 (Figure 5.1). The temperature was maintained at 37°C by a thermostatically controlled water jacket surrounding the receiver chamber. The receiver medium chosen was a mixture of acetonitrile:water (40:60). Although it would be desirable to have a receiving medium that is similar to the physiological condition in the oesophageal lumen, it is also imperative to ensure that the release of the drug can be measured without bias. The most important factor for selection of the receiving medium was the solubility of the active ingredient in the medium. The receiver should provide a diffusional 'sink' for the active ingredient released from the formulation (Thakker et al, 2003). Also the receiving medium should allow sufficient quantities of the active ingredient to be released within a reasonable time period, to ensure accurate analysis of the release rate samples (Thakker et al, 2003). This receiver fluid was chosen to facilitate solution of the drug in the receiver medium, maintaining sink conditions and also facilitated subsequent HPLC analysis of samples of the receiver fluid. The choice of receiver fluid made it suitable for use with all model drugs, hence the receiver medium was kept constant for all formulations.

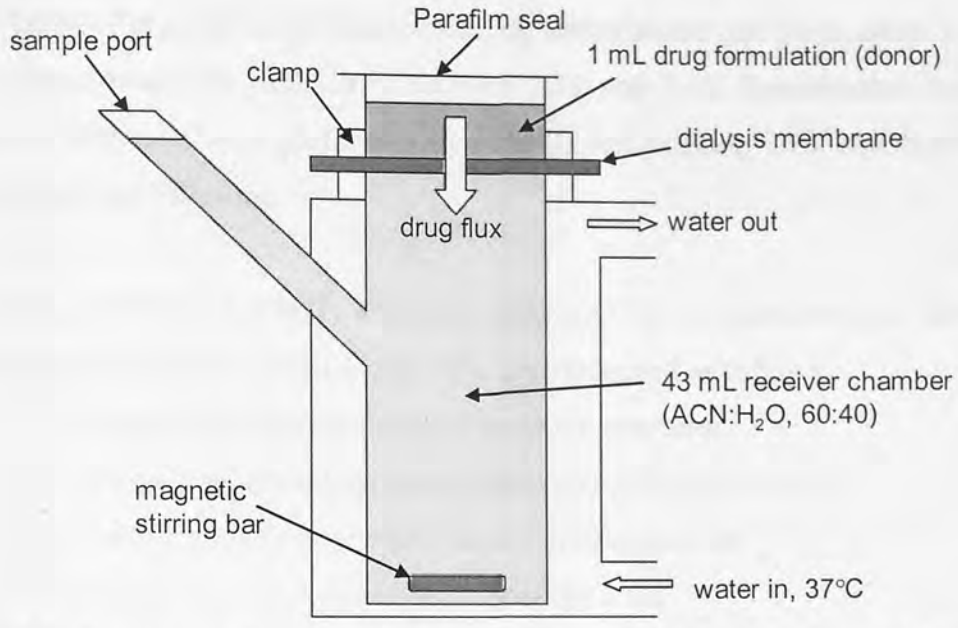


Figure 5.1. Diagrammatic representation of two- chamber diffusion cell used for assessment of drug release.

The receiver medium was stirred by means of a magnetic stirring bar, which greatly increased mixing efficiency and reduced the possibility of a stagnant boundary layer forming next to the membrane surface, hence encouraging sink conditions. The diffusion barrier between donor and receiver chambers was hydrated synthetic dialysis membrane (pore size 12-14 kDa). This was used in preference to oesophageal tissue, which would have brought about variation in the experimental setup. By using a synthetic membrane of known qualities, the membrane was kept constant; hence the only variable for all Franz cell experiments was the composition of the donor formulation. Moreover, these studies were designed to investigate the rate of drug release from a liquid formulation, and not drug permeation through oesophageal tissue. The membrane was selected as an inert holding surface for the formulation, but not a barrier, and was intended to allow drug diffusion into the receiver medium as it was released from the vehicle (Thakker et al, 2003). Dialysis membrane was soaked and rinsed in distilled water before use to ensure pliability and unblocked dialysis pores. Additionally, the membrane was handled wearing non-powdered gloves as powder tended to leave a deposit on the wet membrane, which may block the pores. 1mL formulation containing a known concentration of drug was added to the donor side. The open parts of each Franz cell were covered with Parafilm® to prevent evaporation of the sample over time, and to prevent escape of solvents in the receiver fluid into the atmosphere.

The methods of preparation of drug-containing formulations and the controls for drug release experiments are detailed in sections 2.11 and 2.12. Experiments involving radioactive materials were performed in a designated working area, and appropriate safety procedures followed.

For DEXT, MANN and PROP, known quantities of the formulation (and hence the radioactive counts) added to the Franz cells were achieved as follows:

- Mass of beaker containing formulation measured
- Mass of an unused syringe in clean weigh boat measured
- 1mL formulation syringed into a scintillation tube.
- Mass of used syringe + weigh boat obtained

This provided a known mass of formulation in the scintillation tube, the radioactive counts were obtained and counts/g used as a control from which the Franz cell data could be quantified.

The Franz cells were set up taking care to expel any air bubbles (which would have affected the diffusional surface area), and a tight seal between the donor chamber and membrane was achieved using Parafilm® to prevent leakage of donor material.

- For each Franz cell, a clean syringe was place in a clean weigh boat.
- 1mL formulation was placed into a clean syringe
- Mass of the full syringe in its weigh boat obtained.
- The dose was then placed into the donor compartment of the Franz cell
- Stirring commenced, timing initiated
- Mass of used syringe + weigh boat obtained

The exact mass of the dose placed in the Franz cell was therefore known. The drug release from this Franz cell was quantified using the control sample prepared previously.

For ISDN, NIF and SIL, quantities of the formulation added to the Franz cells were known, as the drug concentration in mg/mL was known for each formulation, and the 1 mL volume added to each Franz cell donor was accurately measured using a Gilson® pipette. Drug release from the Franz cells was therefore quantified from the known formulation volume at the start.

At selected time intervals, 1mL samples were withdrawn from the receptor medium and analysed as described below. The volume of receiver medium was kept constant by addition of equivalent amounts of fresh fluid each time. This dilution factor was taken into account during analysis. Franz cell experiments were repeated three times for each test formulation.

5.3 Determination of drug concentration in receiver

Samples taken from the receiver medium were analysed to determine drug concentration, which was used to calculate how much of the drug had diffused out of the formulation.

5.3.1 ISDN/NIF/SIL analysis

1 mL samples collected directly into HPLC vials were analysed by the HPLC methods described in section 2.4.1. Throughout the experiments, the drug concentration in each sample was low enough such that further dilution was not required prior to injection into the HPLC system.

5.3.2 DEXT/MANN/PROP analysis

1mL samples were directly collected into scintillation tubes and 4 mL scintillation fluid (Optiphase HiSafe) was added to each sample tube, and to the control sample tube. Tubes were sealed and mixed well for 5 seconds each on a vortex mixer. The number of radioactive counts in each sample was then assessed using a β-counter (section 2.5.2). The amount of drug released over time was calculated by comparison of the counts in each sample with the counts of the known sample prepared previously.

5.4 Data manipulation: Calculating drug release from Franz cell samples

5.4.1. Assessing drug release by HPLC

The example described is for release of ISDN (starting concentration 1.0 mg/mL) from LMO emulsion. At the start of each study day an HPLC calibration was carried out. Table 5.1 shows the calibration for ISDN performed on the relevant study day.

[ISDN] mg/mL	HPLC peak area
1	17020
0.1	2081
0.01	210
0.001	21.82
0.0001	2.743
0.5	10103
0.05	1072
0.005	113.9
0.0005	12.8
0.00005	2.193
0.25	5312
0.025	537.6
0.0025	56.09
0.00025	6.157
2.5E-05	1.229

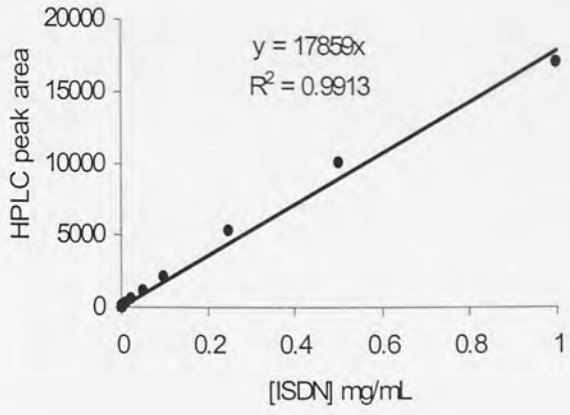


Table 5.1. HPLC calibration for ISDN

Linearity of the calibration was assessed using the R^2 correlation coefficient as calculated by Microsoft Excel®. The calibration was checked to ensure that it remained linear at lower concentrations as well as the full range. Between 0.00025 and 0.025 mg/mL the R^2= 0.9997, and was therefore linear. The calibration between 0.000025 and 0.0001 mg/mL was still linear with an R^2 value of 0.9858. The equation of the best fit straight line generated (y=17859 x in this example) was then used to calculate ISDN concentration from the HPLC peak areas obtained that day.

Samples from the Franz cell receiver fluid were analysed by HPLC as described in section 2.4.1, and the HPLC data was gathered in terms of output peak area for each sample. Franz cells were performed three times for each formulation (repeats A,B, and C). Examples of the HPLC peak outputs are shown in Table 5.2

Franz	Min	HPLC peak area	Franz	Min	HPLC peak area	Franz	Min	HPLC peak area
A	5	41.5	B	5	29.9	C	5	34.1
A	10	30.0	B	10	45.9	C	10	62.9
A	15	58.1	B	15	64.0	C	15	41.5
A	20	74.6	B	20	85.6	C	20	54.2
A	25	91.2	B	25	103.4	C	25	63.3
A	30	107.5	B	30	74.4	C	30	123.4
A	45	162.4	B	45	182.3	C	45	109.1
A	60	206.0	B	60	232.4	C	60	144.2
A	120	408.5	B	120	446.1	C	120	280.9
A	210	695.4	B	210	744.9	C	210	479.4

Table 5.2. HPLC peak areas for ISDN release from LMO - experiments A, B and C.

The mean HPLC peaks areas and standard deviations were then calculated from this data (see Table 5.3)

Min	mean HPLC peak area	st. dev
5	35.165	5.9
10	46.232	16.5
15	54.502	11.6
20	71.450	15.9
25	85.957	20.6
30	101.777	25.0
45	151.246	37.9
60	194.192	45.3
120	378.490	86.6
210	639.908	141.2

Table 5.3. Mean HPLC peak areas for Franz cells – ISDN release from LMO

As the calibration equation was y= 17859x, the HPLC peak areas were divided by 17859 to obtain the concentration of ISDN in mg/mL. For time points after the first five-minute sample, the drug concentrations were then corrected for the dilution factor that was caused by replacing receiver volume with fresh receiver fluid. The data were then expressed in terms of μg ISDN released over time, or alternatively these values were divided by the molecular weight of ISDN (236.136) to give the μmol ISDN diffused. These data could also be used to describe the release in terms of % drug released from the starting donor material.

All data manipulation for the HPLC-analysed drugs nifedipine, isosorbide dinitrate and sildenafil was performed in this way, with a new calibration performed on each study day to nullify the effects of inter-day variations on analysis.

5.4.2. Assessing drug release by measurement of radioactive counts

The example described is for release of MANN (starting concentration 2.5 mg/mL) from 0.1% w/v Noveon AA1 at pH 4.7. The initial radioactivity in the donor formulation for each Franz cell (A,B and C) was calculated using the known reference sample taken from the stock formulation (see Section 5.2). The mass of the reference sample was known (1.003 g in this example), and the amount of drug in this sample (mg) was therefore also a known value (multiply mass (g) by the concentration (mg/mL)). This assumes that the density of each formulation is 1 mg/mL. The counts per minute (CPM) were measured using scintillation equipment, and from this data, the CPM per mg drug in the starting formulation could be calculated.

	mass (g)	mg MANN in reference sample	MEAN CPM	CPM per mg drug
Reference for cells A,B,C	1.0062	2.516	267900	106499.7

Table 5.4. Calculation of CPM per mg drug for the reference sample

The mass of samples placed into the donor compartments at the start of each Franz cell experiment was also known. The mg of drug existing in the starting donor formulation was calculated based on this mass. The reference CPM/mg was then used to calculate the theoretical starting radioactivity (CPM) in the donor of each Franz cell.

Franz cell repeat	mass of donor formulation (g)	mg MANN in donor	total CPM of Franz cell donor
A	0.9689	2.422	257969
B	1.0096	2.524	268805
C	0.9997	2.499	266169

Table 5.5. Calculation of the starting CPM in each Franz cell donor formulation

During the course of the experiment, samples were taken from the receiver of each Franz cell, and these were analysed using scintillation equipment to measure the radioactive CPM in each sample. Using the information generated previously, the data could be analysed in terms of % radioactivity released into the receiver chamber. This was considered to represent the proportion of drug that had diffused from the donor to the receiver chamber. Examples of these calculations are shown in Table 5.6.

	Cell A		Cell B		Cell C	
Min	mean CPM	% start CPM	mean CPM	% start CPM	mean CPM	% start CPM
5	404	0.157	432	0.161	380	0.143
10	476	0.185	682	0.254	658	0.247
15	999	0.387	939	0.350	930	0.349
20	1207	0.468	1263	0.470	1242	0.467
25	1385	0.537	1443	0.537	1257	0.472
30	1570	0.609	1629	0.606	1517	0.570
45	2236	0.867	2230	0.830	2132	0.801
60	2564	0.994	2689	1.000	2530	0.950
90	3293	1.276	3098	1.152	3282	1.233
120	3901	1.512	4664	1.735	3795	1.426
180	5131	1.989	4650	1.730	5146	1.933
240	6231	2.415	5763	2.144	6340	2.382

Table 5.6. Calculation of the percentage of the starting radioactive counts found in receiver samples at different time points.

The percentage total CPM found in each sample was multiplied by the total mass of drug (mg) in the starting donor formulation for that particular Franz cell (Table 5.5) to calculate the amount of drug (mg) found in the receiver sample at a particular time point. This data was again corrected for the dilution factor due to sample removal from the receiver medium and fresh medium replacement.

5.5 Results and Discussion

5.5.1 Shape of drug release profiles

The release data were analysed in terms of cumulative μmol drug diffused per unit area over time (Appendix 5A), to give the amount of drug flux during the studies. One striking characteristic of the majority of release profiles observed is the absence of any lag phase, i.e. delay before drug release. This is demonstrated in Fig 5.2, which shows some examples of profiles.

An absence of lag phase may be confirmed by the y-axis intercept of the straight line of best fit for each profile. A negative y- intercept suggests a lag period is present, whereas a positive y-intercept shows there is no lag phase.

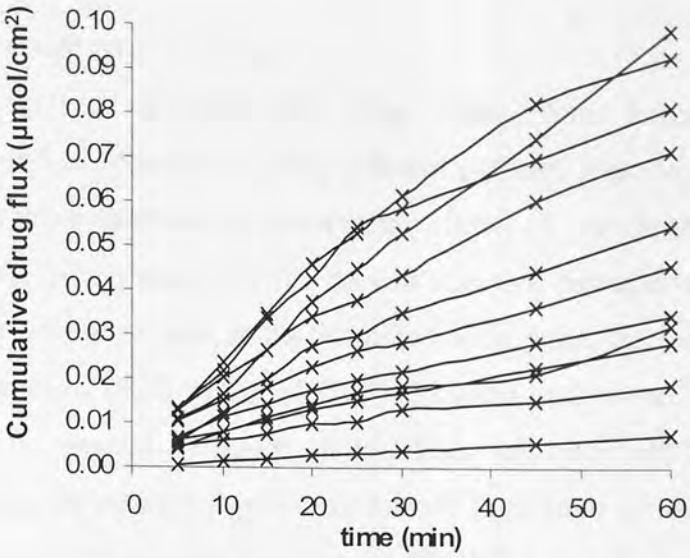


Figure 5.2.Graph showing mean cumulative µmol released per unit area over time. A selection of drugs and formulations are represented (n=3).

Out of all the formulations, drugs and concentrations tested, only a very small number exhibited lag based on the y-intercept analysis (Appendix 5B). The negative intercepts were observed from ISDN 2.5 mg/mL from ACN:H_2O control, DEXT 2.5 mg/mL from HMO, SIL 2.5 mg/mL from Noveon pH 6.0., SIL 1.0 from HMO and Noveon pH 6.0. Nifedipine was the exception, as its release displayed short lag times for all the formulations tested except Smart Hydrogel® pH 3.6 and HMO emulsion. The reason why NIF should experience lag rather than the other drugs has not yet been elucidated. This may be due to the particularly low solubility of NIF, meaning that very little of the drug was in solution in the starting formulation, with the majority being in suspension.

The lack of lag phase in the majority of the formulations is important as it indicates that upon commencement of the study, drug release begins immediately. This immediate release is extremely valuable in the proposed drug delivery system. The residence time of the formulation within the oesophagus is limited by the bioadhesive capability of the formulation. However, even for the strongest-retained formulation, its retention time is still short, with most of the liquid being removed from the oesophagus within a few seconds (Chapters 4 and 6). For the best possible efficacy, drug must be released as quickly as possible from the formulation, and to as large an extent as possible.

5.5.2 Effect of vehicle on drug release

The data were analysed in terms of % drug released from formulation over time (Appendix 5C) and in terms of μg drug diffused per unit area over time (Appendix 5A). These data were assessed to examine the effects of vehicle type of the release rate of each drug. When analysing the data in terms of cumulative amount of drug diffused per unit area over time, at the 30 minute time point, the range of cumulative release varied between 18.33 $\mu g/cm^2$ (PROP 2.5 mg/mL in Noveon® pH 4.7) and 0.17 $\mu g/cm^2$ (for NIF 1.0 mg/mL release from both LMO emulsion and Noveon® pH 6.0). When considering the cumulative % drug release from each formulation over time, the greatest release at 30 minutes was seen for PROP 2.5 mg/mL from LMO emulsion (60.95 ± 6.36 percent) and the poorest release at 30 minutes was shown by NIF 2.5 mg/mL from the water control (0.63 ± 0.18 percent released).

Drug	Initial conc^n (mg/mL)	Formulation from which % release was greatest
DEXT	2.5	high pH Noveon® and Smart Hydrogel®
MANN	2.5	LMO and HMO
ISDN	2.5	low pH Noveon® and Smart Hydrogel®
ISDN	1.0	Noveon® and LMO
SIL	2.5	Low pH Noveon®, LMO and HMO
SIL	1.0	Noveon®, LMO and HMO
NIF	2.5	Noveon® and Smart Hydrogel®
NIF	1.0	LMO, HMO and high pH Noveon®
PROP	2.5	LMO, HMO and Noveon

Table 5.7. Table showing formulations from which percent release after 30 min was greatest for each drug.

Table 5.7 shows, for each drug, the formulation from which percent release after 30 min was greatest. No trends were observed to indicate that certain types of drug (e.g. in terms of lipophilicity or MW) have greatest percent release from certain types of formulation. The most unusual observation was that the largest drug molecule, dextran, had fastest release from the Smart Hydrogel® and Noveon® at pH 6.0. These higher pH formulations have a greater viscosity compared to the lower pH 3.6 and pH 4.7 solutions. It was surprising that dextran appears to diffuse more easily out of the high viscosity formulations than the low viscosity formulations. It would have been expected that a high viscosity medium would slow the diffusion of a large molecule such as dextran through the formulation hence reducing the rate and extent of drug release. For example, in a study performed by Thakker et al. (2003), the release of

retinoic acid was found to be inversely proportional to the amount of viscosity builder in a semi-solid formulation.

It was also unexpected that the drugs showed different release trends depending on their original concentration in a formulation.

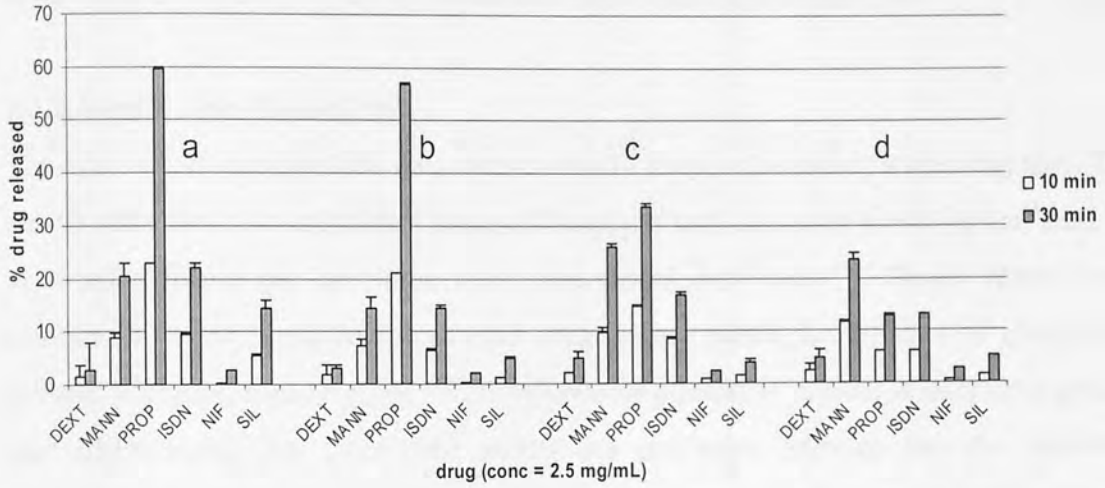


Figure 5.3. Graph showing percent drug release from polymer formulations. a = Noveon® pH 4.7, b = Noveon® pH 6.0, c = Smart Hydrogel® pH 4.7, d = Smart Hydrogel® pH 6.0. Starting drug conc^{n} was 2.5 mg/mL. Percent release after 10 min and 30 min is shown. (Mean ± S.D, n = 3)

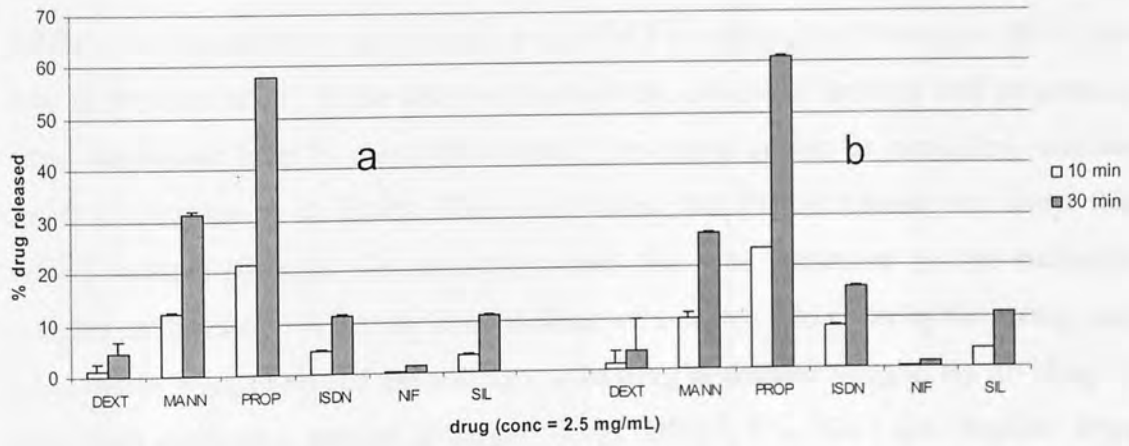


Figure 5.4. Graph showing percent drug release from emulsions. a = HMO, b = LMO. Starting drug conc^{n} was 2.5 mg/mL (except SIL, 2.0 mg/mL). Percent release after 10 min and 30 min is shown. (Mean ± S.D, n = 3).

Figures 5.3. and 5.4 show the percent drug release from different polymer and emulsion formulations. For both types of formulations, it was observed that PROP, MANN and ISDN always had the greatest percent release, after 10 minutes and 30 minutes. DEXT, NIF and SIL consistently demonstrate poorer percentage release at

both time points. This may be due to the molecular sizes of the drugs. MANN, ISDN and PROP were the smallest drug molecules investigated, and NIF, SIL and DEXT were larger (see section 3.6). It is reasonable to assume that smaller molecules might be able to diffuse more easily through a polymer network, or through any viscous medium. These data suggest that smaller drug molecules will have more successful drug release from any of the formulations tested than large molecules.

5.5.3 Steady state flux analysis

The best way to compare the data quantitatively was by the steady state drug flux. The steady state flux was calculated as μg diffused per unit area over time (μg cm^{-2} min^{-1}), or μmol diffused per unit area over time (μmol cm^{-2} min^{-1}). These values were calculated for the linear region of each drug release curve, for the first 60 minutes of release. Some profiles (e.g. for PROP) showed a plateau of release at later time points, and additionally, the later time points are not very relevant for the proposed application (as very little is retained on the oesophagus for longer than 30 minutes). For this reason, the later time points of release were not considered in the calculations.

The formulation which showed the greatest flux for both parameters was the PROP 2.5 mg/mL from LMO emulsion (0.53 μg cm^{-2} min^{-1} and 0.002 μmol cm^{-2} min^{-1}). PROP also demonstrated similar flux from HMO emulsion, and Noveon at pH 4.7 and 6.0. A previous study in the literature reports the release of dextran and propranolol from membrane bags in dissolution media (simulated gastric or simulated intestinal fluid) (Tarvainen et al, 2000). This study found that PROP release was faster than DEXT release through the membrane, and this was attributed to the molecular weights of the drugs. When the formulations were sorted into order by the steady state flux, trends were observed depending on the drug molecular weight. By dividing the drugs into qualitative groups of 'larger' drugs (DEXT, SIL, NIF) and 'smaller' drugs (MANN, ISDN, PROP) based on the MW, it was seen that the small drugs dominated the top half of the rank where steady state release was greatest, and larger drugs completed the lower half (see table in Appendix 5D). This further suggests that molecular weight is an important factor in how fast drug flux from the formulations occurs, and that smaller drug molecules will be more successfully released from any of the formulations tested than large molecules. Additionally, smaller molecules will pass more rapidly through the diffusion membrane.

5.5.4 Effects of pH on release from polymer solutions

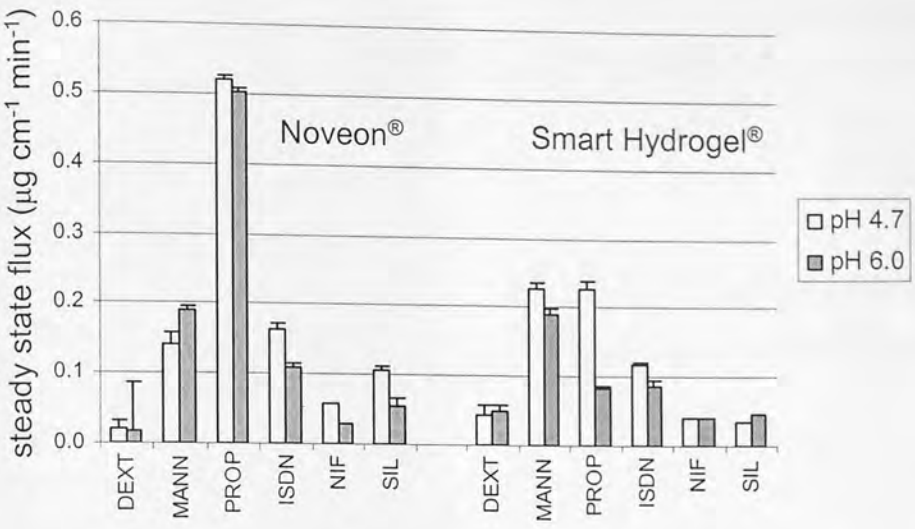


Figure 5.5. Graph showing effect of pH on drug flux (µg cm^{-2} min^{-1}) from 0.01% Noveon® and 2% Smart Hydrogel® solutions. Start drug $conc^{n}$ = 2.5 mg/mL. (Mean + S.D. n = 3).

Figures 5.5 illustrates the steady state drug flux from polymer solutions. The effect of pH on steady state drug flux was not as pronounced as expected. For all formulations, there was only a modest difference between flux from pH 4.7 and pH 6.0 preparations of the same polymer. These difference were most notable for PROP release from Smart Hydrogel® (0.23 vs. 0.08 µg cm^{-2} min^{-1} for pH 4.7 and pH 6.0 respectively), $p<0.01$). This indicates that higher pH polymer solutions may be good drug delivery systems, as their increased pH results in better retention (see section 4.4.2), but the increased viscosity of the formulation does not impede drug release, as might have been expected.

Barcbucci et al. (2000) investigated the effect of pH changes on the water uptake properties of hydrogels. At low pH, the carboxyl groups of poly (acrylic acid) are in the protonated form COOH (Tarvainen et al, 2000). At basic pH, the carboxylic acid groups are dissociated. This leaves the network more open because of the dielectric repulsion between COO^{-} groups (Barbucci et al, 2000). The hydrogels tested by Barbucci's group showed greater water uptake with increasing pH (i.e. an increase from pH 2 to pH 7.4. This was attributed to the hydrogen bonds in the pH 7.4 gel being broken and electrostatic repulsion occurring. The opening up of the structure led to the water uptake. Similarly, in the polymers tested during this study (Noveon

AA1® and Smart Hydrogel®) the change in pH may have affected the tightness of the gel structure. A more open structure could allow drug to diffuse more readily from the drug delivery vehicle, and this might outweigh or counteract the effect of an increased viscosity at higher pH.

