THE RELEASE OF NICOTINE FROM CHEWING GUM FORMULATIONS

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SUMMARY

The first clinically proven nicotine replacement product to obtain regulatory approval was Nicorette® gum. It provides a convenient way of delivering nicotine directly to the buccal cavity, thus, circumventing 'first-pass' elimination following gastrointestinal absorption. Since launch, Nicorette® gum has been investigated in numerous studies (clinical) which are often difficult to compare due to large variations in study design and degree of sophistication. In order to standardise testing, in 2000 the European Pharmacopoeia introduced an apparatus to investigate the *in vitro* release of drug substances from medical chewing gum. With use of the chewing machine, the main aims of this project were to determine factors that could affect release from Nicorette® gum, to develop an *in vitro in vivo* correlation and to investigate formulation variables on release of nicotine from gums.

A standard *in vitro* test method was developed. The gum was placed in the chewing chamber with 40 mL of artificial saliva at 37°C and chewed at 60 chews *per* minute. The chew rate, the type of dissolution medium used, pH, volume, temperature and the ionic strength of the dissolution medium were altered to investigate the effects on release *in vitro*. It was found that increasing the temperature of the dissolution media and the rate at which the gums were chewed resulted in a greater release of nicotine, whilst increasing the ionic strength of the dissolution medium to 80 mM resulted in a lower release. The addition of 0.1% sodium lauryl sulphate to the artificial saliva was found to double the release of nicotine compared to the use of artificial saliva and water alone. Although altering the dissolution volume and the starting pH did not affect the release, the increase in pH may be insufficient to provide optimal conditions for nicotine absorption (since the rate at which nicotine is transported through the buccal membrane was found to be higher at pH values greater than 8.6 where nicotine is predominately unionised).

Using a time mapping function, it was also possible to establish a level A *in vitro in vivo* correlation. 4 mg Nicorette® gum was chewed at various chew rates *in vitro* and correlated to an *in vivo* chew-out study. All chew rates used *in vitro* could be successfully used for IVIVC purposes, however statistically, chew rates of 10 and 20 chews *per* minute performed better than all other chew rates.

Finally a series of nicotine gums was made to investigate the effect of formulation variables on release of nicotine from the gum. Using a directly compressible gum base, in comparison to Nicorette® the gums crumbled when chewed *in vitro*, resulting in a faster release of nicotine. To investigate the effect of altering the gum base, the concentration of sodium salts, sugar syrup, the form of the active drug, the addition sequence and the incorporation of surfactant into the gum, the traditional manufacturing method was used to make a series of gum formulations. Results showed that the time of addition of the active drug, the incorporation of surfactants and using different gum base all increased the release of nicotine from the gum. In contrast, reducing the concentration of sodium carbonate resulted in a lower release. Using a stronger nicotine ion-exchange resin delayed the release of nicotine from the gum, whilst altering the concentration of sugar syrup had little effect on the release but altered the texture of the gum.

Key words: Nicorette®, IVIVC, dissolution, smoking cessation, buccal membrane.

"Om bhur bhuva swah,
tat savitur varenyam,
bhargo devasya dheemahi,
dhiyo yo nah prachodoyat.
Om shanti, shanti, shanti"

To my loving parents

Thank you for believing in me
and encouraging me to always do the best I can,
but, most of all, thank you for your constant love.

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CONTENTS

Figure		Page
Title page		1
Summary		2
Dedication		3
Acknowledgement		4
Contents		5
List of figures		15
List of Tables		24
Abbreviations		31
CHAPTER ONE	**INTRODUCTION**	34
1.1	Use of buccal membrane for the delivery of drugs	34
1.1.1	Anatomy of buccal cavity	34
1.1.2	Routes of transport across the oral mucosa	35
1.1.3	Advantages of delivery *via* the buccal cavity	37
1.1.4	Implications for dug delivery	38
1.1.5	Saliva	40
1.1.5.1	Saliva glands	42
1.1.5.2	Composition of saliva	42
1.1.5.3	Stimulation of saliva flow	43
1.2	Nicotine	44
1.2.1	Chemical profile of nicotine	44
1.2.2	Pharmacokinetics of nicotine	45
1.2.2.1	Absorption of nicotine	45
1.2.2.2	Distribution of nicotine in body tissues	46
1.2.2.3	Elimination of nicotine	48
1.2.3	Medical consequences of nicotine	48
1.3	Smoking Cessation	49
1.3.1	Medical consequences of smoking	52
1.3.2	Nicotine replacement therapy (NRT)	53
1.4	Chewing gum	57
1.4.1	Composition of chewing gum	57

1.4.2 Gum texture 59
1.4.3 Advantages of a chewing gum formulation 60
1.4.4 Use of chewing gum as a drug delivery system 61
1.4.5 Drug release testing from chewing gum 63
1.5 Nicorette® gum 68
1.5.1 Clinical efficacy of Nicorette® gum 69
1.5.2 Pharmacokinetics of Nicorette® gum 69
1.5.3 Formulation of Nicorette® gum 71
1.5.4 Ion-exchange resin 71
1.5.4.1 The use of ion-exchange resins in pharmaceutical formulations... 75
1.5.4.2 Amberlite® IRP 64 77
1.6 Project aims and objectives 79
1.6.1 Factors affecting release of nicotine from chewing gum 80
1.6.2 *In vitro in vivo* correlation 80
1.6.3 Investigating formulation variables on the release of nicotine from the gums..... 80

CHAPTER TWO GENERAL METHODS 82
2.1 HPLC assay of nicotine 82
2.1.1 Experimental 82
2.1.1.1 Materials 82
2.1.1.2 Equipment 82
2.1.1.3 Methods 83
2.1.1.3.1 Standards 83
2.1.1.3.2 Mobile phase 83
2.1.1.3.3 Chromatographic condition 83
2.1.1.3.4 Example of nicotine chromatogram 83
2.2 HPLC method validation 84
2.2.1 Linearity 84
2.2.2 Method precision 85
2.2.3 Instrument precision 85
2.2.4 Accuracy 86
2.2.5 Limit of detection 87

2.2.6 Limit of quantification 87
2.2.7 Selectivity and specificity 88
2.3 Chewing machine 90
2.3.1 Experimental 90
2.3.1.1. Materials 90
2.3.1.2. Equipment 90
2.3.1.3. Methods 92
2.4 Dissolution apparatus 92
2.4.1 Experimental 92
2.4.1.1 Materials 92
2.4.1.2 Equipment 92
2.4.1.3 Methods 93
2.5 Sodium analysis 93
2.5.1 Experimental 93
2.5.1.1 Materials 93
2.5.1.2 Equipment 93
2.5.1.3 Method 94
2.5.1.3.1 Standards 94
2.5.1.3.2 Sample preparation 94
2.5.1.3.3 ICP AES method condition 94
2.6 Total drug loading in gum 95
2.6.1 Experimental 95
2.6.1.1 Materials 95
2.6.1.2 Equipment 95
2.6.1.3 Methods 95
2.7 pH Measurements 95
2.8 Artificial saliva 96
2.9 f_2 calculation 96
2.10 Citrate-phosphate buffer 97
2.11 Texture analysis 97
2.11.1 Experimental 97
2.11.1.1 Material 97
2.11.1.2 Equipment 98

2.11.1.3 Method 98
2.11.1.3.1 Calibration 98
2.11.1.3.2 Test 98
2.11.1.3.3 Data analysis 98
2.12 Directly compressible gums 99
2.12.1 Experimental 99
2.12.1.1 Materials 99
2.12.1.2 Equipment 99
2.12.1.3 Methods 99
2.13 Formulation of a standard nicotine gum 99
2.13.1 Experimental 100
2.13.1.1 Materials 100
2.13.1.2 Equipment 100
2.13.1.3 Method 100
2.14 Ion-exchange resins 101
2.14.1 Experimental 102
2.14.1.1 Materials 102
2.14.1.2 Methods 102
2.14.1.2.1 Loading of Amberlite® IRP69 resin 102
2.14.1.2.2 Release from nicotine Amberlite® IRP69 and Nicotine Polacrilex resin 102

CHAPTER THREE *IN VITRO* CHEWING GUM RELEASE STUDIES 103
3.1 Introduction 103
3.1.1 Factors affecting release from chewing gums 103
3.1.1.1 The active substance 103
3.1.1.2 The chewing gum 105
3.1.1.3 The individual 106
3.1.2 Release from ion-exchange resin 106
3.2 Chewing machine validation 108
3.2.1 Method 108
3.2.1.1 Number of chews *per* minute 108
3.2.1.2 Inter-day and intra-day variation in the number of chews *per*

minute 108
3.2.2 Results and discussion 109
3.2.2.1 Number of chews *per* minute 109
3.2.2.2 Inter-day and intra-day variation in the number of chews *per* minute 110
3.3 Comparison of *in vitro* release using real and artificial saliva 112
3.3.1 Method 112
3.3.1.1 Collection and preparation of human saliva 112
3.3.1.2 *In vitro* chewing 113
3.3.2 Results and discussion 114
3.4 Release from Nicorette® gum 115
3.4.1 Method 116
3.4.2 Results and discussion 116
3.5 Diffusion of nicotine through Nicorette® chewing gum 117
3.5.1 Method 117
3.5.2 Results and discussion 118
3.6 Factors affecting release from Nicorette® gum *in vitro* 120
3.6.1 Materials and Methods 120
3.6.1.1 Chew rate 120
3.6.1.1.1 Chewing machine 120
3.6.1.1.2 Release of nicotine from Nicotine Polacrilex 120
3.6.1.2 Different dissolution media 121
3.6.1.2.1 Chewing gum 121
3.6.1.2.2 Release of nicotine from Nicotine Polacrilex 121
3.6.1.2.3 Release of sodium from chewing gum 121
3.6.1.3 Temperature 121
3.6.1.3.1 Chewing gum 121
3.6.1.3.2 Release of nicotine from Nicotine Polacrilex 121
3.6.1.3.3 Texture analysis 121
3.6.1.4 pH of dissolution medium 122
3.6.1.4.1 Chewing gum 122
3.6.1.4.2 Release of nicotine from Nicotine Polacrilex 122
3.6.1.5 Volume of dissolution medium 122

3.6.1.5.1 Chewing gum 122
3.6.1.5.2 Release of nicotine from Nicotine Polacrilex 122
3.6.1.5.3 Release of sodium from the gum..... 123
3.6.1.6 Ionic strength 123
3.6.1.6.1 Chewing gum 123
3.6.1.6.2 Release of nicotine from Nicotine Polacrilex 123
3.6.2 Results and discussion 123
3.6.2.1 The effect of chew rate on the release of nicotine from gum 123
3.6.2.1.1 Effect of chew rate on the release from 2 mg Nicorette® . 124
3.6.2.1.2 Release of nicotine from Nicotine Polacrilex with different stir rates 128
3.6.2.2 The effect of dissolution media on release from resin and gum ... 131
3.6.2.2.1 Release from 2 mg Nicorette® into different dissolution media..... 132
3.6.2.2.2 Release of nicotine from Nicotine Polacrilex in different dissolution media 134
3.6.2.2.3 Release of sodium from 2mg and 4 mg Nicorette® 137
3.6.2.3 The effect of temperature on the release of nicotine from Nicorette® and Nicotine Polacrilex resin 139
3.6.2.3.1 Release of nicotine from 2 mg Nicorette® at different *in vitro* temperatures 139
3.6.2.3.2 Texture of the gums exposed to different temperatures 141
3.6.2.3.3 The effect of temperature on the release of nicotine from Nicotine Polacrilex resin..... 143
3.6.2.4 The effect of dissolution medium pH on the release from Nicorette® and Nicotine Polacrilex resin 145
3.6.2.4.1 Effect of dissolution media pH on release of nicotine from Nicorette® 145
3.6.2.4.2 Effect of pH on the release of nicotine from Nicotine Polacrilex *in vitro* 148
3.6.2.5 Effect of dissolution volume on release of nicotine from gum and Nicotine Polacrilex 150
3.6.2.5.1 Effect of dissolution media volume on release from

Nicorette® 150
3.6.2.5.2 Concentration of sodium when nicotine was released into the varying dissolution volumes 153
3.6.2.5.3 Effect of dissolution volume on the release of nicotine from Nicotine Polacrilex 154
3.6.2.6 The effect of dissolution medium ionic strength on release of nicotine from gum and Nicotine Polacrilex 156
3.6.2.6.1 Ionic strength of dissolution medium effect on release from Nicorette® 157
3.6.2.6.2 Effect of ionic strength on release of nicotine from Nicotine Polacrilex 160
3.7 *In vitro* chewing of two 2 mg Nicorette® gums 161
3.7.1 Method 162
3.7.2 Results and discussion 162
3.8 Conclusion 163

CHAPTER FOUR *IN VITRO IN VIVO* CORRELATION 165
4.1 Introduction 165
4.2 Experimental 167
4.2.1 *In vivo* chew-out study 167
4.2.1.1 Method 168
4.2.1.1.1 Study design 168
4.2.2 *In vitro* release study 169
4.2.2.1 Material 169
4.2.2.2 Method 169
4.3 Results and discussion 169
4.3.1 Salivary pH and release of nicotine during *in vivo* chew-out 169
4.3.2 *In vitro* chewing study 171
4.3.3 *In vitro in vivo* correction 176
4.4 Conclusion 183

CHAPTER FIVE NICOTINE DIFFUSION THROUGH BUCCAL MUCOSA 184

5.1 Introduction 184

5.1.1 Diffusion of nicotine 184

5.1.2 Diffusion of drug across a membrane 186

5.1.3 *In vitro* diffusion cells 190

5.1.4 Selection of mucosal membrane 192

5.2 Experimental 192

5.2.1 Materials 192

5.2.2 Method 192

5.2.2.1 Determining buccal thickness 192

5.2.2.2 Buccal membrane preparation for microscopy 193

5.2.2.3 Franz cells 193

5.2.2.4 Partitioning study 194

5.3 Results and discussion 194

5.3.1 Buccal thickness 194

5.3.2 *In vitro* diffusion of nicotine through buccal membrane 196

5.3.2.1 Diffusion of nicotine tartrate salt through buccal membrane at various pH values 197

5.3.2.2 Diffusion of nicotine from Nicotine Polacrilex resin through buccal membrane at various pH values 203

5.3.2.3 Diffusion of nicotine from nicotine Amberlite® IRP69 resin through buccal membrane at various pH values 206

5.3.2.4 Diffusion of nicotine from the various sources at the different pH values 209

5.3.3 Partitioning study 214

5.4 Conclusion 215

CHAPTER SIX INVESTIGATING FORMULATION VARIABLE ON THE RELEASE OF NICOTINE FROM GUMS 217

6.1 Introduction 217

6.1.1 Traditional manufacturing of chewing gum 217

6.1.2 Sensory and texture analysis 218
6.2 Directly compressible gum base 220
6.2.1 Method 222
6.2.1.1 Direct compression 222
6.2.1.2 Granulation 222
6.2.1.2.1 Materials 222
6.2.1.2.2 Method 222
6.2.1.3 Compression strength 223
6.2.2 Results and discussion 223
6.2.2.1 Direct compression of Pharmagum® M and S..... 223
6.2.2.2 Granulation of Pharmagum® M and S 227
6.2.2.3 Effect of compression strength of Pharmagum® M and S 232
6.2.2.4 General discussion 236
6.3 Chewing gum formulation 239
6.3.1 Materials and methods 239
6.3.1.1 Materials 239
6.3.1.2 Methods 239
6.3.2 Results and discussion 241
6.3.2.1 Batch reproducibility 241
6.3.2.2 Effect of different gum bases on release 245
6.3.2.3 Effect of different concentrations of sodium carbonate and sodium bicarbonate 250
6.3.2.4 Effect of different concentrations of sugar 259
6.3.2.5 Effect of addition of surfactants 263
6.3.2.6 Effect of different forms of active ingredients 268
6.3.2.7 Effect of time of addition of active drug 272
6.3.2.8 Comparisons of all dissolution profiles 275
6.4 Conclusion 281

CHAPTER SEVEN GENERAL SUMMARY 282
7.1 Factors affecting release from chewing gum 284
7.2 *In vitro in vivo* correlation 287
7.3 Investigation of formulation variables on release from gums 287

7.4 General conclusion 290

References 292

Appendices 301

Appendix 1 HPLC method validation 301

A1.1 Linearity 301

A1.2 Method precision 302

A1.3 Instrument precision 302

A1.4 Accuracy 303

A1.5 Limit of detection 303

A1.6 Limit of quantification 304

A1.7 Selectivity and specificity 305

Appendix 2 Nicotine content of resins 306

A2.1 Nicotine Polacrilex 306

A2.2 Nicotine Amberlite® IRP69 306

Appendix 3 *In vivo* study population 307

A3.1 Inclusion criteria for subjects 307

A3.2 Exclusion criteria 308

LIST OF FIGURES

Figure		Page
1.1	Anatomy of the oral cavity	34
1.2	Cross section of buccal membrane	34
1.3	Schematic showing the trancellular and paracellular routes of drug transport	36
1.4	locations of the parotid, submandibular and sublingual salivary glands	42
1.5	Nicotine $C_{10}H_{14}N_2$ MW 162.23	44
1.6	Blood nicotine concentrations during and after cigarette smoking, oral snuff, chewing tobacco and nicotine gum	46
1.7	Chemical structure of nicotine and major pathways of nicotine metabolism	48
1.8	Number of cigarettes smoked *per* day *per* daily smoker	51
1.9	Texture profile of Liberty bubble gum base at 39 °C using the texture analyser	59
1.10	Texture ranges of commercially available Wrigley gum using the texture analyser	60
1.11	A cross sectional diagram of the release chamber of the chewing machine constructed by Christrup and Moller	65
1.12	Chewing gum machine developed by Rider *et al.*	66
1.13	Technical drawing of the chewing apparatus showing the six chewing modules	66
1.14	Detail of one chewing module	67
1.15	Illustration of how Nicorette® chewing gum should be chewed	68
1.16	Schematic illustrations of nicotine concentrations obtained from single dose administration of nicotine reduction medications and smoking	70
1.17	Resulting structure after polymerisation of the unsaturated monomer CH_2=CHX undergoing a chain reaction	71
1.18	A divinyl compound	72
1.19	Co-polymerisation of styrene and divinyl benzene sulphonated with concentrated sulphuric acid to produce a strongly acidic cationic resin	72
1.20	Chemical structure of Amberlite® IRP64	79

2.1	Typical Nicotine HPLC chromatogram at a concentration of 21.0 µg/mL	84
2.2	Chromatograph illustrating the parameters for calculation of N, and K'	89
2.3	A schematic of the EP chewing apparatus	90
2.4	EP chewing apparatus used for the *in vitro* dissolution of medicated chewing gum	91
2.5	QTS 25 texture analyser	98
2.6	Gum kettle with S" shaped blade used to produce and mix small scale batches of chewing gum	100
2.7	Rolling, scoring and cutting of the formulated gums into 1g pieces	101
3.1	The mode number of chews on the various interval timer settings	109
3.2	4 mg Nicorette® release profile in real and artificial saliva	114
3.3	4 mg Nicorette® pH profile in real and artificial saliva	115
3.4	Release of nicotine from 2 mg and 4 mg Nicorette® in artificial saliva	116
3.5	pH of the artificial saliva during release of nicotine from 2 mg and 4 mg Nicorette®	117
3.6	Nicotine release from 2 and 4 mg Nicorette® gum without mastication	118
3.7	Percentage nicotine release from 2 and 4 mg Nicorette® gum without mastication	119
3.8	Nicotine release from 2 mg Nicorette® chewed *in vitro* at 82, 60, 42, 22, 12, 6 and 4 chews/minute	125
3.9	Profiles with same release rate but different original starting concentration	127
3.10	Profiles with same original starting concentration but different release rates	127
3.11	pH of the artificial saliva as nicotine was released from 2 mg Nicorette® when chewed *in vitro* at 82, 60, 42, 22, 12, 6 and 4 chews/minute	129
3.12	Release of nicotine from Nicotine Polacrilex resin *in vitro* with effect of paddle stirring rate	130

3.13 Release of nicotine *in vitro* from Nicorette® 2 mg gum in various dissolution media 133

3.14 pH of the artificial saliva as nicotine was released *in vitro* from Nicorette® 2 mg gum in various dissolution media 134

3.15 Release of nicotine from Nicotine Polacrilex *in vitro* using various dissolution media 135

3.16 Release of nicotine from Nicotine Polacrilex in 0.1% Tetradecyl trimethyl ammonium bromide demonstrating diffusion controlled release 136

3.17 Sodium ion concentration when 2 mg and 4 mg Nicorette® gum are chewed *in vitro* in water and artificial saliva 138

3.18 Nicotine release from 2 mg Nicorette® *in vitro* when chewed in artificial saliva at 23°C, 37°C and 53°C 140

3.19 pH of the artificial saliva as nicotine was released from 2 mg Nicorette® *in vitro* when chewed in artificial saliva at 23°C, 37°C and 53°C 141

3.20 Time versus load graph of 2 mg Nicorette® exposed to different temperatures 142

3.21 Release of nicotine from Nicotine Polacrilex *in vitro* at various temperatures of the dissolution medium 144

3.22 Release of nicotine from 2 mg Nicorette® *in vitro* using artificial saliva buffered at pH 3.0, 5.0. 7.0 and 9.0 146

3.23 pH of the artificial saliva as nicotine was released from 2 mg Nicorette® *in vitro* using artificial saliva buffered at pH 3.0, 5.0. 7.0 and 9.0 147

3.24 Release of nicotine from Nicotine Polacrilex *in vitro* with effect of dissolution medium pH 148

3.25 Release of nicotine from 2 mg Nicorette® gum *in vitro* in different volumes of dissolution medium 151

3.26 pH of artificial saliva as nicotine was released from 2 mg Nicorette® gum *in vitro* with varying dissolution volume 152

3.27 Sodium released from 2 mg Nicorette® when chewed *in vitro* in 20 mL, 40 mL and 80 mL of artificial saliva 153

3.28	Concentration of sodium ions released from the chewing gum in different volumes of dissolution media	154
3.29	The effect of dissolution volume on the release of nicotine from Nicotine Polacrilex	155
3.30	The effect of ionic strength on nicotine release from 2 mg Nicorette®	157
3.31	Effect of ionic strength on pH of dissolution medium as nicotine was released from 2 mg Nicorette® *in vitro*	159
3.32	Increase in ionic strength with effect of increasing pH as 2 mg Nicorette® was chewed *in vitro*	159
3.33	Effect of ionic strength on the release of nicotine released from Nicotine Polacrilex resin	160
3.34	Nicotine release from 2 mg, two x 2 mg and 4 mg Nicorette®	163
4.1	Salivary pH before and after each gum chewing session	170
4.2	Percentage nicotine released from Nicorette® gum during *in vivo* chew-out	170
4.3	Saliva pH change and saliva pH with proportional nicotine release	171
4.4	Release of nicotine from project 4 mg Nicorette® chewing gum at various chew rates	173
4.5	pH of artificial saliva as nicotine was released from project 4mg Nicorette® chewing gum at the various chew rates	174
4.6	pH change of artificial saliva as the 4 mg Nicorette® gum was chewed at the various chew rates	175
4.7	*In vitro* and *in vivo* release of nicotine from 4 mg Nicorette®	176
4.8	pH of artificial saliva and human saliva as 4 mg Nicorette® was chewed *in vitro* and *in vivo* respectively	177
4.9	Correlation between *in-vivo* release and *in-vitro* release at the various chew rates	178
4.10	Effect of increasing time scale factor from 2.0 to 5.0 at various values of bend point whilst shape scale factor remains constant	179
4.11	Effect of increasing time shape factor from 1.0 to 5 at various value of bend point whilst time scale factor remains constant	180
4.12	*In vitro in vivo* correlation	181
4.13	An example of IVIVC with corresponding times	182

4.14	A nomogram for converting *in vitro* times to *in vivo* times	183
5.1	Molecular structure of nicotine at the two pKa	186
5.2	Fractions of nicotine species as a function of pH	186
5.3	Typical diffusion profile of drug diffusion through a membrane	187
5.4	Concentration profile across homogenous membrane at steady-state zero-order flux	188
5.5	Schematic representation of a vertical Diffusion cell	191
5.6	Light micrograph of porcine buccal mucosa fixed with glutaraldehyde and osmium tetroxide and imbedded in Spurr's resin ..	195
5.7	Photomicrograph of porcine buccal mucosa (x 100 magnification)	196
5.8	Initial diffusion of nicotine hydrogen tartrate salt at various pHs across buccal membrane	198
5.9	The flux of the nicotine diffusion as the percentage of unionised nicotine increased	199
5.10	Diffusion of nicotine hydrogen tartrate salt at various pHs across buccal membrane	200
5.11	*In vitro* diffusion of nicotine through buccal mucosa using nicotine hydrogen tartrate salt at pH 8.6	202
5.12	Initial diffusion of nicotine from Nicotine Polacrilex various pHs across buccal membrane	204
5.13	Diffusion of nicotine from Nicotine Polacrilex at various pHs across buccal membrane	205
5.14.	The flux of nicotine diffusion from Nicotine Polacrilex as a percentage of unionised nicotine	206
5.15	Initial diffusion of nicotine from nicotine Amberlite® IRP69 at various pHs across buccal membrane	207
5.16	Diffusion of nicotine from nicotine Amberlite® IRP69 at various pHs across buccal membrane	208
5.17	The flux of nicotine diffusion from nicotine Amberlite® IRP69 as a percentage of unionised nicotine	208
5.18	The diffusion of nicotine from nicotine tartrate salt, Nicotine Polacrilex and nicotine Amberlite® IRP69 across the buccal membrane at pH 2.2, pH 5.4, pH 7.0 and pH 8.6	210

5.19 The permeation profiles of nicotine through various oral mucosae at pH 8.8 212

5.20 Comparison of nicotine permeation through the non-keratinised mucosa under different pH conditions 213

5.21 Partitioning of nicotine as a function of pH in buccal mucosa 214

5.22 Linear relationship demonstrated by nicotine in buccal mucosa as expected from the pH partition theory 215

6.1 Addition of various solubilisers to control the release of the active substance 218

6.2 Example of taste profile 219

6.3 Change of gum properties during chewing 219

6.4 Compactibility of Pharmagum® S compared to other excipients when compressed using a 15mm Flat Face "F" at a compression speed of 10 mm/minutes 221

6.5 Compressibility of Pharmgum® S compared to other excipients 221

6.6 Release of nicotine from 4 mg Pharmagum® M and S formulations and 4 mg Nicorette® gum 224

6.7 pH of artificial saliva as 4 mg Pharmagum® M and S formulations and 4 mg Nicorette® gum are chewed *in vitro* 225

6.8 Load encountered as a 2 mm diameter probe penetrates 4 mg Nicorette® gum 226

6.9 Load encountered as a 2 mm probe penetrates 4 mg Pharmagum® M and Pharamgum® S formulated gums 226

6.10 Release of nicotine from 4 mg Pharmagum® M and S formulations with effect of granulation of ingredients 228

6.11 pH of artificial saliva as Pharmagum® M and S gums formed using different sized granules were chewed *in vitro* 229

6.12 Load encountered as a 2 mm diameter probe penetrates Pharmagum® M gums made using 1.4 mm granules and 1.7 mm granules 230

6.13 Load encountered as a 2 mm diameter probe penetrates Pharmagum® S gums made from 710 μm granules and 1.4 mm granules 230

6.14 Release of nicotine from 4 mg Pharmagum® M and S formulations compressed with a 1 ton and 5 ton force 233

6.15 pH of artificial saliva as Pharmagum® M and S compressed with a 1 ton and 5 ton compression force were chewed *in vitro* 234

6.16 Load encountered as a 2 mm diameter probe penetrated Pharmagum® M and Pharmagum® S formulated nicotine gums compressed at a pressure of 1 ton 234

6.17 Load encountered as a 2 mm diameter probe penetrated Pharmagum® M and Pharmagum® S formulated nicotine gums compressed at a pressure of 5 ton 235

6.18 Release of nicotine from the standard nicotine gum formulation (Bt 5) and the replicates (Bt 5a and 5b) 241

6.19 pH of the artificial saliva as the standard nicotine gum batch (Bt 5) and the replicated batches (Bt 5a and Bt 5b) were chewed *in vitro* 243

6.20 Load encountered as a 2 mm diameter stainless steel probe penetrated the standard nicotine gum formulation, batch 5, and the replicate batches, 5a and 5b to a depth of 3 mm at 30 mm/minute 244

6.21 Release of nicotine from formulated gums using different gum bases . 246

6.22 pH of the artificial saliva as formulated nicotine gum using different gum bases were chewed *in vitro* 247

6.23 Load encountered as a 2 mm diameter stainless steel probe penetrated batches 11, Dreyco base, 16, Blacktree and 18 Magna T bubble gum base to a depth of 3 mm at 30 mm/minute 248

6.24 Release of nicotine from formulated gums with effect of increasing and decreasing sodium carbonate concentration within the gum 251

6.25 pH of the artificial saliva as nicotine was release from formulated gum with increased and decreased concentration of sodium carbonate 252

6.26 Load encountered as a 2 mm diameter stainless steel probe penetrated batches 7, 8, and 9 to a depth of 3 mm at 30 mm/minute 253

6.27 Release of nicotine from Bt 14, a 2 mg gum with incorporated 30 mg sodium carbonated and Bt 15, a 4 mg gum with incorporated 20 mg sodium carbonate and 10 mg sodium bicarbonate 256

6.28 pH of artificial saliva as batch 14, a 2 mg gum with incorporated 30 mg sodium carbonate and Bt 15, a 4 mg gum with incorporated 20 mg sodium carbonate and 10 mg sodium bicarbonate were chewed *in*

vitro 257

6.29 Texture profile of batch 14 (a) and 15 (b) as a 2 mm diameter stainless steel probe penetrated the gum to a depth of 3mm 258

6.30 Release of nicotine from formulations with different concentrations of sugar syrups (n =3 ± SD) 260

6.31 pH of artificial saliva as Bt 6, gums with 1.0% w/w sugar syrup, Bt 10 gums with 5.5% w/w sugar syrup, Bt 8 gums with 1.0% w/w sugar syrup and 1.0% w/w sodium carbonate and Bt 5 gums with 2.5% sugar syrup were chewed *in vitro* 261

6.32 Texture analysis profile of Bt 6 and 10 as a 2 mm diameter stainless steel probe penetrated the gums to a depth of 3 mm at 30 mm/min 262

6.33 Release of nicotine with the addition of 0.1% w/w Tween 80 to the chewing gum formulations 264

6.34 pH of artificial saliva as nicotine was released from formulated gum with added Tween 80 265

6.35 Texture profiles of Bt 1, Bt 2, Bt 3 and Bt 4 as a 2 mm diameter stainless steel probe penetrated the gums to a depth of 3 mm at 30 mm/min 267

6.36 Release of nicotine from chewing gum containing Nicotine Polacrilex (Bt 5), nicotine Amberlite® IRP69 (Bt 12) and nicotine hydrogen tatrate salt (Bt 13) 268

6.37 pH of the artificial saliva as nicotine from gums containing Nicotine Polacrilex (Bt 5), nicotine Amberlite® IRP69 (Bt 12) and nicotine hydrogen tatrate salt (Bt 13) were chewed *in vitro* 270

6.38 Texture analysis of batch 12 (a) and batch 13 (b) as a 2 mm diameter stainless probe penetrated the gum to a depth of 3 mm at a rate of 30 mm/min 270

6.39 Release of nicotine with the effect of time of addition of the active into the gum formulation 272

6.40 pH of artificial saliva as nicotine was released from Bt 17, gum with nicotine added earlier during the formulation process, Bt 17a, gums with nicotine added later in the formulation process and Bt 5, the standard formulation process 273

6.41 Texture analysis as a 2 mm diameter stainless probe penetrated Bt 17 (nicotine added earlier in the formulation process) and, Bt 17a (nicotine added later in the formulation process) to a depth of 3 mm at a rate of 30 mm/min 274

6.42 Dendrogram using all formulation descriptors and release at t=5, t=15 and t to 50% release 280

A1.1 Nicotine calibration curve 301

A1.2 Calibration curve of nicotine 303

LIST OF TABLES

Table		Page
1.1	Comparison of buccal, nasal and transdermal drug delivery	38
1.2	Available drug products for buccal and/or sublingual use	39
1.3	Constituents of adult human saliva	43
1.4	Flow of parotid saliva in relation to age, sex and stimulation	44
1.5	Human pharmacokinetics of nicotine and cotinine	47
1.6	Distribution of nicotine	47
1.7	Percentage daily smoking prevalence, men and women aged 15 and over, selected regions, early 1990s	50
1.8	Tobacco use is a known or probable cause of death from the conditions listed	52
1.9	Nicotine patch products	55
1.10	Oral nicotine products	56
1.11	Non-nicotine smoking cessation products	56
1.12	Typical chewing gum formulation	57
1.13	Chemical and physical properties of Amberlite® IRP 64	78
2.1	Artificial saliva formulation	96
2.2	Composition of citrate-phosphate buffer at various pH	97
2.3	Standard nicotine gum formulation using directly compressible gum base	99
2.4	Standard nicotine gum formulation *per* gum	101
3.1	Number of chews *per* minute on the different interval timer settings ..	110
3.2	Analysis of variance	110
3.3	Inter-day Tukey HSD,dependent variable: rate	111
3.4	f_2 values indicating similar and dissimilar dissolution curves with effect to chew rate on the release of nicotine from 2 mg Nicorette® ...	126
3.5	f_2 values indicating similar and dissimilar dissolution curves for the effect of paddle speed on the release of nicotine from Nicotine Polacrilex resin	131
3.6	f_2 values indicating similar and dissimilar dissolution curves with the effect of dissolution medium on the release of nicotine from 2 mg Nicorette®	133

3.7	f_2 values indicating similar and dissimilar dissolution curves in different dissolution media for release of nicotine from Nicotine Polacrilex resin	137
3.8	f_2 values indicating similar and dissimilar dissolution curves with the effect of dissolution medium temperature on the release of nicotine from Nicorette® 2 mg	140
3.9	The hardness, adhesive force and load encountered *per* unit time of the gums placed at 4°C, 27°C and 50°C	143
3.10	f_2 values indicating similar and dissimilar dissolution curves for the release of nicotine from Nicotine Polacrilex resin using different temperatures	144
3.11	f_2 values indicating similar and dissimilar dissolution curves for nicotine released in dissolution media of different pH	147
3.12	f_2 values indicating similar and dissimilar dissolution curves for nicotine released from Nicotine Polacrilex resin in dissolution media at different pH	149
3.13	f_2 values indicating similar and dissimilar dissolution curves for the release of nicotine from Nicorette® gum in different volumes of dissolution medium	151
3.14	f_2 values indicating similar and dissimilar dissolution curves for the release of nicotine from Nicotine Polacrilex into different volumes	155
3.15	f_2 values indicating similar and dissimilar dissolution curves for the release of nicotine in different ionic strength	158
3.16	f_2 values indicating similar and dissimilar dissolution curves with the effect of ionic strength on the release of nicotine from Nicotine Polacrilex	161
4.1	Definition of *in vivo* study design	167
4.2	f_2 values for project 4 mg Nicorette® chewing gum	172
4.3	Parameter values for time conversion corresponding to different chew rates in chewing machine	181
5.1	Thickness of buccal membrane	195
5.2	percentage of nicotine species present in solution at pH 2.2, 5.4, 7.0 and 8.6	197

5.3	Flux of nicotine hydrogen tartrate salt diffusion through buccal membrane at the various pH values	199
5.4	Flux of nicotine hydrogen tartrate salt diffusion through buccal membrane at the various pH values later in the diffusion process	200
5.5	First-order rate constants of nicotine diffusion at pH 8.6 using nicotine hydrogen tartrate salt	201
5.6	Amount of nicotine species present in 20 mL of donor solution at pH 2.2, 5.4, 7.0 and 8.6	203
5.7	Flux of nicotine diffusion from Nicotine Polacrilex through buccal membrane at the various pH values	204
5.8	Flux of nicotine diffusion from Nicotine Polacrilex through buccal membrane at the various pH values during 8 hours	205
5.9	Flux of nicotine diffusion through buccal membrane at the various pH values using nicotine Amberlite® IRP69	207
5.10	Flux of nicotine diffusion through buccal membrane at the various pH values using nicotine Amberlite® IRP69 during 8 hours	209
5.11	Flux and amount of nicotine diffused from the various nicotine sources through buccal membrane at pH 2.2, 5.4, 7.0 and 8.6 after 480 minutes	211
5.12	Specific permeability of various nicotine species across selected oramucosae	212
5.13	Specific permeability, partition coefficient and diffusivity of various nicotine species through the non-keratinised buccal mucosa	213
6.1	Characteristics of Pharmagum® S and M	220
6.2	Pharamgums® S and M granules formulae	223
6.3	Hardness, adhesive force and load encountered by the gums *per* unit time for 4 mg Nicorette® and Pharmagum® M and S.....	227
6.4	Hardness, adhesive forces and load encountered *per* unit time for Pharmagum® M and S gums formed from granules	231
6.4a	Significance levels for hardness, adhesive force and load *per* unit time measurements of Nicorette®, and gums formed using Pharmagum® M 1.7 and 1.4 mm granules and Pharmagum® S 1.4 mm and 710μm granules	232

6.5	Hardness, adhesive forces and load encountered by the gum *per* unit time for Pharmagum® M and S gums compressed at 1 ton and 5 ton..	235
6.5a	Significance levels of the hardness, adhesive force and load *per* unit time measurements of Nicorette®, and Pharmagum® M and S compressed at 1 and 5 ton	236
6.6	f_2 values highlighting similar and dissimilar dissolution profiles of different Pharmagum® M and S formulation and Nicorette® gum	238
6.7	Nicotine gum formulations	240
6.8	Concentrations of surfactant, sodium carbonate and maltitol liquid to be added to formulation	239
6.8a	Quantities of surfactants, sodium carbonate and sugar syrup	239
6.9	Concentrations of nicotine added to batches 12 and 13	241
6.10	f_2 values highlighting the similar and dissimilar dissolution profiles of the standard (Bt 5) and reproducible batches (Bt 5a and 5b)	242
6.11	Hardness, adhesive forces and load encountered *per* unit time for the standard nicotine gum batch Bt 5, and the replicates, Bt 5a and Bt 5b .	245
6.11a	Significance levels for hardness, adhesive force and load *per* unit time of the standard nicotine gum Bt 5, and the replicates, Bt 5a and Bt 5b	245
6.12	f_2 values highlighting the similar and dissimilar dissolution profiles of the nicotine gums formulated using different gum bases	247
6.13	Hardness, adhesive forces and load encountered *per* unit time for gums made using the standard gum base (Bt 5), Dreyco base (Bt 11), Blacktree base (Bt 16) and Magna T bubble gum base (Bt 18).....	249
6.13a	Significance level for the hardness, adhesive force and load *per* unit time of gums made using the standard gum base (Bt 5), Dreyco base (Bt 11), Blacktree base (Bt 16) and Magna T bubble gum base (Bt 18).....	249
6.14	f_2 values highlighting the similar and dissimilar dissolution profiles of the nicotine gums formulated using different concentrations of sodium carbonate	252
6.15	Hardness, adhesive forces and load *per* unit time measurements of nicotine gum batches formulated using different concentrations of	

sodium carbonate..... 254

6.15a Significance levels for the hardness, adhesive force and load *per* unit time of gums made using different concentrations of sodium carbonate..... 254

6.16 f_2 values highlighting the similar and dissimilar dissolution profiles of Bt 14, a 2 mg nicotine gum with incorporated 30 mg sodium carbonate and Bt 15 a 4 mg gum with incorporated 20 mg sodium carbonate and 10 mg sodium bicarbonate 256

6.17 Hardness, adhesive forces and load *per* unit time of nicotine gum Bt 14, a 2 mg gums with incorporated 30 mg sodium carbonate and Bt 15, a 4 mg gum with incorporated 20 mg sodium carbonate and 10 mg sodium bicarbonate..... 258

6.17a Significance levels for the hardness, adhesive force and load *per* unit time of Bt 14, a 2 mg gums with incorporated 30 mg sodium carbonate and Bt 15, a 4 mg gum with incorporated 20 mg sodium carbonate and 10 mg sodium bicarbonate..... 259

6.18 f_2 values highlighting the similar and dissimilar dissolution profiles of Bt 6, gums with 1.0% w/w sugar syrup, Bt 10 gums with 5.5% w/w sugar syrup, Bt 8 gums with 1.0% w/w sugar syrup and 1.0% w/w sodium carbonate and Bt 5 gums with 2.5% sugar syrup 260

6.19 Hardness, adhesive forces and load *per* unit time of nicotine gum batches 5, 6, 8 and 10..... 262

6.19a Significance levels for the hardness, adhesive force and load *per* unit time measurements of Bt 6, gums with 1.0% w/w sugar syrup, Bt 10 gums with 5.5% w/w sugar syrup, Bt 8 gums with 1.0% w/w sugar syrup and 1.0% w/w sodium carbonate and Bt 5 gums with 2.5% sugar syrup 263

6.20 f_2 values highlighting the similar and dissimilar dissolution profiles of Bt 1, Bt 2, Bt 3, Bt 4 and Bt 5 264

6.21 Hardness, adhesive forces and load *per* unit time measurements of nicotine gum of Bt 1, standard gums formulated with surfactant, Bt 2, standard gum formulation with 1.0 % w/w sugar syrup and surfactant, Bt 3, standard gum with 1.0% sodium carbonate and surfactant, Bt 4,

standard formulation with 1.0% w/w sodium carbonate, 1.0% w/w sugar syrup and surfactant and Bt 5 the standard gum formulation..... 266

6.21a Significance levels for the hardness, adhesive force and load *per* unit time of Bt 1, standard gums formulated with surfactant, Bt 2, standard gum formulation with 1.0 % w/w sugar syrup and surfactant, Bt 3, standard gum with 1.0% sodium carbonate and surfactant, Bt 4, standard formulation with 1.0% w/w sodium carbonate, 1.0% w/w sugar syrup and surfactant and Bt 5 the standard gum formulation..... 266

6.22 f_2 values highlighting the similar and dissimilar dissolution profiles of gums formulated using Nicotine Polacrilex (Bt 5), nicotine Amberlite® IRP69 (Bt 12) and nicotine hydrogen tartrate salt (Bt 13) 269

6.23 Hardness, adhesive forces and the load encountered *per* unit time of nicotine gums Bt 5, gums with incorporated Nicotine Polacrilex, Bt 12, gums with incorporated nicotine Amberlite® IRP69 and Bt 13, gums with incorporated nicotine hydrogen tartrate salt..... 271

6.23a Significance levels for the hardness, adhesive force and load *per* unit time of Bt 12, gums with incorporated nicotine Amberlite® IRP69, Bt 13, gums with incorporated nicotine hydrogen tartrate salt and Bt 5, gums with incorporated Nicotine Polacrilex..... 271

6.24 f_2 values highlighting the similar and dissimilar dissolution profiles of gums formulated when nicotine was added earlier in the formulation process (Bt 17) and later in the formulation process (Bt 17a) in comparison to the standard formulation process (Bt 5) 273

6.25 Hardness, adhesive forces and load *per* unit time of nicotine gum Bt 17, gum with nicotine added earlier during the formulation process, Bt 17a, gums with nicotine added later in the formulation process and Bt 5, the standard formulation process..... 274

6.25a Significance levels for the hardness, adhesive force and load *per* unit time of Bt 17, gum with nicotine added earlier during the formulation process, Bt 17a, gums with nicotine added later in the formulation process and Bt 5, the standard formulation process..... 275

6.26 f_2 values indicating similarities and differences in the release from the chewing gum formulations 277

6.27 Polynomial coefficients and time to 50% release for each gum batch . 279

A1.1 Nicotine Linear range 301
A1.2 Method Precision 302
A1.3 Instrument precision 302
A1.4 Recovery 303
A1.5 Limit of detection and quantification 304
A1.6 Limit of quantification 304
A1.7 Number of theoretical plates (N), the height equivalent of the theoretical plate (HETP) and column capacity (K') of a nicotine peak 305
A2.1 Nicotine content of Nicotine Polacrilex resin 306
A2.2 Nicotine content of nicotine Amberlite® IRP69 resin 306

ABBREVIATIONS

AS	Artificial saliva
Bt	Batch
cm	Centimetre
Conc^{n}	Concentration
df	Degrees of freedom
ETS	Environmental tobacco smoke
eq	Equivalent
et al	And others
EP	European Pharmacopoeia
FDA	Food and Drug Administration
GSK	GlaxoSmithKline
g	grams
hr	hour
HCl	Hydrochloric acid
HPLC	High performance liquid chromatography
ICH	International Conference on Harmonisation
ICP AES	Inductive coupled plasma atomic emission spectrometer
I.V	Intra venous
IVIVC	*In vitro in vivo* correlation
Kg	Kilogram
Kw	Kilowatts
L	Litres
LOD	Limit of detection
LOQ	Limit of quantification
mEq	milliequivalent
mg	milligram
min	minutes
mL	Millilitre
mm	millimetres
mM	Millimolar
M	Molar
MHC	Moisture holding capacity

MW	Molecular weight
n	number of observations
ng	nanogram
nm	nanometre
N	Newtons
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NRT	Nicotine replacement therapy
OR	Odds ratio
OTC	Over the counter
ppm	Parts *per* million
pKa	Log_{10} dissociation constant
PVP	Polyvinylpyrrolidone
rpm	Revolutions *per* minute
r^2	Correlation coefficient
RSD	Relative standard deviation
s	Seconds
Std	Standard
SD	Standard deviation
SLS	Sodium lauryl sulphate
t	Time
T_{max}	Time to maximum concentration
TTAB	Tetradecyl trimethyl ammonium bromide
UK	United Kingdom
US	United States
USP	United States Pharmacopoeia
vs.	Verses
v/v	volume *per* volume
w/v	weight *per* volume
w/w	weight *per* weight
WHO	World health organisation
°C	Degrees Celsius
µg	Microgram
µmoles	Micromoles

µm	Micrometer
<	Less than
>	More than
%	Percentage
±	Plus or minus

CHAPTER ONE
INTRODUCTION

1.1 Use of buccal membrane for the delivery of drugs

1.1.1 Anatomy of the buccal cavity

All coverings and lining tissues of the body consist of a surface epithelium supported by fibrous connective tissue. The epithelium is well adapted to protect the underlying tissues and organs against mechanical and chemical insults, whilst the connective tissue is an extensive matrix of cells that provide mechanical support and nutrients for the epithelium. The oral cavity is lined by a permeable mucous membrane with underlying connective tissue. Like the skin, the buccal mucosa consists of stratified squamous epithelium supported by connective tissue and lamina propria (Figure 1.1 and Figure 1.2) (Prime, 1989).

Figure 1.1 Anatomy of the oral cavity (Washington, *et al.*, 2001)

Figure 1.2 Cross section of buccal membrane (Washington, *et al.*, 2001)

Although the lining mucosa of the oral cavity is covered by stratified squamous epithelium, three different types of oral mucosa are recognised, masticatory, lining and specialised mucosas. Masticatory mucosa covers gingiva and hard palate. This type of oral mucosa is subject to mechanical forces of mastication such as shearing and abrasions. Lining mucosa: covers the remaining regions, except the dorsal surface of the tongue and provides an elastic, deformable surface capable of stretching with movements such as mastication and speech.

The specialised mucosa has characteristics of both the masticatory and lining mucosa and is found on the dorsum of the tongue

The various types of oral mucosa differ in their relative surface area in the oral cavity. Measurements made by Collins and Dawes, (1987), showed that masticatory mucosa accounts for 25% of the oral mucosa, specialised mucosa, 15% and the lining mucosa, 60%. Both the structure and the relative surface area of the different types of mucosa will influence the delivery of substances across the oral lining.

1.1.2 Routes of transport across the oral mucosa

The fundamental function of the oral mucosa is to produce an effective barrier to potentially harmful substances. This is achieved by presence of an intact stratified squamous epithelium similar to that of skin. However, unlike the skin, the oral mucosa has a moist surface due to the presence of saliva. This increases the permeability of the mucosa as a result of surface hydration (Squier and Johnson, 1975).

Drugs cross the mucosal membrane by either passive or by active processes. The cellular structure of the oral mucosa suggests that there are two permeability barriers. The intercellular spaces and cytoplasm are essentially hydrophilic in character and become a transport barrier for lipophilic compounds. In contrast, the cell membrane is lipophilic and penetration of a hydrophilic compound into the cell membrane is low due to a low partition coefficient (Gibaldi and Kanig, 1965). The existence of the hydrophilic and lipophilic regions in the oral mucosa suggests that there are two routes for drug transport, the paracellular and the transcellular routes (Figure 1.3). All compounds can use these two routes simultaneously, except, one route is usually preferred over the other depending on the physicochemical properties of the drug.

The paracellular route is the primary route for hydrophilic compounds as it is difficult for a hydrophilic compound to penetrate into the lipophilic cell membrane. The flux of drug movement in this route (J_H) can be written as shown by equation 1.1

$$J_H = (D_H \varepsilon / h_H)\, C_D \qquad \text{equation 1.1}$$

Where ε is the fraction of surface area of the paracellular route, D_H is the diffusion coefficient in the intercellular spaces, h_H is the path length of the paracellular route and C_D is the donor drug concentration.

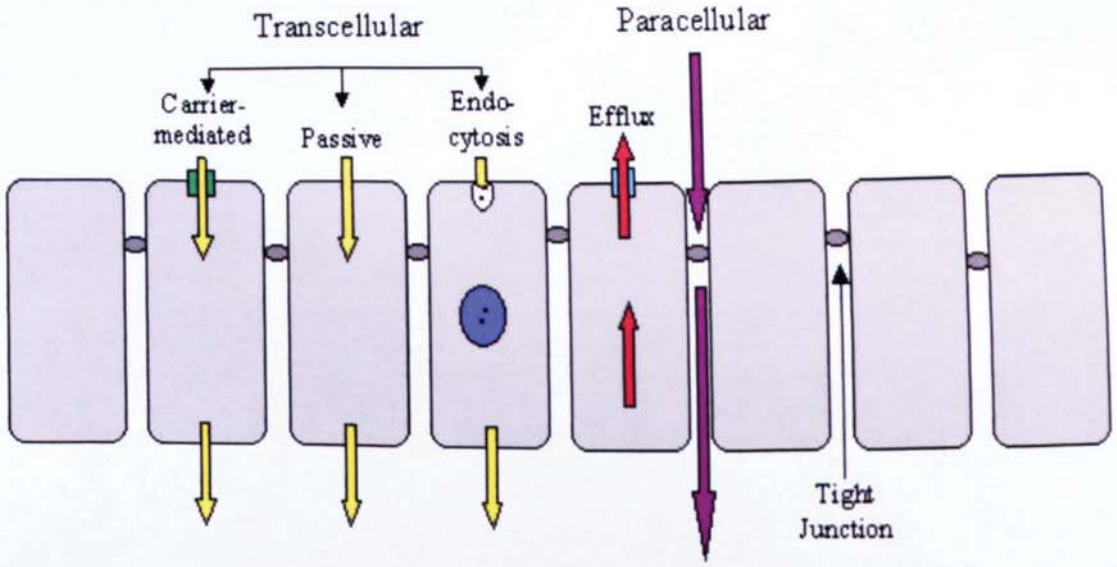


Figure.1.3 Schematic showing the transcellular and paracellular routes of drug transport.

The surface area for diffusion of lipophilic compounds by the transcellular route is large, the partition coefficients are high and the path length for the movement is relatively short. The drug molecules have to move across lipophilic cell membranes and hydrophilic cytoplasm as well as intercellular space. Drug movement through the cytoplasm and intercellular space is relatively rapid hence; the main resistance of this route is presented by the cell membrane. Therefore, the drug flux in the transcellular route (J_L) is expressed by equation 1.2

$$J_L = [(1-\varepsilon)\, D_L\, K_{p/}\, h_L]\, C_D \qquad \text{equation 1.2}$$

Where K_p is the partition coefficient between the lipophilic region and hydrophilic region and h_L is the path length of the transcellular route.

Simple diffusion of the drug through the lipid phase can occur. Here, the absorption pathway is based on the random motion of molecules from a zone of high concentration to a zone of low concentration. At first, this is a rapid process as the concentration gradient is high, however, as the concentration gradient diminishes, the rate decreases thus, rate of penetration is directly proportional to the concentration of the substance.

Active transport is where metabolic energy is required to transport molecules or ions against a concentration or electrochemical gradient. However, this mechanism is unlikely to be involved in the mouth (Lamey and Lewis, 1990). The pertinent factors that determine a drugs ability to penetrate the oral mucosa are its physical and chemical properties. Generally, unionised molecules are absorbed more readily than ionised, thus, the degree of ionisation of the drug is dependent on the pKa and the environmental pH of saliva, as they will determine the degree of ionisation. Small molecules are absorbed more readily than larger molecules and the ability of the drug to dissolve in either non-polar (lipid) or polar (aqueous) solvent is also a major factor (Lamey and Lewis, 1999).

1.1.3 Advantages of delivery *via* the buccal cavity

There are many advantages of delivering drugs *via* the buccal cavity. The buccal route offers the possibility of circumventing the hepatic 'first-pass' elimination that may follow gastrointestinal absorption. It eliminates the exposure of the drug to the acid or digestive enzyme-mediated degradation of the GI fluid; furthermore, there is no delay in the absorption attributable to the presence of food or gastric disease (Beckett and Hossie 1971). Also, there are few proteolytic enzymes, compared to peroral administration and, in addition, the buccal mucosa is highly vascularised (Veuillez *et al.*, 2001). The mucosal lining of the oral cavity has been reported to be more permeable to drugs than the skin (Lecsh *et al.*, 1989). Since the oral mucosa is routinely exposed to various physical forces and a multitude of different foreign substances that are contained in foods and beverages, the oral mucosa has evolved as a robust membrane that is less prone to irreversible damage by drugs, drug dosage forms and formulation excipients (Merkle and Wolany, 1992). These advantages are of great value to facilitate the systemic delivery of drugs that are subject to extensive hepatic clearance and as a means of administering drugs to an unconscious patient.

Although the oral cavity appears to be an ideal site for drug delivery, not all drugs are able to penetrate the oral mucosa. In practice, buccal drug delivery is considered when peroral drug delivery fails. Table 1.1 compares buccal absorption with nasal and transdermal absorption. Due to the lipophilic nature of the buccal tissue, rapid uptake and sustained delivery of lipophilic drugs such as fentanyl, can be accomplished. However, buccal absorption of high molecular weight drugs, such as peptides, proteins and polysaccharides, often result in low permeability within the oral mucosal tissue. Other disadvantages include the maintenance of the dosage form at the buccal or the sublingual site, this is often difficult and in comparison

to the duodenum, both are of small surface area (Lamey and Lewis, 1990), and hence, the dosage form should be optimal both in terms of thermodynamic and adhesion properties. In addition, patient to patient variability and other factors such as salivary flow and the rate of drug absorption all affect drug delivery.

Table 1.1 Comparison of buccal, nasal and transdermal drug delivery (Hoogstraate *et al.*, 1998)

1.1.4 Implications for drug delivery

Many different dosage forms are suitable for buccal delivery, such as tablets, patches, lozenges, sprays, hydrogels, lollypop systems, chewing gum, powders and solution. Table 1.2 contains some buccal and sublingual products that are currently on the market most of which are solid or chewable formulations. The different formulations will allow absorption through various parts of the oral cavity and will provide leakage into the GI tract.

The sites of drug absorption in the oral cavity include the floor of the mouth (sublingual), the inside of the cheeks (buccal) and the gums (gingival). In general, the delivery of a drug requires a delivery mechanism (dosage form) to effectively off load drug present in the oral cavity, to release a drug, which then diffuses through the mucosa into the local blood circulation.

Table 1.2 Available drug products for buccal and/or sublingual use (Hoogstraate *et al.*, 1998)

The buccal and the sublingual region are the two principal sites within the oral cavity that are used for drug absorption. Buccal delivery is where the therapeutic agent is held between the cheeks or the lip, sublingual delivery is where the therapeutic agent is held under the tongue. The oral cavity is lined with a permeable mucous membrane with underlying connective tissue that has an extensive venous and lymphatic drainage. Drainage from the mouth is to the superior *vena cava*; hence, absorbed drug is protected from the rapid first-pass metabolism in the liver. The richness of the blood, lymphatic supply and drainage is a major factor in the rapidity of systemic drug effect following buccal absorption (Li-Lan *et al.*, 1999).

The surface area available for drug absorption in the oral cavity is approximately 200 cm^2 (Lamey and Lewis, 1990). For drug absorption to occur, the drug must be able to dissolve.

The oral cavity is bathed in saliva (pH 6.2-7.4), hence, this favourably facilities drug dissolution to occur.

Salivary flow is also important in determining the rate of dissolution of the drug. The drug must have a bland taste in order to prevent excess saliva flow, which would increase the likelihood of a portion of the drug being swallowed before absorption. In addition, activities such as talking, drinking, smoking should be avoided during drug administration as these activities may effect the period the drug is in contact with the mucosa and therefore overall drug delivery (Lamey and Lewis, 1990).

1.1.5 Saliva

As written by Mandal 1987, humans can manage without saliva. Its loss is not life-threatening in any immediate sense, but it results in a variety of difficulties. There are number of functions served by saliva some of which are highlighted below.

- Digestive function

The contribution of salivary secretions to the digestive process is mainly preparative. The enzymes in saliva, mainly amylase, may be viewed as the first step in the digestion of food. It forms a food bolus which is readily chewed and moved towards the posterior regions of the oral cavity where it is swallowed. The high water content of the parotid secretions and the mucins generated by the submandibular and minor salivary glands, moisten and coat the food to facilitate ingestion.

- Protective function

In comparison to the skin, the oral tissues are inherently weaker, but saliva acts as an effective barrier against friction. The salivary glands supply lubricatory molecules to coat not only the food but the oral soft tissue. This lubricating film allows passage of food by providing a smooth tissue surface that exhibits minimal friction and also saliva protects the cells and tissues of the mouth, preventing them from drying.

- Maintenance of mucous membrane integrity

The salivary mucins posses rheological properties which include high viscosity, elasticity and adhesiveness. The molecular structure of salivary mucins enables them to bind to water

effectively, hence their presence on the surface of the mucous membrane serves as a natural waterproofing and helps maintain these tissues in a hydrated state.

- Soft tissue repair

It is well known that saliva enhances blood-clotting (Mandel, 1987). This effect is one of the body's mechanisms which protects against uncontrolled progression of gum disease. It also promotes wound healing. Saliva speeds blood coagulation both by affecting the anticoagulant directly in blood and by diluting the antithrombin (Mandel, 1987). This is a valuable property in an area where rough food or traumatic injury can induce bleeding and where tissues can bleed readily because of inflammatory disease.

- Maintenance of ecological balance

In colonisation of tissue surfaces, adherence is a critical event for survival of many bacteria. Interference with this process, bacterial clearance, by mechanical, immunological and non-immunological means is one of the major functions of the salivary defence system. The ability of saliva to maintain an appropriate ecological balance in the oral cavity was an important evolutionary force in the long period of human existence before plaque control.

- Direct anti-bacterial

The harmless bacteria normally present in saliva prevents other potentially harmful bacteria from growing and causing diseases. The cells and antibodies in saliva, as well as some of the enzymes and other proteins in saliva also kill foreign bacteria when they enter the mouth.

- Maintenance of pH

Saliva is effective in helping to maintain a relatively neutral pH in the oral cavity. Here, and in the oesophagus, the major regulation of pH during eating and drinking is *via* the salivary bicarbonates, the level of which varies directly with flow rate.

- Maintenance of tooth integrity

Acids in food, *e.g.* in cold drinks and fruits are known to decalcify teeth. Saliva acts as a buffer against this and may remineralise areas which have already suffered decalcification (Dong *et al.*, 1995).

In terms of drug delivery, the fact that saliva is continually secreted into the oral cavity means, that there is an ample supply of solvent to dissolve drugs prior to absorption. However, involuntary swallowing of saliva may result in a major part of the dissolved or suspended released drug being removed from the site of absorption.

1.1.5.1 Saliva glands

Figure 1.4 locations of the parotid, submandibular and sublingual salivary glands (www.entassocoaties.com/salivary-glands).

Saliva is produced predominately by three pairs of salivary glands the parotid, the sublingual and the submandibular (Figure 1.4). The parotid glands are pyramid-shaped, the base is the superficial lobe which is horseshoe-shaped and lies around the ear, the apex is the deep lobe which lies between the jaw bone (mandible) and the inside of the throat. The submandibular glands lie just below the angle of the jaw bones and the sublingual glands lie under the mid portion of the tongue. They all secrete saliva into the mouth, the parotid through tubes that drain saliva, called salivary ducts near the upper teeth, submandibular under the tongue, and the sublingual through its many ducts at the floor of the mouth. Beside these glands, there are many minor salivary glands located on the lips, inner cheek area (buccal mucosa), and extensively in other linings of the mouth, throat, trachea and oesophagus will all aid in the generation of saliva.

1.1.5.2 Composition of Saliva

Saliva is a complex fluid containing 99% water, organic and inorganic material (Table 1.3). The pH of saliva ranges from 5.8-7.4. The saliva of the oral cavity has a low buffering

capacity. This may allow local regions to be modified through formulation to promote the existence of optimal pH for the absorptive non-ionised, drug species.

Table 1.3 Constituents of adult human saliva (Crouch, 1971)

1.1.5.3 Stimulation of saliva flow

There is considerable variation in the flow rate of saliva between individuals, with time of day age, sex and during disease conditions (Table 1.4). The amount of saliva produced throughout the day is approximately 1-1.5 L, but this value is variable with some researchers reporting the total production of saliva to be as low as 0.5-0.6 L *per* day. (Rathbone *et al.*,

1996). Such variable salivary flow characteristics may affect the *in-vivo* release profiles of delivery systems designed for prolonged controlled release.

Table 1.4 Flow of parotid saliva in relation to age, sex and stimulation (Lentner 1981)

1.2 Nicotine

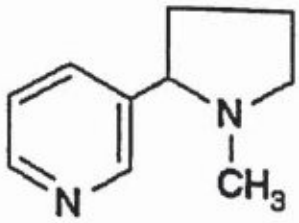


Figure 1.5 Nicotine $C_{10}H_{14}N_2$ MW 162.23

1.2.1 Chemical profile of nicotine

Nicotine (Figure 1.5) is a tertiary amine consisting of a pyridine and pyrrolidine ring. Its chemical names include (S)-3-(1-Methyl-2-pyrrolidinyl)pyridine or 1-methyl-2-(3-pyridyl)pyrrolidine. Nicotine may exist in two different three-dimensional structure called stereoisomers, (S)-nicotine and (R)-nicotine. Tobacco contains only (S)-nicotine (also called l-nicotine), which is the most pharmacologically active form. Tobacco smoke contains the less potent (R)-nicotine (also called d-nicotine) in quantities up to 10% of the total nicotine present within a cigarette (Pool *et al.*, 1985). Nicotine is a naturally occurring liquid alkaloid from the tobacco plant *Nicotiana tabacum*. It is a weak base with a pKa of approximately 3.1 and 8.0 (Benowitz, 1980). In its natural state, nicotine is a colourless to pale yellow, very hygroscopic, oily liquid with an unpleasant pungent odour and sharp burning persistent taste.

When exposed to air, light or burned, the liquid turns brown in colour. The weight of nicotine liquid is approximately 1.01 g *per* mL and it is miscible in water, alcohol and other organic solvents (Martindale, 1975).

1.2.2 Pharmacokinetics of Nicotine

Since nicotine was first identified in the early 1800s, it has been studied extensively and shown to have a number of complex and sometimes unpredictable effects on the brain and the body. To understand the nature and duration of effects of nicotine in humans, one must consider the level of nicotine in body organs, the time course of nicotine in the body with usual patterns of use, and influence of the route of administration on these processes.

1.2.2.1 Absorption of Nicotine

Nicotine is absorbed through the skin, the mucosal lining of the mouth, the nose or by inhalation in the lungs. Bioavailability of nicotine from the gastrointestinal tract (that is, swallowed nicotine) is incomplete because of first-pass metabolism, whereby, after absorption into the portal venous circulation, nicotine is metabolised by the liver before it reaches the systemic venous circulation. This is in contrast to nicotine absorbed through the lungs or oral/nasal mucosa, which reaches the systemic circulation without first passing through the liver.

Nicotine is a weak base with a two pKa values (index of ionic dissociation) of 3.1 and 8.0 (aqueous solution at 25°C). This means that at pH 8.0, 50% of the nicotine is ionised and 50% is non-ionised. In its ionised state, such as in acidic environments nicotine does not rapidly cross the membrane; consequently, its movement across cell membranes depends on pH (Benowitz, 1986).

The pH of smoke from flue-cured tobaccos found in most cigarettes is acidic (pH 5.5). At this pH the nicotine is primarily ionised. As a consequence, there is little buccal absorption of nicotine from cigarette smoke, even when held in the mouth. The pH of smoke from tobacco in pipes and cigars is alkaline (pH 8.5). At this pH, nicotine is mostly unionised and well absorbed from the mouth (Armitage and Turner, 1970). Cigarettes however are a highly engineered drug-delivery system. By inhaling, the smoker can get nicotine to the brain very rapidly with every puff. The lag time between smoking and entry into the brain is only 10 to 20 seconds. This is shorter than after an IV injection. Absorption of nicotine from

smokeless tobacco or nicotine gum is a more gradual process than that which occurs during smoking. Peak levels are achieved over 20-30 minutes (Figure 1.6). The rapid absorption of nicotine during smoking occurs due to nicotine entering into the circulation through the pulmonary rather than the portal or systemic venous circulation and moves into the small blood vessels that line the skin, lungs and mucous membranes. The rapid absorption of nicotine from cigarette smoke through the lung occurs because of the huge surface area of the alveoli and small airways and also due to the dissolution of nicotine at physiological pH (approximately 7.4), which facilitates transfer across cell membranes. From there, it moves to the bloodstream, then to the brain and finally to the rest of the body.

Figure 1.6 Blood nicotine concentrations during and after cigarette smoking, oral snuff, chewing tobacco and nicotine gum. Cigarettes were smoked for 10 minutes. Oral snuff (2 to 5 mg), chewing tobacco (average 7.9 mg, range 0.9 to 17.8 mg) and nicotine gum (two 2 mg gum pieces) were held in the mouth or chewed for 30 minutes. Data represent average value for 10 subjects (Benowitz 1988).

1.2.2.2 Distribution of Nicotine in Body Tissues

After absorption into the blood (pH 7.4), about 69 percent of the nicotine is ionised, 31% nonionised and less than 5% is bound to the plasma protein (Benowitz *et al.*, 1982). The nicotine is distributed extensively to the body tissues with a volume of distribution averaging

180 L (Table 1.5). The pattern of tissue uptake of nicotine has been examined in tissues of rabbits by measuring concentrations of nicotine in various tissues after infusion of nicotine to steady state (Table 1.6). Spleen, liver, lungs, and brain have a high affinity for nicotine, whereas the affinity of adipose tissue is relatively low. After IV injection, concentrations of nicotine decline rapidly because of the tissue uptake of the drug. Shortly after an IV injection, concentrations in arterial blood, lung, and brain are high, while concentrations in tissues such as muscle and adipose (major storage tissues at steady state) are low. The consequence of this distribution pattern is that uptake into the brain is rapid, occurring within 1 or 2 min, and blood levels fall because of peripheral tissue uptake for 20 or 30 min after administration. Thereafter, blood concentrations decline more slowly, as determined by rates of elimination and rates of distribution out of storage tissues.

Table 1.5 Human pharmacokinetics of nicotine and cotinine (Benowitz, *et al.*, 1982)

Table 1.6 Distribution of nicotine (Benowitz *et al.*, 1986a)
Note: Tissue to blood nicotine concentration rations based on 24 hr constant I.V infusion of nicotine in rabbits.

1.2.2.3 Elimination of Nicotine

The elimination half-life of nicotine averages about two hours although there is considerable individual variation (range 1-4 hours). It is metabolised rapidly as it undergoes extensive metabolism primarily in the liver, but also to a small extent in the lung, brain and the kidney. Nicotine levels, however, decline rapidly in the brain and plasma owing to the distribution to peripheral tissues and to elimination (Benowitz, 1986) About 70-80% of nicotine is metabolised to cotinine *via* C-oxidation, and another 4% to nicotine *N'*-oxide (Figure 1.7). There is a considerable inter-individual variability in the rate of metabolism of nicotine to cotinine and smokers have on average slower nicotine clearance compared to non-smokers (Zevin *et al.*, 1998). Renal excretion depends on urinary pH and urine flow, and accounts for 2-35% of the total elimination (Rosenberg *et al.*, 1980)

Figure 1.7 Chemical structure of nicotine and major pathways of nicotine metabolism (Zevin *et al.*, 1998).

1.2.3 Medical consequences of nicotine

Nicotine is a highly toxic substance. Its fatal dose in man is approximately 40-60 mg. In acute poisoning, death may occur within a few minutes due to respiratory failure arising from paralysis of the muscles of respiration (Martindale, 1975)

The medical consequences of nicotine exposure result from the effects of both the nicotine itself and how it is administered. The most deleterious effects of nicotine addition are the results of tobacco use. Although the exact role of nicotine in smoking related disorders is unclear; the evidence would suggest that nicotine is not a direct cause of smoking-related disorders (Hughes 1993). The medical consequences of smoking will be discussed in detail in section 1.3.1.

1.3 Smoking Cessation

It is difficult to quantify accurately the costs of smoking to the economy, as smoking impacts on many different aspects of life. The economic costs, or benefits, of smoking are complex, difficult to determine and vary between countries. The major fact about smoking is that it kills. Awareness of the risks and costs of smoking is only one step on the way to controlling the problem. Much of the problem lies in the fact that cigarettes and other forms of tobacco are addictive substances.

Cigars and cigarettes are today's most popular forms of tobacco consumed world-wide. Currently it is estimated that the total number of smokers in the world is 1.1 billion. That is one–third of the adult population aged 15 years and over smoke. Globally, around 47% of men and 12% of women smoke, although prevalence vary widely internationally (Table 1.7). In many countries, the age at which people begin to smoke is dropping to less than 15 years of age in many regions (WHO 1998). The pace of this growth has been accelerating as smoking has become an established part of life in all countries of the world.

The number of cigarettes that the average smoker consumes daily varies with the maturity of the tobacco market (Figure 1.8). As smoking addiction increase, so does nicotine tolerance. Tolerance to a chemical means that, with regular use, the effects of that chemical are reduced below their original levels. Therefore, in order to compensate, smokers increase the number of cigarettes they smoke to ensure that the effects are maintained.

It is a relatively simple process for someone to stop smoking. However, stopping smoking is often difficult to achieve. Completely unsupported quitting, going 'cold turkey,' is the method that is most commonly tried by smokers who want to quit smoking. This method of quitting has the advantages that it is both cheap and can be started immediately, which means it avoids the risk of people will put off giving up until a better day, which never arrives. Of

smokers who give up by going "cold turkey," only 3% are still non-smokers after 6 months (GSK data on file). Part of the problem is that successful quitting needs careful thought and planning as well as enormous motivation and willpower.

Table 1.7 Percentage daily smoking prevalence, men and women aged 15 and over, selected regions, early 1990s (WHO 1996)
*Smoking prevalence estimates for Africa, are based on very limited information
**Includes countries of Northern Africa, Western Asia and the central Asian Republics of the former Soviet Union.

Figure 1.8 Number of cigarettes smoked *per* day *per* daily smoker (WHO 1996)

Another method, which is not supported with pharmacological agents, is that of giving up gradually. The smoker cuts down the number of cigarettes smoked each day until they eventually reaches zero. The main problem with this method is that of being tempted to smoke just one more cigarette and never really quitting. In addition some smokers compensate for the reduced number of cigarette by inhaling more deeply and holding the smoke in for longer.

A survey commissioned by the Health Education Authority, (Maguire, 2000) asked 2,000 smokers about their experience with stopping smoking. The survey found that too much emphasis was being placed on willpower and not enough on the use of smoking cessation aids such as nicotine replacement therapy (NRT) and amfebutamone (bupropion hydrochloride SR). There were also concerns from smokers about asking for help to stop, as

this was viewed as "weak-willed". Many smokers also viewed NRT products as carcinogenic. One recommendation from this survey was to encourage healthcare professionals to become facilitators rather than educators in the process of change hence, healthcare professionals need to be refined and moderated to make them more effective in supporting smokers to stop (Maguire 2001).

1.3.1 Medical consequences of smoking

Cigarette smoking causes more than three and a half million deaths each year. This translates to nearly ten thousand deaths per day. Based on current trends, this will increase to ten million annual deaths during the 2020 or 2030's (WHO, 1998). Table 1.8 lists some major causes of death by tobacco use.

Table 1.8 Tobacco use is a known or probable cause of death from the conditions listed (WHO, 1998).

It is well documented that smoking accounts for one-third of all cancers (WHO, 1998). Foremost among the cancers caused by tobacco is lung cancer, the number one cancer killer of both men and women. Cigarette smoking has been linked to about 90 percent of all lung

cancer cases. Smoking is also associated with cancers of the mouth, pharynx, larynx, oesophagus, stomach, pancreas, cervix, kidney, urethra, and bladder. The overall rates of death from cancer are twice as high among smokers as among nonsmokers, with heavy smokers having rates that are four times greater than those of non-smokers (www.nih.gov).

In addition to cancer, smoking also causes lung diseases such as chronic bronchitis and emphysema, and it has been found to exacerbate asthma symptoms in adults and children. In the 1940s a relationship between cigarette smoking and coronary heart disease was first reported. Since that time, it has been well documented that smoking substantially increases the risk of heart disease, including stroke, heart attack, vascular disease, and aneurysm. It is estimated that nearly one-fifth of deaths from heart disease are attributable to smoking (NIDA, 2001).

While one often thinks of medical consequences that result from direct use of tobacco products, passive or secondary smoke also increases the risk for many diseases. Environmental tobacco smoke (ETS) is a major source of indoor air contaminants. In America alone, second-hand smoke is estimated to cause approximately 3,000 lung cancer deaths per year among nonsmokers and contributes to as many as 40,000 deaths related to cardiovascular disease. Exposure to tobacco smoke in the home increases the severity of asthma for children and is a risk factor for new cases of childhood asthma. ETS exposure has been linked also with sudden infant death syndrome. Additionally, dropped cigarettes are the leading cause of residential fire fatalities, leading to more than 1,000 such deaths each year (NIDA, 2001).

1.3.2 Nicotine replacement therapy (NRT)

It was recently estimated that in a mature tobacco market, approximately two-thirds of smokers would like to quit (GSK data on file). In addition to the health benefits, this creates an important business opportunity, a growing market for products that will help smokers overcome tobacco dependences. Traditionally, part of the problem with smoking control therapies was their relative lack of efficacy. However, this situation has evolved dramatically with the introduction of nicotine replacement therapy. NRTs can approximately double the rate of success of people who attempt smoking cessation compared to those using willpower alone (Henningfield, 1995).

Ending the regular use of nicotine in nicotine-dependent people produces physical symptoms that vary in intensity depending on demographical variables such as age and sex, personality factors including health, control, drive for achievement, extraversion, and neuroticism, and indices of nicotine use, such as cigarette consumption and the number of years smoked (Parrot, 1995). The main symptoms of the nicotine withdrawal disorder highlighted by www.quit-smoking.net are:-

- Headache.
- Nausea.
- Constipation or diarrhea.
- Falling heart rate and blood pressure.
- Fatigue, drowsiness and insomnia.
- Irritability.
- Difficulty concentrating.
- Anxiety.
- Depression.
- Increased hunger and caloric intake.
- Increased pleasantness of the taste of sweets.
- Tobacco cravings.

NRT partially replaces the nicotine obtained from tobacco. It is intended to replace nicotine only temporarily and helps to reduce withdrawal symptoms, such as anxiety, irritability and restlessness, thereby enabling people to function while they learn to cope without cigarettes. It also helps reproduce some of the effects that the user would normally obtain from cigarettes and reduces the discomfort of the quitting attempt. NRTs partially help satisfy the craving from cigarettes while eliminating the harmful toxins associated with tobacco, with the result that the desire to smoke is reduced.

There are wide variations between countries in terms of the range of nicotine products that are available. In certain countries, some products are currently prescription-only but in others the majority of products are available non-prescription/OTC. There are several nicotine replacement formulations currently commercially available and others are being developed to improve on the efficacy. The world's first NRT product was a gum Nicorette® by Pharmacia & Upjohn. Since this launch there have been several additions to the range of NRT products available. Other NRT gums were launched in the early 1980s. The first NRT

patches were launched in 1990s with the launch of Habitrol® patch (Novartis) in the US. As well as gums and transdermal patches, other products include an oral inhaler, a nasal spray, microtabs (sub-lingual tablets) and, most recently, lozenges (Table 1.9 and Table 1.10). In some countries, there are a number of non-nicotine smoking cessation products available. Some are prescription only, whereas others are sold over the counter (Table 1.11).

The success of smoking cessation products varies worldwide. Some healthcare professionals and smokers inaccurately perceive nicotine as dangerous and so believe that NRT products are equally of harmful, addictive and ineffective substances as cigarettes (GSK Data on file)

Brand name		Delivery determined	Application period	Treatment period	Number of dosage	Patch size	Nicotine content	Nicotine absorbed
Australia/Europe	US	by	(hours)	(weeks)	levels	cm^3	(mg)	approx (mg)
Nicabate	NicoDerm	Rate	16 or 24	8-10	3	22	114	21
NiQuitin	CQ	controlling				15	78	14
		membrane				7	36	7
Nicotinell	Hibitrol	Polymer	24	6-12	3	30	52.5	21
Nicopatch				(8 in US)		20	35	14
						10	17.5	7
Niconil,Nicotrans,	Prostep,	Polymer	24	4-12	2	30	47.3	22
Nicodon, Nicotin,	Nicotine			(6 in US)		15	23.6	11
Nicostop, Nicolan	transdermal							
	system							
Nicorette patch	Nicotrol	Adhesive	16	12	3	30	24.9	15
Nicotrol	15mg only			(6 in US)	(1 in US)	20	16.6	10
						10	8.3	5

Table 1.9 Nicotine patch products (GSK data)

Brand name		*Treatment period (weeks)*	*Nicotine per dose (mg)*	*Approximate amount of nicotine absorbed per dose (mg)*
Europe	*US*			
Nicorette chewing gum	Nicorette	12	2	0.86
			4	1.2
Nicotinell chewing gum	N/A	12	2	0.7
			4	Data not available
Nicotinell lozenge	N/A	12	1	1
Nicorette Microtab	N/A	12	2	2

Table 1.10 Oral nicotine products (GSK Data)

Product	*Dosage form*	*Active(s)*
Zyban	Tablets	Bupropion hydrochloride
Nicobrevin	Capsules	Quinine, camphor, menthyl valerate, Eucalyptus oil
Antinicotium Sine	Suspension	Herbal: Lobelia inflate, Robinia, pseudoacacia, Tabacum
Tabmint	Nasal Spray	Silver acetate
Stopp Mint/Fruit	Gum	-
Nicofree	Capsules	-
Tabecs	Tablets	-

Table 1.11 Non-nicotine smoking cessation products

1.4 Chewing Gum

The history of chewing gum can be traced back as far as the ancient Greeks who chewed mastiche, derived from the resin of the mastic tree although the modern chewing gum was introduced into common usage in the middle of the nineteenth century (www.fordgum.com). Chewing gum was then developed within the confectionary market from the late nineteenth century, with the later introduction of synthetic gum bases and sugar-free versions. Chewing of non-medicated sugar-free gums have been shown to decrease tooth decay, increase plaque pH and stimulate saliva flow (Imfeld, 1999)

1.4.1 Composition of chewing gum

Table 1.12 Typical chewing gum formulation (Conway, 2002)

There have been many different formulations of chewing gum reported in literature (Table 1.12) but the essential component of all chewing gum is the gum base. The gum base may comprise of a complex mixture of elastomers, natural and synthetic resins, fats, emulsifiers, waxes, antioxidants, fillers and flavouring agents. To the base, other components such as sweetening agents, flavouring agents and aromatics are added and, in the case of medicated chewing gums, the active ingredient.

The elastomers such as polyisobutylene and butyl rubber provide elasticity and cohesion to the chewing gum. The resins have two main functions, as a mastication substance and as a binding agent between the elastomers and fillers. They contribute to the balance between the properties of elasticity and plasticity. Glycerol esters from pine resins are an example of a natural resin used in medicated gums because of their lack of taste and good chemical

stability. Polyvinyl acetate is a common example of a synthetic resin that is also used in medicated gums.

Emulsifiers and fats such as glycerol monostearate and lecithin, and partly hardened vegetable and animal fats, *e.g.* hydrogenated soybean oil, are added to soften the mixture of elastomers, resins and fillers to give the required chewing consistency, mouth feel and promote uptake of saliva into the gum during mastication. Microcrystalline waxes serve the same purpose as the fats and emulsifiers; however, the stability of the waxes is often better than that of the fats.

The stability of a medicated chewing gum is generally good as the conditions for growth of microorganisms as well as chemical degradation processes are poor. Drugs formulated into chewing gum are protected from light and oxygen, from the air, in addition to the gum itself containing very little amounts of water. By coating the gum with appropriate substances and/or by suitable packaging, stability can be improved further. Unfavourable interactions between ingredients can also be avoided by separating one individual ingredient from another. This can be achieved by encapsulating one of the ingredients in porous polymeric beads while formulating the other directly in the gum base. Alternatively, antioxidants can be added to protect the gum base and flavours from oxidation.

Sweeteners such as sorbitol, mannitol, aspartame and cane sugars are added to enrich the flavour and provide enjoyable chewing quality. The finest grades of pure powdered cane and beet sugar and corn syrup are widely used in making confectionary chewing gum. The corn syrup not only helps sweeten the gum but also keeps it fresh and flexible. In sugar free varieties of chewing gum aspartame is widely used. It is a highly concentrated sweetener with a taste virtually indistinguishable from sugar and is formed from aspartic acid and phenylalanine, two common amino acids found naturally in many foods.

Fillers are an important ingredient in that they provide the right texture for the gum base. Talc is often used because of its acid resistance in products that have sour tastes, while calcium carbonate is used in most other products. A further consideration when making a medicated gum is the particle size of the solid substance suspended in the chewing gum. To avoid an unpleasant gritty feeling during chewing, or risk of damaging the enamel of the teeth, the particle size should be kept below approximately 100 μm (Rassing, 1994).

1.4.2 Gum texture

When formulating a medicinal chewing gum, the development team should evaluate the required product properties against practical issues to select the right gum base as this determines the basic characteristics of the product. One of the main factors used to evaluate the properties of the gum base is its texture. Different aspects of gum textures include firm *vs* soft, elastic *vs* waxy, silky mouth-feel *vs* coarse mouth-feel, juicy *vs* dry and freeness *vs* tact. Some qualities are physical, and the others are perceptive. By changing the gum formulation one can alter the texture of the gum. For example, more elastomers tend to make the texture firmer whilst more plasticizers would make the gum softer.

Until recently, texture trials were conducted using a trained panel of assessors but, with the development of texture analysis systems, the use of texture analysers provides a unique method of attaining and maintaining agreed quality standards within research and development, production and quality control. In comparison to a trained panel of assessors, texture analysis is inexpensive, convenient and most importantly eliminates the reliance on human judgment.

Figure 1.9 Texture profile of Liberty bubble gum base at 39 °C using the texture analyser (Lee, 2001)

An example of a texture profile produced by a texture analyser can be seen in figure 1.9. A probe penetrates the gum twice at the exact same location at a controlled speed and depth. The force that encountered by the probe is then plotted against time. The first peak height

(A) indicates the initial biting resistance or gum firmness, whilst the negative peak is related to the tack or adhesiveness of the gum. The ratio of the area of second peak (B) to the first peak is related to the cohesiveness of the gum. Many other parameters can also be calculated using the texture analyser based on food texture theories (Rosenthal, 1999). Figure 1.10 shows the ranges of several key texture parameters at the final stage for a number of Wrigley chewing gum formulations available commercially (Lee, 2000).