5.5.5 Release from emulsions

The steady state flux data was also calculated for the emulsions; this is shown in Figure 5.6. The same trends were observed for data in μmol as that for μg release, as expected.

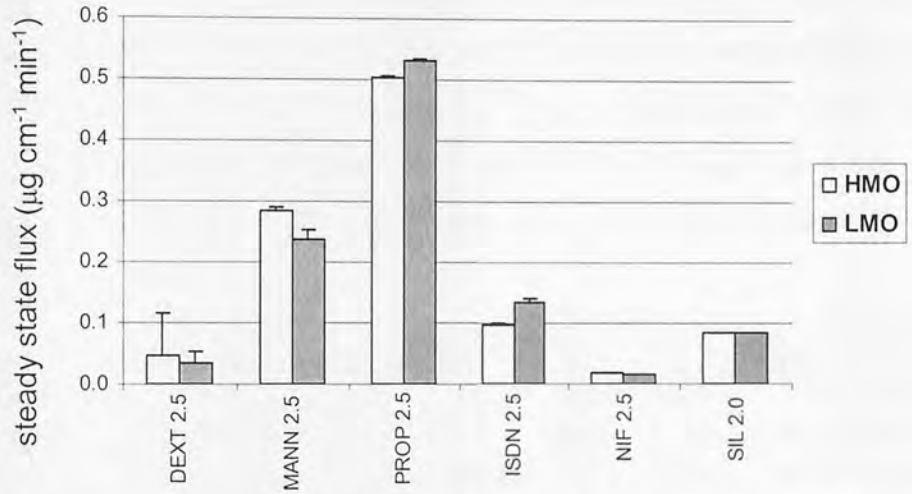


Figure 5.6. Graph showing drug flux (μg cm^{-2} min^{-1}) from HMO and LMO emulsions. Start drug $conc^{n}$ = 2.5 mg/mL. (Mean + S.D. n = 3).

There was no significant difference between drug release from the two emulsions for PROP, MANN, SIL, DEXT and NIF. However, isosorbide dinitrate showed a faster rate of release from the LMO emulsion than the HMO emulsion (0.14 vs. 0.10 μg cm^{-2} min^{-1} respectively). This difference was statistically significant ($p<0.05$). The reason for this difference is uncertain. It may be due to the internal composition of the emulsions, e.g. the internal droplet size, although one would expect to see differences for the other drugs also. The rate of release from the o/w emulsions does not appear to be influenced by the phase into which the drug is incorporated. In preparing the emulsions, hydrophobic drugs (PROP and NIF) were incorporated into the oily phase, and more hydrophilic drugs (MANN, DEXT, ISDN, SIL) placed in the aqueous phase. The fast release of PROP compared to the much slower release of NIF shows that incorporation into the internal rather than the external phase does not influence release per se. However, in a study by Baudonnet et al. (2004), when salicylic acid

was solubilised into the internal phase of o/w emulsions, the presence of the surfactant at the interface between the oil and the water phases appeared to modulate salicylic acid passage to the external phase. These experiments might be repeated using formulations in which the same drug has been incorporated into the lipid or aqueous phase of the emulsion, as this might help to better understand the mechanisms of release from these emulsions.

5.5.7 Modelling drug release kinetics

Graphs were drawn of $\mu g/cm^2$ against time, ln $\mu g/cm^2$ against time, the reciprocal $\mu g/cm^2$ against time, and $\mu g/cm^2$ against square root of time, in order to determine whether the release profiles of the drugs from formulations demonstrated zero, first, second order or Higuchi kinetics (Higuchi, 1960). The linearity of each graph was indicated by the linear R^2 correlation coefficient value. The data (in full in Appendix 5E) were analysed by drug (Table 5.8) and then by formulation (Table 5.9).

Drug	Full 240 min			First 60 min			First 30 min		
mg/mL	**Higuchi**	**zero**	**1st**	**Higuchi**	**zero**	**1st**	**Higuchi**	**zero**	**1st**
DEXT 2.5	0.835	**0.916**	0.945	0.929	**0.973**	0.958	0.918	**0.961**	0.982
MANN 2.5	**0.984**	0.935	0.738	0.957	**0.973**	0.890	**0.959**	0.958	0.926
PROP 2.5	**0.887**	0.765	0.611	**0.988**	0.964	0.839	0.980	**0.989**	0.950
ISDN 1.0	**0.978**	0.961	0.775	0.979	**0.991**	0.890	0.978	**0.983**	0.933
ISDN 2.5	**0.970**	0.937	0.729	0.977	**0.981**	0.864	0.983	**0.984**	0.925
NIF 1.0	0.936	**0.989**	0.834	0.909	**0.977**	0.891	0.905	**0.955**	0.945
NIF 2.5	0.910	**0.984**	0.774	0.940	**0.969**	0.833	0.927	**0.953**	0.920
SIL 1.0	**0.922**	0.909	0.760	0.964	**0.986**	0.888	0.955	**0.973**	0.940
SIL 2.5	**0.950**	0.926	0.719	**0.934**	0.931	0.815	0.923	**0.929**	0.887

Table 5.8. The R^2 linear correlation coefficients for mean µg drug release and its transformations against time, for the different drugs. Bold figures indicate the highest R^2 for each drug at each time point.

When comparing the goodness-of-fit to established models, it was noticed that the kinetics of drug release alter during the time course of the experiments. Data are not displayed for the second order release kinetics (reciprocal $\mu g/cm^2$ against time), as this proved to be a poor model at all time-points examined. Both Higuchi and zero-order models describe drug release acceptably at different time points. Most drugs exhibit a release profile that best fits Higuchi model over the full 240 minutes of the experiment ($R^2 \geq 0.835$), although DEXT and NIF may be more closely modelled to zero order release. However, when only the first 30 minutes of release are examined,

all drugs can be closely fitted to zero order kinetics ($R^2 \geq 0.958$). Zero order release also very closely fits all drugs during the first hour ($R^2 \geq 0.931$). This indicates that drug is still linearly released from the formulations for up to 60 minutes. The overlap of different models seen during this study has been observed previously. Chatchawalsaisin et al. (2004) performed experiments examining the release kinetics of paracetamol from polymer pellets *in vitro*, and found that overlapping release mechanisms occurred. An earlier study by Leucuta found that the release of nifedipine from hydrophilic matrices conformed with both Higuchi and first order release kinetics (Leucuta, 1988).

When examining the strength of fit to established models for the different vehicles (Table 5.9), the kinetics of drug release again altered during the time course of the experiments. As before, the second order release model data (reciprocal $\mu g/cm^2$ against time) was omitted from further analysis, as this proved to be the poorest model at all time-points examined.

Vehicle	Full 240 min			First 60 min			First 30 min		
	Higuchi	**zero**	**1st**	**Higuchi**	**zero**	**1st**	**Higuchi**	**zero**	**1st**
HMO	0.956	**0.971**	0.783	0.959	**0.986**	0.882	0.937	**0.956**	0.937
LMO	0.955	**0.970**	0.797	0.952	**0.987**	0.897	0.962	**0.986**	0.950
Nov 4.7	**0.913**	0.874	0.713	0.938	**0.942**	0.833	0.954	**0.969**	0.920
Nov 6.0	**0.895**	0.894	0.749	0.927	**0.944**	0.858	0.914	**0.922**	0.903
SH 3.7	**0.920**	0.892	0.707	0.963	**0.979**	0.860	0.936	**0.959**	0.926
SH 4.7	**0.963**	0.955	0.775	0.969	**0.984**	0.888	0.963	**0.980**	0.948
SH 6.0	0.965	**0.968**	0.793	0.976	**0.986**	0.871	0.977	**0.983**	0.931

Table 5.9. The R^2 linear correlation coefficients for μg drug release and its transformations against time, for the different formulations.

Both Higuchi and zero-order models describe drug release acceptably at different time points. The Higuchi model best described release from Noveon® solutions and Smart Hydrogel® at pH 3.7 and 4.7 over the full 240 minutes of the experiment, and Smart Hydrogel® pH 6.0 and both emulsions more fitted more closely to zero order release. For both the first hour and the first 30 minutes, release of drugs from all the formulations was best described by a zero order model ($R^2 \geq 0.942$ and 0.922 for 60 min and 30 min respectively). It is also noticed that the data displayed for the hour and half hour periods in Table 5.3 would adequately fit Higuchi kinetics, with $R^2 \geq$

0.927 for the first hour and $R^2 \geq 0.914$ for the first 30 minutes, although this is not the strongest model.

5.5.8 Relevance of amounts of drug released

Based on the rate of drug release from each formulation, calculations were made that assessed how much (μg) of each drug would be available to the oesophageal tissue ten minutes after swallowing. These calculations assumed that the surface area of the human oesophagus is approximately 181 cm^2, that the entire oesophageal surface would be evenly coated with a formulation for this time period, and that the drug release was for the first ten minutes. This was intended to give a better feel for how efficacious the formulations might be in practice.

The surface area of the human oesophagus (assuming a cylindrical structure) was calculated by oesophageal diameter 25mm (Boyce, 1999) × π × oesophageal length 230mm (Li et al, 1994). This gave a value of approximately 181 cm^2. It is important to note that this is likely to be an underestimation of the surface area of the oesophageal lumen, which is not a completely flat surface, but has folds and crevices. The data produced are displayed in full (see Appendix 5F). The greatest estimated amount of drug released over the oesophageal surface area, after ten minutes coverage, was 963 μg for PROP release from LMO emulsion. The lowest estimated drug release after ten minutes was 31 μg for NIF release from LMO. The mean estimated drug release, based on the high concentration formulations (except controls) is shown in table 5.10.

Drug	Mean estimated release (μg)
DEXT	64.436
MANN	382.554
PROP	714.286
ISDN	250.642
NIF	55.619
SIL	126.329

Table 5.10. Mean estimated total release after 10 min for different drugs, based on high concentration formulations, excluding controls.

These results highlight the amounts of drug that might be released from the proposed drug delivery formulation over a useful and feasible time period, although there is a

large amount of variation between formulations. Further experiments should involve the assessment of whether these amounts of drug would be sufficient to have an effect on oesophageal motor function, in order for the system to be an effective treatment for oesophageal motility disorders.

5.6. Conclusions

These experiments help to assess how much of the drug will be available to the target tissue and how quickly, but further studies might also include testing the permeability of the oesophageal mucosa to the candidate drugs, to find out how much drug might be absorbed. This Franz–style diffusion cell had the receiver maintained at 37°C by a circulating water jacket. Using the available equipment, it was not, however, possible to control the temperature of the Franz cell donor compartment. Although the donor material is warmed from by the receiver fluid below to an extent, ideally the donor compartment would be controlled independently. The temperature of the donor is of particular relevance where Smart Hydrogel® is considered as the vehicle, as drug release might be affected by the temperature-induced thermogelling at 37°C. Further studies might include the development of a model which incorporates temperature control within the donor compartment.

It can be concluded from these analyses that drug release observed during these Franz cell release studies is in general well modelled over the first hour by zero order kinetics, with no lag phase encountered by most formulations. The linear release observed is ideal for the proposed drug delivery system, in which rapid drug release is required, without a delay, in order to maximise efficacy of the system.

Chapter 6: The duration of liquid retention on oesophageal tissue –*in vivo* assessment

Chapter 6

The duration of liquid retention on oesophageal tissue –*in vivo* assessment

Introduction

The aim of this project was to develop an oral drug delivery formulation to target the oesophagus for the purpose of treating oesophageal disorders. This is to be achieved using a liquid that exhibits prolonged contact with the epithelium. Following screening to assess retention of formulations on *ex vivo* porcine tissue, the retention of selected Tc99m-labelled candidate vehicles was assessed in humans, by γ-scintigraphy. Due to their promising retention *in vitro* (Chapter 4), the LMO and HMO emulsions, SBE, polymer blends, Smart Hydrogel® and alginate LF120M formulations were selected for evaluation of their oesophageal retention *in vivo*. Correlations between the retention profiles obtained by the two methods were examined, and the influence of viscosity on *in vivo* retention was investigated. The obejctives of this study were to evaluate retention of some formulations *in vivo*, assess the predictive value of the *in vitro* model, and to determine the factors influencing retention *in vivo*.

6.1 Methods for assessing liquid retention in healthy human subjects

6.1.1 Gamma Scintigraphy

Gamma (γ)-scintigraphy provides an extremely valuable non-invasive method of acquiring information about formulation transit under physiological conditions (Wilson et al, 1997; Wilding et al, 2001; Newman et al, 2003; Teran et al, 2005). The quantity of radioactive material required to be incorporated into a formulation to make it suitable for γ-scintigraphy assessment is very small, and does not affect the performance characteristics of the system (Wilding et al, 2001). The head of a γ-camera consists of a large sodium iodide crystal, activated with thallium to promote scintigraphic properties. Coupled to this crystal is an array of photomultiplier tubes which detect light pulses, and γ- rays are focused onto the detector (Wilding et al, 2001). The equipment is encased in lead. The formulation to be tested must be labelled using a γ-emitting radionuclide. Its transit may then be monitored by the γ-camera. Technetium 99m (Tc99m) is often selected as the radiolabel for scintigraphic studies due to its short half life (6 hours), versatile chemistry, and near ideal energy

(140 keV) (Wilding et al, 2001). Tc99m was also used as the label in the *in vitro* retention studies (Chapter 4). This facilitated direct comparison between the retention profiles of the liquids in the *in vitro* and the *in vivo* studies. Scintigraphic methods have been used previously to evaluate the intra-gastric disposition of alginate (Washington et al, 1993), intra-ocular and intra-gastric carbomer retention (Wilson et al, 1998; Jackson et al, 2001), and oesophageal retention of Smart Hydrogel® (Potts et al, 1997a).

6.1.2 Study approval and volunteer recruitment

The *in vivo* scintigraphy studies were designed and carried out in the Nuclear Medicine department of St. Mark's Hospital, Harrow, UK, where eleven healthy subjects were recruited for the study by advertisement. The studies were directed and performed on study days by Research Fellow Dr. Chris Rayner and radiographers Neil Barron and Greg Morris. The protocol was approved by the Harrow Research Ethics Committee and the Administration of Radioactive Substances Advisory Committee, and all eleven volunteers gave written informed consent to participate. Subjects with gastrointestinal symptoms, a history of hiatus hernia, upper gastrointestinal surgery, taking medication known to influence gastro-oesophageal motor function, and those with significant illness were excluded from participating. Women who were pregnant or lactating were also excluded. Volunteers attended the study after having fasted from solids and nutrient liquids for 3 hours prior to the study, and from water for 1 hour prior to the study. Each subject participated in a maximum of 5 study days, and individual study days were separated by at least 48 hours.

6.1.3. Selection and preparation of formulations

The solutions and emulsions required were prepared in the suitably equipped laboratory at Aston University and sent to St. Mark's Hospital for the studies. Formulations were prepared using the same procedures as for the *in vitro* retention experiments (see Section 2.7 – 2.10). The only difference was that ISDN incorporated into formulations for administration to human subjects was of clinical grade rather than laboratory grade material.

The liquids assessed for their retention *in vivo* were selected due to their consistently good retention both at early and late time points in the *in vitro* study (Section 4.4.2). The formulations tested *in vivo* were therefore Alginate LF 120M 2%, Smart Hydrogel® 2% at pH 4.7 and pH 6.0, HMO and LMO emulsions, and Blend A. Of the two blends, the one with the best expected retention based on the *in vitro* model was chosen, and so Blend B was rejected at this stage. Additionally, sucralfate retention was assessed as it was interesting to compare a commercially available formulation with the liquids under development. The silicone blend emulsion was also selected for this study, as it showed such variable retention *in vitro* that its behaviour *in vivo* was subsequently of interest. Formulations were stored in a refrigerator and used within two days of manufacture. The formulations were allowed to equilibrate to room temperature immediately prior to use.

6.1.4 Assessment of liquid transit *in vivo*

Transit of liquid formulations through the oesophagus was assessed in eleven subjects, studied in an upright position. All formulations were tested at an administered single dose of 5 mL volume. As with the *in vitro* studies, formulations were labelled with technetium-99m tin colloid (5 MBq per 5 mL of formulation). The materials prepared at Aston University were transported un-labelled, and the Tc99m was mixed with each test compound at St. Mark's Hospital by vortexing together for 30 seconds, immediately prior to each study. This mixing procedure was sufficient to disperse the radiolabel homogenously throughout the formulation (see section 4.3.2). Each radiolabelled formulation was drawn into a 5 mL syringe (two syringes were used when the dosage volume was to be 10 mL), and a 30-second static image was produced of the syringe before and after the study; this was used to determine the total activity administered to the volunteer.

Doses of 5 mL were administered directly into the oral cavity from the syringe and swallowed as a single bolus, while 10 mL doses were taken as two 5 mL volumes, 15 seconds apart. Dynamic images were acquired from the onset of swallowing at 2 frames per second for 120 seconds. Static images were then acquired at 3, 5, 10, 15, 20, and 30 minutes after administration of the test compound, each for a 30 second period. The field of imaging included the oropharynx, oesophagus, and stomach.

Subjects swallowed the labelled test formulation whilst standing in front of the gamma camera. The formulation was introduced into the oral cavity by the study investigator. During imaging the subject stood in front of the gamma camera but at other times they could move freely, usually choosing to sit down. The field of imaging included the oropharynx, oesophagus and stomach area. At the end of each study, the dynamic images between 90 and 120 seconds after the onset of swallowing were summed, and the resulting image was used to define of anatomical regions of interest (ROIs). ROIs included the whole oesophagus, the upper, middle and lower thirds of the oesophagus, the stomach, and the entire field. Figure 6.1 indicates how the regions of interest were designated for the upper, middle and lower thirds of the oesophageal region.

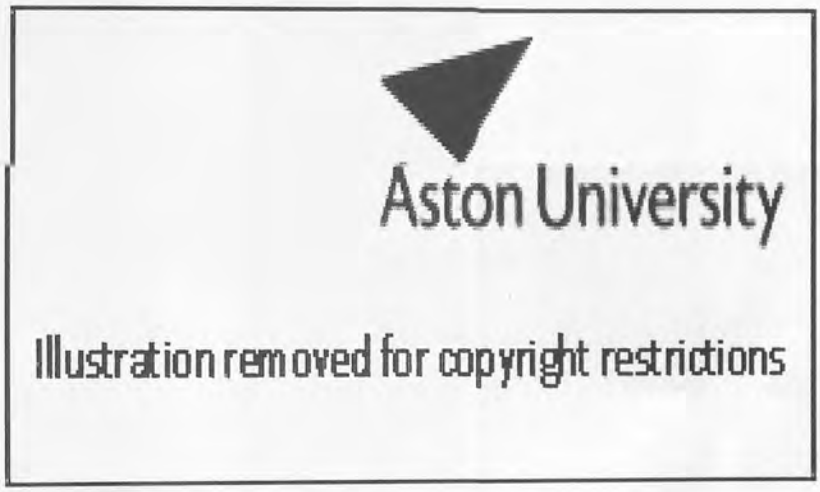


Figure 6.1. Designation of three regions of interest in the oesophagus. Picture courtesy of Yarra Medica Ltd.

The ROIs were transposed onto the other images and used to calculate activity counts for each anatomical region over time. Radioactive counts were corrected for background interference and the decay of the Tc99m over time, based on its half-life.

Retention of test formulation at each time point in the whole oesophagus and each of the upper, middle and lower thirds, was calculated as a percentage of the total activity administered from the syringe(s). Oesophageal retention profiles of liquid formulations were assessed in terms of percent of the administered liquid remaining in

the oesophagus over time. The total area under the curve (AUC 30) was calculated for each retention curve. Relationships between mean AUC 30 values for *in vitro* and *in vivo* studies were examined, and the *in vivo* retention was also compared to the viscosities of the formulations. This analysis was performed by the group at St. Mark's Hospital using statistical software (Statview 5.0 SAS Institute, Cary, North Carolina, USA). AUC 30 values of different formulations were compared using unpaired t-tests, and paired tests were used to compare 5 mL and 10 mL doses of identical formulations. AUC 30 values for the different regions of the oesophagus were compared using analysis of variance measurements. P values below 0.05 were accepted as significant.

6.1.5 Rheometry

Rheological assessment of formulations was achieved using a TA Instruments AR1000 rheometer, and measurements were performed as detailed in section 2.2. Flow rheometry measurements were performed at 25°C, at a shear rate of $10s^{-1}$ to determine the viscosity of each formulation in Pa.S. Additionally oscillatory measurements were used to determine the elastic and viscous moduli of each formulation and hence the loss tangent (see section 2.2.1).

6.2 Results and Discussion

6.2.1. Tissue attenuation of radioactive counts

The mean ratio of counts from the entire field early in the imaging sequence (90-120 s), to the number of counts measured directly from the administering syringes, was 0.39 (± S.D. 0.08) for all studies, 0.43 (± 0.09) for Smart Hydrogel® pH 4.7, and 0.44 (± 0.09) for Smart Hydrogel® pH 6, suggesting considerable attenuation of *in vivo* counts by intervening tissue. This level of attenuation was surprising, as Tc99m is a very high energy γ-emitter, and the γ-radiation produced should easily be able to pass through tissue. However tissue attenuation is a recognised issue in γ-scintigraphy (Pitcairn and Newman, 1997; Lee et al, 2001; Pitcairn et al, 2003). It is important to remember that gamma radiation being emitted will pass easily in all directions 360° around the source (the dose). The counter take images only from the ventral side of the volunteer, and so counts may be lost at this stage.

6.2.2 Dynamic imaging data and comparison of formulations

Dynamic images acquired from the onset of swallowing for 120 seconds revealed that the majority of each formulation passed into the stomach within seconds, and only a minority remained in the oesophagus with the opportunity for prolonged retention. An example of this rapid clearance (for heavy mineral oil emulsion) is shown in Figure

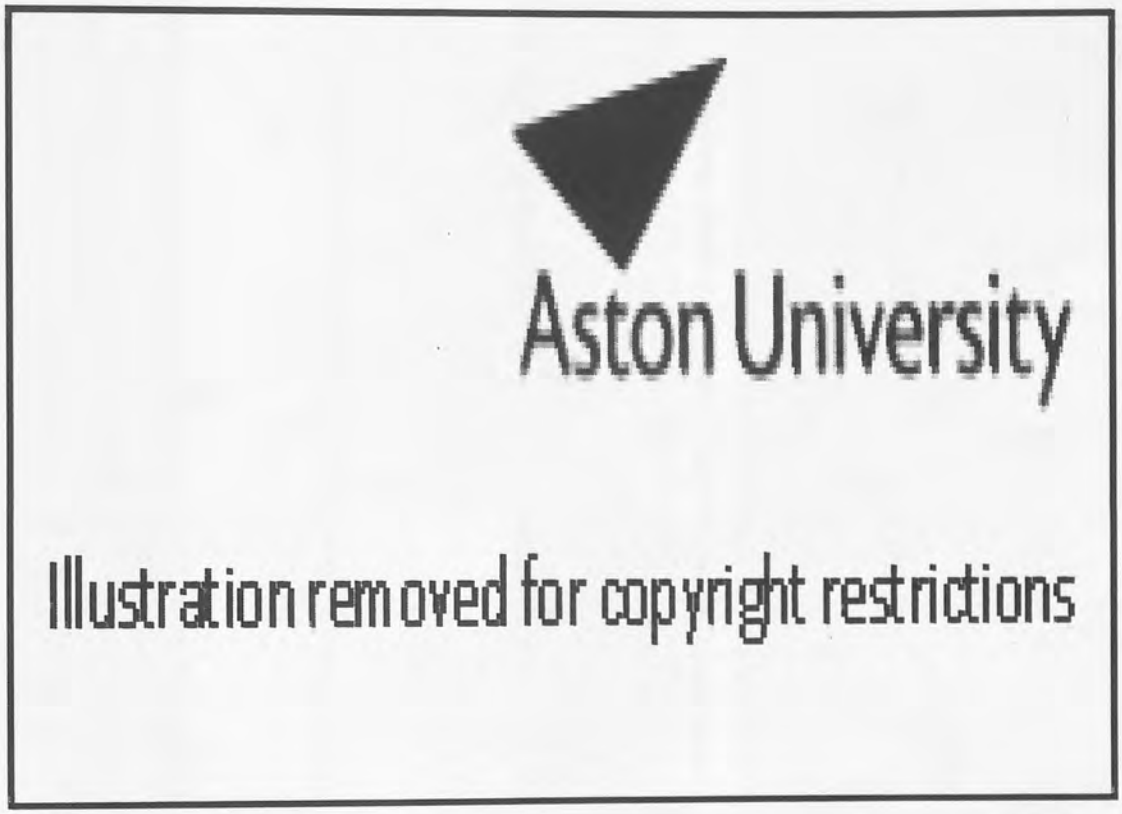


Figure 6.2. Images from one subject showing transit of HMO emulsion over time (images manipulated to adjust for background counts). Picture courtesy of Yarra Medica Ltd.

Data for the static time points revealed that, as with the *in vitro* model, the human studies showed an initial fast loss of material from the total oesophagus during the first 5 to 10 minutes followed by a slower rate of loss thereafter identified by the flatter portion of the curve at later time points. This biphasic removal process was less pronounced for the Smart Hydrogel® formulations at both pH 4.7 and 6.0, which underwent a more constant rate of removal from the tissue over time. The *in vivo* retention profiles are shown in Figure 6.3.

Aston University

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Figure 6.3. Retention profiles of formulations, showing % retention in the total oesophagus over time. (S.H. = Smart Hydrogel®) Mean + S.D. For LMO and Antepsin®, n=1. For SBE, n=2. For all others, n ≥ 4. Graph plotted by Dr. Chris Rayner, Yarra Medica Ltd.

The biphasic clearance observed in humans in this study is in concordance with the *in vitro* porcine model (see section 4.4.1), and with previous *in vitro* studies performed (Riley et al, 2002; Smart et al, 2003; Kockisch et al, 2004; Richardson et al, 2005). The similarity between the shapes of retention profiles produced *in vitro* and *in vivo* is encouraging and helps to validate the *in vitro* model against the human data. The two phases of clearance were also seen in a study by Potts et al. (1997b), in which 65 percent of a aTc99m labelled solution of Smart Hydrogel® was cleared into the stomach within 5 seconds, and a slower loss occurred thereafter. This biphasic clearance might be due to administration of an excess of material, and might be less evident if smaller volumes of formulations were administered. Alternatively the early phase may represent the formulation that does not interact with the mucosa, and the second may represent loss of formulation that has adhered. These possibilities are not mutually exclusive. It would be interesting to further examine the volume effects of Smart Hydrogel® dosage, to see if a volume is reached where the biphasic removal process is more prominent and the profile becomes similar to that of the other formulations.

The prolonged early retention of Smart Hydrogel® (as opposed to a fast initial loss) may be a result of the hydrogel's distinctive properties. Not only does the 2% w/v

Smart Hydrogel® solution gel in response to the increase in temperature as it warms to body temperature, but it also has shear-gelling properties (see Chapter 3). The *in vitro* retention model described in Chapter 4 is a suitable representation of the temperature and humidity in the oesophagus; however it does not incorporate peristaltic simulation. *In vivo*, in healthy subjects for whom peristaltic function is normal, the formulations are subjected to shear forces upon swallowing. Rheological measurements demonstrate that a shear rate of 10 s^{-1}, which approximates the forces associated with swallowing and draining under gravity (Barnes et al, 1989), causing the Smart Hydrogel® solution to form into gel-like clumps or beads (Section 3.3.1). This gelled form of Smart Hydrogel® may have enhanced retention in the oesophagus, perhaps within the tissue folds in the lumen. The shear- and temperature- responsive properties could be the reason for the distinct retention profile shape and exceptional retention *in vivo*.

The AUC 30 data for each formulation, for the total *in vivo* oesophageal counts, is shown in rank order in Figure 6.4. The two Smart Hydrogel® formulations were better retained than any other material ($p>0.05$). There was no significant difference between retention of the pH 4.7 and pH 6.0 Smart Hydrogel® formulations. Blend A was next best retained (although not significantly better than SBE emulsion). The remaining formulations showed similar retention to each other.

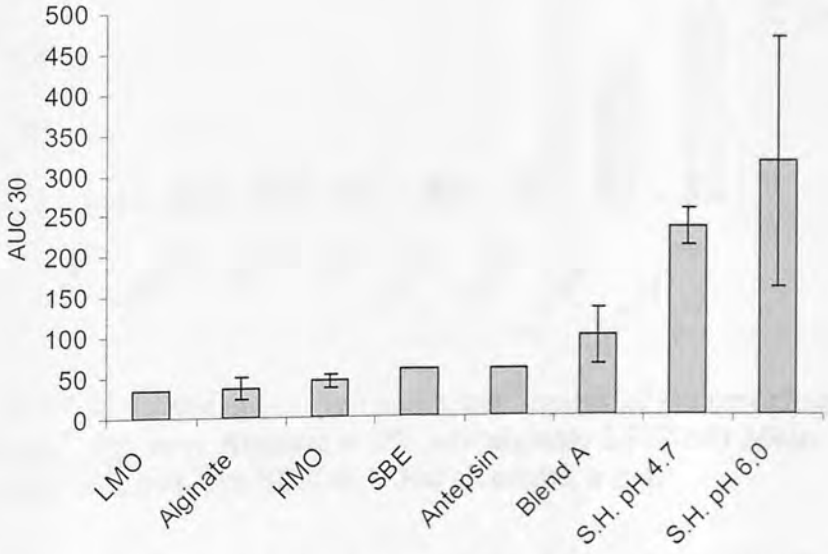

Figure 6.4. AUC 30 of formulation retention, for the total oesophagus. (S.H. = Smart Hydrogel® 2% w/v, alginate = 2% w/v Alginate LF120M) Mean ± S.D. For LMO and antepsin, n=1. For SBE, n=2. For all others, $n \geq 4$.

6.2.3. Regional differences in retention

The *in vivo* studies are valuable as they help to identify the pattern of retention in the different regions of the oesophagus, i.e. whether retention is more prolonged in the upper/middle/lower thirds of the oesophagus. This is information that could help to direct the use of the drug delivery system for the most appropriate therapeutic area. For example, diseases such as achalasia and GORD would particularly benefit from a formulation that is effective in the distal oesophagus, and LOS region.

Regional variation in AUC 30 of oesophageal retention is displayed in Figure 6.5. For Smart Hydrogel®, apparent disproportionate retention was observed between oesophageal regions, with the retention occurring in the upper and middle thirds. However, this failed to reach significance ($p=0.12$ at pH 6, $p=0.32$ at pH 4.7). Blend A also showed regional variation of retention, but for this formulation the retention was significantly better retained in the lower third of the oesophagus than upper third ($P<0.05$). For many of the formulations, there was a large amount of variability in the adhesion data, and this makes statistical interpretation of the data difficult and comparisons potentially unreliable.

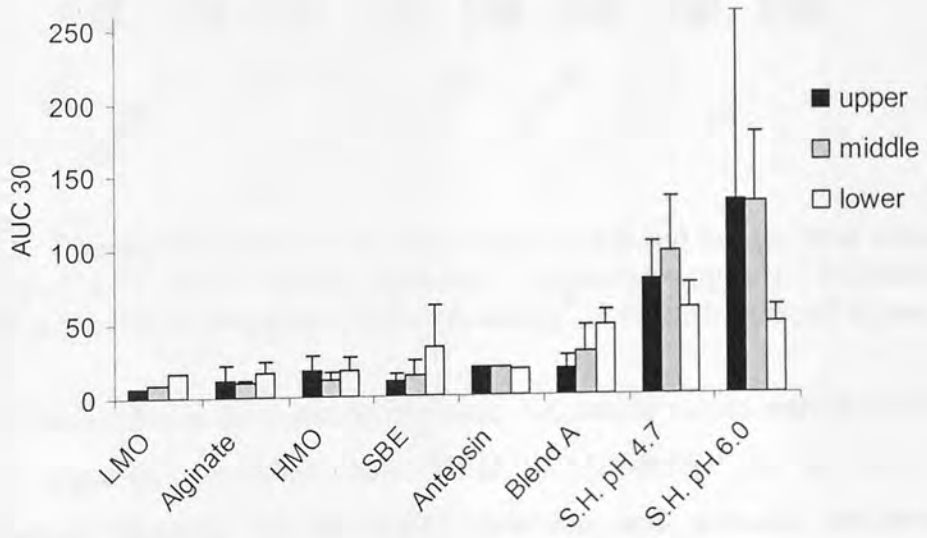

Figure 6.5. AUC 30 for the upper, mid and lower regions of the oesophagus. (S.H. = Smart Hydrogel® 2% w/v, Alginate = 2% w/v alginate LF120M) Mean + S.D. For LMO and Antepsin®, n=1. For SBE, n=2. For all others, $n \geq 4$.

The other formulations tested did not demonstrate a significant difference between distribution over the three areas of interest, for the AUC 30. The LMO emulsion may be better retained in the lower third of the oesophagus, although only one analysis was

carried out for this formulation and therefore the significance of this result is uncertain.

6.2.4. Correlation between *in vitro* and *in vivo* data

Mean AUC 30 values *in vivo* were compared with those obtained from *in vitro* studies (Figure 6.6).

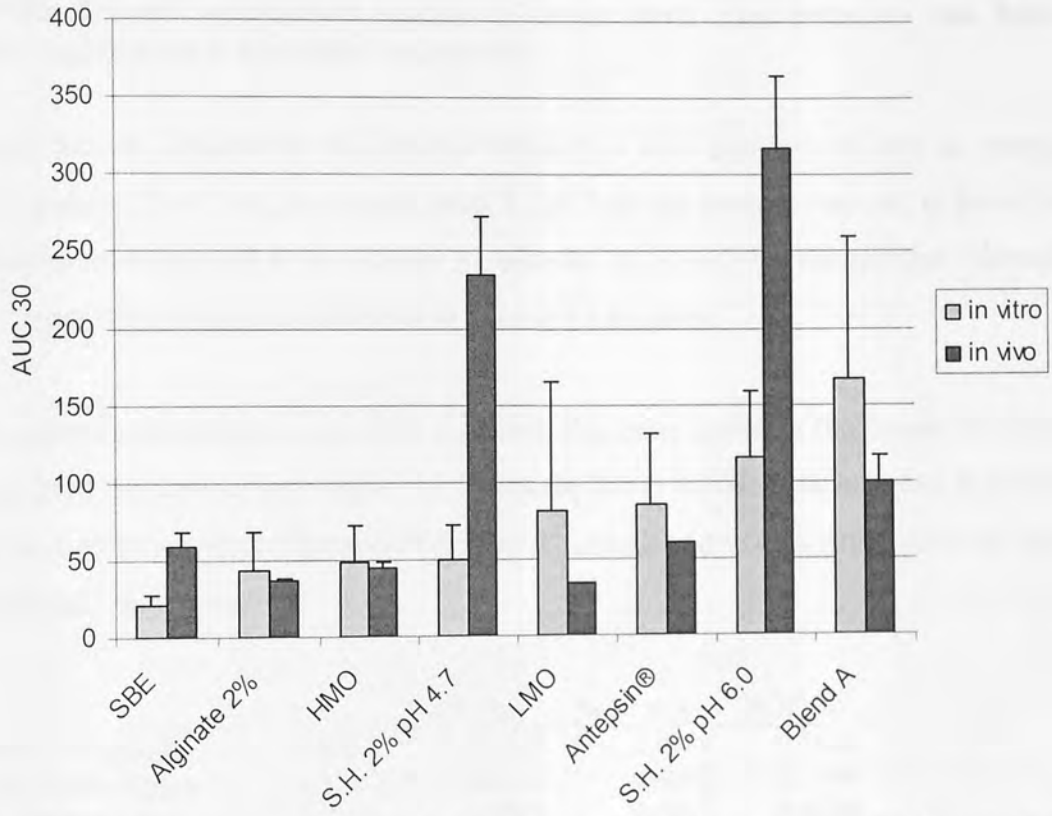

Figure 6.6. Comparison of AUC 30 for *in vitro* model and human total oesophagus data. Mean + S.D. (S.H. = Smart Hydrogel®, Alginate = alginate LF120M) For *in vitro* data, n ≥3. For *in vivo* data, LMO & Antepsin®, n=1; SBE, n=2; all others, n ≥ 4.

When comparing the *in vitro* and human AUC 30, similar values were seen for HMO emulsion, alginate, sucralfate, and Blend A. However, the *in vitro* model overestimated retention of the LMO emulsion and silicone emulsion, and underestimated retention of Smart Hydrogel® at both pH 4.7 and pH 6 when compared to the human data.

Data for percent retention and AUC 30 data for the human retention data was plotted against those values obtained from the *in vitro* porcine retention model. Strength of

linear correlations between *in vivo* and *in vitro* retention data were represented by the R^2 correlation coefficients, shown in Table 6.1.

	% 5 min	% 10 min	% 30 min	AUC 30
total oesophagus	0.0833	0.1416	0.0005	0.0821
upper oesophagus	0.0417	0.1432	0.0007	0.0529
mid oesophagus	0.0740	0.1226	0.0000	0.0658
lower oesophagus	0.1013	0.0590	0.0082	0.1200

Table 6.1. Correlation coefficients obtained from comparison of mean retention data for the different oesophageal regions in humans, with mean retention data from *in vitro* experiments at equivalent time points.

There was no discernible relationship between *in vitro* and *in vivo* data at matching time points. The R^2 values ranged from 2×10^{-6} for the percent retained in the middle oesophagus compared to *in vitro* at 30 minutes, to 0.1432 for the percent retained in the upper oesophagus compared to *in vitro* at 10 minutes.

The above calculations were then repeated, this time omitting the Smart Hydrogel® data from the comparison. Table 6.2 shows the linear correlations between *in vivo* and *in vitro* retention data represented by the R^2 correlation coefficients, with the Smart Hydrogel® data removed.

	% 5 min	% 10 min	% 30 min	AUC 30
total oesophagus	0.2082	0.2118	0.0000	0.5623
upper oesophagus	0.1167	0.0052	0.0002	0.1568
mid oesophagus	0.2519	0.2191	0.0002	0.4628
lower oesophagus	0.4628	0.2165	0.0025	0.3670

Table 6.2. Correlation coefficients obtained from comparison of mean retention data for the different oesophageal regions in humans, with mean retention data from *in vitro* experiments at equivalent time points. Data shown omits Smart Hydrogel® retention.

It can be seen that the Smart Hydrogel® measurements have an important effect on this correlation, as in general the *in vitro* - *in vivo* relationship is stronger without the inclusion of Smart Hydrogel®. The highest R^2 representing the strongest linear relationship was 0.5623, for the total AUC in the total *in vivo* oesophageal region compared to *in vitro* AUC 30. This correlation is shown in Figure 6.7, as an example of the relationships observed.

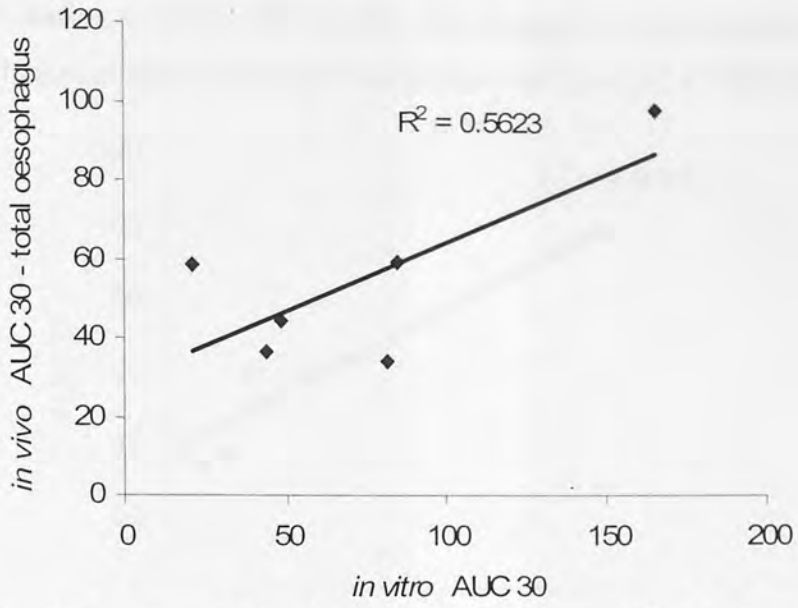

Figure 6.7. Graph showing relationship between the mean AUC 30 of retention curves for *in vitro* and in total oesophageal *in vivo* data, for six formulations, not including Smart Hydrogel®.

It can be concluded from these comparisons that the data obtained from the *in vitro* model correlated poorly with that obtained in the *in vivo* studies. The *in vitro* model appears to be an imperfect tool for predicting retention of the formulations in humans. However, the number of formulations tested in humans was low, and the number of repeats of experiments of some of the materials was also low (for LMO and Antepsin®, n=1, for SBE, n=2, for all others, $n \geq 4$). Furthermore, extensive investigations using γ-scintigraphy might reveal that the *in vitro* model is useful for screening the retention of certain types of formulations with similar characteristics in relation to each other, for example emulsions, or polymer blends. Incorporation of a peristaltic simulation into the *in vitro* model would be valuable in assessing materials with properties like Smart Hydrogel®, which is shear-responsive. The model would need to be developed further in order to achieve this.

6.2.5 Importance of viscosity for *in vivo* retention

It was previously found that retention on *ex vivo* porcine oesophageal tissue did not correlate with the overall viscosity (Chapter 4). The relationship between viscosity of liquids and their retention *in vivo* was also investigated. The overall viscosity (in Pa.s) of the formulations was plotted against the *in vivo* AUC 30 for the total oesophageal region. It was found that the *in vivo* AUC 30 data showed a weak linear correlation

with the formulation viscosity (R^2 = 0.52). The strength of this correlation increased upon the exclusion of Smart Hydrogel® from the correlation (R^2 = 0.90) (Figure 6.8).

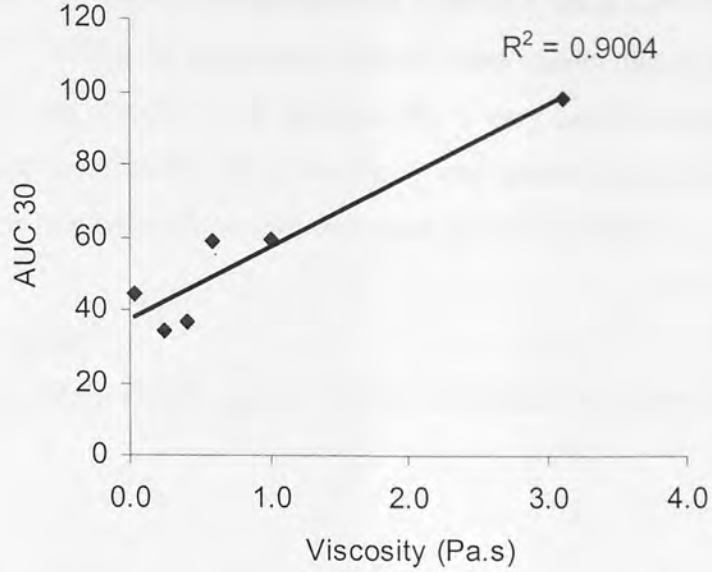

Figure 6.8. Scatter diagram showing relationship between viscosity (Pa.s) and retention AUC 30 for the total oesophagus, for all formulations excluding Smart Hydrogel®.

The viscosity data used to make these correlations is from measurements taken at 25°C. It is known that the physical properties of Smart Hydrogel® change with increasing temperature, and that at body temperature, the 2% w/v solution has a higher viscosity than at 25°C (see section 3.3.2). The Smart Hydrogel® solution is also likely to be affected by the shear forces in the oesophagus, caused by swallowing the formulation (see section 3.3.1). These unusual properties of Smart Hydrogel® may explain why the Smart Hydrogel® does not fit into the strong viscosity correlation observed for other formulations. However, it should be remembered that a much weaker relationship between viscosity and retention was observed *in vitro* (R^2 = 0.40 for AUC 30 vs. viscosity) when the correlation includes all formulations tested *in vitro* (see Chapter 4). The correlation seen *in vivo* may be an artefact of the very small number of formulations tested, and the low number of repeats performed for some experiments. Additionally studies *in vivo* are typically associated with a particularly high variability. Further experiments involving a wider variety of formulations and a higher number of repeats could help to clarify if this correlation does exist.

For the same selection of formulations as those tested *in vivo*, there is also a weak correlation between the *in vitro* AUC data and viscosity, (R^2 = 0.60), which also

increases upon the exclusion of Smart Hydrogel® from the correlation (R^2 = 0.77). When the entire range of liquids tested in the *in vitro* model was included in such a comparison, however, the correlation between total AUC 30 *in vitro* and viscosity was very weak (R^2 = 0.25). In conclusion, the *in vitro* model failed to establish any relationship between viscosity and retention for a very broad spectrum of differing liquids. Any such relationship found in the *in vivo* studies is tentative, and may be misleading as the number of formulations tested *in vivo* is small.

6.2.6. Adverse events

There were no adverse events reported during the studies. All formulations were well tolerated.

6.3 Discussion

The human studies highlight the difficulty of achieving prolonged retention of any formulation in the oesophagus, which in healthy subjects, is able to rapidly clear contents by peristalsis. Smart Hydrogel® was the best retained formulation *in vivo*, however the mean retention at 10 minutes was only approximately 14% of the administered dose, and minimal amounts still remained at 30 minutes. These data are in agreement with a study reported by Potts et al. (1997b), in which 13% of a Tc99m-labelled Smart Hydrogel® solution was still retained 10 minutes after swallowing. It should be noted that these retention values do not make allowance for attenuation of scintigraphic counts by tissue absorption and emission of gamma rays away from the gamma camera, and the mean ratio of counts obtained from the whole upper gastrointestinal field (encompassing the entire swallowed dose) to the counts obtained from the administering syringe was around 0.4 (see section 6.2.1). It is possible that actual retention could be twice the reported value. However it is still possible to use the data to compare formulations.

Smart Hydrogel® differs from the other formulations in undergoing a reversible increase in viscosity when warmed from ambient to body temperature (Bromberg and Ron, 1998; 1999). The other unique property of Smart Hydrogel® was its increase in viscosity when subjected to shear forces. Such forces are generated in the oesophagus during swallowing (Barnes et al, 1989), making the shear-thickening property of the

formulation potentially as important as its ability to gel *in situ* with body heat. These properties of Smart Hydrogel® may account for its disproportionate retention in the upper and middle oesophagus, in contrast to the next most retentive formulation (Blend A). Although these regional differences in retention failed to reach statistical significance for Smart Hydrogel®, the small number of subjects (n=4 at each pH value) makes statistical interpretation difficult and raises the possibility of a type 2 error (where there is a failure to reject the null hypothesis when in fact there is a difference between the groups) (Swinscow, 1997). The unique shear/ gelling properties of Smart Hydrogel® amongst the formulations tested during this study may also account for the underestimation of retention observed in the *in vitro* model. The porcine model described in Chapter 4 might predict the *in vivo* retention of Smart Hydrogel® more accurately if simulation of peristalsis were incorporated.

Blend A was the most viscous of the remainder of the formulations, which could account for its relatively higher retention. Although viscosity has previously been reported to be a factor in oesophageal retention of compounds (Ito et al, 1990), this was not the case in our *in vitro* model, which suggested that a high viscous to elastic ratio (loss tangent) was more important for early retention than other rheological parameters. The preliminary rheological work performed on Blend A (see section 3.3.4) demonstrates that it has some thermally-responsive property, despite the low concentration of the thermally-responsive component poloxamer 407. The thermally-responsive property of Blend A might account for its good retention *in vitro* and *in vivo*.

Areas under the retention curves for HMO emulsion, alginate, sucralfate, and Blend A in the *in vitro* model were similar to those obtained in humans. However, the model failed to accurately predict retention of Smart Hydrogel®, LMO emulsion, and SBE emulsion. A previous report also noted disparity in the retention of film-coated risedronate tablets on porcine oesophageal mucosa when compared to human scinitgraphic data (McCargar et al, 2001). These discrepancies between *in vitro* and *in vivo* adhesion might be improved with further development of the oesophageal model.

6.4 Conclusions

Most of the candidate bioadhesive formulations tested were retained only briefly in the human oesophagus, although Smart Hydrogel® shows potential as a vehicle for topical oesophageal drug therapy. The retention of Smart Hydrogel® *in vivo* far exceed that reported and predicted by the *in vitro* study, this may be due to the shear forces encountered *in vivo* which cause the solution to form a gel. This study demonstrates the potential of a polymer solution to act as a vehicle for topical administration of drugs to the oesophagus. These studies also highlight the scope for improvement of the existing porcine oesophageal retention model.

Chapter 7: The effects of polymer formulations containing isosorbide dinitrate or sildenafil on oesophageal motility in healthy volunteers

Chapter 7

The effects of polymer formulations containing isosorbide dinitrate or sildenafil on oesophageal motility in healthy volunteers

7.1. Introduction

The aim of the project was to develop a drug delivery system with a direct pharmacological action on oesophageal tissue. This involved carrying a drug within a bioadhesive liquid that exhibits prolonged contact with the oesophageal epithelium. The second series of *in vivo* studies aimed to determine the extent of the effects of the formulations containing isosorbide dinitrate, or sildenafil on oesophageal muscle in healthy human subjects. These experiments were also designed to determine whether the formulations were having a systemic effect, or whether they were indeed acting by a local action on oesophageal tissue. The effects of drugs on oesophageal motor function were evaluated by well-established manometric techniques such as those used routinely in clinical practice.

Systemic pharmacological therapy for oesophageal motor disorders such as achalasia and diffuse spasm has limitations such as side effects and lack of efficacy, which might be overcome by using drug formulations with a local effect (see section 1.4). Local drug delivery to the oesophagus via an oral liquid could be enhanced by using a formulation that exhibits prolonged retention in the oesophagus after swallowing. The purpose of this *in vivo* study was to examine the effects on oesophageal motor function of the organic nitrate isosorbide dinitrate, or the phosphodiesterase (PDE) type 5 inhibitor, sildenafil, when administered orally in adhesive Smart Hydrogel® formulations, compared to intra-gastric administration, and to placebo drug-free Smart Hydrogel®, in healthy subjects. This would give an indication of the efficacy of the proposed bioadhesive drug delivery strategy in the form of a Smart Hydrogel® polymer vehicle, compared to normal systemic absorption of the drug.

The synthetic block co-polymer Smart Hydrogel® is thermally responsive when formulated as a 2% w/v aqueous solution, and undergoes gelation when subjected to shear forces (see sections 3.3.1 and 3.3.2) The Smart Hydrogel® displayed retention on *ex vivo* porcine oesophageal tissue (Chapter 4), and has also displayed prolonged oesophageal retention in humans (Chapter 6 and (Potts et al, 1997b)). For these

reasons Smart Hydrogel® was considered an interesting potential vehicle for targeted oesophageal drug delivery, and was used as the vehicle in this *in vivo* study.

A previous study reported that sublingual ISDN (5 mg) decreased both the amplitude of oesophageal peristaltic waves and LOS pressure in healthy subjects, for up to forty minutes (Dantas et al, 1988), and another report found that either 20 mg or 40 mg orally twice daily for 3 days decreased LOS pressure but had no effect on peristaltic wave amplitude (Stacher et al, 1997). Several studies have shown that systemically administered ISDN can reduce lower oesophageal sphincter pressure in patients with achalasia (Gelfond et al, 1981; Gelfond et al, 1982; Rozen et al, 1982; Bortolotti et al, 1994).

A dose of 50 mg SIL administered orally or via an intra-gastric tube in healthy volunteers decreased the amplitude and propagation velocity of pressure waves in the oesophageal body, and decreased basal LOS pressure. Effects have been shown to persist for an hour post-dosing (Rhee et al, 2001; Bortolotti et al, 2001; Eherer et al, 2002). A 50 mg dose of SIL infused directly into the stomach also has been found to decrease LOS pressure (Bortolotti et al, 2000; 2002a) in patients with achalasia or a hypertensive LOS. SIL also decreased the amplitude of oesophageal waves in patients with nutcracker oesophagus and diffuse oesophageal spasm (Eherer et al, 2002; Lee et al, 2003).

Such reports in the literature suggest that ISDN and SIL could be useful in the treatment of oesophageal motility disorders. However, the chronic use of nitric oxide donors or sildenafil in patients with oesophageal motility disorders has been limited by variable efficacy of treatment and adverse effects (Gelfond et al, 1981; Traube and McCallum, 1985; Thomas et al, 1986; Bortolotti, 1999; Richter, 2001; Storr et al, 2001; Eherer et al, 2002). Such side effects include headache with higher doses of nitrates (Stacher et al, 1997). It is proposed that by targeting drugs directly to the oesophageal mucosa, allowing a direct pharmacological action on underlying smooth muscle, systemic exposure would be minimised, thus overcoming these limitations. As the locally-targeted drug delivery system could avoid first pass metabolism, it is hypothesised that a lower drug dose would be required, hence reducing the potential side effects.

7.2 Study design

7.2.1 Subjects

Healthy volunteers aged 25 to 56 were recruited by advertisement, and subjects with gastrointestinal symptoms, a history of hiatus hernia or upper gastrointestinal surgery, those with significant medical illnesses, and those taking medication known to influence gastro-oesophageal motility were excluded from the study. Women who were pregnant or lactating were also excluded. For each study, subjects were asked to fast from solids and nutrient liquids for six hours prior to the study, but were permitted to drink water until one hour prior. Subjects were asked to not to smoke for twelve hours prior to the study.

7.2.3 Ethical and safety considerations

The protocol was approved by the Harrow Research Ethics Committee and the Administration of Radioactive Substances Advisory Committee, and all subjects gave written informed consent. The studies were directed and performed in the Physiology Department of St. Mark's Hospital, Harrow, Middlesex, by Research Fellow, Dr. Chris Rayner.

The placement of the oesophageal manometry catheter involves minor discomfort; this can be minimised by use of local gel anaesthetic in the nose. Anaesthetic was offered to all volunteers but was optional. The methods used during this study to assess oesophageal motility are used routinely in both clinical practice and research studies.

The doses of drugs tested in this study were within the range previously evaluated in human subjects. ISDN has previously been given orally to healthy humans in oesophageal motility studies in doses between 20 and 40 mg (Torrabadella et al, 2004). While nitrates have the potential to lower blood pressure and induce tachycardia, a previous study showed that during three days administration of up to 40 mg isosorbide dinitrate twice daily, neither systolic or diastolic blood pressure nor heart rate were altered significantly in healthy volunteers (Torrabadella et al, 2004). Headache was the only side effect in this study; slight headache was reported in 7 of 12 subjects at a dose of 20 mg twice daily.

Sildenafil has previously been given orally or intra-gastrically to healthy humans in oesophageal motility studies at a dose of 50 mg (Rhee et al, 2001; Bortolotti et al, 2001; Eherer et al, 2002). The drug was well tolerated in all of these studies, with mild headache in a small number of subjects, and either no change, or a very modest decrease, in blood pressure. The mean maximum fall in systolic blood pressure in healthy subjects given 100 mg oral sildenafil is reported to be 8.4 mmHg, maximal 1-2 hours after dosing (Pfizer Inc., 2002).

Cardiovascular effects and other adverse reactions were closely monitored during the study. Blood pressure and heart rate were monitored at regular intervals throughout the study (specifically, at 0, 15, 30, 50, 70, 80, 100, 115, 135, 145, and 165 minutes). Additionally, at 30-minute intervals (specifically, at 5, 35, 65, 95, 130, and 160 minutes), subjects were asked to subjectively score sensations that correspond to recognised drug side effects, namely dizziness/light-headedness, flushing/heat, headache, weakness, nausea, and palpitations, on a numerical scale (0= none, 1= faint, 2= weak, 3= mild, 4= moderate, 5= strong, 6=intense).

7.2.4 Preparation of test formulations

All formulations used in this study were prepared in the suitably equipped laboratory at Aston University and sent to St. Mark's Hospital for the studies. Smart Hydrogel® 2% w/v aqueous solution was used as the drug-free control; this was prepared as described in section 2.9 and adjusted to pH 4.7. The Smart Hydrogel® test formulations incorporating ISDN and SIL were prepared as described in section 2.11.4 and adjusted to pH 4.7. The only difference was that ISDN incorporated into formulations for administration to human subjects was of clinical grade rather than laboratory grade.

7.2.5. Study order

Six subjects attended the laboratory on at least two days each, and study days were separated by at least 48 hours. All subjects received either the SIL or drug-free formulations. Four subjects attended an additional third day and also received the ISDN formulations. The order of the studies was randomised and single blinded. The three study days (x, y, and z) for each subject were arranged as follows:

- Day x: both a low and a high dose of drug given orally, and vehicle only given intra-gastrically.
- Day y: vehicle only given orally, and both doses of drug given intra-gastrically.
- Day z: vehicle only given both orally and intra-gastrically on the remaining day

Low and high doses of ISDN were 5 mg and 25 mg ISDN respectively in 10 mL Smart Hydrogel® 2% w/v solution (pH 4.7).

Low and high doses of SIL were 10 mg and 25 mg SIL respectively in 10 mL Smart Hydrogel® 2% w/v solution (pH 4.7).

The formulations were delivered both intra-gastrically and systemically in order to distinguish between local effects enhanced by the bioadhesive drug delivery system and systemic effects resulting from absorption following direct infusion into the stomach. Six subjects underwent oesophageal manometry at baseline, and after low and high doses of sildenafil or drug-free vehicle formulations. Four of the subjects also received low and high doses of ISDN formulations

7.3 Methods

7.3.1 Oesophageal manometry catheter

Oesophageal manometry involves the use of a thin tube inserted into the oesophagus, which measures pressure inside the lumen. The silicone rubber manometry catheter used (Dentsleeve, Adelaide, Australia) had an external diameter of 3.5 mm, and incorporated a sleeve sensor to enable continuous monitoring of lower oesophageal sphincter pressure. The catheter also had one side-hole to measure intra-gastric pressure, seven oesophageal side-holes at 3 cm intervals beginning at the proximal margin of the sleeve, and a pharyngeal side-hole. An additional central lumen terminated at the distal, intra-gastric end of the catheter, enabling intra-gastric infusion of formulation or vehicle.