Figure 1.10 Texture ranges of commercially available Wrigley gum using the texture analyser (Lee, 2000).

1.4.3 Advantages of a chewing gum formulation

The possible reasons for developing chewing gum formulations as a drug delivery system are, firstly, to improve the bioavailability by delivering the drug *via* the oral mucosa and secondly to formulate a delivery system that is a convenient alternative to capsules and tablets.

As drugs absorbed *via* the oral mucosa directly enter the systemic circulation, it may prove useful for drugs that undergo extensive first-pass metabolism in the liver. Due to the rich vascular supply of the buccal mucosa, measurable concentrations of the active substance may be present in the blood after only a few minutes of chewing hence a fast onset of action is likely to be attained.

Chewing gum as an oral mucosal drug delivery system offers the possibility of releasing drugs in a controlled manner over an extended time for a local or a systemic effect. The release rate of the drug can be carefully controlled through manipulation of the gum formulation, the active form of the drug used or the way the gum is chewed by the individual, hence, allowing extended exposure of the drug in the oral cavity. It has the potential of over-coming the problems of short-lived actions and variations in drug release and retention times, which are associated with conventional systemic oral mucosal drug delivery systems. In addition, chewing gum can be administered anytime, anywhere, without water in a discreet manner. Furthermore the treatment can be terminated if and as required by expelling the chewing gum.

Although some active substances are absorbed very slowly across the buccal mucosa, a chewing gum formulation may still promote fast absorption. Drugs that are released from the gum, and are swallowed, are introduced to the gastrointestinal tract either dissolved or suspended in the saliva. The processes of disintegration and dissolution that slow the onset of action of conventional tablets are by-passed and thus the drug will be present in a bioavailable form. Recently, the rate of absorption and relative bioavailability of caffeine administered in chewing gum and capsules have been compared (Kamimori *et al.*, 2002). The study found that the bioavailability and maximum plasma concentrations were similar for both formulations but the rate of absorption of caffeine from the 200 mg chewing gum formulation (T_{max} 55 min) was faster than that of the 200 mg caffeine capsule (T_{max} 120 min) indicating that the rate of absorption from the chewing gum was significantly faster.

1.4.4 Use of chewing gum as a drug delivery system.

The chewing of gum is a well-adapted and frequently undertaken activity in both adults and children. It is a potentially useful way of delivering drugs both locally or systemically *via* the oral cavity.

In 1869 a dentist, Dr. William F. Semple from Mount Vernon, Ohio, took out the first patent for chewing gum. He considered chewing gum as not only a confectionery but also as having a potential role as a dentifrice. (Rassing, 1994). Later, in 1924, the first medical gum containing aspirin "Aspergum" was marked in the USA.

Improved technology and extended knowledge have made it possible to develop and manufacture medical chewing gum with pre-defined properties, such as release profile for the active substances, taste and texture. Chewing gum is a convenient drug delivery system which is appropriate for a wide range of active substances. Chewing gums are available with nicotine for smoking cessation, ascorbic acid in vitamin/mineral supplements, zinc salts for fresh breath and dimenhydrinate for motion sickness, sodium fluoride and carbamide as well as chlorhexidine acetate for caries prevention, xerostomia alleviation and fresh breath (Ellermann, 2002).

The effect of chewing different non-medicated chewing gums on plaque pH, saliva flow rates and the incidence of dental caries have been the topic of several studies. Chewing gum not only acts on salivary stimulants but may also be a useful vehicle for some agents such as fluoride, chlorhexidine and calcium phosphate (Itthagarun, 1997). The mastication of xylitol chewing gum has been shown to reduce dental caries in children and young adults better than any other sugar-free chewing gum (Tanzer, 1995) hence; chewing sugar-free chewing gum has been increasingly accepted as an adjunct to other oral products and has become part of anti-caries prevention programmes.

Local effects to treat a health condition require that the relevant active substance be available at a therapeutic level near or within the tissue being treated. For the treatment of oral cavity conditions, chewing gum is an ideal drug delivery system. The active substance can be released as the gum is chewed, thus providing the potential for a high level of active substance to obtain local effect. An oral anti-fungal miconazole chewing gum formulation, developed by Pederson, has been used in clinical trials (Pederson *et al.*, 1990). A chewing gum containing a 3.8 mg dose of miconazole was compared to a dose of 100 mg of miconazole gel formulation. It was found that the therapeutic effect of the two preparations was equal despite the fact that a much lower dose was released from the chewing gum formulation.

Drug released from chewing gum can provide a systemic effect in two ways. The drug can be swallowed or it has the potential to be absorbed through the oral mucosa into the systemic circulation. Drugs absorbed directly *via* the membrane lining of the oral cavity avoid metabolism in the gastrointestinal tract and the first-pass effect of the liver hence, it could provide better bioavailability. Chewing gum promotes buccal absorption by releasing active

substances at carefully controlled rates, thus allowing extended exposure in the oral cavity. As mentioned earlier, Aspergum®, a chewing gum containing aspirin has been compared with the bioavailability of an acetylsalicylic acid tablet (Woodford, 1981). Bioavailability parameters were estimated from the recovery of the drug in urine. 434 mg (63.7% of the administered dose) of acetylsalicylic acid was released from the gum after it was chewed for 15 minutes of which 98.2% was recovered in the urine. In contrast 91.5% of the original 648 mg was recovered from the tablet formulation. The study concluded that the chewing gum may provide a faster relief from pain.

Another such study was carried out using vitamin C in chewing gum. Again, the excretion of ascorbic acid in urine after administering a tablet and after chewing an ascorbic acid chewing gum was compared (Christrup, *et al.*, 1988). The relative recovered fraction of vitamin C (chewing gum compared to chewing tablet) was approximately 1.3 indicating a better bioavailability for the chewing gum formulation.

The use of chewing gum as a drug delivery system in smoking cessation is well established. Nicotine and silver acetate are drugs that have been incorporated into chewing gum formulations to help people quit smoking. Tabmint®, the chewing gum containing silver acetate has been used since 1960 for smoking cessation (Rassing, 1994). Silver acetate works by giving the tobacco smoke an unpleasant taste. Also, in the 1960s the idea of nicotine replacement therapy was developed by Ferno and colleagues (Nunn-Thompson, 1989). Ferno developed a chewing gum formulation that allowed adequate absorption of nicotine through the buccal mucosa. Today, the use of nicotine chewing gum is well established as can be seen not only by the current sales numbers but also from the number of articles published.

1.4.5 Drug release testing from chewing gum

The need for and the value of *in vitro* drug testing is well established for different types of pharmaceutical dosage forms. Testing of tablets and capsules are well established and apparatus and standardised methods are described in the pharmacopoeias. Standardised equipment for disintegration, dissolution and drug release testing are available on the market. These apparatus however are not suitable for monitoring drug release from chewing gums since here a continuous mastication is needed to facilitate release of the drug.

The release of drug substances from chewing gum is generally quantified by *in vivo* chew-out studies (Rider *et al.*, 1992). Volunteers are asked to chew a piece of gum and during the mastication process, the drug substance within the gum product should be released into the saliva. The drug is either absorbed through the buccal mucosa or swallowed and absorbed through the gastrointestinal tract. The chewing gum is then removed and analysed for the residual drug substance. Obvious disadvantages to this include the lack of chew control and variations in the flow and composition of subjects' saliva.

At present, only a few publications refer to devices that stimulate the human mastication of chewing gums. Kleber *et al.*, in 1981 constructed a device to study the ability of gum to polish teeth. Dissolution studies carried out using this apparatus demonstrated that it was unsuitable for the *in vitro* investigation of drug release from medicated gums (Rider, *et al.*, 1992). Christup and Moller in 1986 constructed a chewing machine consisting of a temperature-controlled reservoir for the dissolution medium and two pistons which were driven by compressed air (Figure 1.11). During a chewing cycle, the pistons placed at opposite ends, move towards each other and when they meet and press the chewing gum in between they make a twisting movement before returning to the starting point (Rassing, 1994).

In 1992, Rider *et al.*, developed a chewing machine (Figure 1.12) to test the *in vivo-in vitro* correlation of a prototype gum product containing phenylpropanolamine hydrochloride. The chewing machine consisted of a conical Teflon® base and a rotating ribbed Teflon® plunger suspended in a dissolution vessel. The plunger ribs were small indentations cut into the plunger. This allowed for circulation of the dissolution medium within the base. One cycle of the piston consisted of an upward and downward stroke of the piston whilst the plunger rotated simultaneously at 10 to 40 rpm. A series of parameters were evaluated including rotation speed and plunder frequency. It was found that there was a correlation between the *in vitro* release profile at 20 rpm and 30 cycles/ min plunger frequency and an *in vivo* chew out study (Rider, *et al.*, 1992).

Figure 1.11 * A cross-sectional diagram of the release chamber of the chewing machine constructed by Christrup and Moller. A piece of chewing gum is placed in the reservoir (1), the dissolution medium (10-25 mL) is added and the lid (3) put on. The pistons (2) which move against each other then back to the starting point are driven by compressed air. The normal frequency is 60 cycles each minute. Test samples are taken at specified time intervals from the reservoir and the content of drug analysed (Rassing, 1994).
*Diagram duplicated from the original illustration.

Since the introduction of chewing gum apparatuses mentioned above, Kvist *et al.*, in 1999 developed a new chewing machine (Figure 1.13 and 1.14) in order to maximise the versatility of the method. The machine described has adjustable settings of temperature, chewing frequency, chewing time, volume of medium, distance between the jaws and twisting movement (Kvist *et al.*, 2000). The apparatus consisted of six chambers each of which had a thermostatted test cell of glass in which two vertically oriented pistons holding an upper and lower chewing surface, respectively, are mounted. The chewing cells are filled with an appropriate test medium and the chewing gum is loaded onto the lower chewing surface. The chewing procedure consists of the up and down strokes of the lower surface in combination with a twisting movement of the upper surface. The chewing machine was used to test the release of a number of drug products, Nicorette®, Nicotinell® both of which contain the active substance nicotine, Travvell® (dimenhydrinate), V6® (xylitol) and an experimental formulation containing meclizine. The results showed that the apparatus was a suitable tool in the quality control of manufactured medicated chewing gums as well as being a useful tool in research and development.

Figure 1.12 Chewing gum machine developed by Rider *et al.*, (Rider, *et al.*, 1992) (A) Teflon Base, (B) Teflon (C) a dissolution flask support system, (D) a standard dissolution flask, (E) a stainless- steel shaft, (F) a willow block, (G) a motor, (H) a Teflon plate and (I) a piston.

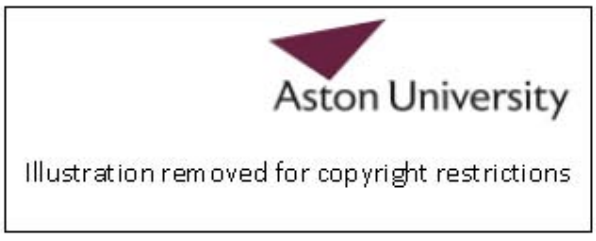


Figure 1.13 Technical drawing of the chewing apparatus showing the six chewing modules (Kvist, *et al.*, 1999)

Figure 1.14 Detail of one chewing module (Kvist, *et al.*, 1999).

The release profile of any drug substance is vital for pharmacokinetics; consequently, focus is on the release profile during the entire development phase. The increasing interest in chewing gums as a drug delivery system therefore calls for development of robust *in vitro* drug release equipment and standardised test methods for medicated gums (Kvist *et al.*, 1999). In 2000, the European Pharmacopoeia (EP) produced a monograph describing a suitable apparatus for studying the *in vitro* release of drugs substances from medicated chewing gums (EP, 2002). The machine consists of a temperature-controlled chewing chamber in which the gum is held in place by two horizontal pistons driven by compressed air. A third vertical piston 'tongue' operates alternatively to the two horizontal pistons to ensure that the gum stays in the right place. The apparatus will be discussed in more detail in section 2.3.

1.5 Nicorette® gum

Nicorette® was the first clinically proven nicotine replacement product to obtain regulatory approval. It is a chewing gum formulation that provides user-controlled nicotine release for oral absorption. The gum is available in two strengths, low (2 mg) and full (4 mg), the latter for highly dependent smokers.

Figure 1.15 Illustration of how Nicorette® chewing gum should be chewed. (www.nicorette.quit.com).

In order to maintain complete abstinence from smoking, Nicorette® gum should be chewed according to the instructions in the package leaflet (Figure 1.15). Whenever there is an urge to smoke, sufficient gum, usually 8-12 pieces but no more than 24 should be used each day. Gum use should continue for up to 3 months, and then be reduced gradually to 1-2 pieces. Highly dependent smokers (≥ 20 cigarettes per day) and those who have failed to give up when using Nicorette® 2 mg should use the 4 mg strength, otherwise patients should use the 2 mg strength. Nicorette® gum is frequently under used by consumers as they are unable to chew the required number of pieces of gum per day which would theoretically replace the nicotine usually provided by their cigarette consumption (Pharmacia, 1999).

Compared with the constituents of cigarette smoke, Nicorette® gum delivers only nicotine, generally at a lower dose and at a considerably slower rate of release than smoking. This clearly makes Nicorette® gum safer than smoking, in addition, the potential adverse effects of Nicorette® gum are generally mild. Post-marketing surveillance indicates that the most common adverse events reported with Nicorette® gums are:

- Headache and dizziness
- Slight throat irritation and excessive salivation
- Gastrointestinal symptoms such as nausea, vomiting, hiccups and flatulence
- Jaw muscle ache, ulcers, denture damage

As discussed earlier (section 1.2.2), nicotine is a highly toxic substance. When using Nicorette® gum, intoxication is unlikely even when several pieces are swallowed as nicotine within the gum is only released when the gum is chewed. Also, the high pre-systemic metabolism of drug prevents the development of nicotine toxicity.

1.5.1 Clinical efficacy of Nicorette® Gum

Nicorette® gum has been investigated in numerous clinical studies. In a meta-analysis of controlled trials on smoking cessation rates with NRT (Silagy *et al.*, 1994), Silagy *et al.*, identified that the use of NRT increased the odds ratio (OR) of abstinence to 1.71 (OR of nicotine gum 1.61) compared with those allocated to the control group. Abstinence rates among nicotine gum users varied from 15% in hospital trials to 36% in smoking cessation clinic trials compared to 11% and 23%, respectively for placebo groups in the same setting. Another more recent meta-analysis (Silagy *et al.*, 2003), at 12 months, showed that 18% of those receiving nicotine gums remained abstinent, compared to 11% in the control group. It was also found that the odds ratio of abstinence increased to 1.63 compared with placebo

Because of large variations in study design and degree of sophistication, it is difficult to compare and contrast trials of clinical efficacy or integrate data from previously published studies as the success may be influenced by several factors including:

- Degree of patient motivation
- Level of nicotine dependence
- Dosage used
- Follow-up procedure
- Concomitant use of behavioral therapy
- Therapist experience

1.5.2 Pharmacokinetics of Nicorette® gum

Release from Nicorette® gum is greater after 20-30 minutes of chewing (GSK data on file). This will however vary depending on factors such as rate and intensity of chewing, saliva flow and whether the saliva is being expectorated or swallowed. Several studies have demonstrated that the release of nicotine from chewing gum is far from 100%. In one study, 0.86 mg was absorbed from the 2 mg strength and 1.2 mg from the 4 mg strength (Benowitz *et al.*, 1987). In another study using just the 4 mg gum it was estimated that approximately

54% was released following a 10 minute chew out (1 chew per second) and 46% released after 10 minutes with a chew rate of 1 chew every 8 second (Nesmeth-Coslett *et al.*, 1988).

Pharmacia's promotional literature states that *in vivo* nicotine extraction efficiency from 2 mg gum is approximately 65%. This variation is mostly determined by how the gum is chewed. After a single dose, the time to maximum concentration (T_{max}) is achieved after approximately 30 min (for 2 mg gum). When one piece is chewed hourly for 12 h, the trough levels were 4.3 ng/mL and 7.9 ng/mL for 2 mg and 4 mg strength, respectively (Benowitz *et al.*, 1987). In reality, gum use is usually within 5-10 pieces/day, and the nicotine concentrations, therefore, rarely exceeds 10 ng/mL for the 2 mg strength and 15 ng/mL for the 4 mg strength (Balfour *et al.*, 1996). The high peak concentrations of nicotine obtained by smoking cannot be obtained with gum or other nicotine reduction medications. Although the rate of nicotine absorption is much slower with Nicorette® gum than with cigarette smoke, plasma nicotine concentrations achieved after chewing one piece of nicotine 4 mg gum for 30 minutes are comparable to those attained after smoking one cigarette (Figure 1.16)

Figure 1.16 Schematic illustrations of nicotine concentrations obtained from single dose administration of nicotine reduction medications and smoking (Balfour *et al.*, 1996).

1.5.3 Formulation of Nicorette® gum

Nicorette® 4 mg gum comprises a gum base, sweetener, flavouring agents, sodium carbonate (30 mg) and Nicotine Polacrilex (nicotine bound to a weakly acidic ion-exchange Amberlite® IRP 64 resin). The formulation for the 2 mg is similar, except that 10 mg of sodium carbonate is replaced by 10 mg of sodium bicarbonate. The ion-exchange resin is said to control the release of nicotine from the gum whilst the alkaline hydrocarbonate buffer increases the oral absorption of nicotine by increasing salivary pH to 8.5 (Pharmacia, 1999).

1.5.4 Ion-exchange resins

Ion-exchange resins are polymers which have active groups in the form of electrically charged sites. At these sites, ions of the opposite charge are attracted but may be replaced by other ions depending on their relative concentrations and affinities for the sites. Two key factors determine the effectiveness of a given ion-exchange resin: favourability of any given ion, and the number of active sites available for this exchange.

The most commonly used polymer matrix for ion-exchange resins are either a polystyrene matrix, the polymerisation of styrene (vinylbenzene) under the influence of catalyst yields linear polystyrene, or, a polyacrylic matrix, polymerisation of an acrylate, a methacrylate or an acrylonitrile cross-linked with divinyl-benzene. Other types of matrix include phenol-formaldehyde resins and polyalkylamine resins.

In the manufacture of ion-exchange resins, polymerisation generally occurs in suspension. Monomer droplets are formed in water and upon completion of the polymerisation process they become hard spherical beads of polymer. An example of this reaction is the polymerisation of the unsaturated monomer of the type CH_2=CHX which undergoes a chain reaction leading to the structure shown in figure 1.17:

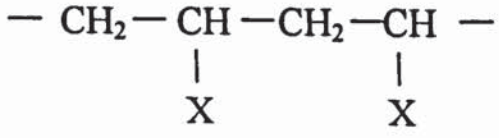


Figure 1.17 Resulting structure after polymerisation of the unsaturated monomer CH_2=CHX undergoing a chain reaction

This chain polymer is still soluble in certain solvents and in order to produce an insoluble resin it is necessary to incorporate a bi-functional monomer, generally a divinyl compound (Figure 1.18)

$$CH_2 = CH - R - CH = CH_2$$

Figure 1.18 A divinyl compound

This produces cross-linking of the polymer chains and renders the product insoluble. An example of cation exchange resin prepared by co-polymerisation is the reaction between styrene C_6H_5-CH=CH and divinyl benzene CH_2=CH-C_6H_4.CH=CH_2. The resultant co-polymers are sulphonated with concentrated sulphuric acid to give the structure shown in figure 1.19.

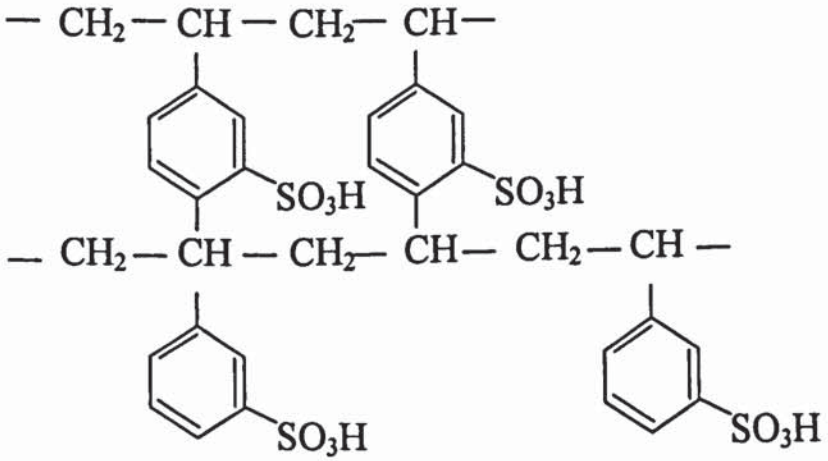


Figure 1.19 Co-polymerisation of styrene and divinyl benzene sulphonated with concentrated sulphuric acid to produce a strongly acidic cationic resin.

The $-SO_3H$ groups attached to the insoluble polymer matrix are ionised so that the H^+ is capable of exchanging with other cations in the solution which are in contact with the resin. The polymerisation of an emulsion or coarse suspension of the monomer produces a resin in the form of beads of controlled size which are convenient for handling both in the processing operations and in the final usage of the product.

The resulting ion-exchange resins formed can be grouped into four general categories depending on the function group attached to the polymer during polymerisation. The resin

could be a strong acid, weak acid, strong base or weak base. Examples of the functional groups include,

- -COOH which is weakly ionised to $-COO^-$
- $-SO_3H$ which is strongly ionised to $-SO_3^-$
- $-NH_2$ that weakly attracts protons to form NH_3^+
- -Secondary and tertiary amines that also attract protons weakly

$-NR_3^+$ that has a strong, permanent charge (R represents any organic group).

The ion-exchange forms the basis of a large number of chemical processes which can be divided into three main categories, substitution, separation and removal. Substitution is said to have taken place when, for example, a toxic ion, *e.g.* cyanide is removed from solution and replaced by a non toxic ion or *vice versa* when, a valuable ion is recovered from solution and replaced by a valueless ion. The separation of drugs within a HPLC column is an example of separation occurring using ion exchange resins. A solution containing a number of different ions is passed through a column containing ion exchange resins where the ions are separated and emerge in order of their increasing affinity for the resin. Removal of ions is a technique that can lead to the demineralisation of a solution. By using a combination of cation resins (in the H^+ form), and anion resins (in the OH^- form), all ions are removed from a solution and replaced by water (H^+ OH^-), thus, the solution is demineralised (Dardel and Arden).

The degree of cross-linking and porosity, exchange capacity, stability, density, particle size and moisture content of the resin will have an effect upon how the system performs (Anand *et al.*, 2001). An increase in the degree of cross-linking produces harder, less elastic resin's which are more resistance to oxidising conditions and also, more resistant to activation as access to the interior of the bead is hindered by the high density of the matrix. Because the resins are harder, osmotic stress cannot be absorbed by the structure, therefore, causing the bead to shatter. Also, the rate of exchange increases in proportion to the mobility of the ions inside the exchanger bead, if the structure is too dense, ionic motion is slowed down, thus, reducing the operating capacity of the resin. Finally, cross-linking can also reduce the retention of water in an ion exchange resin. The volume occupied by water is a measure of the resins porosity hence, the porosity and mechanical strength of the resin can be modified by varying the degree of cross-linking or the amount of solvent added (Dardel and Arden).

The total exchange capacity of a resin represents the number of active sites available on the resin and is expressed in equivalents *per* unit weight or *per* unit volume. Operating capacity, is defined as the proportion of total capacity used during the exchange process and depends on a number of process variables including,

- Concentration and type of ions to be absorbed
- Temperature
- Depth of resin bed and
- Type, concentration and quantity of absorbing ion

The chemical, thermal, mechanical, osmotic stability and resistance to drying also affects the efficacy of the ion-exchange resin. High oxidising conditions (presence of chlorine or chromic acid), can attack the matrix and destroy cross-linking causing the resin to swell until it softens. Polystyrene and polyacrylic resins made by suspension polymerisation are perfect spheres and suffer little damage when used in continuous moving-bed ion exchange plants. However, mechanical strength can vary considerably from one product to another, and resin beads which seem to have many internal cracks under the microscopes are more likely to break under mechanical stress than crack free products. Also, during ion exchange the configuration around each active group in the resin changes. The absorbed ion generally has a different size, shape and more importantly a different hydration layer than the displaced ion. During these volume changes of the hydration layer the resin bead may swell or contract due to the osmotic stress, thus, the moisture content or the moisture holding capacity (MHC) of the resin governs the kinetics, exchange capacity and mechanical strength of the ion-exchange resin and is defined as

$$\mathrm{MHC} = (P_{\mathrm{Hydr}} - P_{\mathrm{Dry}}) / P_{\mathrm{Hydr}} \qquad \text{equation 1.3}$$

Where P_{Hydr} is the weight of the hydrated resin sample and P_{Dry} is the weight of the sample after drying (Dardel and Arden).

The density of the resin has an effect upon how the system performs as it determines the hydrodynamic behaviour in counter flow systems. Generally the density of the dry, water-free resin is smaller for anion exchangers than cation exchangers. The density of water-swollen resins depends on the type of counter ion, swelling capacity, the degree of cross-linking as well as the density of the dry resin. Finally, the smaller the ion-exchange resin

beads the greater the rate of the exchange reaction conversely, the coarser the particles the higher the flow rate of the resin. Standard resins contain particles with diameters from 0.3 to 1.2 mm but, coarser or finer grades are available.

1.5.4.1 The use of ion-exchange resins in pharmaceutical formulation

Ion-exchange resins have been used for a variety of process including, water treatment, chemical detoxification, chemical analysis, therapeutic use as an antacid and for sustained release of cationic drugs, particularly nitrogenous bases (Kril and Fung, 1990). The complexation of drugs with ion-exchange resins has proved promising means of achieving their controlled release, of taste masking, of enhancing stability (Conaghey *et al.*, 1998) and of drug delivery (Irwin *et al.*, 1990). Not only are they used for controlled release but in tablet formulations, ion-exchange resins have been used as a disintegrants because of their swelling ability (Sriwogjanya and Bodmeier, 1998).

In general, the resins that are used in pharmaceutical formulations are powdered with a particle size of approximately 10-150 μm (100-500 mesh) although there are some applications where ion-exchange beads are used for example in gelatine capsules (Bellany, 1996). Compared with the ground-water treatment resins, pharmaceutical resin beads are specially produced using well defined starting material and without the use of recycled solvents or acid.

When used to control the release of drugs, for taste masking, to increase the stability of the drug or as a drug delivery system, the active drug must firstly be loaded onto the drug resin. For complexation to occur, the drug in an ionic form is mixed with the ion-exchange resin to form a complex known as 'resinate'. The performance of resinate is governed by several factors such as

- the pH and temperature of the drug solution
- the molecular weight and charge intensity of the drug and ion exchange resin
- geometry
- mixing speed
- ionic strength of the drug solution
- the degree of cross linking and the particle size of the ion exchange resin
- the nature of the solvent and
- the contact time between the drug species and the ion exchange resin.

Then, when the resinate from the delivery system reaches the site of delivery, an exchange process takes place between the drug ions on the resinate and the ions in environment surrounding the resinate, resulting in the liberation of free-drug ions. Therefore, the ionic strength and the pH at the site of delivery plays a key role in the liberation of immobilised drug from the resinate (Anand, *et al.*, 2001).

Ion-exchange resin systems are very versatile and have been used for a number of pharmaceutical applications and preparations. Ion-exchange technology has been used to sustain the release of drugs in pharmaceutical preparations (Raghunathan, 1989 and Sheumaker, 1988). The release of the drug from the ion-exchange resin depends upon a series of ionic reactions between various body fluids and drug-resin complex. The rate of release is related to the rate of diffusion of the active ingredient through the resin particle which can be changed by altering the particle size and the degree of cross-linking and the chemistry of the resin. Nicorette® chewing gum is an example in which the drug-resin complex is used to control the release of nicotine from the gum base. The nicotine is released only during chewing, thereby providing the minimal supply to facilitate smoking cessation. Other formulations include codeine onto Amberlite® IRP 69 where the drug-resin complex is used in a cough elixir and the use of the anti-asthmatic drug terbutaline loaded ion exchange resin in a controlled-release liquid formulation (Cuna *et al.*, 2000)

The stability of certain pharmaceutical preparations is affected by environmental conditions such as light, temperature pH *etc.* These preparations must be protected by various means to enable the formulation to be stable during formulation, storage and transportation. Vitamin B12 is an example of a pharmaceutical that can deteriorate on storage. The storage stability of vitamin B12 can be prolonged by adsorbing it onto a carboxylic acid exchange-resin, Amberlite® IRP 64 and using the resin-drug complex as a dosage form of the vitamin (Bouchard, *et al.*, 1958)

As the taste of pharmaceutical preparations is an important parameter governing patient compliance and commercial success of the product in the market place, bitter-tasting drugs can be adsorbed onto ion exchange resins, thus, effectively removing them from solution during transit through the mouth. At salivary pH (approx 6.8), resinate can remain intact making the drug unavailable for the taste sensation. As the formulation enters the GI tract, the environment changes to acidic and the drug release can take place (Lu *et al.*, 1991).

Examples of drugs in which this technique has been successfully demonstrated include ranitidine and paroxetine.

Various materials are added to tablets to facilitate disintegration. The choice of these agents must take into account possible adverse reactions between the drug and the disintegration agent. Most disintegrants function through their swelling properties and traditionally, starches, alginic acid, carboxymethyl cellulose and clay have been employed for this purpose, however, the fine particle size ion-exchange resins have shown superiority in several tablet formulations due to their tremendous swelling pressures as they are hydrated (Peppas and Colombo, 1989).

Ion-exchange resins have also been widely used in various topical pharmaceutical preparations. Such formulations are dependent upon the buffering and adsorption characteristics of the preparations and for many years, substances such as clay, talc, zinc oxide and boric acid have been used. The incorporation of ion exchange resins into topical formulation has many inherent advantages which include:-

- High buffering and adsorption capacities due to the high ion exchange capacities of the resins
- Non irritating properties
- Long duration of effectiveness
- Non-diffusion of ion exchange resins beneath the skin level

Other applications for ion-exchange resins include the use of resins in nasal (Illam, 1996) and ophthalmic preparations (Jungherr, 1998), site-specific drug delivery systems (Irwin *et al.*, 1990) as a microencapsulated or coated resinates or even as a simple resinate to improve drug stability (Anand, *et al.* 2001). Ion-exchange resins have also been used in novel release systems such as sigmoid release systems (Narisawa *et al.*, 1994), in iontophoretically assisted transdermal drug delivery systems (Conaghey *et al.*, 1998) and, finally, as a pH and ionic strength-responsive systems (Akerman *et al.*, 1999).

1.5.4.2 Amberlite® IRP 64

Amberlite® IRP 64 resin is derived from a porous copolymer of methacrylic acid and divinylbenzene (Figure 1.20 and Table 1.13). It is an insoluble, weakly acidic, cation exchange resin supplied as a dry, fine powder in the hydrogen form. It is suitable for use in

pharmaceutical applications, primarily as a carrier of certain basic (cationic) drugs and related substances. It is also used to mask objectionable tastes associated with certain basic drugs. It provides the means for binding medicinal agents onto an insoluble polymeric matrix. This houses an effective technique for overcoming problems of taste and odour in oral dosage formulations as well as providing a matrix upon which a sustained or controlled release formulation can be developed.

Table 1.13 Chemical and physical properties of Amberlite® IRP 64 (Rohm Hass, 2003)

One of the best recognised uses of ion-exchange resins as excipients is the use of nicotine loaded onto Amberlite® IRP 64 to produce Nicotine Polacrilex which is the principal ingredient of Nicorette® chewing gum.

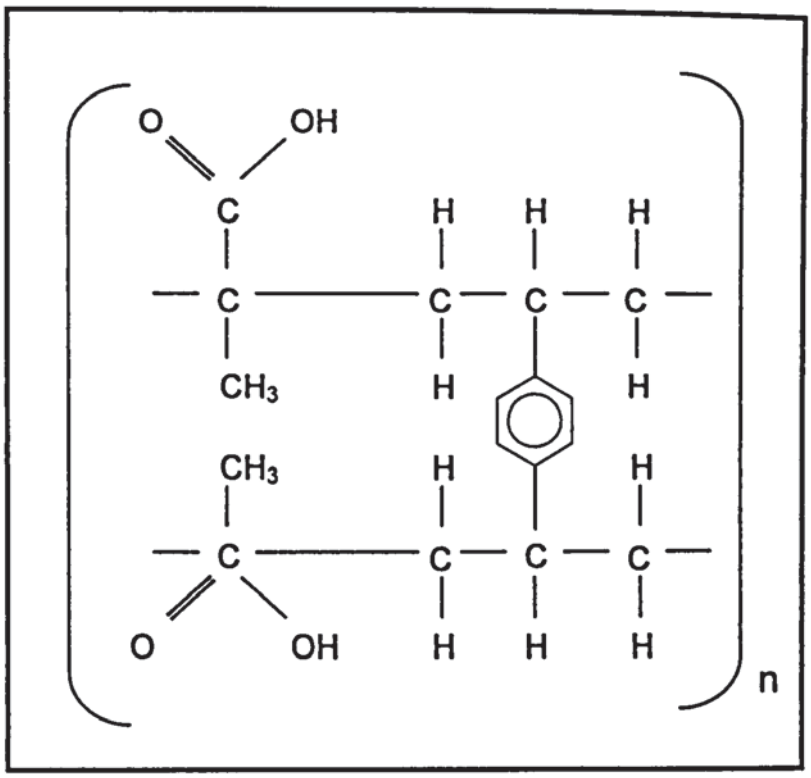


Figure 1.20 Chemical structure of Amberlite® IRP64

1.6 Project aims and objectives

In the past, drug release from chewing gum was quantified by *in vivo* chew-out studies. It is now possible to test the release of drug substances *in vitro* using the official EP chewing apparatus. Although there has been data published using various other chewing devices, to date there has been no published data on work carried out using the EP chewing apparatus.

In several studies carried out on the release of nicotine from chewing gums *in vivo*, it was found that the release of nicotine from the chewing gums was incomplete (Benowitz, 1987, Nesmeth-Coslett, *et al.*, 1988). In one study (Rider, *et al.*, 1992) looked at the release of phenlypropranolamine (a water-soluble basic drug) from a chewing gum formulation assumed to contain no ion exchange resin, > 95% release was achieved after 20 minutes chewing. In Nicorette® gum it is not exactly known what causes the incomplete release of the nicotine from the chewing gum. The result obtained by Rider *et al.*, (1992) suggests that the ion exchange may play an important role on controlling the rate and extent of drug release in the Nicorette® formulations.

The main aim of this study was to use the EP chewing apparatus to quantify *in vitro* release of nicotine from commercially available nicotine gums, to develop a reliable *in vitro-in vivo* correlation and to study variables in formulations which may control release and absorption of nicotine.

1.6.1 Factors affecting release of nicotine from chewing gum

Various publications have reported that there are many factors affecting rate and amount of active released from chewing gum formulations. Rassing, (1994), highlighted that the main factors that affect the release are the aqueous solubility of the drug, the formulation and manufacturing methods used to make the chewing gum formulations. Other variables that could affect the release of the active *in vivo* are the patients. When the individual is chewing the gum it may be regarded as an extraction process. Consequently, the release is related to the time the gum is being chewed, the frequency and intensity by which the individual is chewing, and also on the amount and composition of the individual's saliva. To mimic the conditions of *in vivo* release from medicated chewing gums in this project, factors such as chew rate, temperature, pH, the dissolution medium used and the volume of dissolution medium used were studied using the EP chewing apparatus

1.6.2 *In vitro in vivo* correlation

The development of IVIVC is an area within biopharmaceutics receiving considerable interest within industrial, regulatory and academic sectors. The reasons for this include the reduction of development costs, avoiding excessive use of human volunteers in bioavailability and bioequivalence studies, leading to earlier regulatory filings, hence earlier product launches. The EP chewing apparatus was used to generate *in vitro* chewing data from Nicorette® and this was then correlated to *in vivo* chew-out data based on a mathematical model. The generation of *in vivo* data was not within the scope of the project and it was therefore necessary to utilise data from a parallel project running at Sheffield University.

1.6.3 Investigating formulation variables on the release of nicotine from the gums

When developing a new formulation, the actual physical and chemical properties of the active substance have to be considered carefully. These properties are analysed and matched with the most appropriate release profile to obtain a fully controlled release within the appropriate period of time (Ellermann, 2002). As highlighted earlier, the rate of nicotine

absorption is much slower with Nicorette® gum than with cigarette smoking. The nicotine chewing gum formulation should be designed to have a release rate similar to that of cigarette smoking though maintaining the concentration of the active at a defined level to ensure optimal effects and minimum side effects.

For the successful completion of the project the following objectives were identified:

- To establish a reliable method to measure nicotine release from gum formulations
- To understand the role of the ion exchange resin in nicotine gum formulations and identify the variables that affect rate and extent of release of nicotine from the resin
- To understand the relative importance of gum base and ion exchange resin in controlling the rate and extent of release of nicotine under a range of *in vitro* conditions
- To establish a reliable *in vivo-in vitro* correlation to enable the further design of optimised formulations
- To investigate the rate and extent of release of nicotine from the chewing gum under a variety of standard conditions.
- To prepare trial formulations of gum using different excipients and different drug and resins to determine *in vitro* release profiles

CHAPTER TWO
GENERAL METHODS

This chapter describes the general methods and materials that were routinely utilised for the preparation of this thesis. Specific details and variations to the standard procedures are outlined in the relevant chapters.

2.1 HPLC assay of nicotine

A number of methods are available for the determination of nicotine by HPLC. If available, a pharmacopoeia method is usually a good starting point, as the methods are generally robust having been widely used and refined. The HPLC analysis used here was one adapted from a GSK protocol which was developed further in-house.

2.1.1 Experimental

2.1.1.1 Materials

(-) Nicotine hydrogen tartrate, ammonium phosphate monobasic, ammonium hydroxide, and acetonitrile were supplied by Sigma U.K and were of pharmaceutical, analytical or HPLC grade as appropriate. Double distilled water was generated in-house using a Fison's Fi-Streem still.

2.1.1.2 Equipment

The HPLC system employed comprised of the following set up.

Column	Waters Xterra RP-18 4.6 x150 mm	
Pump	Shimadzu Liquid Chromatograph LC-6A (serial No. 279514LF)	
Detector	Shimadzu UV Spectrophotometric Detector SPD-6A (serial No. 30712)	
Autosampler	Shimadzu Auto-injector SIL-6B (serial No. 90287)	
Intergrator	Shimadzu Chromatopac CR-4AX (serial No. C50102914493)	
Printer	Shimadzu Printer 121LP	
Vials for	Chromacol Vials	: 2SV
autosampler	Chromacol Septum	: 8T02
	Chromacol Caps	: 8-5CJ

2.1.1.3 Methods

2.1.1.3.1 Standards

Working standards using nicotine hydrogen tartrate were prepared by serial dilution of a 3000 µg/mL stock standard to produce 0.12, 0.3, 1.2, 3.0, 12.0, 30.0, 60.0, 120.0, and 300.0 µg/mL solutions of nicotine hydrogen tartrate. All standards were diluted using double distilled water.

2.1.1.3.2 Mobile Phase

The mobile phase used for the assay of nicotine consisted of 70% ammonium phosphate buffer at pH 8.5 and 30% acetonitrile. The ammonium phosphate buffer was prepared in line with the procedure below: -

6.7g Ammonium Phosphate Monobasic made to approximately 950 mL using double distilled water. The pH of this solution was then adjusted to 8.5 using 3.0% Ammonium Hydroxide Solution. The volume was then made up to 1 L with double distilled water.

2.1.1.3.3 Chromatographic condition

Flow rate	1 mL/minute
Mobile Phase	70% ammonium phosphate buffer at pH 8.5:
	30% acetonitrile
Injection volume	20 µm
Wavelength	260 nm
Column	Waters Xterra RP-18 4.6 x150 mm
Retention time	approx 3.4 minutes
Run time	6.0 minutes

2.1.1.4 Example of nicotine chromatogram

Figure 2.1 shows an example of a typical nicotine HPLC chromatogram using the Waters Xterra RP-18 4.6 x 150 mm column.

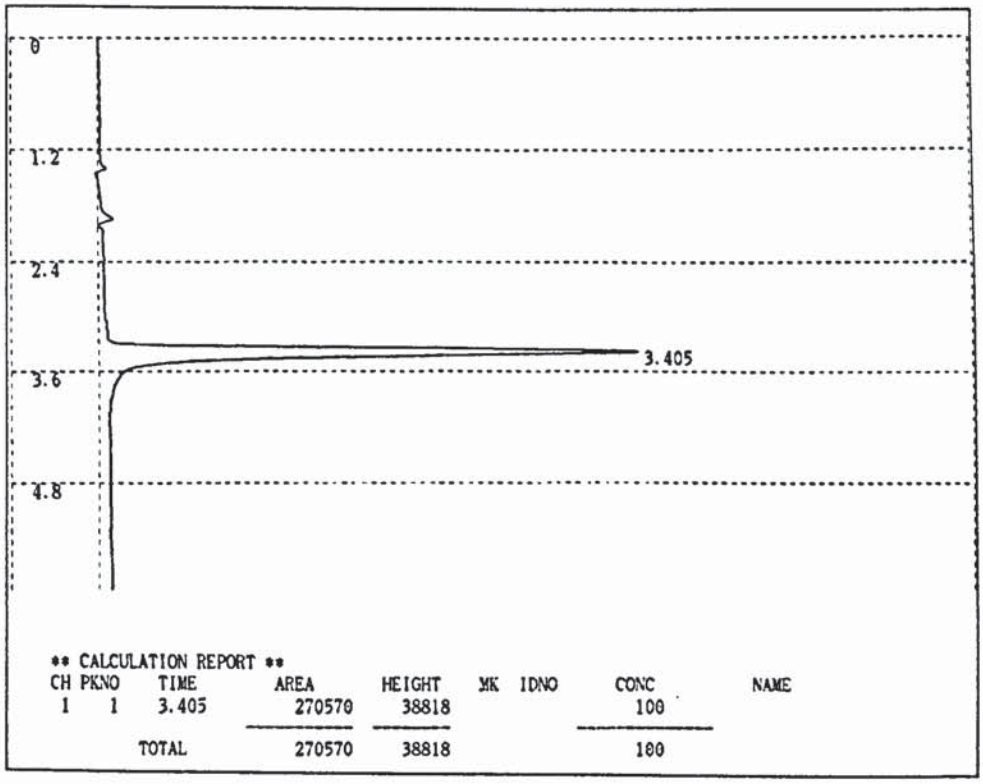


Figure 2.1 Typical nicotine HPLC chromatogram at a concentration of 21.0 µg/mL.

2.2 HPLC method validation

For pharmaceutical HPLC methods, guidelines from the USP (USP, 1994), International Conference on Harmonisation (ICH, 1995) and the FDA (FDA, 1994) provide a framework for performing HPLC validations. All acceptance limits and tests conducted within this section were performed in accordance to the International Conference of Harmonisation (ICH, 1995) and GSK protocol.

2.2.1 Linearity

Linearity is the detectable range that obeys the Beer-Lambert law or Beer's law (equation 2.1) which is defined as the linear relationship between absorbance and concentration of an absorbing species.

$$A=\varepsilon bc \qquad \text{equation 2.1}$$

Where A is absorbance, ε is the molar absorbtivity with units of L mol^{-1} cm^{-1}, b is the path length of the sample (cm) that is, the path length of the cuvette in which the sample is

contained and **c** is the concentration of the compound in solution, expressed in mol/L. The linearity or range of an analytical method is thus described as the concentration interval over which acceptable accuracy and precision are obtained for the analysing species and in turn is dependent on the compound being analysed and the detector used.

The analytical method described in section 2.1 was used to determine the linear range of nicotine. A series of nicotine solutions were made and the peak area from each injection was plotted against the corresponding nicotine free base concentrations to determine the linear range.

2.2.2 Method precision

Method precision is used to determine the precision of the method of analysis. The objective is to ensure that the method will provide the same results when similar samples are analysed. Depending on time and resources, the method can be tested on multiple days, by different analysts and different instruments. Method precision results are used to identify which of the above factors (days, analyst or instrument) contribute significant variability to the final results. In this study, it was impractical to test using different instruments and analysts therefore the method was tested on various days to determine any variation that would be seen from day-to-day using the method described below.

A known concentration sample of 21.05 µg/mL solution of nicotine free base from 60.00 µg/mL nicotine hydrogen tartrate was prepared on six consecutive days. The samples were then analysed according to the HPLC method described in section 2.1 and the response noted. The % RSD was calculated and the result was considered acceptable if a % RSD of < 2% was attained.

2.2.3 Instrument precision

This test was conducted to determine the precision of the HPLC system. In order to obtain meaningful data the precision study was preformed mirroring exactly the sample and standard preparations procedures that were used in the final method. The measurements of precision in this case can be described as a measure of how close the data values for a number of measurements under the same analytical conditions are to each other. The precision, as measured by multiple injections of a homogenous sample, indicates the performance of the HPLC under the pre-defined chromatographic conditions and day of

testing. The percentage relative standard deviation (%RSD) was then used to determine the variation limit of analysis. The narrower the difference, the more precise or sensitive to variation one can expect the results.

To determine the instrument precision, a 4.22 µg/mL solution of nicotine was made and six replicate injections were made from the same vial of the nicotine solution and analysed using the method described in section 2.1. The % RSD was calculated and a value of < 2% RSD was used to deem the results acceptable.

2.2.4 Accuracy

Accuracy is the measure of how close the experimental value is to the true value. Accuracy tests examine the percentage of analyte recovered by the assay. This test evaluates the specificity of the method in the presence of the excipients under the set chromatographic conditions used for the analysis for a given drug product. It can identify recovery problems that may be encountered during the preparation of the sample and in addition, flaws within the chromatographic procedure. The accuracy is usually determined in one of four ways. It can be assessed by analysing a sample of known concentration and comparing the measured value to the true value. The second approach is to compare test results from the new method with results from an existing alternate method that is known to be accurate. A third approach is by spiking analyte in blank matrices. For assay methods, spiked samples are prepared in triplicate at three levels over a range of the target concentration. The analyte levels in the spiked samples are then determined and the percentage recovery calculated. The fourth approach utilised the technique of standard dilutions and is used when it is not possible to prepare a blank sample matrix (*i.e.* no presence of analyte). The method used in this validation was the third approach where known concentrations of nicotine were used to spike the artificial saliva and the % recovery was then calculated.

Artificial saliva (section 2.8) was spiked with three known concentrations of nicotine. Each nicotine sample was analysed in triplicate using the standard HPLC method (section 2.1).

- Triplicate analyses of artificial saliva were spiked at a concentration of 5.3 µg/mL nicotine free base
- Triplicate analyses of artificial saliva were spiked at a concentration of 53.2 µg/mL nicotine free base

- Triplicate analyses of artificial were saliva spiked at a concentration of 106.4 µg/mL nicotine free base

All results of % recovery of ± 2% of the theoretical value and below 2% RSD were used to consider the result acceptable.

2.2.5 Limit of detection

The limit of detection (LOD) is defined as the lowest concentration of an analyte in a sample that can be detected under the stated experimental conditions. It is expressed as a concentration at a specified signal-to-noise ratio. The ICH has listed visual non-instrumental ways in which the LOD can be determined other than the using the signal-to-noise ratio method. The LOD can be determined by techniques such as thin-layer chromatography (TLC) or titration. Another method of calculating the LOD is based on the standard deviation (SD) which is the method used in this study for calculating the LOD.

The method was as follows:-

Six replicate injections at 0.422 µg/mL nicotine free base from 1.2 µg/mL nicotine hydrogen tartrate was analysed using the HPLC method (section 2.1). The LOD was calculated by using equation 2.2

$$LOD = \frac{3.3\ SD}{S}$$

equation 2.2

where SD is the standard deviation of the replicates and S was the slope of the calibration curve.

2.2.6 Limit of quantification

The limit of quantification (LOQ) is the lowest amount of analyte that can be accurately and precisely measured under the stated operational conditions of the method. Like LOD, LOQ is expressed as a concentration and is sometimes determined by multiplying the signal-to-noise ratio by ten. The signal-to-noise ratio method is a good rule of thumb, but it should be noted that the determination of LOQ is a compromise between the concentration and the required precision and accuracy. If better precision is required, a higher concentration must

be reported for LOQ. Like LOD other methods are available to calculate the LOQ. As reported by the ICH, the calculations method is based on multiplying the SD by ten.

Six injections of nicotine free base at a concentration of 0.422 µg/mL from 1.2 µg/mL nicotine hydrogen tartrate, were conducted using the standard HPLC method (section 2.1). The LOQ was then determined using equation 2.3, where SD was the standard deviation of the replicates and S is the slope of the calibration curve.

$$LOQ = \frac{10 \times SD}{S}$$

equation 2.3

Six further replicate injections at the LOQ concentration were then made and the % RSD calculated. The LOQ value was accepted if the %RSD was < 5%.

2.2.7 Selectivity and specificity

Selectivity and specificity is the measure of the degree of interference from substances such as other active ingredients, excipients, impurities and degradation products. The tests ensure that a peak response is due to a single component only and no co-elution exists. Specificity and selectivity can be measured and documented by the resolution, plate count and tailing factor.

Theoretical plate number (N) is a measure of column efficiency, that is, how many peaks can be located *per* unit run-time of the chromatogram. N remains constant for each chromatogram with a fixed set of operating conditions. Parameters that affect N include peak position, particle size in column, flow rate of mobile phase, column temperature, viscosity of mobile phase, and molecular weight of analyte. In general the value for the theoretical plate number should be greater than 2000 *per* column. H or HETP (height equivalent of the theoretical plate), is a better indicator of the column efficiency since it allows for a comparison between columns of different lengths.

Another measure of selectivity is the column capacity K', which is a measure of the sample retention. It is a measure of where the peak of interest is located with respect to the void volume *i.e.*, elution time of the non-retained components often referred to as the solvent front.

A 105.46 µg/mL nicotine free base sample was analysed using the HPLC method described in section 2.1. The chromatogram produced was then evaluated to determine the number of theoretical plates, the HETP and the column capacity. The number of theoretical plates was calculated using equation 2.4, where, N was the plate count (the number of theoretical plate in a chromatographic column), t_A was the retention time of the peak and W_A was the peak width at baseline determined by tangents (Holbrook, 1991) (Figure 2.2).

$$N = 16 \left(\frac{t_A}{W_A}\right)^2$$

equation 2.4

The HETP of the nicotine chromatogram was calculated using equation 2.5 where N was the number of theoretical plates and L was the length of the column in mm (Holbrook, 1991).

$$HETP \quad \frac{L}{N}$$

equation 2.5

To determine the column capacity equation 2.6 was used where t_o, was the time taken for the solvent front to elute and t_A was the retention time of the analyte under investigation (Holbrook, 1991) (Figure 2.2).

$$K' = \frac{t_A - t_o}{t_o}$$

equation 2.6

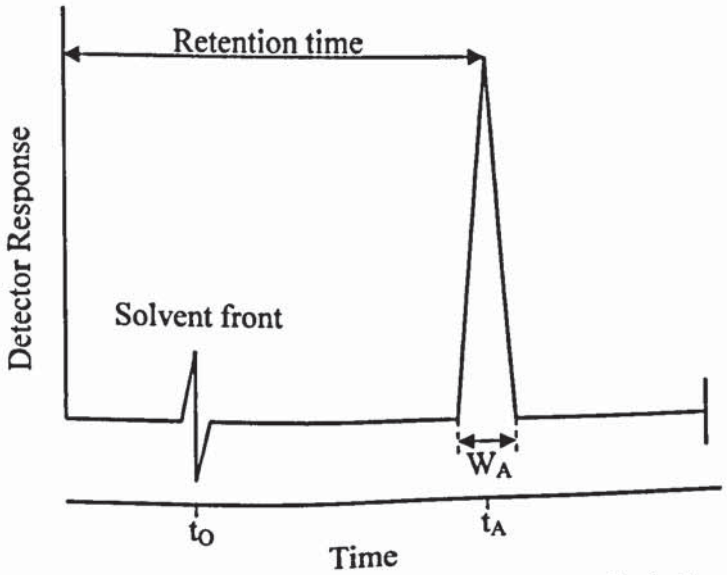


Figure 2.2 Chromatograph illustrating the parameters for calculation of N, and K'

The results of the HPLC method validation can be found in Appendix 1

2.3 Chewing machine

The European Pharmacopoeia recommends using 20 mL of unspecified buffer (with a pH close to 6) in a chewing chamber of 40 mL and a chew rate of 60 strokes *per* minute.

2.3.1 Experimental

2.3.1.1 Materials

Nicorette® chewing gum was used as supplied by Boots Chemist, GSK, Weybridge U.K or made in-house to an approximate 1 g size. Artificial saliva (section 2.8) was freshly prepared in-house. All material was of pharmaceutical, analytical or HPLC grade as appropriate. Double distilled water was generated in-house using a Fison's Fi-Streem still.

2.3.1.2 Equipment

The chewing machine (Figure 2.3 and 2.4) was used as supplied by GSK Weybridge U.K. It consists of a thermostatically controlled chewing chamber where the chewing gum is immersed in the dissolution medium. The gum is held in place within the chewing chamber by two horizontal pistons and a third vertical piston ("tongue"). All the pistons are electronically controlled and driven by compressed air at a minimum pressure of 50 psi supplied by a Bambi DT 30 oil-free compressor. The two horizontal pistons transmit twisting (approximately 90°) and pressing forces to the chewing gum whilst the third vertical piston operates alternately by a downward force to ensure that the gum stays in place. The chew rate is controlled by a 22 VAC electromatic s-system SB175220 analogue 0-18 second interval timer and, the temperature of the chewing chamber can be adjusted using the temperature gauge. The whole unit is encased in a 0.5 inch Perspex case and is placed on a trolley for ease of movement (Figure 2.3 and 2.4).

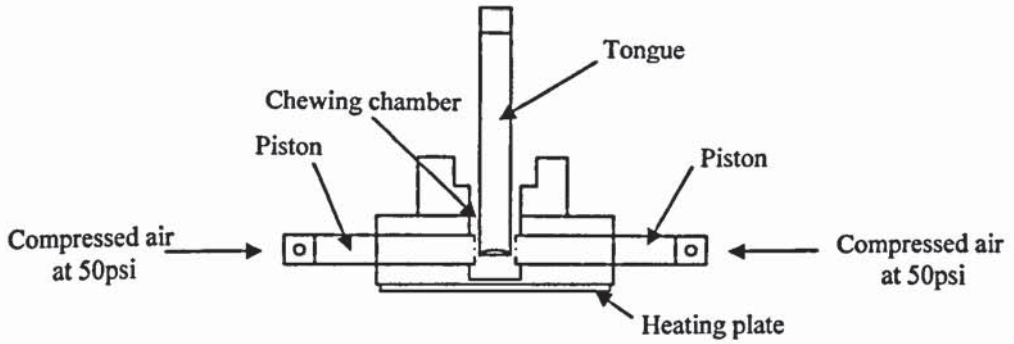
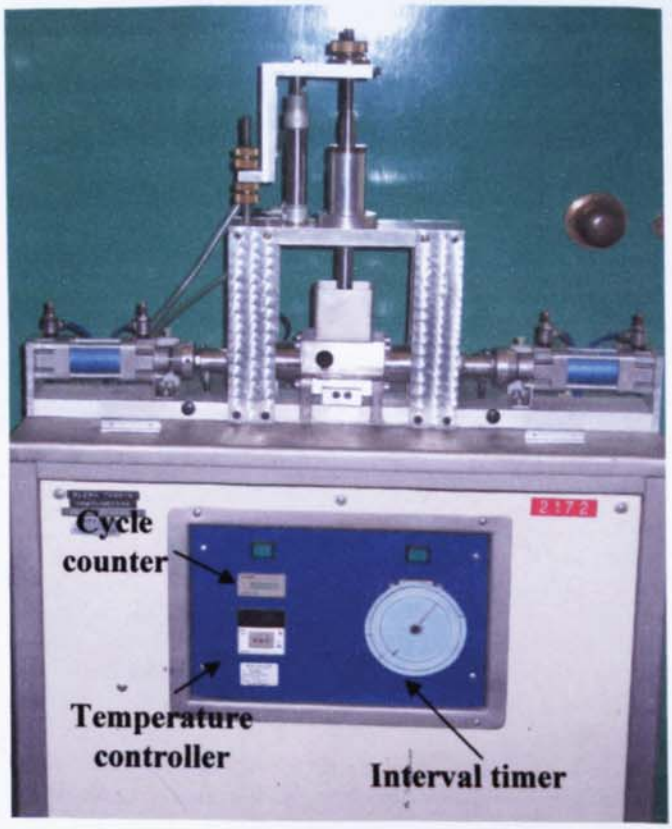
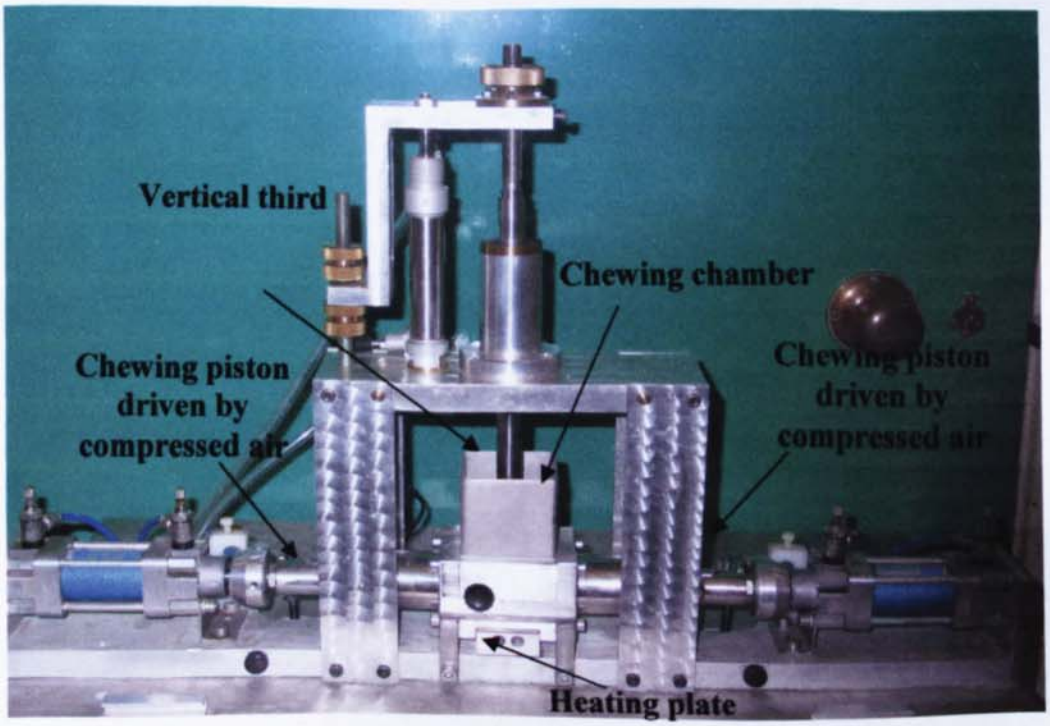


Figure 2.3 A schematic of the EP chewing apparatus



(a)



(b)

Figure 2.4 EP chewing apparatus used for the *in vitro* dissolution of medicated chewing gum. (a) The chewing machine on a trolley containing the temperature controller, interval timer and cycle counter. (b) The chewing machine with the tongue, chewing chamber and the chewing pistons controlled by pressurised air.

2.3.1.3 Method

The gum was placed in the chewing chamber with 40 mL of artificial saliva. The temperature of the chewing chamber was set to 37°C ± 1°C with a chew rate of 60 chews/minute. 2 mL samples were taken at 0, 5, 10, 15, 20, 25 and 30 minutes and replaced with equal amounts of fresh artificial saliva. The samples were filtered through a 0.45 μm filter and the nicotine levels determined using the HPLC method described in section 2.1. Withdrawn samples were replaced by an equal volume of pure dissolution medium. The drug concentration within the medium is therefore diluted progressively by sampling thus, the cumulative amount of drug released was calculated by correcting for this dilution using equation 2.7:

$$M_t[n] = \frac{V_r.C[n] + V_s.\sum_{m=1}^{n-1} C[m]}{1000} \qquad \text{equation 2.7}$$

where

M_t [n] is the current, cumulative mass released from the gum at time t,

C[n] is the current concentration in the dissolution medium

Σ {C[m]} is the summed total of the previous measured concentrations {m=1- (n-1)}

V_r is the volume of dissolution medium and

V_s is the volume of sample removed for analysis

2.4 Dissolution apparatus

Dissolution testing of Nicotine Polacrilex resin was undertaken using a six-vessel tablet dissolution system, fitted with 1L USP round-bottom vessels and stirring paddles.

2.4.1 Experimental

2.4.1.1 Materials

Various dissolution media were prepared all of which were of pharmaceutical grade. Double distilled water was generated in-house using a Fison's Fi-Streem still.

2.4.1.2 Equipment

The dissolution system consisted of a Hanson Research Model SR II 6 flask dissolution bath, equipped with 1 L round-bottomed flasks and paddles conforming to Apparatus II-USP

regulations (USP 1995). The dissolution apparatus was controlled *via* a Hanson Research validate dissolution controller.

2.4.1.3 Method

The dissolution apparatus was set at a temperature of 37 °C ± 0.5 °C and 75 rpm paddle speed. 800 mL of dissolution medium was placed in the round-bottomed flask and allowed to equilibrate. 444 mg* of Nicotine Polacrilex was placed into the flask and 2 mL of sample was removed at 0, 1, 2, 3, 4, 5, 7, 10, 15, 20 and 30 minutes and replaced with equal volumes of fresh dissolution medium. The sample was them filtered through a 0.45 µm filter and assayed by HPLC (section 2.1) and the cumulative amount of drug released calculated using equation 2.7.

* Note that 444 mg of Nicotine Polacrilex in 800 mL dissolution medium was proportional to a 4 mg Nicorette® gum in 40 mL dissolution medium in the chewing machine. 222 mg of Nicotine Polacrilex was used to simulate 2 mg gum in the chewing machine.

2.5 Sodium analysis

Inductively Coupled Plasma Atomic Emission Spectrometer (ICP AES) has been widely used as a quantitative analysis characterisation, and quality control tool in biomedical, pharmaceutical, food and environment for multi- elemental analyses

2.5.1 Experimental

2.5.1.1 Materials

Sodium nitrate and nitric acid was supplied by Sigma. All materials were of pharmaceutical, analytical or HPLC grade as appropriate. Double distilled water was generated in-house using a Fison's Fi-Streem still.

2.5.1.2 Equipment

Sodium analysis was carried out on a Varian Vista AX ICP AES, (Inductive Couple Plasma Atomic Emission Spectrophotometer.

2.5.1.3 Method

2.5.1.3.1 Standards

A stock solution of 1000 ppm sodium ions was made as follows:-

370 mg sodium nitrate made up to 100 mL with 3% v/v nitric acid. Calibration standards were made by diluting the stock standard with 3% v/v nitric acid to 400 ppm, 200 ppm, 100 ppm, 50 ppm, and 20 ppm.

2.5.1.3.2 Sample preparation

All samples were diluted 1:20 with 3% v/v nitric acid. The concentration of sodium in the solution was determined using equation 2.8.

$$M_t[n] = \frac{V_r.C[n]+V_s.\sum_{m=1}^{n-1} C[m]}{1000} - \sum M_A \qquad \text{equation 2.8}$$

Where

M_t [n] is the current, cumulative mass released from the gum at time t (mg),

C[n] is the current concentration of sodium in the dissolution medium

Σ {C[m]} is the summed total of the previous measured concentrations {m=1- (n-1)}

V_r is the volume of dissolution medium

V_s is the volume of sample removed for analysis and

M_A is the mass of sodium in V_s mL added at each replacement (mg)

2.5.1.3.3 ICP AES Method Conditions

Wavelength	568.263 nm
Sample Flow Rate	Internal Peristaltic pump at 15 rpm with 1.2 mm internal diameter tubing
Sample Uptake	45 s
Stab Time	30 s
Pump rate	15 rpm
Flush Time	60 s
Replicates	5

Source Equilibration	1 h
Plasma view	Axial
Plasma Gas Flow	15 L/min
Auxiliary Flow	1.5 L/min
Nebulizer Flow	0.9 L/min
Plasma Power Supply	1.0 kw
Peak Algorithm	Peak area

2.6 Total drug loading in gums

2.6.1 Experimental

2.6.1.1 Materials

Nicorette® chewing gum was as supplied by Boots Chemist or GSK. Other gums used were made in-house to an approximate 1 g size. Tetrahydrofuran and HCl were supplied by Sigma UK. All material was of pharmaceutical, analytical or HPLC grade as appropriate. Double distilled water was generated in-house using a Fison's Fi-Streem still.

2.6.1.2 Equipment

The gum suspension was shaken on a LTE multi shaker and the resulting suspension centrifuged using a Hettich Universal 32 centrifuge.

2.6.1.3 Method

Approximately 10 g of composite gum sample was placed in 1 L volumetric flask with 200 mL tetrahyrdofuran. This was shaken at 135 shakes/min for 90 minutes. 300 mL of 0.1 M HCl was added to the flask and allowed to stand before shaking for a further 5 minutes. The solution was then centrifuged at 3000 rpm for 10 minutes after which a 1.25 mL portion of the solution was taken and diluted to 50 mL using double distilled water. From this, a small volume was taken and filtered through a 0.45 μm polypropylene filter and then assayed by HPLC to determine the nicotine content (section 2.1).

2.7 pH measurements

pH measurements were determined using a Mettler Toledo MP230 pH Meter (3 decimal places) connected to a Mettler Toledo In Lab® 413 combination electrode. The pH meter

was calibrated before use on each occasion using Mettler buffers at pH 4.03, 7.00 and 9.21 supplied by Fisher Scientific.

2.8 Artificial Saliva

The formulation for the artificial saliva was developed by Parker *et al.* in 1999 (Table 2.1). All chemicals used were supplied by Aldrich and were of pharmaceutical grade. The distilled water was generated in-house using a Fison's Fi-Streem still.

Table 2.1 Artificial saliva formulation (Parker et al, 1999)

2.9 f_2 Calculation

Moore and Flanner's f_2 equation 2.9 was used to compare the dissolution curves. The equation is a logarithmic transformation of the average of the squared vertical distance between test and reference mean dissolution values. It evaluates the difference between the percentage drug dissolved *per* unit time to provide a single value describing the closeness of the two dissolution profiles. It is a powerful tool as curve comparisons can evaluate the effects of altering a process variable on the dissolution of the drug. Values of f_2 greater than 50 imply equivalence, whilst values of f_2 lower than 50 show dissimilar curves.

$$F_2 = 50.\log\left(\left[1+\frac{1}{n}.\sum_{t=1}^{n}(R_t - T_t)^2\right]^{-0.5}.100\right)$$

equation 2.9

Where n is the number of dissolution points, R_t is the reference dissolution values at time t. T_t is the test dissolution value at time t and W_t is an optional weight factor.

The equation has been adopted by the FDA in various guidance documents as an acceptable mathematical tool for comparison of reference and post-change products (U.S FDA, 1997). They recommend that f_2 should be used with mean dissolution data when coefficients of variation is not more than 20% at the earlier time points and not more than 10% at other dissolution time points (O'Hara, *et al.*, 1998).

2.10 Citrate-phosphate buffer

All chemicals used were supplied by Sigma Aldrich, U.K and of pharmaceutical grade. The distilled water was generated in-house using a Fison's Fi-Streem still. Two stock solutions were made that were subsequently combined, as highlighted in table 2.2, to produce solutions of desired pH

Stock solution (A) 0.1 M citric acid

Stock solution (B) 0.2 M disodium phosphate.

pH	(A) %	(B) %
2.2	98.00	2.00
3.0	79.45	20.55
5.4	44.25	55.75
7.0	18.15	81.85
8.6	0.80	99.20

Table 2.2 Composition of citrate-phosphate buffer at various pH

2.11 Texture analysis

The texture of the chewing gum formulations and Nicorette® gum were determined using a texture analyser

2.11.1 Experimental

2.11.1.1 Materials

Nicorette® 2 mg and 4 mg gum were used as supplied by Boots Chemist. Other nicotine containing gums were formulated in-house (chapter six). All chemicals used to formulate the chewing gums were of pharmaceutical, food, or analytical grade.

2.11.1.2 Equipment

A CNS Farnell QTS 25 texture analyser was attached to both a QTS controller (Figure 2.5) and a PC running the purpose designed TexturePro™ version 2.0 software.

Figure 2.5 QTS 25 texture analyser

2.11.1.3 Method

2.11.1.3.1 Calibration

The apparatus was calibrated *via* the QTS controller prior to use. The QTS 25 was switched on and the recalibrate loads option selected. The hook screw and hanger was fitted to the load cell and the weight was zeroed. A 5 kg calibration weight was attached and the QTS automatically adjusted and stored the revised calibration factors.

2.11.1.3.2 Test

A compression test was conducted using a 2 mm diameter stainless steel probe attached to the load cell. The probe penetrated the gum to a distance of 3 mm at a test speed of 30 mm/min with a trigger value set to 10 g.

2.11.1.3.3 Data analysis

The hardness, adhesive force, load encountered by the gum *per* unit time were calculated and a graph of load *via* time constructed for the different gums using the TexturePro™ software.

2.12 Directly compressible gums

A directly compressible chewing gum base can has been manufactured by SPI Pharma group. A normal tablet machine can be used to formulate gums.

2.12.1 Experimental

2.12.1.1 Materials

Pharmagum® S and M compressible chewing gum base were used as supplied by SPI Pharma. Sodium carbonate, mannitol, magnesium stearate and sorbitol were bought from Sigma UK and of pharmaceutical grade. Nicotine Polacrilex resin was used as supplied by GSK, Weybridge U.K

2.12.1.2 Equipment

A Manesty single-punch tablet machine was used to prepare 1 g chewing gum pieces.

2.12.1.3 Method

The ingredients (Table 2.3) were mixed together and then added to the hopper of the tablet machine. To ensure an even mix, the ingredients were mixed together in order of their bulk mass. The ingredients with the lowest bulk mass were added first, with subsequent ingredients mixed according to increasing bulk mass.

	4 mg gum Quantity (%)	Quantity for 20 tablets (g)
Pharmagum® S/M	55.77	11.154
Nicotine Polacrilex	2.23	0.446
Sodium Carbonate	3.00	0.600
Sorbitol	26.50	5.300
Mannitol	10.00	2.000
Magnesium Stearate	2.50	0.500

Table 2.3 Standard nicotine gum formulation using directly compressible gum base

2.13 Formulation of a standard nicotine gum

Formulations of standard nicotine gum were produced at the GSK site in Parsippany, New Jersey US.

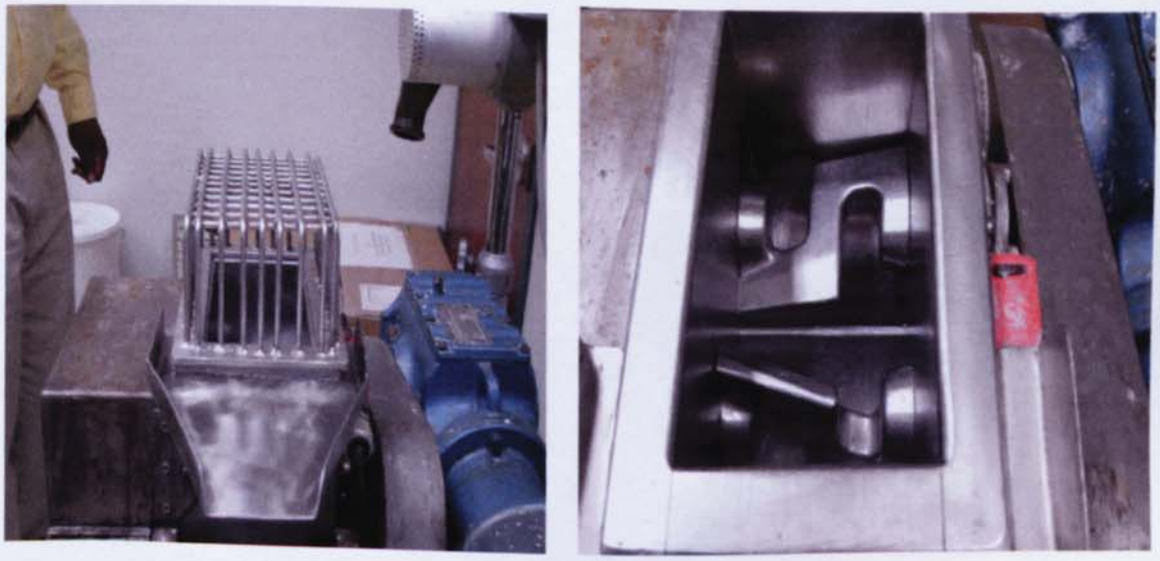
2.13.1 Experimental

2.13.1.1 Materials

All materials used were supplied by GSK, Parsippany and were of pharmaceutical and food grade.

2.13.1.2 Equipment

An A.C Squirrel cage gum kettle (Figure 2.6) was used to produce small batches of gums. It consisted of "S" shaped blades attached to a Bald/win TEFC 145 TC motor and Sew-Eurodrive, S67LP145 gear box. The front blade rotated at approximately 52 rpm whilst the rear blade rotated at 28 rpm.



(a) (b)

Figure 2.6 Gum kettle (a) with S" shaped blade (b) used to produce and mix small scale batches of chewing gum.

2.13.1.3 Method

The standard nicotine gums (Table 2.4) were made using the small gum kettle (Figure 2.6). The gum base was added to the warmed gum kettle and mixed for 10 minutes to soften the base. Sorbitol was then added and mixed for a further 5 minutes, after which a premix of Nicotine Polacrilex and mannitol was added. After 4 minutes of mixing, the flavour was added and further mixed for 1 minute before maltitol liquid was added. A premix of Acesulfame potassium and buffers were added after 1 minute and then mixed for as further minute after which glycerol was poured into the mixture. The gum formulation was mixed for a further 5 minutes before it was removed from the kettle using talc. The gum was then rolled and scored to approximate 1 g pieces (Figure 2.7).

(a) (b)

Figure 2.7 Rolling, scoring (a) and cutting (b) of the formulated gums into 1g pieces.

	Composition %w/w 2 mg	**Composition %w/w 4 mg**
Active Substance		
Nicotine Polacrilex	1.211	2.422
Excipients		
Gum base	49.00	49.00
Calcium carbonate	21.00	21.00
Sorbitol	15.569	14.348
Maltitol liquid	2.5	2.5
Glycerol	0.7	0.7
Acesulfame potassium	0.03	0.03
Optamint Nicomint	2.0	2.0
Mannitol	5.0	5.0
Sodium carbonate	2.0	3.0
Sodium bicarbonate	1.0	-
Talc	q.s*	q.s*
Total	**100**	**100**

Table 2.4 Standard nicotine gum formulation *per* gum
*Talc is added as needed to prevent sticking of the gum

2.14 Ion -exchange resins

A nicotine Amberlite® IRP 69 resin was produced in-house and the nicotine loading on both the nicotine Amberlite® IRP69 and Nicotine Polacrilex determined.

2.14.1 Experimental

2.14.1.1 Material

Nicotine Polacrilex resin was used as supplied by GSK. Amberlite® IRP69 was bought and used as supplied by Aldrich Chemical, UK. Nicotine hydrogen tartrate, hydrochloric acid and sodium hydroxide were all supplied by Sigma and of pharmaceutical grade. Double distilled water was generated in-house using a Fison's Fi-Streem still.

2.14.1.2 Method

2.14.1.2.1 Loading of Amberlite® IRP69 resin

Amberlite® IRR69 reins were purified/activated and loaded by an adaptation of the techniques by Cheng and by Irwin (Cheng *et al.*, 2002; Irwin *et al.*, 1987).

Purification/Activation of resin

40g Amberlite® IR69 resin was soaked in 2 M HCl for 30 minutes. The acid was then removed by vacuum filtration and the resin was rinsed with distilled water. The procedure was repeated with 1.5 M NaOH and again with 2 M HCl after which the resin was washed with distilled water until the pH of the filtrate was greater than pH 4. The activated resin was then freeze-dried using a Virtis Advantage® freeze-drier for 24 hours

Drug-resin complex

40 g of activated Amberilte® IRP69 resin was stirred in 800 mL of 30 mg/mL nicotine solution made using 0.01 M HCl. After 24 hours, the suspension was centrifuged at 4000 rpm for 5 minutes and the resulting supernatant diluted (0.3 mL in 100 mL) with distilled water and analysed to determine the nicotine uptake. The loaded resins were then freeze-dried for 24 hours.

2.14.1.2.2 Release from nicotine Amberlite® IRP69 and Nicotine Polacrilex resin

100 mg of loaded Amberlite® IRP69 resins was placed in 5 mL of 0.1 M HCl and 0.1 M NaCl and stirred for 24 hours. After 24 hours the suspension was centrifuged at 4000 rpm for 5 minutes. The supernatant was removed and diluted using distilled water (0.3 mL in 100 mL) and the nicotine released determined using the HPLC method described in section 2.1. This procedure was also conducted to determine the release of nicotine from Nicotine Polacrilex (Appendix 2).

CHAPTER THREE
IN VITRO CHEWING GUM RELEASE STUDIES

3.1 Introduction

There are a number of factors that determine the rate and amount of drug released from chewing gum both *in vitro* and *in vivo.* Not only is the formulation of the gum of importance but also the properties of the active substance and the individual chewing the gum can determine how quickly or slowly the drug is released from the gum base.

The nicotine within the Nicorette® chewing gum has been formulated as a complex bound to a cation exchange resin which is claimed to prolong nicotine release (Lichtneckert, *et al.*, 1974). The aim of this study was to investigate factors that would affect the release of nicotine from Nicorette® chewing gum *in vitro.* The chewing gum machine was validated and then used to carry out a series of experiments to determine how factors such as chew rate, pH, temperature, ionic strength, volume of dissolution medium, and the use of different dissolution buffers would affect the rate and amount of nicotine released from the gum.

3.1.1 Factors affecting release from chewing gum

3.1.1.1 The active substance

Active substances may be found in the form of salts or compounds with different solubilities *e.g.* prodrugs, thus the compound offering the best properties for achieving optimal release may be selected. It has been reported in the literature that the release rate of a drug from chewing gum is firstly dependent on the solubility of the drug in water/saliva (Rassing and Jacobsen, 2003). Highly hydrophilic substances will be almost completely released from the chewing gum within 10-15 minutes whilst drug substances with solubility in water of less than 0.1-1 g/100 mL will dissolve in the lipophilic components of the gum base and will exhibit a slow and possibly incomplete release. Also, if the solubility of the active substance is affected by the pH, a suitable release rate may be achieved by adding buffer substances to the formulation. The release of the buffer substance should be related to the release of the active substance in order to achieve the optimal effect hence, controlling the release by adding buffer substances may be quite complex (Rassing and Jacobsen, 2003).

The first extensive investigation of the effect of drug solubility on the drug release from gums was conducted by Kassen *et al.* (1973). They investigated the *in vitro* release from chewing gum for six drugs with varying solubilities. The chewing gum base used in this study was Vinnapas®, a synthetic gum base comprising mainly of polyvinylacetate. The relationship between the drug solubility and time to 100% release was observed and it was found that, as the water solubility increased, the time required to obtain 100% release was reduced. However, this was the case for only five of the drugs studied. The sixth drug studied (calcium gluconate) did not follow this relationship. The time to 100% release was short which was not predicted from its water solubility. The investigators explained that complexes were formed between calcium gluconate and sucrose (sweetener) in the chewing gum and this resulted in the drug being released faster than anticipated.

Other studies that were conducted to examine the effect of the solubility of the drug also found similar results. To exemplify:-

- The *in vitro* release of phenylpropanolamine hydrochloride (PPA, aqueous solubility 1:2) was investigated by Rider *et al.* (Rider *et al.*, 1991). They observed a fast release of PPA (5 min-75%; 15 min-90 %) which produced a good correlation between *in vitro* and *in vivo* results.

- Jensen *et al.* (1991) used the mastication device described by Christrup and Moller (1985) to investigate the *in vitro* and *in vivo* release rate of noscapine containing chewing gum. The release of noscapine hydrochloride (aqueous solubility 1:4), embonate (aqueous solubility 1:10000) and free base (slightly soluble in water) after 5 minutes of mastication was 78%, 23% and 8% respectively. Results indicted that the % release appeared to be dependent on solubility.

To obtain an optimal formulation that produces the desired release profile, it is may be necessary to adjust the release rate of the drug from the gum, either to obtain a slower release of readily water-soluble component or to obtain a faster or more complete release of a water–insoluble component.

It is also possible to increase or delay the release of an active substance by changing the physical forms through a variety of coating and encapsulating techniques of the substance particle. The active substance may be encapsulated in hydrophilic or a hydrophobic coating.

To reduce the release rate, a coating with ethyl cellulose can be used (Ellermann, 2002). Patents in this area describe numerous examples (Huzinec, *et al.*, 1999; Wong, *et al.*, 1997); however, many of the methods have only limited use in chewing gum formulations as the coatings can be destroyed during the gum mixing process.

3.1.1.2 The chewing gum

Several methods are available to alter the release of drugs from chewing gum by modification of the gum base. The water content of gum base is very low and the gum binds lipophilic substances very firmly. In order to obtain the optimal formulation, it is possible to

- Decrease the release rate of highly hydrophilic substances
- Increase the release rate of lipophilic substances
- Achieve a more complete release of lipophilic substances
- Prolong the release

Changing the aqueous solubility of the active substance will increase or decrease the release. A similar effect may be obtained by changing the hydrophilic/lipophilic balance of the chewing gum formulation. The simplest way to achieve this is to increase or decrease the amount of gum base. An increase in the gum base will make the formulation more lipophilic and thus reduce the release rate of a given active substance. However, as a high content of standard gum base hinders the manufacturing process, special gum base properties are required for formulations with high gum base content (Ellerman, 2002).

Instead of changing the gum base content, it is far more effective to change the release properties by adding solubilisers to the formulation (Andersen and Pedersen, 1996). This method enables release from the chewing gum of even highly insoluble substances. However, using solubilisers affects the texture of chewing gum which may result in the residual product becoming soft to an unacceptable degree after a very short period of chewing.

Christrup and Moller (1985) investigated the release of ascorbic acid from chewing gum formulations. Ascorbic acid was mixed with hydrophilic components and added to the chewing gum while, in others, ascorbic acid was added with hydrophobic components. Results showed that the latter formulations showed a slower but complete release of drug compared to the former formulation.

Other methods to modify release of drugs from chewing gum comprise granulation of the drug with hydrophilic components/melted lipids or by mixing the drug with a melted polymer (Yang, 1987).

3.1.1.3 The individual

As with other pharmaceutical products, inter-patient variance also occurs for medical chewing gum. In addition to the conventional inter-patient variations when using medicated chewing gums, the individual needs to chew the gum to extract the drug. Consequently, the release is related to the time the gum is chewed, to the frequency and intensity of the chewing and also to the amount and composition of the individual's saliva.

3.1.2 Release from ion-exchange resin

Since the active substance within Nicorette® gum is Nicotine Polacrilex, dissolution studies were also conducted on Nicotine Polacrilex in parallel to the chewing gum studies in order to determine how the volume of dissolution medium, rotation speed, pH, ionic strength, temperature and various dissolution media would affect nicotine release from the Polacrilex resin. This would determine if any changes in release were due to the resin (active) or the chewing gum formulation.