The catheter was perfused with water at 0.15 mL per minute via a low compliance pump. Both the sleeve sensor and the gastric side-hole were perfused continuously; the oesophageal and pharyngeal side-holes were perfused only during the sequences of swallows, in order to minimise the flow of water into the oesophageal lumen.

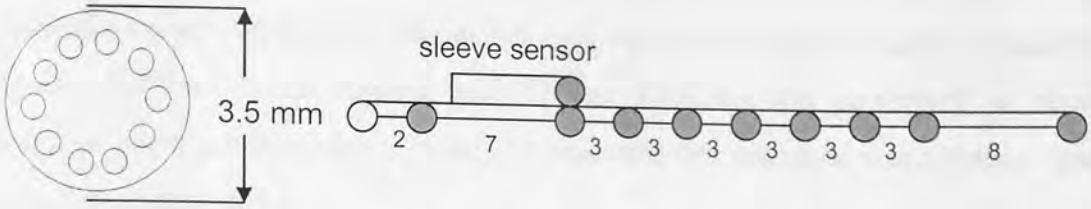

Figure 7.1. Diagram of the silicone rubber manometry catheter used, incorporating a LOS sleeve, oesophageal body side holes, and core channel for intra-gastric infusion.

7.3.2 Positioning of oesophageal manometry catheter

The manometry catheter was passed trans-nasally into the stomach, and withdrawn to a position where the sleeve sensor was positioned at the lower oesophageal sphincter. The nasal cavity was anaesthetised using xylocaine gel if the subject desired. Once the manometry catheter was in place, the subject was positioned in the supine right lateral position thereafter for the duration of the study.

7.3.3. Swallow sequences and formulation administration

After resting for fifteen minutes to accommodate to the presence of the catheter, subjects were asked to perform 10 dry swallows at 30-second intervals. Following this, five 20 mL boluses of air were administered into the oesophageal lumen via a mid-oesophageal side-hole in the manometry catheter. The subject then performed ten water swallows of 5 mL each at 30- second intervals. This was swallow sequence A. For the wet swallows, volumes of drinking water were introduced into the oral cavity using a syringe.

Five minutes after the last water swallow, the manometry catheter was carefully removed. The subject then sat upright and swallowed the low dose of formulation (either ISDN, SIL, or control). For SIL, this was administered as two 5 mL volumes, 15 seconds apart. Immediately afterwards, either the vehicle alone, or a low dose of drug in the Smart Hydrogel® vehicle was infused directly into the stomach via the central intra-gastric lumen of the manometry catheter. Fifteen minutes after the swallowed and infused doses, the catheter was re-inserted and re-positioned as before. Formulations were administered in this way without the catheter *in situ* because the presence of an oesophageal catheter impaired oesophageal retention of radiolabelled

Smart Hydrogel® in preliminary studies (data not available). Once the catheter was in place, the subject returned to the previous supine position and the same sequence of 10 dry swallows, 5 air boluses, and 10 wet swallows was performed as before (swallow sequence B). After a further 15 minutes, this sequence was repeated again (swallow sequenced C).

Five minutes after the last wet swallow in the final sequence, the catheter was removed again and the high dose of formulation was administered. The subject swallowed either the high concentration of drug in the Smart Hydrogel® vehicle, or vehicle only. Immediately afterwards, either the vehicle only, or a high concentration of drug in the Smart Hydrogel® vehicle, was infused directly into the stomach via the central intra-gastric lumen of the manometry catheter. After 15 minutes the catheter was re-inserted, the subject re-positioned, and the sequence of 10 dry swallows, 5 air boluses, and 10 wet swallows was repeated (swallow sequence D) and repeated once again after another 15-minute interval (swallow sequence E). Figure 7.2 shows a timeline of swallow sequences, administration of high and lose dose formulations, and the removal and insertion of the manometry catheter tube.

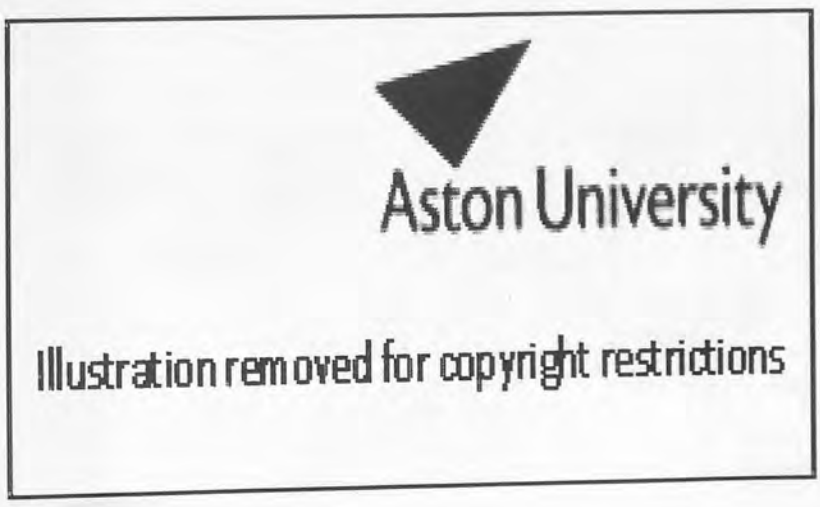

Figure 7.2. Diagram showing timings of the swallow sequences A to E, administration of high and lose dose formulations, and the removal and insertion of the manometry catheter tube (diagram courtesy of Dr. Chris Rayner, St. Mark's Hospital).

Statistical comparisons were made within study days to assess dose-response, and between study days to compare isosorbide dinitrate and sildenafil with control, using analysis of variance (ANOVA) with repeated measures. Where significant differences were observed, a paired t-test was used to determine points of significant difference. P values < 0.05 were considered significant. These statistical tests were performed at St.Mark's Hospital, Harrow, Middlesex.

During each series of wet or dry swallows, basal LOS pressure was measured as the mean of end-expiratory pressures recorded by the sleeve sensor when a stable baseline had been attained prior to each swallow, and was referenced to intra-gastric pressure. The amplitude and duration of peristaltic waves were measured at the levels of each oesophageal side-hole, incorporating measurements for the upper, middle and lower thirds of the oesophagus, and means calculated for each series of 10 dry swallows and 10 wet swallows. The velocity of peristaltic waves was measured between adjacent side-holes, and mean values calculated for each series of swallows. Analysis of manometric data was performed by researchers at St.Mark's Hospital, Harrow, Middlesex.

Blood pressure and heart rate were monitored at regular intervals throughout the study. Following the last wet swallow, the manometry catheter was removed, and both supine and standing blood pressure and heart rate were measured. Provided these measurements remained stable during the study, and there was no substantial postural fall in systolic or diastolic blood pressure ($\geq$ 15 mmHg), the subject was free to leave the laboratory. Adverse reactions were closely monitored during the study. Subjects were asked to subjectively score the intensity of sensations that correspond to recognised drug side effects, i.e. dizziness/light-headedness, flushing/heat, headache, weakness, nausea, and palpitations, on a numerical scale (0= none, 1= faint, 2= weak, 3= mild, 4= moderate, 5= strong, 6= intense). Out of the four subjects who were administered ISDN, headache was reported by two subjects after low dose isosorbide dinitrate (maximum intensity score = 1), and three after the high dose (maximum score = 3).

Statistical comparisons were made within study days to assess dose-response, and between study days to compare orally administered drug with intra-gastric delivered drug and the vehicle-only control.

7.4 Results

7.4.1 Cardioavascular effects and adverse events

Headache is a recognised side effect of ISDN (Abrams, 1983). Eherer reported previously that 50 mg SIL can cause tolerable headaches (Eherer et al, 2002). Headache was not a reported symptom in this study following 10 and 25 mg doses of SIL. Of the six subjects who were administered SIL, two reported a sensation of "indigestion" after low dose, and four after high dose. This feeling of dyspepsia is a known side-effect of SIL in subjects without oesophageal motility disorders and is due to its relaxant effect on the LOS smooth muscle (Lim et al, 2002). This effect indicated that the SIL was having the desired effect on the LOS smooth muscle. In oesophageal motility patients, this effect might be the beneficial desired result of treatment. In healthy subjects, however, relaxing a normal LOS increases the risk of reflux, which may manifest as heartburn, and is considered an adverse effect. This effect may have been aggravated during this study as the subjects had fasted prior to the study, resulting in a decreased pH of the gastric contents, which is more prone to irritating the oesophageal epithelium. It is thought that patients with a hypercontractile LOS are less likely to experience the reflux-associated symptoms caused by the effects of SIL, as the hypertensive LOS would be returned to a 'normal' LOS contractile state, rather than a hypertensive one. Blood pressure and heart rate were monitored at regular intervals throughout the study. Neither ISDN nor SIL had an effect on heart rate, but both drugs were associated with a fall in mean arterial pressure when compared with control (repeated measures ANOVA, $P < 0.05$ for each) (figure 7.3).

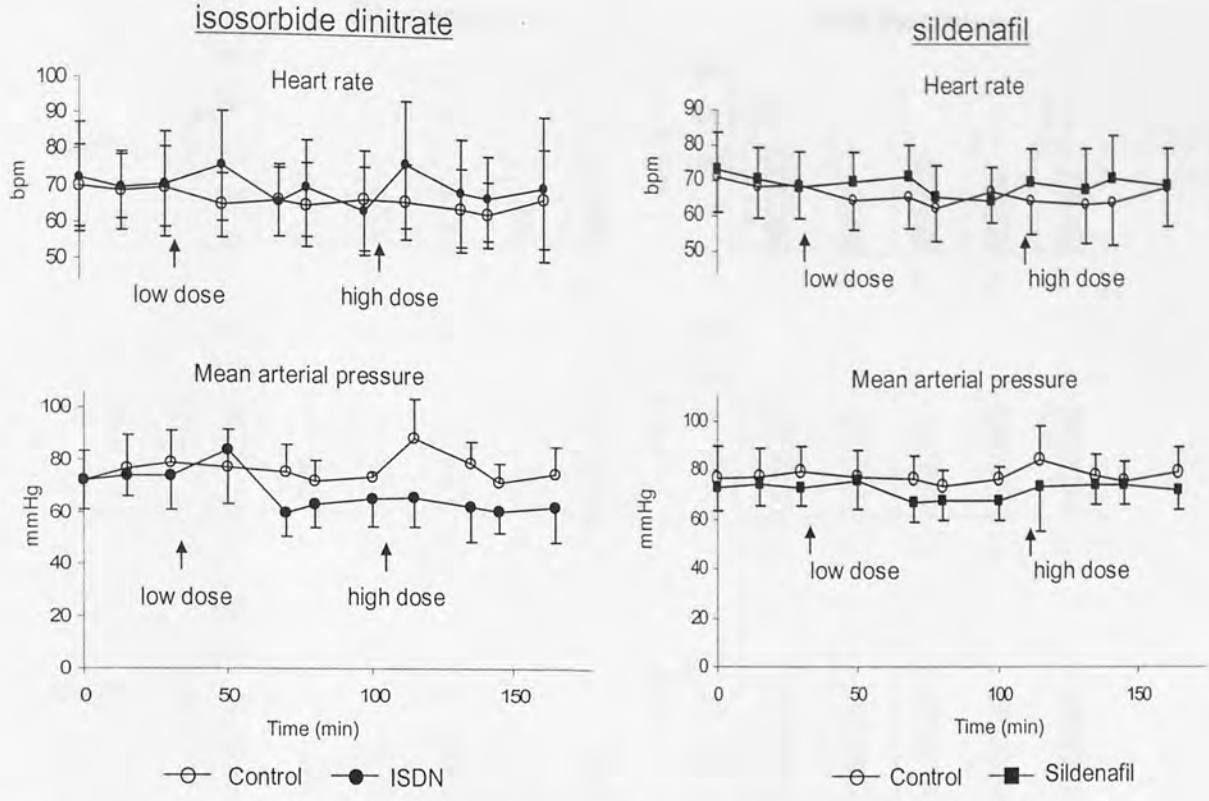

Figure 7.3. The effects of oral ISDN and SIL at both low and high doses, on subject heart rate and mean arterial pressure. Mean ± S.D. ISDN n= 4, SIL n=6.

7.4.2 Effect of formulations on amplitude of peristaltic pressure waves

This study was single blinded, and in most cases the subjects remained unaware of the effects of the administered drug. Upon reading the tracings, for some drug-containing formulations, the effects on peristaltic waves and changes in manometry readings were so pronounced that the study would have been unblinded. Such a situation was also reported by Eherer et al. (2002), where the manometric changes were so obvious that the type of treatment could be determined by the examiner who was blinded and initially unaware of the administered substance.

Isosorbide dinitrate formulations administered orally at both low and high doses did not significantly affect the amplitude of peristaltic waves in the oesophageal body when compared to control Smart Hydrogel® vehicle only (Figure 7.4). The effect of ISDN failed to reach significance for all swallow sequences recorded by all catheter channels.

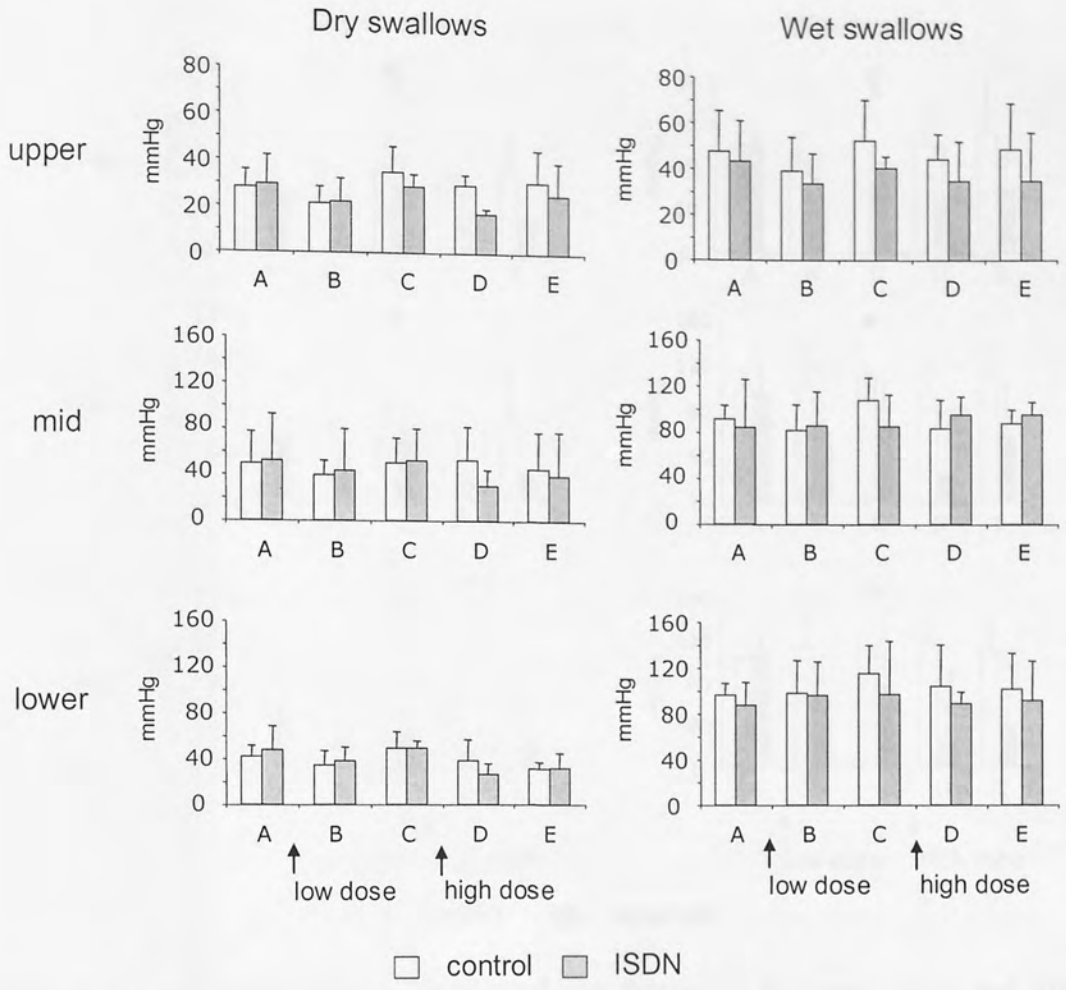

Figure 7.4. Mean amplitude of 10 dry and wet swallows, in upper, mid-, and lower oesophagus (3, 9, and 15 cm above lower oesophageal sphincter), during swallow sequences A to E, after ISDN or control formulations. Mean + S.D. n= 4.

Sildenafil formulations administered orally decreased the amplitude of pressure waves in the lower ($P < 0.05$), mid ($P < 0.05$), and upper ($P = 0.05$) oesophageal body in a dose-dependent manner, when compared with control.

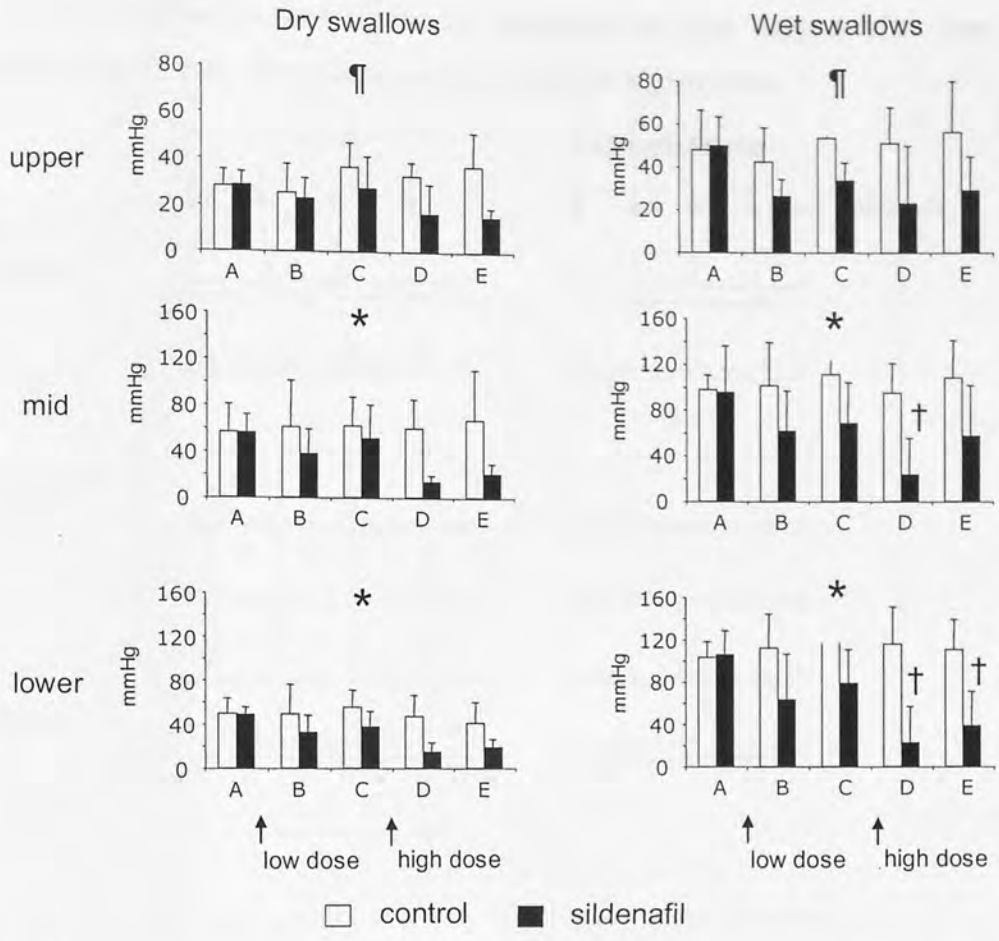

Figure 7.5. Mean amplitude of 10 dry and wet swallows, in upper, mid-, and lower oesophagus (3, 9, and 15 cm above lower oesophageal sphincter), during swallow sequences A to E, after oral SIL or control formulations.
* $P < 0.05$ (repeated measures ANOVA, sildenafil vs. control).
¶ $P = 0.05$ (repeated measures ANOVA, drug vs. control).
† $P < 0.05$ (paired t test, high vs. low dose, ie. period D vs. B, or period E vs. C).

Neither isosorbide dinitrate nor sildenafil was associated with any difference in peristaltic velocity during dry or wet swallows (data not shown). Sildenafil administered orally was associated with a decrease in amplitude of pressure waves in the lower, middle, and upper oesophagus during both dry and wet swallows when compared with control (repeated measures ANOVA; $p = 0.05$ for upper oesophagus; $p < 0.05$ for all other comparisons) (Figure 7.5). This statistical analysis was carried out by the group at St.Mark's Hospital.

The mean amplitude of wet swallows in the lower and mid- oesophagus was lower following sildenafil 25 mg when compared with 10 mg ($P < 0.05$ for each), indicating a dose-dependent effect. It was interesting to note that peristaltic pressure waves in

the oesophageal body were transiently abolished in one subject after low dose sildenafil (Figure 7.6), and in four subjects after the higher dose.

Figure 7.6. Manometric recording of four wet swallows following low dose (10 mg) sildenafil in Smart Hydrogel® formulation or control Smart Hydrogel® vehicle only (data for one subject only, recorded during swallow sequence B). (Trace courtesy of Dr. Chris Rayner, St. Mark's Hospital).

These findings are interesting as no previous reports can be found in the literature of sildenafil having an effect on oesophageal motor function at a dose as low as 10 mg. This suggests that SIL could be useful in treating oesophageal motility disorders at doses lower than those previously considered.

7.4.3. Impact of administration route on the effect of sildenafil on wet swallows

The effect of SIL on wet swallow amplitude was compared for the oral and gastric routes. ISDN administered orally did not have any significant effect on wet swallow amplitude during any of the swallow sequences A to E, and so was omitted from this analysis. Upper oesophagus indicates measurements taken 15 cm above the LOS, mid oesophagus measurements were taken 9 cm above the LOS, and lower oesophagus was measured 3 cm above the LOS sleeve. It was noted with interest that for swallow

sequence B (the first sequence following low dose drug, 10 mg), SIL administered by the oral route caused no significant effect on the wet swallow amplitude in the upper and middle oesophagus, and the reduction in amplitude in the lower oesophagus was only just statistically significant (P=0.05). In the same swallow sequence however, SIL administered via the intra-gastric catheter lumen caused a significant reduction in wet swallow amplitude at upper middle and lower channels. For swallow sequence C (the second sequence following low dose drug), the oral SIL demonstrated a significant effect on peristaltic pressure waves measured by all catheters. This indicates that the oral route, where drug is administered within a bioadhesive vehicle of Smart Hydrogel®, results in a delayed onset of action. For swallow sequence C, the gastric SIL maintains a significant effect in all thirds of the oesophagus, however this significance is of a reduced extent, indicating that the effect occurs early on but quickly begins to subside.

Following a high dose of orally administered SIL (25 mg), a significant reduction in peristaltic pressure waves in the middle and lower oesophagus was observed only at first (sequence D), but later was seen to have a significant effect in all sections of the oesophagus (sequence E). This again indicated a delayed onset. However via the intra-gastric route, SIL caused a significant reduction in amplitude initially, in all oesophageal body regions, but later this effect was lost in the middle oesophagus, where there was no significant difference between control and gastric SIL for swallow sequence E.

The decrease in wet swallow amplitude measured during sequence E for orally administered SIL was significant, and, interestingly, was found to be an even more prominent effect than the decrease in amplitude for gastrically-infused SIL, in all regions of the oesophageal body. This was an import finding in evaluating bioadhesive carriers in comparison to the gastric route. It indicated that with an equivalent drug dose (25 mg SIL), the oral route, using Smart Hydrogel® as bioadhesive carrier, has a greater effect on peristaltic pressure waves than the intra-gastric (and hence systemic) route. The bioadhesive drug delivery vehicle appears to be a valid and effective strategy for delivering SIL to the oesophagus.

7.4.4. Effect of formulations on lower oesophageal sphincter pressure

Both oral ISDN doses caused a decrease in basal lower oesophageal sphincter pressure ($p < 0.05$) when compared with control (Figure 7.7). The difference was statistically significant after higher dose ISDN.

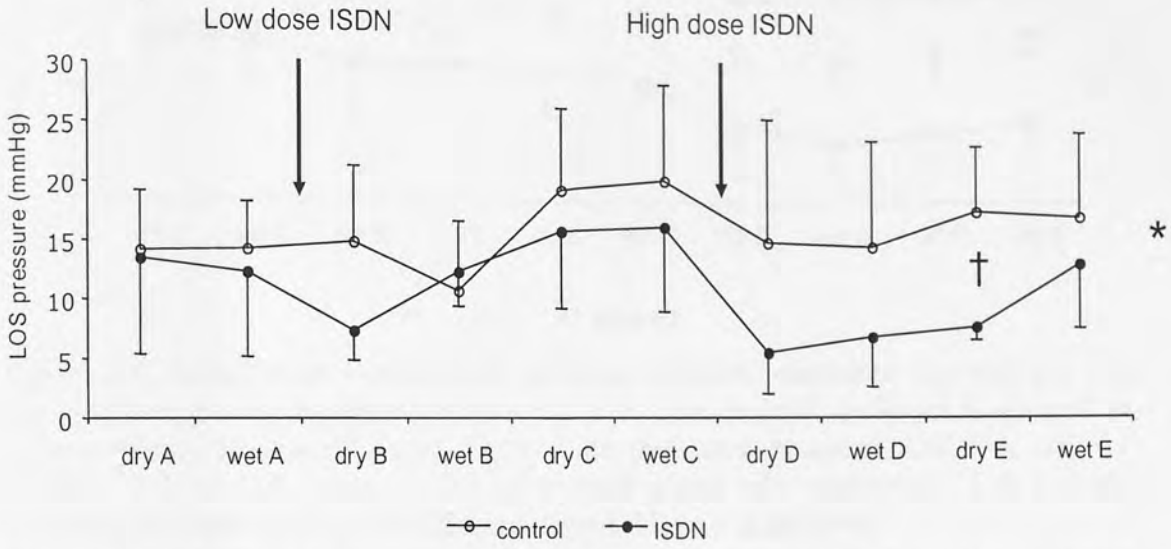

Figure 7.7. Basal LOS pressure measured between dry and wet swallows during swallow sequences A to E, after oral ISDN in Smart Hydrogel® or control (Smart Hydrogel® only). * $P < 0.05$ (repeated measures ANOVA, ISDN vs. control).
† $P < 0.05$ (paired t test, at individual point of comparison). Mean ± S.D. n = 4.

This shows that ISDN could be valuable in achalasia treatment as it reduces the LOS pressure in a dose-dependent manner. The data indicate that ISDN is effective on LOS when administered in an adhesive drug delivery vehicle, i.e. Smart Hydrogel®.

SIL administered orally also caused a decrease in basal LOS pressure ($p < 0.05$) when compared with control (Figure 7.8). The difference was statistically significant after both high and low doses of sildenafil, but was particularly pronounced following the high 25 mg does of SIL. This statistical analysis was carried out by the group at St.Mark's Hospital.

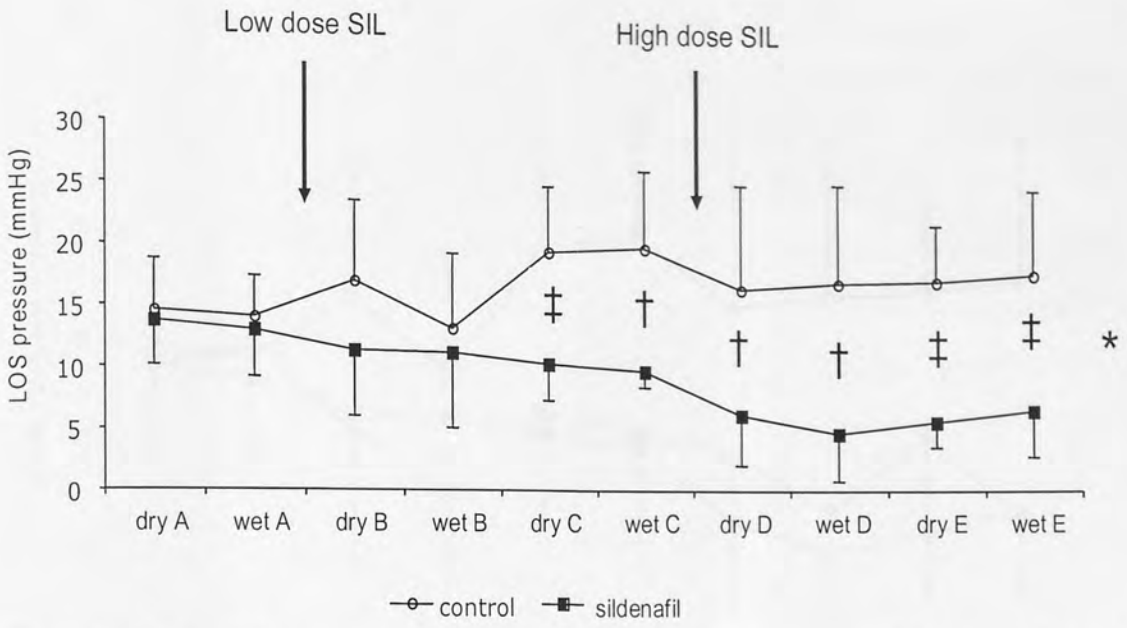

Figure 7.8. Basal lower oesophageal sphincter pressure measured between dry and wet swallows during swallow sequences A to E, after oral SIL in Smart Hydrogel® or control (Smart Hydrogel® only). * $P < 0.05$ (repeated measures ANOVA, SIL vs. control). † $P < 0.05$ (paired t test, individual points of comparison). ‡ $P < 0.005$ (paired t test, individual points of comparison). Mean ± S.D. n = 6.

This study showed that SIL has a relaxant effect on the LOS that is dose dependent, and importantly, SIL is able to have an effect on the LOS at doses lower than previously reported in the literature.

Upon examination of the SIL data for oral and gastric administration, it was seen that there was no significant difference between the effects caused by SIL via the oral route compared to the intra-gastric route (Figure 7.9). For swallow sequences C to E, the LOS pressure was lower following oral SIL than gastric SIL. However, this difference was not significant.

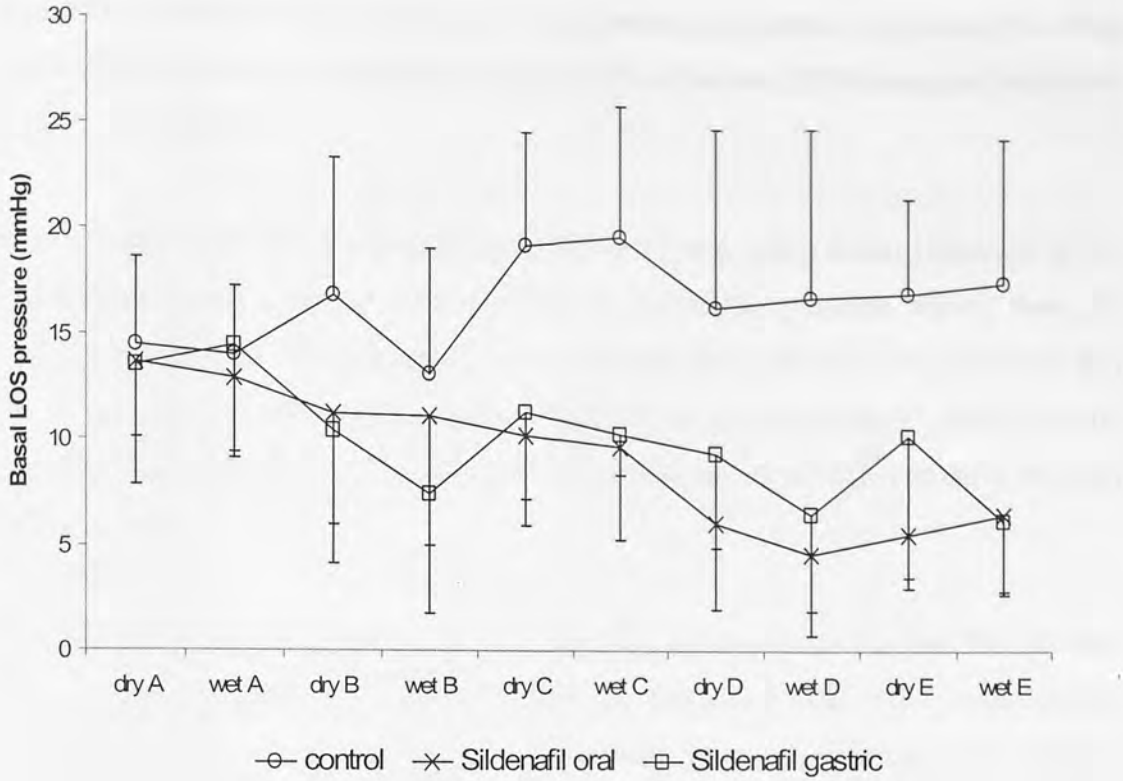

Figure 7.9. Basal lower oesophageal sphincter pressure measured between dry and wet swallows during swallow sequences A to E, after oral or gastric SIL in Smart Hydrogel® or control (Smart Hydrogel® only). Mean ± S.D. n = 6 for oral SIL and controls, n=5 for gastric SIL.

7.5 Conclusions

Ingestion of ISDN formulations at low or high doses (5 mg or 25 mg in a 10 mL volume) did not affect the amplitude of pressure waves in the oesophageal body, but the ISDN formulations did reduce basal lower oesophageal sphincter pressure, particularly after the higher dose. This effect was, however, accompanied by side effects including a drop in blood pressure and mild headache. Ingestion of the formulations containing SIL reduced both the amplitude of pressure waves in the oesophageal body, and basal LOS pressure, even after the low 10 mg dose, although the effects were more marked following the higher dose. Although these positive effects were accompanied by a fall in mean arterial pressure, the sildenafil formulations were well tolerated.

The number of subjects who participated in this study was small. However, some major effects on oesophageal motor function were noted and these were found to be relatively uniform between subjects. It is possible that more subtle effects of

isosorbide dinitrate on the oesophageal body were not detected, given that this drug was evaluated in fewer subjects than sildenafil (n=4 for oral ISDN compared with n=6 for oral sildenafil).

With an equivalent 25mg sildenafil dose, the oral route, using Smart Hydrogel as the bioadhesive carrier, had a greater effect on peristaltic pressure waves than the systemic route. This demonstrates that the concept of bioadhesive drug delivery is a valid and effective strategy to deliver sildenafil to the oesophagus. There was no significant difference between the oral and gastric routes of SIL administration on LOS pressure.

Bioadhesion has been a popular strategy for drug delivery over the last few decades and several bioadhesive formulations have been developed with a view to prolonging contact with, and delivering drugs to the buccal, intestinal, colonic, nasal, vaginal, and ocular surfaces (Lehr, 1994b). Prior to a pioneering report of prolonged retention of Smart Hydrogel® within the human oesophagus (Potts et al, 1997b), there have been few studies of liquids with bioadhesive properties in the oesophagus, where peristalsis and gravity present such a challenge to adhesion and targeted drug delivery. The results reported in Chapter 6 are similar to those described by Potts et al., and confirm the retentive potential of this novel thermally responsive polymer in the healthy oesophagus. In addition to delivering smooth muscle relaxants to the oesophagus as demonstrated in this study, such a vehicle could potentially be used as a barrier or 'bandage' to protect the oesophageal epithelium from gastric reflux, as a topical carrier for anti-fungal agents or photo-sensitising agents used in the ablative therapy of Barrett's oesophagus, or possibly for the systemic delivery of peptide drugs that require pre-gastric absorption.

The disorders of oesophageal motor function, which include achalasia, nutcracker oesophagus, and diffuse oesophageal spasm, often present a therapeutic challenge. Whilst the pharmacology of oesophageal smooth muscle is well understood, and active ingredients can have the desired effect on motility, the pharmacological therapies directed at modifying smooth muscle activity in these disorders, when delivered systemically, have been limited clinically by variable efficacy and side effects (Bortolotti, 1999; Storr et al, 2001). It is proposed that these problems could be

overcome by localised drug delivery, which could require a lower total dose than systemic administration. Topical smooth muscle relaxants including nitrates (Carapeti et al, 1999b), calcium channel antagonists (Carapeti et al, 1999a), and more recently sildenafil (Torrabadella et al, 2004), have been successfully employed on the anal sphincter, but the work reported here is novel in the application of this concept to the oesophagus.

The drug effects observed in this study are similar to those reported after systemic administration of nitrates (Dantas et al, 1988; Stacher et al, 1997) or sildenafil (Rhee et al, 2001; Bortolotti et al, 2001; Eherer et al, 2002) in healthy humans. The proportion of observed effects that were mediated by a local as opposed to a systemic action is still uncertain, although the potency of even a low dose of SIL (10 mg) was remarkable as there have been no previous reports of SIL affecting oesophageal motor function at such a low dose. Oral liquid bioadhesive formulations of either ISDN or SIL might provide useful medical therapy in patients with achalasia who are unfit for pneumatic dilatation or surgery, and might even prove to be more effective in achalasia patients than in healthy subjects if there is greater oesophageal retention of the formulation, as predicted. When studying the effects of the calcium channel blocker diltiazem on oesophageal motor function, Richter et al. (1984) found that although the amplitude and duration of contractions was reduced in nutcracker oesophagus patients, there was no effect on contractions in healthy subjects. This demonstrates the potential discrepancies that can arise between patients and healthy individuals. The data presented here assessed manometric effects of the formulations in healthy volunteers with no history of gastrointestinal symptoms. However, the drugs may have different effects in patients, and so the formulations might be more or less effective in treating the disease state. Trials of these formulations in patient groups would be helpful to determine their therapeutic benefit. In conclusion, bioadhesive formulations of ISDN and SIL may potentially provide a topical medical therapy for oesophageal motor disorders.

Chapter 8: Conclusions

Chapter 8
Conclusions

The aim of this work was to develop a liquid oral drug delivery system to target the oesophagus for the purpose of treating oesophageal disorders. During this project, a wide range of formulations was tested for their bioadhesive potential in vitro and in vivo, and for their drug release properties in vitro. Some formulations such as the emulsions are novel in their application to oesophageal bioadhesion studies. The effect of formulations containing active ingredient on oesophageal motor function in healthy humans was also assessed.

Bioadhesion was measured *in vitro* by the duration of liquid retention on porcine oesophageal mucosa during continuous washing, using an adaptation of the method described by Batchelor et al (2002) (Chapter 4). Bioadhesion *in vivo* was measured using gamma scintigraphy in healthy volunteers (Chapter 6). Release of model drugs from the various formulations was tested *in vitro* using Franz-style diffusion cells. Candidate drugs for the delivery system included isosorbide dinitrate, nifedipine and sildenafil. The effects of promising formulations on healthy oesophageal motor function were assessed *in vivo* manometrically, to investigate the feasibility of the system for treating oesophageal motor disorders.

It was noted that drug incorporation into the vehicles can affect their physical and rheological properties, and affect the extent of retention (Chapter 4.4.6). For example, the 2% w/v Smart Hydrogel® solution showed reduced viscosity upon addition of drugs, although this did not detrimentally impact retention. The most robust vehicles, unaffected by drug incorporation, would be the best candidates for a drug delivery system.

In vitro retention profiles using *ex vivo* porcine tissue showed two distinct clearance phases over 30 minutes, likely related to excess free formulation (fast clearance rate) and adhered formulation (slower clearance rate). This biphasic clearance of liquid formulations from the oesophagus was seen in both the porcine model and during *in vivo* retention studies in humans. This phenomenon has been observed previously in the literature (Kockisch et al, 2004; Richardson et al, 2005). Inevitable variations in

animal tissue and human oesophagi would have affected the consistency of the data, and some retention profiles (e.g. the silicon-based emulsion) therefore were highly variable. Many candidate formulations were retained only briefly in the human oesophagus, although Smart Hydrogel® shows promise as a vehicle for targeted oesophageal drug delivery. The retention of Smart Hydrogel® *in vivo* far exceeded that reported and predicted by the *in vitro* study; this may be due to the shear forces encountered *in vivo*, which help the solution to form a gel more quickly (Section 3.3).

Noveon AA1 solutions at 0.1% w/v were not well retained in the porcine model, and this confirms some of the reports in the literature (Henriksen et al, 1996; Young et al, 2002; Vermani, 2002). The studies demonstrated that both the viscosity and retention of Noveon AA1 and Smart Hydrogel® solutions were pH-dependent. However, viscosity was not closely correlated with retention performance for the entire range of vehicles. For polymers, loss tangent was related to surface tension, and both these parameters correlated with retention *in vitro*. Conversely, emulsions showed no link between loss tangent and surface tensions, and neither parameter was related to retention.

The porcine oesophageal tissue varied between animals, as would be expected. However there were also intra-animal variations, with the distal oesophagus being thicker than the proximal oesophagus for example. It would be very interesting in further *in vitro* work to examine the differences between distal or proximal retention, by using different specific sections of the oesophagus. The different regions of the oesophagus were not differentiated in the current study. The in vitro retention studies might also be enhanced by examining the effect of different volumes of applied liquid, as this may help to find an 'optimal' dose volume.

In vitro tests using the model described may be valuable in screening to identify formulations with good retention in the oesophagus at late time points. These formulations which excel at later time points as well as in the early phase of the experiment will be the most valuable for the proposed drug delivery system. However, the *in vitro* model currently lacks peristaltic simulation which would improve the representation of the *in vivo* situation. Smart Hydrogel® would be an appealing candidate for a drug delivery system within the oesophagus, as the

temperature change during oral dosing, and the shear forces of peristalsis and swallowing, should help the Smart Hydrogel® to gel in situ, and have a prolonged residence time in the oesophagus. These properties may explain the discrepancy between retention values in the *in vitro* and *in vivo* model.

Drug release was measured using diffusion apparatus with the receiver at 37°C. These experiments were used to estimate how much drug might be available to the total oesophagus, and how quickly the drugs were released. Although the donor material is warmed from below by the receiver fluid, ideally the donor compartment would be controlled independently. The temperature of the donor is of particular relevance where Smart Hydrogel® is considered as the vehicle, as drug release might be affected by the temperature-induced thermogelling at 37°C. Further studies might include the development of a model which incorporates temperature control within the donor compartment. As discussed in Chapter 3, shear forces on Smart Hydrogel® cause bead-like gel formations to occur. It would be helpful to examine drug release from these as well as the un-gelled solution. The Franz cell experiments carried out during this project investigate release from the un-sheared form, and this could be different after the gel has been swallowed and subjected to shear forces. New experiments would need to be designed to investigate this.

Drug release observed during Franz cell studies was generally well fitted over the first hour to zero order kinetics, with no lag phase encountered by most formulations. Such linear release is ideal for the proposed drug delivery system, in which rapid drug release is required, without a delay, in order to maximise efficacy of the system. Permeation enhancers could be used in the formulation to increase efficacy by maximising permeation of released drug into oesophageal tissue (He et al, 2005). Drug release rates using Franz cells were used to estimate the total amount of drug that would be available to the oesophagus over a time period.

ISDN formulations at 5 mg or 25 mg in a 10 mL volume did not affect the amplitude or velocity of peristaltic waves in the oesophageal body, but the ISDN formulations did reduce basal lower oesophageal sphincter pressure, in a dose-dependent manner. Ingestion of the formulations containing SIL reduced both the amplitude of pressure waves in the oesophageal body, and basal LOS pressure, in a dose-dependent manner,

even after the low 10 mg dose, although the effects were more marked following the higher dose. This was an exciting result as there are no previous reports in the literature of sildenafil having an effect on oesophageal function as such a low dose. Sildenafil may therefore be useful in treating oesophageal motility disorders at lower doses than previously considered. Despite some minor side effects including headache, the formulations given during this study were well tolerated. Some of the effects on peristaltic waves and changes in manometry readings were extremely pronounced.

The decrease in wet swallow amplitude measured during the final swallow sequence for orally administered SIL was significant, and, importantly, was found to be an even more prominent effect than the decrease for gastrically-infused SIL, in all regions of the oesophageal body. This indicated that with an equivalent drug dose (25 mg SIL), the oral route, using Smart Hydrogel® as a bioadhesive carrier, has a greater effect on peristaltic pressure waves than the systemic route. This demonstrates that use of bioadhesive drug delivery vehicle is a valid and effective strategy for delivering SIL to the oesophagus. It was found that the oral bioadhesive formulations produced effects with a more delayed onset than the gastrically-infused formulations, indictaing that drug is absorbed more quickly through the gastric mucosa than the oesophageal wall.

Nitrates, calcium channel antagonists and sildenafil (Carapeti et al, 1999a; Carapeti et al, 1999b; Torrabadella et al, 2004) have been successfully employed on the anal sphincter as topical smooth muscle relaxants, but this project is novel in the application of this concept to the oesophagus. The proportion of observed effects that were mediated by a local as opposed to a systemic action is still uncertain, although the potency of even a low dose of SIL (10 mg) was remarkable as there have been no previous reports of SIL affecting oesophageal motor function at such a low dose. Oral liquid bioadhesive formulations may be useful in patients with oesophageal motility disorders who are unfit for pneumatic dilatation or surgery, and might even prove to be more effective in achalasia patients than in healthy subjects if there is a longer duration of oesophageal retention of the formulation, as predicted. When studying diltiazem and its effects on the oesophagus, Richter et al. (1984) found that although the amplitude and duration of contractions was reduced in nutcracker oesophagus

patients, there was no effect on contractions in healthy subjects. This demonstrates the potential discrepancies that can arise between patients and healthy individuals. This project assessed manometric effects of the formulations in healthy volunteers only, and these subjects were selected to have no history of gastrointestinal symptoms. It is important to remember that drugs may have different effects in patients, and the formulations might be more or less effective in treating the disease state. Trials of bioadhesive formulations in patient groups would be helpful to determine their therapeutic benefit.

Smart Hydrogel® was found to have thermogelling, shear gelling and bioadhesive properties which may make it an ideal candidate for a targeted drug delivery vehicle. Other novel formulations included o/w emulsions which were stable and well retained, and highly viscous polymer blends. The effects of sildenafil on wet swallow amplitude *in vivo* were enchanced by use of Smart Hydrogel® when compared to systemic administration. In conclusion, bioadhesive formulations containing sildenafil and isosorbide dinitrate may potentially provide a novel topical medical therapy for oesophageal motor disorders.

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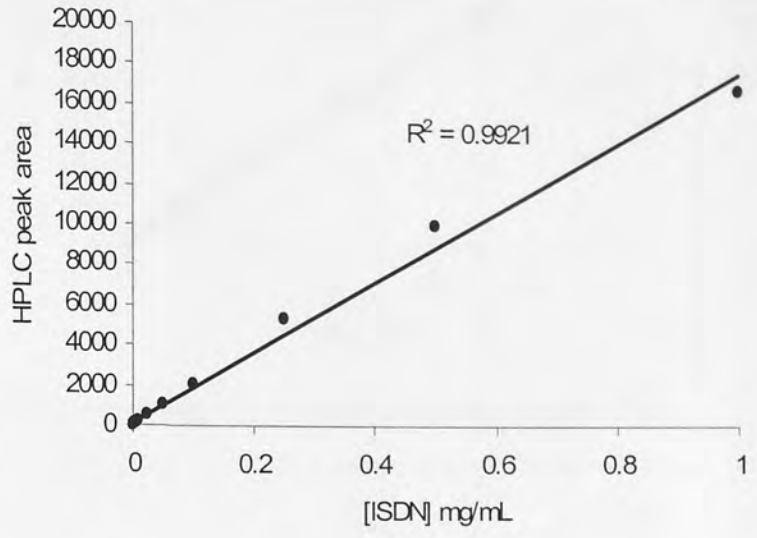
Appendix 2A - Examples of HPLC calibration curves for isosorbide dinitrate, nifedipine and sildenafil

a) Isosorbide dinitrate (performed 8 March 2004)

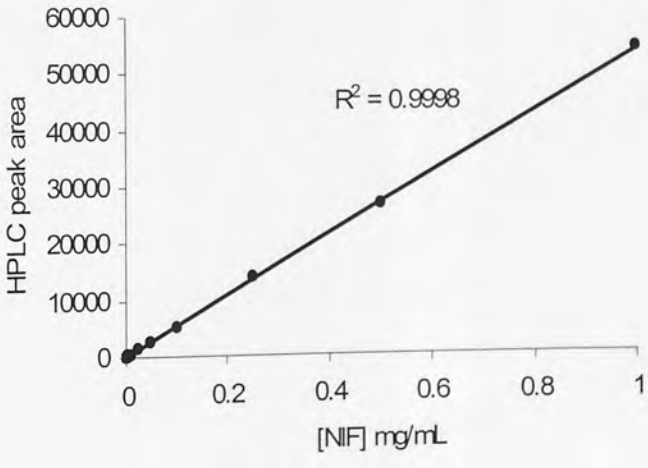
[ISDN]	HPLC peak area
1	17019.8
0.1	2081.07
0.01	209.974
0.001	21.8174
0.0001	2.74283
0.5	10102.6
0.05	1072.15
0.005	113.883
0.0005	12.8032
0.00005	2.19253
0.25	5311.89
0.025	537.633
0.0025	56.09
0.00025	6.1566
0.000025	1.22939



Mobile phase acetonitrile:water (60:40). Flow rate 1 mL/min. Injection volume 25µl. UV detection λ 215 nm.

b) Nifedipine (performed 15 May 2005)

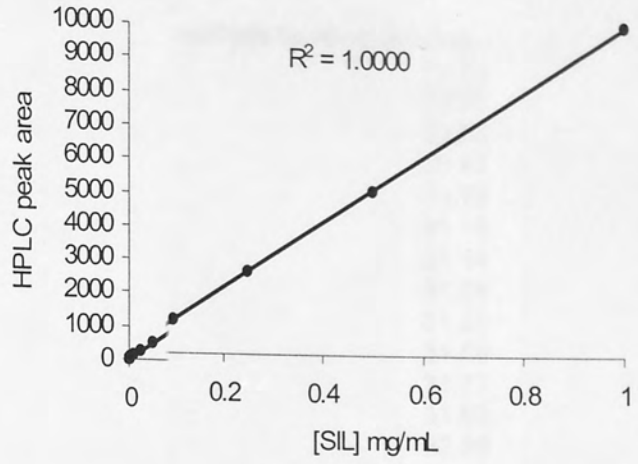
[NIF]	HPLC peak area
1	54448.8
0.1	5243.51
0.01	481.9
0.001	50.2014
0.0001	6.58928
0.5	26549.5
0.05	2450.43
0.005	230.089
0.0005	24.0348
0.25	13808.1
0.025	1326.63
0.0025	129.113
0.00025	13.801



Mobile phase methanol: ammonium acetate (0.04M): acetonitrile: triethylamine (400:400:200:8), Flow rate 1mL/min. Injection volume 25µl. UV detection λ 235nm.

c) Sildenafil (performed 10 May 2005)

[SIL]	HPLC peak area
1	9936.58
0.1	961.457
0.01	100.392
0.001	11.8885
0.0001	1.31172
0.5	4933.79
0.05	486.06
0.005	48.21
0.0005	5.2329
0.25	2460.59
0.025	246.266
0.0025	24.5829
0.00025	2.88222



Mobile phase acetonitrile:water (95:5). Flow rate 1.5 mL/min. Injection volume 10μL, UV detection λ 245 nm.

Appendix 3A - Measured surface tensions of formulations (mN/m)

Formulation	surface tension (mN/m)
LMO + ISDN 1.0 mg/mL	29.73
LMO 2000 rpm	29.96
LMO + ISDN 2.5 mg/mL	30.58
LMO+ NIF 2.5 mg/mL	30.65
HMO + NIF 1.0 mg/mL	30.79
LMO 200 rpm	31.10
HMO 2000 rpm	31.14
HMO 200 rpm	31.24
HMO	31.25
HMO + NIF 2.5 mg/mL	31.56
HMO + ISDN 2.5 mg/mL	31.77
LMO	31.83
HMO + ISDN 1.0 mg/mL	32.26
LMO + NIF 1.0 mg/mL	34.62
Poloxamer 407 20% w/v	37.86
Smart Hydrogel® 2% pH 4.7 SIL 2.5 mg/mL	41.82
Smart Hydrogel® 2% pH 4.7 SIL 1.0 mg/mL	43.30
Smart Hydrogel® 2% pH 4.7 ISDN 1.0 mg/mL	46.00
Alginate LF120M 2% w/v	55.02
Glycerol	61.83
Noveon AA1® 0.1% pH 4.7+ NIF 1.0	62.29
Antepsin®	63.15
Noveon AA1® 0.1% pH 6.0 + ISDN 2.5	64.74
Noveon AA1® 0.1% pH 4.7 + ISDN 2.5	69.66
Noveon AA1® 0.1% pH 6.0 + NIF 1.0	71.41
Noveon AA1® 0.1% pH 4.7 + ISDN 1.0	71.97
distilled water	72.28
Noveon AA1® 0.1% pH 4.7	72.82
Noveon AA1® 0.1% pH 4.7+ NIF 2.5	72.85
Noveon AA1® 0.1% pH 6.0 + ISDN 1.0	73.02
Noveon AA1® 0.1% pH 6.0 + NIF 2.5	73.56
Noveon AA1® 0.1% pH 6.0	73.62

Appendix 4A - Validation of use of Tc99m - Examples of homogeneity of radiolabel in formulations following 15 seconds vortexing.

LMO emulsion

Mass (g)	Counts (Cps)	cps/g	% of mean cps/g
0.2365	428813	1813163	99.8198999
0.234	430648	1840376	101.318055
0.2267	413201	1822678	100.343728
0.2357	428841	1819436	100.165247
0.1142	257616	1786519	98.3530702
	mean cps/g =	1816434	

Noveon AA1 0.1%w/v solution, pH 6.0

Mass (g)	Counts (Cps)	cps/g	% of mean cps/g
0.2363	708085	2996551	96.6021871
0.2454	778564	3172632	102.242175
0.2154	685351	3181760	102.536625
0.1883	578024	3069697	99.0217173
0.2383	733840	3079480	99.3172955
	mean cps/g =	3100024	

Appendix 4B - Table showing mean percent vehicle retained *in vitro* on porcine oesophageal tissue over time – Vehicles only (no drug)

Minutes	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	22	24	26	28	30	no. of repeats
HMO emulsion																					
mean	5.84	3.91	2.96	2.52	2.16	1.89	1.72	1.55	1.37	1.26	1.18	1.03	0.94	0.84	0.82	0.72	0.67	0.71	0.62	0.64	6
st dev	2.80	1.79	1.30	1.07	0.83	0.66	0.57	0.45	0.41	0.38	0.36	0.35	0.33	0.26	0.32	0.22	0.22	0.32	0.19	0.31	
LMO emulsion																					
mean	9.47	7.78	7.06	5.21	4.65	4.23	3.94	3.75	3.56	3.38	1.49	1.36	2.84	1.18	2.55	1.05	1.00	2.31	0.88	2.11	7
st dev	7.08	6.65	6.43	6.12	5.51	4.94	4.66	4.52	4.38	4.17	0.90	0.81	3.65	0.69	3.28	0.61	0.59	3.03	0.52	2.76	
SBE emulsion																					
mean	22.09	14.79	12.52	10.87	9.14	7.63	6.80	6.28	5.87	5.47	4.82	4.00	3.48	3.10	2.81	2.56	2.39	2.23	2.09	2.13	9
st dev	21.13	13.70	12.57	11.54	9.36	7.41	6.57	6.00	5.59	5.16	4.48	3.83	3.39	3.04	2.74	2.50	2.34	2.19	2.02	1.93	
Smart Hydrogel 2% w/v pH 4.7																					
mean	15.00	6.69	4.31	3.37	2.59	2.25	1.86	1.70	1.51	1.37	1.17	1.07	0.97	0.90	0.83	0.78	0.73	0.71	0.67	0.55	4
st dev	7.58	2.54	0.89	0.67	0.78	0.72	0.80	0.77	0.76	0.75	0.66	0.59	0.54	0.49	0.48	0.46	0.45	0.44	0.43	0.54	
Smart Hydrogel 2% w/v pH 6.0																					
mean	17.52	11.37	8.94	7.52	6.85	6.16	5.70	5.11	4.77	4.35	3.62	3.25	2.70	2.36	2.12	1.93	1.76	1.62	1.49	1.38	3
st dev	6.75	3.73	2.72	1.68	1.57	1.37	1.35	1.23	1.21	1.18	1.35	1.45	1.43	1.41	1.36	1.29	1.19	1.09	1.03	0.97	
Blend A																					
mean	47.96	26.06	15.38	10.70	8.57	6.76	5.83	5.11	4.39	4.04	3.40	3.14	2.94	2.81	2.64	2.72	2.64	2.58	2.52	2.47	9
st dev	17.56	10.69	8.55	6.02	5.06	4.11	3.57	3.17	2.81	2.60	2.31	2.23	2.15	2.10	2.01	1.69	1.65	1.64	1.62	1.60	
Antespin ®																					
mean	20.30	13.17	9.70	7.39	5.34	4.28	3.62	3.05	2.69	2.32	1.91	1.65	1.46	1.31	1.16	1.00	0.89	0.77	0.70	0.64	5
st dev	9.32	6.76	5.16	3.89	2.82	2.28	2.02	1.83	1.67	1.46	1.24	1.04	0.87	0.79	0.67	0.49	0.42	0.32	0.29	0.28	
Noveon AA1 0.1% w/v pH 7.0																					
mean	2.66	1.56	1.20	1.04	0.93	0.86	0.81	0.77	0.73	0.70			0.58		0.46			0.35		0.24	3
st dev	0.43	0.51	0.45	0.44	0.39	0.37	0.36	0.34	0.33	0.32			0.29		0.25			0.22		0.17	
Noveon AA1 0.1% w/v pH 6.0																					
mean	2.55	1.70	1.08	0.86	0.73	0.59	0.54	0.51	0.46	0.43	0.39	0.37	0.33	0.31	0.29	0.27	0.26	0.25	0.23	0.22	3
st dev	1.30	0.92	0.61	0.48	0.39	0.33	0.28	0.25	0.25	0.23	0.21	0.19	0.16	0.13	0.12	0.12	0.11	0.11	0.10	0.10	
Noveon AA1 0.1% w/v pH 4.7																					
mean	2.37	1.37	1.06	0.95	0.88	0.82	0.75	0.70	0.67	0.64	0.59	0.56	0.50	0.48	0.46	0.44	0.42	0.41	0.39	0.38	3
st dev	0.38	0.11	0.13	0.16	0.15	0.15	0.18	0.19	0.19	0.20	0.19	0.18	0.16	0.14	0.13	0.13	0.13	0.12	0.12	0.11	
Blend B																					
mean	57.31	28.51	16.05	10.62	7.90	6.12	5.18	4.46	3.89	3.59	3.21	2.96	2.77	2.45	2.29	2.21	2.10	2.03	1.98	1.81	5
st dev	8.48	5.72	4.09	3.19	2.58	2.33	2.27	2.06	2.02	1.92	1.77	1.61	1.47	1.16	1.02	0.96	0.90	0.85	0.80	0.65	