Drug release from the ion exchange resin is initiated by the penetration of cations into the resin which will compete with bound drugs for sulphonate ionic sites. The whole process is diffusion-based and is described by the model

$$F_t = 1 - \frac{6}{\pi^2} \cdot \sum_{n=1}^{\infty} \frac{1}{n^2} \cdot \exp\left[- \frac{4\pi^2 n^2 D.t}{d^2} \right]$$

equation 3.1

with a rate constant B defined as :

$$B = \frac{4\pi^2 D}{d^2}$$

equation 3.2

Practically, this can be calculated from:

$$F_t = 1 - \frac{Q_t}{Q_o} = \frac{M_t}{M_\infty}$$

equation 3.3

Where:

F_t	=	fraction of drug released from resinate at time t
Q_o	=	initial drug content of the resinate (g g^{-1})
Q_t	=	drug content of resinate at time t (g g^{-1})
M_t	=	drug released from resinate at time t (g)
M_∞	=	total drug released from resin at exhaustion (g)
D	=	Diffusion coefficient of drug within resin (m^2 min^{-1})
d_p	=	mean diameter of resin particles (m)
t	=	time into dissolution (minutes)
n	=	Summation variable incrementing from unity to infinity.

The variables in this equation which control the rate of drug release are the particle diameter (d_p) and the diffusion coefficient of the drug (D) in the resin. The rate constant B may be calculated from the product *B.t*, represented in the following equations:

When $F_t \leq 0.85$

$$B.t = 2\pi - \frac{\pi^2 F_t}{3} - 2\pi \sqrt{1 - \frac{\pi F_t}{3}}$$

equation 3.4

or when $F_t \geq 0.85$

$$B.t = - \ln(1-F_t) - 0.04977$$

equation 3.5

where a plot of *B.t vs t* gives a linear plot of slope *B* (Irwin, 2002).

3.2 Chewing machine validation

The chewing machine used for testing the release of active drug from the gum was validated to ensure that it was reliable, robust and above all reproducible. The chewing machine used in this study was an EP chewing machine and has been described previously in section 2.3. The main concern regarding the use of the machine was the performance of the interval timer as this component will control the rate at which the gum was chewed. In previous studies, it has been shown that chew rate has an effect on the release of drug substances from the chewing gum (Kvist, *et al.*, 1999). Thus, it is important to ensure that chew rate obtained by the chewing machine is consistent as well as being reproducible.

The design of the interval timer on the chewing machine used in this study was an analogue 0-18 second timer. The analogue dial had numerical increments of 0, 0.8, 3, 6, 9, 12, 15 and 18. Since the chew rate plays such an important role in the *in vitro* release of drug, a test was conducted on the various increments of the interval timer to establish, firstly, the chew rate at each increment and, secondly, any inter-day and intra-day variation in the chew rate at each increment of the analogue timer.

3.2.1 Method

3.2.1.1 Number of chews *per* minute

The chewing machine was switched on and the temperature of the chewing chamber was allowed to equilibrate. The interval timer setting was selected, the chewing pistons switched on and the number of chews every minute was noted for 30 minutes. After each run the chewing machine was switched off and was left for 5 minutes. The whole process was then repeated for each interval timer setting.

Gum used	Nicorette® 2 mg
Temperature of chewing chamber	37°C ± 1°C
Dissolution medium	40 mL 0.1% w/v sodium lauryl sulphate (SLS) solution

3.2.1.2 Inter-day and intra-day variation in the number of chews *per* minute

The number of chews every minute for 10 minutes was noted for each interval setting twice a day for four days.

Gum used	Nicorette® 2 mg
Temperature of chewing chamber	37°C ± 1°C

Dissolution medium 40 mL 0.1% w/v SLS solution

An analysis of variance was conducted to determine if there was any inter-day or intra-day variation in the number of chews *per* minute.

3.2.2 Results and discussion

3.2.2.1 Number of chews *per* minute

The number of chews *per* minute on the various increments on the interval timer was investigated and the mode number of chews on each setting determined (Figure 3.1, Table 3.1). The variation in the chew rate on a continuous run of 30 minutes for each of the interval timer setting was small, SD < 2.0, this suggested that there was very little variation in the number of chews *per* minute on the interval timer settings. Conversely, looking at the % RSD (relative standard deviation) of the various timer settings it was found that as the interval timer increased and the number of chews *per* minute decreased the % RSD increased (Table 3.1). This suggested that, at the lower interval timer settings, there was the likelihood of variations in the number of chews *per* minute.

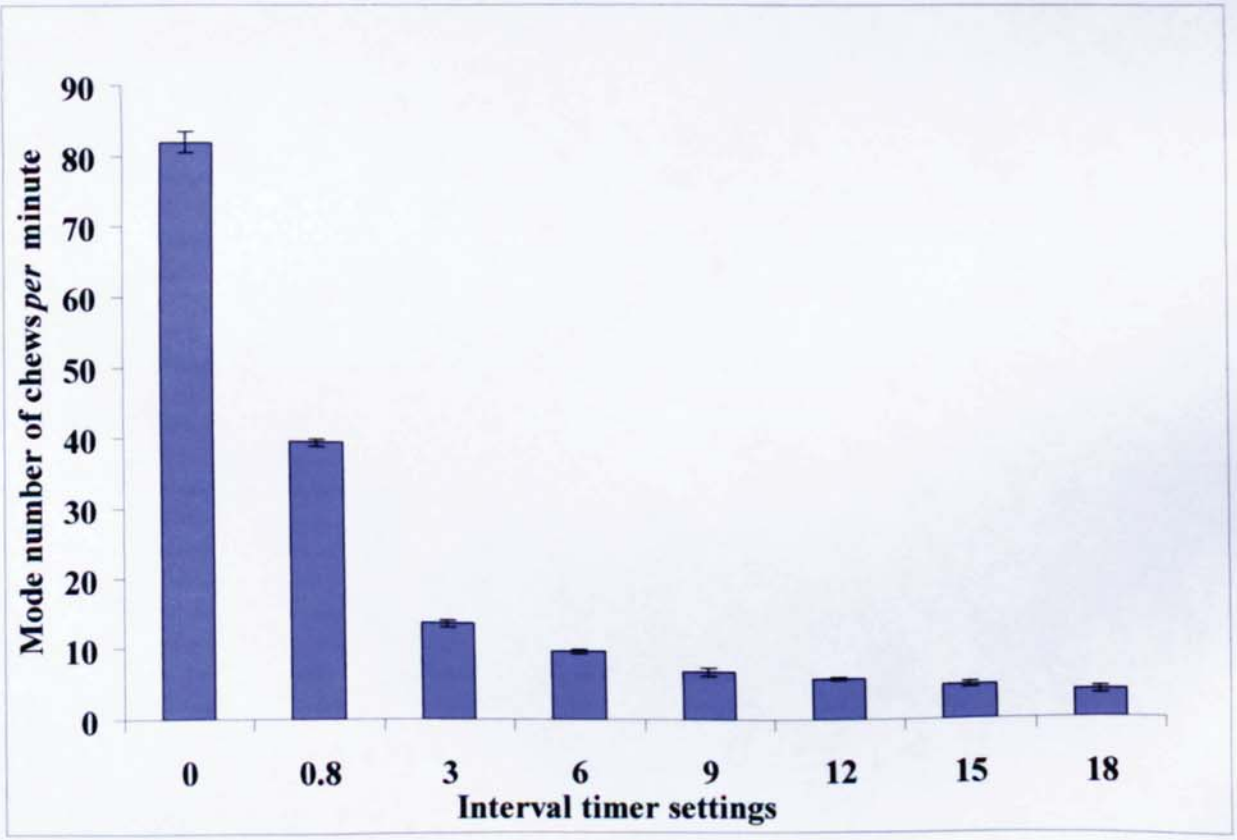


Figure 3.1 The mode number of chews *per* minute on the various interval timer settings (n=30 ± SD).

	Interval Timer settings							
	0	0.8	3	6	9	12	15	18
Mean	81.20	39.70	14.40	9.87	7.43	6.03	4.77	3.60
Mode	82	40	14	10	7	6	5	4
SD	1.540	0.535	0.498	0.346	0.568	0.183	0.430	0.498
% RSD	1.9	1.3	3.5	3.5	7.6	3.03	9.01	13.8

Table 3.1 Number of chews *per* minute on the different interval timer settings

3.2.2.2 Inter-day and intra-day variation in the number of chews *per* minute

Analysis of variance was conducted to determine if there was any inter-day and intra-day variation in the number of chews *per* minute on the different interval timer settings (Table 3.2).

Source	Type III Sum of squares	df	Mean square	F	Sig.
Corrected Model	405909.461[a]	63	6443.007	19745.684	0.000
Intercept	282487.385	1	282487.385	865730.32	0.000
Setting	405641.580	7	57948.797	177593.88	0.000
Inter-day	38.415	3	12.805	39.243	0.000
Intra-day	0.111	1	0.111	0.341	0.560
Setting* inter-day	139.919	21	6.663	20.419	0.000
Setting* intra-day	4.791	7	0.684	2.098	0.042
Inter-day*intra-day	12.010	3	4.003	12.269	0.000
Setting * inter-day* intra-day	43.492	21	2.071	6.347	0.000
Error	187.622	575	0.326		
Total	689368.000	639			
Corrected total	406097.083	638			

Table 3.2 Analysis of variance. Dependent variable: rate
Output from SPSS 11.0
a. R Squared =1.000 (Adjusted R Squared = 0.999)

Results indicate that, within the same day there was no difference in the chew rate (F = 0.341, Significance = 0.560). However, between days, results suggest that there was some

difference in the chew rate (F = 39.24, significance = 0.0). The main source of variation was seen when comparing the different interval timer settings of the chewing machine (F = 177593.88, Significance = 0.00); however, this was what one would have expected as each setting of the interval timer gives a different chew rate. Post Hoc tests were conducted on the inter-day and setting data to highlight differences. Post Hoc tests were not preformed for intra-day as there were no differences seen in the chew rate within the same day.

(I) Inter-day	(J) Intra-day	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence interval Lower Bound	95% Confidence interval Upper Bound
1.00	2.00	0.7282*	6.397E-02	0.000	0.5639	0.8926
	3.00	0.6845*	6.397E-02	0.000	0.5201	0.8488
	4.00	0.4282*	6.397E-02	0.000	0.2639	0.5926
2.00	1.00	-0.7282*	6.397E-02	0.000	-0.8926	-0.5639
	3.00	-4.3750E-02	6.387E-02	0.903	-0.2078	0.1203
	4.00	-0.3000*	6.387E-02	0.000	-0.4641	-0.1359
3.00	1.00	-0.6845*	6.397E-02	0.000	-0.8499	-0.5201
	2.00	4.375E-02	6.387E-02	0.903	-0.1203	0.2078
	4.00	-0.2562*	6.387E-02	0.000	-0.4203	-9.2179E-02
4.00	1.00	-0.4282*	6.397E-02	0.000	-0.5926	-0.2639
	2.00	0.3000*	6.387E-02	0.000	0.1359	0.4641
	3.00	0.2562*	6.387E-02	0.000	9.218E-02	0.4203

Table 3.3 Inter-day Tukey HSD,dependent variable: rate
Calculations based on observed means. * The mean difference is significant at the 0.05 level.

Post Hoc tests confirmed that there were differences between the different interval timer settings of the chewing machine, (significant value = 0.00 indicating that all settings were different to each other). Tests conducted on inter-day variations confirmed that the chew rates were different on each day except days 2 and 3 where no difference was observed (Table 3.3). Results highlight that the day-to-day variation of the chewing machine was significant, however the spread of data was very narrow hence absolute variation was still quite small.

It was concluded that on each day of testing the chewing machine would be calibrated and set to the appropriate chew rate so that the exact chew rate would be known and hence minimising variations as statistical analysis indicated no differences in intra-day chew rates.

3.3 Comparison of *in vitro* release using real and artificial saliva

As detailed in section 1.1.5.3, there is considerable variation in the flow rate of saliva between individuals, with time of day, age, sex and during disease conditions. In this project, whilst testing the *in vitro* release from medicated chewing gum, it was decided that artificial saliva would be used as a dissolution medium. Although real human saliva would be an ideal dissolution medium to use, there were many disadvantages regarding its use. A large volume of real saliva would be needed to undertake the many studies. The method of collecting and preparing real saliva is lengthy and time-consuming, hence, would be impractical to use. Other disadvantages of using real saliva include the risk of contamination thus the need to sterilise equipment between chewing cycles, and the subject to subject variability in the saliva characteristics could affect the *in vitro* release profiles resulting in high levels of variability between identical cycles.

A study was conducted to confirm the suitability of artificial saliva during the chewing gum dissolution cycles. The release from Nicorette® 4 mg gum in artificial saliva was compared with that of the release in real saliva. During the course of this project, the chewing machine pistons were changed which affected the release profile of the gums. The dissolution profile of the gum in real saliva was conducted using the replacement set of pitons but, release in artificial saliva comparisons were made pre- and post- piston change.

3.3.1 Method

3.3.1.1 Collection and preparation of human saliva

The method used for collection and preparation of human saliva was based on a method supplied by GSK.

Exclusion criteria for saliva donors

- Smokers.
- Anyone with a current infection, *e.g.* colds, flu, stomach bug, etc.
- Anyone that was currently being treated with antibiotics.

- Anyone who has been identified as having glandular fever within the last six months.
- Anyone under 18 or over 65 years of age.
- Anyone with tender, sore or bleeding gums.

Method of saliva donation

- No food or drinks (except water) were consumed for one hour prior to saliva donation or during the donation period.
- Teeth were cleaned with water for two minutes prior to saliva donation.
- Orbit sugar-free chewing gum, a sterile container and an ice bucket was provided to each donor.
- The chewing gum was then chewed and the saliva was dispensed into the sterile container and stored on ice

Note that chewing gum was given to the saliva donors to stimulated saliva production (Table 1.4).

Saliva Filtration

- The collected saliva was pooled and centrifuged in a Hettich® Universal 32 centrifuge at 4000 rpm for 15 minutes
- The supernatant was pooled and then filtered through a 0.45 µm vacuum Costar filter in a Class II safety cabinet.
- The filtrate is stored on ice until use

3.3.1.2 *In vitro* chewing

Artificial saliva was made and used as described in section 2.8 and real saliva collected (section 3.5.1.1). The standard *in vitro* chewing gum dissolution method (section 2.3) was used to chew 4 mg Nicorette® in the real and artificial saliva. Samples were taken and the pH value (section 2.7) of all the samples determined prior to analysis. The release curves pre- (piston set 1) and post-piston change (piston set 2) in artificial saliva were then compared to release using real saliva using Moore and Flanner's f_2.

Note: - Release in real saliva was conducted using piston set 2 only.

3.3.2 Results and discussion

Comparing the dissolution profiles of the real saliva to that of artificial saliva (1) and artificial saliva (2) gave f_2 values of 58.8 and 67.4 respectively. This indicates that both release profiles in artificial saliva were similar to the release profile of 4 mg Nicorette® in real saliva (Figure 3.2). A faster initial release of nicotine was seen from the Nicorette® chewing gum chewed using piston set 2 compared to that of set 1, but f_2 comparisons showed that the release was similar (f_2 = 51.38). In spite of the difference in the initial release of nicotine from the gum, all three dissolution runs gave the same release of nicotine after 30 minutes of chewing.

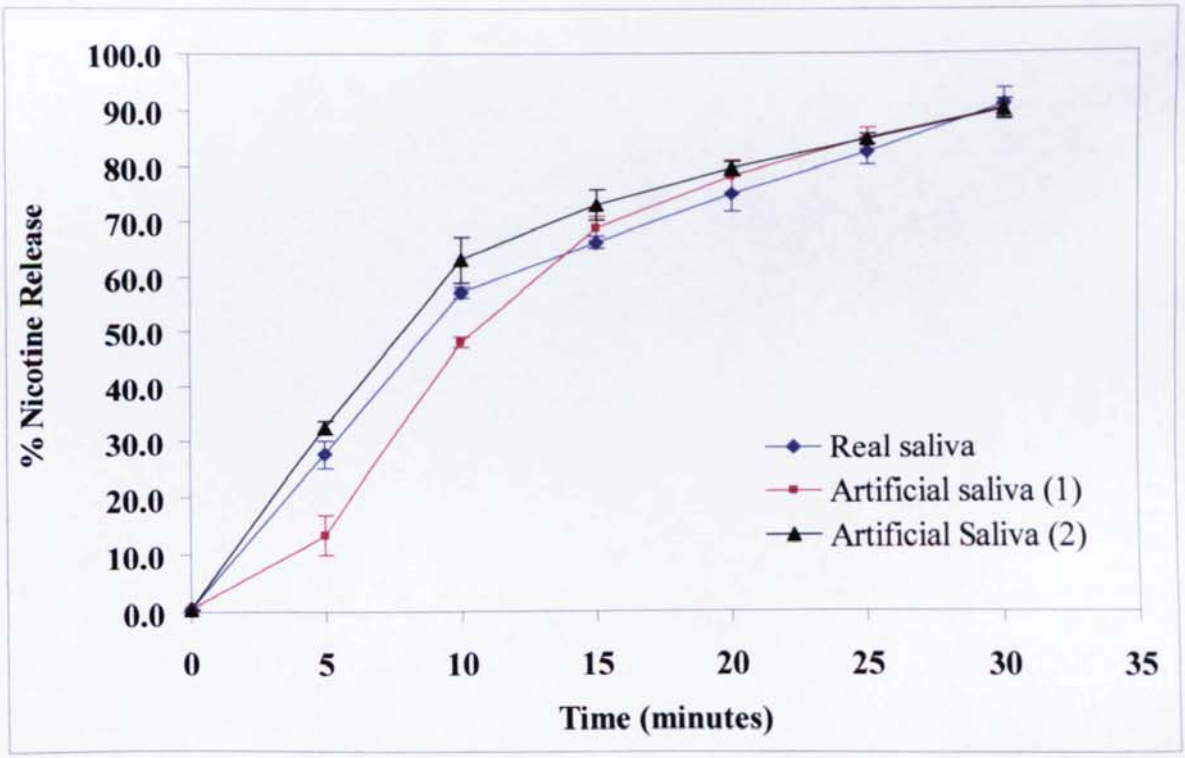


Figure 3.2 4 mg Nicorette® release profile in real and artificial saliva (n = 3 ± SD). (1) indicates results pre-piston change, (2) indicates post-piston change.

Within 10 minutes of chewing (artificial saliva (1)), a linear increase in pH was observed which then levels at about pH 8.4 (Figure 3.3). Final pH value observed using real saliva and artificial saliva (2) were 8.7 and 8.6 respectively indicating pH changes of about 1.1 pH units, hence, showing similar properties of both real and artificial saliva in terms of buffering capacity.

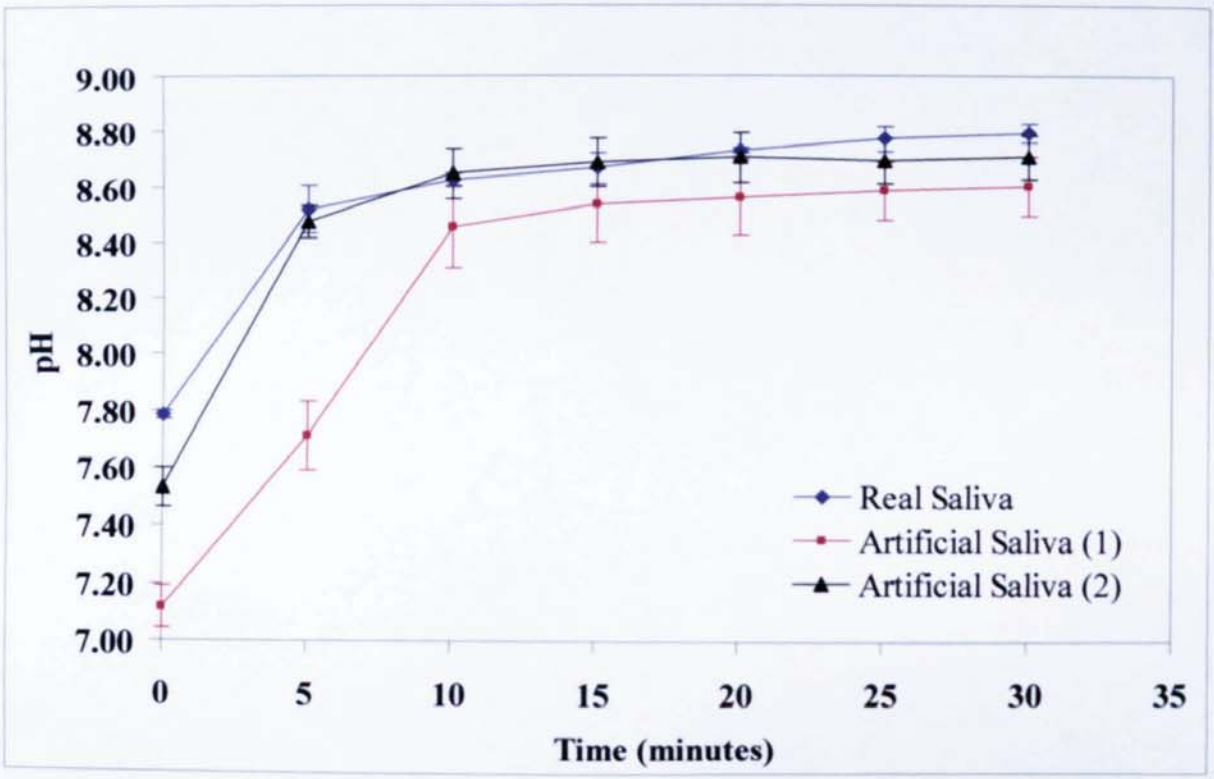


Figure 3.3 4 mg Nicorette® pH profile in real and artificial saliva (n = 3 ± SD). (1) indicates results pre-piston change, (2) indicates post-piston change.

Results showed that there was little difference in the release of nicotine from Nicorette® 4 mg gum in real and artificial saliva as f_2 values confirmed similarities in the release profiles. It was therefore concluded that using artificial saliva would not affect or alter the release of nicotine from gums significantly, thus, it was acceptable to use as an alternative to real saliva for the *in vitro* testing of chewing gum.

3.4 Release from Nicorette® gum

Several studies have demonstrated that the release of nicotine from Nicorette® chewing gum was incomplete. Pharmacia's promotional literature states that *in vivo* nicotine extraction efficiency from the 2 mg gum is approximately 65%. In one independent report (Benowitz *et al.*, 1987), it was estimated that 53% of nicotine was extracted from a 2 mg gum and 72% from a 4 mg gum following a 20 minute chew-out study. In another report (Nesmeth-Coslett *et al.*, 1988) using just the 4 mg gum, it was estimated that approximately 54% was released following a 10 minute chew out (1 chew/second) and 46% was released after 10 minutes with a chew rate of 1 chew every 8 seconds. Here, a study was conducted using the EP chewing apparatus to determine whether the *in vitro* release of nicotine from 2 mg and 4 mg Nicorette® gum were similar to that found during the *in vivo* chew-out studies.

3.4.1 Method

The standard chewing gum dissolution method (section 2.3) was used to determine the dissolution profile in artificial saliva (section 2.8). The pH (section 2.7) of all the samples was determined prior to analysis. Results of the percentage release from the 2 mg and 4 mg Nicorette® gums were compared using Moore and Flanner's f_2 equation.

3.4.2 Results and discussion

Using the chewing apparatus, approximately 41% of nicotine was released from the 2 mg Nicorette® gum and 86% from the 4 mg after 30 minutes of chewing at 60 chew/minute (Figure 3.4). Comparison of the dissolution curve using Moore and Flanner's f_2 equation indicates that the dissolution profiles from the 2 mg and 4 mg Nicorette® were dissimilar (f_2 = 24.23). The results correspond to the earlier reported *in vivo* chew out studies where greater extraction efficiency was reported from the 4 mg gum (72%) in comparison to the 2 mg gum (53%) (section 3.4).

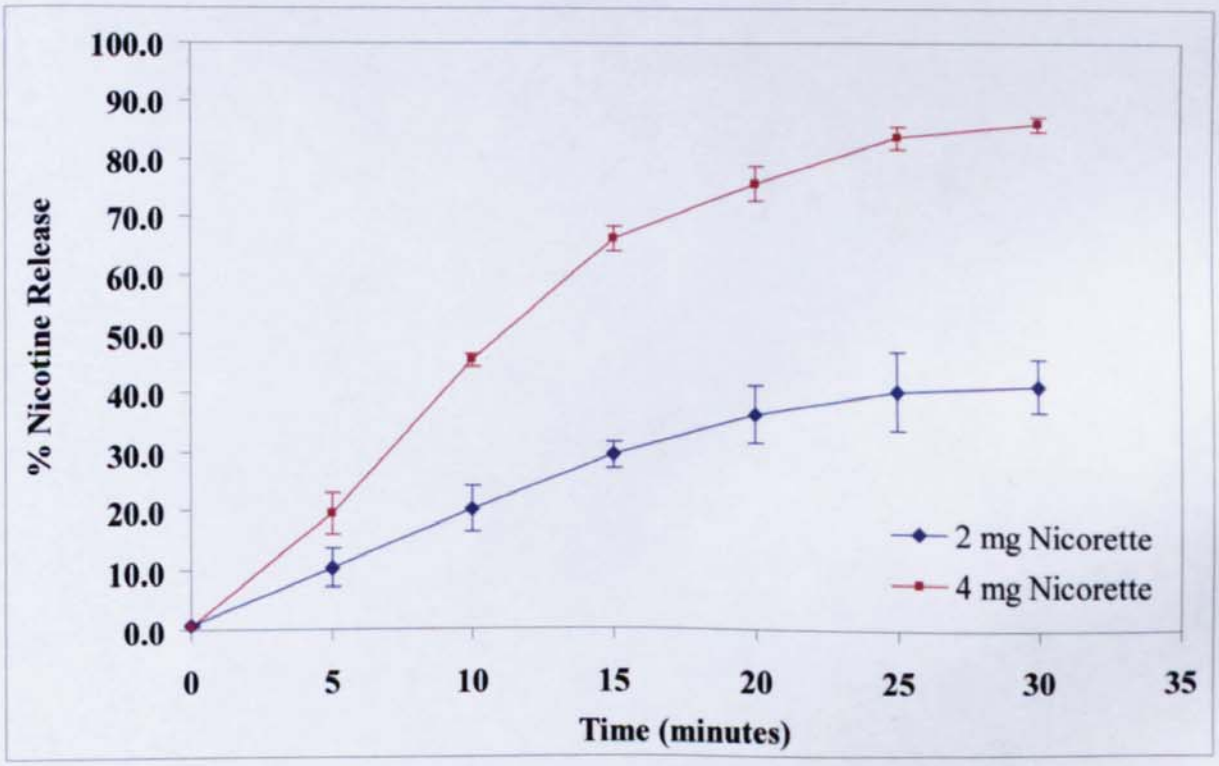


Figure 3.4 Release of nicotine from 2 mg and 4 mg Nicorette® in artificial saliva f_2 = 24.23 (n = 3 ± SD)

A pH change of 1.481 ± 0.044 pH units from 4 mg Nicorette® in comparison to a pH change of 1.232 ± 0.112 pH units when 2 mg Nicorette® was chewed showed that there was a significant difference in the pH changes (p = 0.023) (Figure 3.5). 4 mg Nicorette® contains

30 mg sodium carbonate (13 mg sodium salt) in comparison to the 2 mg gum which contains 20 mg sodium carbonate and 10 mg sodium bicarbonate (11.42 mg sodium salt). pH changes were therefore greater when the 4 mg gum were chewed as more sodium salts was released.

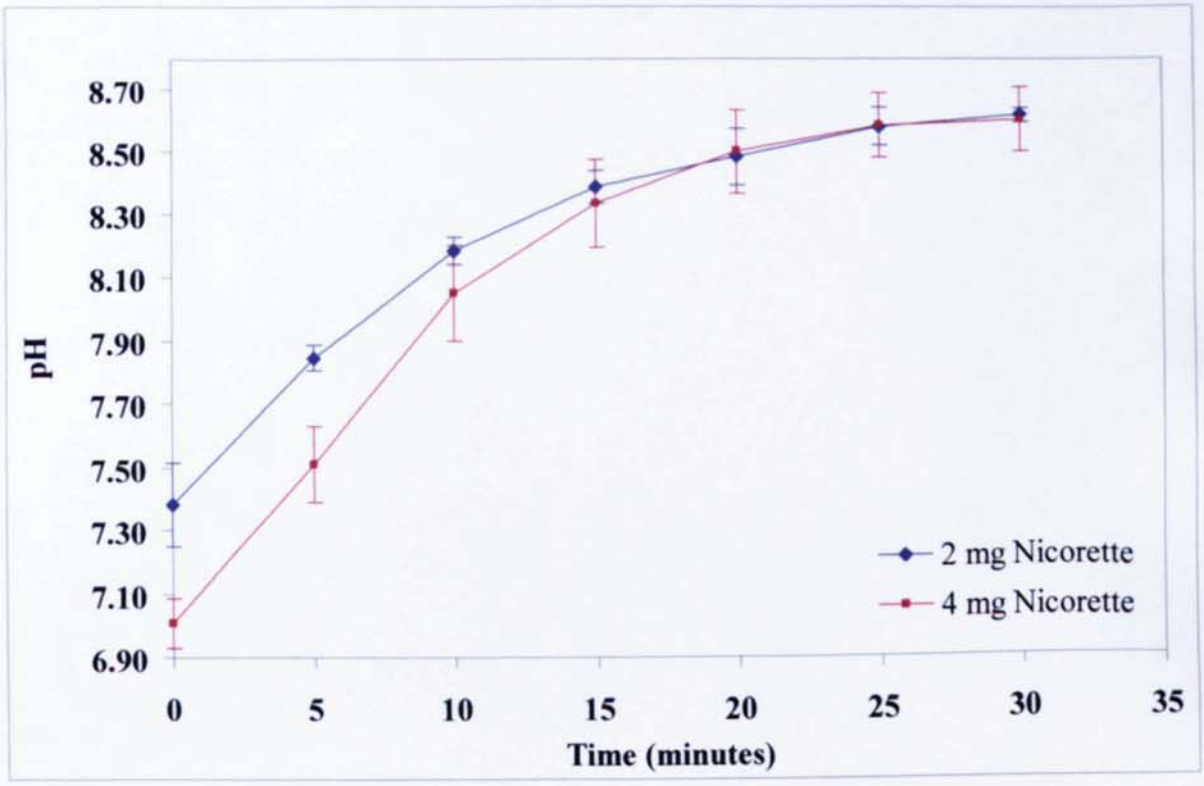


Figure 3.5 pH of the artificial saliva during release of nicotine from 2 mg and 4 mg Nicorette® (n = 3 ± SD)

Nicorette® patient information leaflets state that the gum should be chewed for approximately half and hour. Both reported *in vivo* (Benowizt, *et al.*, 1987; Nesmeth-Coslettet, *et al.* 1988) and this experimental *in vitro* data shows that after 30 minutes of chewing the release of nicotine for the gums was incomplete.

3.5 Diffusion of nicotine through Nicorette® chewing gum

For drug to be released from medicated chewing gums, the gums need to be chewed to allow the drug to be released. A study was conducted to test the diffusion of the nicotine through the gum base without any mastication.

3.5.1 Method

Nicorette® 2 and 4 mg strength were used as supplied from a commercial source. The total amount of nicotine present in the gum was determined for each batch of gums (section 2.6).

The gum was placed in 20 mL artificial saliva and stirred for 24 hours at rate of 300 rpm using a magnetic stirrer bar. 1 mL samples were removed at 0, 5, 10, 20, 30 and 60 minutes and finally after 24 hours and medium was replaced with equal amounts of fresh buffer. The nicotine content in each sample was determined by HPLC. Note: - Three replicates were conducted for both 2 and 4 mg gums.

3.5.2 Results and Discussion

The amount of nicotine released from both 2 and 4 mg gum within 60 minutes of diffusion was very similar (Figure 3.6). A steady increase in the amount of nicotine released from the gum was observed. Within the first 5 minutes release from both 2 and 4 mg gum was almost identical after which a greater release was observed from the 2 mg gum which continues to rise. After 60 minutes 0.062 mg and 0.064 mg nicotine was released from 2 mg and 4 mg gums. After 24 hours, 0.266 ± 0.068 mg of nicotine (13.3%) was released from the 2 mg gum whilst 0.249 ± 0.044 mg of nicotine (6.23%) was released from the 4 mg gum indicting that the release from both 2 and 4 mg gum without chewing was in each case very low and found to be similar ($p = 0.735$).

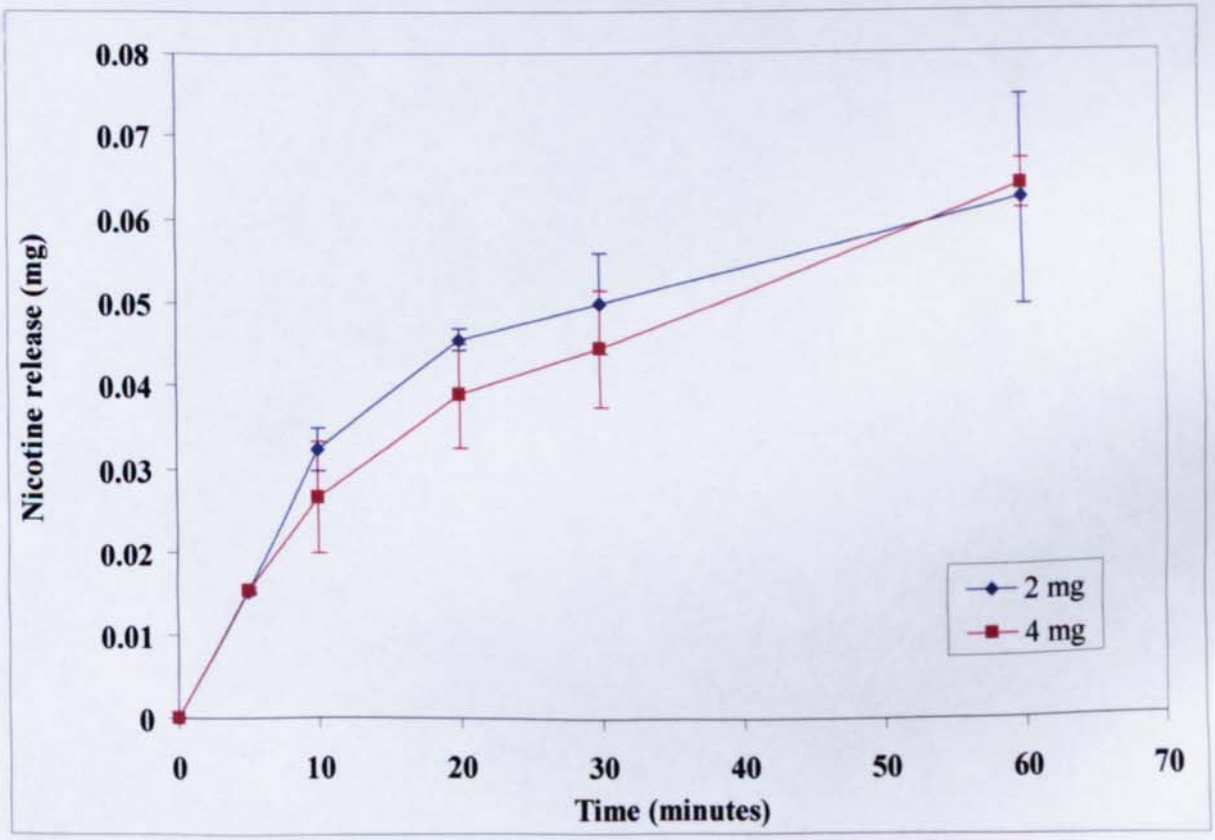


Figure 3.6 Nicotine release from 2 and 4 mg Nicorette® gum without mastication (n = 3 ± SD)

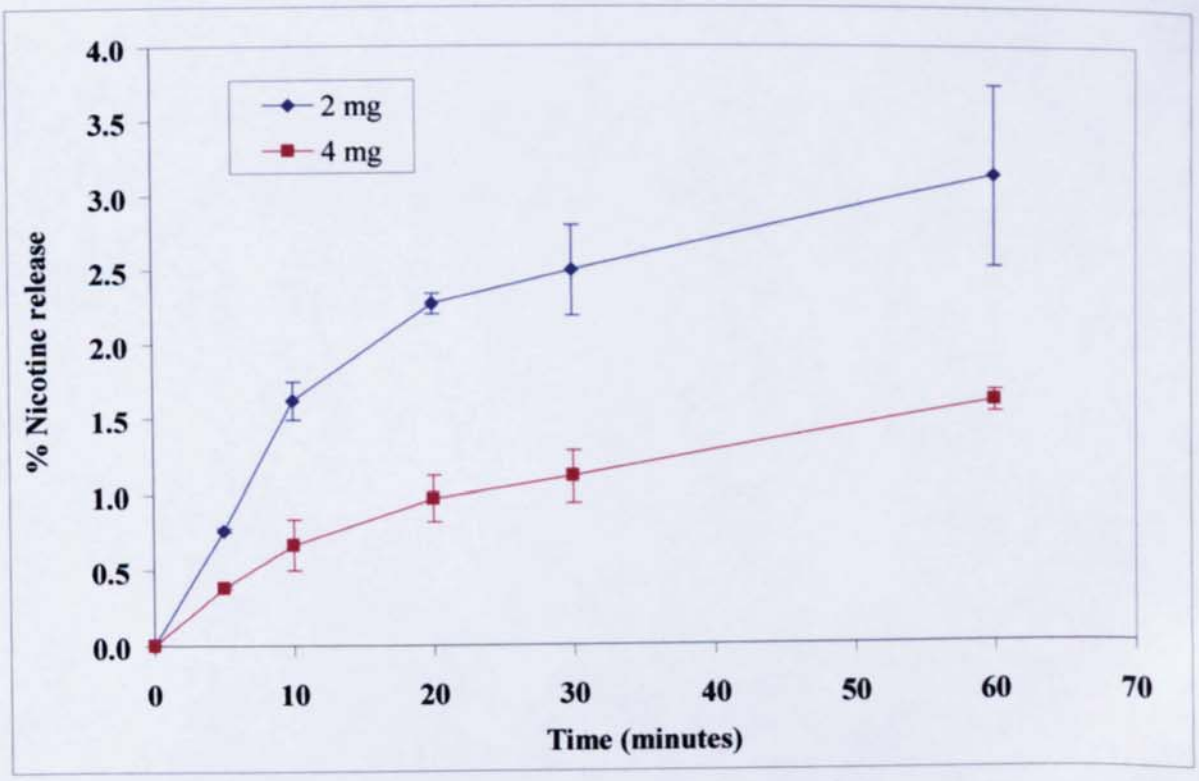


Figure 3.7 Percentage nicotine release from 2 and 4 mg Nicorette® gum without mastication (n = 3 ± SD)

When comparing the percent release of nicotine from the gums, nicotine release from the 2 mg gum was almost twice that released from 4 mg gum (Figure 3.7). After 24 hours 13.30 % ± 3.43 of nicotine was released from the 2 mg gum whilst only 6.22% ± 1.09 of nicotine was released from the 4 mg gum.

Nicorette® 4 mg gum contains double the loading of nicotine compared to the 2 mg gum. The release of nicotine from the gums without mastication showed that although 4 mg gum contains more nicotine, the same amount of nicotine was released from the 2 mg gum. Chewing gum base is a water-insoluble solid that is made with a mixture of elastomers, plasticiser, waxes, lipids, emulsifers and other texture agents added to enhance the overall texture and mouth feel of the gum. Generally, diffusion of a drug through the gum base would be slow as gum needs to be masticated before significant drug is released. Observations here were that a small percentage of nicotine was released from both the 2 mg and 4 mg gum by simple diffusion. Since the same amount of drug was released from both gums a possible explanation for the release could be that nicotine was released from the surface of the chewing gum only and was not diffusing through the gum base. Both 2 and 4 mg Nicorette® gum pieces are the same size and shape thus possessing the same surface

area. When the gums were in contact with the artificial saliva, any nicotine resin at the surface of the gum piece would release bound nicotine. Also, nicotine could diffuse through any micropores that are present within the gum matrix but, generally it can be concluded that diffusion through the gum was low with less than 4% release within 60 minutes therefore chewing is essential to facilitate release.

3.6 Factors affecting release from Nicorette® gum *in vitro*

3.6.1 Materials and Methods

A series of experiments were conducted to investigate *in vitro* dissolution properties affecting the release of nicotine from Nicorette® (2 mg), purchased and use as supplied from a commercial source. For each experiment, the conditions used in the standard method for *in vitro* testing of gums (section 2.3) were adapted. Since the active drug in Nicorette® is Nicotine Polacrilex, dissolution experiments were also conducted using Nicotine Polacrilex supplied by GSK to determine if the factors investigated using the chewing machine had an effect on the gum or on the release of nicotine from the resin. For each experiment the dissolution method described in section 2.4 was adapted to obtain the required conditions for the experiment.

3.6.1.1 Chew rate

3.6.1.1.1 Chewing machine

The standard method for *in vitro* chewing gum testing (section 2.3) was used to chew 2 mg Nicorette® at 82, 60, 42, 22, 12, 6 and 4 chews/minute. Samples were then analysed using the HPLC method described in section 2.1 and the release curves compared using the f_2 equation (section 2.9).

3.6.1.1.2 Release of nicotine from Nicotine Polacrilex

The standard dissolution method (section 2.3) was used to determine the effect of paddle speed on the release of nicotine from Nicotine Polacrilex. 444 mg of Nicotine Polacrilex (80 mg nicotine) was added to 800 mL of 40 mM sodium chloride stirred at a rate of 150, 75 and 20 rpm.

3.6.1.2 Different dissolution media

3.6.1.2.1 Chewing gum

Release of nicotine from the gum was tested in 40 mL of artificial saliva, distilled water and artificial saliva with 0.1% w/v sodium lauryl sulphate (SLS). Samples were analysed using the HPLC method described in section 2.1 and the release curves compared using the f_2 equation.

3.6.1.2.2 Release of nicotine from Nicotine Polacrilex

444 mg of Nicotine Polacrilex was added to 800 mL of artificial saliva, water, 0.1% w/v Tween 20, 0.1% w/v SLS and 0.1% w/v tetradecyl trimethyl ammonium bromide (TTAB) and stirred at 75 rpm.

3.6.1.2.3 Release of sodium from chewing gum.

To determine the release of sodium as the gums were chewed *in vitro*, standard chewing run were conducted using 2 mg and 4 mg gum Nicorette® in water and artificial saliva. Samples were taken at 0, 5, 10, 15, 20, 25 and 30 minutes and then analysed to determine the sodium content using the method described in section 2.5.

3.6.1.3 Temperature

3.6.1.3.1 Chewing gum

Release of nicotine from 2 mg Nicorette® gum was tested in 40 mL of artificial saliva at 23°C, 37°C and 53°C. Samples were analysed using the HPLC method described in section 2.1 and the release curves compared using the f_2 equation. Note that the temperature selected (23°C and 53°C) were the lowest and highest temperature that the chewing chamber could maintain.

3.6.1.3.2 Release of nicotine from Nicotine Polacrilex

444 mg of Nicotine Polacrilex (80 mg nicotine) was added to 800 mL of artificial saliva at 30°C, 37°C and 45°C and was stirred at 75 rpm.

3.6.1.3.3 Texture analysis

Textures of gums exposed to temperatures of 4°C, 27°C and 50°C were studied. Pieces of 2 mg Nicorette® gums removed from the blister pack were stored in the fridge at 4°C for 1 hour. For exposure to high temperature, Nicorette® 2 mg gums were stored in an oven

maintained at 50°C for 1 hour and finally the gums removed from the blister pack were allowed to stand at room temperature for 1 hour. The general texture analysis method (section 2.11) was used to determine the hardness, apparent modulus and adhesiveness of the gum.

3.6.1.4 pH of dissolution medium

3.6.1.4.1 Chewing gum

Release of nicotine from the gum was tested in 40 mL of artificial saliva adjusted to pH 3.0, 5.0, 7.0 and 9.0 using HCl or NaOH as appropriate. The release curves then compared using the f_2 equation.

3.6.1.4.2 Release of nicotine from Nicotine Polacrilex

The standard dissolution method (section 2.4) was used to determine the effect of pH on the release of nicotine from Nicotine Polacrilex resin. 444 mg of Nicotine Polacrilex was added to 800 mL of 150 mM NaCl buffered to pH 3.0, 5.0, 7.0 and 9.0 using HCl or NaOH as appropriate.

3.6.1.5 Volume of dissolution medium

3.6.1.5.1 Chewing gum

Release of nicotine from the gum was tested in 20 mL 40 mL and 80 mL of artificial saliva. The samples were analysed using the HPLC method described in section 2.1 and the release curves compared using the f_2 equation.

Note: - due to problems with the chewing piston, the pistons on the chewing machine were changed and it was found that release using the new pistons was higher than release from the old set of pistons (section 3.3).

3.6.1.5.2 Release of nicotine from Nicotine Polacrilex

444 mg of Nicotine Polacrilex was added to 400 mL, 800 mL and 1000 mL of 40 mM NaCl which was stirred at 75 rpm. Samples were removed and analysed using the HPLC method described in section 2.1 and the dissolution curves compared using the f_2 equation.

3.6.1.5.3 Release of sodium from the gum

Sodium analysis was conducted on the *in vitro* dissolution of nicotine from chewing gum with effect of dissolution volume. Standard chewing runs were conducted using 2 mg Nicorette® gum in 20 mL, 40 mL and 80 mL of artificial saliva. Samples were taken at 0, 5, 10, 15, 20, 25 and 30 minutes and then analysed to determine the sodium content using the method described in section 2.5.

3.6.1.6 Ionic strength

3.6.1.6.1 Chewing gum

Release of nicotine from the gum was tested in 40 mL of artificial saliva at an ionic strength of approximately 30 mM, 40 mM, and 80 mM and water. The ionic strength of the artificial saliva was altered by changing the concentration of the sodium chloride. The addition of 50 mM sodium chloride to the artificial saliva formulation increased the ionic strength to 80 mM. To decrease ionic strength, the sodium chloride was omitted from the formulation.

3.6.1.6.2 Release of nicotine from Nicotine Polacrilex

444 mg of Nicotine Polacrilex was added to water, 10 mM, 40 mM, 150 and 250 mM NaCl at 37°C and stirred at 75 rpm. The amount of nicotine released was determined using HPLC and the dissolution curves compared using the f_2 equation.

3.6.2 Results and discussion.

3.6.2.1 The effect of chew rate on the release of nicotine from gums

The rate and intensity at which the gum is chewed should determine the amount of drug released from medicated gums. Studies were conducted by Nesmeth-Coslett *et al.*, (1987) to assess the effects of varying the rate at which a single piece of 4 mg nicotine gum was chewed. Six volunteers were asked to chew the gum for 10 minutes at varying rates during four sessions. Blood samples and the chewed gum were analysed for nicotine. Additionally, a measure of masticatory pressure was employed to assess the intensity of chewing and to empirically verify the number of chews. A weak but direct relationship between chew rate and the amount of nicotine extracted was found. Also, whilst measuring masticatory pressure and verifying the number of chews, it was found that the subjects showed compensatory changes in behaviour by chewing slower than instructed at the high rate and

faster than instructed in the low rate condition thus, despite instructions to vary chew rates across an 8-fold range, actual chew rate varied by only 2.2 fold. The findings concluded that the rate of chewing nicotine gum can make a difference in the amount of nicotine extracted from the gum.

In another such study, Dong *et al.* (1995) examined the effects of chewing frequency and duration on flow-rate of saliva and sucrose release from chewing gum. Twelve volunteers chewed Wrigley's spearmint chewing gum containing sucrose at chewing frequencies of 35, 50, 70, 90 and 130 chews/minute. It was found that salivary flow-rate was not strongly influenced by chew frequency and was independent of chew frequency over the range of chewing frequencies studied. It was also found that the higher chewing frequencies failed to cause a more rapid release of sucrose from chewing gum. It was reported that this may have been due to less intense clenching and the shorter clenching time at the higher chewing frequencies.

A series of experiments were conducted here to determine the effect of the chew rate on the release of nicotine from Nicorette® *in vitro*. The normal physiological chewing rate ranges from 40 to 80 chew/minutes (Kerr, 1961, Neil, 1967, Louridis *et al.*, 1970). The chew rates used *in vitro* ranged from 4 to 82 chews/minute as this was the maximum and minimum chew rate obtainable from the chewing machine. Also, as the active drug in Nicorette® is Nicotine Polacrilex resin, the effect of paddle speed (rpm) on the release of nicotine from the resin was also conducted to determine if increase agitation of the resins would increase nicotine release.

3.6.2.1.1 Effect of chew rate on release from 2 mg Nicorette®

Drug diffusion through the gum was relatively slow without chewing. Approximately 0.266 mg of nicotine was released from a 2 mg gum (13.3%) and 0.249 mg from a 4 mg gum (6.2%) in 24 hours (section 3.5) thus, for drug to be released from the gum the gum needs to be chewed. As the gum is chewed, new surfaces are exposed which allow drug dissolution to occur.

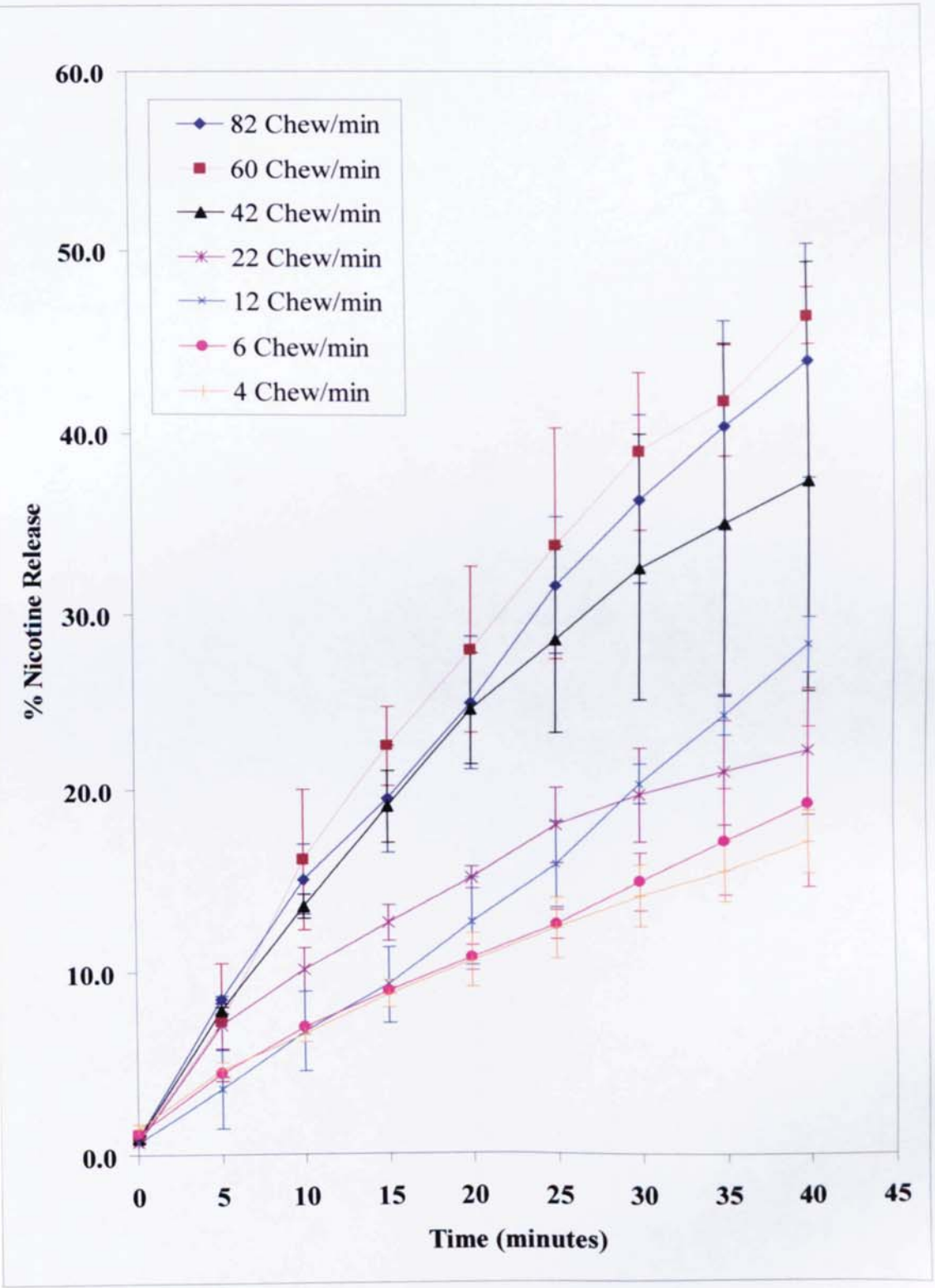


Figure 3.8 Nicotine release from 2 mg Nicorette® chewed *in vitro* at 82, 60, 42, 22, 12, 6 and 4 chews/minute (n = 3 ± SD).

Results showed that as chew rate increased, a greater % of nicotine was released from the gum (Figure 3.8). At the faster chew rates, greater release of drug from the chewing gum is due to a larger surface area exposed. Two patterns can be seen when observing the graph. The first trend was followed by the release at 82, 60 and 42 chews/minutes and the other trend seen by 22, 12, 6 and 4 chews/minute. At the higher chew rates of 82, 60 and 42 chews/minute, a greater percentage release was observed than at the lower chew rates (22, 12, 6 and 4 chew/minute).

Number of chews *per* minute	Number of chews *per* minute						
	82	**60**	**42**	**22**	**12**	**6**	**4**
82		81.31	72.83	44.27	45.26	39.23	38.20
60	81.31		64.33	41.38	42.12	36.86	35.95
42	72.83	64.33		50.31	50.85	43.72	42.53
22	44.27	41.38	50.31		73.49	70.47	67.31
12	45.26	42.12	50.85	73.49		66.85	63.05
6	39.23	36.86	43.72	70.47	66.85		93.17
4	38.20	35.95	42.53	67.31	63.05	93.17	

Key

Dissolution curves not similar

Dissolution curves similar

Dissolution curves not compared

Table 3.4 f_2 values indicating similar and dissimilar dissolution curves with effect to chew rate on the release of nicotine from 2 mg Nicorette®. The mean of the replicates was used to compare the dissolution.

Chew rates of 82, 60 and 42 chews/minute had similar release curves (f_2 greater than 50) and chew rates of 22, 12, 6 and 4 produced similar release curves as f_2 values were all greater than 50 (Table 3.4). f_2 values of 50.31 and 50.85 when comparing release curves of 42 with 22 and 12 chews/minutes respectively showed that the release profiles were similar although very close to the critical value, whilst, graphical representation would suggest that they were dissimilar. Moore and Flanner's f_2 is a mathematical dissolution curve comparison method

(Moore and Flanner, 1996). It is a logarithmic transformation of the sum of squared errors. It takes the average sums of squares of the difference between test and reference profiles and fits the results between 0 and 100. The fit factor (f_2) is 100 when the test and reference profiles are identical and approaches zero as the dissimilarity increases. Values greater than 50 are considered to be similar. Here, the values are just above 50, indicting that they are similar but also highlighting that the similarities are weak. Figures 3.9 and 3.10 illustrate the effect on the f_2 value on generated first-order dissolution curves when the release rate was constant but the A_0 (original starting concentration) differs (Figure 3.9) or when A_0 remains constant and the release rate differ (Figure 3.10). The figures showed how two dissolution curves can appear graphically different but are similar when f_2 is applied.

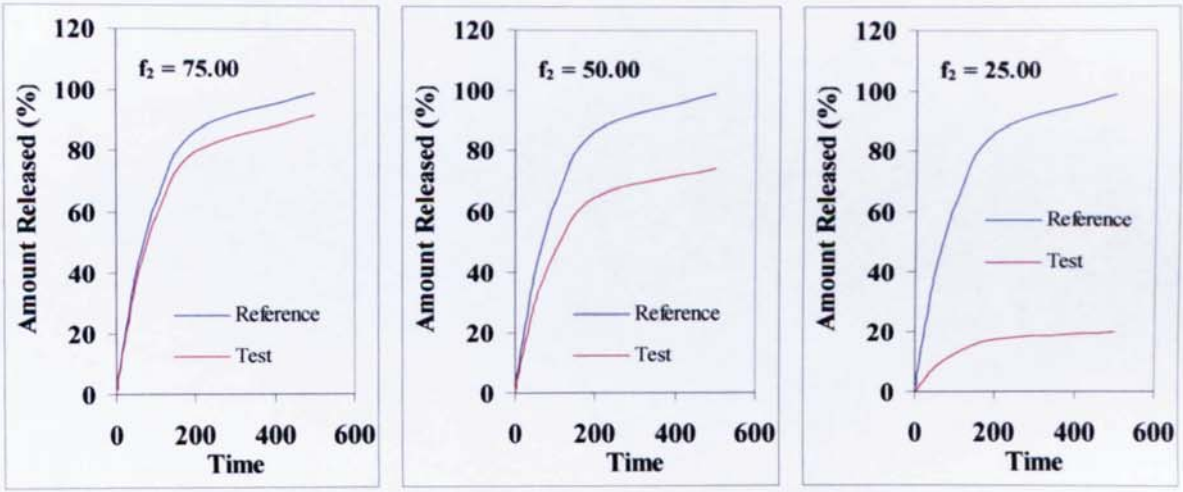


Figure 3.9 Profiles with same release rate but different original starting concentration.

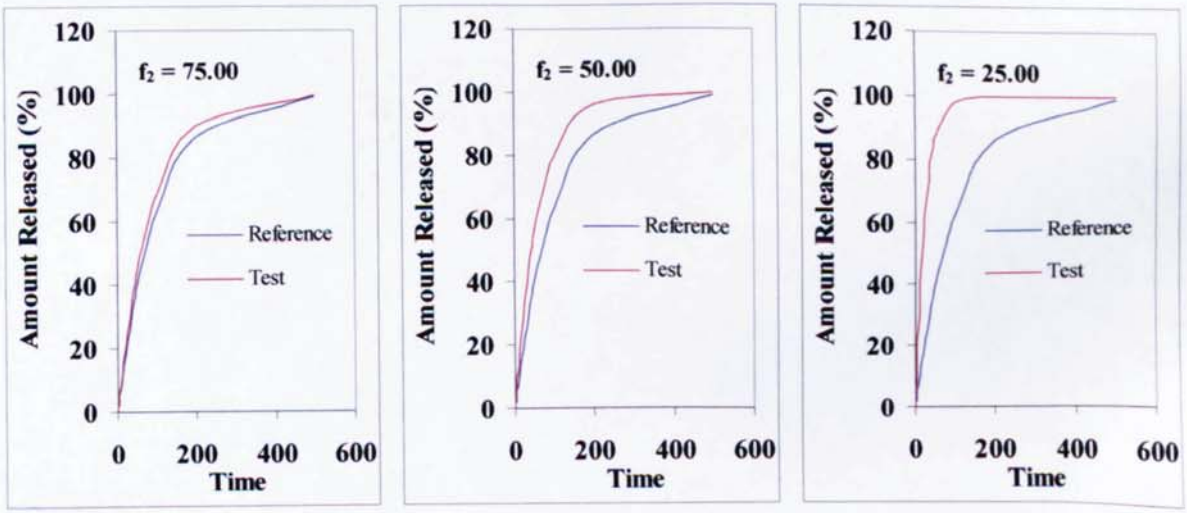


Figure 3.10 Profiles with same original starting concentration but different release rates

As the gum was chewed, the pH of the artificial saliva also increased (Figure 3.11). Chewing the gum not only releases the active component but also releases other soluble excipients within the gum. The increase in pH was a result of the release of sodium bicarbonate and carbonate contained within the gum. As it will be found later (chapter five), the diffusion of nicotine through the buccal membrane was promoted with increasing pH hence, the release of the alkaline buffers from the chewing gum is an important factor to consider as the overall pH of the oral cavity will influence the absorption of nicotine through the buccal membrane.

At time zero, a large variation in the starting pH was observed due the variations in the initial starting pH of the artificial saliva used. The greatest increase in pH was observed at 82 chews/minute (an increase 1.457 pH units) whilst the smallest pH change was seen at 4 chew/minute (an increase of 0.668 pH units). The pH of human saliva ranges from 5.8-7.4 (section 1.1.5.2), thus the alkaline buffers are included to raise the pH of the saliva to allow optimised nicotine diffusion conditions. At lower chew rates (22, 12, 6 and 4 chews/minute), pH increase was less than 1 pH unit. This suggested that the increase in pH in some cases may not be adequate for optimised nicotine diffusion. Thus, the rate at which the gum is chewed not only governs how much nicotine is released from the gum but, also, how rapidly it would diffuse through the membrane. Chewing at a faster rate not only increases nicotine release but it also increases the release of buffering agents, hence, enhancing conditions for nicotine diffusion across the buccal membrane.

3.6.2.1.2 Release of nicotine from Nicotine Polacrilex resin with different stir rates

Ion-exchange resins are generally used in formulations to control the release or for taste masking the active drug. When investigating the effect of stir rate on the dissolution of nicotine from Nicotine Polacrilex, nicotine was released rapidly from the resin (Figure 3.12). The expected slow diffusion of nicotine from the resin was not seen, thus equations 3.1 – 3.5 were not used to calculate the amount of nicotine released. Possible reasons for the rapid diffusion could be due to the physical nature of the ion-exchange resin. Properties such as the particle size of the resin, the porosity, the degree of cross linking, the contact time between the drug species and the ion-exchange resin when loading and the strength of the resin will determine how quickly the drug is released. Due to the small particle size distribution (70% resin was < 75μm) and the fact that Polacrilex is a weak exchange could explain why nicotine was rapidly released from the resin.

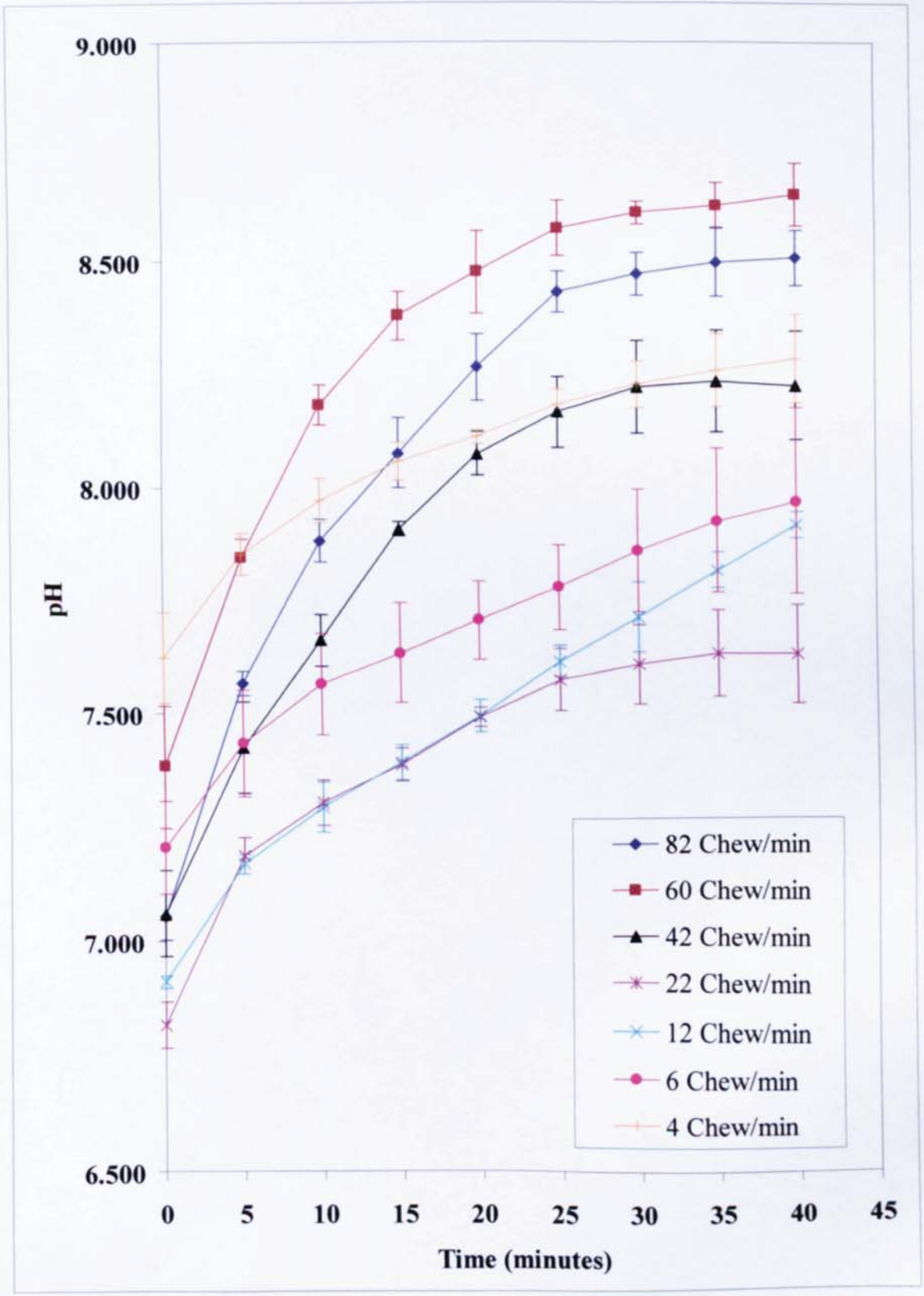


Figure 3.11 pH of the artificial saliva as nicotine was released from 2 mg Nicorette® when chewed *in vitro* at 82, 60, 42, 22, 12, 6 and 4 chews/minute (n = 3 ± SD).

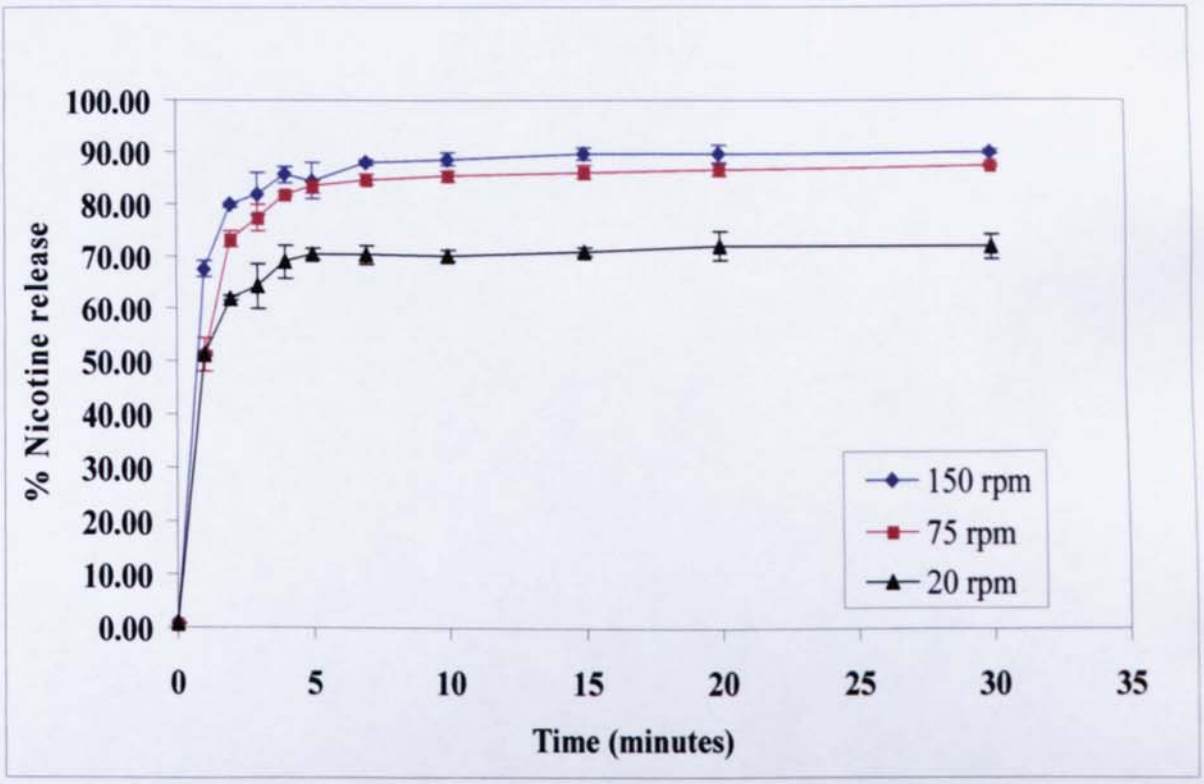


Figure 3.12 Release of nicotine from Nicotine Polacrilex resin *in vitro* with effect of paddle stirring rate (n=3, ± SD).

With increasing paddle speed (rpm), the percentage nicotine released from the resin increased. Within the first minute of dissolution, with stirring rates of 75 and 20 rpm approximately 50% of the nicotine was released from the Polacrilex resin. At 75 rpm, the percentage nicotine released increased to 73% after 2 minutes and continued to rise at a steady rate to give a final release of 88% at 30 minutes, whilst, at 20 rpm nicotine was released at a slower rate (62% released after 2 minutes and 72% at 30 minutes). When the paddle speed was increased to 150 rpm a greater percentage release of nicotine (90% after 30 minutes) was observed in comparison to that at 20 and 75 rpm. Increasing the agitation of the resins not only seemed to increase the rate at which the nicotine was released but also the amount of nicotine released. At higher paddle speeds, the higher degree of agitation of the resins may possibly increase the release of nicotine molecules embedded within the resin matrix thus providing a greater and faster release of nicotine than that observed at the lower paddle speeds.

Dissolution curve comparisons using the f_2 equation (Table 3.5) showed that at 150 and 75 rpm the release of nicotine from the resin was similar (f_2=60.70) whilst, at 20 rpm, the release of nicotine from the resin was dissimilar to release obtained at both 70 and 150 rpm.

		RPM		
		20	**75**	**150**
RPM	**20**		44.93	39.12
	75	44.93		60.70
	150	39.12	60.70	

Key

Dissolution curves not similar

Dissolution curves similar

Dissolution curves not compared

Table 3.5 f_2 values indicating similar and dissimilar dissolution curves for the effect of paddle speed on the release of nicotine from Nicotine Polacrilex resin. The mean of the replicates was used to compare the dissolution.

It can be concluded that the results obtained during this study were similar to those reported earlier (section 3.6.2.1). Increasing *in vitro* chew rate resulted in a greater release of nicotine from the gums, therefore, the rate at which Nicorette® gum is chewed affects the amount of nicotine released. It was also found that increasing the degree of agitation during dissolution of Nicotine Polacrilex not only increased the rate of nicotine release but also the amount of nicotine released from the resin. When using a 40 mM sodium chloride solution (similar ionic strength to artificial saliva) for investigating the release of nicotine from Nicotine Polacrilex, a rapid release of nicotine was observed within the first minute of dissolution. This suggests that the release of nicotine was not controlled by the Nicotine Polacrilex resin, hence, suggesting that the resins could be incorporated into the gum formulation as a drug stabilising agent.

3.6.2.2 The effect of dissolution media on release from resins and gums

It is important that the most appropriate dissolution medium is selected to conduct any *in vitro* dissolution study. Ideally, physiological conditions at the site of administration should be taken into account when selecting the *in vitro* dissolution/ release test conditions (Siewert, *et al.*, 2003). The dissolution tests conducted on chewing gum throughout this report have been carried out using artificial saliva at pH 6.7. Nicotine release data from 2 mg Nicorette®

showed that approximately 50% of nicotine was released in artificial saliva when using the EP chewing apparatus. In a previous study (Kwist *et al.*, 1999), approximately 90% release was reported from 2 mg Nicorette® gum after 40 minutes of *in vitro* testing using 0.1% sodium lauryl sulphate (SLS) as the dissolution medium.

Experiments were conducted to study the effect on release of nicotine from Nicorette® with the addition of SLS to the artificial saliva or replacing it with distilled water. Further experiments were performed using Nicotine Polacrilex resin to determine whether the addition of also surfactant effected release from the resin. For nicotine to be released from the resin, ion exchange would have to take place whereby a cation from the surrounding dissolution medium would be exchanged with the nicotine ion attached to the resin thus one would expect little release of nicotine in water.

3.6.2.2.1 Release from 2 mg Nicorette® into different dissolution media

Release of nicotine from 2 mg Nicorette® in artificial saliva and water appear to be very similar compared to release in AS with 0.1% w/v SLS which gave the greatest release (Figure 3.13). After 5 minutes of chewing, double the amount of nicotine was released from the AS + 0.1% SLS (15.32%) compared to the gums placed in AS and water alone (7.25% and 7.26% respectively). At the end of the chewing run (at 40 minutes), 81.25% of nicotine was released from the gum placed in AS spiked with 0.1% w/v SLS compared to only 46.49% released in artificial saliva alone and 53.19% in water. This showed that the addition of SLS to the dissolution medium almost double the release of the nicotine from the gums.

Further comparison of the dissolution curve using the f_2 equations indicates that water and AS resulted in similar release profiles (f_2 = f_2 68.25), while, the addition of SLS to the artificial saliva resulted in an increased release therefore giving a dissimilar release profile to water and AS (Table 3.6).

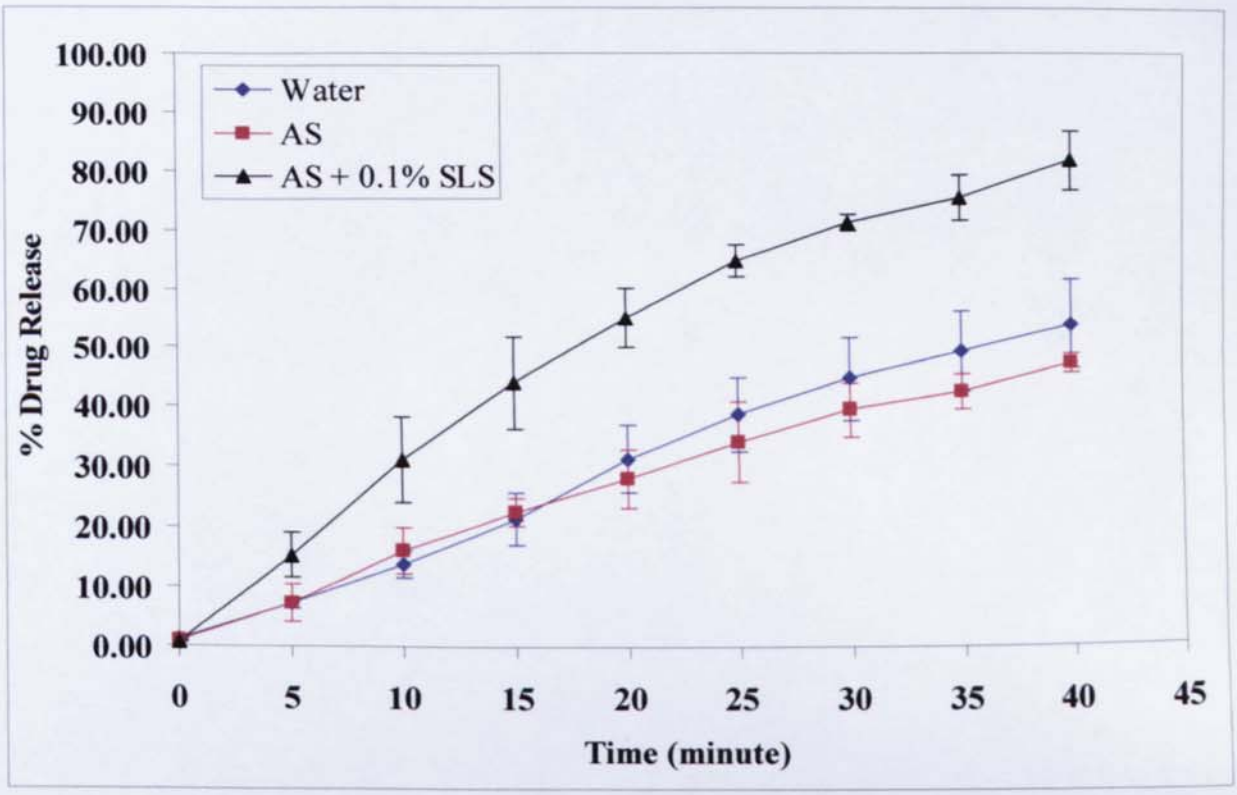


Figure 3.13 Release of nicotine *in vitro* from Nicorette® 2 mg gum in various dissolution media (n=3, ± SD).

		Dissolution medium		
		Water	AS	AS + SLS
Dissolution medium	Water		68.25	33.07
	AS	68.25		29.90
	AS+SLS	33.07	29.90	

Key

- Dissolution curves not similar
- Dissolution curves similar
- Dissolution curves not compared

Table 3.6 f_2 values indicating similar and dissimilar dissolution curves with the effect of dissolution medium on the release of nicotine from 2 mg Nicorette®. The mean of the replicates was used to compare the dissolution curves.

As the 2 mg gum was chewed in water, within the first five minutes of chewing there was a sharp rise in the pH of the dissolution medium. The pH increases by 3.789 pH units and then plateaued. This rise was significantly higher than that observed for gum chewed in AS and AS + 0.1% SLS (Figure 3.14). At the end of 40 minutes, pH rises of 1.270 and 1.259 pH units were seen from the gum chewed in AS and AS +0.1% SLS respectively. This confirmed that the AS and AS +0.1% SLS exhibited buffering capacity as pH was maintained close to pH 8.5.

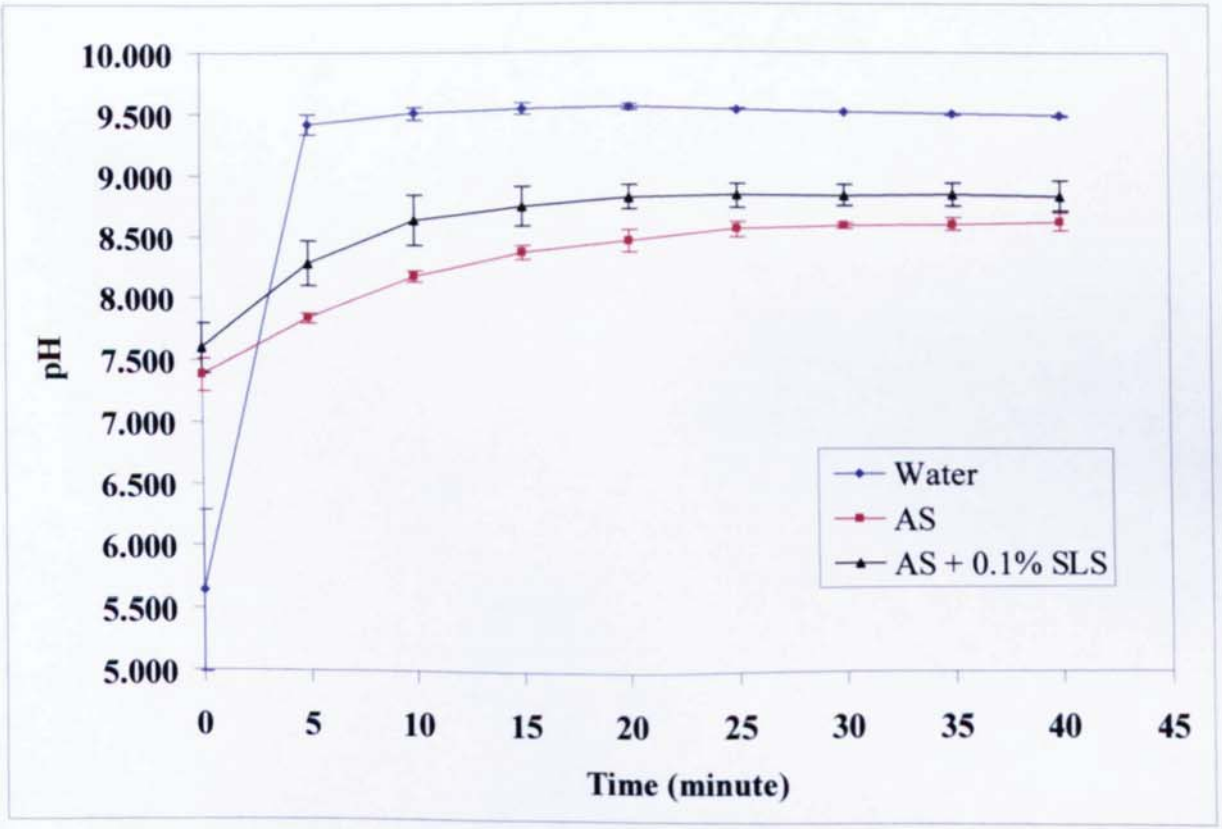


Figure 3.14 pH of the artificial saliva as nicotine was released *in vitro* from Nicorette® 2 mg gum in various dissolution media (n=3, ± SD).

3.6.2.2.2 Release of nicotine from Nicotine Polacrilex in different dissolution media.

The release of nicotine from Nicotine Polacrilex in the different dissolution media was different to that observed by the gums. The highest release from Nicotine Polacrilex was observed when using AS as the dissolution medium (Figure 3.15). After 5 minutes, 92.10% of nicotine was released from the resin after which the rate of release slowed to give a final release of 98.68% after 30 minutes. Little drug was released when the resin was placed in water. Only 15.73% of the nicotine was released from the resin after 30 minutes with the

majority of release occurring within the first minute of dissolution (11.05% ± 0.58). Tween 20, a non-ionic surfactant, gave slightly higher release than water. After 30 minutes 20.89% of nicotine was released from the resin of which half was released within the first minute of the experiment. For nicotine to be released from Nicotine Polacrilex resins, ions would have to be present in the surrounding solution to facilitate exchange. Therefore, in theory, no nicotine should be released from the resins in water and Tween 20. However, as observed, approximately 21% and 16% of nicotine was released in Tween 20 and water respectively (Figure 3.15). This could have been due to nicotine which was not strongly bound to the ion-exchange resin and hence was released into the dissolution medium initially. However, thereafter release was stagnant due to the lack of ions in solution to facilitate release.