Minutes	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	22	24	26	28	30	no. of repeats
Alginate LF 120M 2% w/v																					
mean	10.71	4.38	2.68	2.02	1.68	1.49	1.37	1.30	1.25	1.22	1.18	1.14	1.10	1.09	1.07	1.05	1.03	1.02	1.01	1.00	3
st dev	0.86	0.54	1.01	1.04	0.99	0.94	0.88	0.86	0.87	0.86	0.86	0.84	0.82	0.80	0.80	0.79	0.79	0.79	0.79	0.79	
Distilled water																					
mean	3.94	2.56	1.94	1.67	1.50	1.38	1.29	1.21	1.14	1.07	0.99	0.94	0.90	0.85	0.81	0.78	0.75	0.73	0.70	0.67	5
st dev	2.44	1.58	1.35	1.30	1.26	1.21	1.15	1.08	1.02	0.95	0.89	0.84	0.91	0.75	0.73	0.70	0.69	0.67	0.66	0.64	
LMO prepared using Silverson mixer - low viscosity and stability																					
mean	3.30	2.08	1.60	1.37	1.22	1.11	1.04	1.01	0.96	0.90	0.83	0.78	0.74	0.70	0.66	0.63	0.60	0.57	0.54	0.52	2
st dev	2.08	1.47	1.04	0.86	0.75	0.68	0.64	0.65	0.62	0.59	0.57	0.55	0.52	0.50	0.49	0.48	0.48	0.46	0.45	0.44	
HMO prepared using Silverson mixer - low viscosity and stability																					
mean	2.99	1.91	1.45	1.14	0.96	0.84	0.75	0.68	0.63	0.53	0.44	0.41	0.38	0.35	0.33	0.31	0.30	0.28	0.27	0.26	2
st dev	1.90	1.01	0.73	0.57	0.47	0.41	0.38	0.37	0.35	0.25	0.20	0.18	0.17	0.16	0.15	0.14	0.13	0.13	0.13	0.12	
Poloxamer 407 20% w/v																					
mean	10.39	6.11	4.50	3.79	3.11	2.76	2.51	2.31	2.12	1.98	1.72	1.62	1.52	1.39	1.30	1.23	1.14	1.09	0.97	0.85	3
st dev	6.73	5.03	3.60	3.06	2.28	1.96	1.78	1.65	1.54	1.44	1.16	1.10	1.07	0.93	0.85	0.81	0.75	0.70	0.57	0.44	
Glycerol																					
mean	2.75	1.69	1.15	0.91	0.81	0.76	0.73	0.70	0.67	0.63	0.61	0.58	0.56	0.55	0.53	0.51	0.50	0.49	0.48	0.46	3
st dev	1.93	0.87	0.29	0.28	0.34	0.37	0.38	0.38	0.39	0.40	0.41	0.41	0.41	0.42	0.42	0.42	0.42	0.43	0.43	0.43	
Smart Hydrogel 2% w/v pH 3.6																					
mean	12.12	7.92	6.73	5.47	4.52	4.13	3.79	3.36	2.93	2.69	2.39	2.23	2.03	1.84	1.70	1.59	1.44	1.30	1.24	1.14	3
st dev	8.94	4.76	4.34	3.20	2.39	2.12	1.91	1.47	1.05	0.89	0.74	0.71	0.57	0.38	0.28	0.21	0.14	0.05	0.05	-	
HMO emulsion homogenisation speed @ 200 rpm																					
mean	3.94	2.17	1.56	1.21	1.01	0.89	0.81	0.75	0.70	0.65	0.59	0.49	0.43	0.39	0.37	0.36	0.34	0.33	0.32	0.31	3
st dev	1.74	0.85	0.76	0.68	0.67	0.62	0.61	0.59	0.57	0.56	0.53	0.42	0.34	0.31	0.30	0.29	0.29	0.28	0.27	0.27	
HMO emulsion homogenisation speed @ 800 rpm																					
mean	7.05	4.81	3.66	2.79	2.48	2.24	2.00	1.83	1.63	1.52	1.38	1.29	1.22	1.16	1.09	1.03	0.98	0.94	0.91	0.88	3
st dev	0.37	0.61	0.93	0.77	0.90	0.95	1.05	1.03	0.89	0.89	0.87	0.86	0.86	0.84	0.81	0.77	0.73	0.71	0.69	0.67	
HMO emulsion homogenisation speed @ 2000 rpm																					
mean	4.96	3.02	2.07	1.68	1.41	1.26	0.98	0.87	0.78	0.67	0.57	0.50	0.40	0.35	0.32	0.30	0.28	0.26	0.20	0.18	3
st dev	0.19	0.22	0.38	0.46	0.47	0.47	0.37	0.30	0.27	0.21	0.17	0.14	0.07	0.04	0.02	0.01	0.01	0.02	0.06	0.07	
LMO emulsion homogenisation speed @ 800 rpm																					
mean	5.62	3.43	2.45	1.95	1.61	1.44	1.30	1.20	1.04	0.96	0.84	0.76	0.69	0.62	0.57	0.53	0.50	0.46	0.43	0.41	3
st dev	1.34	1.05	0.97	0.80	0.63	0.59	0.56	0.53	0.45	0.41	0.36	0.33	0.31	0.27	0.24	0.22	0.19	0.19	0.18	0.17	
LMO emulsion homogenisation speed @ 200 rpm																					
mean	2.91	2.00	1.64	1.48	1.25	1.05	0.91	0.84	0.80	0.76	0.70	0.66	0.62	0.59	0.55	0.53	0.51	0.49	0.43	0.39	3
st dev	1.39	1.08	0.87	0.77	0.55	0.40	0.48	0.48	0.48	0.47	0.45	0.43	0.42	0.41	0.38	0.37	0.36	0.35	0.34	0.34	
LMO emulsion homogenisation speed @ 2000 rpm																					
mean	5.83	4.10	3.34	2.88	2.70	2.36	2.19	2.09	2.01	1.78	1.64	1.53	1.45	1.40	1.36	1.33	1.31	1.28	1.27	1.24	3
st dev	1.20	1.25	1.10	0.88	0.85	0.43	0.27	0.24	0.23	0.37	0.31	0.26	0.24	0.23	0.23	0.23	0.24	0.24	0.24	0.23	

Appendix 4C - Table showing mean percent formulation retained *in vitro* on porcine oesophageal tissue over time – Drug-loaded vehicles only

Minutes	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	22	24	26	28	30	no. of repeats
NIF (2.5 mg/mL) in HMO emulsion																					
mean	4.34	2.36	1.53	1.15	0.91	0.76	0.66	0.57	0.50	0.45	0.39	0.35	0.32	0.28	0.25	0.23	0.21	0.20	0.19	0.17	3
st dev	0.87	0.42	0.40	0.42	0.38	0.31	0.29	0.22	0.17	0.15	0.13	0.11	0.09	0.06	0.05	0.05	0.04	0.03	0.02	0.02	
NIF (1.0 mg/mL) in HMO emulsion																					
mean	3.28	2.10	1.53	1.19	0.97	0.82	0.72	0.64	0.59	0.52	0.45	0.40	0.37	0.33	0.30	0.29	0.27	0.26	0.25	0.24	3
st dev	0.53	0.64	0.54	0.45	0.36	0.32	0.29	0.26	0.25	0.23	0.19	0.15	0.13	0.10	0.08	0.07	0.07	0.07	0.07	0.07	
NIF (2.5 mg/mL) in LMO emulsion																					
mean	10.66	1.93	1.37	1.14	0.99	0.86	0.79	0.73	0.67	0.62	0.55	0.51	0.48	0.45	0.42	0.40	0.38	0.37	0.35	0.34	3
st dev	14.06	1.01	0.66	0.50	0.42	0.39	0.35	0.32	0.29	0.27	0.25	0.24	0.23	0.23	0.22	0.22	0.21	0.20	0.20	0.19	
NIF (1.0 mg/mL) in LMO emulsion																					
mean	6.62	4.32	3.85	3.51	3.35	2.30	2.10	1.66	1.40	1.29	1.15	1.05	0.97	0.91	0.87	0.83	0.79	0.77	0.74	0.72	3
st dev	1.29	1.45	1.53	1.40	1.40	0.75	0.71	0.14	0.18	0.25	0.32	0.34	0.33	0.34	0.33	0.33	0.33	0.33	0.32	0.32	
NIF (2.5 mg/mL) in Noveon AA1 (0.1% w/v) pH 4.7																					
mean	4.29	2.55	1.89	1.64	1.45	1.30	1.18	1.12	0.95	0.87	0.79	0.72	0.68	0.64	0.61	0.58	0.56	0.54	0.52	0.51	3
st dev	0.55	0.10	0.23	0.21	0.22	0.24	0.24	0.21	0.27	0.27	0.25	0.23	0.22	0.21	0.20	0.19	0.19	0.19	0.19	0.18	
NIF (1.0 mg/mL) in Noveon AA1 (0.1% w/v) pH 4.7																					
mean	4.19	2.57	1.79	1.52	1.35	1.23	1.16	1.09	1.05	1.01	0.95	0.91	0.88	0.85	0.83	0.81	0.79	0.77	0.76	0.75	3
st dev	2.08	1.34	0.80	0.69	0.64	0.59	0.57	0.56	0.56	0.55	0.55	0.54	0.54	0.54	0.53	0.53	0.53	0.53	0.53	0.53	
NIF (2.5 mg/mL) in Noveon AA1 (0.1% w/v) pH 6.0																					
mean	4.06	2.68	2.14	1.95	1.84	1.76	1.50	0.98	0.80	0.73	0.61	0.56	0.51	0.48	0.44	0.42	0.40	0.38	0.36	0.35	3
st dev	3.93	2.83	2.33	2.21	2.12	2.05	1.71	0.88	0.63	0.54	0.39	0.34	0.30	0.27	0.24	0.22	0.21	0.20	0.20	0.19	
NIF (1.0 mg/mL) in Noveon AA1 (0.1% w/v) pH 6.0																					
mean	1.32	0.84	0.69	0.61	0.57	0.52	0.50	0.47	0.45	0.44	0.42	0.40	0.38	0.37	0.36	0.34	0.33	0.32	0.30	0.28	3
st dev	0.54	0.22	0.13	0.11	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.09	0.08	0.08	0.08	0.08	0.07	0.06	
ISDN (2.5 mg/mL) in HMO emulsion																					
mean	4.34	2.84	2.07	1.69	1.43	1.24	1.12	1.03	0.96	0.90	0.82	0.76	0.73	0.70	0.67	0.65	0.62	0.59	0.56	0.54	3
st dev	2.04	1.41	0.96	0.74	0.62	0.54	0.50	0.47	0.45	0.42	0.37	0.35	0.34	0.33	0.32	0.31	0.29	0.25	0.23	0.21	
ISDN (1.0 mg/mL) in HMO emulsion																					
mean	3.78	2.12	1.47	1.24	1.04	0.91	0.80	0.74	0.66	0.59	0.52	0.47	0.44	0.41	0.38	0.37	0.35	0.33	0.31	0.30	3
st dev	1.75	0.93	0.57	0.52	0.42	0.36	0.28	0.24	0.20	0.17	0.13	0.11	0.10	0.09	0.09	0.08	0.08	0.08	0.08	0.08	
ISDN (2.5 mg/mL) in LMO emulsion																					
mean	2.96	2.09	1.71	1.44	1.13	1.04	0.96	0.91	0.85	0.80	0.74	0.68	0.62	0.57	0.54	0.50	0.48	0.46	0.44	0.43	3
st dev	1.21	1.03	0.93	0.77	0.55	0.53	0.51	0.48	0.46	0.43	0.41	0.38	0.35	0.33	0.32	0.31	0.31	0.31	0.30	0.30	

Minutes	1	2	3	4	5	6	7	8	9	10	12	14	16	18	20	22	24	26	28	30	no. of repeats
ISDN (1.0 mg/mL) in LMO emulsion																					
mea n	3.09	1.78	1.30	1.06	0.92	0.81	0.75	0.68	0.63	0.59	0.53	0.49	0.45	0.42	0.40	0.38	0.36	0.35	0.33	0.32	3
st dev	1.42	0.82	0.57	0.45	0.38	0.32	0.29	0.25	0.22	0.20	0.17	0.15	0.13	0.12	0.11	0.11	0.10	0.09	0.09	0.08	
ISDN (2.5 mg/mL) in Noveon AA1 (0.1% w/v) pH 4.7																					
mean	6.30	5.10	4.32	3.86	3.58	3.34	3.13	3.00	2.80	2.67	2.46	2.25	2.10	1.96	1.85	1.75	1.67	1.60	1.53	1.44	3
st dev	7.11	5.96	4.98	4.46	4.16	3.90	3.66	3.53	3.26	3.13	2.85	2.57	2.38	2.21	2.06	1.93	1.82	1.73	1.66	1.56	
ISDN (1.0 mg/mL) in Noveon AA1 (0.1% w/v) pH 4.7																					
mean	2.05	1.05	0.78	0.63	0.52	0.45	0.40	0.37	0.34	0.32	0.30	0.27	0.25	0.23	0.22	0.21	0.19	0.18	0.18	0.17	3
st dev	0.58	0.30	0.27	0.25	0.22	0.21	0.19	0.18	0.18	0.17	0.16	0.15	0.15	0.14	0.14	0.13	0.12	0.12	0.12	0.11	
ISDN (2.5 MG/ML) in Noveon AA1 (0.1% w/v) pH 6.0																					
mean	2.31	1.27	0.98	0.83	0.74	0.67	0.63	0.59	0.56	0.54	0.51	0.48	0.46	0.44	0.42	0.41	0.39	0.38	0.37	0.36	3
st dev	0.92	0.31	0.20	0.15	0.15	0.16	0.15	0.16	0.16	0.16	0.16	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.15	0.15	
ISDN (1.0 mg/mL) in Noveon AA1 (0.1% w/v) pH 6.0																					
mean	4.73	2.62	1.96	1.53	1.27	1.10	0.84	0.76	0.70	0.66	0.60	0.56	0.53	0.50	0.48	0.45	0.43	0.41	0.40	0.39	3
st dev	3.08	1.25	0.87	0.62	0.50	0.42	0.37	0.33	0.30	0.28	0.23	0.21	0.19	0.17	0.16	0.12	0.11	0.10	0.10	0.10	
ISDN (2.5 mg/mL) in Smart Hydrogel® 2% w/v pH 4.7																					
mean	33.37	19.88	11.84	9.49	8.11	7.07	6.35	5.85	5.52	5.11	4.63	4.30	3.98	3.73	3.87	3.35	3.47	3.05	2.91	2.76	4
st dev	13.27	10.64	6.93	5.06	4.04	3.62	3.33	3.22	3.14	2.86	2.69	2.60	2.42	2.25	2.59	2.09	2.28	1.81	1.73	1.62	
ISDN (0.5 mg/mL) in Smart Hydrogel® 2% w/v pH 4.7																					
mean	17.87	13.41	12.18	10.87	9.35	8.25	7.59	6.87	7.53	5.80	4.43	4.95	4.62	4.33	4.09	3.88	3.71	3.58	3.42	3.70	3
st dev	4.20	3.22	3.00	2.28	2.94	3.21	2.92	2.43	0.74	1.87	1.43	1.77	1.62	1.45	1.34	1.26	1.20	1.15	1.10	0.94	
SIL (2.5 mg/mL) in Smart Hydrogel® 2% w/v pH 4.7																					
mean	10.51	7.24	5.73	4.41	3.80	3.42	2.97	3.35	2.59	2.43	2.10	1.93	1.73	1.56	1.43	1.31	1.23	1.14	1.08	1.00	4
st dev	1.73	1.50	1.41	1.95	1.87	1.76	1.58	1.11	1.41	1.32	1.11	1.07	0.99	0.91	0.85	0.79	0.76	0.73	0.71	0.83	
SIL (1.0 mg/mL) in Smart Hydrogel® 2% w/v pH 4.7																					
mean	15.46	9.93	8.16	6.87	6.21	5.44	5.03	4.69	4.40	4.14	3.75	3.45	3.13	2.96	2.78	2.66	2.55	2.45	2.37	2.97	3
st dev	4.12	3.05	2.82	2.43	2.55	2.45	2.29	2.28	2.24	2.24	2.25	2.23	2.27	2.25	2.21	2.18	2.15	2.13	2.11	2.42	
SIL (2.5 mg/mL) in Smart Hydrogel® 2% w/v pH 6.0																					
mean	95.37	86.85	76.35	53.07	31.95	30.74	29.42	28.48	27.53	26.85	25.65	24.70	23.94	23.44	23.04	22.62	22.26	21.94	21.63	21.21	3
st dev	2.55	6.50	4.51	28.90	33.03	32.93	32.17	31.77	31.51	31.29	30.95	30.57	30.03	29.67	29.38	28.98	28.60	28.27	27.91	27.37	
ISDN (2.5 mg/mL) in Smart Hydrogel® 2% w/v pH 6.0																					
mean	77.68	72.04	65.50	61.90	59.72	57.80	56.17	54.35	52.84	50.78	46.83	43.73	40.33	36.87	34.03	31.27	27.86	24.93	22.37	20.44	3
st dev	33.67	36.11	42.59	42.12	41.55	40.64	39.94	38.79	37.72	36.29	33.77	31.81	29.73	27.88	26.41	24.44	22.12	19.88	17.55	15.82	
SIL (2.5 mg/mL) in Smart Hydrogel® 2% w/v pH 4.7																					
mean	89.26	80.45	74.70	70.11	66.99	64.48	47.31	43.72	41.86	40.22	37.33	34.73	32.15	25.63	24.27	22.56	21.27	19.62	18.33	17.25	3
st dev	10.61	16.91	18.78	18.31	18.03	17.91	23.44	24.88	23.68	22.52	20.65	18.58	16.46	9.34	8.65	7.97	7.61	7.62	7.93	8.20	
NIF (2.5 mg/mL) in Smart Hydrogel® 2% w/v pH 6.0																					
mean	79.52	58.47	55.57	31.85	30.30	29.30	28.57	27.40	26.13	25.15	23.31	22.07	20.42	19.30	18.23	16.82	15.92	15.17	14.24	13.69	3
st dev	20.39	27.31	26.63	11.70	12.14	12.12	12.20	12.46	11.86	11.41	10.41	10.27	9.76	9.43	9.67	10.42	10.59	10.43	10.05	9.97	

HMO homogenisation speed @ 2000 rpm																							
mean %	4.96	3.02	2.07	1.68	1.41	1.26	0.98	0.87	0.78	0.67	0.57	0.50	0.40	0.35	0.32	0.30	0.28	0.26	0.20	0.18			
st dev %	0.19	0.22	0.38	0.46	0.47	0.47	0.37	0.30	0.27	0.21	0.17	0.14	0.07	0.04	0.02	0.01	0.01	0.02	0.06	0.07			
mean AUC		3.99	2.54	1.88	1.55	1.34	1.12	0.93	0.82	0.72	1.24	1.07	0.90	0.75	0.67	0.62	0.57	0.53	0.46	0.38	22.08	14.89	7.92
st dev AUC		0.20	0.30	0.42	0.47	0.47	0.42	0.33	0.28	0.24	0.38	0.31	0.21	0.11	0.06	0.03	0.02	0.02	0.07	0.12	4.48	3.14	1.58
LMO homogenisation speed @ 800 rpm																							
mean %	5.62	3.43	2.45	1.95	1.61	1.44	1.30	1.20	1.04	0.96	0.84	0.76	0.69	0.62	0.57	0.53	0.50	0.46	0.43	0.41			
st dev %	1.3417	1.0517	0.9747	0.7973	0.633	0.587	0.561	0.531	0.445	0.409	0.355	0.333	0.309	0.275	0.24	0.216	0.193	0.188	0.181	0.175			
mean AUC		4.52	2.94	2.20	1.78	1.53	1.37	1.25	1.12	1.00	1.79	1.59	1.44	1.31	1.19	1.10	1.03	0.96	0.89	0.85	29.86	17.71	13.16
st dev AUC		1.20	1.01	0.89	0.71	0.61	0.57	0.55	0.49	0.43	0.76	0.69	0.64	0.58	0.51	0.46	0.41	0.38	0.37	0.36	11.62	6.46	5.59
LMO homogenisation speed @ 200 rpm																							
mean %	2.91	2.00	1.64	1.48	1.25	1.05	0.91	0.84	0.80	0.76	0.70	0.66	0.62	0.59	0.55	0.53	0.51	0.49	0.43	0.39			
st dev %	1.3878	1.0811	0.8687	0.7739	0.55	0.402	0.479	0.481	0.479	0.471	0.448	0.435	0.42	0.407	0.385	0.372	0.357	0.347	0.341	0.34			
mean AUC		2.45	1.82	1.56	1.37	1.15	0.98	0.88	0.82	0.78	1.46	1.36	1.28	1.21	1.14	1.08	1.04	1.00	0.92	0.82	23.12	11.81	12.09
st dev AUC		1.23	0.97	0.82	0.66	0.48	0.44	0.48	0.48	0.47	0.92	0.88	0.86	0.83	0.79	0.76	0.73	0.70	0.69	0.68	13.88	6.04	8.31
LMO homogenisation speed @ 2000 rpm																							
mean %	5.83	4.10	3.34	2.88	2.70	2.36	2.19	2.09	2.01	1.78	1.64	1.53	1.45	1.40	1.36	1.33	1.31	1.28	1.27	1.24			
st dev %	1.2002	1.249	1.0992	0.881	0.851	0.435	0.275	0.241	0.232	0.366	0.311	0.259	0.242	0.23	0.235	0.234	0.239	0.237	0.235	0.229			
mean AUC		4.97	3.72	3.11	2.79	2.53	2.27	2.14	2.05	1.89	3.42	3.17	2.98	2.85	2.76	2.70	2.64	2.59	2.55	2.51	53.65	25.48	30.07
st dev AUC		1.22	1.17	0.99	0.87	0.64	0.35	0.26	0.24	0.30	0.68	0.57	0.50	0.47	0.46	0.47	0.47	0.48	0.47	0.46	11.09	6.05	5.34

Appendix 4E - Use of sodium fluorescein as a marker for bioadhesive studies

At the start of the project, liquid vehicles were mixed thoroughly with a small volume (10 μL in 1 mL) of 0.01% sodium fluorescein solution. The bioadhesive experiments were carried out using the same apparatus as described in Chapter 4, however the eluate from the washings at different time periods was analysed spectrophotometrically. The amount of fluorescein in the eluate was proportional to the amount of liquid formulation washed off the oesophageal tissue.

This method of labelling was shortly abandoned as it was deemed inappropriate for this particular study. When opaque liquids were investigated, the opacicity of the eluate affected the UV readings, and unusually high measurements were recorded. Additionally, as the surface used was animal tissue, some particulate cellular matter was washed off the tissue during the experiment and this also affected the readings. A decision was made that the data obtained using a fluorescent label gave incorrect readings as an artefact of the method.

The Tc99m labelling method was developed to solve some of these problems.

Appendix 5A - Tables showing cumulative drug release per unit area over time from different formulations (Franz cell studies)

Mean µg drug diffused per unit area over time (µg cm^{-2} over time)

			Time (min)	5	10	15	20	25	30	45	60
drug	[drug]	Formulation	pH								
DEXT	2.5 mg/mL	smart hydrogel 2%	pH 4.7	0.51	0.62	0.82	0.97	1.29	1.49	2.03	3.00
DEXT	2.5 mg/mL	smart hydrogel 2%	pH 6.0	0.71	0.80	1.06	1.13	1.47	1.55	2.47	3.21
DEXT	2.5 mg/mL	LMO emulsion	--	0.27	0.38	0.51	0.67	0.92	1.09	1.15	2.24
DEXT	2.5 mg/mL	HMO emulsion	--	0.33	0.38	0.56	0.79	0.96	1.38	2.06	2.85
DEXT	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.61	0.51	0.52	0.64	0.74	0.87	1.27	1.74
DEXT	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.77	0.60	0.66	0.73	0.90	0.98	1.29	1.58
DEXT	2.5 mg/mL	ACN:H2O	--	1.01	0.95	1.03	1.09	1.21	1.39	1.88	2.33
DEXT	2.5 mg/mL	H2O	--	0.82	0.86	0.89	0.88	0.92	0.98	1.26	1.35
MANN	2.5 mg/mL	smart hydrogel 2%	pH 4.7	2.02	3.06	4.83	6.27	6.92	7.98	11.12	13.16
MANN	2.5 mg/mL	smart hydrogel 2%	pH 6.0	2.12	3.53	4.86	6.05	6.04	7.03	10.65	12.95
MANN	2.5 mg/mL	LMO emulsion	--	1.82	3.01	4.17	5.38	6.82	8.17	11.81	15.48
MANN	2.5 mg/mL	HMO emulsion	--	2.38	3.77	4.84	6.85	8.26	9.69	13.68	18.18
MANN	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	1.79	2.77	3.63	4.43	5.67	6.32	11.31	10.84
MANN	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	1.74	2.28	3.31	4.28	5.35	4.37	9.82	10.29
MANN	2.5 mg/mL	ACN:H2O	--	2.35	4.07	5.75	7.67	8.72	9.87	13.22	14.41
MANN	2.5 mg/mL	H2O	--	1.78	3.68	4.95	6.80	7.61	10.05	14.65	16.74
PROP	2.5 mg/mL	smart hydrogel 2%	pH 4.7	3.06	4.48	5.96	8.23	8.94	10.40	13.22	16.28
PROP	2.5 mg/mL	smart hydrogel 2%	pH 6.0	1.42	1.86	2.28	2.96	3.16	3.96	4.45	5.70
PROP	2.5 mg/mL	LMO emulsion	--	2.14	3.33	4.63	5.97	7.57	8.73	12.96	14.64
PROP	2.5 mg/mL	HMO emulsion	--	2.36	3.28	3.21	5.82	6.45	7.91	10.43	12.89
PROP	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	3.86	7.06	10.34	13.63	15.75	18.33	24.65	27.80
PROP	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	3.99	6.50	9.99	12.74	16.15	17.47	20.80	24.40
PROP	2.5 mg/mL	ACN:H2O	--	4.95	5.28	6.87	6.64	7.39	8.09	9.30	10.79
PROP	2.5 mg/mL	H2O	--	14.49	16.64	19.07	21.56	23.51	26.21	29.63	33.47
ISDN	1.0 mg/mL	smart hydrogel 2%	pH 3.6	0.85	1.37	1.85	2.29	2.58	2.92	4.04	5.03
ISDN	1.0 mg/mL	smart hydrogel 2%	pH 4.7	0.70	1.08	1.43	1.75	1.97	2.28	2.98	3.94
ISDN	1.0 mg/mL	smart hydrogel 2%	pH 6.0	0.55	0.98	1.22	1.51	1.62	1.94	2.72	3.19
ISDN	1.0 mg/mL	LMO	--	1.04	1.40	1.65	2.16	2.60	3.08	4.56	5.87
ISDN	1.0 mg/mL	HMO	--	0.50	0.82	1.22	1.45	1.71	2.09	2.98	3.91
ISDN	1.0 mg/mL	Noveon AA-1 0.1%	pH 4.7	1.07	1.84	2.50	3.18	3.71	4.13	5.37	6.60
ISDN	1.0 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.77	1.51	1.80	2.27	2.67	2.96	4.06	5.07
ISDN	1.0 mg/mL	ACN:H2O	--	0.08	0.15	0.21	0.27	0.34	0.39	0.53	0.65
ISDN	1.0 mg/mL	H2O	--	0.43	0.68	0.96	1.19	1.38	1.53	1.91	2.10
ISDN	2.5 mg/mL	smart hydrogel 2%	pH 3.6	1.67	3.53	5.00	6.62	7.70	8.76	12.11	14.25
ISDN	2.5 mg/mL	smart hydrogel 2%	pH 4.7	1.54	2.66	3.35	4.15	4.67	5.22	6.77	8.22
ISDN	2.5 mg/mL	smart hydrogel 2%	pH 6.0	1.06	1.88	2.50	3.16	3.62	4.02	5.37	6.71
ISDN	2.5 mg/mL	LMO	--	1.96	2.53	2.60	3.40	4.13	4.88	7.30	9.22
ISDN	2.5 mg/mL	HMO	--	0.79	1.37	1.90	2.39	2.82	3.47	5.10	6.88
ISDN	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	1.35	2.91	4.28	5.35	6.29	6.79	8.70	10.87
ISDN	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	1.45	1.96	2.65	3.36	4.01	4.44	5.02	7.66
ISDN	2.5 mg/mL	ACN:H2O	--	0.46	0.89	1.28	1.70	2.10	2.52	3.84	5.45
ISDN	2.5 mg/mL	H2O	--	2.43	4.31	5.76	7.06	8.25	9.18	11.87	13.40
NIF	1.0 mg/ml	smart hydrogel 2%	pH 3.6	0.06	0.11	0.18	0.31	0.41	0.55	0.96	1.71
NIF	1.0 mg/mL	smart hydrogel 2%	pH 4.7	0.14	0.15	0.18	0.31	0.41	0.53	0.85	1.13
NIF	1.0 mg/mL	smart hydrogel 2%	pH 6.0	0.08	0.13	0.24	0.30	0.35	0.47	0.57	0.74
NIF	1.0 mg/mL	LMO	--	0.04	0.05	0.08	0.10	0.12	0.17	0.23	0.35
NIF	1.0 mg/mL	HMO	--	0.05	0.08	0.11	0.13	0.15	0.18	0.28	0.33
NIF	1.0 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.02	0.05	0.10	0.21	0.23	0.37	0.83	1.38

NIF	1.0 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.01	0.02	0.05	0.09	0.09	0.17	0.37	0.64
NIF	2.5 mg/mL	smart hydrogel 2%	pH 3.6	0.09	0.21	0.40	0.50	0.71	0.85	1.32	1.96
NIF	2.5 mg/mL	smart hydrogel 2%	pH 4.7	0.25	0.28	0.40	0.55	0.64	0.77	1.37	1.68
NIF	2.5 mg/mL	smart hydrogel 2%	pH 6.0	0.09	0.18	0.37	0.50	0.63	0.80	1.37	1.44
NIF	2.5 mg/mL	LMO	--	0.03	0.08	0.14	0.24	0.28	0.38	0.63	0.78
NIF	2.5 mg/mL	HMO	--	0.05	0.12	0.20	0.32	0.40	0.45	0.74	1.00
NIF	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.03	0.13	0.27	0.39	0.62	0.85	1.28	2.20
NIF	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.04	0.13	0.23	0.28	0.42	0.63	0.78	1.49
NIF	2.5 mg/mL	ACN:H2O	--	0.14	0.29	0.44	0.56	0.70	0.84	1.26	1.78
NIF	2.5 mg/mL	H2O	--	0.03	0.05	0.08	0.12	0.14	0.20	0.37	0.70
SIL	1.0 mg/mL	smart hydrogel 2%	pH 3.6	0.20	0.30	0.44	0.54	0.71	0.80	1.07	1.30
SIL	1.0 mg/mL	smart hydrogel 2%	pH 4.7	0.20	0.26	0.37	0.46	0.62	0.68	0.77	1.04
SIL	1.0 mg/mL	smart hydrogel 2%	pH 6.0	0.16	0.22	0.32	0.37	0.47	0.49	0.69	0.86
SIL	1.0 mg/mL	LMO	--	0.24	0.39	0.61	0.76	0.95	1.15	1.71	2.30
SIL	1.0 mg/mL	HMO	--	0.18	0.32	0.48	0.59	0.73	0.89	1.35	1.80
SIL	1.0 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.26	0.53	0.69	0.84	1.00	1.07	1.53	2.01
SIL	1.0 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.24	0.42	0.61	0.86	1.12	1.40	2.34	3.28
SIL	2.5 mg/mL	smart hydrogel 2%	pH 3.6	0.39	0.86	1.38	1.89	2.20	2.72	4.01	5.06
SIL	2.5 mg/mL	smart hydrogel 2%	pH 4.7	0.22	0.43	0.68	0.81	1.06	1.26	1.88	2.52
SIL	2.5 mg/mL	smart hydrogel 2%	pH 6.0	0.33	0.49	0.75	1.07	1.34	1.58	2.29	2.89
SIL	2.0 mg/mL	LMO	--	0.45	0.87	1.37	1.75	2.10	2.63	3.91	5.07
SIL	2.0 mg/mL	HMO	--	0.43	0.86	1.38	1.85	2.30	2.70	3.82	5.03
SIL	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.48	1.09	1.62	1.51	1.17	1.09	0.97	0.70
SIL	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.20	0.42	0.47	0.51	0.45	0.52	0.62	0.68
SIL	2.5 mg/mL	ACN:H2O	--	0.82	1.34	2.04	2.81	3.32	3.87	5.89	7.90
SIL	2.5 mg/mL	H2O	--	2.05	3.80	5.72	7.82	8.91	9.99	13.54	15.93

Standard deviation of µg drug diffused per unit area over time (µg cm^{-2} over time)

			Time (min)	5	10	15	20	25	30	45	60
drug	[drug]	Formulation	pH								
DEXT	2.5 mg/ml	smart hydrogel 2%	pH 4.7	0.10	0.04	0.02	0.05	0.04	0.04	0.36	0.28
		smart hydrogel 2%	pH 6.0	0.09	0.08	0.19	0.30	0.10	0.16	0.37	0.54
		LMO emulsion	--	0.01	0.04	0.01	0.06	0.02	0.11	0.73	0.21
		HMO emulsion	--	0.04	0.02	0.00	0.05	0.11	0.22	0.58	0.58
		Noveon AA-1 0.1%	pH 4.7	0.05	0.07	0.06	0.03	0.10	0.07	0.15	0.29
		Noveon AA-1 0.1%	pH 6.0	0.31	0.17	0.13	0.16	0.19	0.14	0.15	0.18
		ACN:H2O	--	0.07	0.06	0.12	0.07	0.18	0.12	0.18	0.26
		H2O	--	0.02	0.02	0.03	0.04	0.10	0.11	0.02	0.09
MANN	2.5 mg/ml	smart hydrogel 2%	pH 4.7	0.13	0.56	0.20	0.14	0.48	0.29	0.30	0.42
		smart hydrogel 2%	pH 6.0	0.28	0.26	0.66	0.73	2.53	2.33	0.32	1.12
		LMO emulsion	--	0.26	0.05	0.14	0.11	0.26	0.31	1.01	0.41
		HMO emulsion	--	0.35	0.51	0.40	0.31	0.81	1.00	1.52	2.19
		Noveon AA-1 0.1%	pH 4.7	0.17	0.19	0.43	0.41	0.83	1.01	0.85	1.17
		Noveon AA-1 0.1%	pH 6.0	0.22	0.43	0.35	0.30	0.17	1.99	0.23	1.46
		ACN:H2O	--	0.13	0.10	0.09	0.36	0.52	1.03	2.54	4.24
		H2O	--	0.04	0.51	0.32	0.40	0.84	0.85	1.38	2.62
PROP	2.5 mg/ml	smart hydrogel 2%	pH 4.7	0.24	0.05	0.59	0.50	0.23	0.42	0.47	0.61
		smart hydrogel 2%	pH 6.0	0.34	0.37	0.30	0.34	0.48	0.43	0.63	0.81
		LMO emulsion	--	0.08	0.21	0.15	0.20	0.15	0.54	0.41	0.25
		HMO emulsion	--	0.44	0.48	2.41	0.64	0.34	0.33	1.55	1.84
		Noveon AA-1 0.1%	pH 4.7	0.45	0.59	0.98	1.00	0.23	1.63	0.62	1.83
		Noveon AA-1 0.1%	pH 6.0	0.40	0.56	0.51	1.01	1.86	0.15	0.83	1.50
		ACN:H2O	--	0.41	0.13	0.97	0.48	0.52	0.95	0.26	0.55
		H2O	--	0.34	0.88	0.87	0.58	2.00	1.91	2.10	1.17
ISDN	1.0 mg/ml	smart hydrogel 2%	pH 3.6	0.14	0.18	0.30	0.22	0.31	0.29	0.37	0.36
		smart hydrogel 2%	pH 4.7	0.08	0.17	0.27	0.32	0.38	0.27	0.44	0.39
		smart hydrogel 2%	pH 6.0	0.09	0.28	0.24	0.25	0.47	0.34	0.56	0.60
		LMO	--	0.17	0.49	0.36	0.48	0.62	0.76	1.14	1.37
		HMO	--	0.08	0.11	0.05	0.13	0.15	0.19	0.32	0.40
		Noveon AA-1 0.1%	pH 4.7	0.20	0.28	0.47	0.54	0.67	0.78	1.09	0.73
		Noveon AA-1 0.1%	pH 6.0	0.03	0.27	0.22	0.28	0.34	0.42	0.38	0.43
		ACN:H2O	--	0.01	0.01	0.02	0.02	0.02	0.02	0.12	0.23
		H2O	--	0.02	0.02	0.02	0.03	0.02	0.04	0.11	0.13
ISDN	2.5 mg/ml	smart hydrogel 2%	pH 3.6	0.10	0.31	0.55	0.41	0.99	1.68	1.55	1.82
		smart hydrogel 2%	pH 4.7	0.11	0.27	0.29	0.36	0.37	0.25	0.36	0.63
		smart hydrogel 2%	pH 6.0	0.11	0.05	0.19	0.21	0.31	0.40	0.49	0.38
		LMO	--	0.19	0.35	0.14	0.09	0.12	0.12	0.27	0.43
		HMO	--	0.04	0.13	0.12	0.16	0.16	0.24	0.40	0.88
		Noveon AA-1 0.1%	pH 4.7	0.18	0.20	0.38	0.51	0.62	0.71	0.89	1.02
		Noveon AA-1 0.1%	pH 6.0	0.65	0.37	0.44	0.48	0.67	0.62	2.31	1.03
		ACN:H2O	--	0.02	0.08	0.09	0.16	0.23	0.22	0.46	0.66
		H2O	--	0.16	0.19	0.13	0.20	0.18	0.27	0.20	0.12
NIF	1.0 mg/ml	smart hydrogel 2%	pH 3.6	0.04	0.02	0.03	0.06	0.07	0.12	0.13	0.07
		smart hydrogel 2%	pH 4.7	0.03	0.02	0.01	0.02	0.04	0.03	0.07	0.02
		smart hydrogel 2%	pH 6.0	0.01	0.00	0.02	0.01	0.01	0.09	0.08	0.06

		LMO	--	0.02	0.01	0.01	0.03	0.03	0.04	0.09	0.11
		HMO	--	0.04	0.05	0.05	0.04	0.05	0.07	0.07	0.09
		Noveon AA-1 0.1%	pH 4.7	0.01	0.01	0.02	0.05	0.08	0.09	0.09	0.26
		Noveon AA-1 0.1%	pH 6.0	0.00	0.00	0.00	0.01	0.02	0.02	0.07	0.03
NIF	2.5 mg/ml	smart hydrogel 2%	pH 3.6	0.01	0.04	0.06	0.08	0.13	0.18	0.25	0.37
		smart hydrogel 2%	pH 4.7	0.12	0.12	0.17	0.18	0.18	0.11	0.21	0.37
		smart hydrogel 2%	pH 6.0	0.01	0.03	0.05	0.01	0.05	0.07	0.10	0.10
		LMO	--	0.00	0.01	0.04	0.06	0.06	0.07	0.13	0.33
		HMO	--	0.02	0.01	0.02	0.02	0.03	0.02	0.06	0.00
		Noveon AA-1 0.1%	pH 4.7	0.01	0.03	0.06	0.14	0.16	0.17	0.23	0.26
		Noveon AA-1 0.1%	pH 6.0	0.01	0.02	0.03	0.04	0.09	0.13	0.18	0.27
		ACN:H2O	--	0.01	0.01	0.03	0.04	0.06	0.09	0.15	0.20
		H2O	--	0.00	0.01	0.02	0.03	0.02	0.05	8.00	0.26
SIL	1.0 mg/ml	smart hydrogel 2%	pH 3.6	0.05	0.06	0.05	0.01	0.06	0.11	0.11	0.19
		smart hydrogel 2%	pH 4.7	0.02	0.02	0.03	0.03	0.04	0.04	0.13	0.07
		smart hydrogel 2%	pH 6.0	0.03	0.07	0.14	0.18	0.27	0.30	0.47	0.62
		LMO	--	0.03	0.08	0.11	0.12	0.12	0.17	0.22	0.34
		HMO	--	0.02	0.04	0.07	0.09	0.06	0.13	0.22	0.27
		Noveon AA-1 0.1%	pH 4.7	0.03	0.05	0.03	0.03	0.05	0.06	0.07	0.13
		Noveon AA-1 0.1%	pH 6.0	0.03	0.05	0.03	0.11	0.13	0.17	0.26	0.24
SIL	2.5 mg/ml	smart hydrogel 2%	pH 3.6	0.03	0.06	0.06	0.05	0.02	0.01	0.06	0.19
		smart hydrogel 2%	pH 4.7	0.02	0.08	0.08	0.11	0.21	0.17	0.33	0.47
		smart hydrogel 2%	pH 6.0	0.04	0.06	0.09	0.12	0.19	0.23	0.38	0.48
		LMO	--	0.03	0.06	0.04	0.08	0.20	0.05	0.21	0.13
		HMO	--	0.09	0.09	0.11	0.09	0.09	0.10	0.12	0.17
		Noveon AA-1 0.1%	pH 4.7	0.02	0.05	0.10	0.05	0.09	0.06	0.09	0.10
		Noveon AA-1 0.1%	pH 6.0	0.01	0.12	0.08	0.13	0.02	0.11	0.10	0.04
		ACN:H2O	--	0.11	0.09	0.10	0.12	0.09	0.19	0.33	0.38
		H2O	--	0.24	0.41	0.73	1.00	1.23	1.25	1.42	1.82

Appendix 5B - Tables showing y-intercepts for all linear steady state flux graphs based on first 60 minutes.

These data were used to determine if a lag time was present (indicated by a negative value).

drug	[drug]	formulation	y- intercept
DEXT	2.5	Smart 4.7	0.21
DEXT	2.5	Smart 6.0	0.33
DEXT	2.5	LMO	0.02
DEXT	2.5	HMO	-0.09
DEXT	2.5	Noveon 4.7	0.31
DEXT	2.5	Noveon 6.0	0.45
DEXT	2.5	ACN:H_20 (60:40)	0.61
DEXT	2.5	H_2O only	0.61
MANN	2.5	Smart 4.7	1.15
MANN	2.5	Smart 6.0	2.04
MANN	2.5	LMO	0.82
MANN	2.5	HMO	0.90
MANN	2.5	Noveon 4.7	1.66
MANN	2.5	Noveon 6.0	0.73
MANN	2.5	ACN:H_20 (60:40)	1.18
MANN	2.5	H_2O only	0.25
PROP	2.5	Smart 4.7	2.79
PROP	2.5	Smart 6.0	1.14
PROP	2.5	LMO	2.30
PROP	2.5	HMO	2.04
PROP	2.5	Noveon 4.7	2.24
PROP	2.5	Noveon 6.0	1.98
PROP	2.5	ACN:H_20 (60:40)	4.32
PROP	2.5	H_2O only	14.37
ISDN	1.0	Smart 3.7	0.66
ISDN	1.0	Smart 4.7	0.53
ISDN	1.0	Smart 6.0	0.59
ISDN	1.0	LMO	0.39
ISDN	1.0	HMO	0.13
ISDN	1.0	Noveon 4.7	0.70
ISDN	1.0	Noveon 6.0	0.96
ISDN	1.0	ACN:H_20 (60:40)	0.06
ISDN	1.0	H_2O only	0.24
ISDN	2.5	Smart 3.6	1.00
ISDN	2.5	Smart 4.7	1.51
ISDN	2.5	Smart 6.0	1.24
ISDN	2.5	LMO	0.96
ISDN	2.5	HMO	0.53
ISDN	2.5	Noveon 4.7	1.53
ISDN	2.5	Noveon 6.0	1.06
ISDN	2.5	ACN:H_20 (60:40)	-0.05
ISDN	2.5	H_2O only	1.45
NIF	1.0	Smart 3.6	-0.16
NIF	1.0	Smart 4.7	-0.02
NIF	1.0	Smart 6.0	-0.06
NIF	1.0	LMO	-0.14
NIF	1.0	HMO	0.01
NIF	1.0	Noveon 4.7	-0.14
NIF	1.0	Noveon 6.0	-0.19
NIF	2.5	Smart 3.6	0.01
NIF	2.5	Smart 4.7	-0.33

NIF	2.5	Smart 6.0	-0.31
NIF	2.5	LMO	-0.12
NIF	2.5	HMO	-0.05
NIF	2.5	Noveon 4.7	-0.77
NIF	2.5	Noveon 6.0	-0.26
NIF	2.5	ACN:H_20 (60:40)	-0.06
NIF	2.5	H_2O only	-0.15
SIL	1.0	Smart 3.8	0.16
SIL	1.0	Smart 4.7	0.12
SIL	1.0	Smart 6.0	0.13
SIL	1.0	LMO	0.17
SIL	1.0	LMO repeat	0.06
SIL	1.0	HMO	-0.06
SIL	1.0	Noveon 6.0	-0.20
SIL	2.5	Smart 3.8	0.16
SIL	2.5	Smart 4.7	0.18
SIL	2.5	Smart 6.0	0.15
SIL	2.0	LMO	0.09
SIL	2.0	HMO	0.11
SIL	2.5	Noveon 4.7	0.87
SIL	2.5	Noveon 6.0	-0.12
SIL	2.5	ACN:H_20 (60:40)	0.58
SIL	2.5	H_2O only	0.30

Appendix 5C - Tables showing cumulative percent drug release over time from different formulations (Franz cell studies)

Mean % drug release from formulations

			Time(min)	5	10	15	20	25	30	45	60
drug	[drug]	formulation	pH								
DEXT	2.5 mg/mL	smart hydrogel 2%	pH 4.7	1.64	2.00	2.68	3.15	4.19	4.83	6.58	9.76
DEXT	2.5 mg/mL	smart hydrogel 2%	pH 6.0	2.30	2.60	3.44	3.66	4.78	5.03	8.02	10.42
DEXT	2.5 mg/mL	LMO emulsion	--	0.89	1.24	1.64	2.18	2.97	3.55	3.72	7.27
DEXT	2.5 mg/mL	HMO emulsion	--	1.08	1.27	1.86	2.60	3.17	4.56	6.77	9.37
DEXT	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	1.99	1.65	1.69	2.08	2.40	2.82	4.14	5.64
DEXT	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	2.49	1.94	2.15	2.37	2.91	3.18	4.20	5.14
DEXT	2.5 mg/mL	ACN:H_2O	--	3.27	3.07	3.36	3.54	3.94	4.51	6.09	7.57
DEXT	2.5 mg/mL	H_2O	--	2.65	2.79	2.89	2.86	2.99	3.20	4.08	4.37
MANN	2.5 mg/mL	smart hydrogel 2%	pH 4.7	6.54	9.93	15.68	20.35	22.46	25.91	36.12	42.74
MANN	2.5 mg/mL	smart hydrogel 2%	pH 6.0	7.03	11.75	16.17	20.13	20.17	23.45	35.49	43.20
MANN	2.5 mg/mL	LMO emulsion	--	5.91	9.79	13.55	17.48	22.14	26.54	38.34	50.27
MANN	2.5 mg/mL	HMO emulsion	--	7.67	12.15	15.61	22.09	26.65	31.26	44.10	58.62
MANN	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	5.82	9.00	11.79	14.40	18.43	20.52	36.73	35.19
MANN	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	5.69	7.49	10.84	14.04	17.53	14.39	32.20	33.76
MANN	2.5 mg/mL	ACN:H_2O	--	7.62	13.23	18.68	24.90	28.33	32.06	42.94	46.79
MANN	2.5 mg/mL	H2O	--	5.78	11.94	16.07	22.10	24.72	32.63	47.58	54.35
PROP	2.5 mg/mL	smart hydrogel 2%	pH 4.7	9.93	14.56	19.34	26.72	29.04	33.77	42.92	52.85
PROP	2.5 mg/mL	smart hydrogel 2%	pH 6.0	4.63	6.04	7.39	9.60	10.27	12.85	14.44	18.51
PROP	2.5 mg/mL	LMO emulsion	--	12.86	23.49	34.39	45.32	52.34	60.95	81.93	92.45
PROP	2.5 mg/mL	HMO emulsion	--	13.20	21.52	33.12	42.15	53.66	57.91	68.91	80.84
PROP	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	12.55	22.93	33.57	44.25	51.16	59.51	80.04	90.28
PROP	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	12.96	21.14	32.48	41.41	52.50	56.79	67.62	79.34
ISDN	1.0 mg/mL	smart hydrogel 2%	pH 3.6	6.89	11.14	15.01	18.57	20.93	23.71	32.80	0.00
ISDN	1.0 mg/mL	smart hydrogel 2%	pH 4.7	5.71	8.81	11.63	14.19	15.96	18.47	24.16	0.00
ISDN	1.0 mg/mL	smart hydrogel 2%	pH 6.0	4.49	7.92	9.88	12.23	13.12	15.77	22.08	25.89
ISDN	1.0 mg/mL	LMO emulsion	--	8.47	11.33	13.39	17.51	21.10	25.00	37.00	47.62
ISDN	1.0 mg/mL	HMO emulsion	--	4.08	6.66	9.92	11.80	13.92	16.98	24.22	31.77
ISDN	1.0 mg/mL	Noveon AA-1 0.1%	pH 4.7	8.72	14.92	20.31	25.84	30.12	33.52	43.63	53.55
ISDN	1.0 mg/mL	Noveon AA-1 0.1%	pH 6.0	6.29	12.22	14.62	18.41	21.68	24.05	32.92	41.13
ISDN	1.0 mg/mL	ACN:H_2O	--	0.66	1.18	1.69	2.20	2.75	3.19	4.32	5.32
ISDN	1.0 mg/mL	H2O	--	3.51	5.55	7.76	9.70	11.21	12.42	15.52	17.01
ISDN	2.5 mg/mL	smart hydrogel 2%	pH 3.6	5.42	11.45	16.22	21.49	25.00	28.44	39.31	0.00
ISDN	2.5 mg/mL	smart hydrogel 2%	pH 4.7	5.00	8.62	10.89	13.49	15.16	16.94	21.97	0.00
ISDN	2.5 mg/mL	smart hydrogel 2%	pH 6.0	3.45	6.09	8.11	10.26	11.74	13.07	17.43	21.78
ISDN	2.5 mg/mL	LMO emulsion	--	6.36	8.22	8.44	11.04	13.40	15.83	23.69	29.95
ISDN	2.5 mg/mL	HMO emulsion	--	2.56	4.45	6.17	7.75	9.14	11.26	16.55	22.35
ISDN	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	4.38	9.44	13.89	17.37	20.44	22.04	28.24	35.28
ISDN	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	4.72	6.37	8.60	10.92	13.01	14.41	16.31	24.86
ISDN	2.5 mg/mL	ACN:H_2O	--	1.48	2.88	4.16	5.53	6.83	8.18	12.46	17.68
ISDN	2.5 mg/mL	H2O	--	7.89	14.00	18.71	22.93	26.78	29.81	38.53	43.52
SIL	1.0 mg/mL	smart hydrogel 2%	pH 3.6	1.64	2.46	3.61	4.38	5.77	6.53	8.69	10.52
SIL	1.0 mg/mL	smart hydrogel 2%	pH 4.7	1.58	2.15	3.00	3.74	5.00	5.55	6.22	8.44
SIL	1.0 mg/mL	smart hydrogel 2%	pH 6.0	1.30	1.82	2.57	3.02	3.79	4.01	5.62	7.02
SIL	1.0 mg/mL	LMO emulsion	--	2.10	4.31	5.62	6.84	8.08	8.69	12.40	16.33
SIL	1.0 mg/mL	HMO emulsion	--	1.47	2.60	3.86	4.83	5.95	7.26	10.96	14.61
SIL	1.0 mg/mL	Noveon AA-1 0.1%	pH 4.7	1.91	3.18	4.93	6.14	7.75	9.32	13.89	18.65
SIL	1.0 mg/mL	Noveon AA-1 0.1%	pH 6.0	1.97	3.42	4.94	6.94	9.09	11.34	18.98	26.63
SIL	2.5 mg/mL	smart hydrogel 2%	pH 3.6	1.27	2.81	4.49	6.13	7.15	8.84	13.02	16.43
SIL	2.5 mg/mL	smart hydrogel 2%	pH 4.7	0.71	1.38	2.19	2.63	3.43	4.11	6.09	8.17
SIL	2.5 mg/mL	smart hydrogel 2%	pH 6.0	1.06	1.60	2.43	3.48	4.34	5.12	7.43	9.39
SIL	2.0 mg/mL	LMO emulsion	--	1.84	3.53	5.56	7.10	8.53	10.69	15.89	20.58
SIL	2.0 mg/mL	HMO emulsion	--	1.75	3.48	5.59	7.53	9.33	10.95	15.52	20.40

SIL	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	2.72	5.39	8.47	10.51	9.58	14.44	19.53	22.80
SIL	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	1.57	3.53	5.27	4.91	3.80	3.53	3.14	2.26
SIL	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.66	1.35	1.52	1.67	1.46	1.68	2.03	2.20
SIL	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.88	1.17	2.09	2.78	3.62	5.00	10.07	9.68
SIL	2.5 mg/mL	ACN:H_2O	--	2.65	4.34	6.62	9.14	10.79	12.55	19.14	25.65
SIL	2.5 mg/mL	H2O	--	6.66	12.35	18.58	25.38	28.92	32.44	43.97	51.74
NIF	1.0 mg/mL	smart hydrogel 2%	pH 3.6	0.47	0.91	1.47	2.48	3.35	4.45	7.82	13.92
NIF	1.0 mg/mL	smart hydrogel 2%	pH 4.7	1.10	1.23	1.45	2.49	3.36	4.26	6.88	9.17
NIF	1.0 mg/mL	smart hydrogel 2%	pH 6.0	0.68	1.03	1.94	2.46	2.85	3.83	4.60	5.97
NIF	1.0 mg/mL	LMO emulsion	--	0.33	0.39	0.63	0.79	0.94	1.34	1.86	2.88
NIF	1.0 mg/mL	HMO emulsion	--	0.41	0.69	0.88	1.02	1.18	1.43	2.31	2.70
NIF	1.0 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.14	0.39	0.84	1.72	1.86	2.97	6.72	11.22
NIF	1.0 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.07	0.20	0.41	0.77	0.76	1.40	3.02	5.19
NIF	2.5 mg/mL	smart hydrogel 2%	pH 3.6	0.30	0.68	1.31	1.64	2.31	2.77	4.29	6.35
NIF	2.5 mg/mL	smart hydrogel 2%	pH 4.7	0.81	0.92	1.31	1.80	2.09	2.50	4.43	5.45
NIF	2.5 mg/mL	smart hydrogel 2%	pH 6.0	0.28	0.60	1.19	1.63	2.04	2.60	4.44	4.68
NIF	2.5 mg/mL	LMO emulsion	--	0.08	0.26	0.44	0.79	0.92	1.22	2.04	2.54
NIF	2.5 mg/mL	HMO emulsion	--	0.18	0.37	0.64	1.03	1.31	1.46	2.41	3.24
NIF	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.11	0.41	0.89	1.27	2.01	2.76	4.17	7.13
NIF	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.14	0.42	0.74	0.91	1.38	2.06	2.54	4.85
NIF	2.5 mg/mL	H2O	--	0.09	0.18	0.27	0.37	0.47	0.63	1.21	2.27
NIF	2.5 mg/mL	ACN:H_2O	--	0.45	0.94	1.42	1.83	2.27	2.73	4.10	5.78