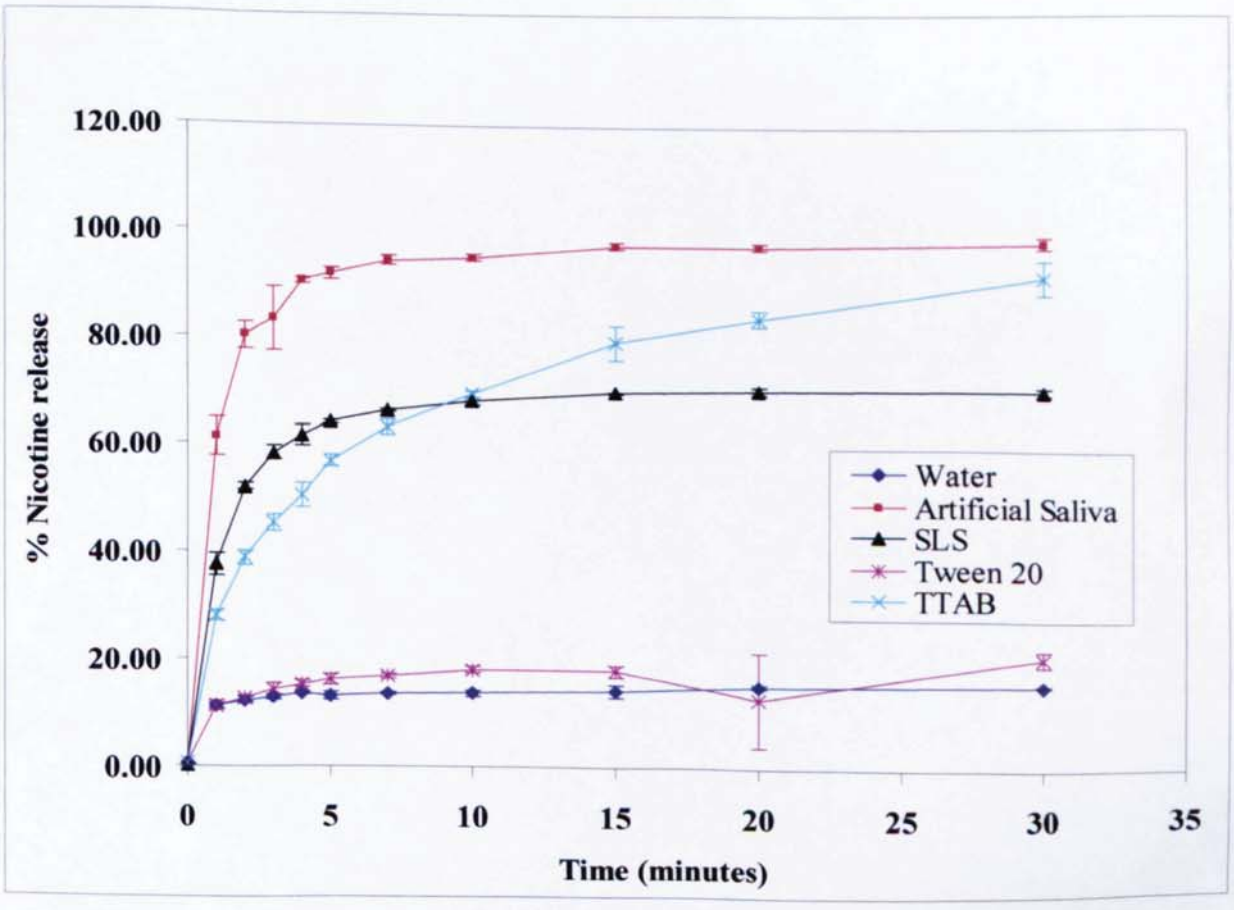
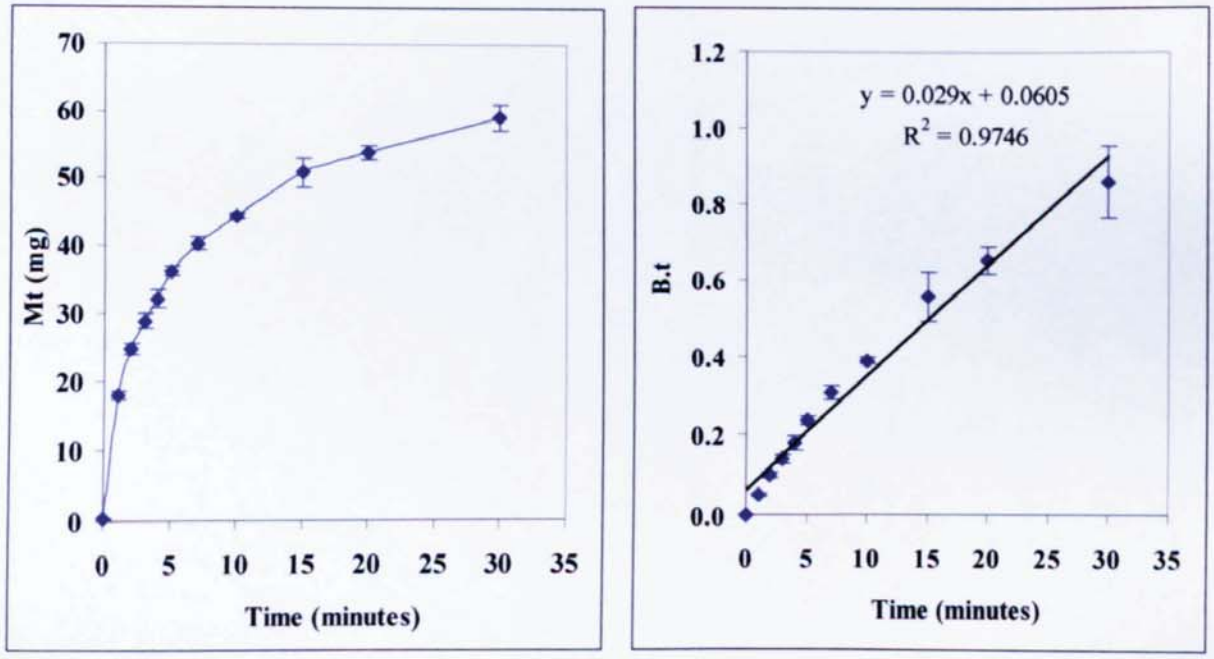


Figure 3.15 Release of nicotine from Nicotine Polacrilex *in vitro* using various dissolution media (n=3, ± SD).

The addition of SLS to the artificial saliva when testing the release of nicotine from chewing gum, almost doubled the release however, when the nicotine was released from the resin in 0.1% SLS, 37.47% ± 2.02 was released within the first minute of dissolution which slowly increased and began to plateau after 10 minutes to give a total release of 70.01% at 30 minutes. SLS is an anionic surfactant. A small amount of sodium ions may be present in

solution for ions exchange to occur. The sodium ions are relatively small and will readily exchange with the nicotine ions. Once all the sodium ions present in solution have been depleted, the release of nicotine will stop as there will be fewer ions for exchange.

The use of TTAB, a cationic surfactant, in the dissolution medium produced a diffusion-based controlled release which could be described by equation 3.1 (Figure 3.16). The rate constant was calculated from the product of B.t, (equation 3.4) and was determined as 0.061 mg min^{-1}. As TTAB is a large cationic molecule (MW 336.4) compared to nicotine, the molecule may slowly diffuse into the resin and bind slowly but strongly to the Polacrilex resin. This results in a controlled release compared to use of other dissolution media.



(a) (b)

Figure 3.16 Release (a) of nicotine from Nicotine Polacrilex in 0.1% Tetradecyl trimethyl ammonium bromide demonstrating diffusion controlled release (b) (n=3, ± SD).

Comparison of the dissolution curves showed that the release in water and Tween 20 were similar (f_2 = 75.86). All other dissolution curves gave different release profiles with the most dissimilar release being that of AS and water, and AS and Tween 20 which gave similarity values of 4.17 and 4.72 respectively (Table 3.7). This result differed to that obtained earlier in the chewing machine when the release of nicotine from Nicorette® in water was similar to the release in AS (Table 3.6). The chewing gum formulation contains buffers which are released as the gum is chewed. The ions that are therefore present in solution (mostly sodium ions from sodium bicarbonate and carbonate) provide enough ions for ion exchange

to take place and therefore release is not hindered by the lack of availability of exchanging ions.

Dissolution Medium						
		SLS	TTAB	Tween 20	Water	AS
Dissolution Medium	SLS		47.38	17.71	16.48	22.25
	TTAB	47.38		17.07	15.77	20.86
	Tween 20	17.71	17.07		75.86	4.72
	Water	16.48	15.77	75.86		4.17
	AS	22.25	20.86	4.72	4.17	

Key

- Dissolution curves not similar
- Dissolution curves similar
- Dissolution curves not compared

Table 3.7 f_2 values indicating similar and dissimilar dissolution curves in different dissolution media for release of nicotine from Nicotine Polacrilex resin. The mean of the replicates was used to compare the dissolution.

3.6.2.2.3 Release of sodium from 2 mg and 4 mg Nicorette®

The concentration of sodium ions in water and artificial saliva as 2 mg and 4 mg gum were chewed was determined (Figure 3.17). Sodium ions in the artificial saliva are supplied by sodium chloride and the sodium orthophosphate dihydrate. In a 40 mL sample of artificial saliva approximately 13.63 mg of sodium ion would be present. In the gum formulations, 30 mg of sodium carbonate (13 mg of sodium ions) is present in the 4 mg formulation. 2 mg Nicorette® contains 20 mg of sodium carbonate and 10 mg of sodium bicarbonate providing a total of 11.42 mg of sodium ions.

Sodium was released from the Nicorette® gum gradually. A total of 10.07 mg (88.17%) was released from 2 mg gum in water after 30 minutes of chewing. Release of sodium from the 4 mg formulation was very similar to that of the 2 mg; 10.22 mg was released after 30 minutes. Since the 4 mg formulation contains more sodium ions, only 78.61% of the total sodium ions present in the gum was released. The total amount of sodium ions that would be present in

the chewing chamber if all the sodium is released from the gum when chewed in artificial saliva would be 26.65 mg and 25.04 mg for 4 and 2 mg gum respectively. After 30 minutes at a chew rate of 60 chews/minute, 26.06 mg ± 1.85 (97.79%) and 24.98 mg ± 0.59 (99.79%) of sodium was detected for 4 mg and 2 mg respectively.

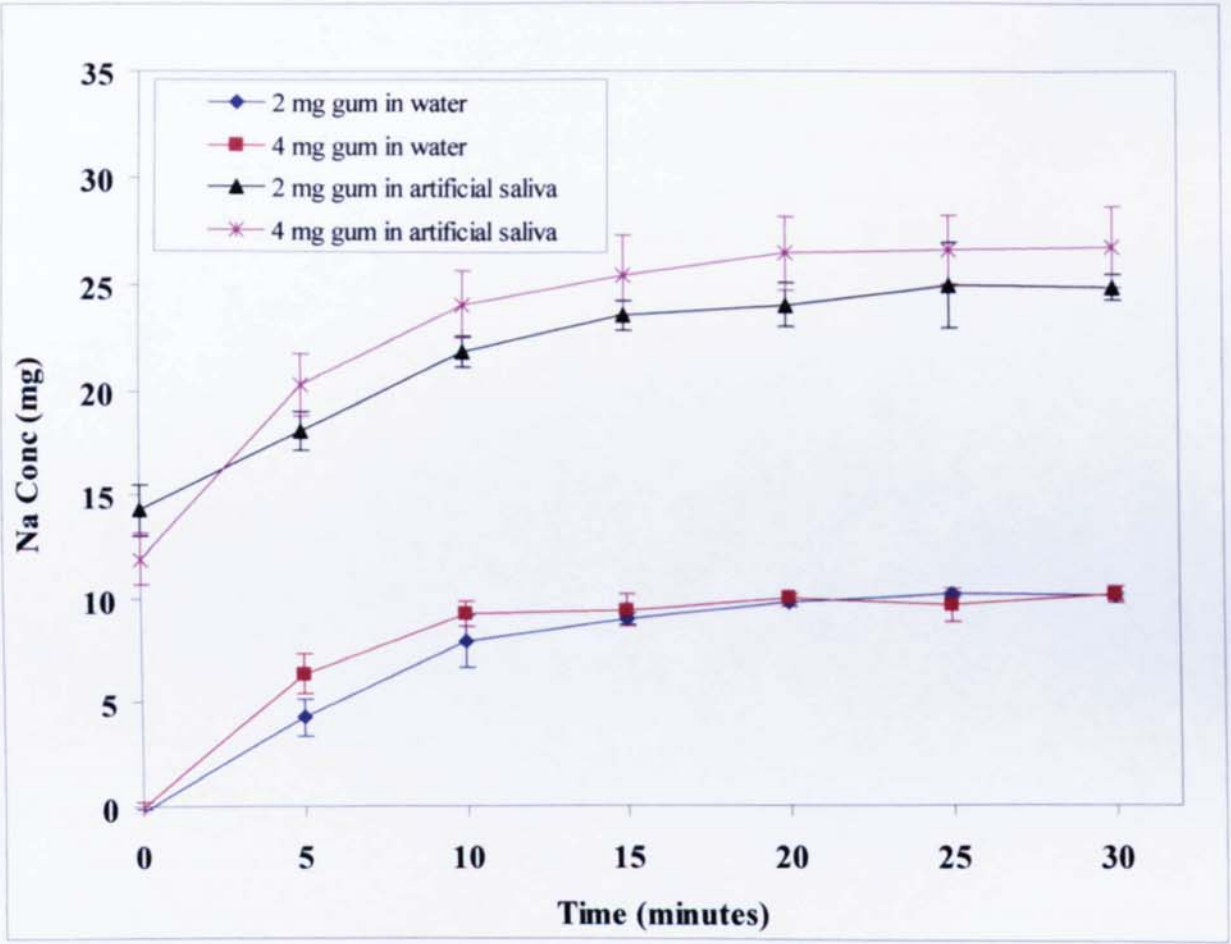


Figure 3.17 Sodium ion concentration when 2 mg and 4 mg Nicorette® gum are chewed *in vitro* in water and artificial saliva (n = 3 ± SD).

The addition of 0.1% w/v SLS (an anionic surfactant) to artificial saliva almost doubled the release of nicotine from the chewing gum, but when 0.1% SLS was used to determine the release from ion-exchange resins approximately 70% of nicotine was released compared to 98.68% released using AS. The presence of ions in the dissolution medium was also an important factor to consider when determining the release from ion-exchange resins. When resins were placed in water or Tween 20 (a non-ionic surfactant) solution, less than 20% of the nicotine was released. In comparison a similar release of nicotine from Nicorette® gum in water was observed to AS. Thus, the incorporation of sodium bicarbonate and carbonates into the gum formulations provides ions for ion exchange. Approximately 10 mg of sodium,

from both 2 and 4 mg gums was released thus providing enough ions for ion exchange to occur.

3.6.2.3 The effect of temperature on the release of nicotine from Nicorette® and Nicotine Polacrilex resin.

As with any chewing gum formulation, whether it is for confectionery use or medicinal use, the temperature at which the gum is to be stored is of great importance. Gums exposed to high temperatures or placed in direct sunlight often undergo textural changes. The gums become soft and sticky and other excipients within the formulation will generally begin to be release and dissolve in the softened gum base leading to an undesirable product.

For any equipment used for dissolution testing of chewing gums, it is of the utmost importance that the temperature is thoroughly controlled since the texture of the formulation changes rapidly with the temperature of the test medium thus, the temperature of the test medium is of fundamental importance for obtaining reproducible release.

Experiments were conducted to determine the effect of the test medium temperature on the release of nicotine from Nicorette® and Nicotine Polacrilex resin. Texture analysis of the gums exposed to high and low temperatures was also conducted to demonstrate how this impacts on the hardness, adhesiveness and the overall texture of the gum.

3.6.2.3.1 Release of nicotine from 2 mg Nicorette® at different *in vitro* temperatures

Within the first 5 minutes, five times as much nicotine was released from the gums chewed in the dissolution medium at 53°C than at either 37°C and 23°C. 38.94% was released from the gums chewed in artificial saliva at 53°C in comparison to 7.25% and 8.59% released from gums chewed at 37°C and 23°C respectively (Figure 3.18). After 40 minutes of chewing at 53°C, almost all of the nicotine within the gum was released (99.65% ± 3.85). In contrast to the gums chewed at 37°C and 23°C which both showed similar release profile and gave less than half the release after 40 minutes (approximately 40%).

Dissolution curve comparison using the f_2 equation suggested that gums chewed at 37°C and 23°C showed similar release profiles (f_2=65.05), whilst gums chewed at 53°C gave a much greater release than those at 37°C and 23°C and were therefore dissimilar (f_2 = 14.06 and 12.40 respectively) (Table 3.8).

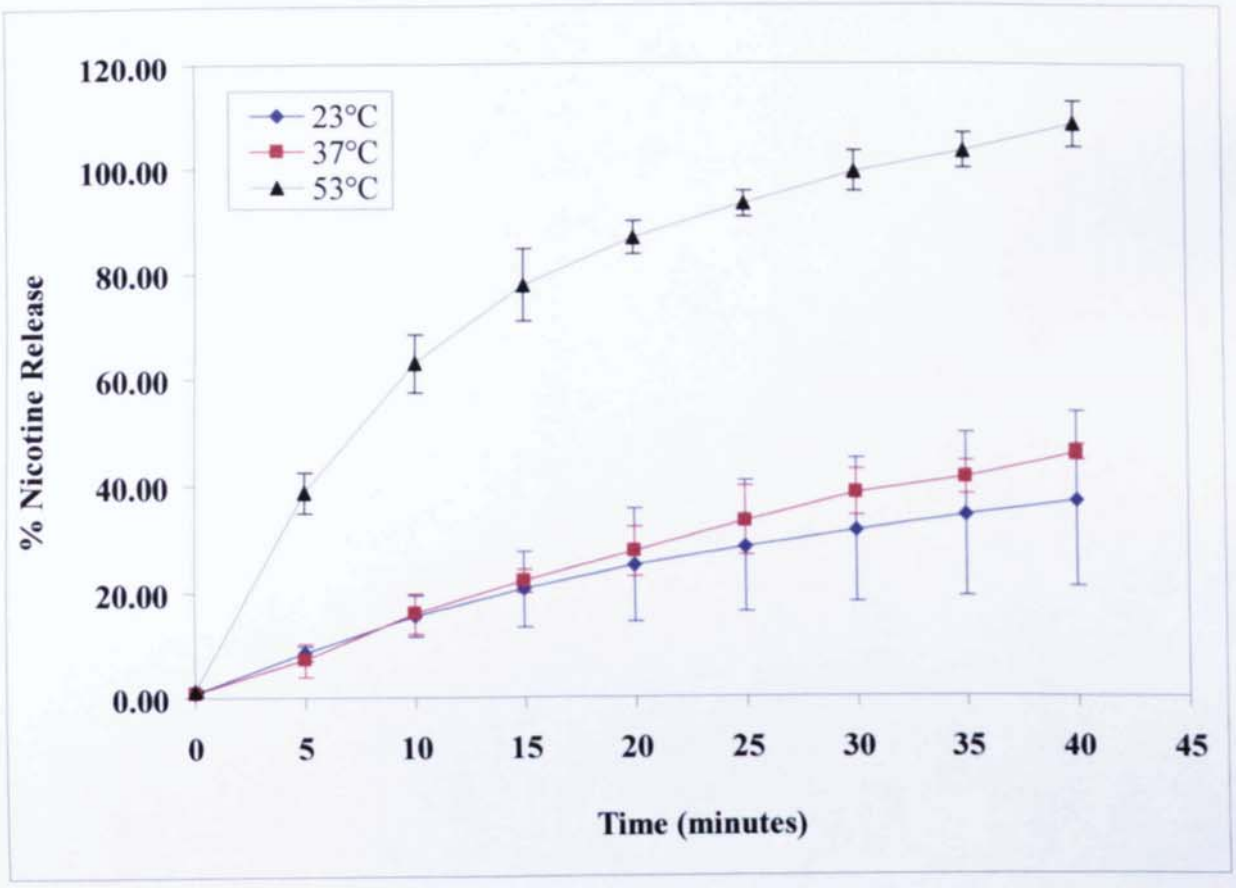


Figure 3.18 Nicotine release from 2 mg Nicorette® *in vitro* when chewed in artificial saliva at 23°C, 37°C and 53°C (n = 3 ± SD).

Temperature (°C)	53	37	23
53		14.06	12.40
37	14.06		65.05
23	12.40	65.05	

Key

- Dissolution curves not similar
- Dissolution curves similar
- Dissolution curves not compared

Table 3.8 f_2 values indicating similar and dissimilar dissolution curves with the effect of dissolution medium temperature on the release of nicotine from Nicorette® 2 mg. The mean of the replicates were used to compare the dissolution curves

As nicotine was released at a faster rate from the gums chewed at 53°C, it was also anticipated that the pH of the artificial saliva would also increase rapidly due release of sodium carbonate and bicarbonate. After 5 minutes, the pH of the artificial saliva at 53°C increased by 1.17 pH units after which slowed to give a final pH of 8.838 (increase of 1.468 pH units) after 40 minutes (Figure 3.19). At 23°C, the overall pH change at the end of 40 minutes chewing was 0.993 pH units, the overall lowest pH change. Within the first ten minutes the pH increased rapidly, and then slowly increased to pH 8.570 after 40 minutes. At 37°C, the pH increased steadily from pH 7.382 to pH 8.653 after 40 minutes giving an overall rise of 1.271 pH units.

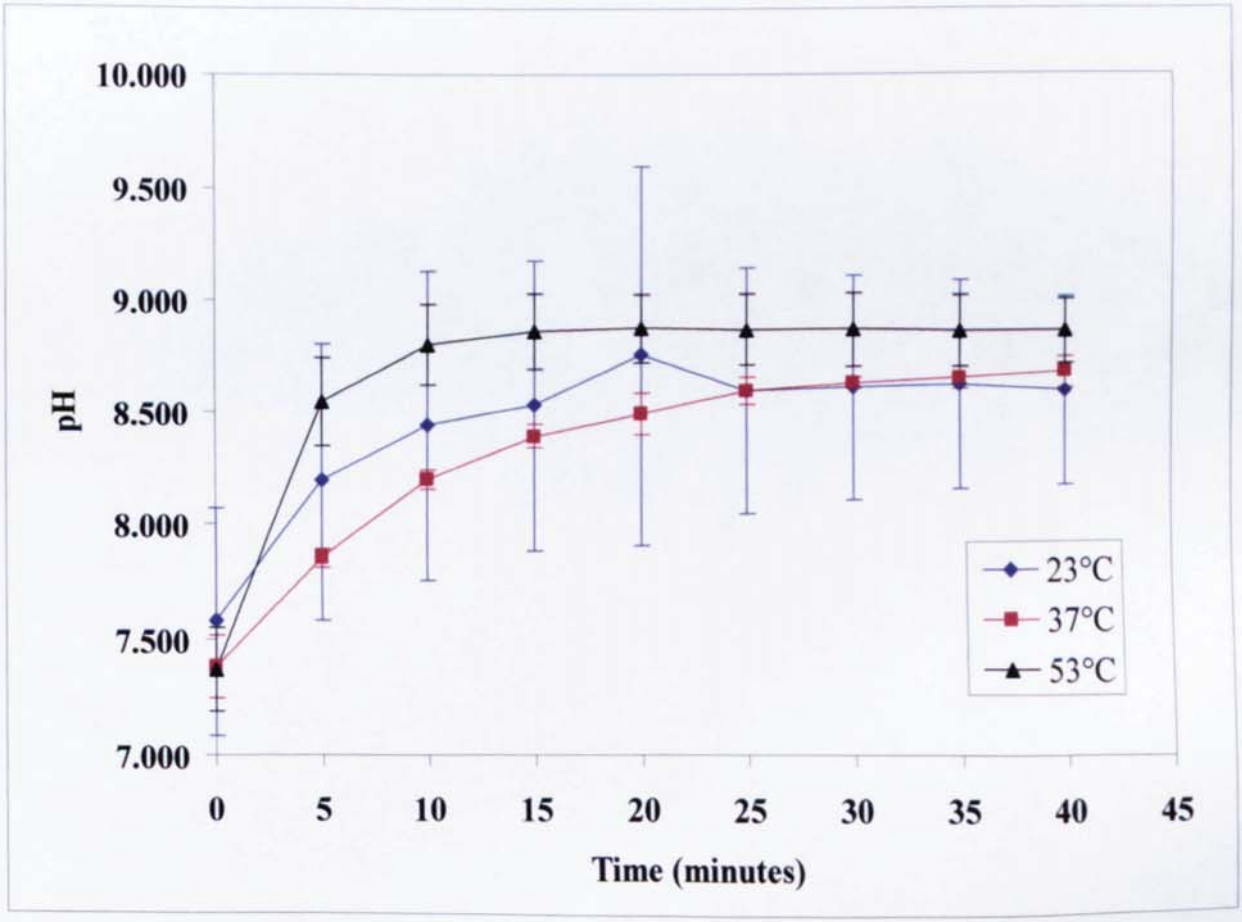


Figure 3.19 pH of the artificial saliva as nicotine was released from 2 mg Nicorette® *in vitro* when chewed in artificial saliva at 23°C, 37°C and 53°C (n = 3 ± SD).

3.6.2.3.2 Texture analysis of the gums exposed to different temperatures

The release of the nicotine from the gums can be explained in terms of the texture of the gums at the different temperatures. After one hour, gums placed at 50°C were softer than those tested at 4°C and 27°C (Figure 3.20). As the 2 mm diameter probe penetrates the gums stored at 4°C to a depth of 3 mm, a sharp initial rise in the force needed to penetrate the gum

was observed. As the probe traveled through the gum, the force required reduced. The reduction in the load required to penetrate the gum indicated that the outer-most layers of the gum had become hard compared to the centre of the gum piece. The probe required less force to penetrate gums stored at room temperature (27°C) than those stored at 4°C. As the probe traveled through the gum, an even load was encountered indicating that the gum was of equal hardness throughout. The lowest force required was presented by gums stored at 50°C. The load required to penetrate the gums increased as the probe entered further into the gum, thus, suggesting that the outer layers of the gum piece were softer than the centre. Gums stored at 4°C required more than double the load (indicated by hardness values) compared to gums stored at 27°C, which, were ten times harder than the gums stored at 50°C (Table 3.9).

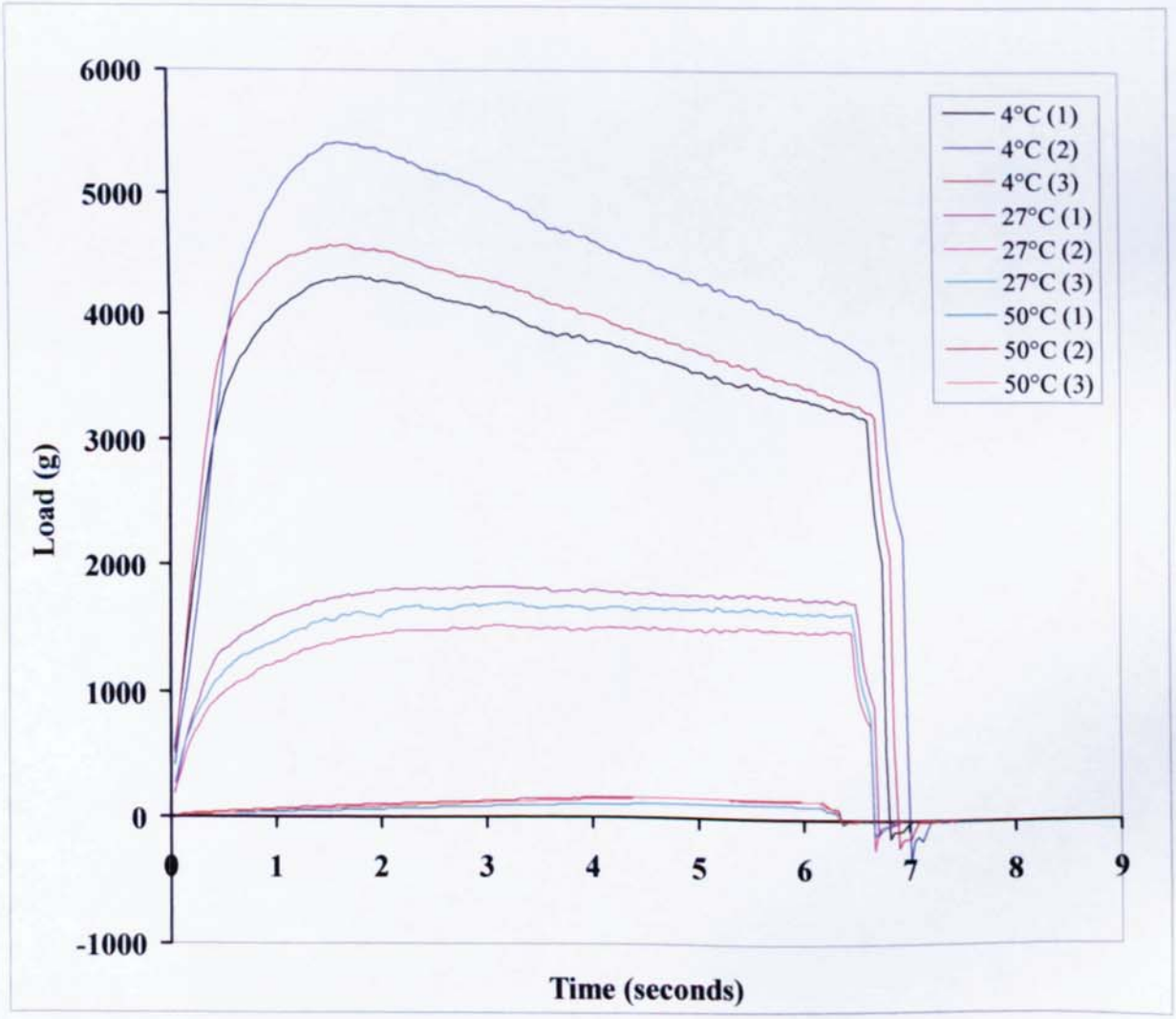


Figure 3.20 Time versus load graph of 2 mg Nicorette® exposed to different temperatures.

Adhesive forces denote the force required to pull the probe away from the sample (negative force generated during upstroke of probe). A mean of -0.18 N suggested that the force required to remove the probe from gums stored at 50°C was low. The load *per* unit time is a measure of the load encountered by the gum *per* unit time and indicates the resistance of the gum to deformation. Calculated values showed that as temperature increased, the resistance of the gum was reduced.

	Hardness (N)		Adhesive force (N)		Load *per* unit time (g/s)	
Temperature	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
4°C	46.65	4.58	-2.23	0.77	7634.89	622.33
27°C	16.51	1.23	-1.51	0.57	1527.63	330.56
50°C	1.48	0.20	-0.18	0.08	33.66	6.85

Table 3.9 The hardness, adhesive force and load encountered *per* unit time of the gums placed at 4°C, 27°C and 50°C.

At the high temperature of 53°C the gums became softer and less rigid. This could be due to the melting of the waxes within the gum base formulation, thus altering the properties of the gum base to form a less rigid and softer gum. The loss of rigidity of the gum will also enable the release any excipients or drug entrapped within the gum matrix. Dissolved soluble excipients within the gum base will furthermore increase the porosity of the gum base allowing the incorporated drug to be released readily.

When Nicorette® gum was placed in the chewing chamber at 53°C, a greater release of nicotine was observed from the gum as the gum was softer and less rigid thus, allowing nicotine to be released more readily.

3.6.2.3.3 The effect of temperature on the release of nicotine from Nicotine Polacrilex resin

The release of nicotine from Nicotine Polacrilex was similar at all temperatures tested (Figures 3.21 and Table 3.10). From 0 to 5 minutes there was a steady rise in release which then began to plateau to give a release of 87% after 30 minutes for resins placed in dissolution media at 37°C, and 91.04% and 91.87% at 30°C and 45°C respectively. Dissolution curve comparisons also showed that all release profiles were similar ($f_2 > 50$). Results therefore showed that the temperature of the dissolution medium had no effect on the release of nicotine from Polacrilex resin.

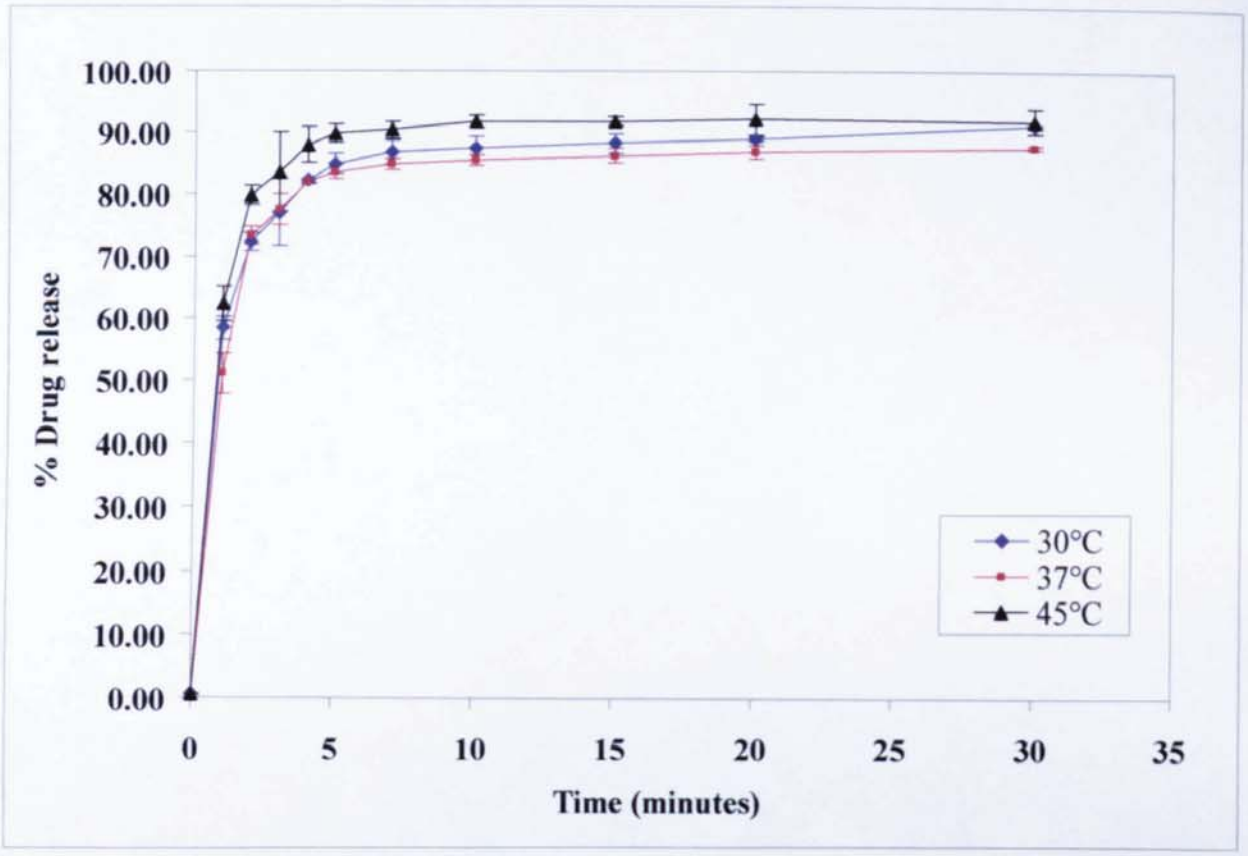


Figure 3.21 Release of nicotine from Nicotine Polacrilex *in vitro* at various temperatures of the dissolution medium (n=3, ± SD).

Temperature (°C)	30	37	45
30		76.41	66.48
37	76.41		59.96
45	66.48	59.96	

Key

- Dissolution curves not similar
- Dissolution curves similar
- Dissolution curves not compared

Table 3.10 f_2 values indicating similar and dissimilar dissolution curves for the release of nicotine from Nicotine Polacrilex resin using different temperatures. The mean of the replicate was used to compare the dissolution curves

Altering the temperature of the dissolution media resulted in differences in release of nicotine from chewing gum, but, not Nicotine Polacrilex. At the higher temperatures due to the changes in the physical properties of the gum, the amount of nicotine released was almost double that observed at the lower temperatures of 37°C and 23°C, whilst, the release from the resins were similar. Careful consideration needs to be made when selecting temperatures for *in vitro* testing of chewing gum. The temperature range should be accurate and precise as fluctuation could cause problems in the release reproducibility. Also, patients should be advised not to ingest any hot or cold substances prior to the use of nicotine gum as fluctuations in the temperature of the oral cavity will also have similar effects to that observed *in vitro*.

3.6.2.4 The effect of dissolution medium pH on the release from Nicorette® and Nicotine Polacrilex resin

In literature, many studies have been reported on the effect of pH on nicotine absorption. The effects of drinking coffee and carbonated beverages was studied by Henningfield, *et al.*, (1990) who reported that intermittent mouth rising with coffee or cola substantially reduced salivary pH and nicotine absorption. The authors concluded by recommending that patients do not ingest any substances during, immediately before or after the use of Nicotine Polacrilex gum.

In 1977, Axelsson and Brantmark compared the use of 4 mg nicotine gum buffered with sodium carbonate and bicarbonate (to increase salivary pH level) to gums without buffering agents (Tomar and Henningfield, 1997). It was found that peak blood nicotine levels in subjects using the buffered gum were two and half times higher than in those using unbuffered gum (10 ng/mL *vs* 4 ng/mL).

Although other factors can influence nicotine absorption, manipulation of pH appears to be the primary means by which the rate of nicotine absorption is controlled. *In vitro* experiments were conducted to determine if pH alone had an effect on the release of nicotine from chewing gum and ion-exchange resins.

3.6.2.4.1 Effect of dissolution media pH on release of nicotine from Nicorette®

At pH 3.0, 5.0. 7.0 and 9.0 for the first 10 minutes, release of nicotine was similar, approximately 10% nicotine was released from the gums. After 40 minutes, release from

gums chewed at pH 7.0 and 5.0 were similar (37.21% and 37.37% respectively) and release from pH 9.0 and 3.0 appear similar (30.85% and 30.85% respectively) (Figure 3.15). The release of nicotine at all pH values were steady and followed the same release mechanism.

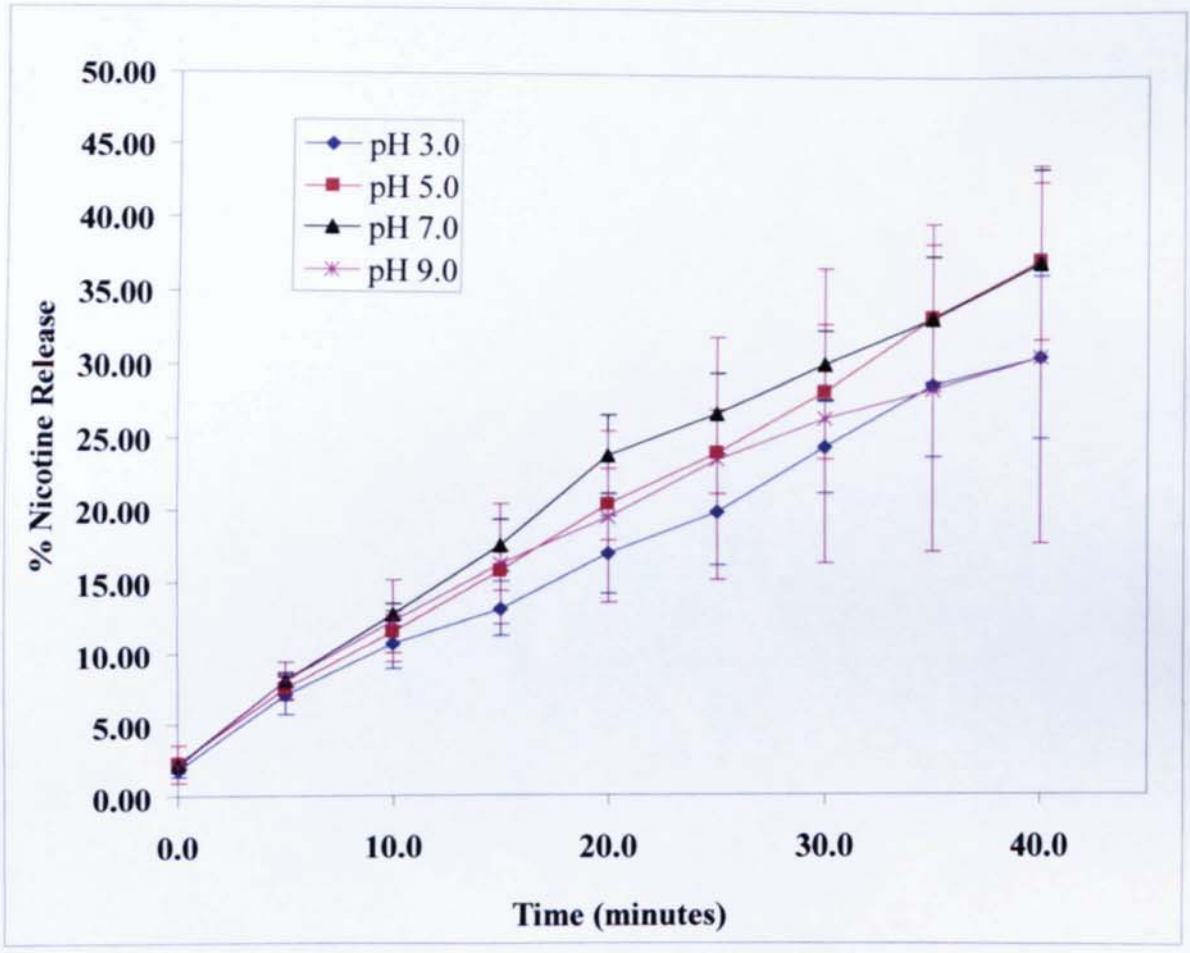


Figure 3.22 Release of nicotine from 2 mg Nicorette® *in vitro* using artificial saliva buffered at pH 3.0, 5.0. 7.0 and 9.0 (n=3, ± SD).

Mathematical dissolution curve comparison using the f_2 equation showed that at all pH values, the release of nicotine was similar (Table 3.11). Results showed that the pH of the dissolution media did not have an effect on the release of nicotine from the gums. However, as highlighted earlier in this section, the pH of the oral cavity could affect the absorption of nicotine through the buccal membrane (see chapter five).

		pH			
		3.0	5.0	7.0	9.0
pH	3.0		71.37	65.17	82.08
	5.0	71.37		84.82	76.12
	7.0	65.17	84.82		72.15
	9.0	82.08	76.12	72.15	

Key

- Dissolution curves not similar
- Dissolution curves similar
- Dissolution curves not compared

Table 3.11 f_2 values indicating similar and dissimilar dissolution curves for nicotine released in dissolution media of different pH. The mean of the replicates were used to compare the dissolution

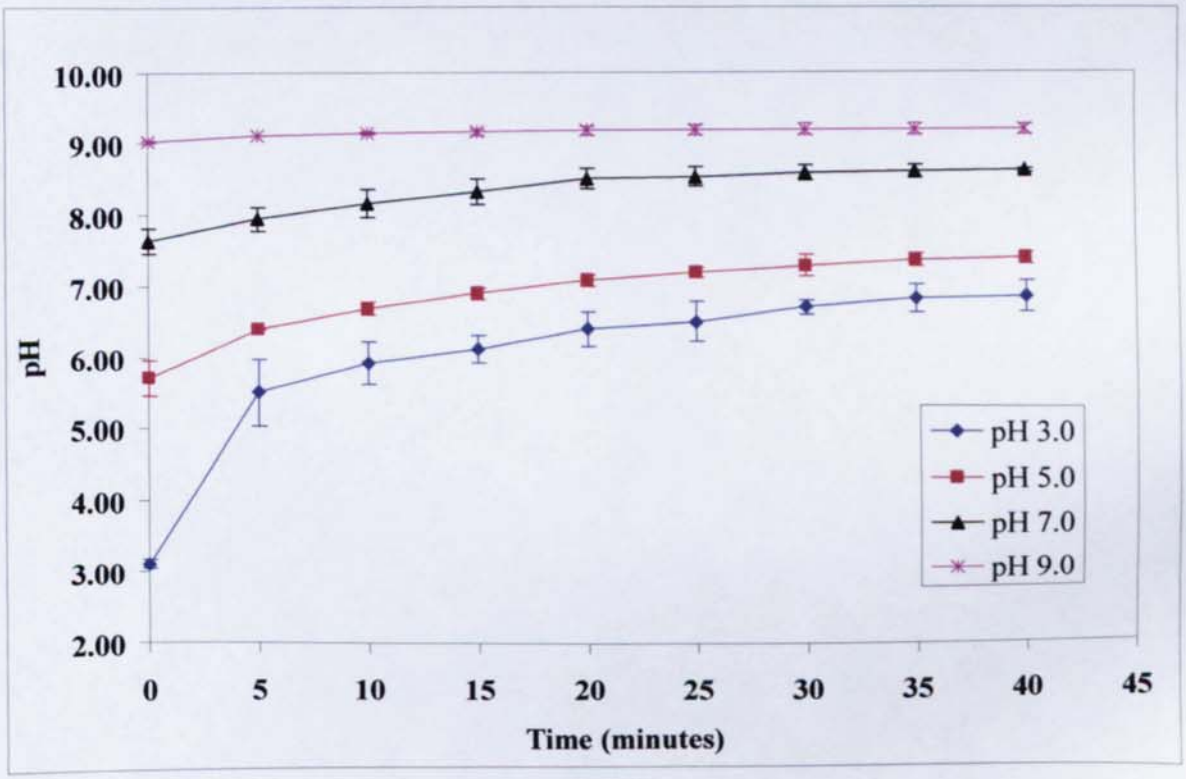


Figure 3.23 pH of the artificial saliva as nicotine was released from 2 mg Nicorette® *in vitro* using artificial saliva buffered at pH 3.0, 5.0. 7.0 and 9.0 (n=3, ± SD).

Artificial saliva buffered at pH 3.0 showed the highest rise in pH after 40 minutes (rise of 3.757 pH units), whilst, increases of 0.156 and 0.958 pH units for pH 9.0 and 7.0 respectively were lower (Figure 3.23). A final pH of 6.869 and 7.392 for pH 3.0 and 5.0 suggested that nicotine released from the gum would predominately be present in the mono-protonated state; therefore, diffusion through buccal membrane would be slower than when nicotine was released at pH 9.0 and 7.0.

3.6.2.4.2 Effect of pH on the release of nicotine from Nicotine Polacrilex *in vitro*

Nicotine Polacrilex is a weakly acidic cation ion-exchanger. In the hydrogen form, the resin neutralises alkalinity by exchanging for any cation, thus at higher pH values it is expected that nicotine release from Polacrilex resin would be greater than at lower pH values. However, results obtained showed that release at the lower pH was greater than that at the higher pHs (Figure 3.24).

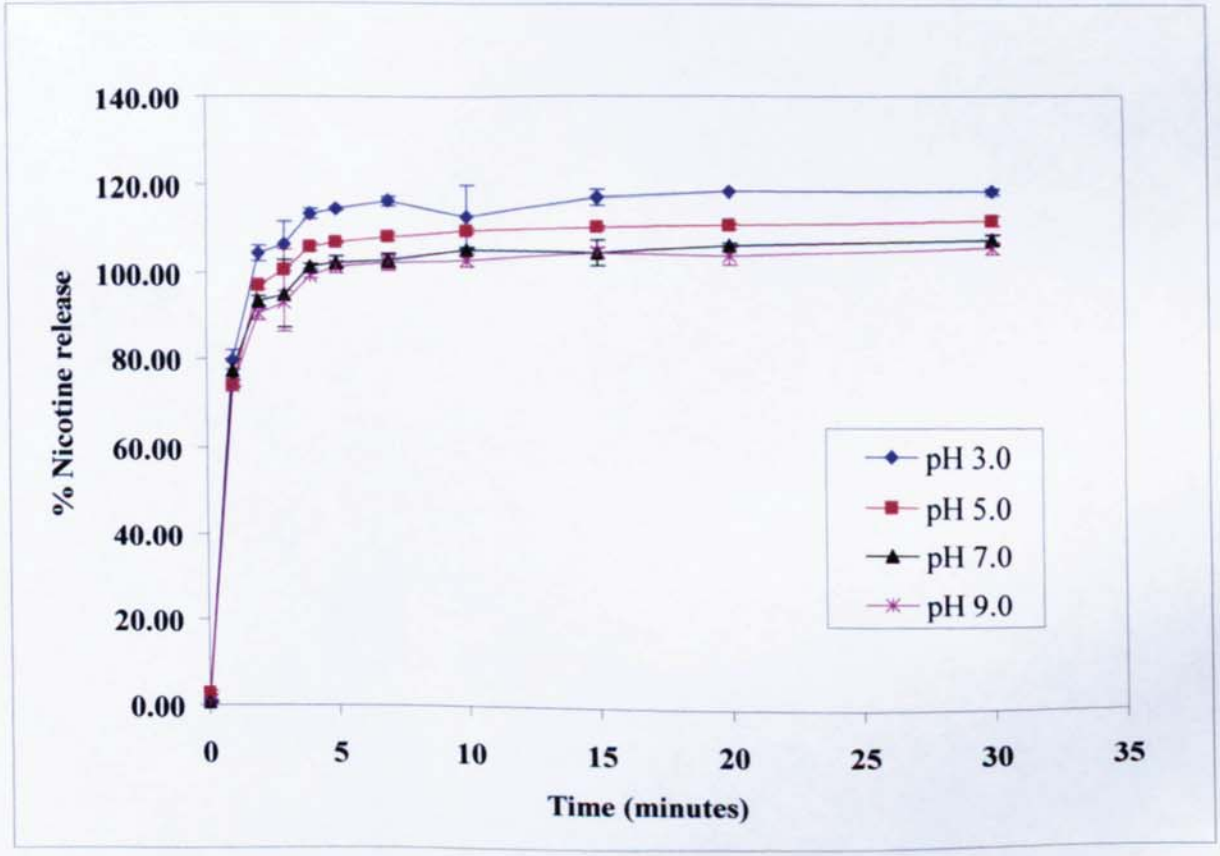


Figure 3.24 Release of nicotine from Nicotine Polacrilex *in vitro* with effect of dissolution medium pH (n=3, ± SD).

The dissolution media used contained 150 mM sodium chloride adjusted to the appropriate pH using HCl or NaOH. At the lower pH, a sufficient number of sodium ions are present in

the media for exchange, thus, at the lower pH similar release was observed to that at the higher pH.

Comparison of the dissolution using f_2 equation showed that the dissolution at pH 3.0 and 5.0 were similar (f_2 = 58.87). Release at pH 7.0 and 9.0 were dissimilar to pH 3.0 (f_2 = 48.70 and 45.75 respectively) but similar to pH 5.0 (f_2 = 66.70 and 61.49 respectively) (Table 3.12).

		pH			
		3.0	**5.0**	**7.0**	**9.0**
pH	**3.0**		58.87	48.70	45.75
	5.0	58.87		66.70	61.49
	7.0	48.70	66.70		82.21
	9.0	45.75	61.49	82.21	

Key

- Dissolution curves not similar
- Dissolution curves similar
- Dissolution curves not compared

Table 3.12 f_2 values indicating similar and dissimilar dissolution curves for nicotine released from Nicotine Polacrilex resin in dissolution media at different pH. The mean of the replicates was used to compare the dissolution curves

The pH of the dissolution media had little effect on the release of nicotine from the resin and the gum as dissolution curves were similar. However, pH could alter the absorption of nicotine through the buccal membrane. Beverages vary in pH, the most acidic being cola (pH 2.30-2.76), followed by grape juice (pH 2.9-3.4) and lemon-lime soda (pH 3.22-3.28). The pH of coffee ranges from 2.9-3.3 tea (pH 4.2) and beer from pH 4.00-4.60 (www.thejcdp.com/issue001). If these beverages were administered prior to or during Nicorette® use, the pH of the oral cavity may hinder the absorption of nicotine through the buccal membrane since the overall increases in salivary pH may not be sufficient enough for optimised nicotine delivery (see chapter five).

3.6.2.5 Effect of dissolution volume on release of nicotine from gum and Nicotine Polacrilex

The volume and flow of saliva within the mouth can vary with factors such as age, sex, time of day and disease state. Flow can range from 0.06-0.104 mL/min to 0.43-0.66 mL/min whilst chewing fruit-flavored chewing gum. The use of lemon juice can further stimulate the flow of saliva to 1.15-1.99 mL/min (Table 1.4). Changes in saliva flow may influence the release of drug from medicated gums.

The EP recommends that 20 mL of an unspecified buffer at approximately pH 6.0 is used to test the release of the active from chewing gum using the chewing apparatus (EP, 2001). However, due to the size of the chewing chamber, the volume usually used in the chewing machine here was 40 mL. The volume of the dissolution medium may affect the *in vitro* release of nicotine from Nicorette®. It was postulated that, at a lower dissolution volume, the rate of nicotine release during the latter stages may be hindered by the higher concentration of nicotine present in the dissolution medium in comparison to greater volumes where the concentration is lower. Experiments were conducted to determine if an increase or decrease in the volume of dissolution medium would affect the release of nicotine from the Nicorette® 2 mg gum and Polacrilex resin. Further experiments were conducted to determine the concentration of sodium carbonate and bicarbonate released. Not only does the released buffer provide ions for exchange but also increases the alkalinity of the oral cavity to enable optimised nicotine absorption; therefore the effect of dissolution volume on sodium release was determined.

3.6.2.5.1 Effect of dissolution media volume on release from Nicorette®

An almost linear increase in release was observed for gum chewed in the different volumes of artificial saliva (Figure 3.25). Although the release from the gums at all volumes was similar, gums chewed in 80 mL gave the lowest release after 30 minutes (80.60%). Highest release was from gums chewed in 40 mL of artificial saliva (95.59%) and 88.32% from gum in 20 mL. Dissolution curve comparison using the f_2 equation, gave values greater then 50 for all compared release profile (Table 3.13), suggesting that although the volume used differed the release from the gums was similar.

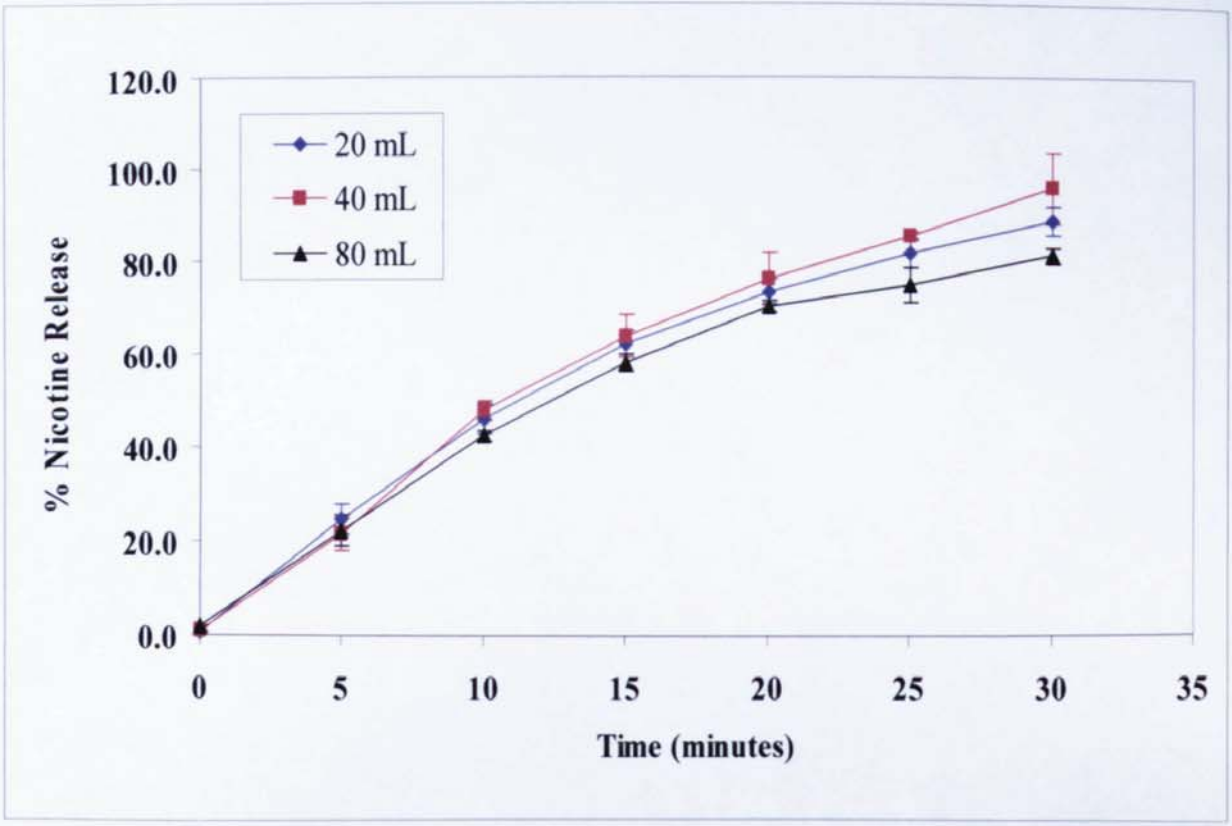


Figure 3.25 Release of nicotine from 2 mg Nicorette® gum *in vitro* in different volumes of dissolution medium (n=3, ± SD).

Volume (mL)	20	40	80
20		71.38	65.68
40	71.38		54.77
80	65.68	54.77	

Key

Dissolution curves not similar

Dissolution curves similar

Dissolution curves not compared

Table 3.13 f_2 values indicating similar and dissimilar dissolution curves for the release of nicotine from Nicorette® gum in different volumes of dissolution medium. The mean of the replicates was used to compare the dissolution curves

The dissolution chamber of the chewing machine was large and required 30-40 mL of dissolution media to ensure that the gum was always in contact with the medium. At the lower volumes, when the chewing machine was stationary, the gum was not always in contact with the medium. However, during the chew cycle, the agitation of the medium as a result of the chewing action of the pistons ensured that the gum was in contact with the medium at all times.

At all volumes, the addition of fresh artificial saliva when samples were removed from the chewing chamber ensured that there was no loss of sink conditions, therefore, the volume of dissolution medium did not seem to have an effect on the release of nicotine from the chewing gum.

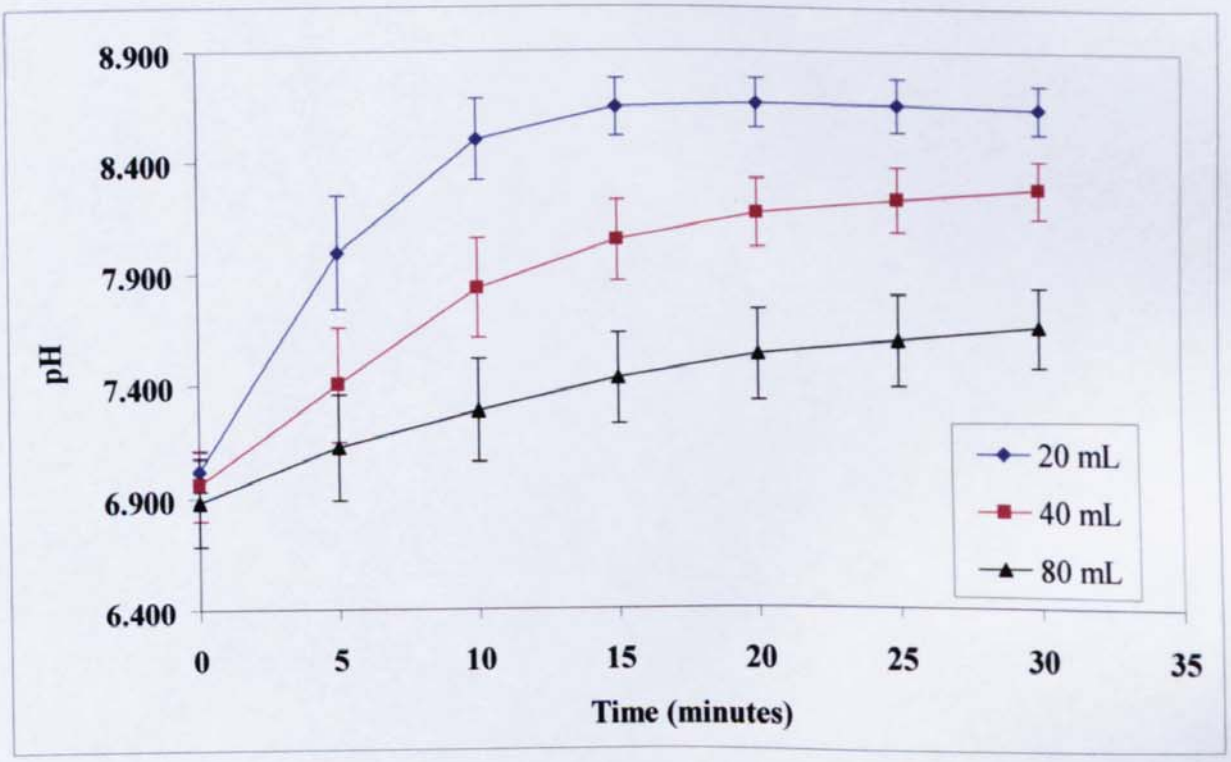


Figure 3.26 pH of artificial saliva as nicotine was released from 2 mg Nicorette® gum *in vitro* with varying dissolution volume (n=3, ± SD).

The pH of the artificial saliva increased as the volume was reduced (Figure 3.26). Within 10 minutes, the pH of the 20 mL artificial saliva rose rapidly from an initial pH of 7.0 to pH 8.5. The rate of pH change then slowed to give a total pH increase of 1.612 pH units at the end of the chew run. Smallest pH changes were observed when gums were chewed in 80 mL of artificial saliva. At the end of 30 minutes, the pH increased by 0.746 pH units compared to an increase of pH 1.307 observed with gum chewed in 40 mL of artificial saliva. The

difference in pH was due to the concentration of buffers that are released from Nicorette® gum when chewed. Higher concentrations of buffers are present in lower dissolution volumes as opposed to larger volumes.

3.6.2.5.2 Concentration of sodium when nicotine was released into the varying dissolution volumes

Sodium carbonate and bicarbonate are the buffers contained within the 2 mg Nicorette® gum, thus analysis of sodium would quantify how much of the buffer was released. Analysis of the sodium content showed the amount of sodium released from the gums chewed in the different volumes of artificial saliva was the same (Figure 3.27). Approximately 11.42 mg of sodium is contained in a 2 mg Nicorette® gum. The sodium was released at a steady rate which after 10 minutes slowed down to give a final release of 9.79 mg, 11.36 mg and 11.51 mg from gums chewed in 20 mL, 40 mL and 80 mL of artificial saliva respectively.

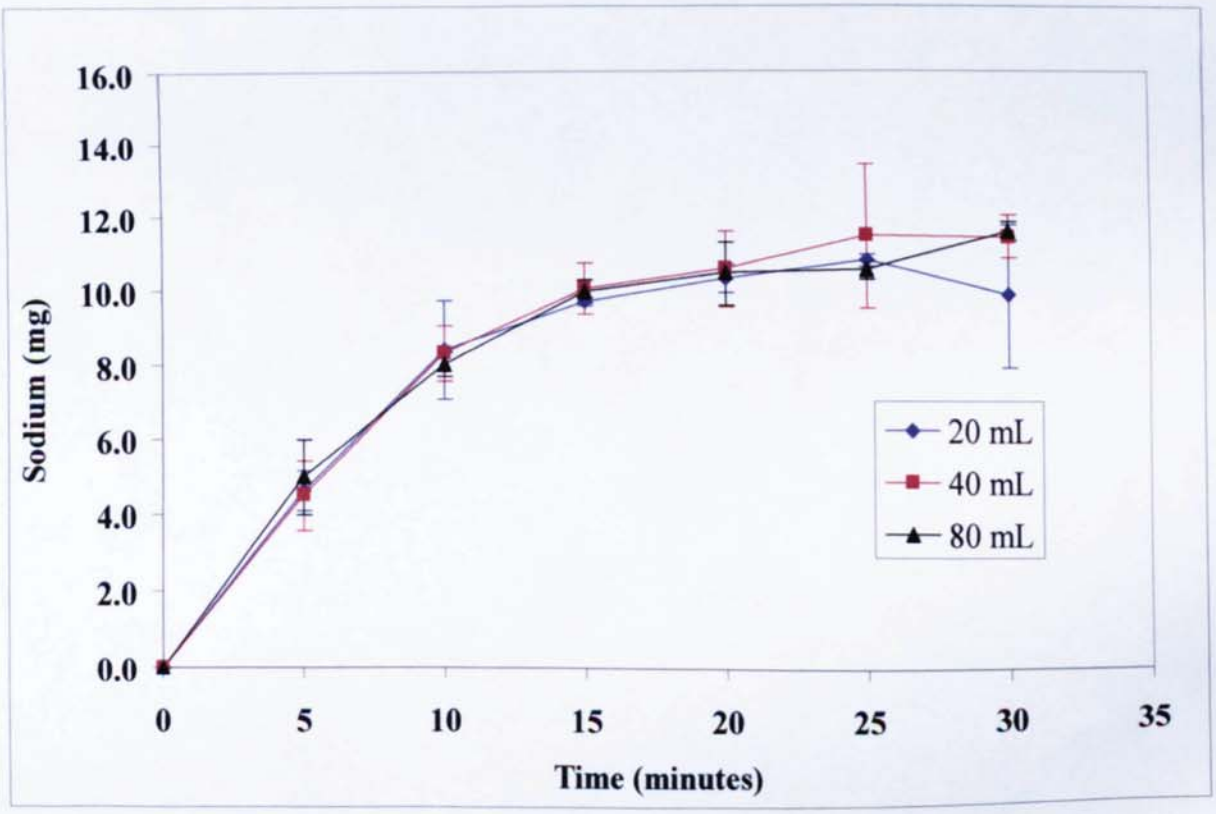


Figure 3.27 Sodium released from 2 mg Nicorette® when chewed *in vitro* in 20 mL, 40 mL and 80 mL of artificial saliva (n=3, ± SD).

Although the release of the buffers was the same from the gums, the pH of the artificial saliva at the lower volume was greater than at the higher volume (Figure 3.26). The reason for this was due to the concentration of the released buffer. At the lower volumes there were

higher concentrations of sodium salts present than at higher volumes (Figure 3.28), therefore more OH^- in solution to raise the pH.

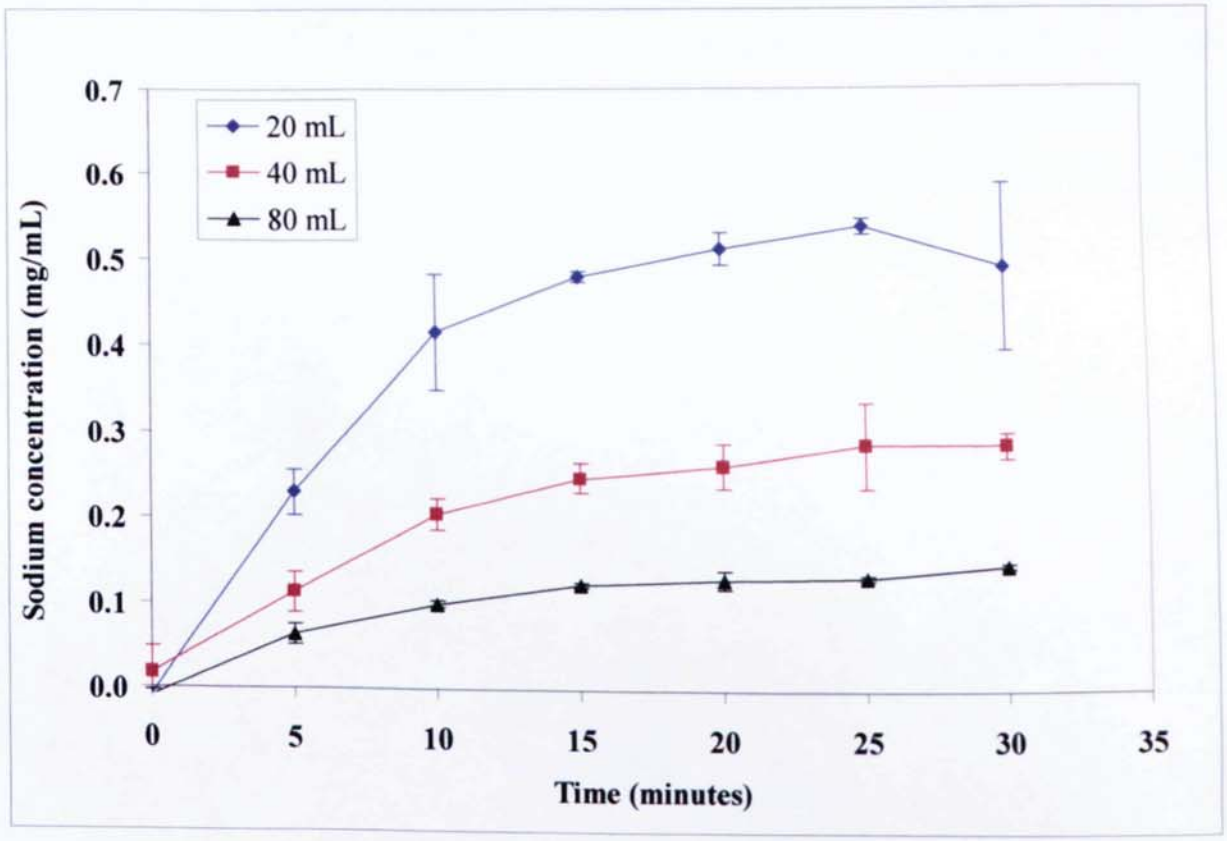


Figure 3.28 Concentration of sodium ions released from the chewing gum in different volumes of dissolution media (n=3, ± SD).

3.6.2.5.3 Effect of dissolution volume on the release of nicotine from Nicotine Polacrilex.

As the volume of dissolution medium increased, the amount of nicotine from the resin also increased (Figure 3.29). At all volumes, within the first 5 minutes the majority of the nicotine was released from the resin (86.39%, 83.59% and 75.49% for 1000 mL, 800 mL and 400 mL respectively), which then plateaued to give a final release of 90.49% in 1000mL, 87.54% and 78.10% in 800 mL and 400 mL respectively. Dissolution curve comparison showed that the release from resin in 400 mL and 800 mL and 800 mL and 1000 mL were similar (f_2 = 55.62 and 67.88 respectively), whilst, release in 400 mL and 1000 mL was dissimilar (f_2 = 49.39) (Table 3.14).

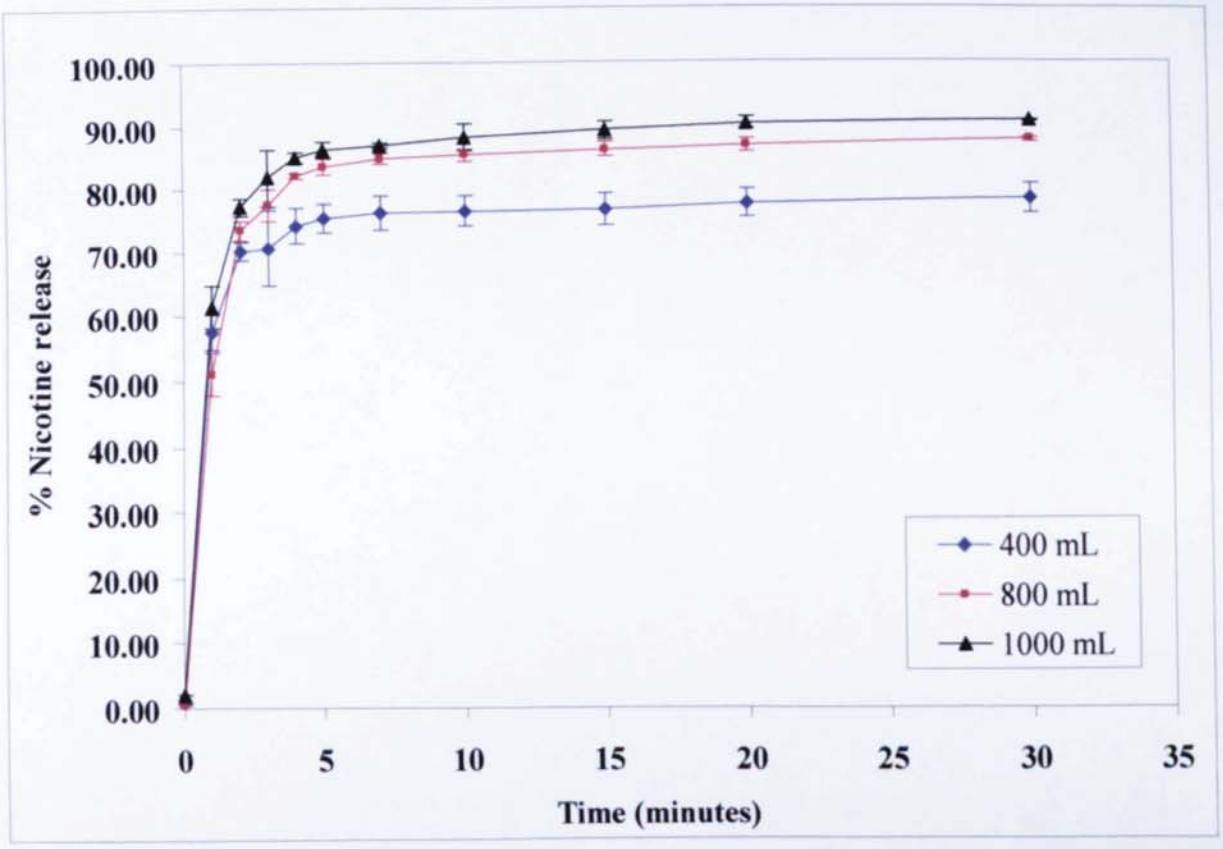


Figure 3.29 The effect of dissolution volume on the release of nicotine from Nicotine Polacrilex (n = 3, ± SD).

		Volume (mL)		
		400	800	1000
Volume (mL)	400		55.62	49.39
	800	55.62		67.88
	1000	49.39	67.88	

Key

Dissolution curves not similar

Dissolution curves similar

Dissolution curves not compared

Table 3.14 f_2 values indicating similar and dissimilar dissolution curves for the release of nicotine from Nicotine Polacrilex into different volumes. The mean of the replicates was used to compare the dissolution curves

In theory, release of nicotine from the resins in the different volumes of dissolution media should have been similar, due to a high quantity of ions present (40 mM sodium chloride) in the dissolution media for exchange to occur. A smaller percentage of nicotine was released from the resin at the lower volume (400 mL) in comparison to 800 mL and 1000 mL suggesting that the volume of dissolution media used did affect the release from the resin.

It can be concluded that, when testing the *in vitro* release of nicotine and sodium salts (sodium carbonate and bicarbonates) from chewing gum, the volume of dissolution medium did not have a major influence on the amount released from the gum. At the lower volumes, although the amount released was the same as at the higher volumes, the concentration of the sodium salt was higher, thus increasing the pH of the dissolution media greater than that observed at higher volumes. Changes in saliva flow may also alter the dose effect of Nicorette® gum *in vivo* as pH plays an important role in the absorption of nicotine through the buccal membrane. If saliva flow is high, the concentration of buffers released will be lower. As nicotine diffusion through buccal membrane is favorable at higher pHs (pH > 8), at lower concentrations of buffer the pH may hinder nicotine absorption. Release of nicotine from Polacrilex resin showed that at greater volumes of 1 L the release of nicotine was increased than that observed at the lower volumes.

3.6.2.6 The effect of dissolution medium ionic strength on release of nicotine from gum and Nicotine Polacrilex

As nicotine in the chewing gum is released *via* ion exchange, the ionic strength of the dissolution medium could increase or decrease the release of nicotine from the gum. The ionic strength of the artificial saliva used in the dissolution testing was determined by equation 3.6 as 38.80 mM at pH 6.70.

$$\mu = \tfrac{1}{2} \Sigma C_i Z_i^2 \qquad \text{equation 3.6}$$

Where

μ = ionic strength (mM)

C_i = Concentration of the ions (mM)

Z_i = Charge of the ion.

Experiments were conducted using both Nicorette® 2 mg gum and Nicotine Polacrilex to ascertain the effect that changing ionic strength would have on the release of nicotine.

3.6.2.6.1 Ionic strength of dissolution medium effect on release from Nicorette®

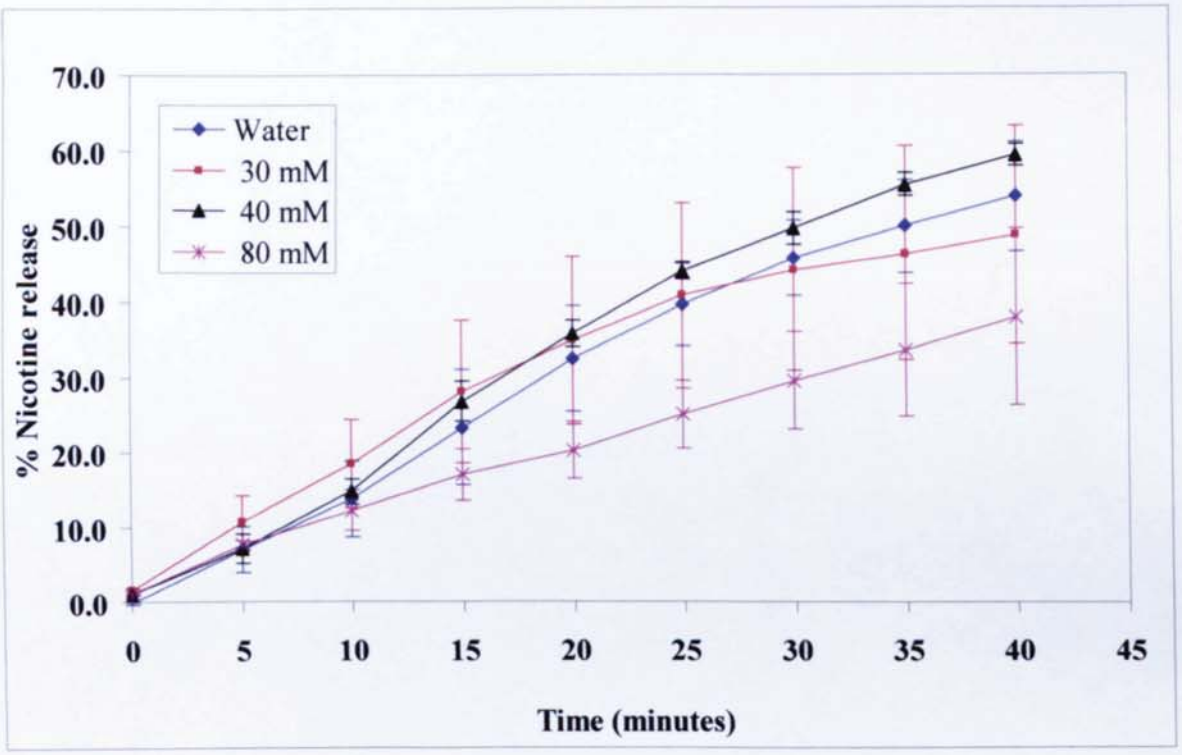


Figure 3.30 The effect of ionic strength on nicotine release from 2 mg Nicorette® (n=3 ± SD).

At a higher ionic strength, a greater amount of nicotine released from the gum would be expected as more ions are present in solution for exchange to occur. Results obtained showed that, at a higher ionic strength (80 mM), the amount of nicotine released was lower than at 30 and 40 mM (Figure 3.30). At the end of 40 minutes, gums chewed at an ionic strength of 40 mM (artificial saliva) gave a total release of 59.41% compared to 49.15% (30 mM), 38.47% at 80 mM and 54.12% in water.

Dissolution curve comparison using the f_2 equation showed that release of nicotine from gum placed in water, 30 mM and 40 mM dissolution medium were similar, whilst, the release at 80 mM differed (Table 3.15). As the gum contains sodium bicarbonate and carbonate, the ionic strength of the water increases as the buffers are released from the gum. If all the buffers were released, the ionic strength of the dissolution medium would approximately be

17 mM, highlighting that at lower ionic strength, the release of nicotine from the gum was greater than at higher ionic strengths.

Ionic Strength (mM)	Water	30	40	80
Water		72.08	71.18	47.03
30	72.08		63.31	47.49
40	71.18	63.31		41.18
80	47.03	47.49	41.18	

Key

- Dissolution curves not similar
- Dissolution curves similar
- Dissolution curves not compared

Table 3.15 f_2 values indicating similar and dissimilar dissolution curves for the release of nicotine in different ionic strength. The mean of the replicates was used to compare the dissolution curves

The pH of the dissolution media was similar for gums chewed in artificial saliva with ionic strengths of 30 mM, 40 mM and 80 mM. At the end of 40 minutes, a total pH change of less than 1.3 pH units was observed from 30, 40 and 80 mM ionic strengths. Higher pH increases were produced by gums chewed in water. The pH rose by 1.783 pH units within 5 minutes and then slowed to give a total pH increase of 1.885 pH units after 40 minutes (Figure 3.31). The rise in pH will affect the ionic strength of the solution thus, as the pH of the dissolution buffer increased the ionic strength increased accordingly (Figure 3.32). The initial ionic strength of the solutions was 28.80 mM, 38.80 mM and 78.80 mM at pH 6.70. Due to the release of sodium carbonate and bicarbonates, the pH of the dissolution medium increased therefore, increasing the ionic strength to approximately 36.45 mM ± 0.03, 46.69 mM ± 0.02 and 86.56 mM ± 0.30 respectively.

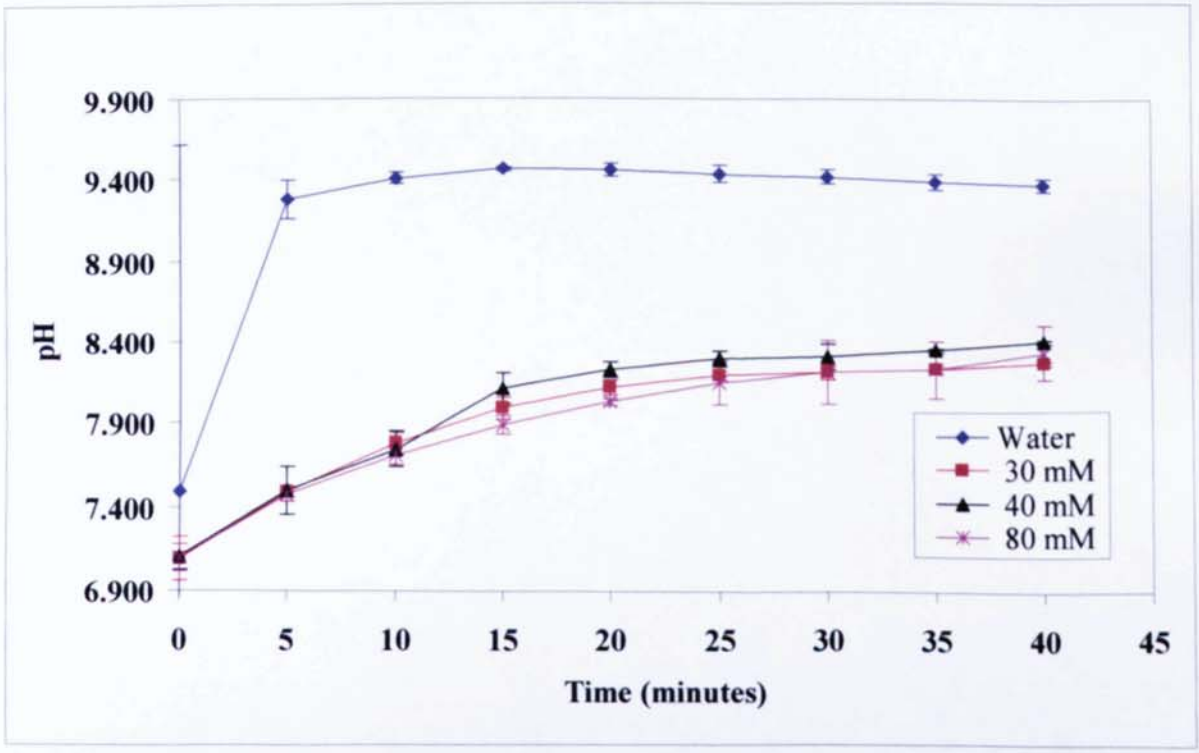
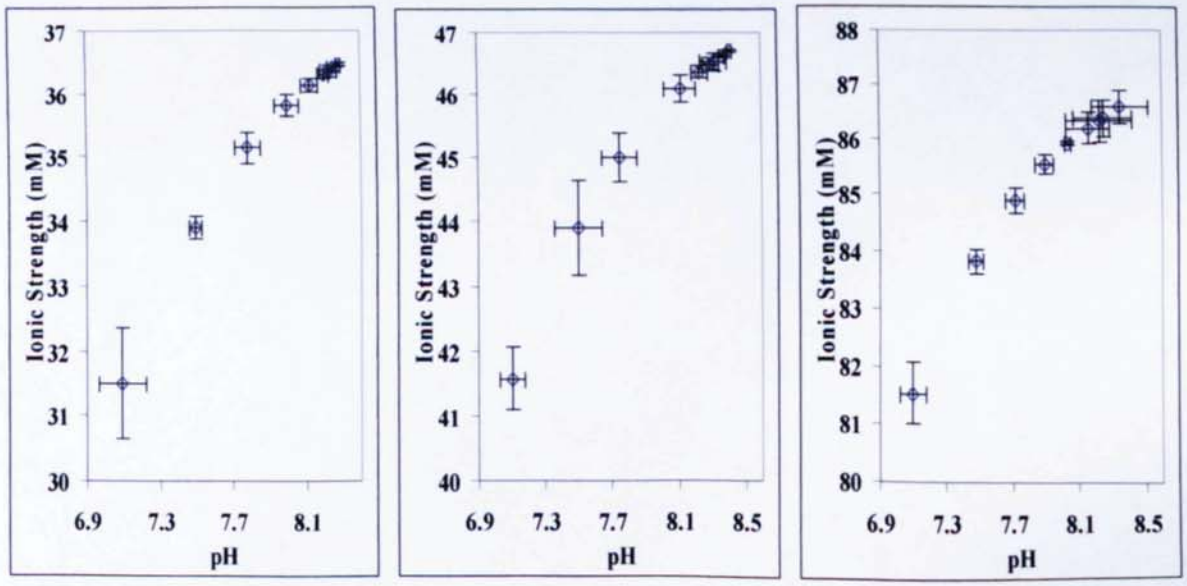


Figure 3.31 Effect of ionic strength on pH of dissolution medium as nicotine was released from 2 mg Nicorette® *in vitro* (n=3 ± SD).