Standard deviation of % drug release from formulations

			Time(min)	5	10	15	20	25	30	45	60
drug	[drug]	formulation	pH								
DEXT	2.5 mg/mL	ACN:H_2O	--	0.23	0.18	0.38	0.21	0.60	0.40	0.60	0.85
DEXT	2.5 mg/mL	H2O	--	0.07	0.06	0.10	0.14	0.31	0.35	0.08	0.29
DEXT	2.5 mg/mL	smart hydrogel 2%	pH 6.0	0.31	0.27	0.61	0.97	0.32	0.50	1.21	1.76
DEXT	2.5 mg/mL	smart hydrogel 2%	pH 4.7	0.31	0.14	0.05	0.15	0.13	0.14	1.16	0.90
DEXT	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.31	0.27	0.61	0.97	0.32	0.50	1.21	1.76
DEXT	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.15	0.22	0.18	0.08	0.32	0.24	0.50	0.93
DEXT	2.5 mg/mL	HMO emulsion	--	0.14	0.11	0.04	0.12	0.33	0.78	1.83	1.87
DEXT	2.5 mg/mL	LMO emulsion	--	0.03	0.14	0.03	0.18	0.07	0.36	2.36	0.56
MANN	2.5 mg/mL	ACN:H_2O	--	0.42	0.33	0.30	1.17	1.69	3.36	8.24	13.77
MANN	2.5 mg/mL	HMO emulsion	--	1.05	1.63	1.14	0.74	2.44	3.07	4.60	6.58
MANN	2.5 mg/mL	H2O	--	0.12	1.65	1.03	1.31	2.72	2.77	4.47	8.52
MANN	2.5 mg/mL	smart hydrogel 2%	pH 6.0	0.67	0.38	1.88	2.03	8.47	7.75	2.49	5.00
MANN	2.5 mg/mL	smart hydrogel 2%	pH 4.7	0.42	1.82	0.57	0.44	1.54	0.94	0.97	1.36
MANN	2.5 mg/mL	LMO emulsion	--	0.85	0.15	0.45	0.37	0.85	0.97	3.25	1.30
MANN	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.54	0.61	1.39	1.30	2.69	3.27	2.77	3.81
MANN	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.75	1.53	1.19	1.05	0.84	6.82	1.20	4.99
ISDN	2.5 mg/mL	H2O	--	0.52	0.62	0.44	0.66	0.57	0.88	0.66	0.38
ISDN	2.5 mg/mL	smart hydrogel 2%	pH 3.6	0.32	1.02	1.80	1.34	3.22	5.45	5.03	0.00
ISDN	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.60	0.63	1.22	1.65	2.00	2.31	2.88	3.32
ISDN	2.5 mg/mL	smart hydrogel 2%	pH 4.7	0.35	0.89	0.93	1.18	1.19	0.81	1.17	0.00
ISDN	2.5 mg/mL	LMO emulsion	--	0.63	1.14	0.46	0.29	0.40	0.38	0.87	1.40
ISDN	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	2.11	1.21	1.44	1.57	2.16	2.01	7.49	3.35
ISDN	2.5 mg/mL	smart hydrogel 2%	pH 6.0	0.36	0.16	0.60	0.68	0.99	1.30	1.61	1.22
ISDN	2.5 mg/mL	HMO emulsion	--	0.13	0.41	0.38	0.53	0.51	0.78	1.29	2.87
ISDN	2.5 mg/mL	ACN:H_2O	--	0.06	0.25	0.30	0.51	0.73	0.71	1.49	2.14
ISDN	1.0 mg/mL	Noveon AA-1 0.1%	pH 4.7	1.64	2.23	3.83	4.42	5.40	6.35	8.86	5.94
ISDN	1.0 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.21	2.22	1.82	2.24	2.75	3.37	3.06	3.53
ISDN	1.0 mg/mL	LMO emulsion	--	1.42	4.00	2.90	3.90	5.04	6.13	9.26	11.12
ISDN	1.0 mg/mL	smart hydrogel 2%	pH 3.6	1.11	1.48	2.44	1.81	2.51	2.34	3.00	0.00
ISDN	1.0 mg/mL	smart hydrogel 2%	pH 4.7	0.65	1.42	2.16	2.61	3.12	2.23	3.55	0.00
ISDN	1.0 mg/mL	smart hydrogel 2%	pH 6.0	0.70	2.26	1.91	2.00	3.81	2.78	4.53	4.85
ISDN	1.0 mg/mL	HMO emulsion	--	0.65	0.89	0.40	1.05	1.25	1.56	2.63	3.25
ISDN	1.0 mg/mL	H2O	--	0.20	0.20	0.20	0.21	0.13	0.34	0.92	1.08
ISDN	1.0 mg/mL	ACN:H_2O	--	0.05	0.07	0.16	0.19	0.18	0.15	0.99	1.84
SIL	2.5 mg/mL	H2O	--	0.76	1.32	2.39	3.26	3.98	4.05	4.60	5.91
SIL	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.27	0.44	0.49	0.97	4.98	0.94	1.19	1.79
SIL	2.5 mg/mL	ACN:H_2O	--	0.35	0.30	0.33	0.39	0.30	0.63	1.07	1.24
SIL	2.0 mg/mL	LMO emulsion	--	0.13	0.24	0.18	0.33	0.80	0.21	0.87	0.54
SIL	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.05	0.15	0.31	0.15	0.28	0.20	0.30	0.34
SIL	2.0 mg/mL	HMO emulsion	--	0.35	0.36	0.45	0.37	0.36	0.41	0.51	0.70
SIL	2.5 mg/mL	smart hydrogel 2%	pH 3.6	0.11	0.21	0.19	0.16	0.06	0.03	0.21	0.61
SIL	2.5 mg/mL	smart hydrogel 2%	pH 6.0	0.13	0.21	0.30	0.40	0.63	0.74	1.25	1.56
SIL	2.5 mg/mL	smart hydrogel 2%	pH 4.7	0.07	0.25	0.27	0.35	0.69	0.55	1.08	1.52
SIL	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.02	0.38	0.27	0.43	0.08	0.37	0.33	0.12
SIL	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.25	0.31	0.65	0.73	0.82	1.38	2.08	3.31
SIL	1.0 mg/mL	LMO emulsion	--	0.24	0.38	0.22	0.21	0.43	0.48	0.57	1.03
SIL	1.0 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.25	0.39	0.27	0.86	1.02	1.39	2.11	1.95
SIL	1.0 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.28	0.64	0.92	0.99	1.00	1.37	1.76	2.79
SIL	1.0 mg/mL	HMO emulsion	--	0.16	0.31	0.59	0.76	0.52	1.05	1.75	2.23
SIL	1.0 mg/mL	smart hydrogel 2%	pH 3.6	0.44	0.47	0.39	0.12	0.51	0.90	0.92	1.52
SIL	1.0 mg/mL	smart hydrogel 2%	pH 4.7	0.16	0.20	0.27	0.23	0.30	0.31	1.03	0.57
SIL	1.0 mg/mL	smart hydrogel 2%	pH 6.0	0.20	0.58	1.17	1.46	2.19	2.47	3.81	5.07

NIF	2.5 mg/mL	ACN:H_2O	--	0.03	0.04	0.11	0.13	0.21	0.28	0.48	0.65
NIF	2.5 mg/mL	smart hydrogel 2%	pH 4.7	0.40	0.38	0.55	0.59	0.57	0.36	0.70	1.21
NIF	2.5 mg/mL	smart hydrogel 2%	pH 3.6	0.03	0.12	0.19	0.25	0.43	0.57	0.81	1.21
NIF	2.5 mg/mL	smart hydrogel 2%	pH 6.0	0.04	0.09	0.15	0.04	0.15	0.23	0.31	0.34
NIF	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.02	0.06	0.11	0.13	0.29	0.41	0.60	0.86
NIF	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.04	0.11	0.20	0.46	0.51	0.56	0.76	0.85
NIF	2.5 mg/mL	HMO emulsion	--	0.05	0.05	0.07	0.07	0.08	0.05	0.20	0.02
NIF	2.5 mg/mL	LMO emulsion	--	0.01	0.02	0.12	0.20	0.19	0.21	0.42	1.06
NIF	2.5 mg/mL	H2O	--	0.01	0.04	0.07	0.09	0.06	0.18	0.40	0.86
NIF	1.0 mg/mL	smart hydrogel 2%	pH 4.7	0.28	0.17	0.07	0.14	0.29	0.28	0.55	0.20
NIF	1.0 mg/mL	smart hydrogel 2%	pH 6.0	0.08	0.02	0.17	0.05	0.08	0.71	0.68	0.51
NIF	1.0 mg/mL	smart hydrogel 2%	pH 3.6	0.31	0.18	0.21	0.49	0.55	0.95	1.04	0.55
NIF	1.0 mg/mL	HMO emulsion	--	0.29	0.43	0.42	0.30	0.40	0.57	0.58	0.76
NIF	1.0 mg/mL	Noveon AA-1 0.1%	pH 4.7	0.12	0.07	0.14	0.39	0.62	0.76	0.71	2.10
NIF	1.0 mg/mL	LMO emulsion	--	0.15	0.07	0.10	0.20	0.22	0.32	0.75	0.88
NIF	1.0 mg/mL	Noveon AA-1 0.1%	pH 6.0	0.00	0.03	0.02	0.09	0.12	0.16	0.55	0.21
PROP	2.5 mg/mL	LMO emulsion	--	1.69	2.38	3.87	4.08	0.56	6.36	3.29	7.60
PROP	2.5 mg/mL	Noveon AA-1 0.1%	pH 4.7	1.45	1.91	3.18	3.23	0.63	5.25	1.99	5.95
PROP	2.5 mg/mL	HMO emulsion	--	1.06	1.20	2.05	2.06	7.78	2.25	1.72	3.81
PROP	2.5 mg/mL	Noveon AA-1 0.1%	pH 6.0	1.28	1.83	1.53	3.29	5.83	0.45	2.63	4.81
PROP	2.5 mg/mL	smart hydrogel 2%	pH 4.7	0.77	0.15	1.93	1.60	0.71	1.34	1.50	1.93
PROP	2.5 mg/mL	smart hydrogel 2%	pH 6.0	1.10	1.21	0.97	1.11	1.56	1.39	2.04	2.62

Appendix 5D - Steady state flux (μg cm^2 min^{-1}) for drug release from different formulations, based on the first 60 min of each experiment

formulation	drug	[drug]	mean μg cm^{-2} min^{-1}	standard deviation μg cm^{-2} min^{-1}
Smart 3.7	ISDN	1.0	0.075	0.004
Smart 3.6	ISDN	2.5	0.259	0.072
Smart 3.6	NIF	1.0	0.025	0.002
Smart 3.6	NIF	2.5	0.013	0.002
Smart 3.8	SIL	1.0	0.019	0.003
Smart 3.8	SIL	2.5	0.081	0.006
Smart 4.7	DEXT	2.5	0.044	0.006
Smart 4.7	MANN	2.5	0.228	0.011
Smart 4.7	PROP	2.5	0.228	0.014
Smart 4.7	ISDN	1.0	0.057	0.006
Smart 4.7	ISDN	2.5	0.117	0.008
Smart 4.7	NIF	1.0	0.018	0.001
Smart 4.7	NIF	2.5	0.041	0.010
Smart 4.7	SIL	1.0	0.018	0.004
Smart 4.7	SIL	2.5	0.034	0.005
Smart 6.0	DEXT	2.5	0.047	0.011
Smart 6.0	MANN	2.5	0.189	0.020
Smart 6.0	PROP	2.5	0.082	0.010
Smart 6.0	ISDN	1.0	0.042	0.010
Smart 6.0	ISDN	2.5	0.086	0.008
Smart 6.0	NIF	1.0	0.034	0.007
Smart 6.0	NIF	2.5	0.040	0.004
Smart 6.0	SIL	1.0	0.018	0.001
Smart 6.0	SIL	2.5	0.045	0.008
HMO	DEXT	2.5	0.048	0.012
HMO	MANN	2.5	0.286	0.033
HMO	PROP	2.5	0.503	0.068
HMO	ISDN	1.0	0.063	0.004
HMO	ISDN	2.5	0.098	0.004
HMO	NIF	1.0	0.006	0.001
HMO	NIF	2.5	0.018	0.002
HMO	SIL	1.0	0.033	0.005
HMO	SIL	2.0	0.083	0.003
Noveon 4.7	DEXT	2.5	0.019	0.003
Noveon 4.7	MANN	2.5	0.141	0.025
Noveon 4.7	PROP	2.5	0.520	0.012
Noveon 4.7	ISDN	1.0	0.115	0.031
Noveon 4.7	ISDN	2.5	0.163	0.017
Noveon 4.7	NIF	1.0	0.018	0.004
Noveon 4.7	NIF	2.5	0.057	0.007
Noveon 4.7	SIL	2.5	0.107	0.010
Noveon 6.0	DEXT	2.5	0.018	0.002
Noveon 6.0	MANN	2.5	0.189	0.033
Noveon 6.0	PROP	2.5	0.503	0.067
Noveon 6.0	ISDN	1.0	0.062	0.006
Noveon 6.0	ISDN	2.5	0.110	0.008
Noveon 6.0	NIF	1.0	0.014	0.003
Noveon 6.0	NIF	2.5	0.029	0.004
Noveon 6.0	SIL	1.0	0.056	0.002
Noveon 6.0	SIL	2.5	0.055	0.005
ACN:H20 (60:40)	DEXT	2.5	0.027	0.004
ACN:H20 (60:40)	MANN	2.5	0.298	0.021

ACN:H20 (60:40)	PROP	2.5	0.116	0.012
ACN:H20 (60:40)	ISDN	1.0	0.012	0.001
ACN:H20 (60:40)	ISDN	2.5	0.092	0.010
ACN:H20 (60:40)	NIF	2.5	0.031	0.002
ACN:H20 (60:40)	SIL	2.5	0.117	0.003
H_2O only	DEXT	2.5	0.014	0.003
H_2O only	MANN	2.5	0.319	0.040
H_2O only	PROP	2.5	0.328	0.048
H_2O only	ISDN	1.0	0.046	0.002
H_2O only	ISDN	2.5	0.275	0.013
H_2O only	NIF	2.5	0.014	0.007
H_2O only	SIL	2.5	0.364	0.056

Appendix 5E – Correlation coefficients for drug release models

Tables showing R^2 correlation coefficient values for Higuchi, zero, first and second order kinetic models of drug release from formulations. Based on graphs were drawn of $\mu g/cm^2$ against time, ln $\mu g/cm^2$ against time, the reciprocal $\mu g/cm^2$ against time, and $\mu g/cm^2$ against square root of time. Data shown for full 240 minutes, and the first 60 and 30 minutes. (SH = Smart Hydrogel®, Nov = Noveon AA1, LMO = light mineral oil emulsion, HMO – heavy mineral oil emulsion).

		full time period				first hour				first 30 minutes			
		Higuchi	zero	1st	2nd	Higuchi	zero	1st	2nd	Higuchi	zero	1st	2nd
DEXT 2.5	LMO	0.872	0.926	0.915	0.734	0.872	0.926	0.915	0.734	0.934	0.980	0.995	0.938
DEXT 2.5	HMO	0.929	0.988	0.948	0.775	0.929	0.988	0.948	0.775	0.879	0.943	0.990	0.961
DEXT 2.5	Nov 4.7	0.826	0.920	0.946	0.734	0.939	0.984	0.990	0.942	0.917	0.953	0.963	0.964
DEXT 2.5	Nov 6.0	0.712	0.830	0.969	0.800	0.976	0.996	0.978	0.927	0.943	0.969	0.980	0.985
MANN 2.5	SH 4.7	0.997	0.967	0.744	0.407	0.993	0.983	0.850	0.635	0.987	0.983	0.929	0.832
MANN 2.5	SH 6.0	0.993	0.965	0.775	0.447	0.973	0.988	0.884	0.653	0.978	0.945	0.873	0.769
MANN 2.5	LMO	0.989	0.937	0.729	0.402	0.971	1.000	0.906	0.664	0.974	0.998	0.967	0.850
MANN 2.5	HMO	0.955	0.853	0.670	0.404	0.971	0.999	0.909	0.689	0.972	0.995	0.970	0.873
MANN 2.5	Nov 4.7	0.983	0.929	0.730	0.442	0.930	0.935	0.891	0.708	0.981	0.996	0.964	0.868
MANN 2.5	Nov 6.0	0.984	0.961	0.777	0.485	0.907	0.935	0.900	0.743	0.864	0.832	0.852	0.830
PROP 2.5	SH 4.7	0.962	0.852	0.666	0.424	0.993	0.982	0.868	0.682	0.982	0.988	0.953	0.873
PROP 2.5	SH 6.0	0.991	0.977	0.823	0.560	0.980	0.975	0.899	0.764	0.958	0.984	0.982	0.936
PROP 2.5	Nov 4.7	0.796	0.612	0.475	0.289	0.996	0.966	0.810	0.571	0.995	0.994	0.924	0.785
PROP 2.5	Nov 6.0	0.800	0.620	0.481	0.303	0.985	0.932	0.779	0.581	0.985	0.990	0.941	0.827
ISDN 1.0	SH 3.7	0.994	0.966	0.764	0.448	0.989	0.995	0.884	0.664	0.998	0.989	0.926	0.817
ISDN 1.0	SH 4.7	0.982	0.985	0.815	0.493	0.981	0.995	0.902	0.697	0.997	0.993	0.940	0.841
ISDN 1.0	SH 6.0	0.989	0.934	0.751	0.434	0.987	0.988	0.873	0.644	0.991	0.978	0.903	0.778
ISDN 1.0	LMO	0.955	1.000	0.862	0.536	0.951	0.996	0.959	0.815	0.953	0.990	0.993	0.949
ISDN 1.0	HMO	0.952	1.000	0.804	0.404	0.959	0.992	0.909	0.711	0.917	0.958	0.954	0.885
ISDN 1.0	Nov 4.7	0.983	0.903	0.689	0.396	0.998	0.982	0.842	0.616	0.998	0.990	0.924	0.804

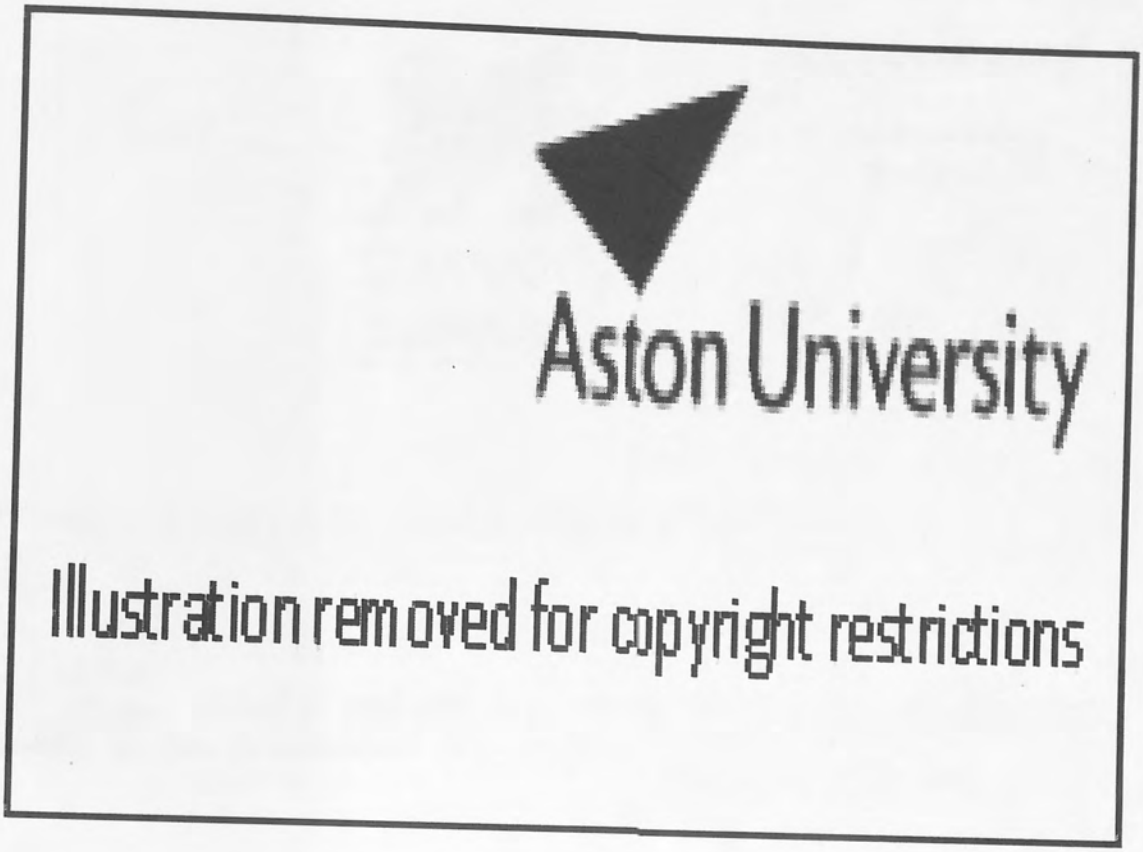
ISDN 1.0	Nov 6.0	0.989	0.942	0.743	0.408	0.990	0.991	0.857	0.605	0.995	0.981	0.893	0.752
		full time period				first hour				first 30 minutes			
		Higuchi	zero	1st	2nd	Higuchi	zero	1st	2nd	Higuchi	zero	1st	2nd
ISDN 2.5	SH 3.7	0.996	0.934	0.677	0.360	0.998	0.978	0.804	0.530	0.998	0.989	0.896	0.732
ISDN 2.5	SH 4.7	0.963	0.975	0.748	0.370	0.998	0.982	0.850	0.630	0.999	0.982	0.907	0.787
ISDN 2.5	SH 6.0	0.989	0.937	0.744	0.407	0.995	0.988	0.851	0.615	0.999	0.986	0.911	0.785
ISDN 2.5	LMO	0.961	0.998	0.868	0.585	0.940	0.990	0.966	0.859	0.920	0.966	0.978	0.954
ISDN 2.5	HMO	0.966	0.998	0.788	0.367	0.962	0.999	0.911	0.652	0.985	0.997	0.946	0.815
ISDN 2.5	Nov 4.7	0.945	0.810	0.544	0.250	0.997	0.965	0.770	0.505	0.996	0.973	0.868	0.709
ISDN 2.5	Nov 6.0	0.966	0.905	0.733	0.448	0.950	0.967	0.897	0.728	0.982	0.995	0.971	0.907
NIF 1.0	SH 3.7	0.968	0.989	0.750	0.308	0.900	0.977	0.916	0.574	0.927	0.977	0.977	0.816
NIF 1.0	SH 4.7	0.957	0.995	0.820	0.497	0.918	0.984	0.944	0.794	0.854	0.927	0.960	0.961
NIF 1.0	SH 6.0	0.888	0.978	0.848	0.287	0.948	0.995	0.857	0.502	0.970	0.994	0.927	0.733
NIF 1.0	Nov 4.7	0.961	0.993	0.981	0.201	0.896	0.968	0.861	0.427	0.907	0.954	0.933	0.683
NIF 1.0	Nov 6.0	0.906	0.988	0.769	0.215	0.883	0.964	0.880	0.441	0.871	0.921	0.930	0.686
NIF 2.5	SH 3.7	0.968	0.988	0.789	0.382	0.984	0.977	0.828	0.597	0.957	0.983	0.950	0.838
NIF 2.5	SH 4.7	0.895	0.981	0.908	0.553	0.928	0.984	0.957	0.807	0.943	0.982	0.979	0.946
NIF 2.5	SH 6.0	0.899	0.980	0.803	0.282	0.962	0.967	0.815	0.500	0.972	0.995	0.929	0.744
NIF 2.5	LMO	0.934	0.998	0.700	0.174	0.965	0.994	0.806	0.421	0.960	0.988	0.910	0.662
NIF 2.5	HMO	0.933	0.992	0.733	0.250	0.943	0.929	0.752	0.490	0.839	0.802	0.819	0.712
NIF 2.5	Nov 4.7	0.894	0.986	0.700	0.143	0.908	0.976	0.823	0.379	0.920	0.972	0.922	0.624
NIF 2.5	Nov 6.0	0.849	0.964	0.784	0.191	0.887	0.954	0.853	0.443	0.901	0.952	0.931	0.674
SIL 1.0	SH 3.7	0.613	0.486	0.511	0.386	0.947	0.961	0.871	0.704	0.831	0.876	0.898	0.853
SIL 1.0	SH 4.7	0.985	0.988	0.813	0.496	0.974	0.962	0.862	0.697	0.959	0.989	0.982	0.921
SIL 1.0	SH 6.0	0.983	0.994	0.833	0.513	0.984	0.995	0.905	0.721	0.983	0.986	0.953	0.879
SIL 1.0	LMO	0.974	0.989	0.811	0.400	0.981	0.993	0.852	0.572	0.996	0.970	0.867	0.719
SIL 1.0	LMO	0.966	0.933	0.749	0.395	0.965	1.000	0.901	0.640	0.979	0.998	0.956	0.831
SIL 1.0	HMO	0.965	0.981	0.809	0.402	0.964	0.999	0.901	0.628	0.983	0.999	0.948	0.810
SIL 1.0	Nov 6.0	0.970	0.991	0.794	0.386	0.933	0.992	0.927	0.646	0.955	0.992	0.975	0.846
SIL 2.5	SH 3.7	0.982	0.991	0.751	0.327	0.959	0.986	0.857	0.548	0.905	0.939	0.910	0.737
SIL 2.5	SH 4.7	0.964	0.898	0.682	0.333	0.970	0.999	0.874	0.574	0.983	0.996	0.931	0.770

SIL 2.5	**SH 6.0**	0.985	0.980	0.772	0.409	0.978	0.996	0.885	0.657	0.965	0.994	0.973	0.878
SIL 2.0	**LMO**	0.985	0.982	0.739	0.344	0.974	0.999	0.871	0.576	0.983	0.997	0.934	0.776
SIL 2.0	**HMO**	0.992	0.984	0.732	0.326	0.985	0.997	0.843	0.548	0.988	0.999	0.930	0.768
SIL 2.5	**Nov 4.7**	0.914	0.837	0.637	0.379	0.844	0.761	0.679	0.496	0.923	0.916	0.861	0.724
SIL 2.5	**Nov 6.0**	0.861	0.781	0.616	0.458	0.861	0.781	0.616	0.458	0.723	0.621	0.573	0.528
SIL 2.5	**Nov 6.0**	0.916	0.956	0.826	0.503	0.900	0.929	0.897	0.682	0.916	0.969	0.985	0.904

Appendix 5F - Table showing predicted total drug release (µg) over the oesophageal surface area, after 10 minutes, based on steady state flux data and a calculated oesophageal area of 181 cm2.

drug	[drug]	formulation	pH	mean µg released after 10 min
ISDN	1.0	smart hydrogel 2%	3.6	134.8
ISDN	2.5	smart hydrogel 2%	3.6	469.1
NIF	1.0	smart hydrogel 2%	3.6	45.5
NIF	2.5	smart hydrogel 2%	3.6	24.1
SIL	1.0	smart hydrogel 2%	3.6	35.2
SIL	2.5	smart hydrogel 2%	3.6	147.1
DEXT	2.5	smart hydrogel 2%	4.7	80.1
MANN	2.5	smart hydrogel 2%	4.7	412.2
PROP	2.5	smart hydrogel 2%	4.7	412.1
ISDN	1.0	smart hydrogel 2%	4.7	102.6
ISDN	2.5	smart hydrogel 2%	4.7	211.0
NIF	1.0	smart hydrogel 2%	4.7	32.7
NIF	2.5	smart hydrogel 2%	4.7	75.0
SIL	1.0	smart hydrogel 2%	4.7	32.5
SIL	2.5	smart hydrogel 2%	4.7	62.3
DEXT	2.5	smart hydrogel 2%	6.0	85.9
MANN	2.5	smart hydrogel 2%	6.0	342.6
PROP	2.5	smart hydrogel 2%	6.0	148.9
ISDN	1.0	smart hydrogel 2%	6.0	75.3
ISDN	2.5	smart hydrogel 2%	6.0	155.5
NIF	1.0	smart hydrogel 2%	6.0	60.7
NIF	2.5	smart hydrogel 2%	6.0	71.6
SIL	1.0	smart hydrogel 2%	6.0	31.8
SIL	2.5	smart hydrogel 2%	6.0	81.7
DEXT	2.5	LMO	--	64.9
MANN	2.5	LMO	--	427.3
PROP	2.5	LMO	--	962.7
ISDN	1.0	LMO	--	163.1
ISDN	2.5	LMO	--	245.6
NIF	1.0	LMO	--	23.9
NIF	2.5	LMO	--	31.0
SIL	1.0	LMO	--	65.6
SIL	1.0	LMO	--	57.0
SIL	2.0	LMO	--	150.3
DEXT	2.5	HMO	--	87.7
MANN	2.5	HMO	--	517.2
PROP	2.5	HMO	--	910.4
ISDN	1.0	HMO	--	114.0
ISDN	2.5	HMO	--	178.2
NIF	1.0	HMO	--	10.9
NIF	2.5	HMO	--	31.9
SIL	1.0	HMO	--	59.8
SIL	2.0	HMO	--	150.4
DEXT	2.5	Noveon AA1 0.1%	4.7	35.2
MANN	2.5	Noveon AA1 0.1%	4.7	254.6

PROP	2.5	Noveon AA1 0.1%	4.7	940.7
ISDN	1.0	Noveon AA1 0.1%	4.7	208.8
ISDN	2.5	Noveon AA1 0.1%	4.7	295.6
NIF	1.0	Noveon AA1 0.1%	4.7	32.8
NIF	2.5	Noveon AA1 0.1%	4.7	102.5
SIL	2.5	Noveon AA1 0.1%	4.7	193.7
DEXT	2.5	Noveon AA1 0.1%	6.0	32.9
MANN	2.5	Noveon AA1 0.1%	6.0	341.3
PROP	2.5	Noveon AA1 0.1%	6.0	910.9
ISDN	1.0	Noveon AA1 0.1%	6.0	112.8
ISDN	2.5	Noveon AA1 0.1%	6.0	199.5
NIF	1.0	Noveon AA1 0.1%	6.0	25.5
NIF	2.5	Noveon AA1 0.1%	6.0	53.2
SIL	1.0	Noveon AA1 0.1%	6.0	102.2
SIL	2.5	Noveon AA1 0.1%	6.0	98.9
DEXT	2.5	ACN:H_20 (60:40)	--	48.9
MANN	2.5	ACN:H_20 (60:40)	--	538.8
PROP	2.5	ACN:H_20 (60:40)	--	209.9
ISDN	1.0	ACN:H_20 (60:40)	--	21.5
ISDN	2.5	ACN:H_20 (60:40)	--	165.9
NIF	2.5	ACN:H_20 (60:40)	--	56.2
SIL	2.5	ACN:H_20 (60:40)	--	211.3
DEXT	2.5	H_2O only	--	25.6
MANN	2.5	H_2O only	--	576.7
PROP	2.5	H_2O only	--	594.4
ISDN	2.5	H_2O only	--	498.5
ISDN	1.0	H_2O only	--	83.2
NIF	2.5	H_2O only	--	26.1
SIL	2.5	H_2O only	--	658.1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