(a) (b) (c)

Figure 3.32 Increase in ionic strength with effect of increasing pH as 2 mg Nicorette® was chewed *in vitro*. Initial ionic strength of the solutions were 28.80 mM (a) 38.80 mM (b) and 78.80 mM (c) at pH 6.70 (n =3 ± SD).

The results attained were not as expected. At the higher ionic strength (80 mM), due to a greater number of ions present in the dissolution media, it was expected that the release would be greater. However, release of nicotine was lower at 80 mM than 30 and 40 mM. In addition, since release in water was similar to 30 and 40 mM, it can be postulated that the

presence of ions in the dissolution media when determining the release of nicotine from gums will not increase release, as the gums contain buffers (sodium bicarbonate and carbonate), which are released from the gum on chewing, thus providing ions for exchange. It can be speculated that at higher salt concentrations, the properties of the gum was altered such that the release was hindered.

3.6.2.6.2 Effect of ionic strength on release of nicotine from Nicotine Polacrilex.

The release of nicotine from the resin was increased with increasing ionic strength (Figure3.33) with greatest release observed at an ionic strength of 150 mM. Dissolution curve comparisons (f_2) showed that the release from resins at the different ionic strengths were dissimilar (Table 3.16).

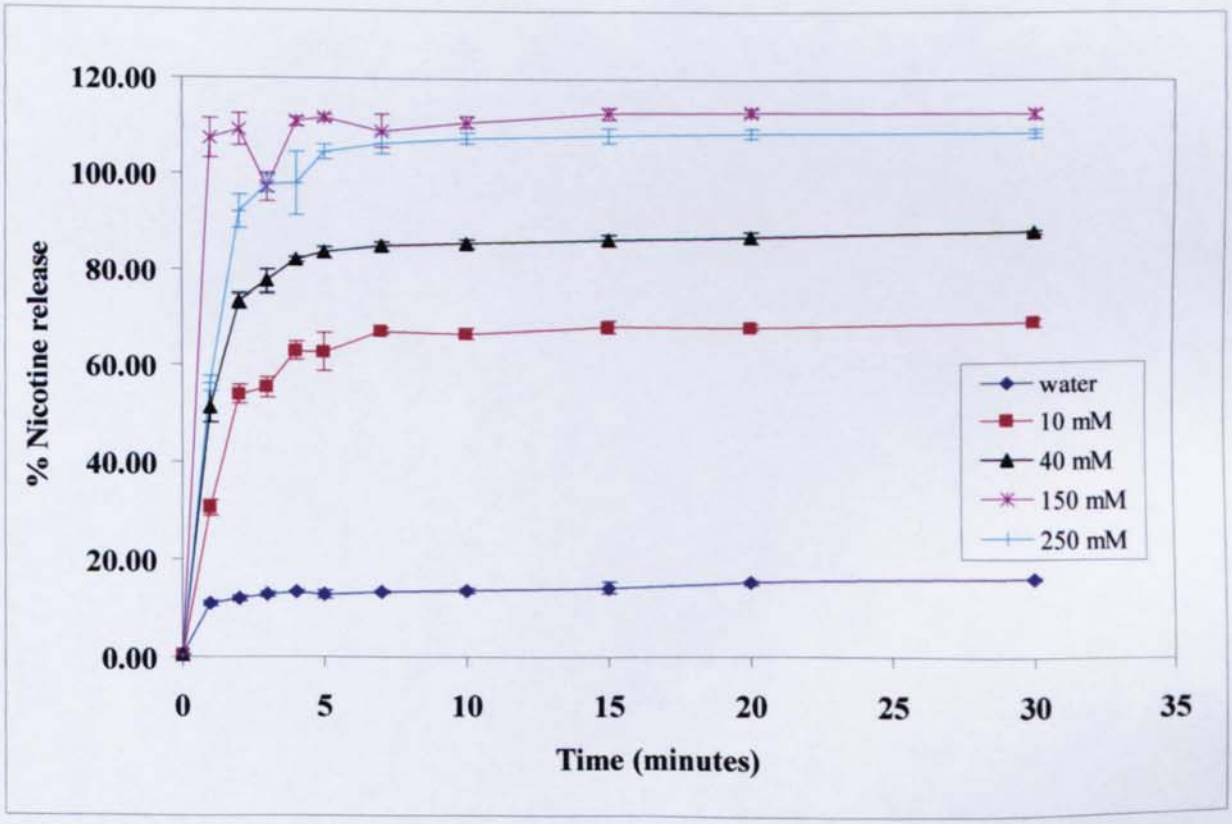


Figure 3.33 Effect of ionic strength on the release of nicotine released from Nicotine Polacrilex resin (n=3 ± SD).

A study conducted by Akerman *et al.* (1999), showed how the ionic strength of the adsorption medium and dissolution medium effected drug adsorption onto and release from a acrylic acid grafted poly(vinylidene fluoride) (PAA-PVDF). They found that drug adsorbed at high ionic strengths (0.4 M), decreased the release rate of the drug in low ionic strengths (0.05 M), whilst, drugs adsorbed at a low ionic strength, showed greater release in low ionic

strength solutions. The explanation by investigation group was that, drug adsorbed onto the PAA-PVDF at higher salt concentrations, the ion exchange capacity of the PAA-PVDF was more saturated with salt ions, therefore the cation exchange process, releases the drug slowly at low ionic strengths.

		Ionic Strength (mM)				
		Water	**10**	**40**	**150**	**250**
Ionic Strength (mM)	**Water**		17.00	9.72	1.90	4.21
	10	17.00		36.42	16.02	21.46
	40	9.72	36.42		26.35	36.49
	150	1.90	16.02	26.35		38.33
	250	4.21	21.46	36.49	38.33	

Key

Dissolution curves not similar

Dissolution curves similar

Dissolution curves not compared

Table 3.16 f_2 values indicating similar and dissimilar dissolution curves with the effect of ionic strength on the release of nicotine from Nicotine Polacrilex. The mean of the replicates was used to compare the dissolution curves

Results show that the increase in the ionic strength, up to 150 mM, resulted in a greater release of nicotine from the Polacrilex resin, however at 250 mM, the release from the resin was lower. Release from gums also showed that at a high ionic strength, release was reduced compared to ionic strengths of 40 and 30 mM. . It can be postulated from the results that increasing ionic strength results in an increase in release, however if the ionic strength is too high release from Nicotine Polacrilex resin can be hindered due to the high concentration of sodium ions in the dissolution medium.

3.7 *In vitro* chewing of two 2mg Nicorette® gums

Changing the hydrophilic/lipophilic balance of the chewing gum formulation can change the release of the drug from the gum. The simplest way to achieve this is to increase or decrease the amount of gum base. An increase in the gum base will make the formulation more lipophilic and thus reduce the release rate of a given active substance.

Without changing the gum formulations, an experiment was conducted to determine if a greater mass of gum base would alter the release of nicotine. In section 3.4 it was shown that the release from 4 mg was greater than the 2 mg formulation. Here, two, 2 mg gum pieces were chewed simultaneously in the chewing chamber to see if this would produce a slower release than that observed by the 4 mg gum.

3.7.1 Methods

The standard *in vitro* chewing gum release method was used to chew 4 mg, 2 mg and two x 2 mg Nicorette®. Sample were taken and analysed by HPLC and the nicotine levels determined. The release was then compared using the f_2 metric.

3.7.2 Results and Discussion

4 mg Nicorette® gum gave a greater release compared to 2 mg gum and two x 2 mg gum (Figure 3.34). Both 2 mg and 4 mg release differed from the two x 2 mg release (f_2 30.25 and 30.36 respectively). 2 mg gum showed a slower steady increase in release with time, whilst, within the first 10 minutes a faster release was observed from 4 mg gum after which the release rate decreased. The same mechanism of release was observed from the two x 2 mg, but, the total amount of drug released was lower.

It was expected that increasing the amount of gum base would reduce the release of nicotine from the gums. The results (Figure 3.34) were therefore as expected. Although the 4 mg gum and two x 2 mg contain the same amount of drug, (4 mg in total), the release from the two x 2 mg was slower. This highlighted that increasing gum base could reduce the rate of drug release. However, as a high content of gum base encumbers the manufacturing process, special gum base properties are required for formulations with high gum base content. It is however possible to manufacture a product with a lower proportion of gum base, thus increase the release (increase due to a reduction in the lipophilic portion of the gum), but, in practice, a piece of chewing gum containing less than 20% gum base will have inferior chewing properties.

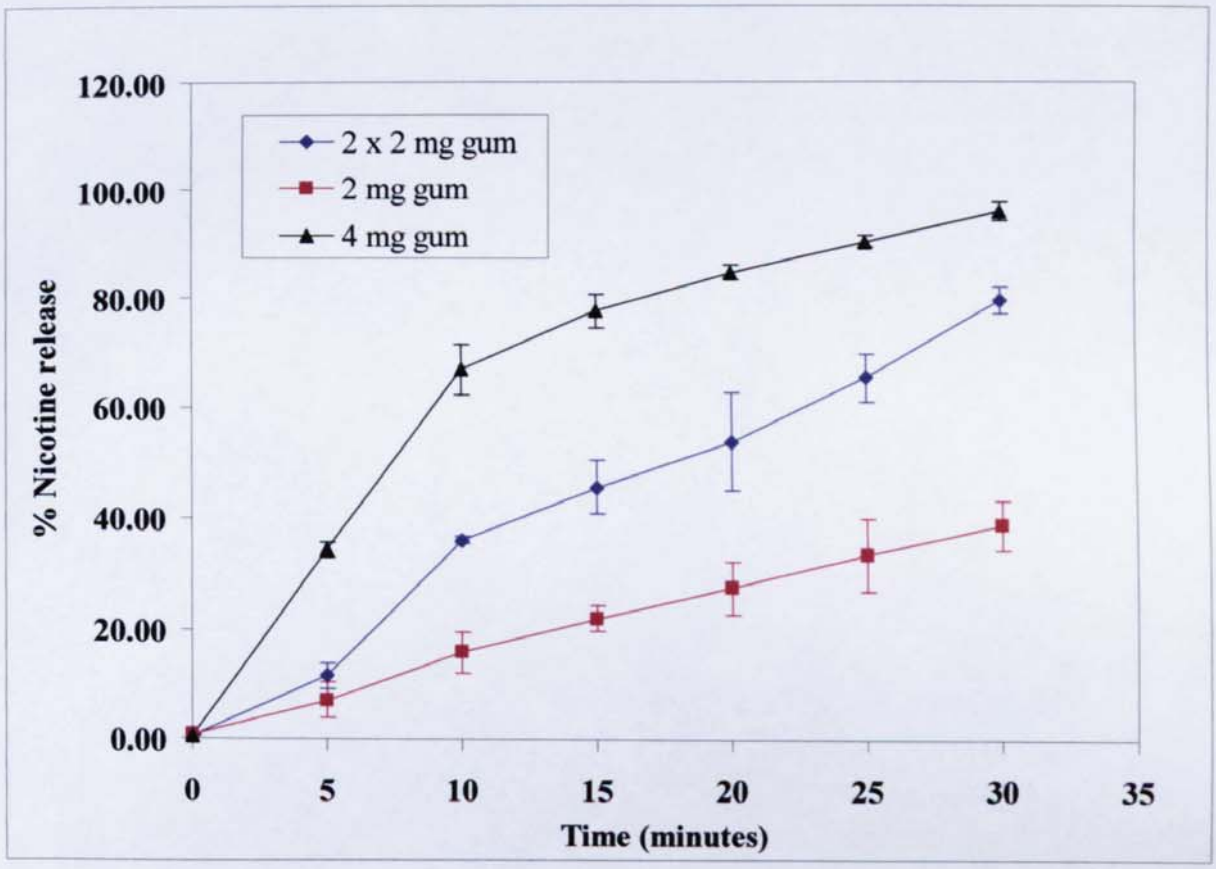


Figure 3.34 Nicotine release from 2 mg, two x 2 mg and 4 mg Nicorette® (n =3 ± SD)

3.8 Conclusion

Validation of the *in vitro* chewing apparatus for release testing from gums showed that, on each day of use, the machine should be calibrated to ensure that chew rates were reproducible. Further, studies on the comparison of real saliva to artificial saliva showed similar release of nicotine, therefore validating the use of artificial saliva as a dissolution medium for release testing of chewing gum. The use of the chewing machine for *in vitro* testing of nicotine from gums, established that release from 4 mg gum was greater than from the 2 mg gum and that the release profiles obtained were similar to those seen in *in vivo* chew-out studies.

The diffusion of nicotine through the gum without chewing was found to be minimum thus highlighting the need for the gum to be masticated when determining the dissolution of drugs from medicated gums. *In vitro* testing of Nicorette® gums highlighted that factors such as chew rate, the type of dissolution medium used and the temperature of the dissolution medium would affect the release of the nicotine from the gum whilst, volume and pH did not appear to impair release. The ionic strength of the dissolution medium also plays a vital role

in release as at high ionic strengths the release from gum and Nicotine Polacrilex can be reduced due to the high concentration of ions in the dissolution medium. Therefore, consideration should be taken when selecting conditions for the *in vitro* testing of drugs from medicated chewing gum. To obtain similar *in vitro* results to those observed *in vivo*, conditions should mimic those expected to be found *in vivo* to reduce variation between *in vivo* and *in vitro* conditions.

CHAPTER FOUR
IN VITRO IN VIVO CORRELATION

4.1 Introduction

As described in section 1.6, *in vitro-in vivo correlation (IVIVC) is* an area within biopharmaceutics receiving considerable interest. It refers to the relationships between *in vitro* dissolution and *in vivo* input rate. Generally, the *in vitro* property is the rate or extent of drug dissolution or release while the *in vivo* response is the plasma drug concentration or amount of drug absorbed.

In an effort to minimise unnecessary human testing, correlations between *in vitro* dissolution and *in vivo* bioavailability (IVIVC) are increasingly becoming an integral part of extended-release drug product development (Uppoor, 2001). The increased activity in developing IVIVCs indicates the value of IVIVCs to the pharmaceutical industry hence, the US Food and Drug Administration published some guidelines in September 1997 entitled, "Extended Release Oral Dosage forms: Development Evaluation and Application of *In vitro In vivo* Correlations". Within this guidelines, the FDA defines IVIVCs as "a predictive mathematical model describing the relationship between an *in vitro* property of a dosage form and an *in vivo* response" (U.S Department of Health and Human Services, 1997). The United States Pharmacopoeia (USP) also defines IVIVC as "the establishment of a relationship between a biological property, or a parameter derived from a biological property produced from a dosage form, and a physicochemical property of the same dosage form" (Leeson, 1995).

Generally, *in vitro* dissolution testing serves as a guidance tool to the formulator regarding product design and in quality control. It is of specific importance for modified-release dosage forms, which are intended to prolong, sustain, or extend the release of drugs (Dressman, *et al.*, 1999). Also, *in vitro* dissolution testing although a powerful and useful method for determining product quality, it is sometimes used to evaluate the clinical performance of the dosage forms, though it is not known whether one can predict the *in vivo* performance from *in vitro* data alone.

The first and main role of establishing an IVIVC is the use of dissolution testing (*in vitro* data) as a surrogate for human studies. The main benefit of this is to minimise the number of bioequivalence studies performed during the initial approval process and during the scale-up and post-approval changes. In order to successfully develop an IVIVC, *in vitro* dissolution has to be the rate-limiting step in the sequence of events leading to appearance of the drug in the systemic circulation following oral or other routes of administration. Furthermore, to utilize the dissolution test as a surrogate for bioequivalence (where a relatively simple *in vitro* test is used in place of human testing), the IVIVC must be predictive of *in vivo* performance of the product (Sunkar and Chilukuri, 2003; Uppor, 2001).

In the past decade, significant medical advances have been made in the area of drug delivery with the development of novel dosage forms and delivery devices such as contact lenses used to deliver ocular pharmaceutical agents and delivery systems offering patients with needle free medicine *i.e.* PowderJect®. Scientists and pharmaceutical companies are constantly developing new formulations to improve on the efficacy of well established products that are currently on the market. The rational development of a delivery system is sensible but expensive. Formulation development and optimisation involves varying excipients levels, processing methods, identifying discriminating dissolution methods and subsequent scale up of the final product (Sunkar and Chilukuri, 2003). In this regard, the use of *in vitro* data to predict *in vivo* bio performance can be considered as the rational development of controlled-release products as it enables the scientist to predict the *in vivo* effect with the added advantages of reducing development costs, avoiding excessive use of human volunteers in bioequivalence studies as well as speeding up the submission dates of the drug.

The aim of this study was to establish a level A correlation from *in vitro* and *in vivo* release of nicotine from 4 mg Nicorette® chewing gum. All *in vitro* work was conducted using the EP chewing apparatus and *in vivo* work carried out by an *in vivo* investigation team at the University of Sheffield. Nicorette® 4mg gum was chewed at various chew rates *in vitro* in artificial saliva. For *in vivo* study Nicorette® 4 mg was chewed using a standardised chewing procedure in a single-centre, open-label, four-phase cross-over study on 16 volunteers (Table 4.1).

Table 4.1 Definition of *in vivo* study design (Adis International, 2004)

4.2 Experimental

4.2.1 *In vivo* chew-out study

Study design/protocol, experimental work, results for all *in vivo* data and the construction of the IVIVC was conducted by the University of Sheffield.

Principal investigators

Dr Wilfred W Yeo

Professor Geoffrey T Tucker

Co investigators

Dr Amin Rostami-Hodjegan

Dr Karen Rowland

Dr Philemon

Dr Joseph Yikona

4.2.1.1 Method

4.2.1.1.1 Study design

The study was a single-centre, open-label, four-phase cross-over design with a minimum interval of 24 hours between each phase. The volunteers (Appendix 3) attended the clinic on approximately 8 occasions and chewed 4 mg Nicorette® gum batch A1080A using a standard chewing protocol for the prescribed time period of 2, 5, 7, 10, 15, 20, 25 and 30 minutes.

A schedule was drawn up such that two chewing sessions, separated by one hour, took place on each of the four study visits. The chew-out periods were paired as follows; 2 and 30 minutes, 5 and 25 minutes, 7 and 20 minutes, and 10 and 15 minutes. On each occasion, the shorter chew-out period was completed first. The volunteers were randomised to receive each of the four phases.

On the day of the study, the subject refrained from drinking any alcoholic or caffeine-containing beverages, or smoking, for 12 hours prior to dosing until after the last chewing session. Early in the morning of the test day the subjects ate a light breakfast (two slices of buttered toast and a drink of water/orange juice) and were advised to fast for two hours prior to study drug administration.

Standardised gum chewing procedure

4 mg Nicorette® Batch A10804 was administered and chewed for the designated period of time as dictated by the schedule in the protocol. The gum was chewed once every 4 seconds (15 chews/minute). The rhythm of the chewing was provided by an audible sound. The subject chewed the gum for 30 seconds on one side of the mouth and then moved the gum to the other side of the mouth, alternating the side of the mouth every 30 seconds. Subjects were instructed to swallow at verbal command every 30 seconds. If the subject swallowed

inadvertently prior to verbal command, the subject was encouraged to swallow when instructed to do so. At the end of the chew interval, each chewed gum piece was collected and analysed for any residual nicotine.

Salivary pH measurements

The pH measurements of buccal salivary samples were measured immediately prior to the commencement of each chew-out session and immediately after completion of the specified period. Before the gum was placed in the mouth, saliva was expectorated into a glass vial and the pH recorded using an Orion 520 pH meter and glass MI-410 micro-combination electrode.

4.2.2 *In vitro* release study

All *in vitro* work was conducted at Aston University.

4.2.2.1 Material

Nicorette® 4 mg chewing gum Batch A1080A expiry date March 2002 was used as supplied by GSK, Weybridge.

4.2.2.2 Method

The total nicotine content of the gum was determined using the method described in section 2.5. *In vitro* release from Nicorette® 4 mg gum was determined using the standard chewing machine method (section 2.2) at chew rates of 82, 60, 40, 30, 20, 10 and 5 chews/minute. Samples were removed and replaced with fresh artificial saliva (section 2.7), filtered, and the pH (section 2.6) of the sample recorded pre analysis (section 2.1).

4.3 Results and discussion

4.3.1 Salivary pH and release of nicotine during *in vivo* chew-out

After 2 minutes of chewing, the mean salivary pH increased from an initial value of 6.79 ± 0.34 to 7.78 ± 0.31. A maximum pH of 7.87 ± 0.20 was attained after 7 minutes and this value decreased to 7.42 ± 0.18 on completion of the 30 minutes chew-out (Figure 4.1).

Salivary pH for the 16 subjects ranged from pH 5.67 to 7.10 before the 7 minute chew-out period and from pH 7.43 to 8.27 after the chewing session, hence, indicating that salivary pH

tended to be lower before chewing compared to after chewing, however the differences between pH before and after chewing lessen with greater chewing times.

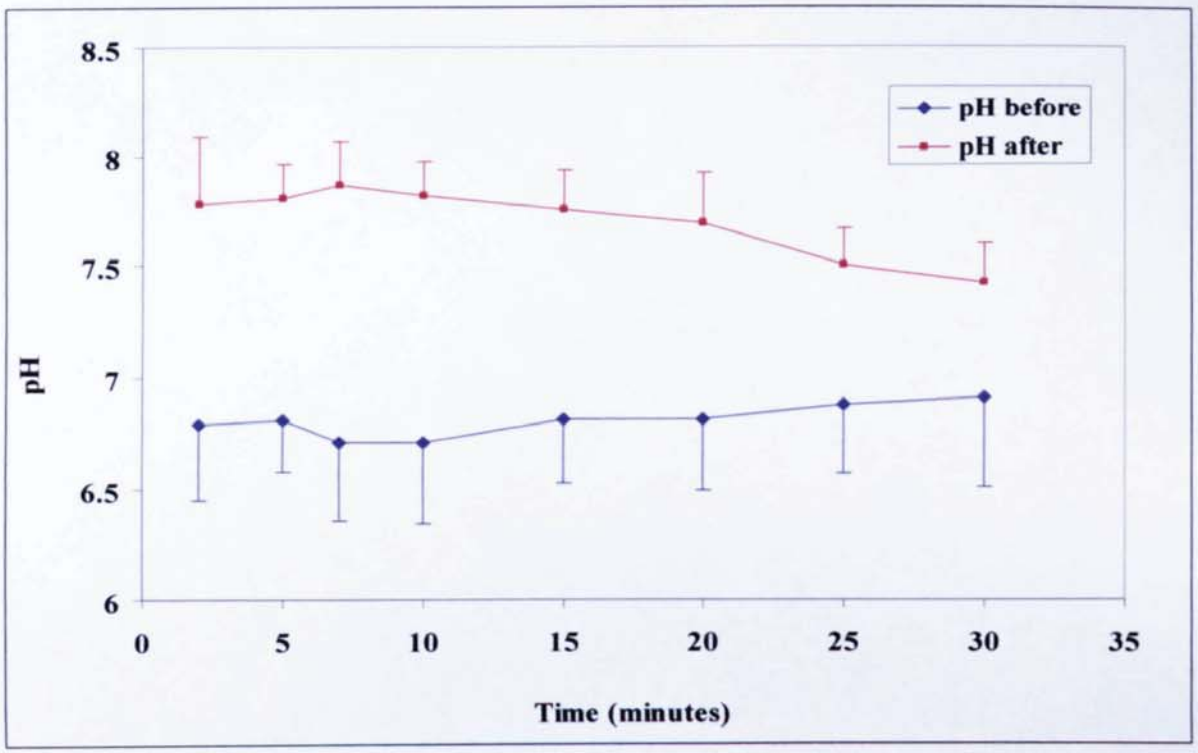


Figure 4.1 Salivary pH before and after each gum chewing session (n=16, ± SD)

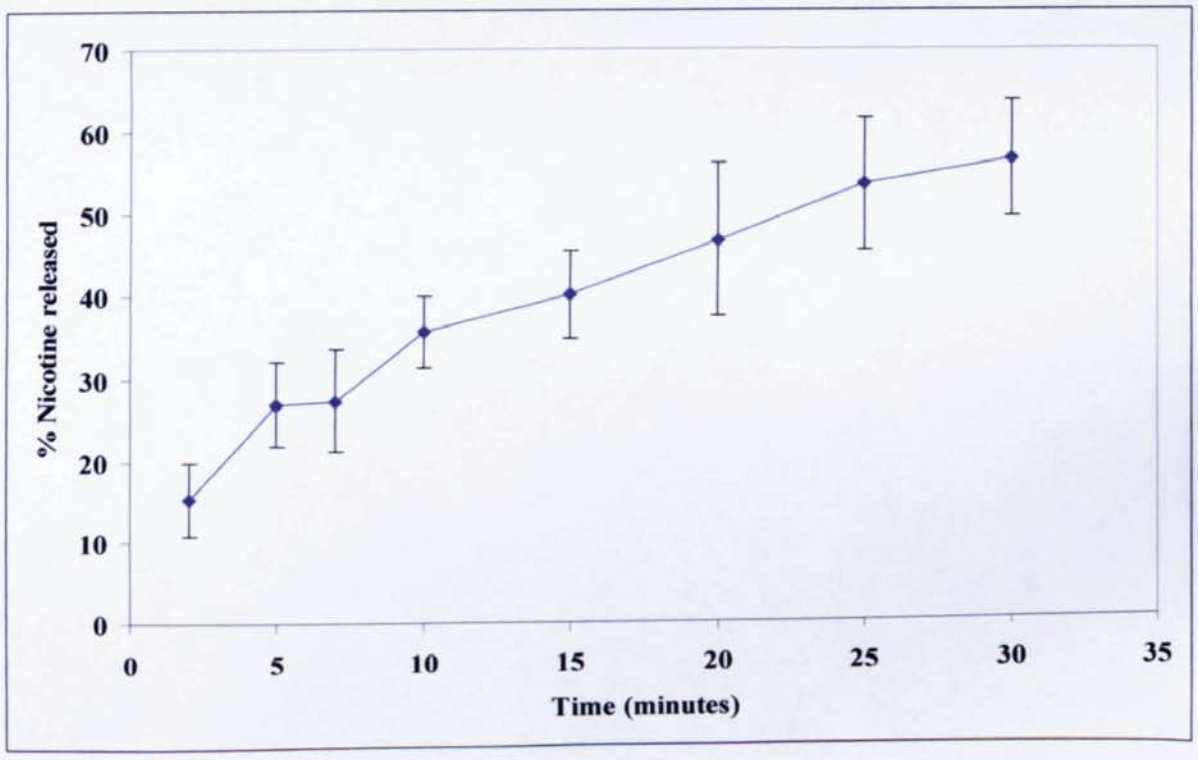


Figure 4.2 Percentage nicotine released from Nicorette® gum during *in vivo* chew-out (n=16, ±SD)

The data from the analysis of the residual nicotine in gum after each chewing session was plotted as percentage nicotine released (Figure 4.2). A gradual rise in release was observed to a maximum mean percentage release of 56.8 % after 30 minutes of chewing at a rate of 15 chew/minute. The range of release from the 16 subjects showed that 32 to 59% of the 4 mg dose remained in the gum after 30 minutes of chew out, giving percentage release of 41 to 68%.

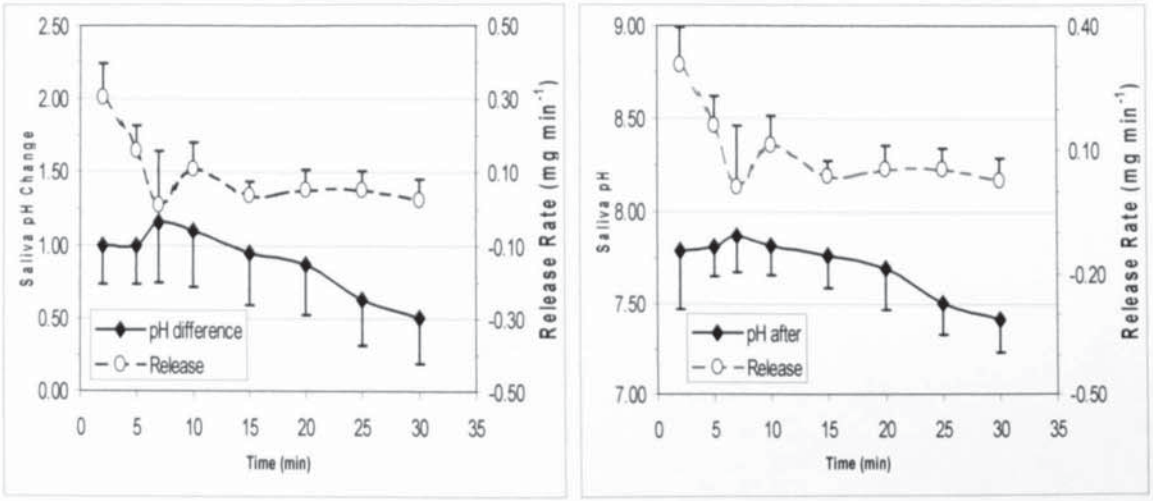


Figure 4.3 Saliva pH change and saliva pH with proportional nicotine release.

An association between absolute saliva pH with release rate was suggested by graphical representation (Figure 4.3). It was clear that chewing gum caused changes in saliva pH and it can be postulated that this is possibly due to the release of sodium carbonate from the gum.

4.3.2 In vitro chewing study

The *in vitro* release of nicotine from 4 mg Nicorette® was investigated at various different chew rates (Figure 4.4). A general pattern observed where-by the amount of nicotine released from the gum increased as the chew rate increased. The shapes of the dissolution curves varied for the different chew rates. The dissolution curves for 82, 60 and 40 chews *per* minute showed similar release profiles whilst, the dissolution curves for 30 and 20 chews *per* minute showed similar release profiles. This was also confirmed using Moore and Flanner's f_2 metric dissolution curve similarity equation whereby two dissolution curves are considered to be similar if f_2 is greater than 50. The f_2 values showed that the dissolution profiles of 82, 60 and 40 chews *per* minute were similar in release as they all had values greater than 50 (Table 4.2). Chew rates of 30 and 20 chews *per* minute showed similar

dissolution profiles (f_2 =62.91) whilst those of 10 and 5 chews *per* minutes were dis-similar to all other chew rates. The differences in the release profiles could be due to the amount of surface area of the gum exposed during the chewing process. As the gum was chewed new chewing gum surfaces are exposed for drug release to occur. At the faster chew rates a greater number of surfaces are exposed hence more surfaces available for drug release to take place compared to that of lower chew rates.

Chew Rate	82	60	40	30	20	10	5
82		74.57	61.52	33.95	29.32	20.94	15.59
60	74.57		75.37	37.36	32.01	22.68	16.86
40	61.52	75.37		40.75	34.59	24.27	18.04
30	33.95	37.36	40.75		62.91	36.67	25.87
20	29.32	32.01	34.59	62.91		44.04	29.77
10	20.94	22.68	24.27	36.67	44.04		44.15
5	15.59	16.86	18.04	25.87	29.77	44.15	

Dissolution curves not similar

Dissolution curves similar

Dissolution curves not compared

Table 4.2 f_2 values for 4 mg Nicorette® chewing gum

At all *in vitro* chew rates the pH of the artificial saliva increased as the gum was chewed (Figure 4.5). Within the first 10 minutes of chewing greater pH rises were observed at 82, 60 and 40 chews *per* minute after which the change in pH decreased and plateaued after about 20 minutes of chewing (Figure 4.6). At chew rates of 30 and 20 chews/minute peak pH changes occurred after 15 minutes of chewing which again plateaued after 25 minutes. At 30 minutes of chewing the final pH change for all chew rates appeared similar ranging from 1.45-1.57 presenting only a difference of 0.12 pH units.

4 mg Nicorette® contains sodium carbonate which is added to the gum to increase the pH of the buccal environment to aid absorption of the nicotine through the buccal membrane. At the lower chew rates, the rate at which the sodium carbonate is released is possibly slower than that at the faster chew rates of 82, 60 and 40 chews/minute.

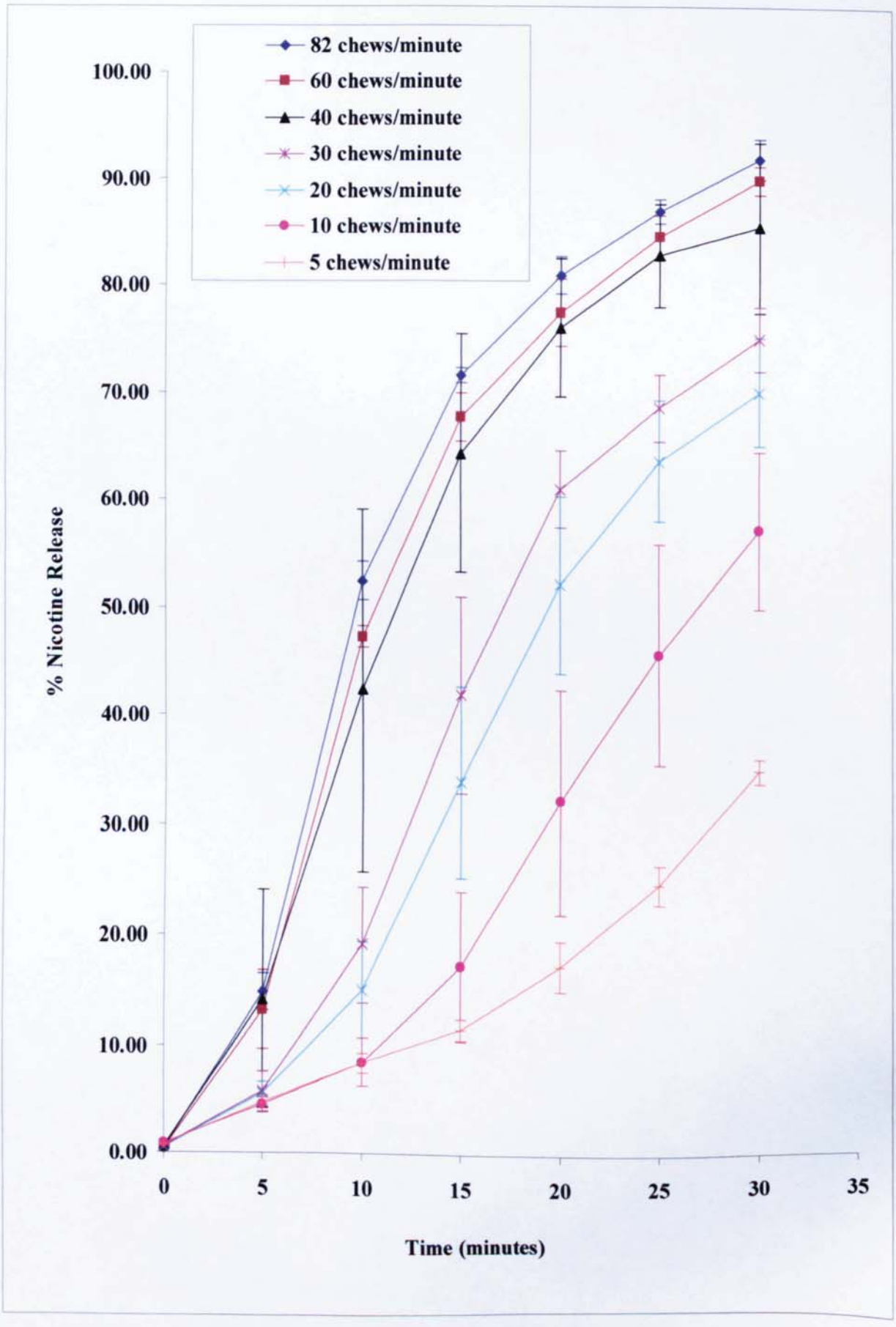


Figure 4.4 Release of nicotine from 4 mg Nicorette® chewing gum at various chew rates (n=3, ± SD).

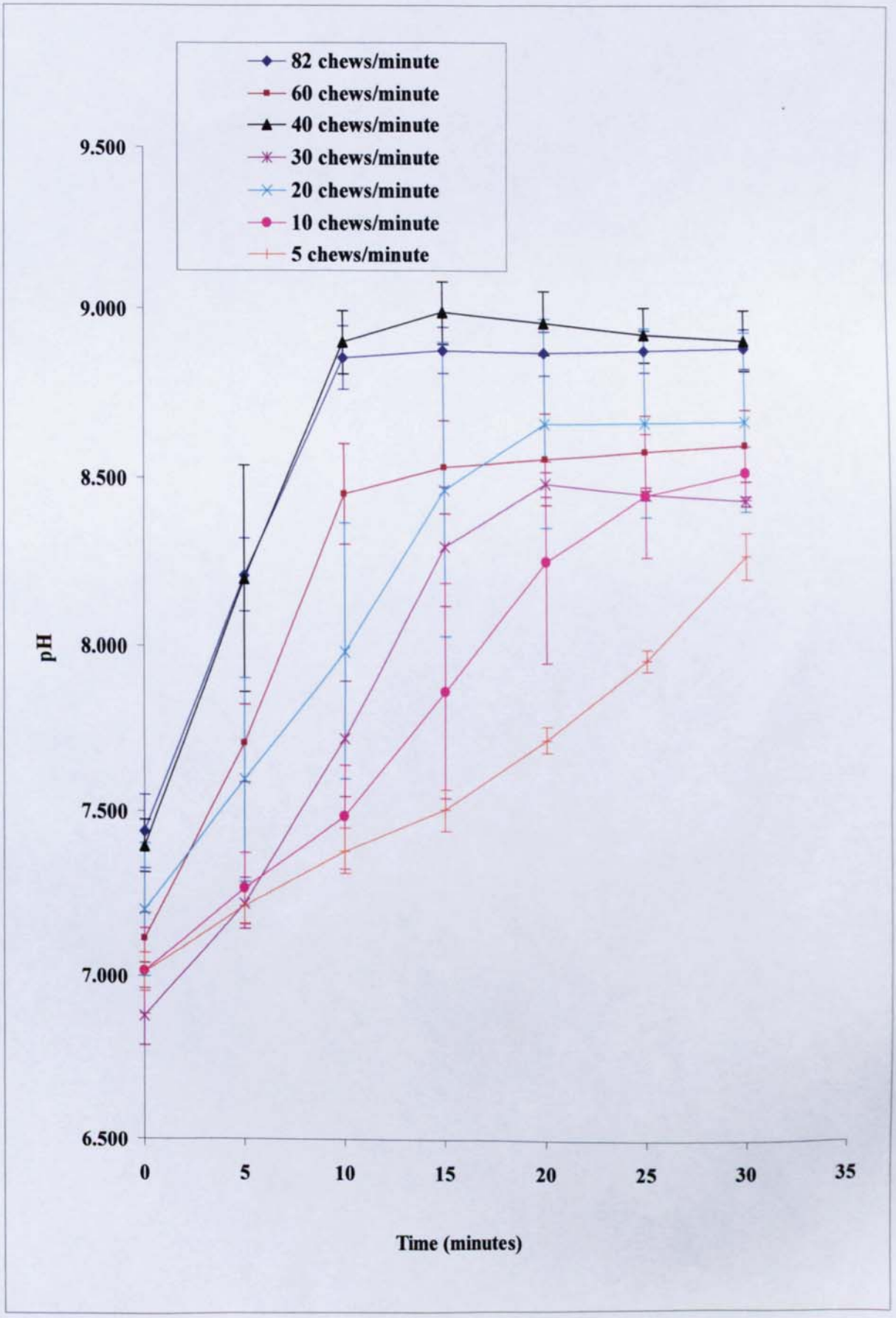


Figure 4.5 pH of artificial saliva as nicotine was released from 4mg Nicorette® chewing gum at the various chew rates (n=3, ± SD).

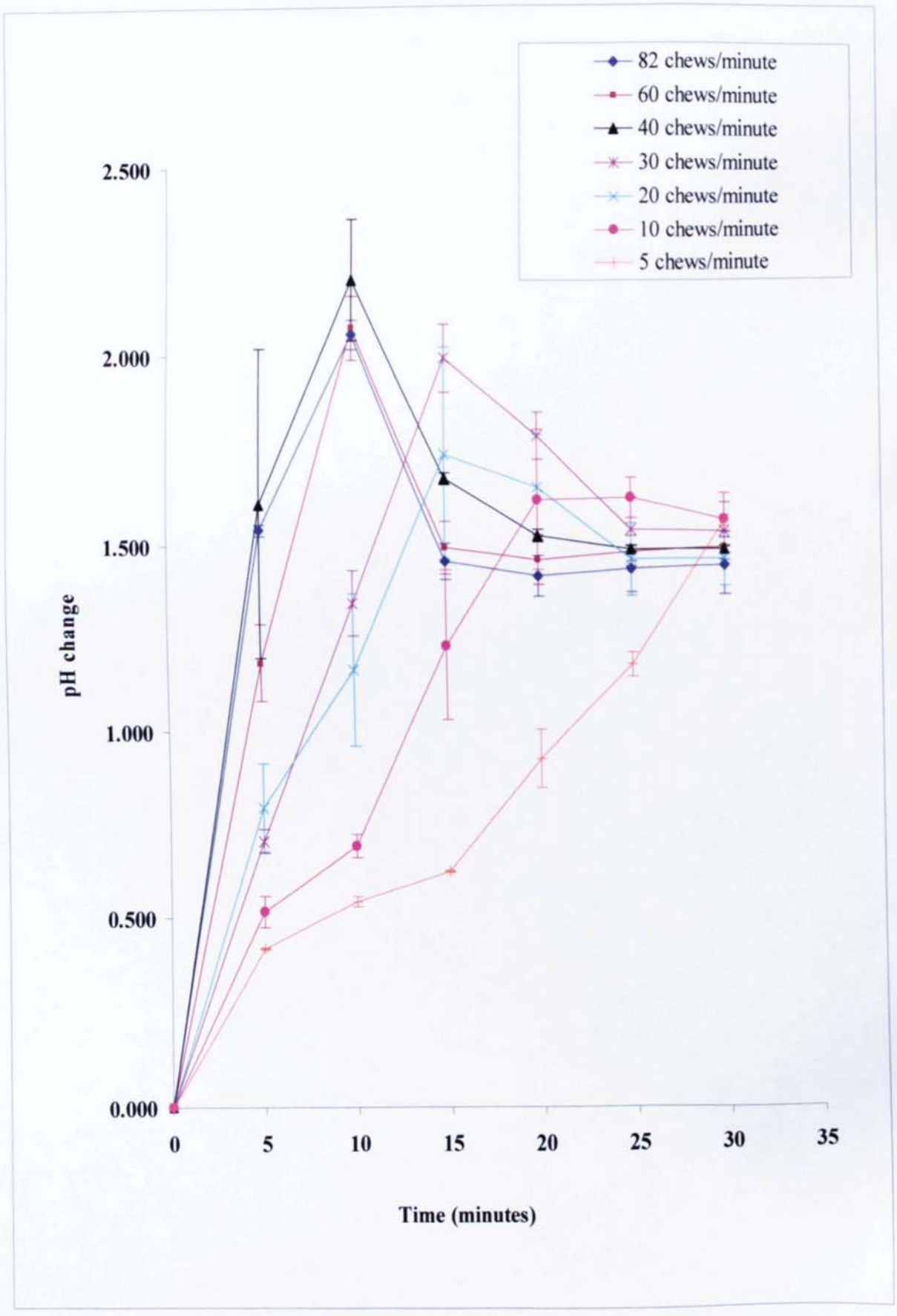


Figure 4.6 pH change of artificial saliva as the 4 mg Nicorette® gum was chewed at the various chew rates

4.3.3 *In vitro in vivo* correlation

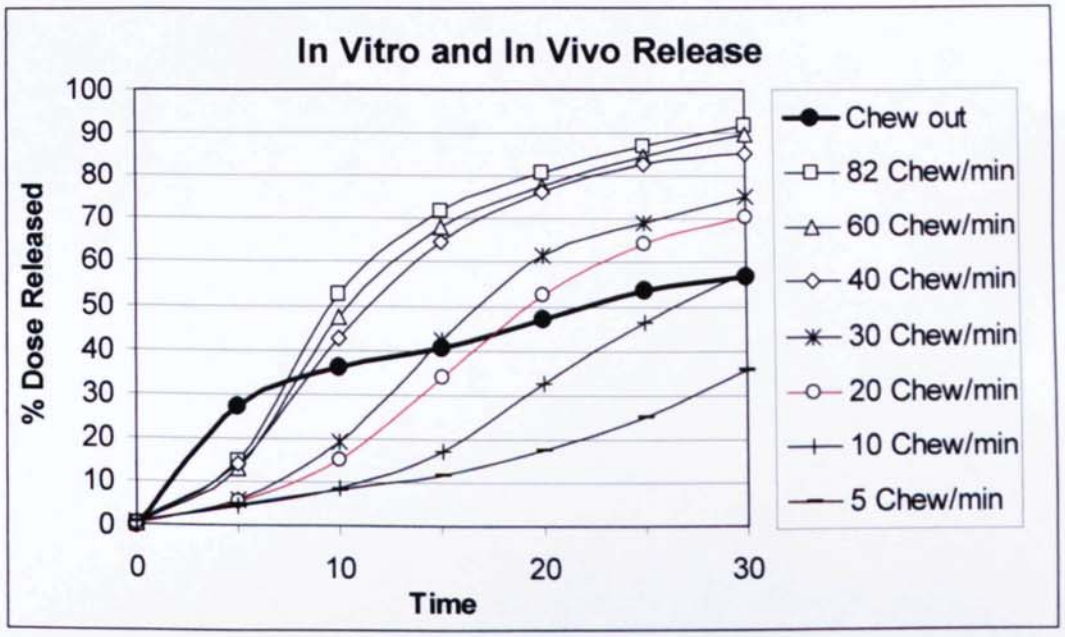


Figure 4.7 *In vitro* and *in vivo* release of nicotine from 4 mg Nicorette®

The relationship between the *in vitro* release data and the *in vivo* data generated by the chew-out study was examined to see if an IVIVC existed between the two sets of data. Compared to *in vitro,* a faster initial *in vivo* release was observed during the chew-out study. After approximately 7 minutes of chewing, the nicotine release *in vivo* began to slow down while a greater *in vitro* nicotine release occurred at the faster chew rates of 82, 60 and 40 chews/minute. At the lower *in vitro* chew rates of 10 and 5 chews/minute the percentage dose released from the gum was relatively lower than that observed *in vivo* throughout the 30 minutes (Figure 4.7)

When comparing the pH of the artificial saliva during the *in vitro* chewing study and the saliva during the chew-out study at the end of the 30 minutes chew-out session, the total pH change in the saliva was approximately 0.63 compared to the range of 1.45-1.57 seen *in vitro*. The greatest pH rise *in vivo* was seen at 7 minutes of chewing where the pH rose by 1.08 units; however, the overall rise in pH was still less than that observed *in vitro* (Figure 4.8). Possible explanation for the difference could be that as buffers were lost by swallowing and as fresh saliva replaces old, buffer capacity was maintained *in vivo*, thus resulting in a lower pH change.

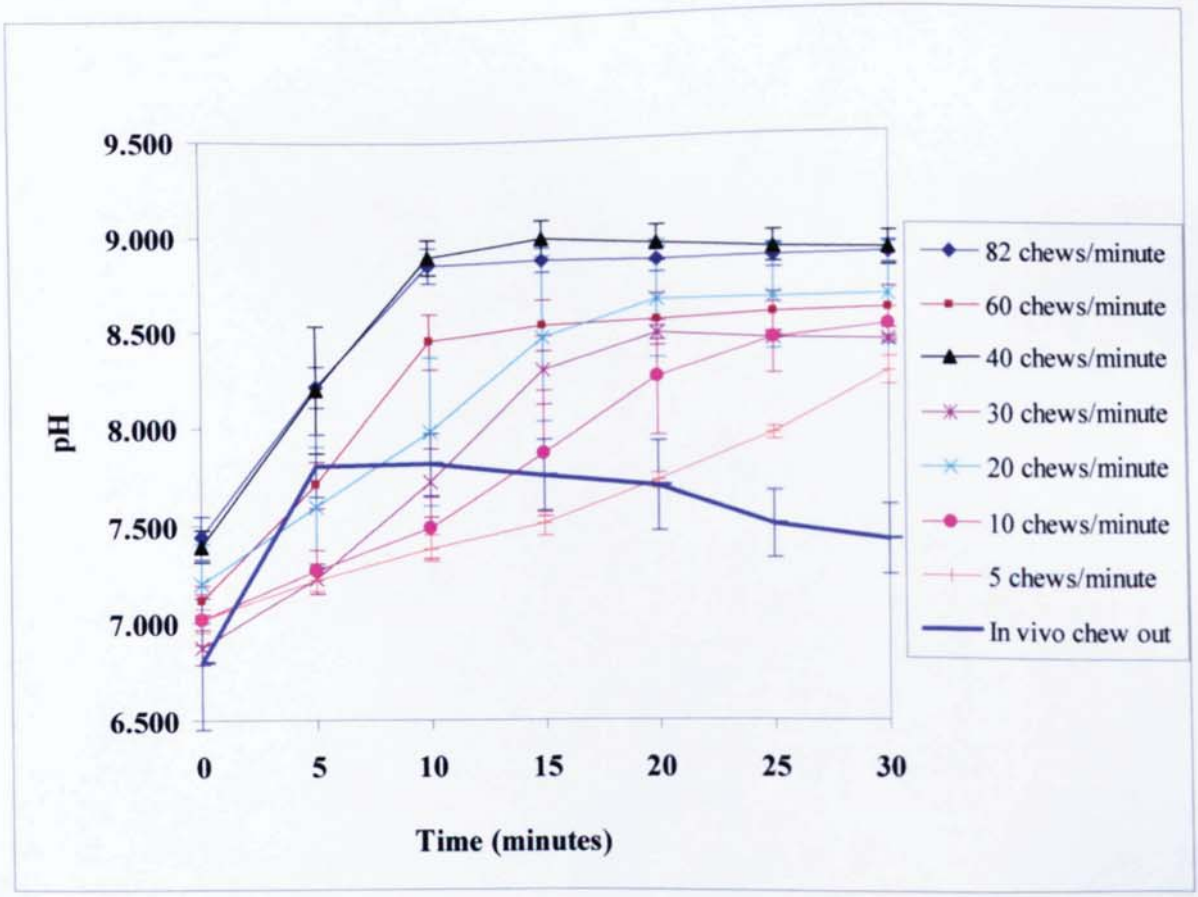


Figure 4.8 pH of artificial saliva and human saliva as 4 mg Nicorette® was chewed *in vitro* and *in vivo* respectively.

None of the *in vitro* release profiles obtained in the chewing machine showed similar pattern/shape of release to that of *in vivo* chew-out release study (Figure 4.8), showing that there was a mechanistic difference between the two types of release. However, correlation between *in vivo* release and *in vitro* releases, regardless of chewing speed in the machine, were significant (Figure 4.9). The highest correlation of *in vivo* release was observed with the *in vitro* studies at chew rates 82, 60 and 40 chews/min (r^2 =0.90).

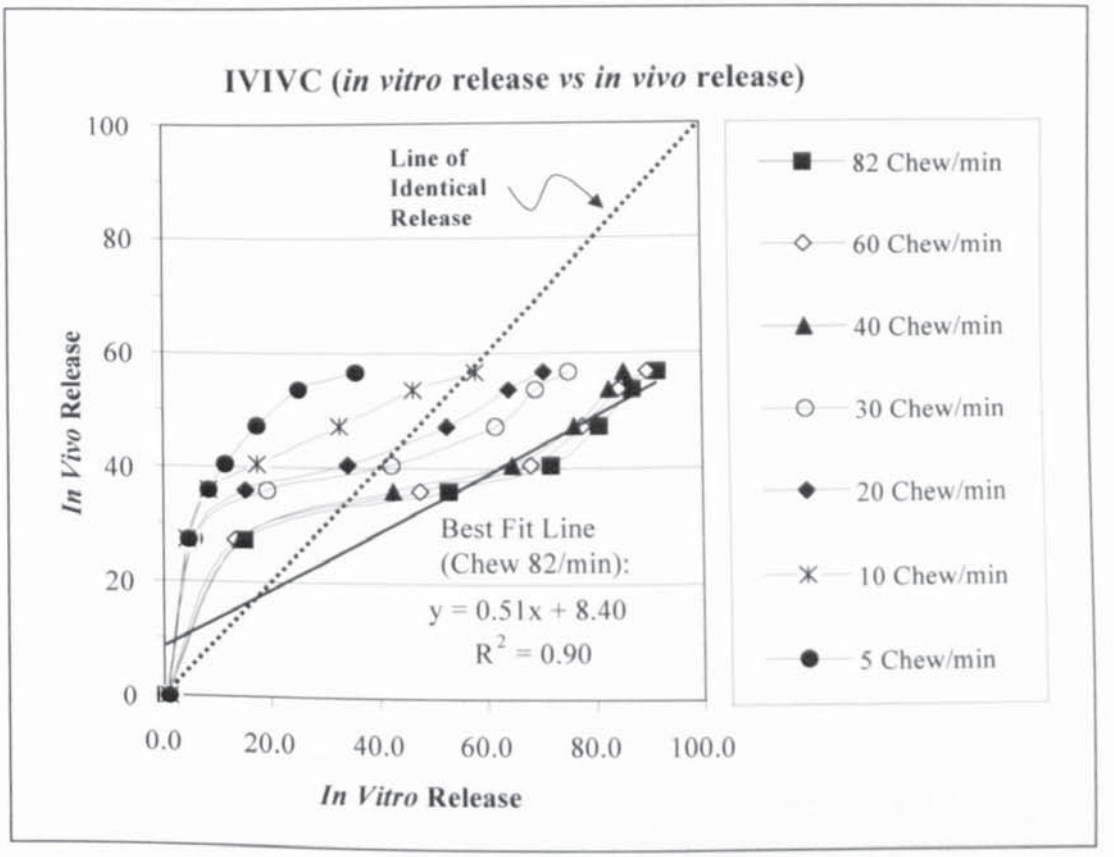


Figure 4.9 Correlation between *in-vivo* release and *in-vitro* release at the various chew rates

Although the *in vivo-in vitro* correlation values were good (r^2=0.90) for the higher *in vitro* chew rates, the data was not randomly distributed around the best fit line, hence the above correlation was not acceptable and time mapping was necessary to improve the correlation between the *in vivo* and *in vitro* data. Earlier work conducted by Rostami-Hodjegan *et al.*, described a mapping process used in the construction of an IVIVC for Panadol® Actifast (Rostami-Hodjegan *et al.*, 2002). This involved finding a suitable function that converts the *in vitro* sampling time to *in vivo* time of release (and *vice versa*). Accordingly, an *in vitro* release at time "t" can be equated to *in vivo* release at time "f(t)" where the "f" describes a conversion function. The most simple functions include models with just lag time (*e.g.* $t_{(in\ vivo)} = t_{(in\ vitro)} + t_{lag}$) or a time factoring (*e.g.* $t_{(in\ vivo)} = 3.t_{(in\ vitro)}$). However, if the shape of release is different for *in vitro* and *in vivo* profiles then more complex functions might be necessary.

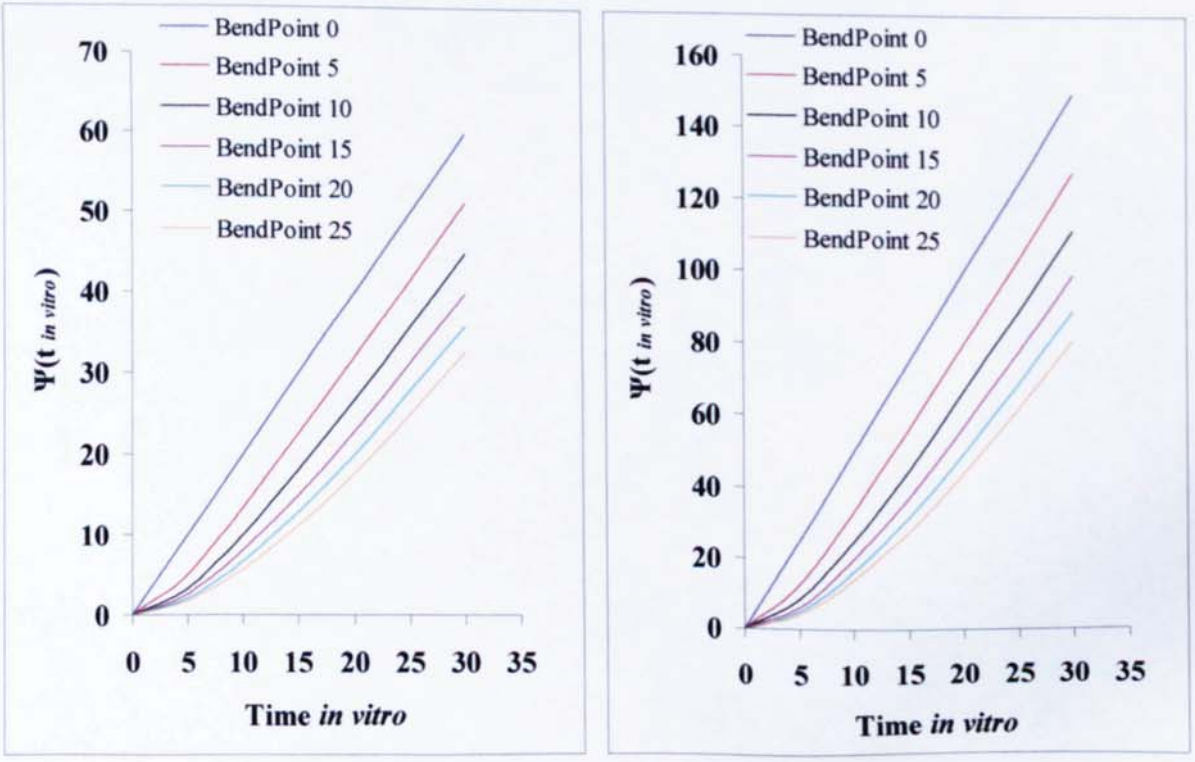
The *in vivo* and *in vitro* nicotine release curves have different release profiles in that a greater initial release was observed *in vivo* which slows down earlier. After 10 minutes at higher *in vitro* chew rates (82, 60 and 40 chews/minute), a greater release compared to *in vivo* chew out data was observed (Figure 4.9). Inspection of Figure 4.9 indicates the need for a

sigmoidal function to link the *in vivo* and *in vitro* data such that early *in vitro* release samples equate to the *in vivo* release samples from later than the corresponding *in vitro* time. This was reversed for later samples where corresponding *in vivo* time was earlier. Therefore, equation 4.1 was used to convert *in vitro* times of sampling to corresponding *in vivo* time:

$$\Psi(t_{in\ vitro}) = \left(\frac{(t_{in\ vitro})^{Shape}}{(BendPoint)^{Shape} + (t_{in\ vitro})^{Shape}} \right) \times scale \times t_{in\ vitro} \qquad \text{equation 4.1}$$

Note:- Solver, within Excel, was used to obtain the best values for conversion parameters "scale", "shape" and "Bend Point".

The calculated time link function (Ψ) comprised of a time scale factor (scale) and a time shape factor (shape). Increasing the time scale factor results in corresponding increase in the Ψ ($t_{in\ vitro}$) value (Figure 4.10) whilst, increasing the time shape factor will interact with bend point and move the curve to the right (Figure 4.11).



(a) (b)

Figure 4.10 Effect of increasing time scale factor from 2.0 (a) to 5.0 (b) at various values of bend point whilst shape scale factor remains constant.

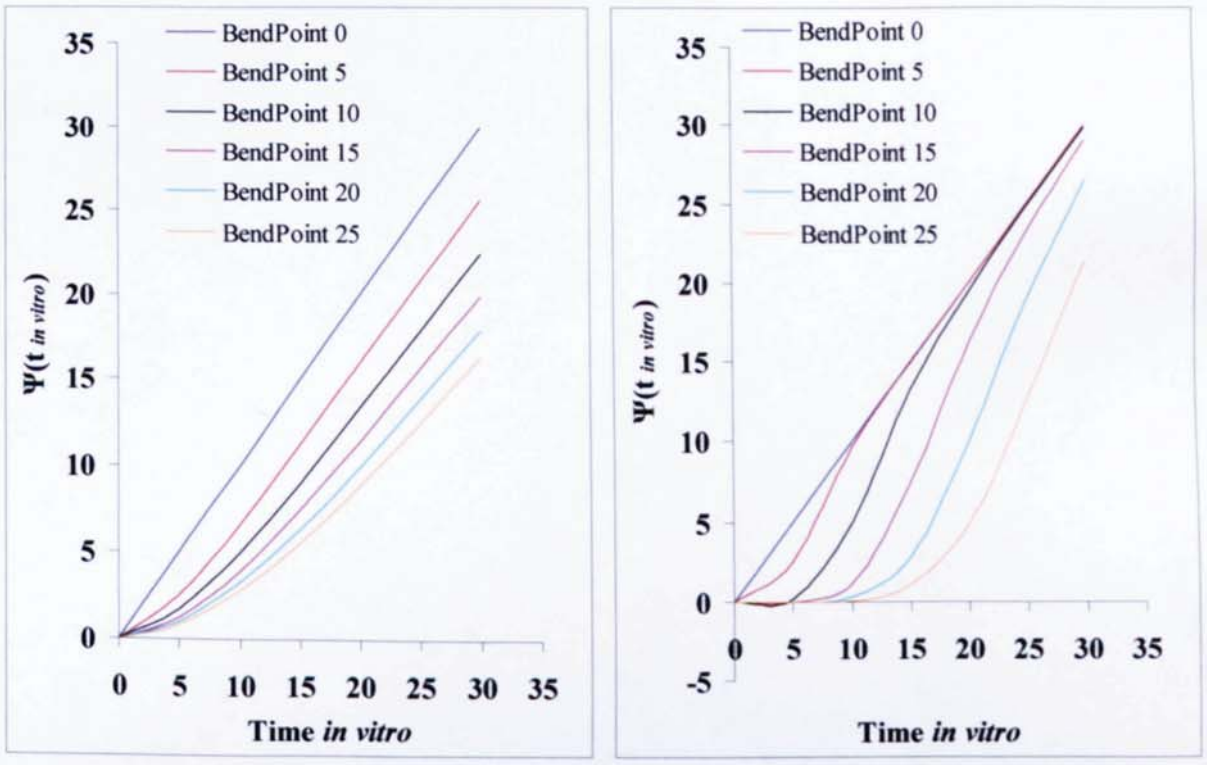


Figure 4.11 Effect of increasing time shape factor from 1.0 (a) to 5 (b) at various value of bend point whilst time scale factor remains constant.

The calculated link function (Ψ) was then used to minimise the sum of squares to form the link between *in vitro* and *in vivo* data (equation 4.2):

$$F_{in\ vivo}(t_{in\ vivo}) = F_{in\ vivo}(\Psi(t_{in\ vitro})) = F_{in\ vitro}(t_{in\ vitro}) \qquad \text{equation 4.2}$$

Note: - $F_{in\ vitro}$ and $F_{in\ vivo}$ are the fractions of drug released during the *in vitro* dissolution test and the fraction absorbed *in vivo* respectively.

Using the mapping function, it was seen that all chew rates used in the *in vitro* study could be used successfully for IVIVC purposes (Figure 4.12). However, statistically, chewing rates of 10 and 20 chews/minute performed better than all other chew rates, as indicated by the minimum sum of squares value of 12.22 and 9.75 respectively (Table 4.3). An additional advantage of these two experiments was that the corresponding *in vivo* release at later sampling times were true values rather than those simulated based on the mechanistic model of *in vivo* release beyond the last sample. In Figure 4.12 all such samples (t >30 min *in vivo*)

are shown in red to indicate that the *in vivo* release for that part of curve is simulated with assumption of individuals continuing chewing for more than 30 minutes.

Chews/minute	82	60	40	30	20	10	5
Minimum Sum of Squares obtained	43.06	25.28	46.23	17.89	**12.22**	**9.75**	13.42
Scale	2.69	2.52	2.38	1.76	**1.61**	**1.81**	436.89
Bend	6.62	7.42	7.83	13.51	**15.82**	**28.22**	1175.17
Shape	6.36	5.14	4.19	4.68	**4.06**	**3.21**	1.95

Table 4.3 Parameter values for time conversion corresponding to different chew rates in chewing machine

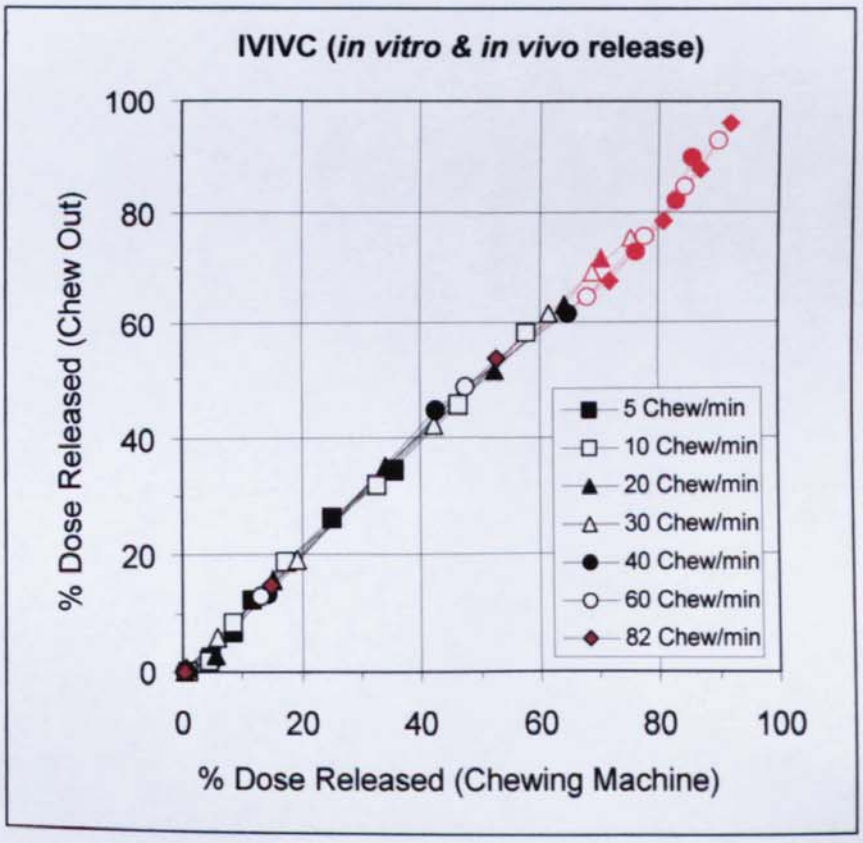


Figure 4.12 *In vitro-in vivo* correlation

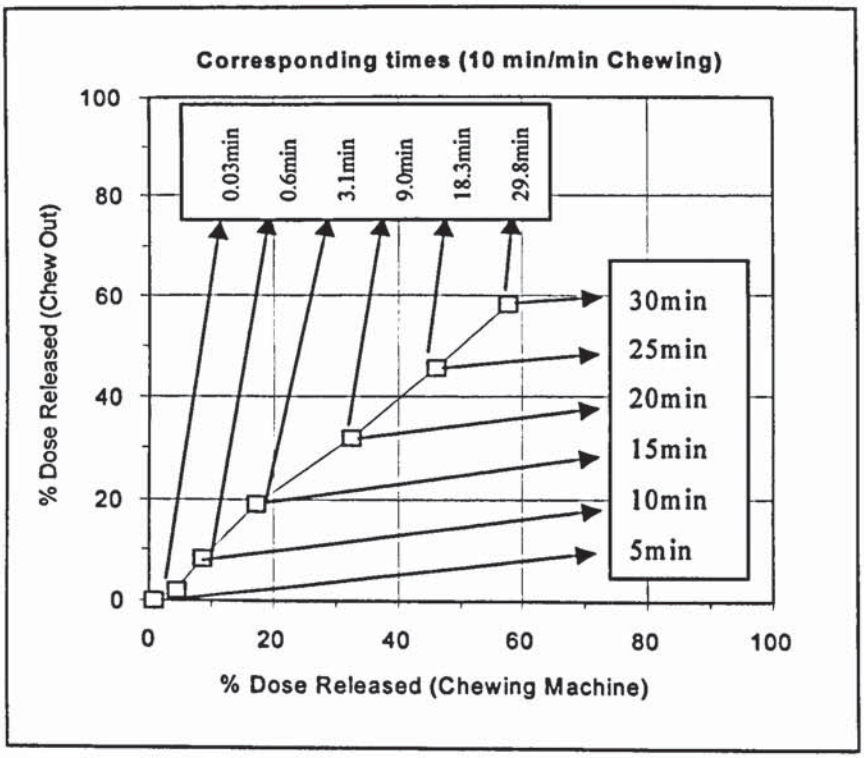


Figure 4.13 An example of IVIVC with corresponding times

An example of IVIVC with corresponding times is shown in Figure 4.13. At a rate of 10 chews/minute, 5 minutes of *in vitro* chewing was similar to 0.03 minutes of *in vivo* chewing or 30 minutes *in vitro* chewing was similar to 29.8 minutes of *in vivo* chewing.

The nomogram (Figure 4.14) can indicate the *in vitro* experimental time required at each chew rate to produce the same result as one would expect *in vivo* during a chew out study. For example to cover a 30 minute *in vivo* chew out period the gum must be chewed in the *in vitro* chewing machine for 30 minutes at 10 chews/minute and for 20 minutes at 30 chews/minute after which one can easily estimate the percentage that will be released *in vivo* from the gum at any time.

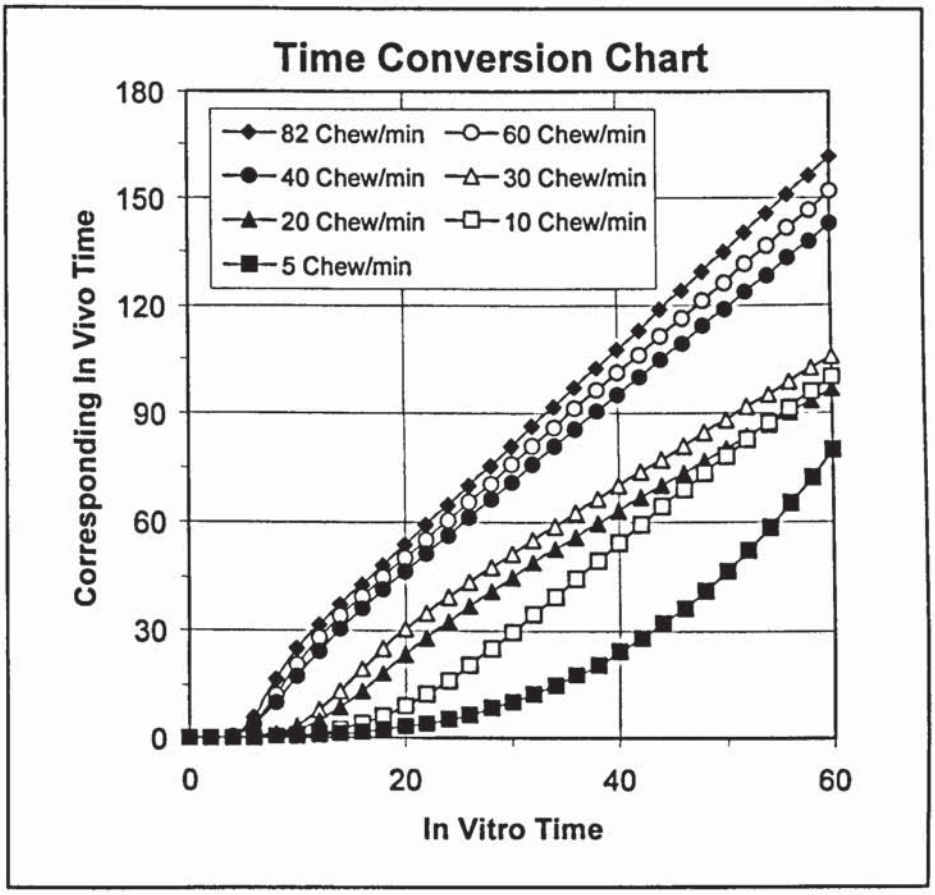


Figure 4.14 A nomogram for converting *in vitro* times to *in vivo* times

4.4 Conclusion

On initial comparison of the *in vitro* and *in vivo* data sets, there appears to be little correlation in terms of either nicotine release or pH. On using the time mapping process, developed by Rostami-Hodjegan *et al.*, the correlation was good. A one-to-one (level A) relationship exists after time conversion with the best chew rate for *in vitro* studies being around 20 chews/minute which will cover the 30 minutes of *in vivo* (according to the standardised chewing protocol used in this clinical study).

On this basis, this technique can be used in future studies on experimental gum formulations to predict their *in vivo* nicotine release profiles. This should therefore speed up the development of new formulations as it should circumvent the need to carry out numerous and expensive chew out studies in volunteers.

CHAPTER FIVE
NICOTINE DIFFUSION THROUGH BUCCAL MUCOSA

5.1 Introduction

The membrane plays an important part in the absorption of a drug as it is often found that the diffusion of the drug through the membrane is the rate-limiting step of the process. Tests are conducted *in vitro* to determine the release mechanism but, one must also consider the absorption of the released drug at the appropriate site of action as only then will the therapeutic effect of the drug be delivered.

5.1.1 Diffusion of nicotine

Diffusion can be defined as the movement of molecules or ions from regions of high chemical potential to a region of low chemical potential. It represents the ability of the molecule to perform work. In the case of diffusion, this work is seen as the random movement of the molecules from a high concentration to a low concentration through the medium across a concentration gradient.

With the on-going research to improve and development new nicotine smoking cessation products, scientists are trying to understand and enhance the mechanisms that allow the diffusion of nicotine into the systemic circulation. Numerous studies have been conducted to determine the *in vitro* diffusion of nicotine through oral mucosa and skin. Here is a summary of the main findings of some of these studies:

- When studying the permeation and partitioning of nicotine as a function of pH within various regions in the mouth, the permeability of nicotine across buccal mucosa was found to follow the pH-partitioning theory for passive diffusion *i.e.* a greater diffusion occurs with the unionised form of nicotine (Nair *et al.*, 1997; Chen, *et al.*, 1999)

- In another study, non-ionised nicotine was found to permeate mainly *via* the transcellular pathway, whereas the mono- and di-protonated molecules permeate *via* the paracellular (more hydrophilic) pathway (Oakley and Swarbrick, 1987).

- Some reports conclude that the permeability of nicotine decreases with increasing concentration indicating that the flux of nicotine can also be determined by other factors such as the partition coefficient and not just the concentration gradient (Zorin *et al.*, 1999; Markowska *et al.*, 1993).

- Studies conducted to investigate and compare the effect of pH and drug concentration on nicotine permeability concluded that the apparent permeability of nicotine across both TR 146 cell culture and buccal mucosa increased significantly with increasing pH. They also concluded that, with increasing concentrations of nicotine, the apparent permeability values decreased, due to a combined effect of decreased diffusion in the epithelium and the partitioning to the epithelium (Neilsen and Rassing, 2002).

- Santi *et al.*, (1991) found that nicotine did not follow the pH partitioning hypothesis when portioned between isopropyl myristate (IPM) and McIlvaine buffers, (Smith and Irwin, 2000).

The formulations of Nicorette® 4 mg and 2 mg chewing gum contain alkaline buffers (sodium bicarbonate and sodium carbonate). It is not known exactly what role the alkaline buffers perform but, presumably they are two-fold, *i.e.* to supply a source of cations to facilitate the release of nicotine from the resinate and also to ensure an alkali environment in the buccal cavity to optimise nicotine absorption. In this study, the *in vitro* diffusion of nicotine through buccal mucosa was investigated at various pHs. Nicotine hydrogen tartrate salt, Nicotine Polacrilex and nicotine Amberlite® IRP69 (section 2.14), were used to determine how the diffusion of nicotine was affected by pH.

The physical and chemical properties of a drug are the most important factors that determine its ability to penetrate the oral mucosa. Generally, unionised molecules are absorbed more readily than ionised. The degree of ionisation of the drug and its ability to penetrate the oral mucosa effectively is dependent on the pH of the surrounding thus in terms of buccal delivery the pH of the oral cavity and mainly saliva. Small molecules are absorbed more readily than larger molecules and the ability of the substance to dissolve in either non-polar (lipid) or polar (aqueous) solvent is also a major factor (Lamey and Lewis, 1999).

Figure 5.1 Molecular structure of nicotine at the two pKa (Nielsen and Rassing, 2002)

Nicotine (Figure 5.1) is a diacidic base with pKa values of approximately 3 and 8 and therefore mainly mono-protonated in saliva (pH 6-8) (Young and Cook, 1996). The absorption of nicotine follows the pH partition hypothesis, so the theoretical relative proportions of the different charged species at any particular pH can be determined by the Henderson-Hasselbalch equation (Figure 5.2)

Figure 5.2 Fractions of nicotine species as a function of pH (Nair *et al.*, 1997)

5.1.2 Diffusion of drug across a membrane

A theoretical diffusion profile of a drug through mucosa is shown in Figure 5.3. Typically, the diffusion of a drug across a membrane comprises two distinct stages; a period of non steady-state condition followed by steady-state penetration which is linear and corresponds to a net balance in the rate of entry and exit of drug into and out of the membrane layer.

Figure 5.3 Typical diffusion profile of drug diffusion through a membrane (Amin, 2001)

The simplest way of modelling the diffusion process of a drug through a membrane is to employ Fick's first law of diffusion to the steady-state phase. The law states that the rate of transfer of a diffusing substance through a unit area of a section (the flux) is proportional to the concentration gradient across the entire barrier phase.

$$J = -D\frac{dC}{dx} \qquad \text{equation 5.1}$$

Where J (μmoles cm^{-2} min^{-1}) is the rate of transfer per unit area (the flux), dC/dx is the concentration gradient across the membrane and D (cm^2 min^{-1}) is the diffusion coefficient. The negative sign signifies that diffusion occurs in the direction of decreasing concentration of penetrant, thus the flux is always a positive quantity and corresponds to the slope of the steady-state diffusion curve (Figure 5.3).

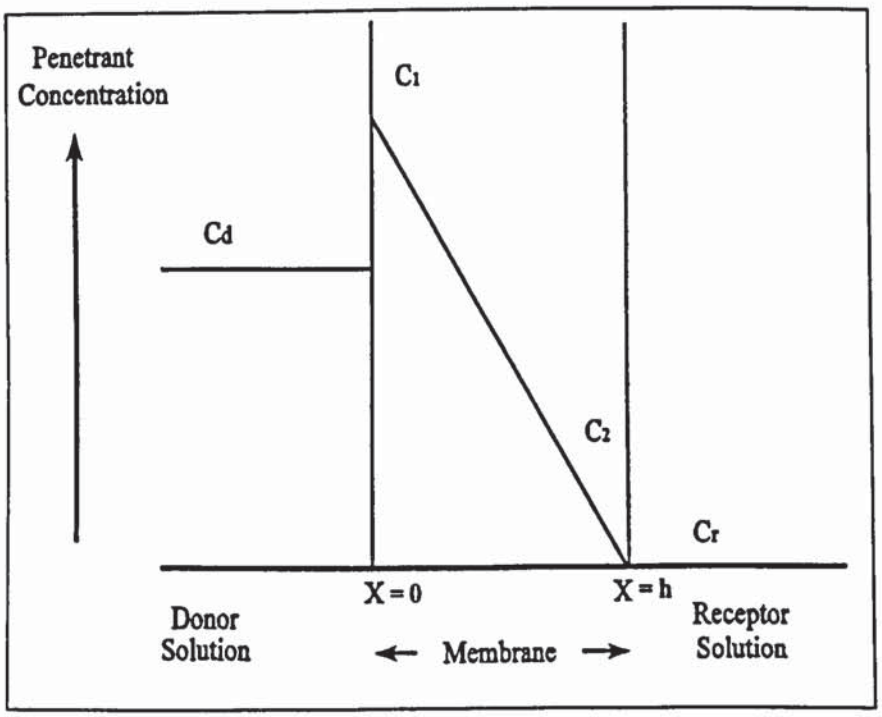


Figure 5.4 Concentration profile across homogenous membrane at steady-state: zero-order flux

For a drug diffusing across a membrane of thickness, h, the classical description of the transport process is highlighted in Figure 5.4. The concentration gradient may be therefore written as

$$\frac{C_1 - C_2}{-h} = -\frac{\Delta C}{h}$$

equation 5.2

Where C_d is the concentration at the donor side of the membrane and C_r is the concentration entering the receiver compartment. By substituting *dc/dx* into equation 5.1 it can be shown that

$$J = \frac{D.\Delta C}{h}$$

equation 5.3

If however we consider the interactions that exist between the drug, the vehicle and the mucosal membrane, which is not an inert barrier to diffusion, we can see that the partition behaviour is very important. For this reason the parameter *K*, the membrane-vehicle partition coefficient is introduced (equation 5.4).

$$K = \frac{C_1}{C_d} = \frac{C_2}{C_r}$$ equation 5.4

where C_1 and C_2 are the concentrations within the membrane, at the donor and receiver compartment side of the membrane respectively. From equation 5.4 it follows that as:

$$dc = C_1\text{-}C_2 = KC_d\text{-}KC_r = K(C_d\text{-}C_r) = K\Delta C$$ equation 5.5

Therefore, the flux (J) across the membrane can be rewritten as:

$$J = \frac{D.K.\Delta C}{h}$$ equation 5.6

If sink conditions apply, *i.e.* the concentration in the receiver compartment is negligible, ΔC approximates to C_d, the applied drug concentration remains constant and the concentration in the receiver phase remains effectively zero (solute concentration at any time (t), within the innermost membrane layer (x = h), is assumed to be negligible ($C_d >> C_r$)) hence equation 5.6 becomes

$$J = \frac{D.K.C_d}{h}$$ equation 5.7

The flux of the penetrant can be calculated from the slope of the steady-state diffusion curve. In some cases, it is not possible to determine D, K, or h independently, therefore, they can be collected into a single variable, the permeability coefficient k_p, defined as:

$$k_p = \frac{K.D}{h}$$ equation 5.8

The units of k_p are cm min^{-1}. Substitution of k_p into equation 5.7 gives,

$$J = k_p C_d$$ equation 5.9

Using this equation it is possible to determine the permeability coefficient k_p by dividing the steady-state slope of Figure 5.3 by the initial concentration of drug applied to the donor compartment.

When the steady-state portion of the line is extrapolated to the time axis, the point of intersection is known as the lag time, t_L, which is dependent on the membrane diffusion coefficient (D) and the thickness of the membrane (h),

$$t_L = \frac{h^2}{6D}$$

equation 5.10

The determination of the lag time permits the estimation of the diffusion coefficient, providing there is no binding. It must be remembered, however, that the above equations are only applicable for steady-state conditions and will not be valid if there are significant interactions, such as binding between the drug and components of the buccal membrane (Holbrook, 1991and Shaw, 2001).

5.1.3 *In vitro* diffusion cells

There are many different types of diffusion cells available. The basic variations include a different orientation (either horizontal or vertical) and either a static or flow-through receptor compartment. The first vertical cell was designed by Coldman *et al.*, in 1969 which was later modified by Franz in 1975, Chowhan and Prichard, 1978, Chien and Valia, 1984, and Gummer *et al.*, 1987 (Amin, 2001). The vertical cells consist of two chambers, the top chamber (donor cell) contains the solution under investigation whilst the bottom chamber (receptor cell) contains the receiving solution which is generally a buffer solution or in many cases just water (Figure 5.5). The solution in the receiver compartment is usually continuously agitated using a magnetic stirrer bar and is maintained at a constant temperature by a temperature controlled water jacket. Attached to the side of the receptor compartment is also a sampling port from which samples of diffused drug for analysis are taken at specific time points. The two compartments of the vertical cell are separated by a horizontal membrane (membrane under investigation) which is mounted between the two halves of the cell and allows the diffusion of the drug from the donor compartment to the receptor. The orientation of the membrane means that the donor chamber may be exposed to

ambient temperature and humidity, while the lower chamber can be maintained at physiological temperature, thus mimicking *in vivo* conditions.

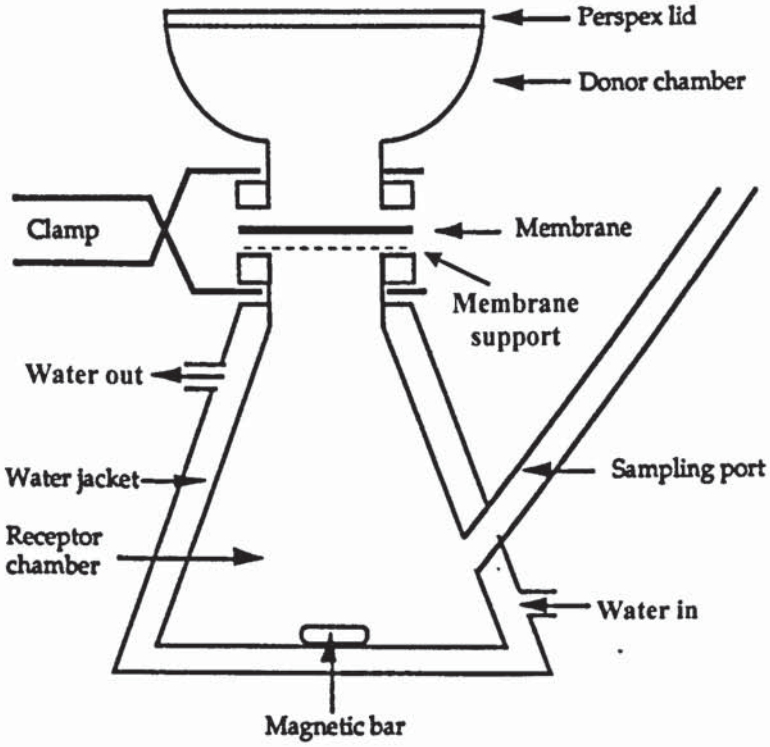


Figure 5.5 Schematic representation of a vertical Diffusion cell

Horizontal diffusion cells are similar to vertical cells in that the donor and receptor compartment are separated by a membrane; however, the membrane is placed in the vertical position as apposed to the horizontal orientation found in the vertical cells. Whilst donor and receptor cells of the vertical diffusion cells can be of different size, in horizontal diffusion cells the donor and receptor compartments are of the same size and shape. Other differences between the two types of cells include the ability to agitate the solutions. In the vertical cell, only the receptor solution is usually agitated, however, in the horizontal cells the donor solution can also be simultaneously agitated to ensure the homogeneity of the formulation under investigation. However, a disadvantage of this type of cell is that a large volume of donor solution is required to cover the membrane surface completely.

One of the most important aspects of setting up an *in vitro* diffusion cell is agitation of the receptor solution. It must be sufficient enough to minimize the diffusion boundary layers at the interfaces between the donor and receptor phase and the absorption barrier. In

comparative work, the rate of agitation should be monitored and maintained at a uniform rate to eliminate boundary layer effects (Ackerman and Flynn, 1987).

In this study, a vertical cell (Figure 5.5) with an average diffusional surface area of 1.77 cm^2 and receptor volume of approximately 30 mL was used to conduct diffusional studies of nicotine.

5.1.4 Selection of mucosal membrane

As it is not practical to obtain buccal mucosa from humans, an alternative animal model is required. The animal model selected should ideally meet the following criteria. It should be readily available in sufficient quantities; it should be well characterised and frequently used by other workers as well as being from an animal where the physiology is similar to man. The considerable similarities between pig and human with respect to anatomy, metabolism, disease and wound healing make this animal a very attractive model. The oral mucosa probably resembles that of a human more closely than any other animal in terms of structure and composition (Chetty, *et al.*, 2001). It also has been reported that pig buccal mucosa has also been largely used in many *in vitro* experiments (Ceschel, *et al.*, 2000).

5.2 Experimental

5.2.1 Materials

Cheeks from freshly slaughtered pigs (female) were obtained from the abattoir (Dawkins International Limited, Nuneaton). The buccal membrane was removed and stored at -70°C until required for use. All chemicals used were obtained from Sigma Aldrich UK and were of pharmaceutical grade.

5.2.2 Method

5.2.2.1 Determining buccal thickness

Three replicate buccal samples were removed from four different pig cheeks. The thickness of the buccal membrane was determined by sandwiching the membrane between two rubber rings. The total thickness of the sandwich was established using a Bestool-Kanon 0-25 mm, 0.01 mm tablet thickness measurer. The buccal thickness was then calculated as being the

total sandwich thickness minus the thickness of the rubber rings. The thickness of the buccal membrane was confirmed by microscopy (section 5.3)

5.2.2.2 Buccal membrane preparation for microscopy.

The buccal tissue was kept at -70 °C until sectioning using a Bright OTF cryost (Bright Instruments Company Ltd., UK). The frozen buccal tissue was placed on a large droplet of OCT compound (BDH Laboratory Supplies, UK) used to fix the buccal tissue onto the aluminium chuck. After allowing a period of half to one hour for the buccal tissue temperature to equalise to the cryostat chamber temperature, sectioning was carried out. The cryosections were collected on gelatine-coated glass microscope slides (Superfrost plus, 25 mm x 75 mm, BDH Laboratory Supplies) and stored at room temperature for further processing.

The mounted tissue was then observed under Zeiss microscope, which was fitted with a high-pressure mercury source at x 100 magnification. The section was then photographed using an Olympus camera containing Jessops SHR400 colour film, which was attached to the microscope with an adapter.

5.2.2.3 Franz cells

Solutions of 100 µg/mL nicotine using, nicotine hydrogen tartrate salt (285 µg/mL), Nicotine Polacrilex (556 µg/mL) and nicotine Amberlite® IRP69 (502 µg/mL) were prepared at pH 2.2, 5.4, 7.0 and 8.6 using citrate phosphate buffer (section 2.10). The concentration of 100 µg/mL represented the conditions found in the chewing chamber of the chewing machine during a normal run (4 mg *per* 40 mL). The receptor compartment of the diffusion cells contained approximately 30 mL of the appropriate pH buffer and was maintained at 37.0°C by means of a water jacket surrounding the cell. Buccal sections of surface area 1.77 cm^2 were cut and vertical Franz cells were assembled as illustrated in figure 5.5, after which, 20 mL of the donor solution was added. To ensure sink conditions, 1 mL of sample was taken from the receiver solution and replaced with an equal volume of fresh buffer at sampling time 0, 5, 10, 20, 30, 60, 120, 240 and 480 minutes. The samples were analysed using the general HPLC method (Section 2.1) and the amount of nicotine diffused through the buccal membrane at each sample point was determined using equation 2.7 to correct for dilutions during sample replacement.

5.2.2.4 Partitioning study

The method used to determine the partitioning of nicotine was adapted from that used by Nair, *et al.*, (1997). Buccal mucosa samples of known thickness and surface area were soaked in 10 mL of 100 µg/mL nicotine solutions at different pHs for 24 hours in a Grants OLS 200 shaking water bath at 37 °C which was shaken at 80 shakes/minute (solutions prepared using nicotine hydrogen tartrate salt in citrate buffer at pH 2.2, 5.4, 7.0 and 8.6). As a control, the nicotine stability at each selected pH, in the absence of buccal mucosa was also conducted in parallel. At the end of 24 hours, the nicotine concentrations at each pH were analysed by HPLC to determine the amount lost from the solution.

The partition coefficient (K) was calculated based on the following equation,

$$K = \frac{(C_b - C_a)}{C_a} \qquad \text{equation 5. 11}$$

where C_b and C_a are the respective overall aqueous concentrations of the drug before and after partitioning into nicotine solution (Ungphaiboon and Maitani, 2001).

5.3 Results and discussion

5.3.1 Buccal thickness

The rate of permeation across a membrane is a function of drug permeability which, in turn is directly proportional to the partition coefficient and diffusion coefficient of the drug across a membrane and inversely proportional to the membrane thickness (equation 5.7).

As the thickness of the buccal membrane is important when determining the rate of permeation across the membrane the average thickness of the buccal membrane was determined as 192 µm ± 57.18 with a range of 100-280 µm (Table 5.1). Photomicrographs of buccal membrane confirm that the thickness of the membrane measured was similar to that reported in literature (Hoogstraate and Wertz, 1998) (Figure 5.6 and Figure 5.7). Figure 5.6 highlights the differentiated epithelium found in the buccal membrane.

Cheek	Thickness (μm)			Mean	SD
	1	2	3		
1	200	170	100	157	51.32
2	280	130	180	197	76.38
3	230	170	270	223	50.33
4	250	190	130	190	60.00
Total				**191.67**	**57.18**

Table 5.1 Thickness of buccal membrane.

Figure 5.6 Light micrograph of porcine buccal mucosa fixed with glutaraldehyde and osmium tetroxide and imbedded in Spurr's resin. SB=stratum basale, SF=stratum filamentosum; SD=stratum distendum (Hoogstraate, and Wertz, 1998).

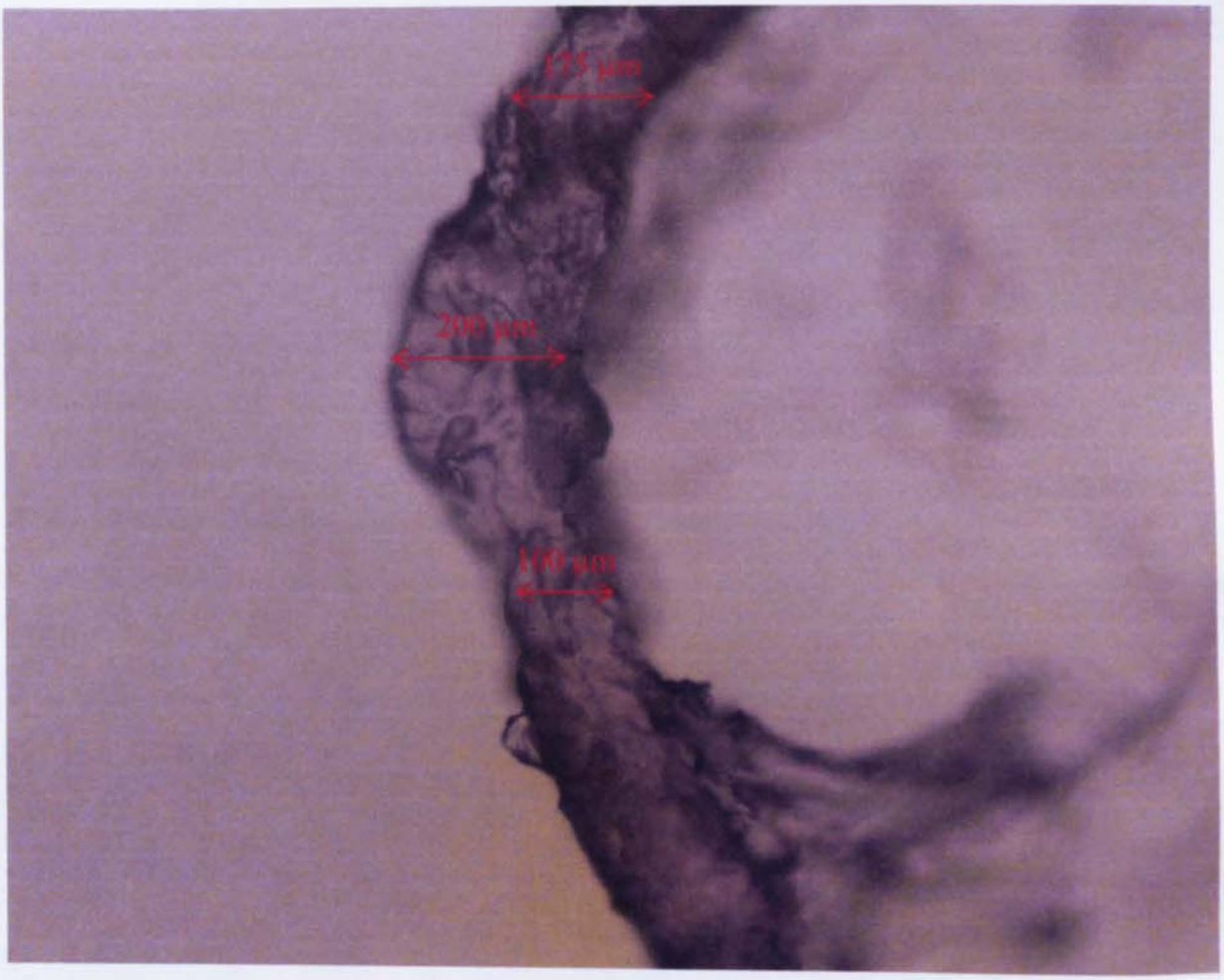


Figure 5.7 Photomicrograph of porcine buccal mucosa (x 100 magnification).

5.3.2 *In vitro* diffusion of nicotine through buccal membrane

Nicotine has two dissociation constants of approximately 3 and 8 which can be described by equation 5.12.

$$H^{+}NNH^{+} \xleftrightarrow[3.1]{pK_{a1}} NNH^{+} \xleftrightarrow[8.0]{pK_{a2}} NN$$

Di-protonated Mono-protonated Unionised

equation 5.12

Depending upon the pH of the solution, various ratios of the different nicotine species can be present in solution (Figure 5.2). At solutions buffered at less than pH 2, almost 100% of the nicotine species present in the solution is di-protonated. At approximately pH 3, equimolar concentrations of di-protonated and mono-protonated species are present in the solution and similarly, at approximately pH 8 an equal quantities of mono-protonated and unionised

nicotine species present. Two pH units above and below the two pKa values of 3 and 8 respectively (at approximately pH 5.5) the solution comprises of mono-protonated nicotine species. As the pH of the solution increases from pH 5.5 to pH 8, there is a gradual increase in unionised nicotine. As the pH of the solution increases further to above pH 8 an increase in unionised nicotine in the solution which is then exclusively present in solution at approximately pH 10.

At the various pHs used in this study, the percentage ionisation of the nicotine species can be seen in Table 5.2. At pH 2.2, nicotine is predominately in the di-protonated state with a small percentage (11.18%) present in the mono-protonated state. At pH 5.4 and 7.0 the nicotine is primarily in the mono-protonated state with 9.09% being unionised at pH 7.0, also, at pH 8.0, 79.92% of nicotine is unionised with 20.08% present in the mono-protonated state. If the diffusion of nicotine through the buccal membrane follows the pH partitioning theory, a greater permeation would be observed at the higher pH value when nicotine is predominately unionised.

	% Nicotine species present		
pH	**Di-protonated (H^+NNH^+)**	**Mono-protonated (NNH^+)**	**Unionised (NN)**
2.2	88.82	11.18	0
5.4	0.5	99.25	0.25
7.0	0.01	90.0	9.99
8.6	0	20.08	79.92

Table 5.2 Percentage of nicotine species present in solution at pH 2.2, 5.4, 7.0 and 8.6

5.3.2.1 Diffusion of nicotine tartrate salt through buccal membrane at various pH values

If pH-partitioning theory holds for membrane diffusion, greater amounts of unionised species of nicotine will result in an increased permeation. On the other hand, for more acidic media, the nicotine will be present in the mono-protonated or di-protonated state, therefore resulting in a lower diffusion of nicotine.

The initial diffusion of nicotine through the buccal membrane is of great interest in this study as Nicorette® gum is not intended to be chewed for more than 30 minutes. Generally, as a

drug diffuses through a membrane a lag phase is observed (Figure 5.3). During this non-steady-state phase (lag phase), the drug slowly enters into the membrane, diffuses through the membrane and then partitions out into the receiving solution.

Within the first 30 minutes, at pH 8.6, greater permeation was observed in comparison to that at lower pH values (Figure 5.8). At pH 8.6 and 7.0, no lag phase was observed, suggesting that the partitioning of nicotine into the membrane occurs rapidly due to a greater percentage of unionised nicotine present in solution. In comparison, at pH 5.4 and 2.2, a lag phase of approximately 5 minutes was observed.

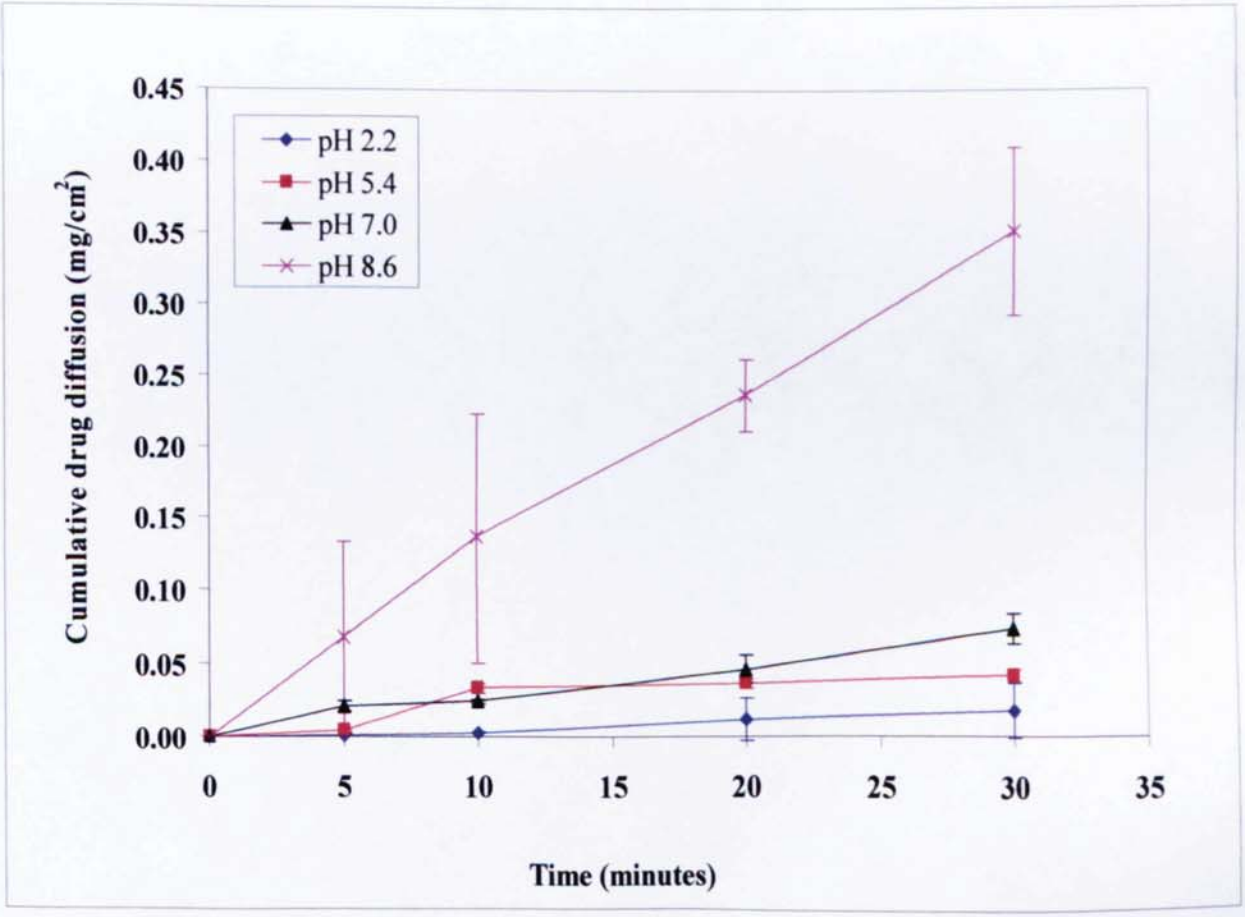


Figure 5.8 Initial diffusion of nicotine hydrogen tartrate salt at various pHs across buccal membrane (n= 4 ± SD).

With a linear increase in diffusion of nicotine at pH 8.6, 7.0 and 2.2 within the first 30 minutes, the flux (determined by the gradient of the line) for nicotine at pH 8.6 showed that the permeation of nicotine was more than five times greater than that observed at pH 7.0 and, more than 18 times greater when nicotine was predominantly di-protonated (Table 5.3). The linear graphical representation of the increase in flux with percentage of unionised nicotine

species showed evidence that, unionised nicotine was the principal diffusing species (Figure 5.9). Results therefore highlighted that with increasing pH, the amount of nicotine diffusion also increased, due to the higher percentage of unionised nicotine present in solution.

pH	Flux ($\mu g\ cm^{-2}\ min^{-1}$)	r^2
2.2	0.613	0.9864
5.4	1.379	0.8766
7.0	2.232	0.9898
8.6	11.504	0.9984

Table 5.3 Flux of nicotine hydrogen tartrate salt diffusion through buccal membrane at the various pH values

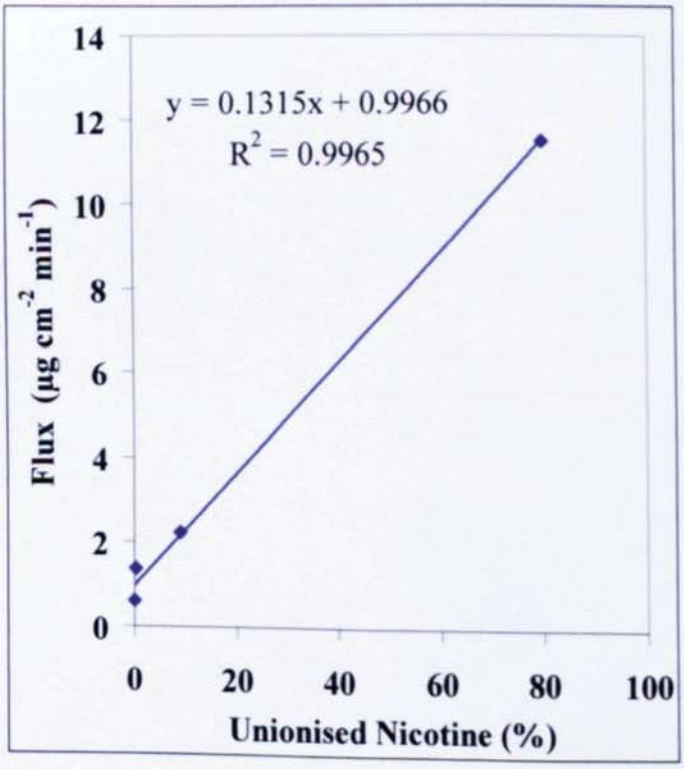


Figure 5.9 The flux of the nicotine diffusion as the percentage of unionised nicotine increased.

The diffusion of nicotine hydrogen tartrate salt at the various pHs over the 8 hour period showed that, as pH of the donor solution increased, the amount of nicotine diffusing through the buccal membrane also increased (Figure 5.10). At pH 8.6, greatest diffusion of nicotine occurred in comparison to nicotine at pH 7.0, 5.4 and 2.2 which produced the lowest amount of permeation.

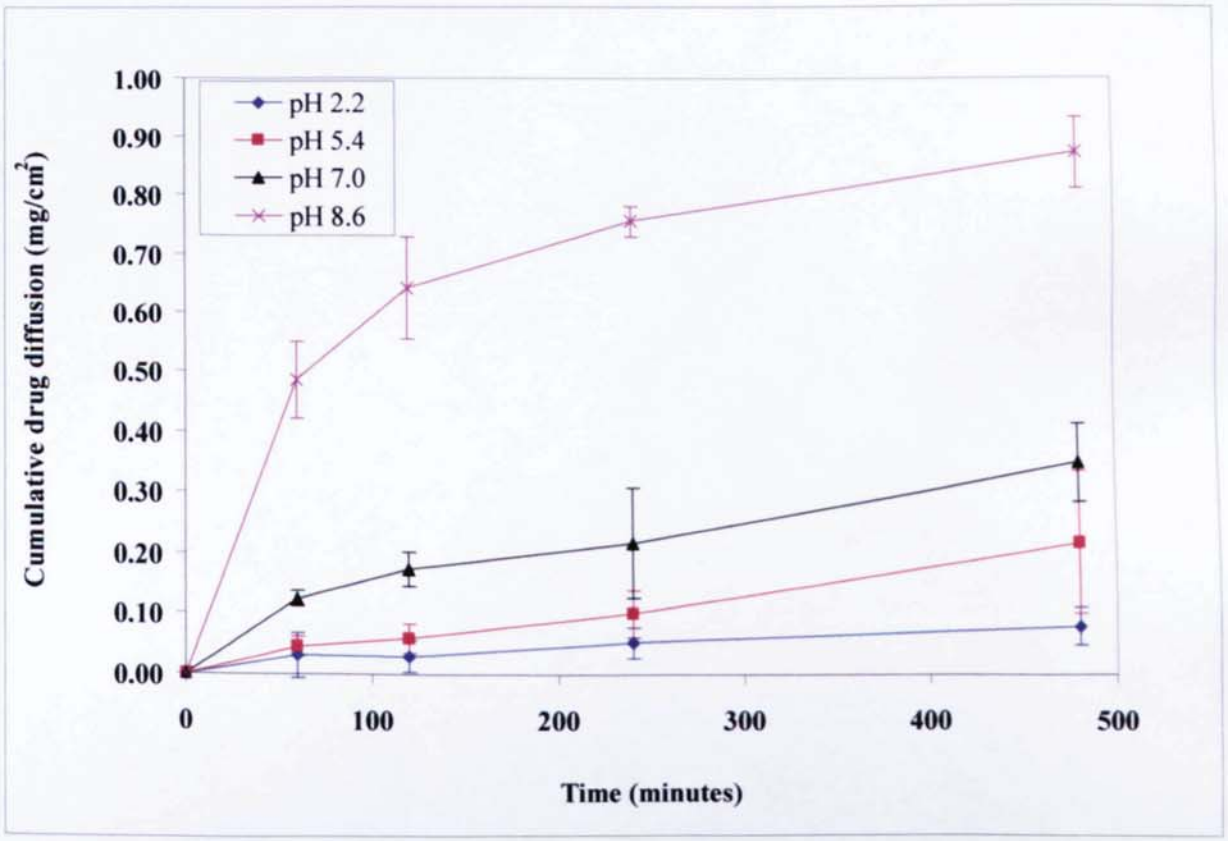


Figure 5.10 Diffusion of nicotine hydrogen tartrate salt at various pHs across buccal membrane (n= 4 ± SD).

pH	Flux (μg cm^{-2} min^{-1})	r^2
2.2	0.128	0.9845
5.4	0.422	0.9932
7.0	0.515	0.9958
8.6	1.686	0.8578

Table 5.4 Flux of nicotine hydrogen tartrate salt diffusion through buccal membrane at the various pH values later in the diffusion process

In comparison to the flux of nicotine diffusion within the first 30 minutes at all pH values (Table 5.3), during the latter stages of diffusion the flux reduced (Table 5.4). At pH 8.6, the correlation coefficient of 0.8578 showed that the diffusion of nicotine did not follow steady-state. It was postulated that at this higher pH, diffusion of nicotine followed first-order kinetics as apposed to the steady-state diffusion observed at the lower pH values. If the diffusion of nicotine followed first-order kinetics, the loss of nicotine from the donor solution would be governed by equation 5.13

$$\frac{dM}{dt} = - k M$$

equation 5.13

where *dM/dt* is the rate of diffusion, *M* is the concentration of the drug and *k* is the first-order rate constant where,

$$k = \frac{A.D.K}{V.h}$$

equation 5.14

and *A* is the surface area, *D* is the diffusion coefficient, *K* the partition coefficient, *V* is the volume of reservoir and *h* is the membrane thickness, and

$$ln\ (M_o - M_t) = ln\ (M_o) - k.t$$

equation 5.15

where, M_t is the mass of drug transferred at time *t* from initial amount M_o. Therefore, a graph of $ln\ (M_o - M_t)$ *vs* time will produce a linear profile with the gradient representing the rate of nicotine loss from the donor solution.

Fitting a first-order model on the diffusion profile obtained using nicotine tartrate salt at pH 8.6 showed that the rate of diffusion followed first-order kinetic, where the rate constant was calculated as 7.533×10^{-3} cm^{-2} min^{-1} (Figure 5.11 and Table 5.5). Therefore at pH 8.6 using the first-order rate constant the flux was calculated (equation 5.13) and found to be 1.507 μg cm^{-2} min^{-1}.

Diffusion cell	k (cm^{-2} min^{-1})	r^2
Cell 1	9.162×10^{-3}	0.9909
Cell 2	9.944×10^{-3}	0.9976
Cell 3	5.053×10^{-3}	0.9867
Cell 4	6.418×10^{-3}	0.9930
Mean	**7.533×10^{-3}**	
SD	**2.153×10^{-3}**	

Table 5.5 First-order rate constants of nicotine diffusion at pH 8.6 using nicotine hydrogen tartrate salt.

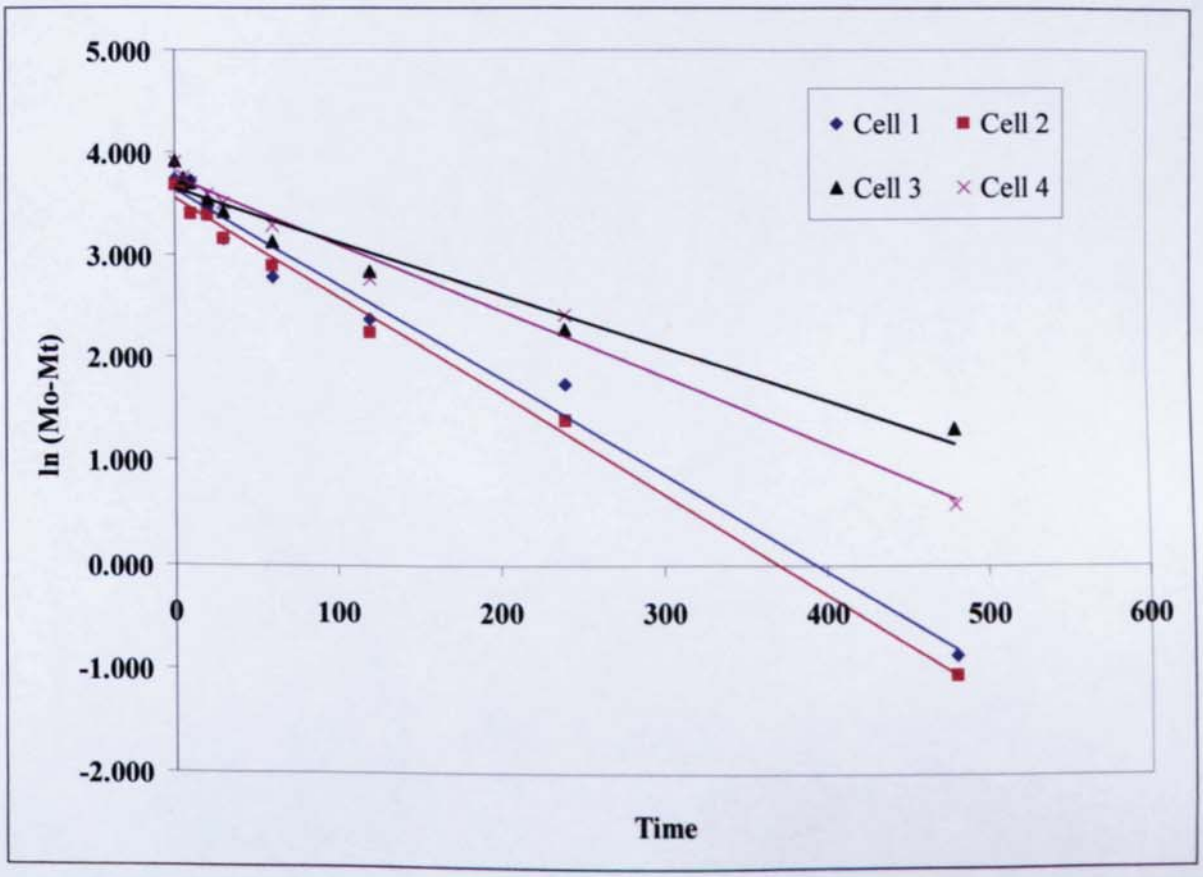


Figure 5.11 *In vitro* diffusion of nicotine through buccal mucosa using nicotine hydrogen tartrate salt at pH 8.6.

From the results it was found that with the use of nicotine hydrogen tartrate salt, the diffusion of nicotine appeared to hold for the pH-partitioning theory, whereby a greater amount of unionised nicotine resulted in an increased permeation. In comparison to all other pH values, at pH 8.6 diffusion followed first-order kinetics apposed to the zero-order diffusion observed at the lower pH values. A possible explanation for the different diffusion mechanism could be explained in terms of the nicotine species present in solution. At pH 8.6, 79.92% of the nicotine in solution was present in the unionised state and, 20.08% in the mono-protonated. The donor solutions used in these experiments contained approximately 2 mg nicotine (20 mL at 100 µg/mL), thus at pH 8.6, 1.598 mg of nicotine in the donor solution would be present in the unionised state whilst, 0.402 mg in the mono-protonated state (Table 5.6). After 8 hour (480 minutes), 0.875 ± 0.065 mg/cm^2, (1.548 mg) of both the mono-protonated and unionised nicotine present in solution diffused though the membrane. The reduction in the flux of nicotine, thus the first-order kinetic diffusion profile could be due to the depletion of unionised nicotine species present in solution.

pH	Nicotine species present (mg)		
	Di-protonated (H^+NNH^+)	Mono-protonated (NNH^+)	Unionised (NN)
2.2	1.776	0.224	0.000
5.4	0.010	1.985	0.005
7.0	0.002	1.800	0.198
8.6	0.000	0.402	1.598

Table 5.6 Amount of nicotine species present in 20 mL of donor solution at pH 2.2, 5.4, 7.0 and 8.6

At the lower pH values, a considerably higher amount of nicotine was present in the donor solution after 8 hours. At pH 2.2, 0.143 mg of nicotine diffused, through the membrane in comparison to 0.389 mg and 0.620 mg at pH 5.4 and 7.0, thus diffusion followed zero-order kinetics as a higher percentage of drug remained in the donor solution.

5.3.2.2 Diffusion of nicotine from Nicotine Polacrilex resin through buccal membrane at various pH values

Nicotine in Nicorette® chewing gum is present in the form of Nicotine Polacrilex resin. For the diffusion of nicotine from the Nicotine Polacrilex resin to occur, the nicotine must firstly be released from the resin during ion exchange. The released nicotine must then dissolve in the donor solution before partitioning into the membrane. Once in the membrane the nicotine must diffuse through the membrane and finally partition out into the receptor solution.

Within the first 30 minutes of diffusion, in comparison to the lower pH values, a greater permeation was seen at pH 8.6 when nicotine was primarily unionised (Figure 5.12). At pH 8.6, 2.2 and 5.4, a lag period of approximately 3 minutes was observed in comparison to approximately 7 minutes observed at pH 7.0, suggesting that the partitioning of nicotine at pH 7.0 was lower than at the other pH values. Also, as observed with nicotine hydrogen tartrate, due to nicotine being predominantly di-protonated at pH 2.2, a lower diffusion was expected than at the higher pH values of 5.4, 7.0 and 8.6. However, at the end of 30 minutes, a greater permeation was observed at pH 2.2 compared to diffusion at pH 5.4 and 7.0. Values of the flux, determined from the slope of the linear portions of the graph showed that, the flux of the diffusion species was greater at pH 2.2 in contrast to pH 5.4 and 7.0. Possible explanation in the increased flux could be explained in terms of the release of nicotine from

the Polacrilex resin. In section 3.6.2.4.2, a greater release of nicotine from the Nicotine Polacrilex resins was observed in comparison to the release at the higher pH values. It could be that at the lower pH values, the rate at which nicotine was released from the ion-exchange resin affects the rate of diffusion across the buccal membrane. Therefore at pH 2.2, nicotine could be released from the resin at a faster rate compared to resins at pH 5.4 and 7.0, resulting in a greater initial diffusion of nicotine at pH 2.2.

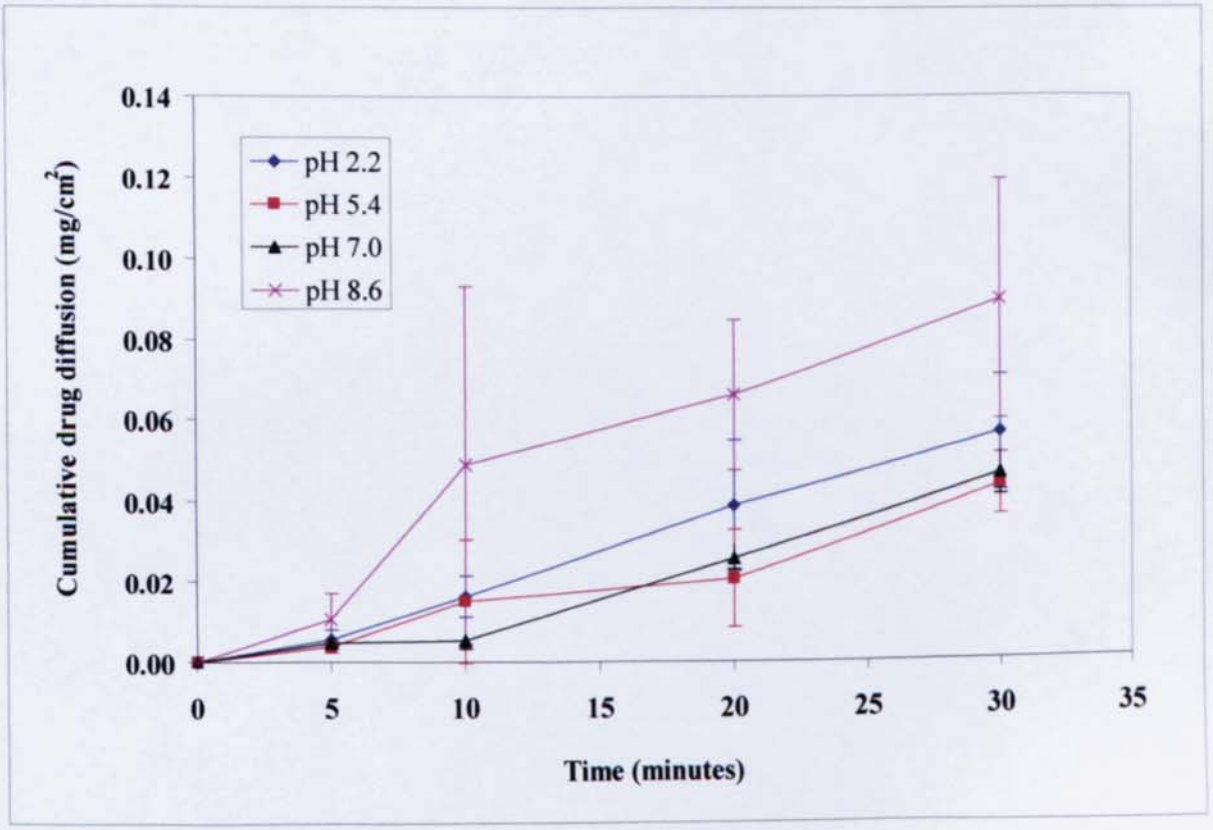


Figure 5.12 Initial diffusion of nicotine from Nicotine Polacrilex at various pH values across buccal membrane (n= 4 ± SD).

pH	Flux (μg cm^{-2} min^{-1})	r^2
2.2	1.952	0.9969
5.4	1.391	0.9765
7.0	1.570	0.9769
8.6	3.011	0.9697

Table 5.7 Flux of nicotine diffusion from Nicotine Polacrilex through buccal membrane at the various pH values

At the end of 8 hours, as seen with nicotine tartrate salt, at the higher pH value of 8.6, a greater amount of nicotine diffused through the buccal membrane (Figure 5.13). However,

final diffusion values of 0.354 mg, 0.664 mg, 0.892 mg and 0.998 mg at pH 2.2, 5.0, 7.0 and 8.6 respectively showed that, less than 50% of the nicotine that was present in the donor solution (2 mg) diffused through the membrane. The flux of nicotine diffusion calculated at each pH value, showed that in comparison to initial flux (Table 5.7), during the later stages of diffusion the flux was lower (Table 5.8). Also, at pH 2.2, initial diffusion showed a greater amount of diffusion in comparison to pH 5.4 and pH 7.0. However, within 8 hours, diffusion at pH 2.2 was lower than the diffusion at all other pH values.

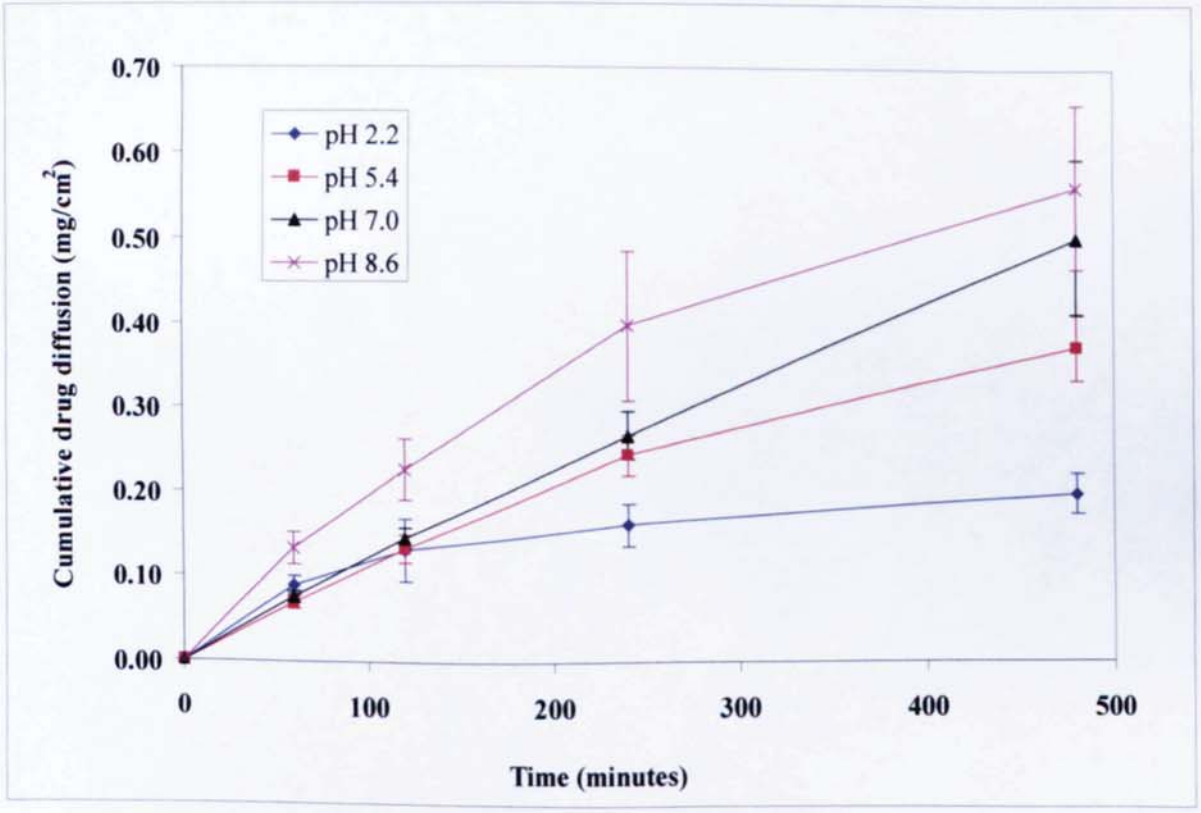


Figure 5.13 Diffusion of nicotine from Nicotine Polacrilex at various pHs across buccal membrane (n= 4 ± SD).

pH	Flux (μg cm^{-2} min^{-1})	r^2
2.2	0.238	0.9518
5.4	0.714	0.9875
7.0	1.012	0.9997
8.6	1.001	0.9804

Table 5.8 Flux of nicotine diffusion from Nicotine Polacrilex through buccal membrane at the various pH values during 8 hours.

Although an increased amount of diffusion was observed at pH 8.6, graphical representation of the rate of diffusion *vs* the percentage of unionised nicotine present showed that a linear relationship was not observed. This highlighted that a greater amount of unionised nicotine did not substantially increase the diffusion of nicotine through the buccal membrane when using Nicotine Polacrilex (Figure 5.14). In terms of diffusion of nicotine from the Nicotine Polacrilex resin through the buccal membrane, the rate of diffusion is not only governed by the pH of the solution, but also the rate of release of nicotine from the Nicotine Polacrilex resin. It can therefore be postulated that for nicotine diffusion to occur with use of Nicotine Polacrilex resin, two effects could be occurring simultaneously; the release of nicotine from the resin at the various pH values, and the diffusion of nicotine though the membrane at the different pH values.

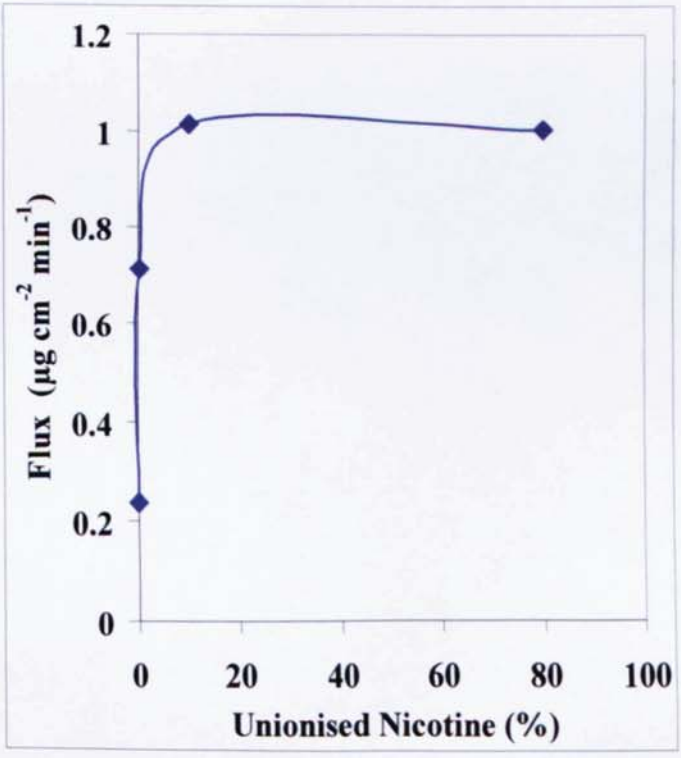


Figure 5.14 The flux of nicotine diffusion from Nicotine Polacrilex as a percentage of unionised nicotine

5.3.2.3 Diffusion of nicotine from nicotine Amberlite® IRP69 resin through buccal membrane at various pH values

With the use of nicotine Amberlite® IRP69 resin initial diffusion curve highlighted a lag phase of less than 4 minutes at all pH values (Figure 5.15). Initial calculations of the flux using the linear portion of the diffusion profile (5-10 minutes) highlighted that at pH 8.6 the flux was ten times greater than that observed at the lower pH values (Table 5.9).

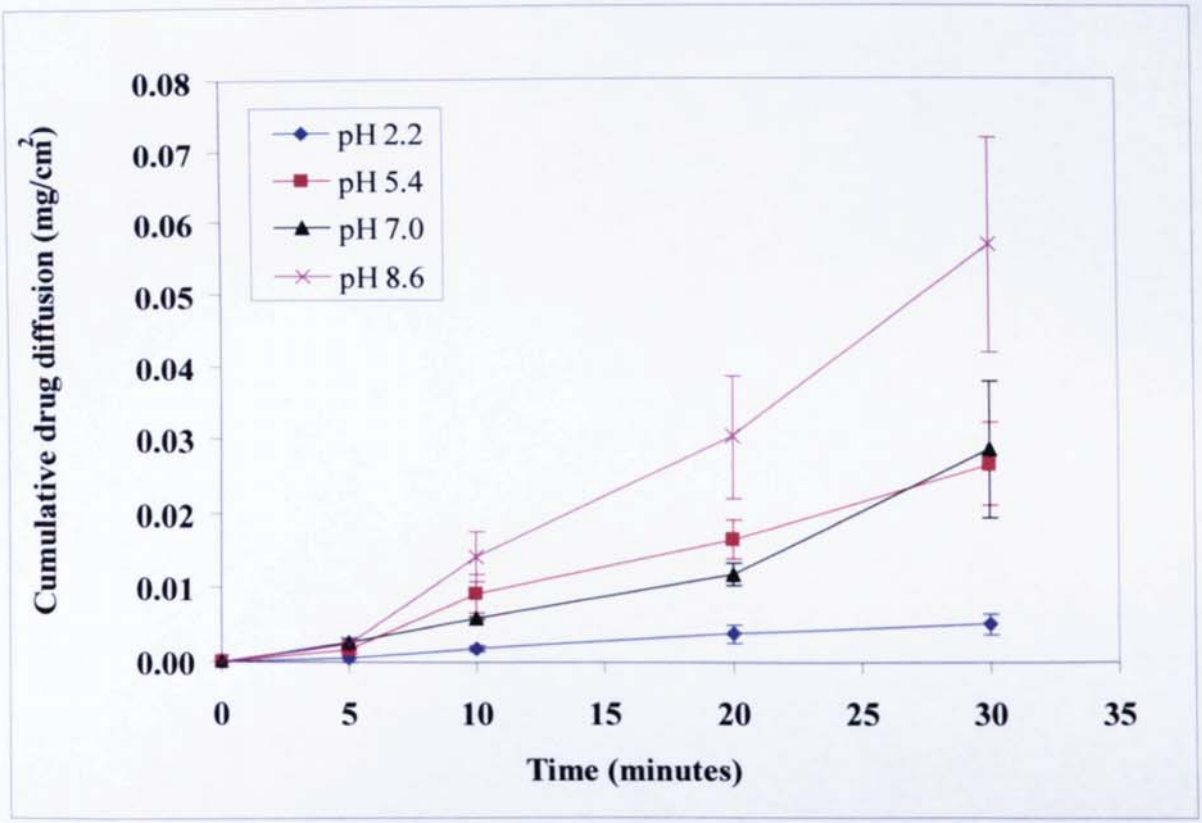


Figure 5.15 Initial diffusion of nicotine from nicotine Amberlite® IRP69 at various pHs across buccal membrane (n= 4 ± SD).

pH	Flux (μg cm^{-2} min^{-1})	r^2
2.2	0.180	0.9936
5.4	0.907	0.9931
7.0	0.913	0.9662
8.6	1.922	0.9878

Table 5.9 Flux of nicotine diffusion through buccal membrane at the various pH values using nicotine Amberlite® IRP69.

The overall, diffusion of nicotine from nicotine Amberlite ® IRP69 showed that after 8 hours, diffusion of nicotine at pH 8.6 was greater than at the lower pH values (Figure 5.16). As the pH increased, the flux of nicotine also increased such that, the flux at pH 8.6 was eight times greater than the flux at pH 2.2 (Table 5.10). In comparison to Nicotine Polacrilex, it was also found that the flux of nicotine diffusion increased linearly with increasing percentage of unionised nicotine (Figure 5.17), thus suggesting that the pH-partitioning theory holds, whereby a greater permeation was observed when nicotine was predominately unionised.

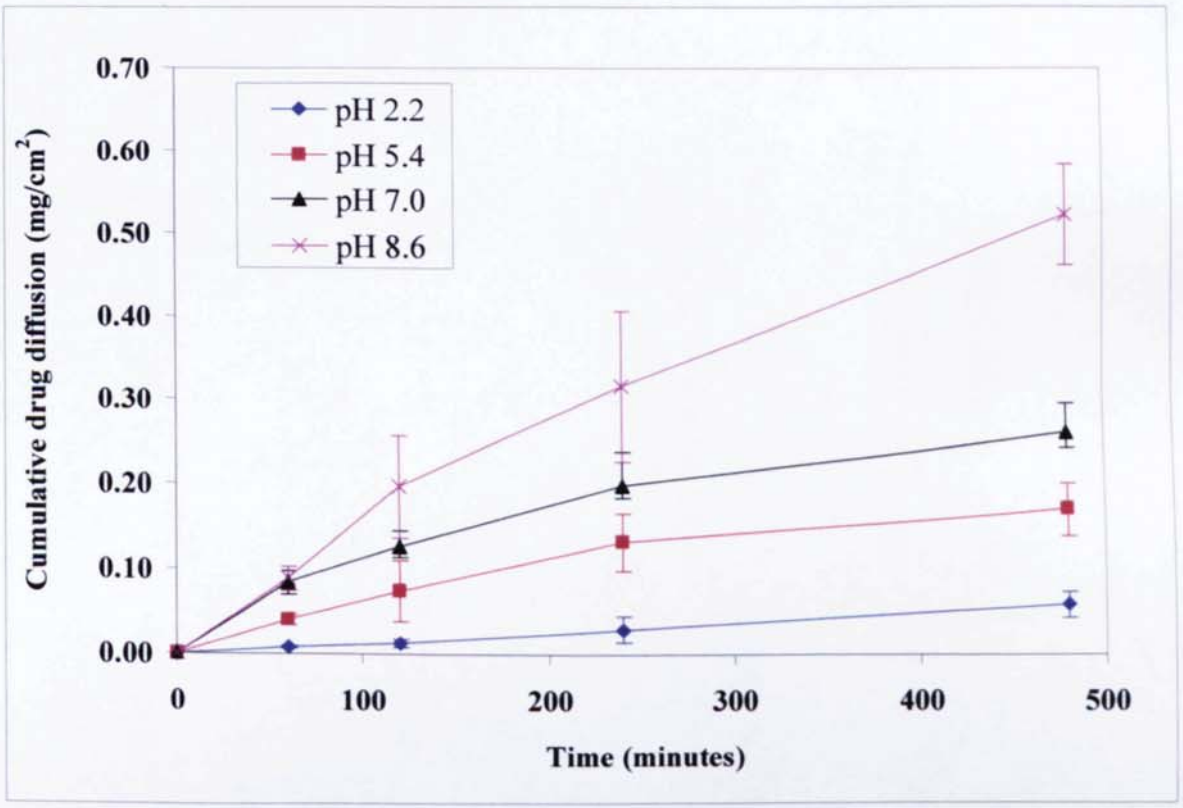


Figure 5.16 Diffusion of nicotine from nicotine Amberlite® IRP69 at various pHs across buccal membrane (n= 4 ± SD).

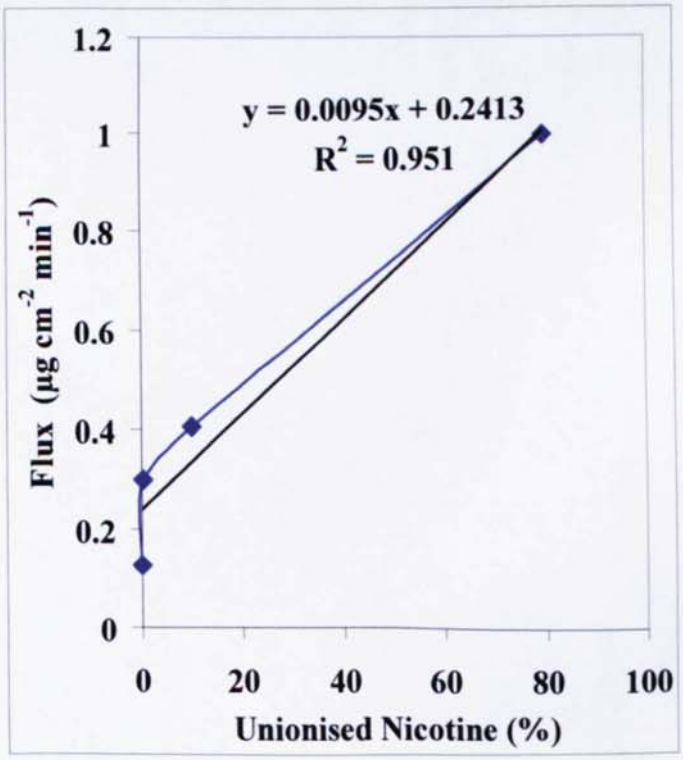


Figure 5.17 The flux of nicotine diffusion from nicotine Amberlite® IRP69 as a percentage of unionised nicotine

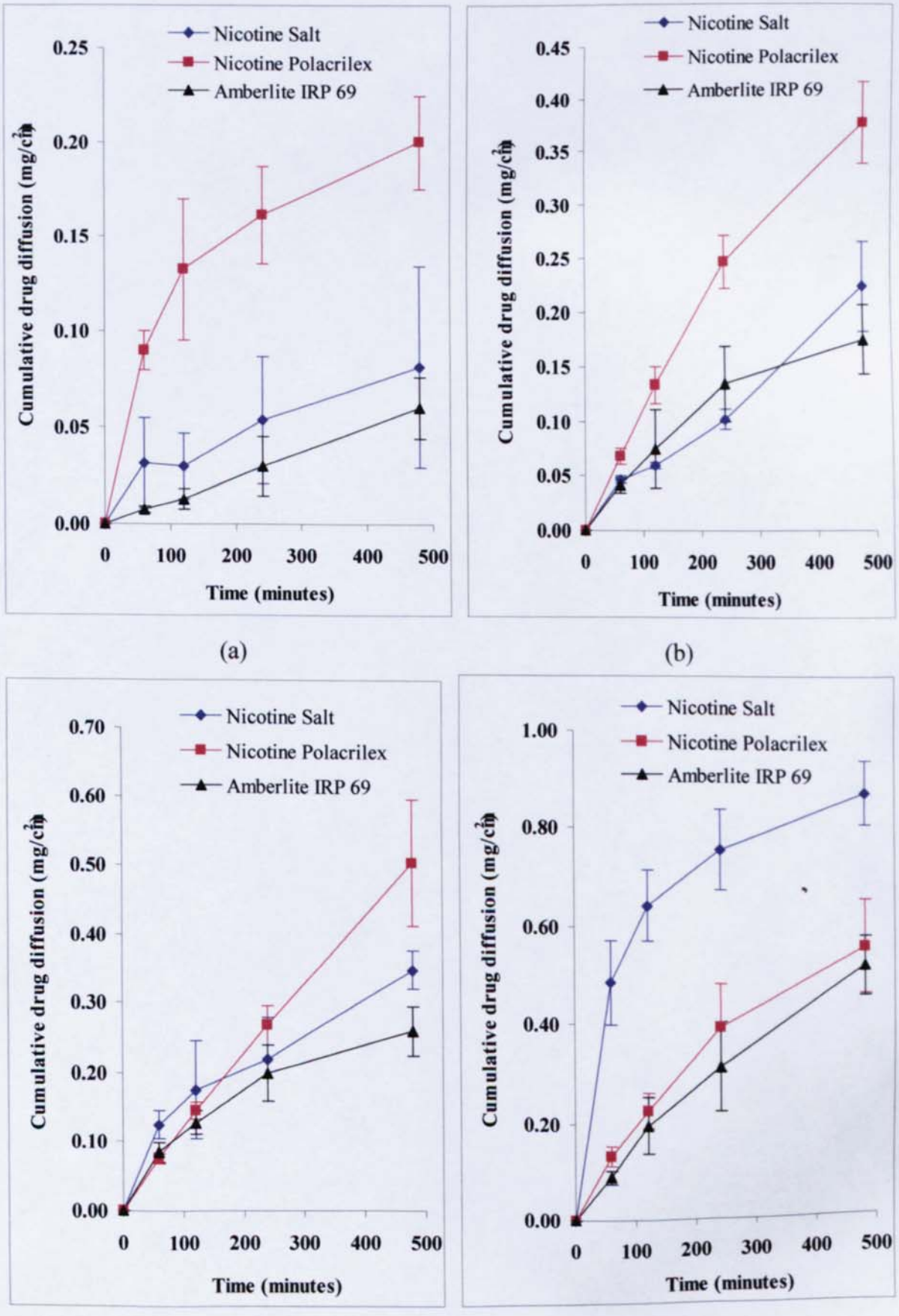
pH	Flux ($\mu g\ cm^{-2}\ min^{-1}$)	r^2
2.2	0.127	0.9992
5.4	0.299	0.9553
7.0	0.405	0.9704
8.6	0.993	0.9913

Table 5.10 Flux of nicotine diffusion through buccal membrane at the various pH values using nicotine Amberlite® IRP69 during 8 hours.

The pH of the solution, hence the species of nicotine present in solution appeared to have a significant effect of the permeation of nicotine through the buccal membrane. Ion-exchange resins, in this case, nicotine Amberlite® IRP69 and the commercially available Nicotine Polacrilex are usually added to formulations to control the release of nicotine (Rohm Hass 1999 and Rohm Hass, 2003). Amberlite® IRP69 is a strongly acidic resin, whilst Nicotine Polacrilex consists of Amberlite® IRP64, a weakly acidic ion exchange resin. It is expected that diffusion of nicotine through buccal membrane would be relatively quicker for nicotine salt than the resin at all pH values. For diffusion of nicotine from ion-exchange resin to occurs cationic exchange must occurs to enable nicotine to be released, after which, dissolution of the nicotine occurs in the surrounding solution and finally partitioning into the membrane. Therefore, diffusion through the membrane using nicotine salt should be quicker and more advantageous as nicotine is already in solution.

5.3.2.4 Diffusion of nicotine from the various sources at the different pH values

The comparisons of nicotine diffusion from the different sources of nicotine (nicotine tartrate salt, Nicotine Polacrilex and nicotine Amberlite® IRP69) at all pH values were compared to each other (Figure 5.18). At pH 2.2 when nicotine was predominately di-protonated, a significantly higher diffusion was observed from Nicotine Polacrilex in comparison to nicotine hydrogen tartrate salt and nicotine Amberlite® IRP69 ($p < 0.0001$), whilst diffusion with nicotine tartrate salt and nicotine Amberlite® IRP69 were similar ($p = 0.2216$) . The flux of nicotine across the buccal membrane quantified by the slope of the linear portion of the graph also showed that with use of Nicotine Polacrilex, the flux was almost double that observed with nicotine hydrogen tartrate and nicotine Amberlite® IRP69, showing a fast rate of nicotine diffusion with the use of Nicotine Polacrilex (Table 5.10).



(a) (b)

(c) (d)

Figure 5.18 The diffusion of nicotine from nicotine tartrate salt, Nicotine Polacrilex and nicotine Amberlite® IRP69 across the buccal membrane at pH 2.2 (a), pH 5.4 (b), pH 7.0 (c) and pH 8.6 (d) (n = 3 ± SD).

pH	Nicotine source	Flux ($\mu g\ cm^{-2}\ min^{-1}$)	r^2	Amount diffused at 480 min (mg)
2.2	Nicotine hydrogen tartrate salt	0.128	0.9845	0.1433 ± 0.052
	Nicotine Polacrilex	0.238	0.9518	0.3540 ± 0.025
	Nicotine Amberlite® IRP69	0.127	0.9992	0.1062 ± 0.016
5.4	Nicotine hydrogen tartrate salt	0.422	0.9932	0.3894 ± 0.041
	Nicotine Polacrilex	0.714	0.9875	0.6638 ± 0.039
	Nicotine Amberlite® IRP69	0.299	0.9553	0.3026 ± 0.031
7.0	Nicotine hydrogen tartrate salt	0.515	0.9958	0.6195 ± 0.029
	Nicotine Polacrilex	1.012	0.9997	0.8920 ± 0.091
	Nicotine Amberlite® IRP69	0.404	0.9704	0.4602 ± 0.035
8.6	Nicotine hydrogen tartrate salt	1.507*	0.9920	1.5488 ± 0.065
	Nicotine Polacrilex	1.001	0.9804	0.9983 ± 0.096
	Nicotine Amberlite® IRP69	0.993	0.9913	0.9275 ± 0.061

Table 5.11 Flux and amount of nicotine diffused from the various nicotine sources through buccal membrane at pH 2.2, 5.4, 7.0 and 8.6 after 480 minutes.

At pH 5.4 and 7.0, when nicotine was predominately mono-protonated, a similar situation was observed whereby a greater diffusion of nicotine occurred with use of Nicotine Polacrilex resin as appose to nicotine hydrogen tartrate and nicotine Amberlite® IRP69 (Figure 5.18). Comparisons of the flux of nicotine diffusion through the membrane also showed that, the flux of nicotine diffusion using Nicotine Polacrilex was almost double that of nicotine hydrogen tartrate and nicotine Amberlite® IRP69 at both pH 5.4 and 7.0 (Table 5.11). Finally, in comparison to nicotine Amberlite® IRP69 a greater diffusion occurred at both pH values for nicotine tartrate salt ($p < 0.05$).

Diffusion at pH 8.6 when 79.92% of the nicotine was in the unionised state showed that the amount and rate of nicotine diffusion using nicotine hydrogen tartrate salt was greater in comparison to Nicotine Polacrilex and nicotine Amberlite® IRP69 which had similar diffusion profiles ($p = 0.259$).

As previously discussed at the beginning of this section, it was expected that at all pH values, diffusion using the nicotine resins would be lower in comparison to the salt. Results showed that at the lower pH values of 2.2, 5.4 and 7.0, a significantly higher diffusion was observed

from the Nicotine Polacrilex resin in comparison to nicotine Amberlite® IRP69 and nicotine tartrate salt. The results were therefore not as expected in terms of diffusion of nicotine from Nicotine Polacrilex resin. However, in comparison to nicotine hydrogen tartrate, the diffusion of nicotine from nicotine Amberlite® IRP69 was as anticipated at all pH values.

In previous studies conducted on the diffusion of nicotine through oral mucosa, it was found that for unionised nicotine, significantly higher permeation was seen through gingival mucosa followed by sublingual and finally buccal mucosa (Chen *et al.*, 1999). This trend was seen throughout all pH values indicating that within the oral cavity buccal mucosa has the lowest permeation (Table 5.12 and Figure 5.19)

Table 5.12 Specific permeability of various nicotine species across selected oramucosae (Chen *et al.*, 1999)

Figure 5.19 The permeation profiles of nicotine through various oral mucosae at pH 8.8 (Chen, *et al.*, 1999).

In the same study, Chen *et al.* (1999) also looked at the effect of pH on the rate of permeation of the nicotine through the non-keratinised mucosa (Figure 5.20). 50 mg/mL nicotine solutions were made using citrate buffer and the diffusion of nicotine at the various pH values through a 1.3 mm x 0.64cm^2 buccal membrane determined. Zero-order kinetics was observed by the different nicotine species with significantly higher permeation at pH 8.8 followed by the mono-protonated nicotine species (pH 5.4) and finally at pH 2.0 where nicotine was predominately di-protonated (Table 5.13).

Table 5.13 Specific permeability, partition coefficient and diffusivity of various nicotine species through the non-keratinised buccal mucosa (Chen *et al.*, 1999).

Figure 5.20 Comparison of nicotine permeation through the non-keratinised mucosa under different pH conditions (Chen, *et al.*, 1999).

In comparison to the studies conducted in-house, results obtained by Chen *et al.*, (1999) were similar. From both experiments, it was found that the diffusion of nicotine through the buccal membrane using nicotine tartrate salt followed the pH-partitioning theory whereby, a greater diffusion was observed at the higher pH values. However, a comparison of the diffusion profiles showed that a more linear, zero-order diffusion was observed from Chen,

et al., study apposed to that observed during this study. Difference in the diffusion mechanism could be due to the difference in the membrane thickness and the concentration of nicotine used in the donor solution. A higher nicotine donor concentration (50 mg/mL) and a thicker buccal membrane (1.3 mm) was used was used during Chen's study in comparison to that used in this study (0.1 mg/mL and 0.194 mm).

5.3.3 Partitioning study

The partition coefficients of nicotine as a function of pH using nicotine in citrate buffer and buccal mucosa were determined (Figure 5.21). A pH effect was observed on the partitioning of nicotine into the buccal membrane. As the pH of the buffer increased, the partition coefficient also increased.

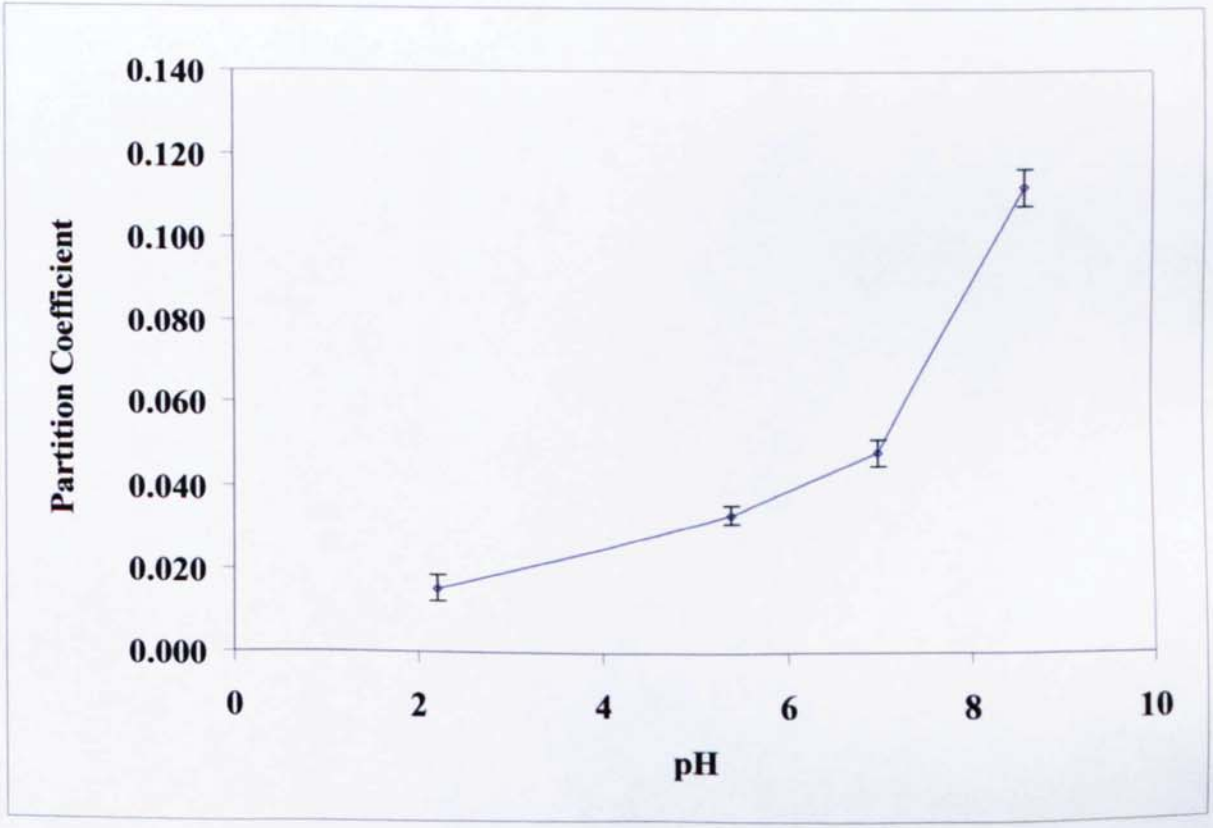


Figure 5.21 Partitioning of nicotine as a function of pH in buccal mucosa

To determine if the partition rate of nicotine across the buccal mucosa follows the pH-partition theory, the flux of nicotine at each pH was plotted against the partition coefficient (Note the flux used was that obtained for nicotine hydrogen tartrate salt at the various pH). A linear relationship was observed (Figure 5.22) confirming that the permeation of nicotine across buccal membrane follows pH-partition hypothesis.

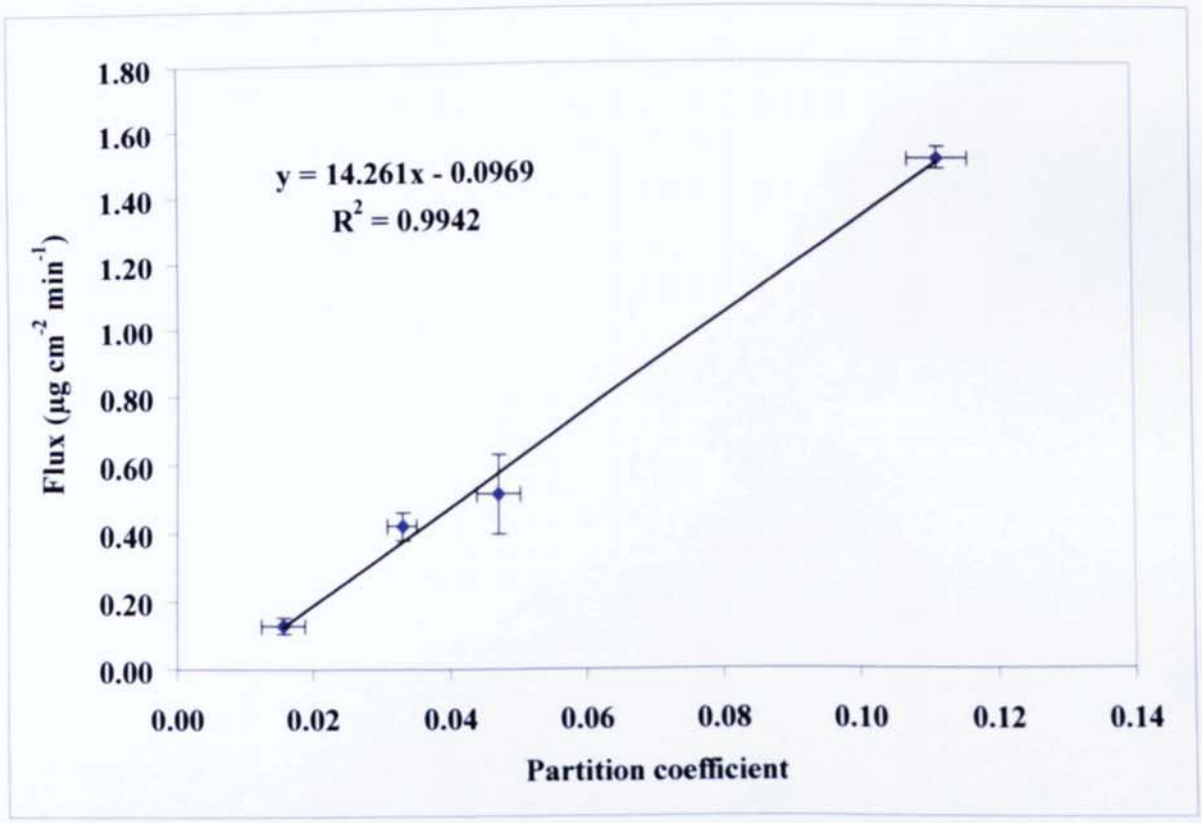


Figure 5.22 Linear relationship demonstrated by nicotine in buccal mucosa as expected from the pH partition theory.

5.4 Conclusion

Results showed that using nicotine hydrogen tartrate, Nicotine Polacrilex and nicotine Amberlite® IRP69 as a source of nicotine, with increasing pH, the amount of nicotine diffusing through the buccal membrane also increased. The unionised species of nicotine was more permeable than mono-protonated which in turn was more permeable than di-protonated nicotine species. It was expected that the diffusion of nicotine using nicotine in the salt form (nicotine hydrogen tartrate) would be greater at all pH values in comparison to nicotine ion-exchange resins; however, at the lower pH values when nicotine was predominantly mono- and di-protonated, a greater diffusion was observed from Nicotine Polacrilex resin. In comparison with use of nicotine Amberlite® IRP69, the flux of nicotine diffusion was slower than nicotine tartrate salt at all pH values, thus resulting in a lower amount of nicotine transported through the buccal membrane.

As with Nair, *et al.*, (1997) and Chen, *et al.*, (1999) a zero-order release mechanism was observed except for nicotine diffusion using nicotine hydrogen tartrate at pH 8.6, where a first-order release mechanism was observed. It was postulated that the difference in the

release mechanism was due to the depletion of unionised nicotine present in solution, therefore during the later stages of diffusion, a decrease in flux.

Finally, the permeation of nicotine across buccal mucosa was found to follow pH-partitioning theory as evidenced by the linear relationship obtained between the flux and partition coefficient. Also, linear relationship observed by the percentage of unionised nicotine and, the flux from nicotine Amberlite® IRP69 and nicotine hydrogen tartrate salt also suggested that nicotine diffusion followed pH-partitioning theory. With use of Nicotine Polacrilex, a linear relationship was not observed, therefore suggesting that with use of Nicotine Polacrilex the pH-partitioning did not hold. However, since the amount of nicotine diffusion increased with increasing pH, pH was still considered to be an important factor when determining the permeation of nicotine through the buccal membrane with use of Nicotine Polacrilex resin.

The present studies have shown that for buccal permeability of nicotine, control pH within the oral cavity is important after release of nicotine from a drug formulation such as chewing gum. For optimal delivery of nicotine, a pH greater than 8.6 would be desirable whereby nicotine is predominately in the unionised state.

CHAPTER SIX
INVESTIGATING FORMULATION VARIABLES ON THE RELEASE OF NICOTINE FROM GUMS

6.1 Introduction

Chewing gum consists of a neutral and tasteless masticatory gum base and several non-masticatory ingredients such as fillers, softeners, sweeteners and flavouring agents. An example of a chewing gum formulation and the various compositions has been discussed earlier (Section 1.4).

6.1.1 Traditional manufacturing of chewing gum

Most chewing gums are manufactured in a similar manner using a gum kettle mixer. The gum base is melted in the large, steam-jacketed, gum kettle mixer, which heats and mixes the gum base at about 50-70°C. Powdered sweeteners, syrups, active ingredients and other formulation additives are then added to the molten gum mixture at pre-determined times. The formulation is then mixed for the exact amount of time required by the formulation and is then sent through a series of rollers that forms the gum into a thin, wide ribbon. Each pair of consecutive rollers is set closer together than the previous pair therefore, gradually reducing the thickness of the gum. A light coating of finely powdered sugar or sugar substitute is then added to keep reduce sticking and to enhance the flavour of the gum. Finally, the gum is scored in a pattern of single sticks or pellets and allowed to cool in a room where the temperature and humidity are carefully controlled to ensure that the finished gum will stay fresh.

When developing a medicated chewing gum formulation, consideration of the release profile and the taste has to be made. The release profile of the chewing gum formulation must be tailor-made to the active substance and to the market needs. For active substances, where a local effect in the oral cavity is required, the chewing gum formulation should be designed to have a release-rate that maintains the concentration of the active drug in saliva at a defined level to ensure optimal effect and minimum side effects. This also applies to release profiles for substances for buccal absorption *e.g.* nicotine lozenge. To achieve the optimal release profile, different release controlling excipients can be used *i.e.* use of solubilisers and encapsulation techniques (Figure 6.1). It is claimed that the release profiles may be designed with a prolonged release, with a peak release, or with combined prolonged and peak release.

Figure 6.1 Addition of various solubilisers to control the release of the active substance (www.fertin.com).

6.1.2 Sensory and texture analysis

In contrast to standard tablets, a chewing gum formulation releases the active drug over time and thus provides a long contact time with the oral mucosa and taste buds. The taste of active substances is often unpleasant (bitter, astringent or even metallic). During the entire formulation process, taste evaluations are made and new formulations are developed until satisfactory results are achieved (Ellerman, 2002). To obtain reliable sensory parameters of chewing gum, descriptive sensory analysis parameters are utilized. The relevant parameters for the product in question are selected and used in taste trials and the expression of the quantitative description analyses constructed (Figure 6.2).

Extensive knowledge and experience is necessary to develop a medical chewing gum formulation. During the development stages, tests are conducted with several gum bases to ensure development of the ideal product with optimal properties. Basic characteristics such as the texture of the gum (softness, hardness, elasticity, crumbleness, stickiness and mouthfeel), the release profile (*i.e.* how fast or slow the active substances and flavours are released from the gum) and the stability of the gum are important and are determined primarily by the type of gum base used (Ellerman 2002). Thus, sensory and texture analysis is conducted on the gums throughout the development phase.

Figure 6.2 Example of taste profile. (Ellerman, 2002).

When gum is chewed, its texture is also changing (Figure 6.3). Initially, the gum is firm in order to maintain a stable shape during storage. Sensory tests have shown that as the gum is chewed, in the first 1-3 minutes, the gum becomes much softer due to the hydration of the gum base by saliva. After 3 minutes, the release of sweeteners and flavouring agents begins to reduce and thus the gum becomes firmer (Lee, 2001).

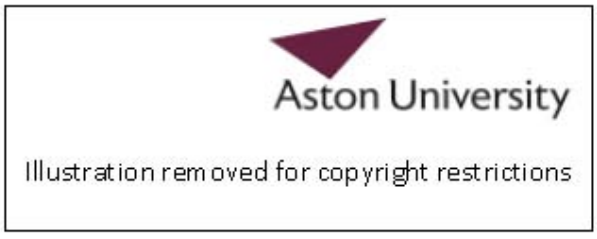


Figure 6.3 Change of gum properties during chewing (Lee, 2001)

Until recently, sensory evaluations of chewing gum relied on panellist chewing. However, texture analysers show promise in providing objective and precise measurements of gum texture (section 1.4.3).

The aim of this study was to develop a number of different nicotine gum formulations to determine the effect of different gum bases, a different form of the active drug and increasing or decreasing the concentration of excipients on the release of nicotine from chewing gum. The standard chewing gum manufacturing method (section 2.13) was adapted to prepare different gum formulations. The gums were then analysed by texture analysis and the *in vitro* release of nicotine determined.

6.2 Directly compressible gum base

SPI Pharma has developed a direct compression gum base (Pharmagum®) which can be compacted into a chewing gum using a standard tablet press. This enables a quicker and more cost effective way of formulating gum compared to traditional methods. Pharmagum® M and S is a mixture of a polyol(s)/or sugars with a chewing gum base to which other ingredients can be added and then compressed.

Tests conducted by SPI Pharma on Pharmagum® S using a Lloyd LR30K tensile machine showed that Pharmagum® S, in terms of its compactability and compressibility, was comparable to other excipients commonly used as diluents in tablet formulations (Figure 6.4 and 6.5). Further tests conducted on the flowability of the powdered gum base also concluded that Pharmagum® S and M were free-flowing powders with physical characteristics that allow for easy handling and compactability in high speed tablet presses (Table 6.1).

Table 6.1 Characteristics of Pharmagum® S and M (www.spipharma.com).
*Carr's index is a simple test to evaluate the flowability of a powder by comparing their bulk density and tapped density. Carr's index = (Tapped Density – Bulk density)/ Tapped Density (Wells and Aulton, 1988).

Figure 6.4 Compactibility of Pharmagum® S compared to other excipients when compressed using a 15mm Flat Face "F" at a compression speed of 10 mm/minutes (www.spipharma.com)

Figure 6.5 Compressibility of Pharmagum® S compared to other excipients (www.spipharma.com)

Here, a number of nicotine gum formulations were made using Pharmagum® S and M and compared to the standard Nicorette® gum to determine if there was a difference between standard gum formulations and gums produced *via* direct compression using a tablet press.

6.2.1 Methods

The total nicotine content of the different gum formulations was verified using the method described in section 2.6. The gums were then tested for release of nicotine using the *in vitro* chewing machine method (section 2.3). The samples were removed and analysed using the HPLC method described in section 2.1 and the texture of the gum was determined using the texture analyser (section 2.11).

6.2.1.1 Direct compression

The method described in section 2.12 was used to prepare 4 mg Pharmagum® M and Pharmagum® S gums samples by direct compression.

6.2.1.2 Granulation

To further improve compressibility and reduce segregation of excipients the formulation was granulated

6.2.1.2.1 Materials

Pharmagum® S and M compressible chewing gum bases were used as supplied by SPI Pharma. Sodium carbonate, mannitol, magnesium stearate, sorbitol, polyvinylpyrrolidone (PVP) and glycerin were bought from Sigma UK and were of pharmaceutical grade. Nicotine polacrilex resin was used as supplied by GSK, Weybridge U.K. Doubled distilled water was generated in house using a Fison's Fi-Streem still

6.2.1.2.2 Method

The powders (Table 6.2) were mixed together using a Peerless mixer in order of their bulk mass *i.e.* after weighing the ingredients, the powders with the lowest bulk mass were mixed together first and so forth to ensure an even mix. Once all the powders were mixed, glycerin was added and mixed for a further minute. 10% PVP solution was then added to the mixture in small quantities until granules were formed.

Ingredients	4 mg gum Quantity (%)
Pharmagum® S/M	80.0
Nicotine Polacrilex	4.5
Glycerin	2.2
Magnesium stearate	2.0
Sorbitol	8.3
Sodium Carbonate	3.0
10% PVP Solution	qs*

Table 6.2 Pharmagum® S and M granules formulae

*PVP solution was added until granules were formed.

To obtain two different sizes of granules for each gum, Pharmagum® S granules were passed through a 710 μm and a 1.4 mm sieve whilst granules formed by Pharmagum® M were passed through a 1.4 mm and 1.7 mm sieves using a plastic spatula. The granules were then placed on a steel tray and left to dry for 24 hours at room temperature. The granules were then compressed using a Manesty single-punch tablet machine to form a compressed chewing gum.

6.2.1.3 Compression strength

4 mg Pharmagum® S and Pharmagum® M formulations as described in section 2.12 were prepared and 1 g samples were compressed at 1 ton and 5 tons for 5 minutes using a Specac 15.011 vacuum die press.

The total nicotine content for all the formulated gums was determined using the method described in section 2.6. The release profile of nicotine from the gum was determined *in vitro* using the chewing machine (section 2.3) and the texture of the formulated gum was established using the texture analyser (section 2.11).

6.2.2 Results and discussion

6.2.2.1 Direct compression of Pharmagum® M and S

Pharmagum® M and S have a similar release profile, faster than that of Nicorette® (Figure 6.6).

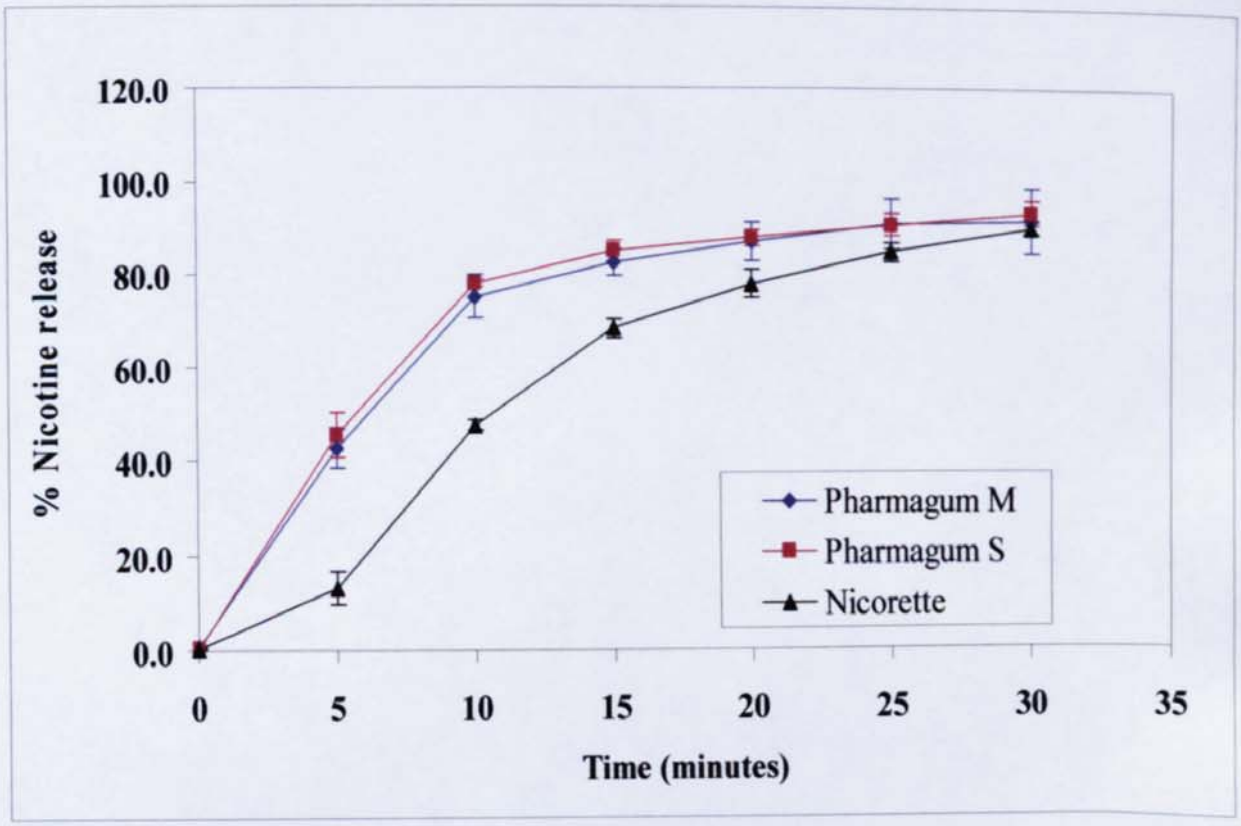


Figure 6.6 Release of nicotine from 4 mg Pharmagum® M and S formulations and 4 mg Nicorette® gum (n =3 ± SD).

After 5 minutes of chewing, 42.46% and 45.50% of nicotine was released from Pharmagum® M and S respectively whilst only 13.23% was released from 4 mg Nicorette®. The release of nicotine from Pharmagum® M and S continued to rise at a steady rate for 10 minutes after which the release began to plateau to give approximately 90% release after 30 minutes. Dissolution curve comparison using the f_2 equation showed that the release profiles of Pharmagum® M and S were similar (f_2 = 82.39) whilst release of nicotine from Nicorette® gum was dissimilar (f_2 = 38.92 and 36.61 respectively).

The pH of the artificial saliva increased whilst the gums were chewed *in vitro* (Figure 6.7). After 30 minutes, the pH rose by 1.346, 1.470 and 1.481 pH units for Pharmagum® M, S and Nicorette® 4 mg gum respectively. The greatest increase was observed within the first 10 minutes of chewing after which the pH increased at a steady rate to give final pH values of pH 8.349 and pH 8.513 for Pharmagum® M and S respectively and pH 8.599 for 4 mg Nicorette®.

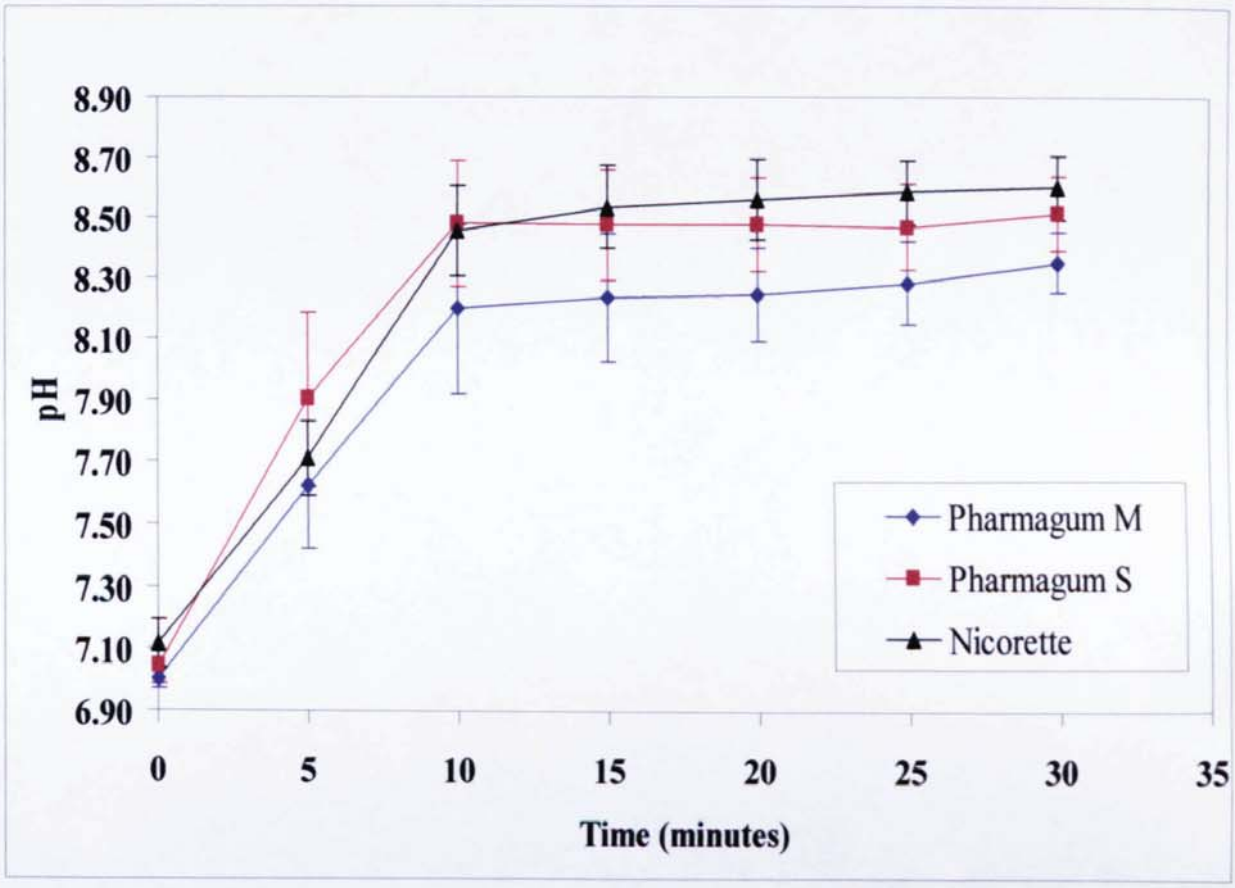


Figure 6.7 pH of artificial saliva as 4 mg Pharmagum® M and S formulations and 4 mg Nicorette® gum are chewed *in vitro* (n =3 ± SD).

The difference in release can be explained in terms the gum's texture. Pharmagum® M and S are similar to a tablet in appearance. When the formulated Pharmagum® M and S were chewed *in vitro* using the chewing machine, within the first minute the gum crumbled like a tablet and formed individual gum pieces which then began to come together. The crumbling of the gum allowed the nicotine to be released from multiple small particles with a large surface area thus providing a faster release rate compared to the Nicorette® gum which remained intact during the chewing process.

When the gums were analysed using the QTS 25 texture analyser, as the probe penetrated the Nicorette® 4 mg gum, a small constant load (g) was required to reach the depth of 3 mm. Once the required depth was reached, on withdrawal a negative peak was observed indicating the adhesiveness of the gum (Figure 6.8). When the same probe penetrated Pharmagum® M and S, a greater force was needed to penetrate the gums which caused the gum to crumble, thus, resulting in a sharp drop in the load *vs.* time plot (Figure 6.9).

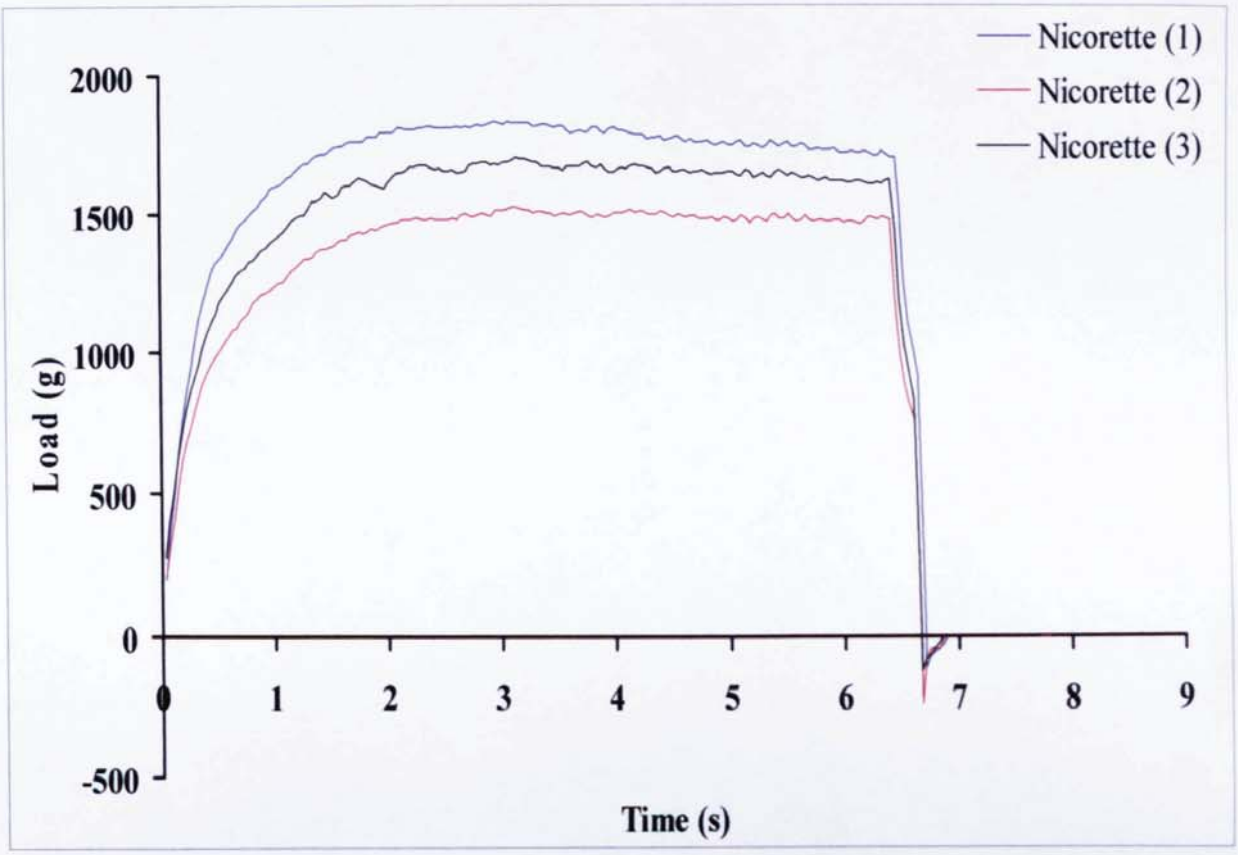
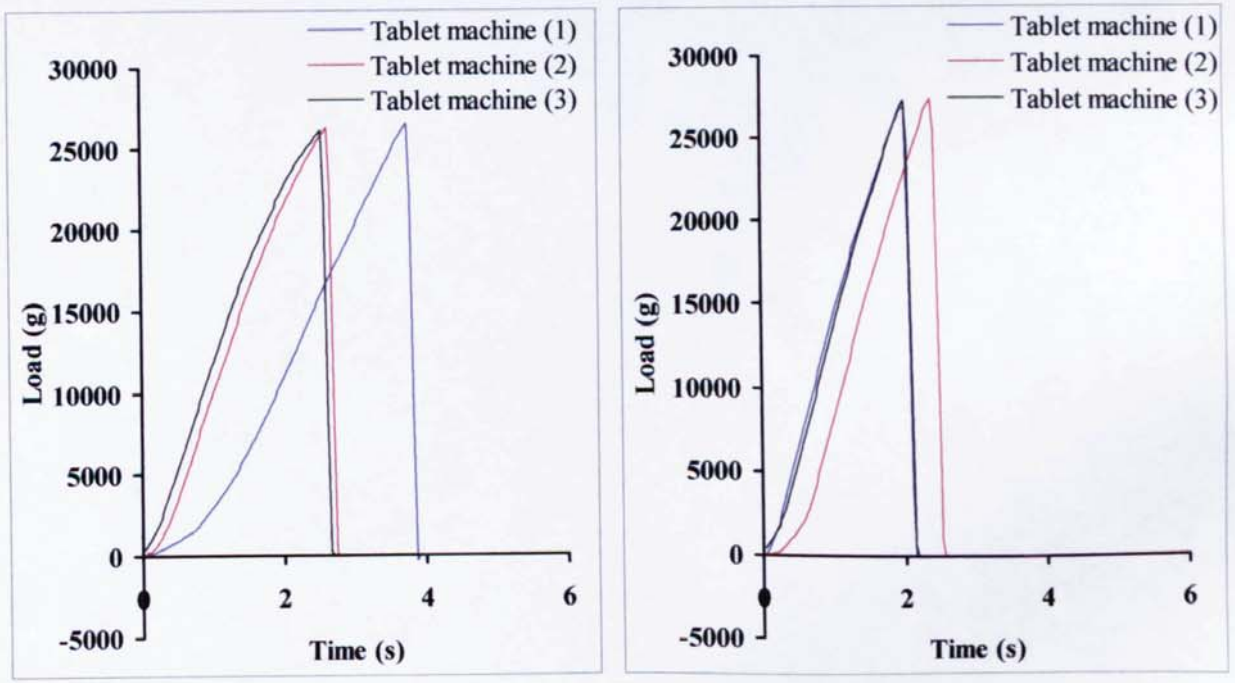


Figure 6.8 Load encountered as a 2 mm diameter probe penetrates 4 mg Nicorette® gum



(a) (b)

Figure 6.9 Load encountered as a 2 mm probe penetrates 4 mg Pharmagum® M (a) and Pharmagum® S (b) formulated gums.

The force required to penetrate the gums showed that Pharmagum® M and S were more than ten times harder than Nicorette®. Values obtained for adhesive forces, demonstrating the force required to pull the probe away from the gum sample, showed that, Nicorette® gum was a more adhesive gum in comparison to Pharmagum® M and S (Table 6.3). Statistical analysis of the textural data using one-way analysis of variance, (Tukey-Kramer multiple comparison test) highlighted that the hardness and adhesiveness of Nicorette® gum was significantly different from Pharmagum® M and S ($P < 0.001$), whilst Pharmagum® M and S were similar ($P > 0.05$). Measurements of the load *per* unit time denoting the resistance of the gum to deformation not only showed that the directly compressible gums, Pharmagum® M and S were ten times more resistant than 4 mg Nicorette® ($P < 0.001$), but also highlighted that there was a significant difference in the resistance of Pharmagum® M and S ($P < 0.001$), in that Pharmagum® S was a more rigid gum in comparison to Pharmagum® M. The increase rigidity of Pharmagum® S is because of the composition of the directly compressible gum base. Pharmagum® M contains 50% more gum base in comparison to Pharmagum® S, therefore producing a less rigid gum.

	Hardness (N)		**Adhesive force (N)**		**Load *per* unit time (g/s)**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Nicorette®	16.51	1.23	-1.51	0.57	1527.63	330.56
Pharmagum® M	208.12	14.13	-0.032	0.03	12589.78	227.14
Pharmagum® S	213.62	17.20	-0.010	0.008	14077.60	198.82

Table 6.3 Hardness, adhesive force and load encountered by the gums *per* unit time for 4 mg Nicorette® and Pharmagum® M and S.

6.2.2.2 Granulation of Pharmagum® M and S

Softeners such as glycerin and other vegetable oil products are included in gum formulations to help blend the ingredients in the gum and keep the gum soft and flexible. To reduce the crumbling effect of the Pharmagum® S and M that was observed when the gums were formulated by direct compression, glycerin was added and the formulation was further granulated to, firstly, reduce segregation of the ingredients and improve the flow properties of the mix and, secondly, to further improve the compression characteristics of the mix.

It was found that, after addition of glycerin and further granulation, the granules formed did not flow as well as the directly compressible mix and crumbled when testing *in vitro* as before. Release from Pharmagum® M and S, irrespective of the granule size, gave similar results ($f_2 > 50$) (Figure 6.10). At 5 minutes, 47.84% and 53.69% of nicotine was released from Pharmagum® M gum made using 1.7 mm and 1.4 mm granules respectively and 52.34% and 45.09% from Pharmagum® S made using 1.4 mm and 710 µm granules respectively. After 5 minutes, the release rate slowed to give a gradual increase in nicotine. At the end of 30 minutes approximately 100% of the nicotine within all the Pharmagum® was released in comparison to only 89.74% released from Nicorette® gum. Comparison of the dissolution profiles showed that the release profiles of Pharmagum® M and S gums formed using different granules sizes were similar ($f_2 > 50$), but were dissimilar to Nicorette® gum ($f_2 < 50$).

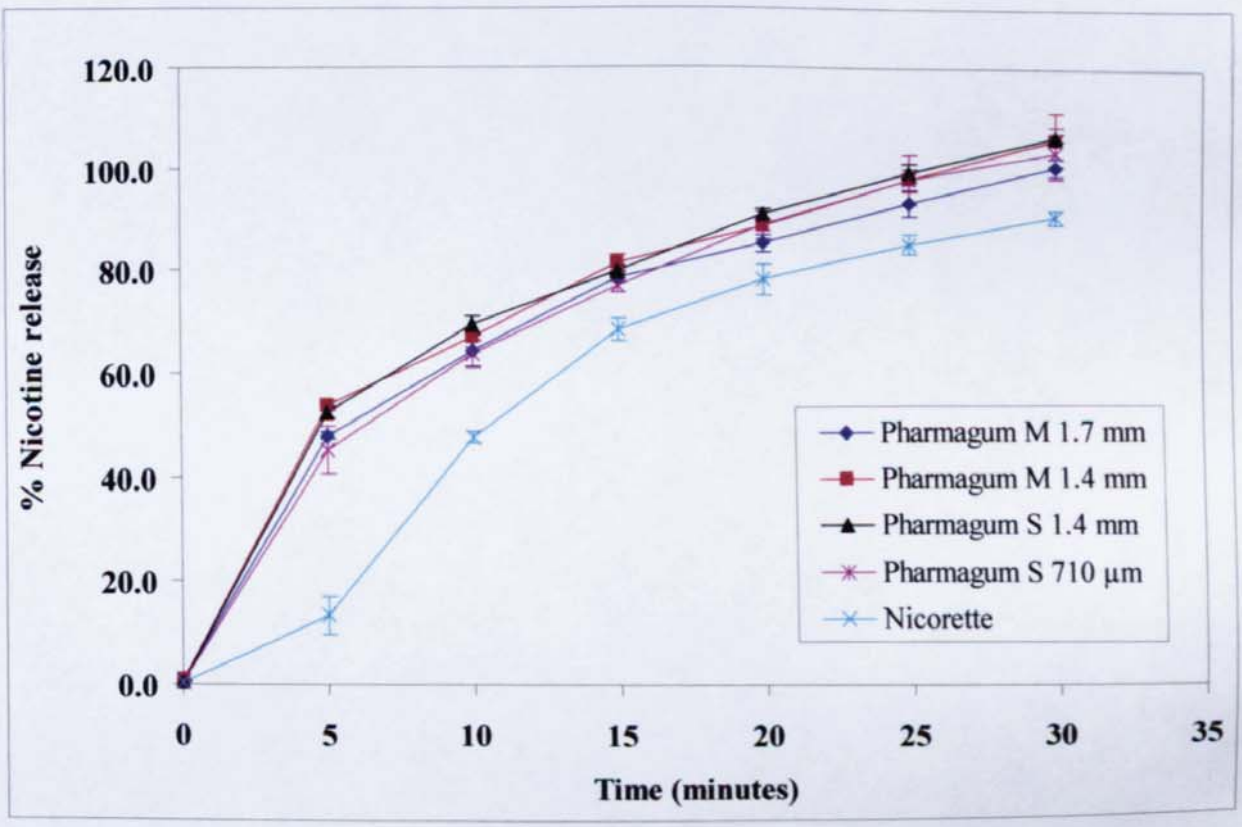


Figure 6.10 Release of nicotine from 4 mg Pharmagum® M and S formulations with effect of granulation of ingredients (n =3 ± SD).

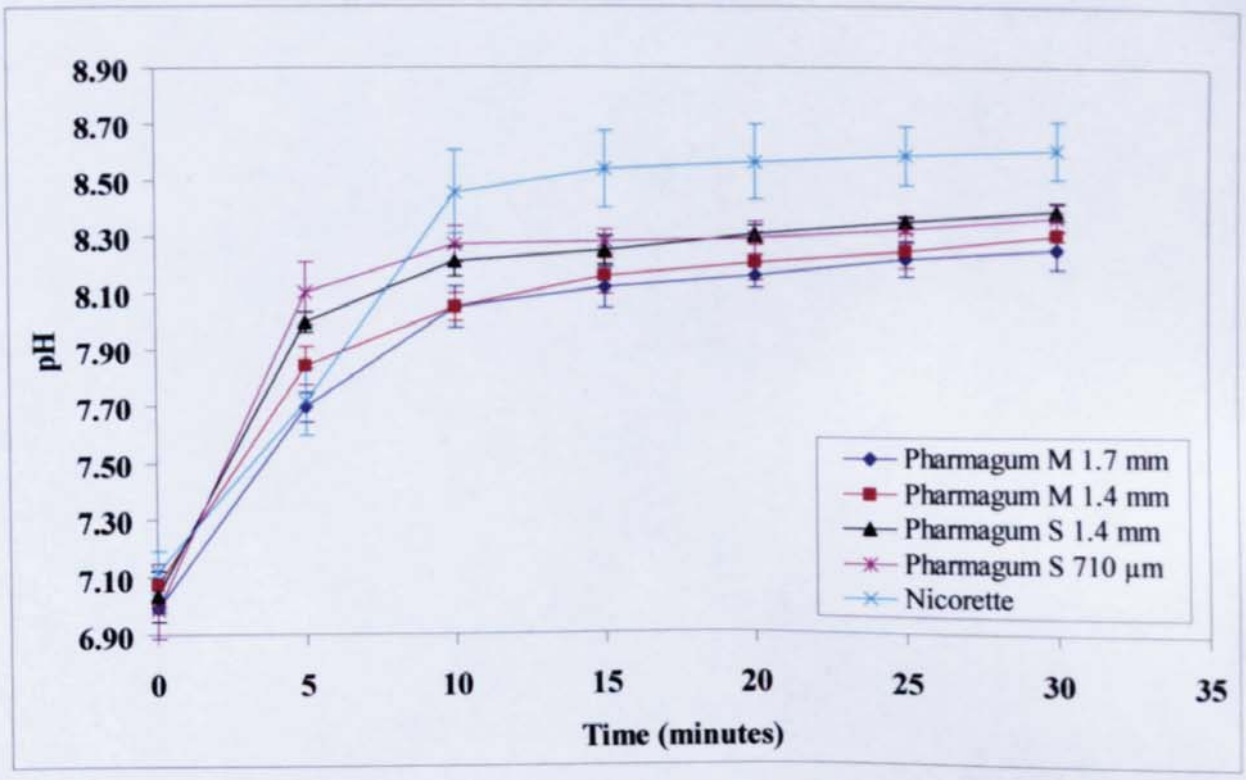
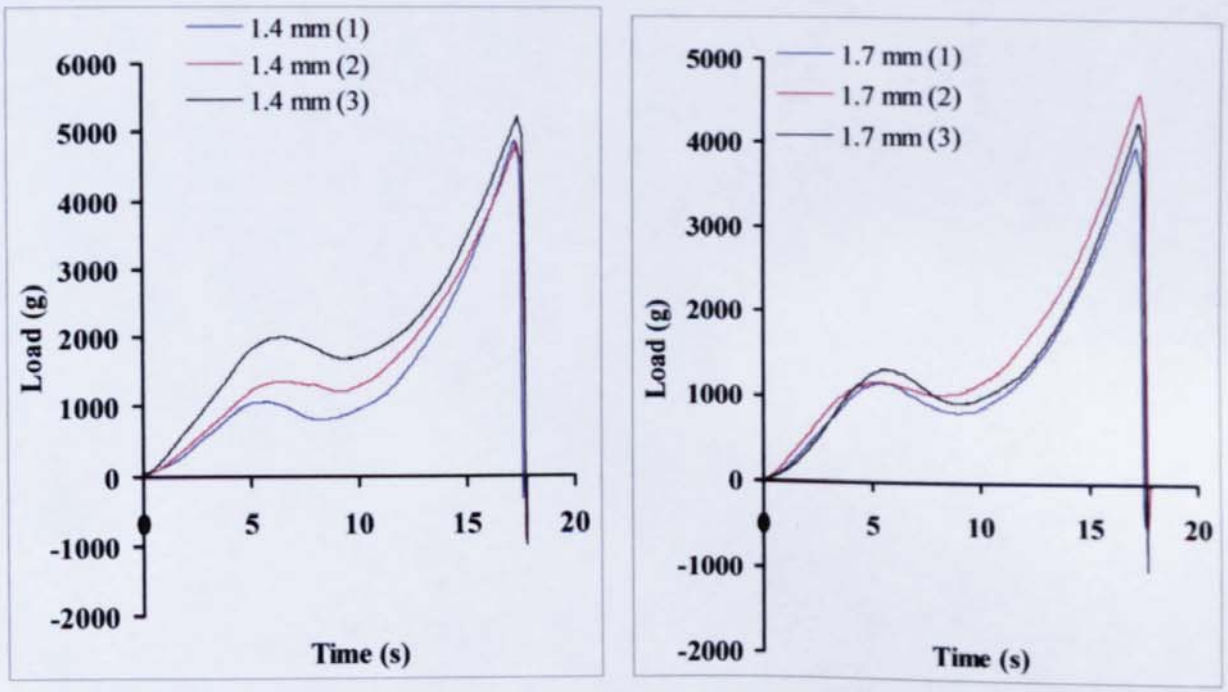


Figure 6.11 pH of artificial saliva as Pharmagum® M and S gums formed using different sized granules were chewed *in vitro* (n =3 ± SD).

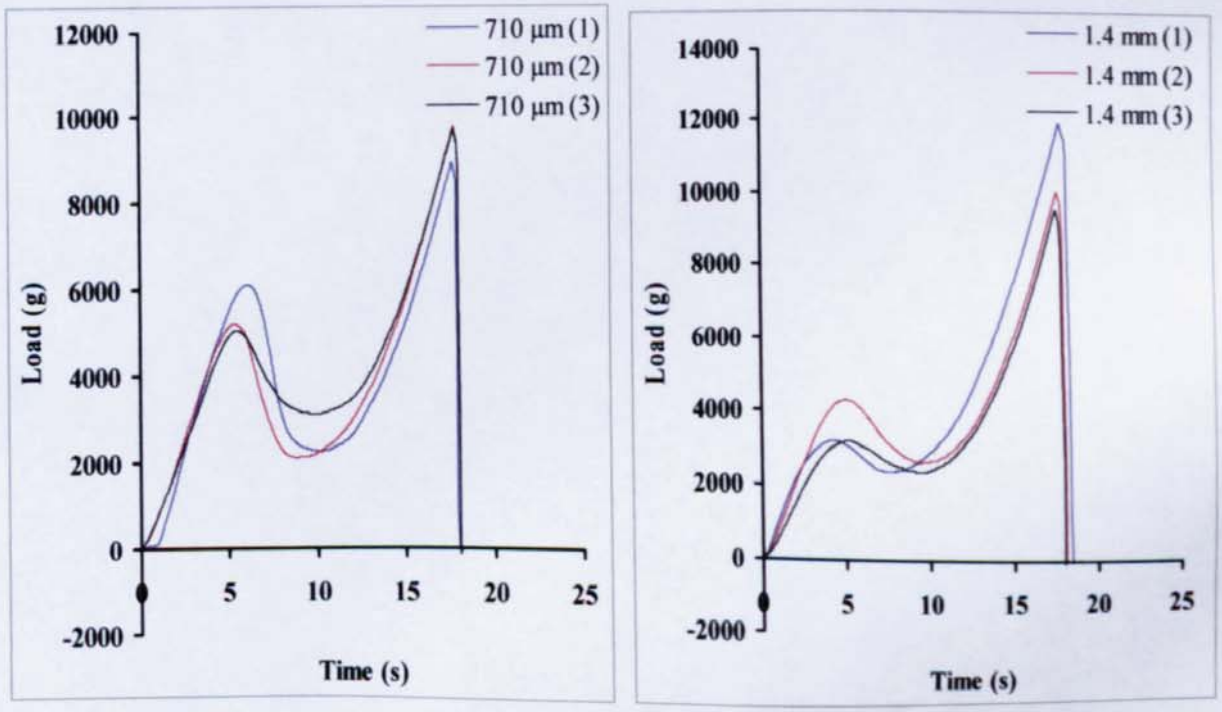
As the gums were chewed, the pH of the artificial saliva within the chewing machine also increased (Figure 6.11). pH rises from all the Pharmagum® formulations appeared similar. Initially, within the first 5 minutes, the pH of the artificial saliva increased by 0.703, 0.768, 0.634 and 1.113 pH units when chewing Pharmagum® M gum made using 1.7 mm, 1.4 mm granules and Pharmagum® S gums made using 1.4 mm and 710 µm granules respectively. At the end of 30 minutes, final pH values of pH 8.242 (Pharmagum® M 1.7 mm), pH 8.291 (Pharmagum® M 1.4 mm), pH 8.380 (Pharmagum® S 1.4 mm) and pH 8.357 (Pharmagum® S 710 µm) was observed in comparison to an increase of 1.482 pH units giving a final pH of 8.599 by chewing Nicorette® gum.

Texture analysis showed that the texture of the granulated Pharmagum® M and S gums (Figure 6.12 and 6.13) were different to the textures of direct compressed Pharmagum® M and S (Figure 6.9) and Nicorette® gum (Figure 6.8). As the 2 mm diameter probe initially traveled through the gum an increasing force was required to penetrate the gum. After 5 seconds, the force required falls after approximately 10 seconds shown by the dip in the load *vs* time graph, and then, as the probe penetrates the gum further, the force increased and peaks after approximately 17 seconds when the required depth was reached.



(a) (b)

Figure 6.12 Load encountered as a 2 mm diameter probe penetrates Pharmagum® M gums made using 1.4 mm granules (a) and 1.7 mm granules (b).



(a) (b)

Figure 6.13 Load encountered as a 2 mm diameter probe penetrates Pharmagum® S gums made from 710 µm granules (a) and 1.4 mm granules (b).

Although the release of nicotine from the granulated Pharmagum® M and S gums was similar, texture analysis of the gums highlighted differences in the gum textures (Table 6.4). Gums produced from Pharmagum® S granules required twice as much force to penetrate the gum to a depth of 3 mm than those produced using Pharmagum® M granules which were softer and more adhesive in nature. This showed that Pharmagum® S gums were twice as hard and less adhesive than gums formed using the different sized Pharmagum® M granules. In comparison to the standard Nicorette® formulation, the granulated gums were harder but less rigid and, with respect to granulated Pharmagum® M gums more adhesive. Results also showed that granulation of Pharmagum® M and S produced softer more adhesive and less rigid gum compared to Pharmagum® M and S gums formulated *via* direct compression (Table 6.3).

	Hardness (N)		**Adhesive force (N)**		**Load *per* unit time (g/s)**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Pharmagum® M 1.7 mm granules	41.85	2.57	-6.40	2.31	212.76	28.28
Pharmagum® M 1.4 mm granules	48.73	1.74	-7.31	2.84	252.56	16.78
Pharmagum® S 1.4 mm granules	102.80	10.00	-0.54	0.39	441.07	43.40
Pharmagum® S 710 µm granules	92.870	3.68	-0.63	0.52	396.60	14.24
Nicorette®	16.51	1.23	-1.51	0.57	1527.63	330.56

Table 6.4 Hardness, adhesive forces and load encountered *per* unit time for Pharmagum® M and S gums formed from granules.

Statistical comparison suggested that, although the granule size differed, there was no significant difference in the texture of the gums formed using the same Pharmagum® ($P > 0.05$) (Table 6.4a). In terms of the gums hardness, although granulation produced softer Pharmagums® compared to direct compression, the Pharmagum® M and S formulations were still significantly harder than Nicorette® ($P < 0.001$), however the adhesiveness of granulated Pharmagum® S gums were not significantly different to Nicorette® ($P > 0.05$).

Granulation of the Pharmagums ® also showed that the Nicorette® gum was significantly more resistant to deformation than the Pharmagum® S and m gums as $P < 0.001$. Results therefore showed that although granulation had changed the texture of the gums by becoming softer and less rigid, granulation of Pharmagum® M and S still did not stop the crumbling effect observed when chewed using the chewing machine.

Comparsion	Hardness P values	Adhesive force P value	Load *per* unit time P value
Nicorette® *vs.* Pharma M 1.7	< 0.001	< 0.05	< 0.001
Nicorette® *vs.* Pharma M 1.4	< 0.001	< 0.05	< 0.001
Nicorette® *vs.* Pharma S 1.4	< 0.001	> 0.05	< 0.001
Nicorette® *vs.* Pharma S 710	< 0.001	> 0.05	> 0.05
Pharma M 1.7 *vs* Pharma M 1.4	> 0.05	> 0.05	> 0.05
Pharma M 1.7 *vs.* Pharma S 1.4	<.0.001	<0.05	> 0.05
Pharma M 1.7 *vs.* Pharma S 710	<.0.001	<0.05	> 0.05
Pharma M 1.4 *vs.* Pharma S 1.4	<.0.001	< 0.01	> 0.05
Pharma M 1.4 *vs.* Pharma S 710	<.0.001	< 0.01	> 0.05
Pharma S 1.4 *vs* Pharma S 710	>.0.05	> 0.05	> 0.05

Table 6.4a Significance levels for hardness, adhesive force and load *per* unit time measurements of Nicorette®, and gums formed using Pharmagum® M 1.7 and 1.4 mm granules and Pharmagum® S 1.4 mm and 710μm granules. Output calculated using Instat Tukey-Kramer multiple comparison test.

6.2.2.3 Effect of compression strength of Pharmagum® M and S

Pharmagum® M and S formulations were compressed using forces of 1 ton and 5 ton. The hypothesis was that if the powdered gum formulations were compressed using a greater force, the individual gum particles that caused the crumbling may compact together and, thus, eliminate the crumbling effect. However, when the gums were chewed *in vitro,* the gums still crumbled into the small gum constituents.

The nicotine release profiles produced from the gums were similar to those observed from granulated and directly compressed Pharmagum® (f_2 >50). After 5 minutes, 46.89% and 45.92% nicotine was released from Pharmagum® M compressed at 1 ton and 5 ton respectively and 43.53%, 45.65% from Pharmagum® S compressed at 1 ton and 5 ton respectively (Figure 6.14). After 5 minutes, as with the granulated gum formulation, the

release of nicotine reduced to give a final release of approximately 95% using Pharmagum® S and, 98% from Pharmagum® M. In comparison, the initial release from Nicorette® was slow which then increased between 10-20 minutes to give a final release of approximately 90% after 30 minutes.

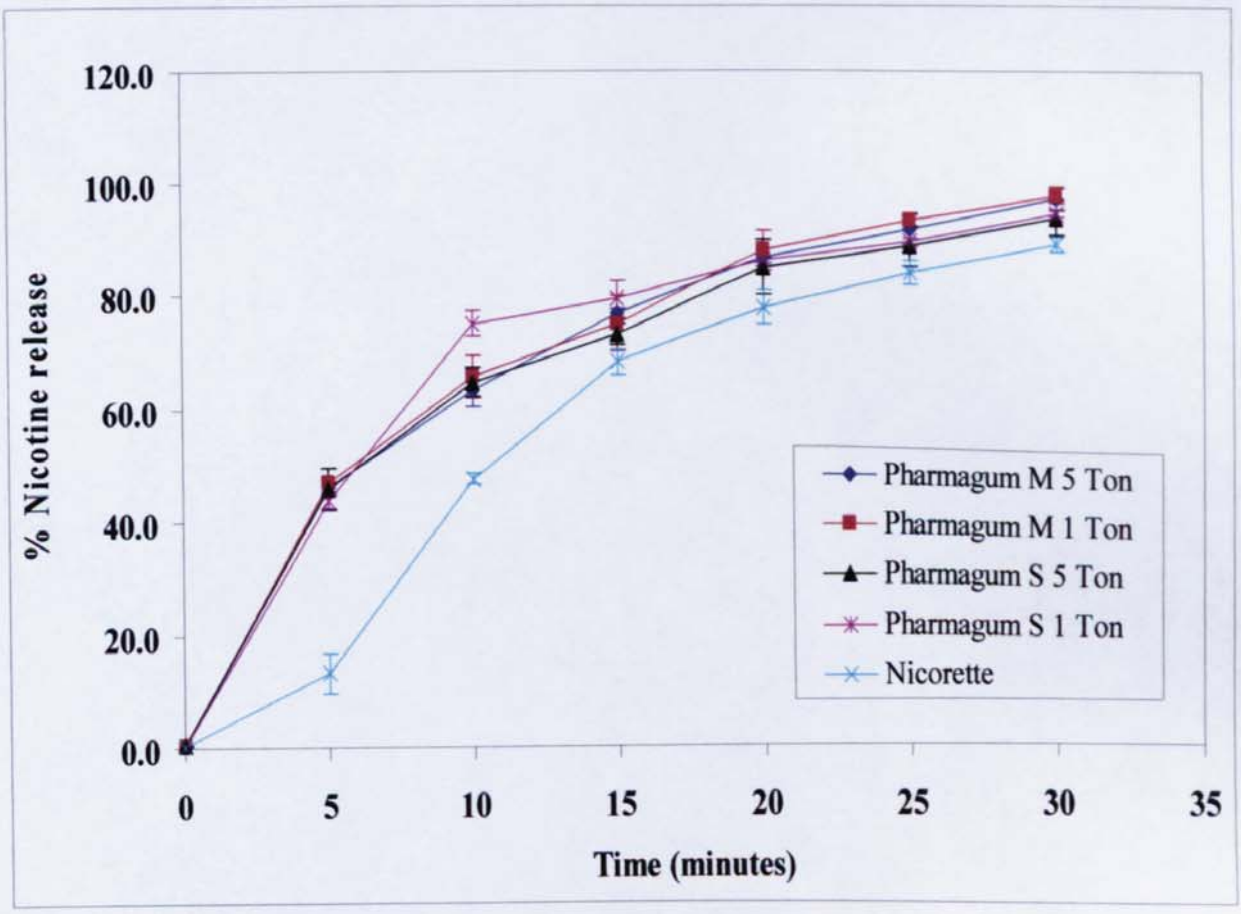


Figure 6.14 Release of nicotine from 4 mg Pharmagum® M and S formulations compressed with a 1 ton and 5 ton force (n =3 ± SD).

For all Pharmagum® formulations, the greatest pH increases were observed within the first 5 minutes of chewing (Figure 6.15). An approximate rise of 1.30 pH units was observed from Pharmagum® M formulations, whilst an increase of approximately 1.0 pH unit was observed when chewing Pharmagum® S formulations. After 5 minutes, the pH increased at a very slow but steady rate to give final pH values of pH 8.473, pH 8.472, pH 8.327 and pH 8.323 when chewing Pharmagum® M, 1 ton, 5 ton and Pharmagum® S 1 ton and 5 ton respectively.

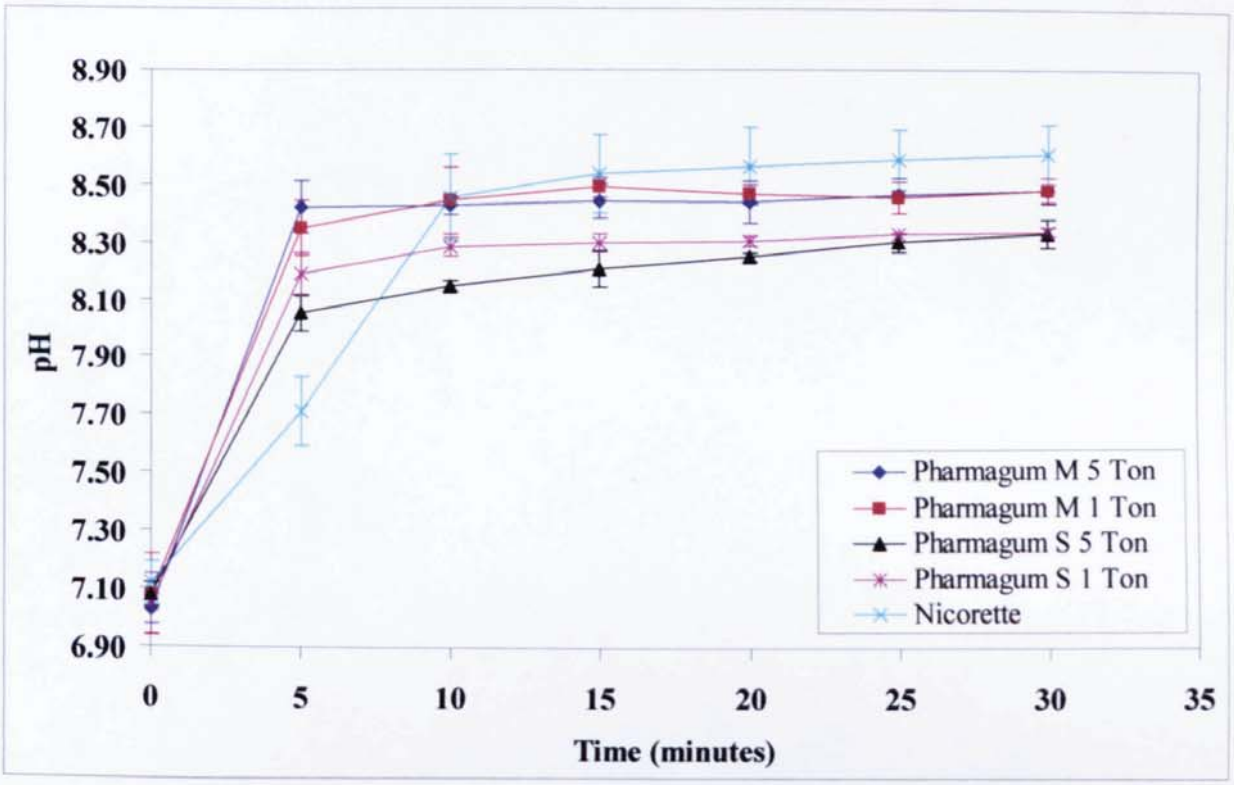
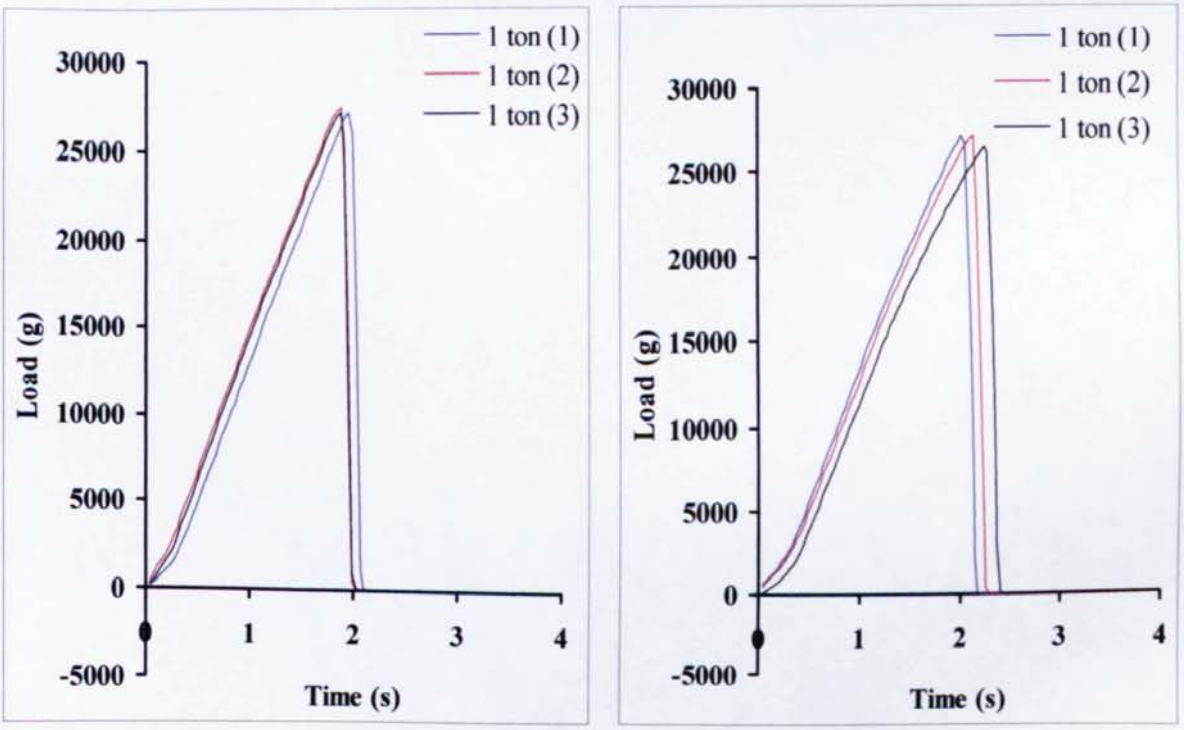


Figure 6.15 pH of artificial saliva as Pharmagum® M and S compressed at 1 ton and 5 ton were chewed *in vitro* (n =3 ± SD).



(a) (b)

Figure 6.16 Load encountered as a 2 mm diameter probe penetrated Pharmagum® M (a) and Pharmagum® S (b) formulated nicotine gums compressed at 1 ton.

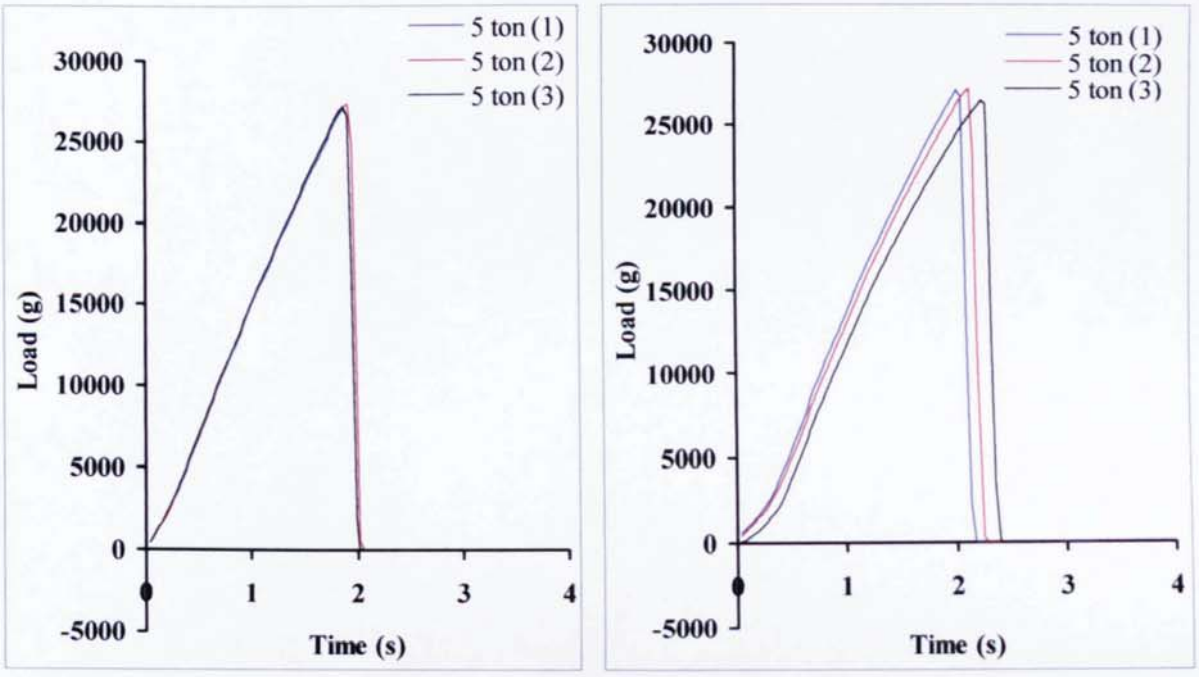


Figure 6.17 Load encountered as a 2 mm diameter probe penetrated Pharmagum® M (a) and Pharmagum® S (b) formulated nicotine gums compressed at 5 ton.

	Hardness (N)		Adhesive force (N)		Load *per* unit time (g/s)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Pharmagum® M 1 ton	266.91	1.16	-0.04	0.00	16128.79	86.16
Pharmagum® M 5 ton	266.49	1.42	-0.06	0.00	15718.87	267.71
Pharmagum® S 1 ton	263.75	3.19	-0.04	0.00	14358.48	659.91
Pharmagum® S 5 ton	263.96	2.26	-0.04	0.00	14518.97	168.60
Nicorette®	16.51	1.23	-1.51	0.52	1527.63	330.56

Table 6.5 Hardness, adhesive forces and load encountered by the gum *per* unit time for Pharmagum® M and S gums compressed at 1 ton and 5 ton.

The texture profiles of Pharmagum® S and M compressed at 1 and 5 ton were similar to the directly compressed Pharmagum® produced using the tablet press (Figures 6.16, 6.17 and 6.9). As the probe penetrated into the gums, after 2 seconds the gums crumbled, thus a sharp drop was observed in the load *vs.* time graphs. Texture analysis results highlighting hardness, adhesiveness and the load encountered *per* unit area of the gums also showed that they were similar to the gums prepared in the tablet press (Table 6.5). In comparison to the standard Nicorette® gum, Pharmagum® M and S compressed at a force of 1 and 5 ton were more than 10 times harder, less adhesive and more rigid ($P < 0.001$). Statistically it was also found that there were no differences in the texture of gums compressed using the different compression forces *i.e.* Pharmagum® M compressed at 1 ton and 5 ton were statistically similar and Pharmagum® S compressed at 1 and 5 ton were similar (Table 6.5a).

Comparsion	Hardness P values	Adhesive force P value	Load *per* unit area P values
Nicorette® *vs.* Pharma M 1 ton	< 0.001	< 0.001	< 0.001
Nicorette® *vs.* Pharma M 5 ton	< 0.001	< 0.001	< 0.001
Nicorette® *vs.* Pharma S 1 ton	< 0.001	< 0.001	< 0.001
Nicorette® *vs.* Pharma S 5 ton	< 0.001	< 0.001	< 0.001
PharmaM 1 ton *vs* Pharma M 5 ton	> 0.05	> 0.05	> 0.05
Pharma M 1 ton *vs.* Pharma S 1 ton	> 0.05	> 0.05	< 0.001
Pharma M 1 ton *vs.* Pharma S 5 ton	> 0.05	> 0.05	< 0.01
Pharma M 5 ton *vs.* Pharma S 1 ton	> 0.05	> 0.05	< 0.01
Pharma M 5 ton *vs.* Pharma S 5 ton	> 0.05	> 0.05	< 0.05
Pharma S 1 ton *vs* Pharma S 5 ton	> 0.05	> 0.05	> 0.05

Table 6.5a Significance levels of the hardness, adhesive force and load *per* unit time measurements of Nicorette®, and Pharmagum® M and S compressed at 1 and 5 ton. Output calculated using Instat Tukey-Kramer multiple comparison test.

6.2.2.4 General discussion

Dissolution curve comparison using f_2 equation showed that the release of nicotine from directly compressed, granulated, and increased compression strength Pharmagum® M and S formulations were similar ($f_2 > 50$). When the Pharmagum® formulations were compared to the standard Nicorette® gum, all dissolution curves were dissimilar (Table 6.6). The dissimilarity in the dissolution was a result of an increased release of nicotine from the

Pharmagum® formulation caused by the crumbling of the gum when chewed *in vitro*. This enabled a faster release of nicotine due to a multiple number of small particles with a large surface area. The crumbling could be due to the formulation method of the gums. Pharmagum® S and M are both a directly compressible free-flowing gum powders with a mixture of polyols (mixture of mannitol, sorbitol and xylitol). During granulation, the gum particles are surrounded by any incorporated ingredients such as magnesium stearate, or any insoluble ingredient added as a glident to increase the flowibility of the granules. As the gum granules are compressed, between each gum particle, a small thin layer of incorporated material (insoluble or soluble) will be present thus separating individual gum particles. When the gum was chewed in the chewing machine, the gum will thus crumble like a tablet into the individual granules/gum particles.

				Pharmagum® M				Pharmagum® S				
				granules		compression			granules		compression	
			Nicorette®	Std	1.7 mm	1.4 mm	5 ton	1 ton	Std	1.4 mm	710 µm	5 ton	1 ton
		Nicorette®		38.92	39.70	35.24	41.01	39.88	36.61	35.01	39.65	41.90	38.94
Pharmagum® M		Std	38.92		60.72	54.35	61.91	62.04	82.39	54.64	56.80	62.41	83.35
	Granules	1.7 mm	39.70	60.72		68.70	86.88	82.45	58.69	65.73	76.10	72.57	64.32
		1.4 mm	35.24	54.35	68.70		63.81	65.70	55.29	86.51	69.16	57.27	56.96
	Compre-sion	5 ton	41.01	61.91	86.88	63.81		88.39	58.62	62.38	75.22	79.20	65.13
		1 ton	39.88	62.04	82.45	65.70	88.39		59.23	65.47	77.37	76.77	66.23
Pharmagum® S		Std	36.61	82.39	58.69	55.29	58.62	59.23		55.69	55.05	58.08	77.07
	Granules	1.4 mm	35.01	54.64	65.73	86.51	62.38	65.47	55.69		69.39	56.45	57.48
		710 µm	39.65	56.80	76.10	69.16	75.22	77.37	55.05	69.39		64.44	59.72
	Compre-ssion	5 ton	41.90	62.41	72.57	57.27	79.20	76.77	58.08	56.45	64.44		66.06
		1 ton	38.94	83.35	64.32	56.96	65.13	66.23	77.07	57.48	59.72	66.06	

Dissolution curves not similar | Dissolution curves similar | Dissolution curves not compared

Table 6.6 f_2 values highlighting similar and dissimilar dissolution profiles of different Pharmagum® M and S formulation and Nicorette® gum.

6.3 Chewing gum formulation

6.3.1 Materials and Methods

6.3.1.1 Materials

Tween 80, and nicotine hydrogen tartrate salt were used as supplied by Sigma UK. Nicotine Amberlite IRP69 was made as detailed in section 2.14. Blacktree gum base was used as supplied by GSK, Weybridge. Dreyco base and Magna T (bubble gum base) was used as supplied by LA Dreyfus, Parsippany, New Jersey. All other ingredients were supplied by GSK, Parsippany, New Jersey.

6.3.1.2 Methods

The standard chewing gum formulation (section 2.13) was adapted to produce a series of formulations (Table 6.7) with high, standard or low concentrations of sodium carbonate, sugar syrup and Tween 80 (Table 6.8 and 6.8a). In two formulations, the Nicotine Polacrilex was substituted for nicotine Amberlite IRP69 and nicotine hydrogen tartrate salt (Table 6.9). Note: - When altering the various concentration of nicotine, sodium carbonate, surfactant and syrup the amount of sorbitol in the standard formulation was adjusted accordingly.

	High (%w/w)	Standard (%w/w)	Low (%w/w)
(Tween 80)		0.1	
Sodium Carbonate	5.0	3.0	1.0
Maltitol liquid	5.5	2.5	1.0

Table 6.8 Concentrations of surfactant, sodium carbonate and maltitol liquid to be added to formulation

Formulation Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Surfactant	+	+	+	+	-	-	-	-	-	-
Sodium Carbonate	+	+	-	-	+	+	-	-	++	+
Sugar Syrup	+	-	+	-	+	-	+	-	+	++

Table 6.8a Quantities of surfactants, sodium carbonate and sugar syrup

- none added/reduced quantity added, + standard, ++ increased quantity added (Table 6.8)

Batch	Description
Bt 1	Standard formulation with standard surfactant
Bt 2	Standard formulation with standard surfactant and low syrup
Bt 3	Standard formulation with standard surfactant and low sodium carbonate
Bt 4	Standard formulation with standard surfactant, low sodium carbonate and low syrup
Bt 5	Standard gum formulation with 4 mg nicotine
Bt 5a	Standard gum formulation replicate 1
Bt 5b	Standard gum formulation replicate 2
Bt 6	Standard gum formulation with low syrup
Bt 7	Standard gum formulation low sodium carbonate
Bt 8	Standard gum low sodium carbonate and low syrup
Bt 9	Standard formulation with high sodium carbonate concentration
Bt 10	Standard formulation with high syrup concentration
Bt 11	Standard formulation with use of Dreyco Base as gum base
Bt 12	Standard formulation with addition of nicotine Amberlite IRP-69 resin
Bt 13	Standard formulation with addition of nicotine hydrogen tatrate salt
Bt 14	30mg sodium carbonate in a 2 mg formulation
Bt 15	20mg sodium carbonate and 10mg sodium bicarbonate in a 4 mg formulation
Bt 16	Standard formulation with use of Blacktree gum base
Bt 17a	Nicotine Polacrilex added with the gum base
Bt 17b	Nicotine Polacrilex added with the buffers
Bt 18	Standard formulation with the use of Magna T (bubble gum base) instead of a gum base

Table 6.7 Nicotine gum formulations

Note: - All formulations were made as a 4 mg gum except formulation 14. High, low, and standard value and concentration of the actives for formulation 12 and 13 can be found in Table 6.8 and 6.9

Active Substance	Composition %w/w 2 mg	Composition %w/w 4 mg
Nicotine Amberlite IRP-69 (19.94% loaded)	1.003	2.006
Nicotine Hydrogen Tartrate Salt	0.570	1.141

Table 6.9 Concentrations of nicotine added to batches 12 and 13

The total nicotine content for the gums was determined using the method described in section 2.6. *In vitro* release studies were conducted using the chewing machine (section 2.3) and the texture of the formulated gum was established using the texture analyser (section 2.11).

6.3.2 Results and discussion

6.3.2.1 Batch reproducibility

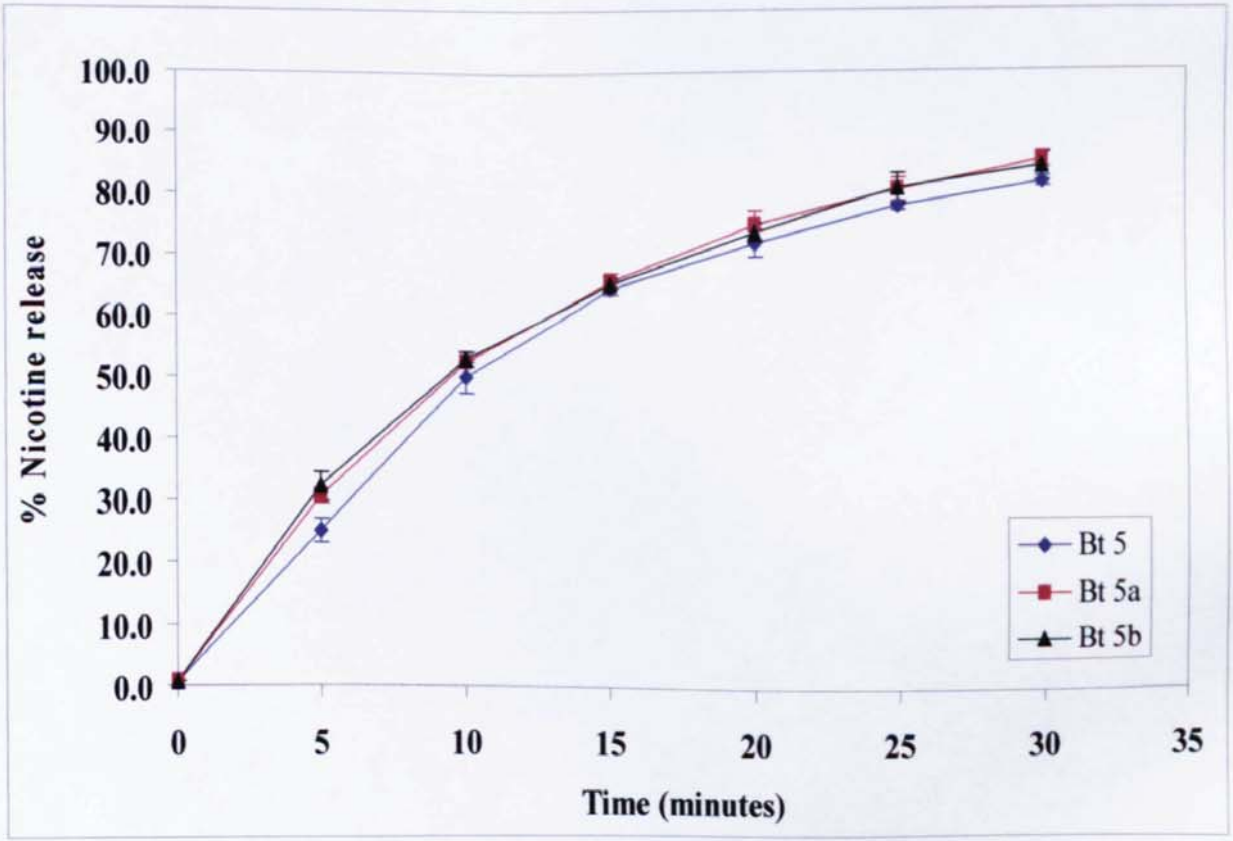


Figure 6.18 Release of nicotine from the standard nicotine gum formulation (Bt 5) and the replicates (Bt 5a and 5b) (n =3 ± SD).

The reproducibility of the formulation method used to make the gum was tested by preparing two replicate formulations (Bt 5a and Bt 5b) of the standard nicotine gum (Bt 5). Release *in vitro* using the chewing machine showed that the release of nicotine from batches 5, 5a, and 5b were similar as highlighted by the f_2 dissolution comparison equation (Table 6.10). After 10 minutes, approximately 50% of the nicotine was released from the standard nicotine gum formulations. At the end of 30 minutes, 81.45%, 85.05% and 83.96% was released from batch 5, 5a and 5b respectively (Figure 6.18).

		Reproducible Batches		
		Bt 5	**Bt 5a**	**Bt 5b**
Reproducible Batches	**Bt 5**		74.51	72.80
	Bt 5a	74.51		93.77
	Bt 5b	72.80	93.77	

Key

- Dissolution curves not similar
- Dissolution curves similar
- Dissolution curves not compared

Table 6.10 f_2 values highlighting the similar and dissimilar dissolution profiles of the standard (Bt 5) and reproducible batches (Bt 5a and 5b).

As previously discussed (section 5.3), the increase in salivary pH when the gum is chewed is important in terms of the absorption of nicotine through the buccal membrane, thus the increase in pH as the gum was chewed *in vitro* was determined. Within the first five minutes of *in vitro* chewing of Bt 5, 5a, and 5b, the standard gum and replicates, the pH of the artificial saliva increased by 0.969, 0.997 and 0.814 pH units with respect to batches 5, 5a and 5b respectively (Figure 6.19). After 30 minutes of chewing final pH values were pH 8.360 (Bt 5), pH 8.386 (Bt 5a) and pH 8.471 (Bt 5b), therefore suggesting that the nicotine present in the artificial saliva was predominately in the unionised form after 10 minutes, and thus could be readily absorbed through the buccal mucosa.

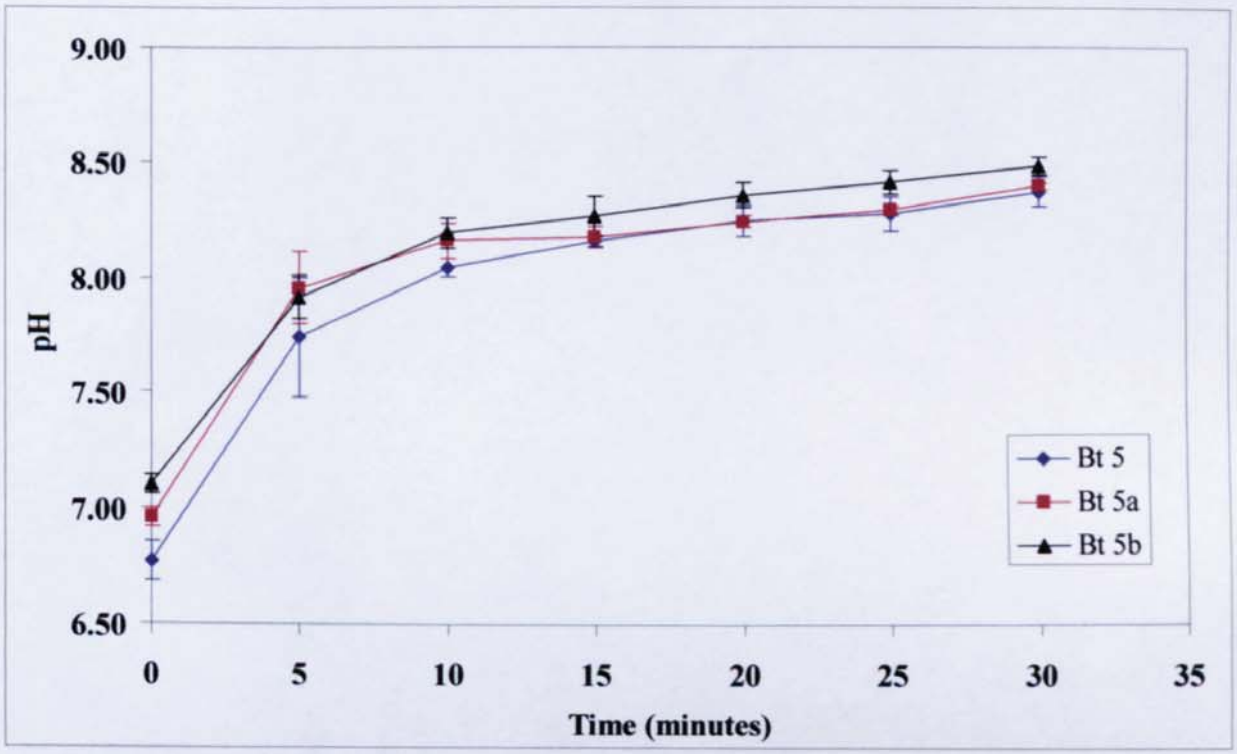
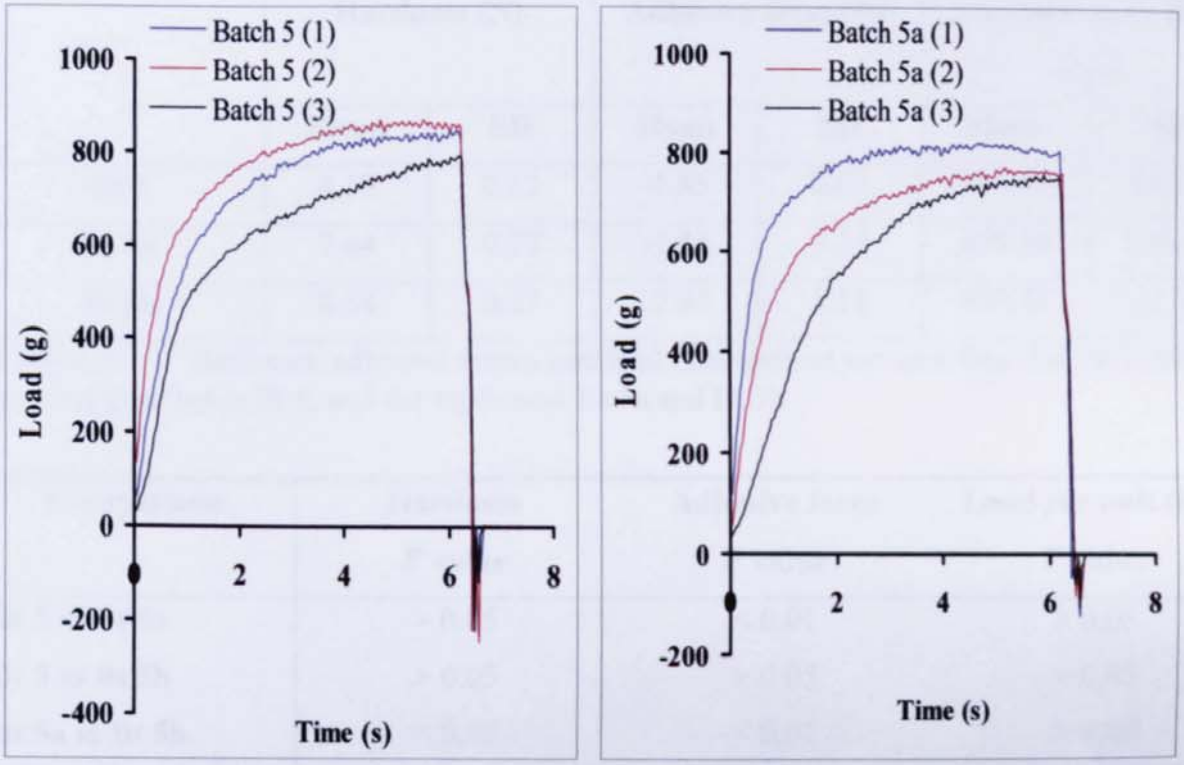


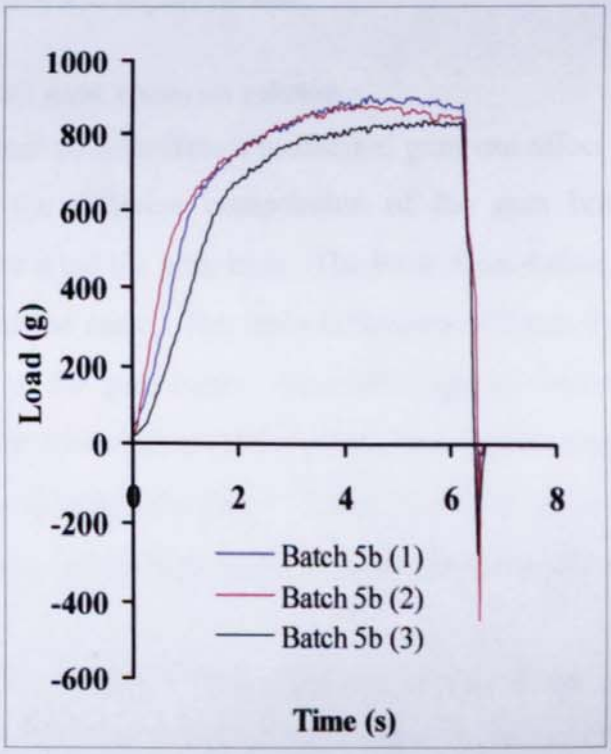
Figure 6.19 pH of the artificial saliva as the standard nicotine gum batch (Bt 5) and the replicated batches (Bt 5a and Bt 5b) were chewed *in vitro* (n=3 ± SD)

Although release of nicotine from the gums was similar, texture analysis showed that there were some variations in the texture of the gums (Table 6.11 and Figure 6.20). As the 2 mm diameter probe penetrated the gums, the gums remained intact and required a small increasing force to reach the depth of 3 mm. Measured hardness showed that batch 5b gums were somewhat harder than batches 5 and 5a as the force required to penetrate the gums was greater (8.54 N in comparison to 8.49 N and 7.64 N respectively). Tukey-Kramer multiple comparison tests conducted showed that Bt 5a was significantly softer than Bt 5b, but was similar in terms of hardness to Bt 5 (Table 6.11a). Significance values for adhesive force and load *per* unit time measurements showed that Bt 5b and Bt 5a and, Bt 5 and 5a were significantly different from each other in terms of their adhesiveness whilst in terms of rigidity, there was no significant difference between the standard gum and the replicate gums.



(a)

(b)



(c)

Figure 6.20 Load encountered as a 2 mm diameter stainless steel probe penetrated the standard nicotine gum formulation, batch 5 (a), and the replicate batches, 5a (b) and 5b (c) to a depth of 3 mm at 30 mm/minute.

	Hardness (N)		Adhesive force (N)		Load *per* unit time (g/s)	
	Mean	SD	Mean	SD	Mean	SD
Bt 5	8.19	0.32	-1.85	0.63	345.59	86.93
Bt 5a	7.64	0.27	-1.04	0.14	450.34	220.77
Bt 5b	8.54	0.27	-2.91	1.11	407.48	21.79

Table 6.11 Hardness, adhesive forces and load encountered *per* unit time for the standard nicotine gum batch Bt 5, and the replicates, Bt 5a and Bt 5b

Comparison	Hardness P value	Adhesive force P value	Load *per* unit time P value
Bt 5 *vs* Bt 5a	> 0.05	< 0.01	> 0.05
Bt 5 *vs* Bt 5b	> 0.05	> 0.05	> 0.05
Bt 5a *vs* Bt 5b	< 0.05	< 0.01	> 0.05

Table 6.11a Significance levels for hardness, adhesive force and load *per* unit time of the standard nicotine gum Bt 5, and the replicates, Bt 5a and Bt 5b. Output calculated using Instat Tukey-Kramer multiple comparison test.

6.3.2.2 Effect of different gum bases on release

The type of gum base used to formulate a medicated gum can affect the release of medicate from the gum due to the different composition of the gum base. Batch 18 (Bt 18) incorporated nicotine into a bubble gum base. The basic formulation for a bubble gum and a chewing formulation was the same. The main difference between the two types of products lies in the composition of the gum bases. Generally, natural rubber and styrene-butadiene rubber are the choices for formulating bubble gums bases whilst chewing gum base consists mainly of butyl rubber and polyisobutylene. Batch 11 and 16 were formulated with the use of other chewing gum base of which the properties and formulations were not known.

Release from batches 11, 16 and 18 were different to that of the standard gum (batch 5). Generally, a greater release was observed from batches 11, 16 and 18 in comparison to batch 5 (Figure 6.21). Within 5 minutes of chewing *in vitro*, approximately 50% of the nicotine incorporated into Bt 11, 16 and 18 was released from the gum in comparison to 25.21% released from the standard gum formulation. Batch 11, nicotine gum made from Dreyco base, gave a steady increase in release with time. At the end of 30 minutes of *in vitro* chewing, approximately 95% of the nicotine within the gum was released. After 15 minutes,

a decrease in release (86%-80%) was observed when chewing Bt 18, gums made using bubble gum base. A possible explanation for this could be that the nicotine released from the bubble gum begins to absorb back onto the gum due to different polymers used in the bubble gum base. Gum formulated using the Blacktree gum base (Bt 16) again showed a different release profile. Initially a rapid increase in nicotine was observed which, after 15 minutes plateaued to give a release of approximately 80% nicotine between 15 and 30 minutes. Dissolution curve comparisons using the f_2 equation showed that the different gum bases had dissimilar release profiles when compared to the standard gum formulation (Table 6.11). When comparing the release profiles of Bt 16 (Blacktree gum) to Bt 11 (Dreyco base) and Bt 18 (Magna T bubble gum base), f_2 values indicated that the release profiles were similar, whilst release from Bt 18 and Bt 11 were dissimilar.

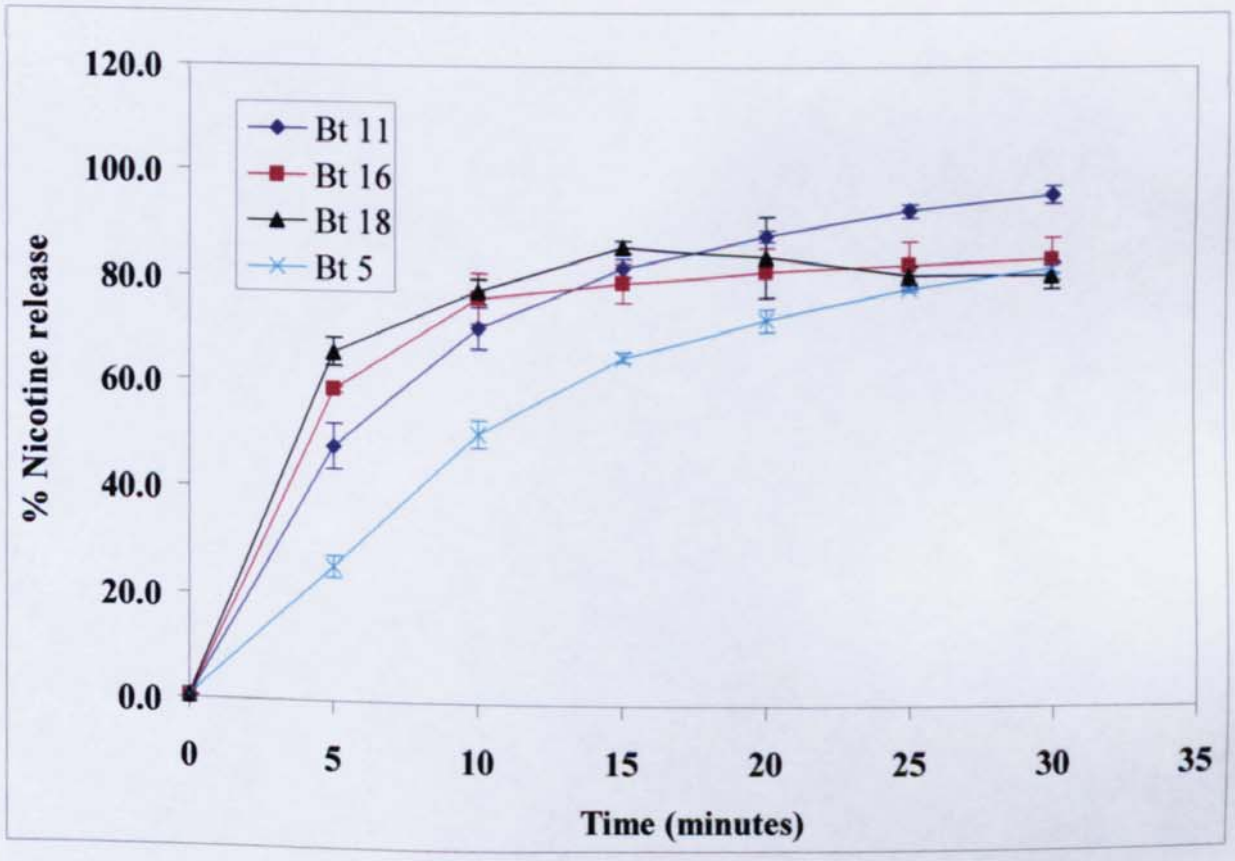


Figure 6.21 Release of nicotine from formulated gums using different gum bases (n =3 ± SD). Bt 11 = Dreyco Base, Bt 16 = Blacktree gum base, Bt 18 = Magna T bubble gum base and Bt 5 was the standard gum base.

		Different gum base			
		Bt 11	Bt 16	Bt 18	Bt 5
Different Gum Base	Bt 11		54.40	48.57	39.31
	Bt 16	54.40		68.01	38.32
	Bt 18	48.57	68.01		34.39
	Bt 5	39.31	38.32	34.39	

Key

Dissolution curves not similar

Dissolution curves similar

Dissolution curves not compared

Table 6.12 f_2 values highlighting the similar and dissimilar dissolution profiles of the nicotine gums formulated using different gum bases.

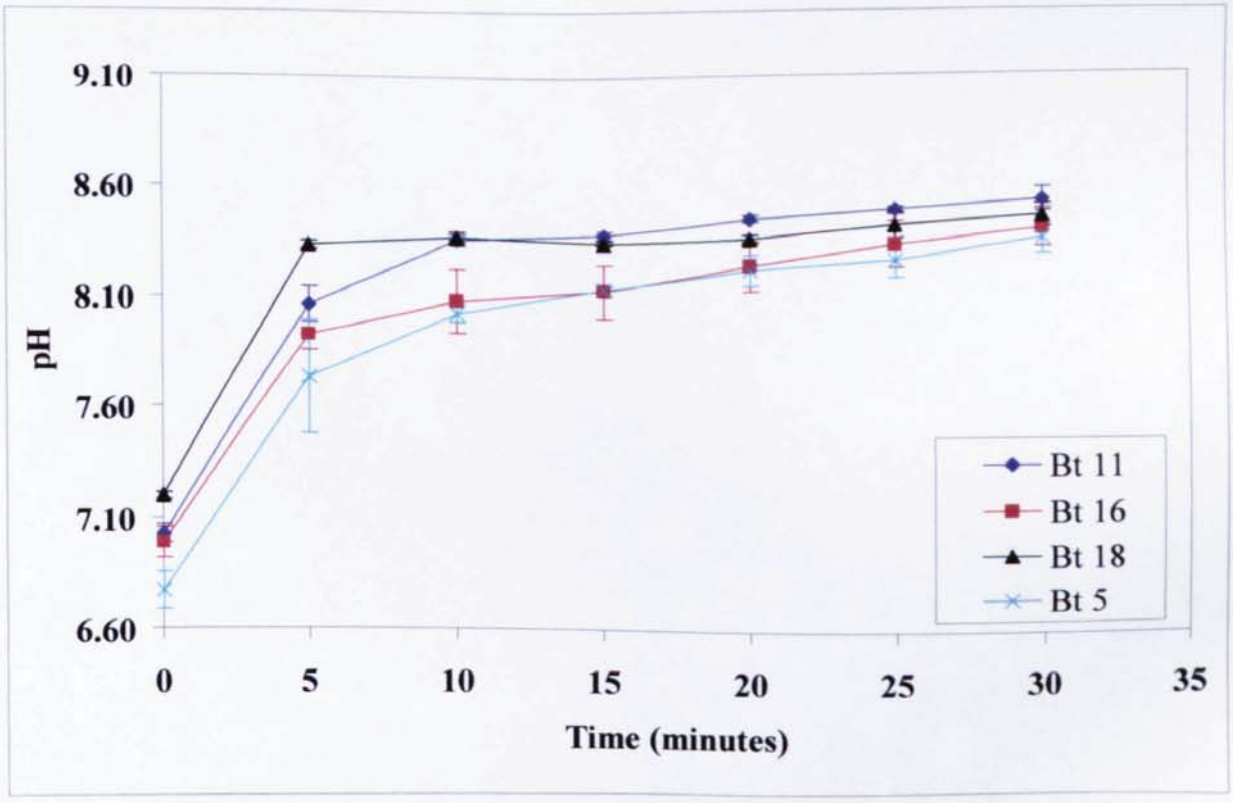


Figure 6.22 pH of the artificial saliva as formulated nicotine gum using different gum bases were chewed *in vitro* (n=3 ± SD). Bt 11 = Dreyco Base, Bt 16 = Blacktree gum base, Bt 18 = Magna T bubble gum base and Bt 5 was the standard gum base

Increases in the pH of the artificial saliva whilst nicotine was released showed similar profiles (Figure 6.22). Rapid pH increases were observed after 5 minutes where the pH increased by approximately 1.0 pH units and then slowly plateaued to give an overall increase of approximately pH 1.5.

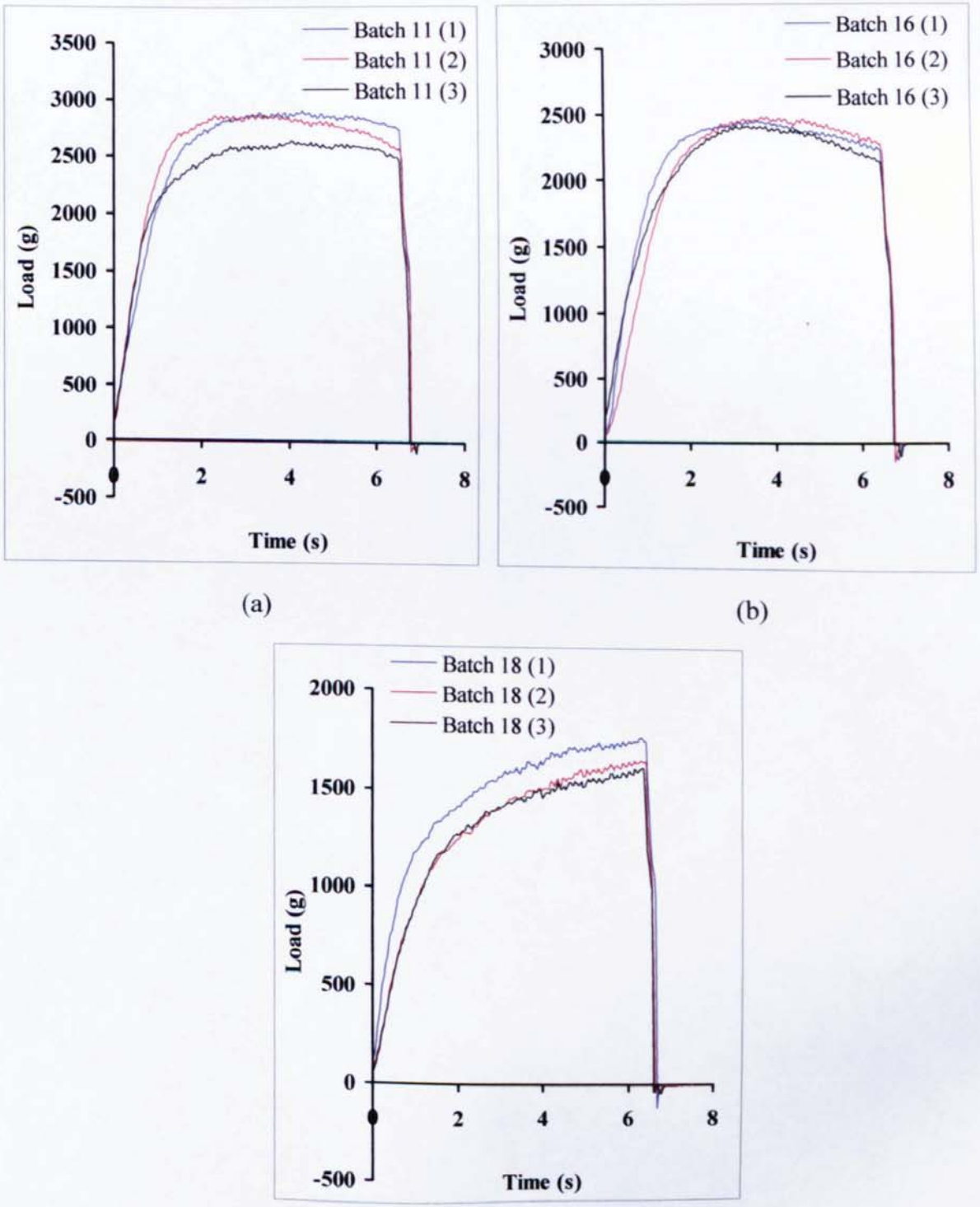


Figure 6.23 Load encountered as a 2 mm diameter stainless steel probe penetrated batches 11, Dreyco base (a), 16, Blacktree (b) and 18 Magna T bubble gum base (c) to a depth of 3 mm at 30 mm/minute.

Texture analysis conducted with the QTS 25 showed slight differences in the texture of the gums. A consistent force was required to penetrate Bt 11 and 16 gums to reach a depth of 3 mm (Figure 6.23). In contrast, as the probe penetrated Bt 18, an increasing force was required the further the probe travelled highlighting that the outer most layers of the gum were softer than the inner layers. In comparison to the standard gum formulation (Bt 5), the use of the different gum bases produced harder, less adhesive and more rigid gums (Table 6.13 Statistically, it was found that in terms of the gum hardness, all the gums were significantly different from each other with Bt 11 being the hardest and most resistant to deformation (Table 6.13a). When considering the resistance to deformation, statistically Bt 18 was similar to the standard gum base, whilst all other formulations were significantly different.

	Hardness (N)		Adhesive force (N)		Load *per* unit time (g/s)	
	Mean	SD	Mean	SD	Mean	SD
Bt 5	8.19	0.32	-1.85	0.63	345.59	86.93
Bt 11	27.55	1.14	-0.81	0.13	2145.45	337.30
Bt 16	23.92	0.28	-1.18	0.19	1527.84	178.08
Bt 18	16.47	0.63	-0.71	0.35	511.14	48.45

Table 6.13 Hardness, adhesive forces and load encountered *per* unit time for gums made using the standard gum base (Bt 5), Dreyco base (Bt 11), Blacktree base (Bt 16) and Magna T bubble gum base (Bt 18).

Comparison	Hardness P value	Adhesive force P value	Load *per* unit time P value
Bt 5 *vs* Bt 11	< 0.001	< 0.05	< 0.001
Bt 5 *vs* Bt 16	< 0.001	> 0.05	< 0.001
Bt 5 *vs* Bt 18	< 0.001	< 0.05	> 0.05
Bt 11 *vs*. Bt 16	< 0.001	> 0.05	< 0.05
Bt 11 *vs*. Bt 18	< 0.001	> 0.05	< 0.001
Bt 16 *vs*. Bt 18	< 0.001	> 0.05	< 0.01

Table 6.13a Significance level for the hardness, adhesive force and load *per* unit time of gums made using the standard gum base (Bt 5), Dreyco base (Bt 11), Blacktree base (Bt 16) and Magna T bubble gum base (Bt 18). Output calculated using Instat Tukey-Kramer multiple comparison test.

Results obtained from the release profiles (Figure 6.21) and texture analysis (Table 6.13) shows that the harder, more rigid gums gave faster nicotine release. With the exception of the gum base, the formulation of the gums was the same, thus the increased release and different textures of the gum was as a result of the use of different gum bases used. Gum base is a mixture of elastomers, plasticizer, texture agents, waxes, lipids, emulsifiers and other ingredients such as colourants and antioxidants. The exact formulation and mixture of these ingredients in the different gum bases are not known as they are company-kept secrets. Simply mixing ingredients together does not make a good gum base mix. To make a good gum base mix, the ingredients are selected in such a way that they are miscible with each other at a molecular level, but presumably also controlling physicochemical and technological properties. The control of the phase mix (mixing of the raw ingredients of the gum base) will have an impact on the release of sweeteners, flavors or other pharmaceutical ingredients thus; gum bases are formulated in consideration of their intended purpose (Lee, 2001). The gum bases used within batches 11, 16 and 18 are gum bases usually used for confectionary products. Addition of nicotine and the other formulation additives could have brought about changes in the properties of the gum base, thus different release profiles were obtained with the use of different gum bases.

6.3.2.3 Effect of different concentration of sodium carbonate and sodium bicarbonate

Sodium carbonate and sodium bicarbonate are added to nicotine formulation to increase the pH of the oral cavity which will increase the absorption of nicotine through the buccal membrane (section 5.3). Thus, the concentration of sodium bicarbonate and sodium carbonate are important factors in controlling absorption.

The effect of increasing and decreasing the concentration of sodium carbonate on the release of nicotine from formulated nicotine gum chewing gum was studied (Figure 6.24). The release from the different formulations was found to differ depending on the concentration of sodium salt added. Generally, it can be seen that at the higher sodium carbonate concentration a greater release of nicotine was observed. After 30 minutes of *in vitro* chewing, greatest release was observed from Bt 9, gums with the highest concentration of added sodium carbonate, followed by, Bt 5, the standard gum formulation, Bt 7, addition of a lower concentration of sodium carbonate (1.0% w/w) and finally, Bt 8 (1.0% w/w sodium carbonate plus lower sugar syrup concentration). Initially release from the standard formulation (Bt 5) was greater than the other gum formulations as approximately 65% was

released from Bt 5 compared to 43%, 26% and 58% released from Bt 7, 8 and 9 respectively at 15 minutes. After 15 minutes, the rate of release from Bt 5 reduced whilst release from Bt 9, 8 and 7 continued to increase to give final release values of 94.04% (Bt 9), 40.16% (Bt 8), 66.40% (Bt 7) and 81.45% (Bt 5). Dissolution curve comparison using the f_2 equation showed that Bt 7 and Bt 8 were different to all other formulation ($f_2 < 50$), whilst using a higher concentration of sodium carbonate (Bt 9) gave a similar release profile to the standard nicotine gum (Table 6.14).

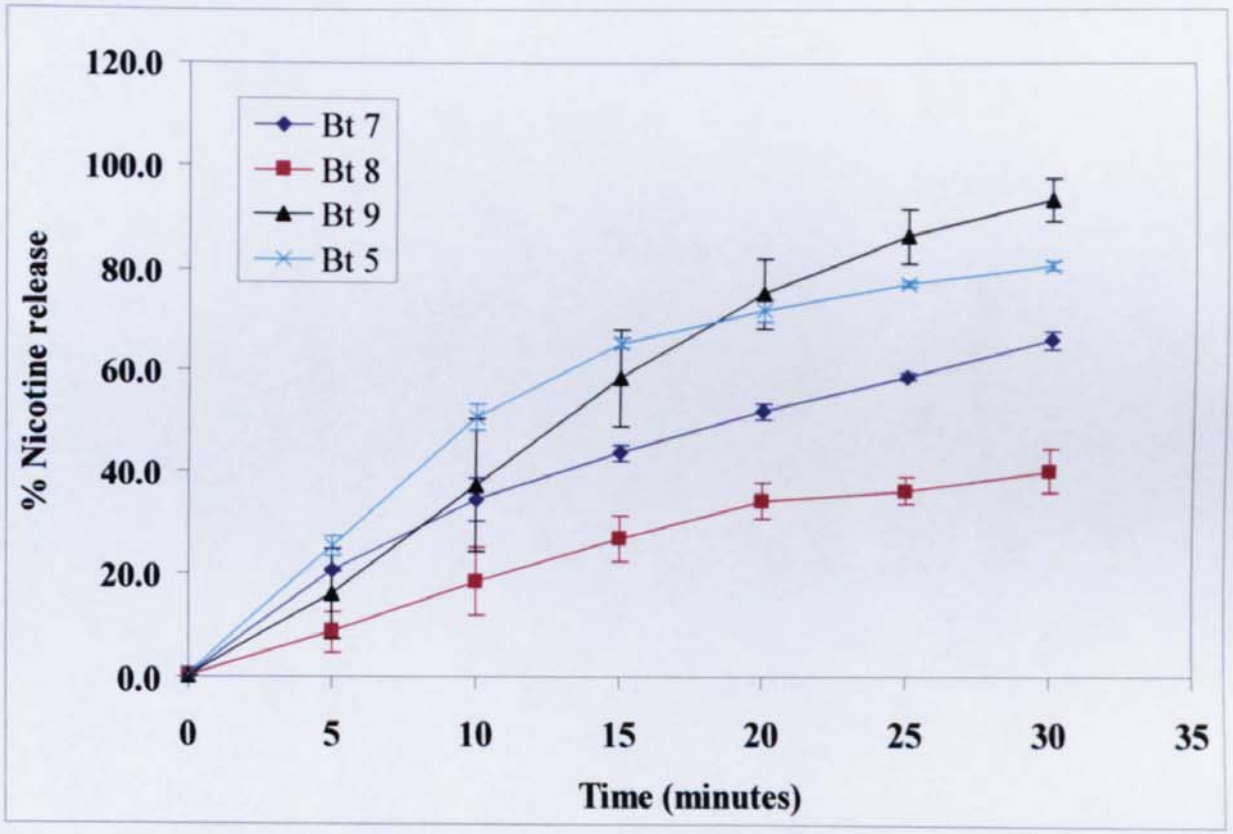


Figure 6.24 Release of nicotine from formulated gums with effect of increasing and decreasing sodium carbonate concentration within the gum (n =3 ± SD). Bt 9 = higher concentration of sodium carbonate (5% w/w), Bt 8 = lower concentration of sodium carbonate plus a lower concentration of sugar syrup (1.0% w/w + 1.0% w/w), Bt 7 = lower concentration of sodium carbonate (1.0% w/w) and Bt 5 = standard concentration (3% w/w).

When the gums were chewed *in vitro,* the pH of the artificial saliva increased for each batch (Figure 6.25). Greatest increase in pH was observed from Bt 9 (pH increase of 2.097 pH units after 30 minutes) due to the higher concentration of sodium carbonate within the formulation. Lowest pH increase was observed using Bt 8 (approximately pH 1.1) whilst Bt 7, although containing a lower concentration of sodium carbonate gave a similar increase in pH to Bt 5 (approximately 1.5 pH units).

		Sodium carbonate concentration			
		Bt 7	**Bt 8**	**Bt 9**	**Bt 5**
Sodium carbonate concentration	**Bt 7**		37.43	36.83	40.08
	Bt 8	37.43		22.74	23.96
	Bt 9	36.83	22.74		52.06
	Bt 5	40.08	23.96	52.06	

Key

Dissolution curves not similar

Dissolution curves similar

Dissolution curves not compared

Table 6.14 f_2 values highlighting the similar and dissimilar dissolution profiles of the nicotine gums formulated using different concentrations of sodium carbonate.

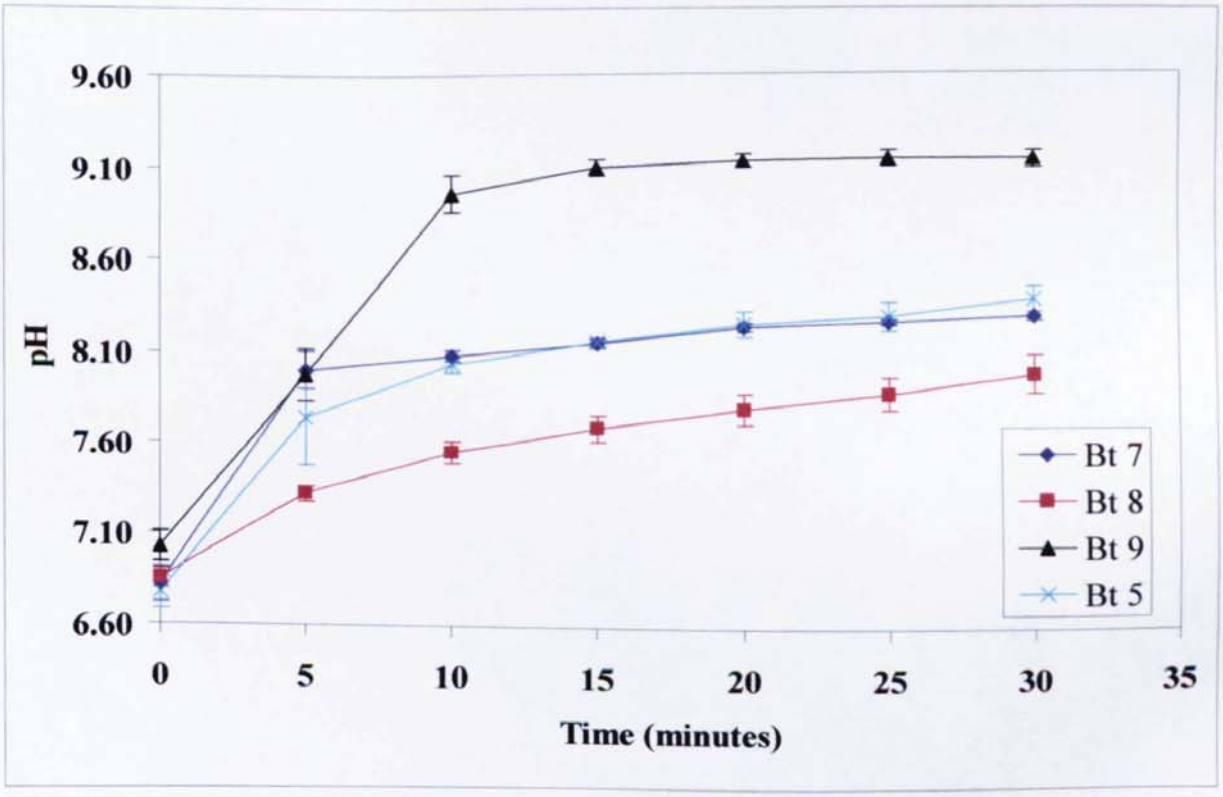
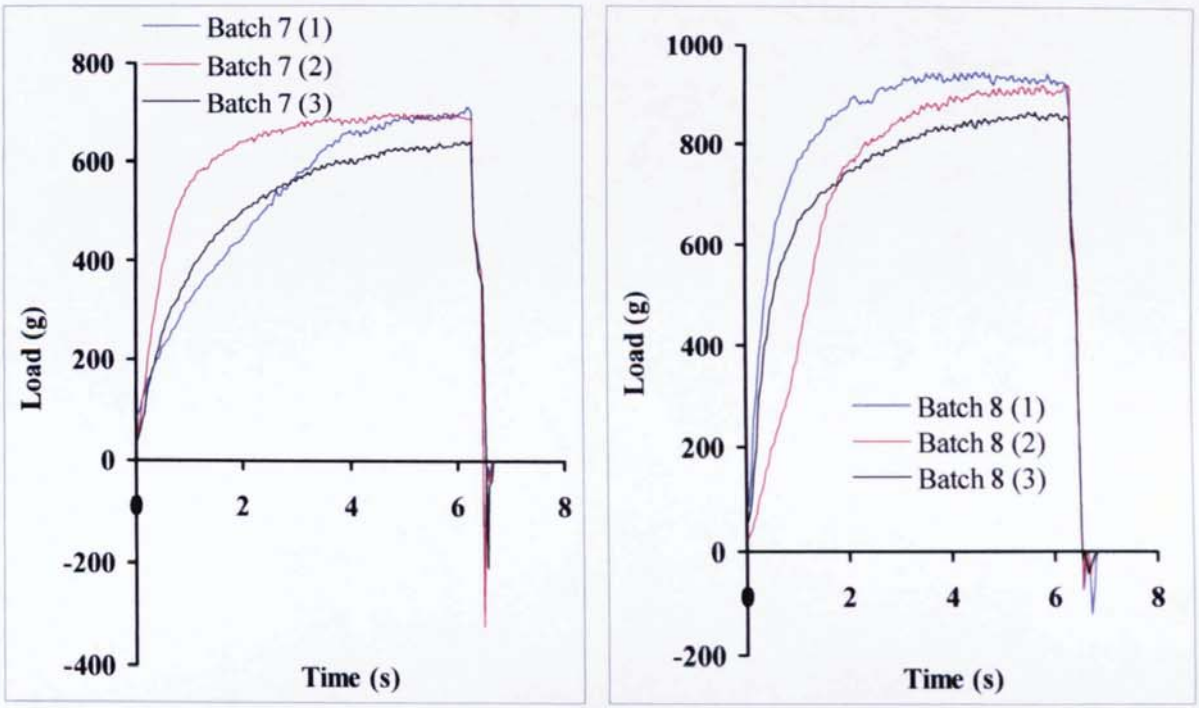
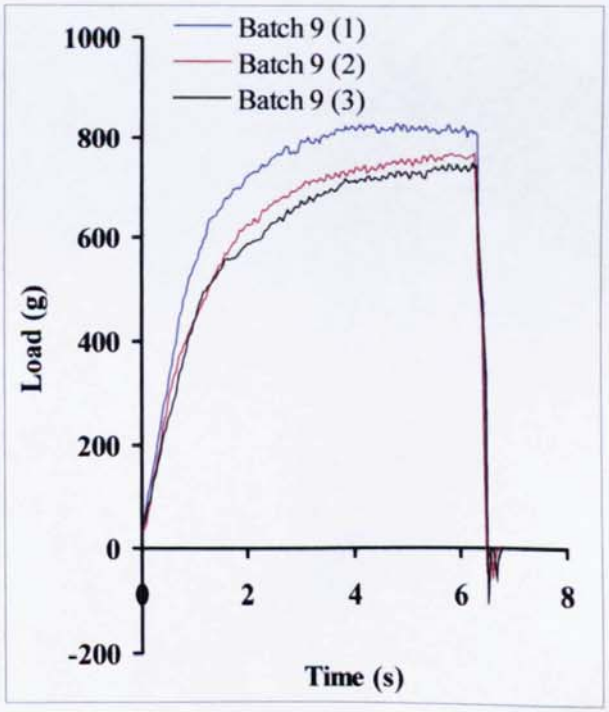


Figure 6.25 pH of the artificial saliva as nicotine was release from formulated gum with increased and decreased concentration of sodium carbonate (n =3 ± SD). Bt 9 = higher concentration of sodium carbonate (5% w/w), Bt 8 = lower concentration of sodium carbonate plus a lower concentration of sugar syrup (1.0% w/w + 1.0% w/w), Bt 7 = lower concentration of sodium carbonate (1.0% w/w) and Bt 5 = standard concentration (3% w/w).



(a)

(b)



(c)

Figure 6.26 Load encountered as a 2 mm diameter stainless steel probe penetrated batches 7 (a), 8 (b) and 9 (c) to a depth of 3 mm at 30 mm/minute.

Texture analysis showed small differences in the texture of the chewing gums (Figure 6.26 and Table 6.15). As the texture analysis probe penetrates the gum, the load *vs.* time profile suggests that the gums were of uniform hardness as the force encountered by the probe showed very little variation. Generated hardness, adhesive force and load *per* unit time

values showed that Bt 8 with reduced quantities of sodium carbonate and sugar syrup, produced a harder, firmer and less adhesive gum. Statistically it was found that in terms of hardness, Bt 8 was significantly different from Bt 7 and Bt 9, but, similar to the standard gum (Table 6.15a). Addition of a lower concentration of sodium carbonate without altering the concentration of the sugar syrup gave a softer, less rigid and more adhesive formulation compared to the standard gums whilst, increasing the concentration of sodium carbonate produced a less adhesive and rigid gum in comparison to the standard formulation. Although the values indicating the gums rigidity differed for the different formulations, statistical comparison tests showed that they were similar for all formulations whilst, in terms of adhesiveness, Bt 7 was significantly more adhesive than Bt 8 and 9, but showed no difference to Bt 5.

	Hardness (N)		**Adhesive force (N)**		**Load *per* unit time (g/s)**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Bt 5	8.19	0.32	-1.85	0.63	345.59	86.93
Bt 7	6.67	0.29	-2.56	0.46	274.04	143.83
Bt 8	8.93	0.33	-0.76	0.32	489.54	109.06
Bt 9	7.68	0.33	-0.74	0.20	309.66	79.95

Table 6.15 Hardness, adhesive forces and load *per* unit time measurements of nicotine gum batches formulated using different concentrations of sodium carbonate. Bt 9 = higher concentration of sodium carbonate (5% w/w), Bt 8 = lower concentration of sodium carbonate plus a lower concentration of sugar syrup (1.0% w/w + 1.0% w/w), Bt 7 = lower concentration of sodium carbonate (1.0% w/w) and Bt 5 = standard concentration (3% w/w).

Comparison	**Hardness P value**	**Adhesive force P value**	**Load *per* unit time P value**
Bt 5 *vs* Bt 7	< 0.01	> 0.05	> 0.05
Bt 5 *vs* Bt 8	> 0.05	> 0.05	> 0.05
Bt 5 *vs* Bt 9	> 0.05	> 0.05	> 0.05
Bt 7 *vs*. Bt 8	< 0.001	< 0.01	> 0.05
Bt 7 *vs*. Bt 9	< 0.05	< 0.01	> 0.05
Bt 8 *vs*. Bt 9	< 0.01	> 0.05	> 0.05

Table 6.15a Significance levels for the hardness, adhesive force and load *per* unit time of gums made using different concentrations of sodium carbonate. Output calculated using Instat Tukey-Kramer multiple comparison test.

Possible explanations for the differences observed in the release profiles and the texture of the different formulation can be explained in terms of the concentration of sodium carbonate added to the formulations. When the gums are chewed *in vitro* or are in contact with water, water-soluble sodium carbonate will dissolve resulting in the formation of micro-pores within the matrix of the gum. The formation of the micro-pores will provide small channels within the gum matrix for nicotine to be released. As the concentration of sodium carbonate increases the number of micro-pores will consequently increase thus providing a larger number of pores from which nicotine can be released. The concentration of added excipients will also affect the overall texture of the gums. Generally, as soluble ingredients are added to the gum base mix, due to their hydrophilic nature the overall hardness of the gum was reduced. Because of the reduced quantities of sodium carbonate and sugar syrup within Bt 8, the gums formed were harder, more rigid and less adhesive than the standard formulations. As a greater ratio of wet (sugar syrup) to dry (sodium carbonate) ingredients were added into formulation Bt 7, the resulting gum was softer and more adhesive. It can be said that the concentration of sodium carbonate added to the gum not only affected the release of nicotine from the formulation but also produced small changes in the gum texture.

As reported previously (section 3.4), the release of nicotine from 2 mg and 4 mg Nicorette® *in vivo* and *in vitro* was found to give different release profiles, with a greater % release with use of 4 mg Niocrette. Differences in the two gums are evident in their formulation. Not only is the concentration of nicotine added to the gum different but, also, the incorporated buffers used in the gum formulation differ. The hypothesis was that the difference in release rate from these chewing gums was due to the different buffers used in the gum formulations. Formulation Bt 15 was a 4 mg gum made using the buffer usually incorporated into a 2 mg formulation (10 mg sodium bicarbonate and 20 mg sodium carbonate) whilst Bt 14 was a 2 mg gum with 30 mg of sodium carbonate normally added to a 4 mg gum.

Release of nicotine from Bt 15 and Bt 5 (standard formulation with 4 mg nicotine) was similar (f_2 = 71.79) whilst Bt 14 was dissimilar (f_2 = 47.05) (Figure 6.27 and Table 6.16). For the first 20 minutes of chewing, Bt 14 gave a slower, but steady increase in release whilst initial release from Bt 15 was faster which, after 15 minutes, reduced such that at the end of 30 minutes approximately 76% was released from both Bt 14 and 15. Comparison of the dissolution curved showed that Bt 15 and 14 were similar (f_2 = 51.28).

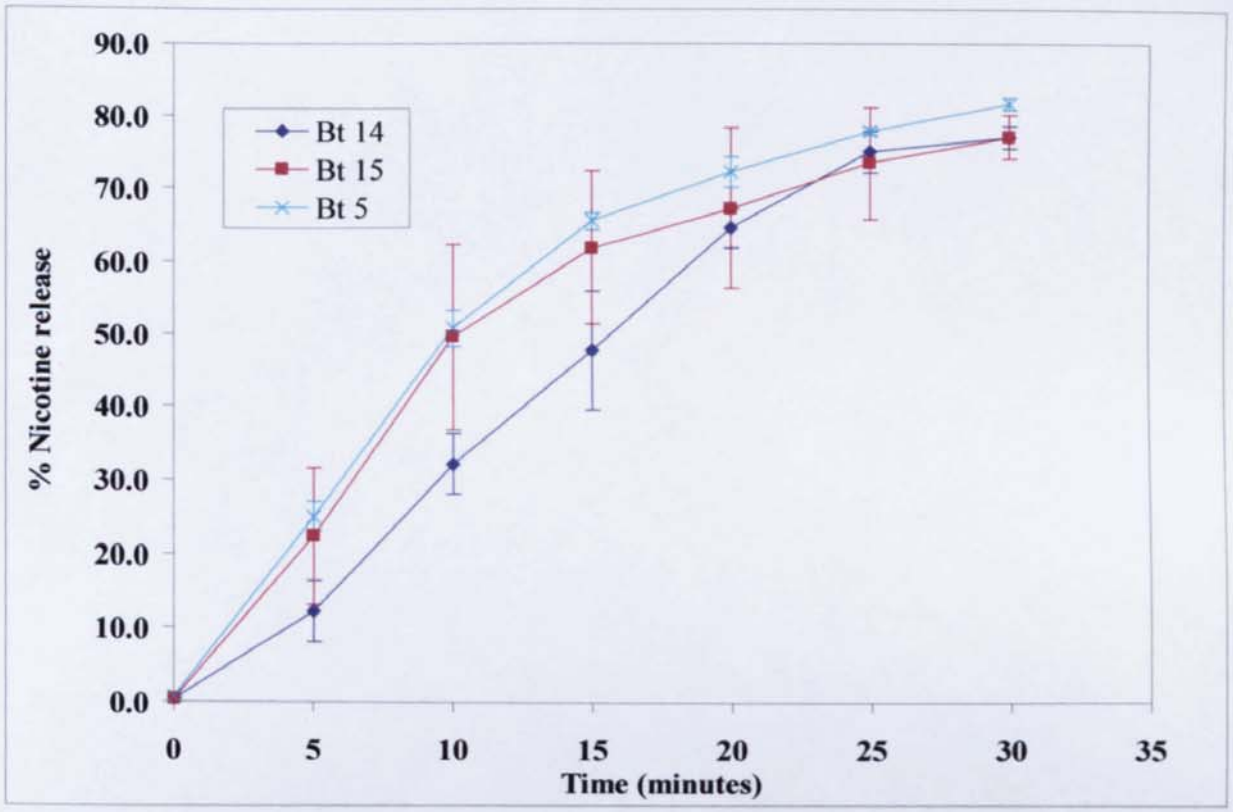


Figure 6.27 Release of nicotine from Bt 14, a 2 mg gum with incorporated 30 mg sodium carbonate and Bt 15, a 4 mg gum with incorporated 20 mg sodium carbonate and 10 mg sodium bicarbonate (n =3 ± SD).

		2 mg and 4 mg gums		
		Bt 5	**Bt 14**	**Bt 15**
2 mg and 4 mg gums	**Bt 5**		47.05	71.79
	Bt 14	47.05		51.28
	Bt 15	71.79	51.28	

Key

Dissolution curves not similar

Dissolution curves similar

Dissolution curves not compared

Table 6.16 f_2 values highlighting the similar and dissimilar dissolution profiles of Bt 14, a 2 mg nicotine gum with incorporated 30 mg sodium carbonate and Bt 15 a 4 mg gum with incorporated 20 mg sodium carbonate and 10 mg sodium bicarbonate.

Artificial saliva showed a greater pH change from Bt 14 (2 mg gum with 30 mg sodium carbonate) compared to the standard gum formulation and Bt 15 (Figure 6.28). Within the first 5 minutes, the pH of the artificial saliva was similar (approximately pH 7.7), but after 10 minutes increase in pH from Bt 15 and Bt 5 began to plateau whilst pH observed from Bt 14 increased.

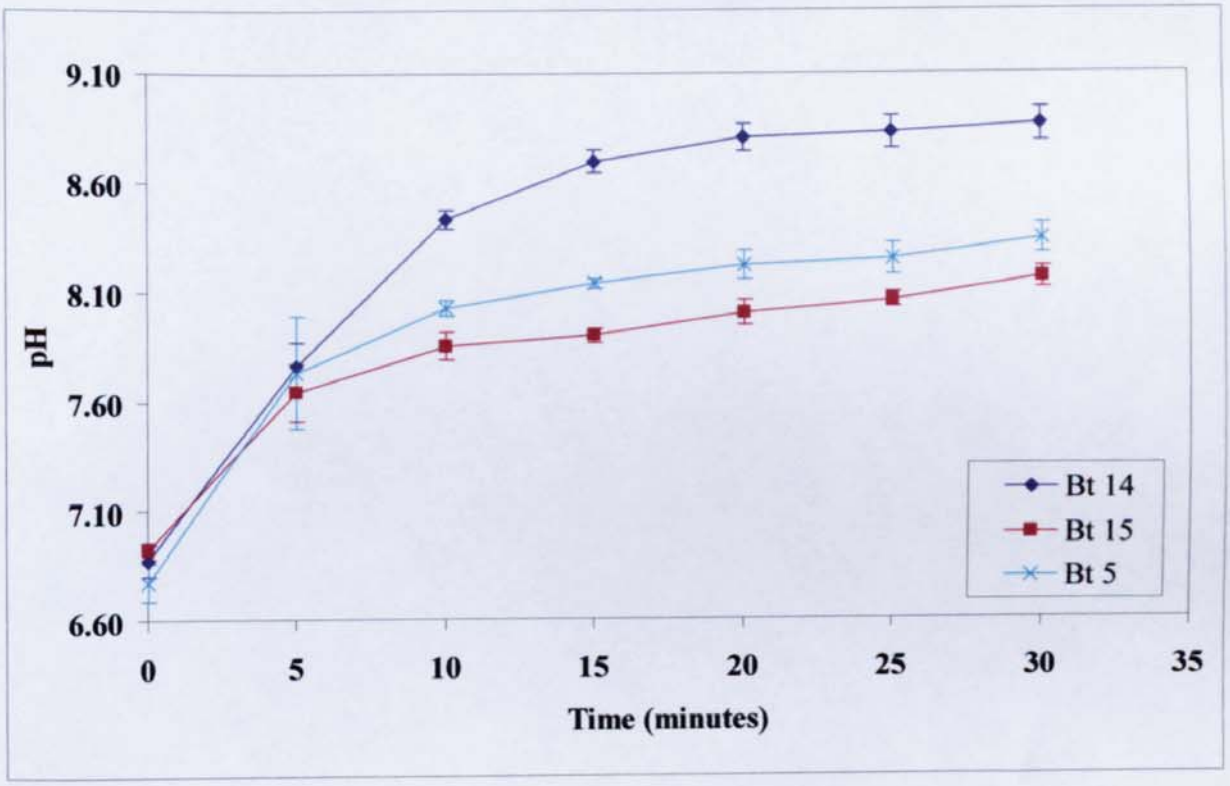
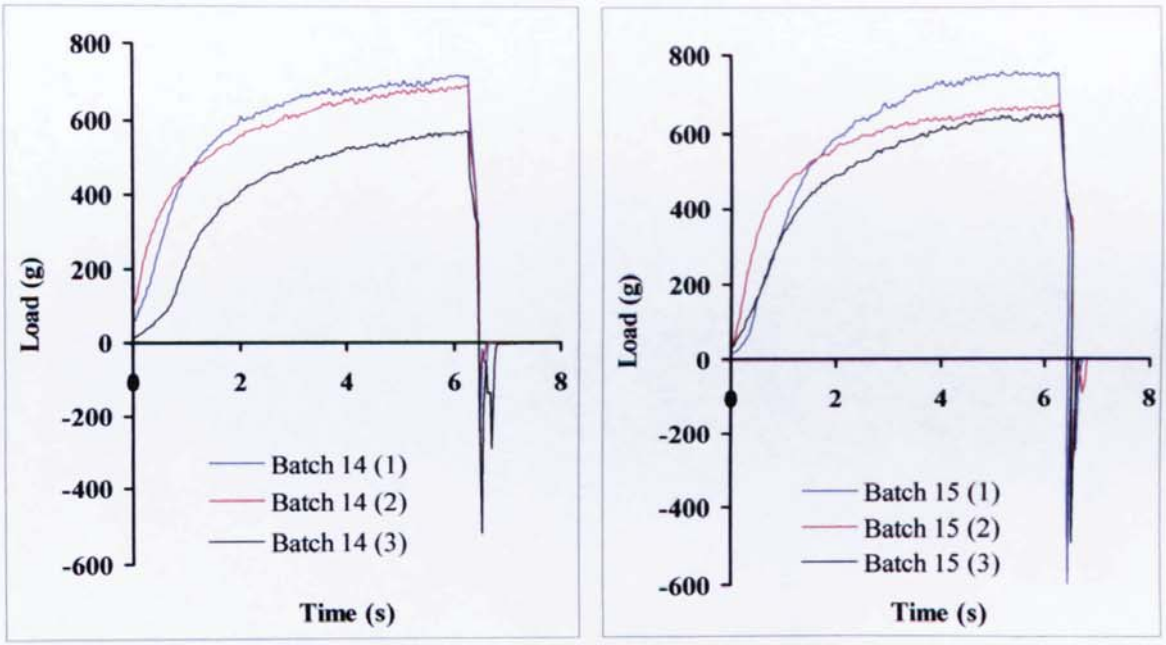


Figure 6.28 pH of artificial saliva as batch 14, a 2 mg gum with incorporated 30 mg sodium carbonate and Bt 15, a 4 mg gum with incorporated 20 mg sodium carbonate and 10 mg sodium bicarbonate were chewed *in vitro* (n=3 ± SD).

The reason for the greater increase in pH can be explained in terms of the alkaline buffers used in the formulations. The pH of a 0.1 M sodium bicarbonate and sodium carbonate solution has been reported as pH 8.4 and pH 11.6 respectively (http://chemed.chem.purdue.edu). The intended function of the buffers is presumably two-fold, *i.e.* to increase the pH of the oral cavity to optimise nicotine absorption and to provide a source of cations to facilitate the release of nicotine from the resinate. As nicotine salt is a weak base, the release of nicotine into solution will result in a decrease in pH. To compensate for the decrease in pH, 30 mg of sodium carbonate (stronger of the two alkaline salts) was added to the 4 mg Nicorette® formulation to increase the pH and, at the lower concentration of nicotine (2 mg gum), a lesser alkaline buffer was required to increase the pH. Thus, a mixture of 20 mg

sodium carbonate and 10 mg sodium bicarbonate (weaker alkaline salt) was added to increase the pH. Results obtained from Bt 14 showed a greater increase in pH as 30 mg of sodium carbonate was added to lower nicotine strength.

Texture profiles of the gum shows that there were large variations in replicate samples in terms of hardness and adhesiveness (Figure 6.29 and Table 6.17). Gums from Bt 14 and 15 were generally more adhesive and softer than the standard nicotine gum formulation and values of the load *per* unit time also indicate that the standard nicotine gum formulation was more resistant deformation than Bt 14 and 15.



(a) (b)

Figure 6.29 Texture profile of batch 14 (a) and 15 (b) as a 2 mm diameter stainless steel probe penetrated the gum to a depth of 3mm.

	Hardness (N)		Adhesive force (N)		Load *per* unit time (g/s)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Bt 5	8.19	0.32	-1.85	0.63	345.59	86.93
Bt 14	6.50	0.62	-2.85	1.69	236.02	42.19
Bt 15	6.81	0.44	-4.61	1.14	243.31	31.79

Table 6.17 Hardness, adhesive forces and load *per* unit time of nicotine gum Bt 14, a 2 mg gums with incorporated 30 mg sodium carbonate and Bt 15, a 4 mg gum with incorporated 20 mg sodium carbonate and 10 mg sodium bicarbonate.

Comparison	Hardness	Adhesive force	Load *per* unit time
	P value	P value	P value
Bt 5 *vs* Bt 14	< 0.05	> 0.05	> 0.05
Bt 5 *vs* Bt 15	< 0.05	> 0.05	> 0.05
Bt 14 *vs* Bt 15	> 0.05	> 0.05	> 0.05

Table 6.17a Significance levels for the hardness, adhesive force and load *per* unit time of Bt 14, a 2 mg gums with incorporated 30 mg sodium carbonate and Bt 15, a 4 mg gum with incorporated 20 mg sodium carbonate and 10 mg sodium bicarbonate. Output calculated using Instat Tukey-Kramer multiple comparison test.

Comparisons of the texture data, using the Tukey-Kramer multiple comparison test showed there was no significant difference in the adhesiveness and resistance to deformation between Bt 5, Bt 14 and Bt 15, however hardness values showed both Bt 14 and Bt 15 were significantly softer compared to the standard gum formulation (Table 6.17a).

Results showed that the release of nicotine from Bt 14 (2 mg gum) and the standard gum were dissimilar but was similar to Bt 15 although different buffers were incorporated into the gum formulation. However, a greater pH increase was observed when chewing Bt 14 (2 mg gum with 30 mg sodium carbonate). It can be hypothesised that the difference in the buffer composition in the commercial Nicorette® gum was to ensure a similar pH increase from both 2 mg and 4 mg nicotine gum in the oral cavity to allow optimised conditions for nicotine absorption.

6.3.2.4 Effect of different concentrations of sugars

The effects of increasing and decreasing the concentration of sugar syrup in chewing gum formulations showed little effect on the release of nicotine from the gum (Figure 6.30). Bt 6 (1.0% w/w sugar syrup) and Bt 10 (5.5% w/w sugar syrup) produced very similar release profiles to standard gum formulations which contained 2.5% w/w sugar syrup (Bt 5). After 30 minutes, 91.15% and 88.07% were released from Bt 10 and Bt 6 respectively in comparison to 81.45% from the standard gum formulations. Dissolution curve comparison showed that release from Bt 6 and Bt 10 was similar to the standard gum ($f_2 > 50$; Table 6.18). Bt 8, gums formulated with a lower concentration of sugar syrup and a lower concentration of sodium carbonate, showed that less than half the nicotine was released from this formulation compared to Bt 5, 6, and 10 ($f_2 < 50$).

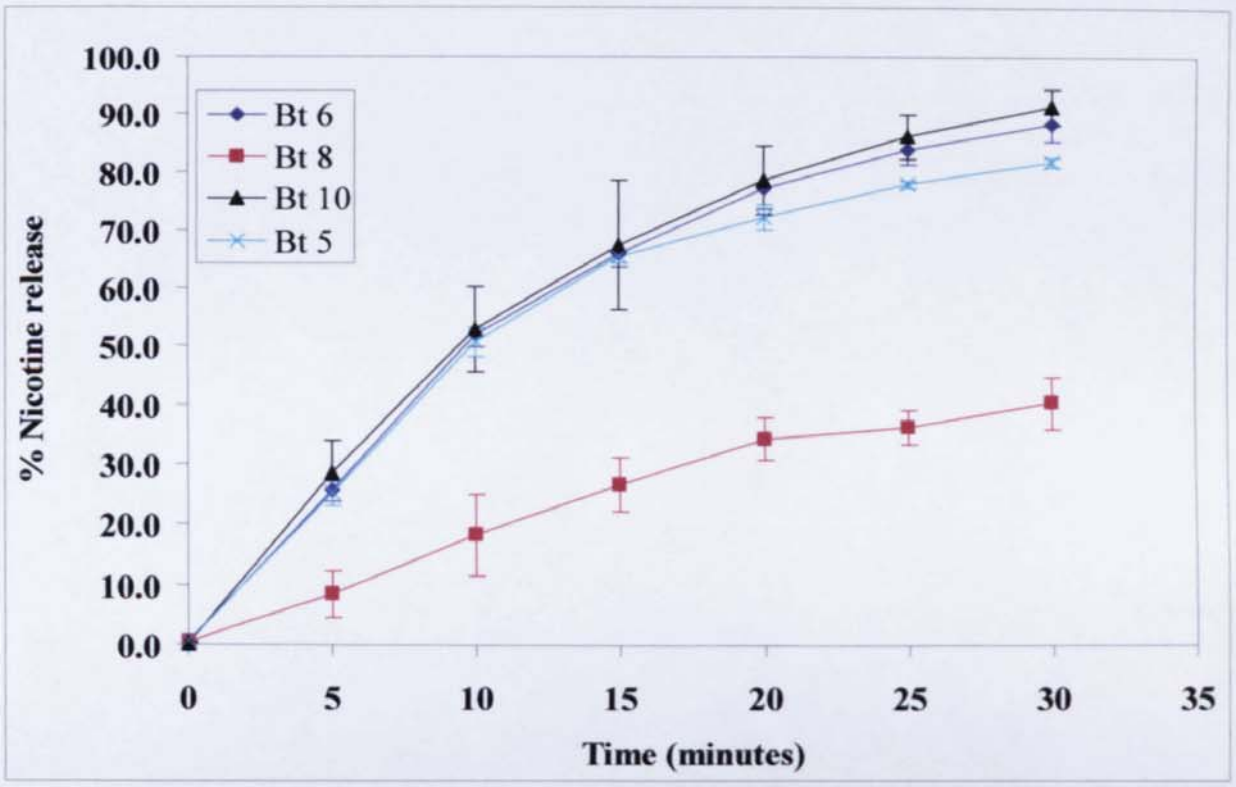


Figure 6.30 Release of nicotine from formulations with different concentrations of sugar syrups (n =3 ± SD). Bt 6 = 1.0% w/w sugar syrup, Bt 10 = 5.5% w/w sugar syrup, Bt 8 = 1.0% w/w sugar syrup and 1.0% w/w sodium carbonate and Bt 5 = 2.5% sugar syrup.

		Sugar concentration			
		Bt 6	**Bt 8**	**Bt 10**	**Bt 5**
Sugar concentration	**Bt 6**		21.86	82.24	69.85
	Bt 8	21.86		20.81	23.96
	Bt 10	82.24	20.81		62.08
	Bt 5	69.85	23.96	62.08	

Key

Dissolution curves not similar

Dissolution curves similar

Dissolution curves not compared

Table 6.18 f_2 values highlighting the similar and dissimilar dissolution profiles of Bt 6, gums with 1.0% w/w sugar syrup, Bt 10 gums with 5.5% w/w sugar syrup, Bt 8 gums with 1.0% w/w sugar syrup and 1.0% w/w sodium carbonate and Bt 5 gums with 2.5% sugar syrup.

Increases in pH also show that the Bt 6 and 10 were similar (Figure 6.31). At 30 minutes the pH of the artificial saliva increased by 1.387 and 1.458 pH units when Bt 6 and 10 were chewed respectively in comparison to an overall pH increase of 1.589 pH units observed from the standard nicotine gum formulation. Bt 8 had the lowest change in pH with an overall increase of approximately pH 1.1.

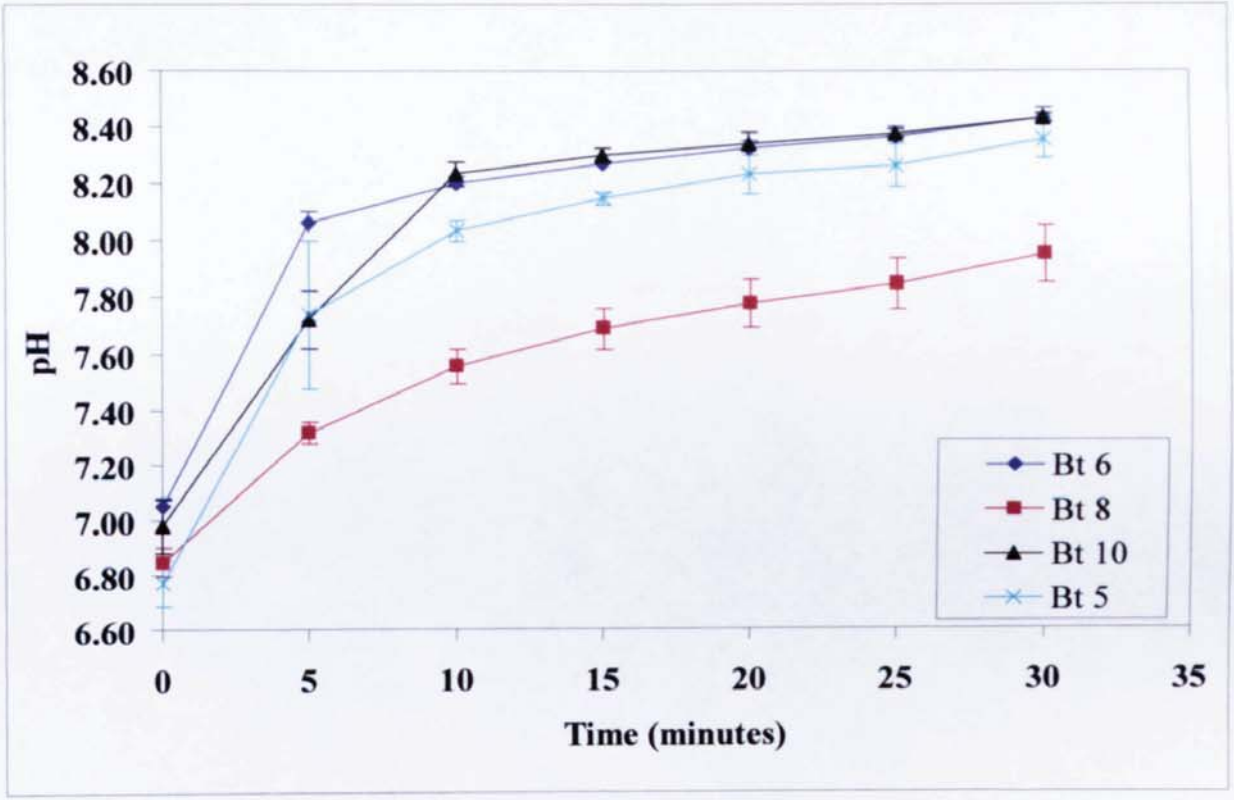
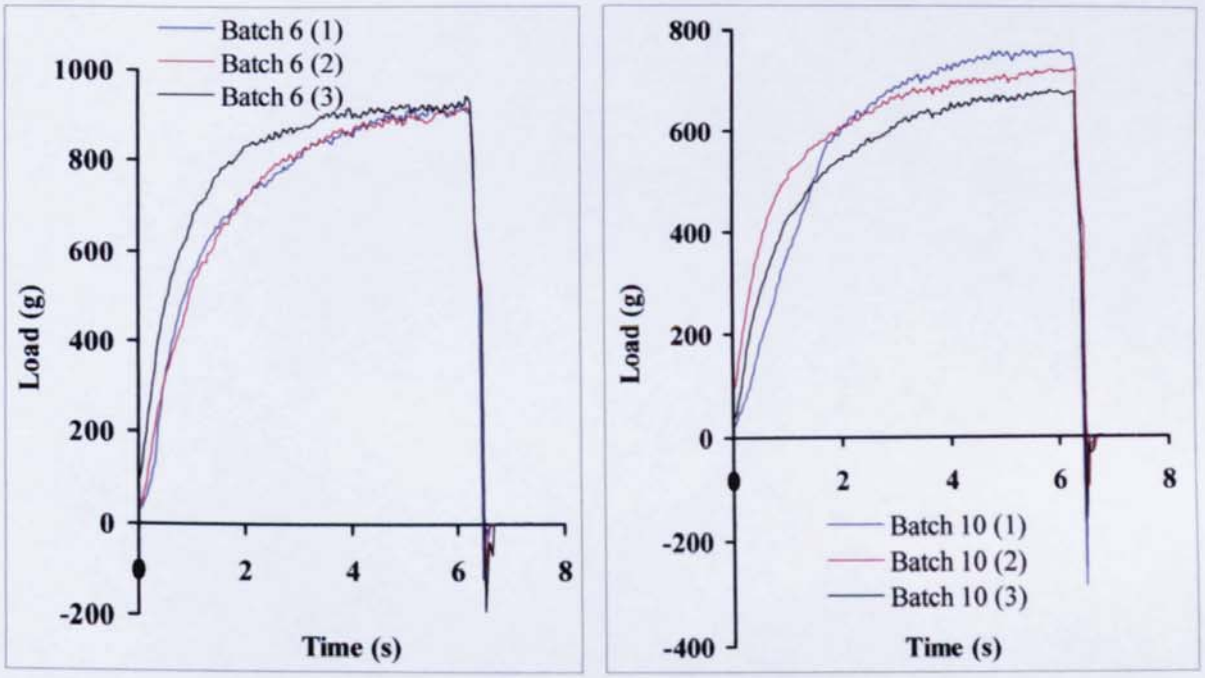


Figure 6.31 pH of artificial saliva as Bt 6, gums with 1.0% w/w sugar syrup, Bt 10 gums with 5.5% w/w sugar syrup, Bt 8 gums with 1.0% w/w sugar syrup and 1.0% w/w sodium carbonate and Bt 5 gums with 2.5% sugar syrup were chewed *in vitro* (n =3 ± SD).

Sweeteners and sugar syrups are added to gum formulations to enrich the flavour and provide enjoyable chewing quality but also to help soften the gum and keep it fresh and flexible. Release data highlight that altering the concentration of the sugar syrup did not appear to have an effect on the release of nicotine from the gum but influence the texture of the gums (Figure 6.32 and Table 6.19). Increasing the concentration of the sugar syrup (Bt 10) produced a softer, less rigid gum whilst decreasing the concentration (Bt 6) resulted in a harder more rigid gum. Reducing the concentration of sodium carbonate or sugar syrup separately did not appear to affect the release of nicotine to a great extent. However, a combination of the two (reduced sodium carbonate and sugar syrup) resulted not only in a lower release of nicotine but a harder more rigid and less adhesive gum.



(a) (b)

Figure 6.32 Texture analysis profile of Bt 6 (a) and 10 (b) as a 2 mm diameter stainless steel probe penetrated the gums to a depth of 3 mm at 30 mm/min.

	Hardness (N)		Adhesive force (N)		Load *per* unit time (g/s)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Bt 5	8.19	0.32	-1.85	0.63	345.59	86.93
Bt 6	9.05	0.11	-1.32	0.41	351.70	64.11
Bt 8	8.93	0.33	-0.76	0.32	489.54	109.06
Bt 10	7.10	0.32	-1.75	0.78	264.78	21.79

Table 6.19 Hardness, adhesive forces and load *per* unit time of nicotine gum batches 5, 6, 8 and 10

Comparison of the texture profiles of the gum found that, statistically, all the gums with the exception of Bt 6 and 8 were significantly different in term of hardness ($P < 0.005$), whilst Bt 6 and Bt 8, gums with a lower concentration of sugar syrup produced harder gums which were not similar (Table 6.19a). Statistical comparisons of the adhesive force and the resistance to deformation showed that there was no difference between the different gum formulations ($P > 0.05$) with the exception of Bt 8 being significantly more rigid than Bt 10.

Comparison	Hardness	Adhesive force	Load *per* unit time
	P value	P value	P value
Bt 5 *vs* Bt 6	< 0.05	> 0.05	> 0.05
Bt 5 *vs* Bt 8	< 0.05	> 0.05	> 0.05
Bt 5 *vs* Bt 10	< 0.01	> 0.05	> 0.05
Bt 6 *vs* Bt 8	> 0.05	> 0.05	> 0.05
Bt 6 *vs* Bt 10	< 0.001	> 0.05	> 0.05
Bt 8 *vs* Bt 10	< 0.001	> 0.05	< 0.05

Table 6.19a Significance levels for the hardness, adhesive force and load *per* unit time measurements of Bt 6, gums with 1.0% w/w sugar syrup, Bt 10 gums with 5.5% w/w sugar syrup, Bt 8 gums with 1.0% w/w sugar syrup and 1.0% w/w sodium carbonate and Bt 5 gums with 2.5% sugar syrup. Output calculated using Instat Tukey-Kramer multiple comparison test.

6.3.2.5 Effect of addition of surfactants

Previous experiments illustrated that the addition of surfactant to the dissolution medium increased release of nicotine *in vitro* from the chewing gum but had little effect on the release of nicotine from the resin (section 3.6.2.2). It was therefore proposed that the surfactant had an effect on the gum base which allowed nicotine to be released from the gum at a faster rate.

When Tween 80 (a non ionic surfactant) was incorporated into a chewing gum formulation, irrespective of the sugar syrup and sodium carbonate concentration, the release from the gums were similar (Figure 6.33). Release from Bt 8 (section 6.3.2.4) with reduced quantities of sodium carbonate and sugar syrup showed approximately 40% release after 30 minutes. The same formulation but with addition of 0.1% w/w Tween 80 surfactant (Bt 4) gave 91.98% release after 30 minutes; thus, the incorporation of surfactant increased the release of nicotine from the gums dramatically. Dissolution curve comparison showed that with the addition of 0.1% Tween 80, irrespective of the reduction of sugar syrup or sodium carbonate the release profiles were similar ($f_2 > 50$; Table 6.20). When compared to the standard gum formulation (Bt 5), release from Bt 1 and Bt 2 was greater and were found to be dissimilar.

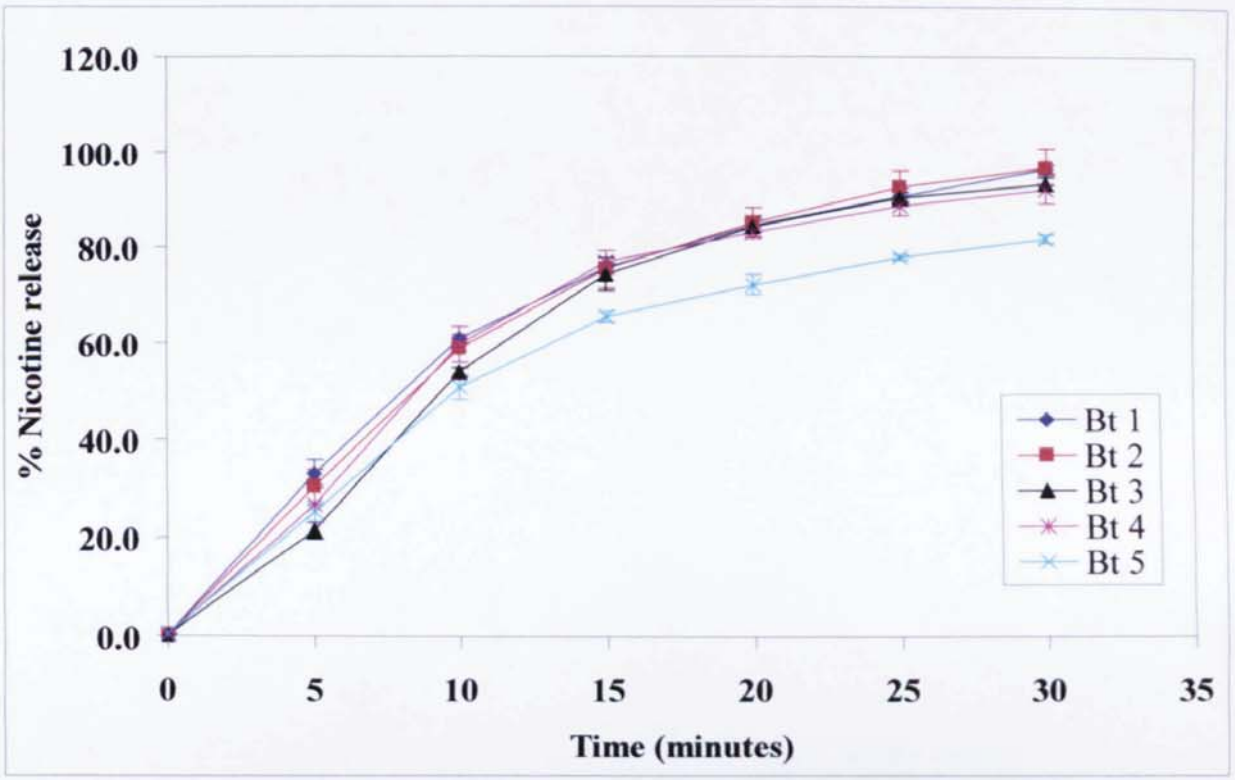


Figure 6.33 Release of nicotine with the addition of 0.1% w/w Tween 80 to the chewing gum formulations (n =3 ± SD). Bt 1 = standard gum with surfactant, Bt 2 = standard gum formulation with 1.0 % w/w sugar syrup and surfactant, Bt 3 = standard gum with 1.0% sodium carbonate and surfactant, Bt 4 = standard formulation with 1.0% w/w sodium carbonate, 1.0% w/w sugar syrup and surfactant and Bt 5 = standard gum formulation.

		Surfactant concentration				
		Bt 1	**Bt 2**	**Bt 3**	**Bt 4**	**Bt 5**
Surfactant concentration	**Bt 1**		86.79	63.59	74.92	48.59
	Bt 2	86.79		68.31	75.99	48.41
	Bt 3	63.59	68.31		73.35	52.73
	Bt 4	74.92	75.99	73.35		52.40
	Bt 5	48.59	48.41	52.73	52.40	

Key

Dissolution curves not similar

Dissolution curves similar

Dissolution curves not compared

Table 6.20 f_2 values highlighting the similar and dissimilar dissolution profiles of Bt 1, Bt 2, Bt 3, Bt 4 and Bt 5.

Increases in the pH of the artificial saliva when the different gum formulations were chewed showed how the pH profiles of Bt 1, 2 and 5 were similar (Figure 6.34). Within the first 5 minutes, there was a rapid increase in pH which then reduced to give final pHs of pH 8.272 (Bt 1), pH 8.298 (Bt 2) and pH 8.360 (Bt 5). Lower increases in pH were observed from Bt 3 and Bt 4 which both contained a lower quantity (1.0% w/w) of sodium carbonate. Bt 3 and 4 pH profiles show that between 5 to 15 minutes the pH remained steady (approximately pH 7.5) and then increased slowly to give final pH values of pH 7.839 and pH 7.878 for Bt 3 and Bt 4 respectively.

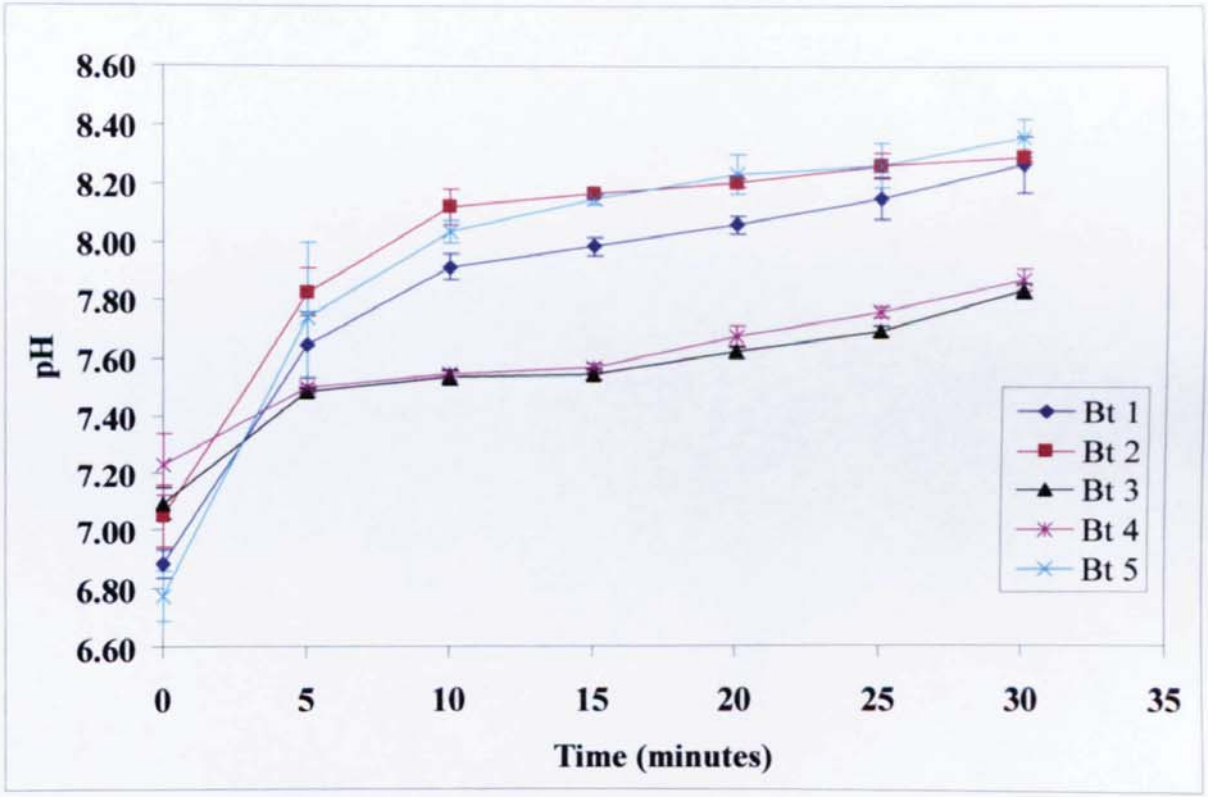


Figure 6.34 pH of artificial saliva as nicotine was released from formulated gum with added Tween 80 (n =3 ± SD). Bt 1 = standard gum with surfactant, Bt 2 = standard gum formulation with 1.0 % w/w sugar syrup and surfactant, Bt 3 = standard gum with 1.0% sodium carbonate and surfactant, Bt 4 = standard formulation with 1.0% w/w sodium carbonate, 1.0% w/w sugar syrup and surfactant and Bt 5 = standard gum formulation.

Texture analysis of the formulated gums highlighted that the addition of 0.1% w/w Tween 80 reduced the hardness of the gums (Table 6.21 and Figure 6.35). Tween 80 behaves in a similar way to sugar syrup with respect to the texture of the gum. It is a viscous liquid and when added to the gums will soften and increase the flexibility of the gum base thus reducing the rigidity and hardness of the gum compared to the standard formulation. Statistical comparison of the texture data showed that there was no significant difference in the

adhesiveness and load *per* unit time measurements of the gums with added surfactant, and also, Bt 3 and Bt 1 were significantly softer in comparison to the standard gums (Table 6.21a).

	Hardness (N)		Adhesive force (N)		Load *per* unit time (g/s)	
	Mean	SD	Mean	SD	Mean	SD
Bt 1	7.09	0.26	-0.79	0.37	208.90	18.56
Bt 2	7.44	0.29	-0.37	0.13	351.43	67.54
Bt 3	6.06	0.21	-1.11	0.55	264.83	4.13
Bt 4	8.16	0.54	-3.19	1.67	445.14	133.68
Bt 5	8.19	0.31	-1.85	0.63	345.59	86.93

Table 6.21 Hardness, adhesive forces and load *per* unit time measurements of nicotine gum of Bt 1, standard gums formulated with surfactant, Bt 2, standard gum formulation with 1.0 % w/w sugar syrup and surfactant, Bt 3, standard gum with 1.0% sodium carbonate and surfactant, Bt 4, standard formulation with 1.0% w/w sodium carbonate, 1.0% w/w sugar syrup and surfactant and Bt 5 the standard gum formulation.

Comparison	Hardness P value	Adhesive force P value	Load *per* unit time P value
Bt 5 *vs* Bt 1	< 0.05	> 0.05	> 0.05
Bt 5 *vs* Bt 2	> 0.05	> 0.05	> 0.05
Bt 5 *vs* Bt 3	< 0.001	> 0.05	> 0.05
Bt 5 *vs* Bt 4	> 0.05	> 0.05	> 0.05
Bt 1 *vs* Bt 2	> 0.05	> 0.05	> 0.05
Bt 1 *vs* Bt 3	< 0.05	> 0.05	> 0.05
Bt 1 *vs* Bt 4	< 0.05	< 0.05	< 0.05
Bt 2 *vs* Bt 3	< 0.01	> 0.05	> 0.05
Bt 2 *vs* Bt 4	> 0.05	< 0.05	> 0.05
Bt 3 *vs* Bt 4	< 0.001	> 0.05	> 0.05

Table 6.21a Significance levels for the hardness, adhesive force and load *per* unit time of Bt 1, standard gums formulated with surfactant, Bt 2, standard gum formulation with 1.0 % w/w sugar syrup and surfactant, Bt 3, standard gum with 1.0% sodium carbonate and surfactant, Bt 4, standard formulation with 1.0% w/w sodium carbonate, 1.0% w/w sugar syrup and surfactant and Bt 5 the standard gum formulation. Output calculated using Instat Tukey-Kramer multiple comparison test.

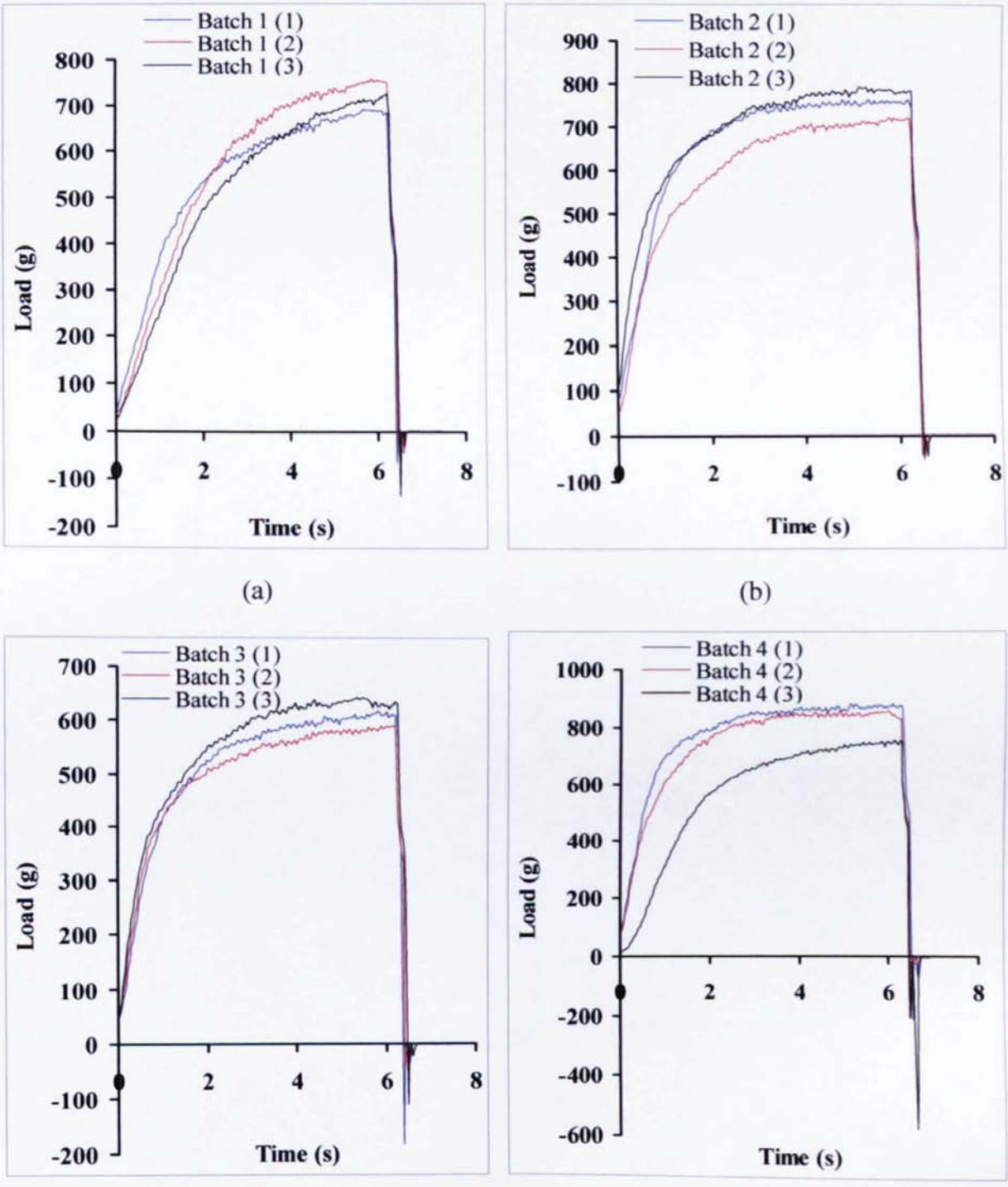


Figure 6.35 Texture profiles of Bt 1 (a), Bt 2 (b), Bt 3 (c) and Bt 4 (d) as a 2 mm diameter stainless steel probe penetrated the gums to a depth of 3 mm at 30 mm/min.

Formulations with lower concentrations of sodium carbonate were found to reduce the release of nicotine from the gums (section 6.3.2.3). In comparison with the same formulation which had surfactants incorporated into them, the release of nicotine from the gums increased. The exact role that the surfactant plays in increasing the release from the gums is not known. In a previous study (Anderson, *et al.,* 1990), the addition of Tween 60 to a chewing gum containing Nystatin increased the release by a factor of 50. Since Nystatin is a

poorly soluble drug in both water and saliva, the author suggested that the increase in release was due to the addition of Tween 60 which is a solubilising agent, thus promotes the release of the water insoluble Nystatin from the gum. Due to their amphipathic nature, surfactants are often employed as emulsifying agents, detergents, solubilising agents, suspension stabilisers, in drug absorption and as wetting agents in dosage forms as they alter the conditions prevailing at an interface, causing, for example, a marked decrease in the surface tension of water. It is possible that in this case the increase of nicotine from the gum could be a result of the surfactant altering the properties of the gum base by interacting with the components and making them softer to increase the release from the gum.

6.3.2.6 Effect of different forms of active ingredient

It is possible to increase or delay the release of an active drug substance by changing its physical form (Ellerman, 2002). Nicotine incorporated into chewing gum formulation in the form of Nicotine hydrogen tartrate salt (Bt 13), Nicotine Polacrilex (Bt 5) and nicotine Amberlite IRP 69, a strongly acidic ion exchange resin (Bt 12), showed that the release from the stronger resin was slower than that from the incorporated salt and Polacrilex resin (Figure 6.36).

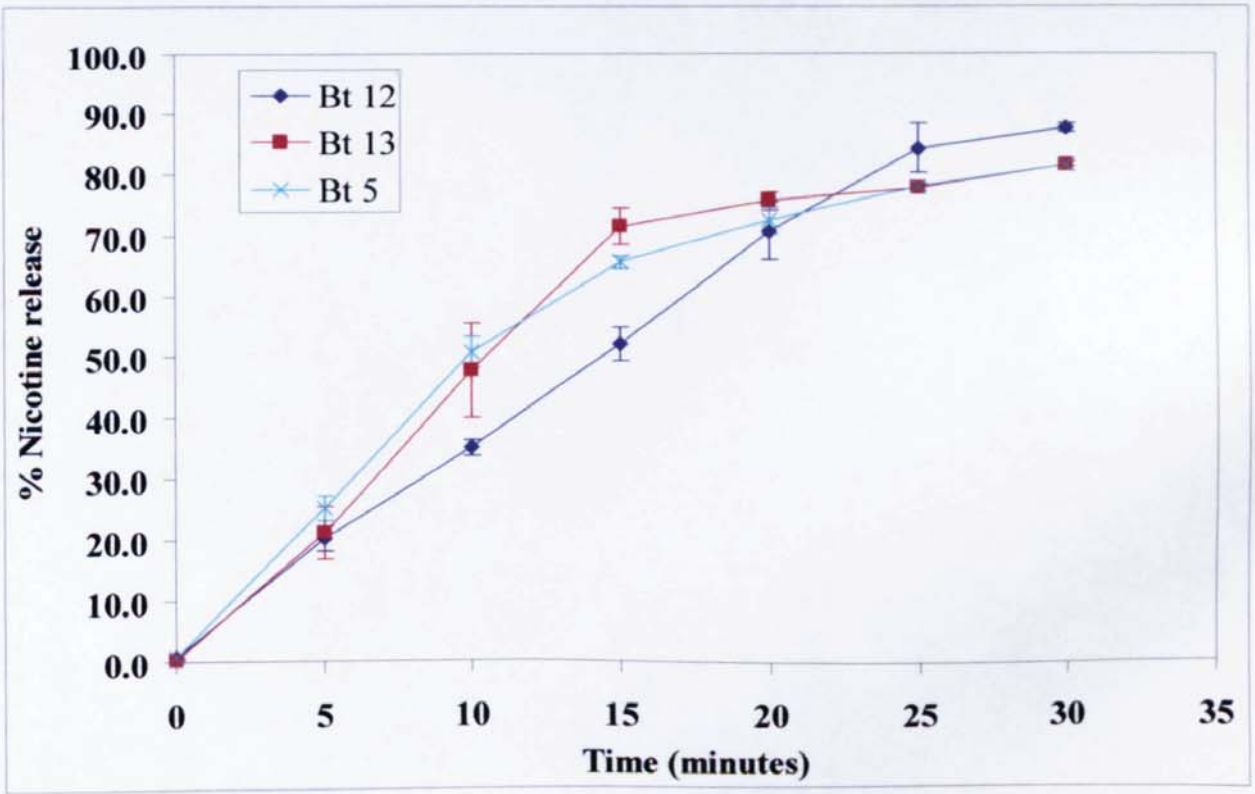


Figure 6.36 Release of nicotine from chewing gum containing Nicotine Polacrilex (Bt 5), nicotine Amberlite® IRP69 (Bt 12) and nicotine hydrogen tatrate salt (Bt 13) (n =3 ± SD).

Within the first 5 minutes of *in vitro* chewing, the release from the gums was similar, (approximately 20-25% released). A linear release of nicotine at a rate of 3.343% min^{-1} (r^2 = 0.9983) was observed from 0-25 minutes of chewing Bt 12 (stronger resin), after which, the rate decreased to give a final release of 87.55% after 30 minutes. The use of nicotine salt (Bt 13) increased release from the gum in comparison to the use of the stronger resin (Bt 12). After 15 minutes 71.35% of nicotine was released from the gum compared to 51.87% and 65.48% released from Bt 12 and Bt 5 (Nicotine Polacrilex) respectively. Dissolution curve comparisons showed that the release from all the gums were similar (Table 6.22).

		Different active form		
		Bt 12	**Bt 13**	**Bt 5**
Different active form	**Bt 12**		50.76	52.70
	Bt 13	50.76		73.82
	Bt 5	52.70	73.82	

Key

- Dissolution curves not similar
- Dissolution curves similar
- Dissolution curves not compared

Table 6.22 f_2 values highlighting the similar and dissimilar dissolution profiles of gums formulated using Nicotine Polacrilex (Bt 5), nicotine Amberlite® IRP69 (Bt 12) and nicotine hydrogen tartrate salt (Bt 13).

pH measurements of the artificial saliva when Bt 13 (nicotine hydrogen tartrate salt) was chewed *in vitro* increased by 2.171 pH units after 10 minutes and then decreased to give a final pH of 9.023 (Figure 6.37). In comparison, a steady increase in pH was observed from Bt 12 and Bt 5 which at the end of 30 minutes gave an overall pH increase of 1.889 and 1.590 pH units respectively.

The change in the physical form of the active drug added to the gums also resulted in textural differences in the gum (Figure 6.38 and Table 6.23). Gums formulated using the nicotine salt was softer than gums formulated using Nicotine Polacrilex and nicotine Amberlite® IRP69 resins. The use of nicotine Amberlite IRP 69 resin resulted in a harder and more rigid gum than those formulated using Nicotine Polacrilex. Statistical comparison showed that the

in terms of the gum hardness, all batches were significantly different, whilst, adhesive and rigidity comparisons showed no significant difference between the different batches (Table 6.23a).

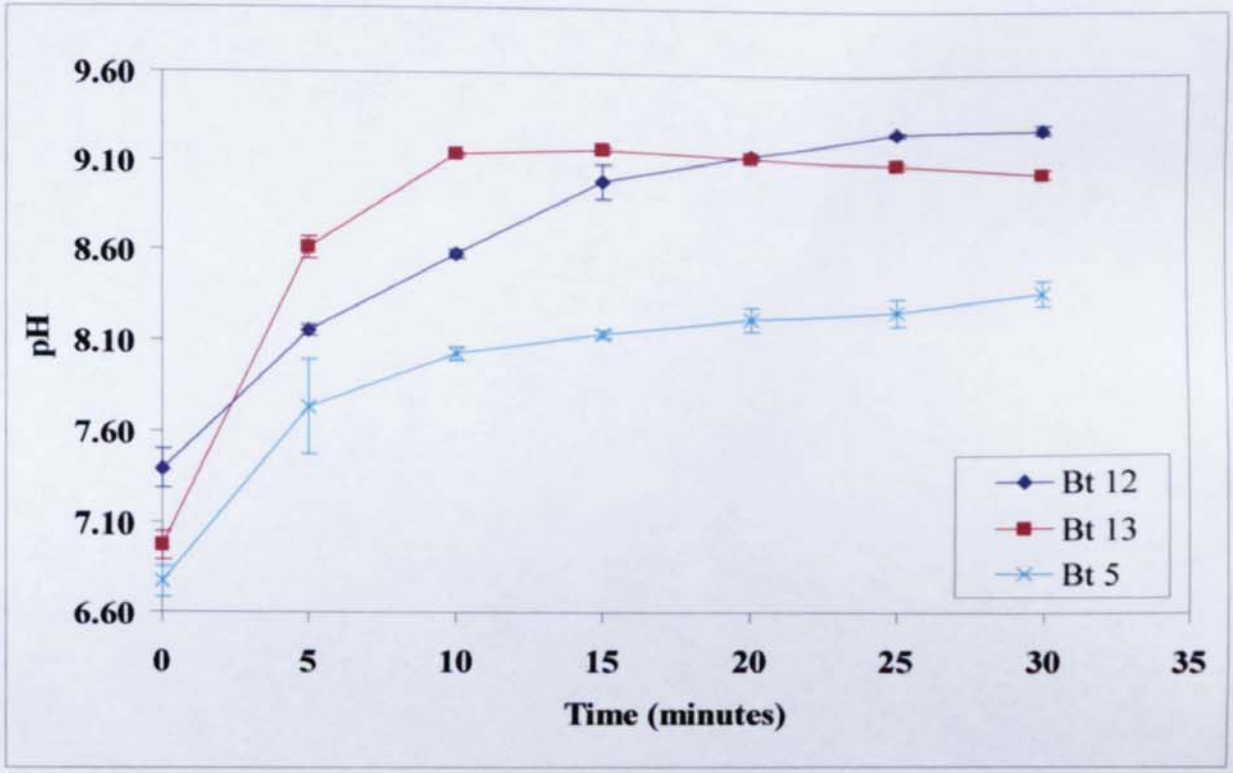
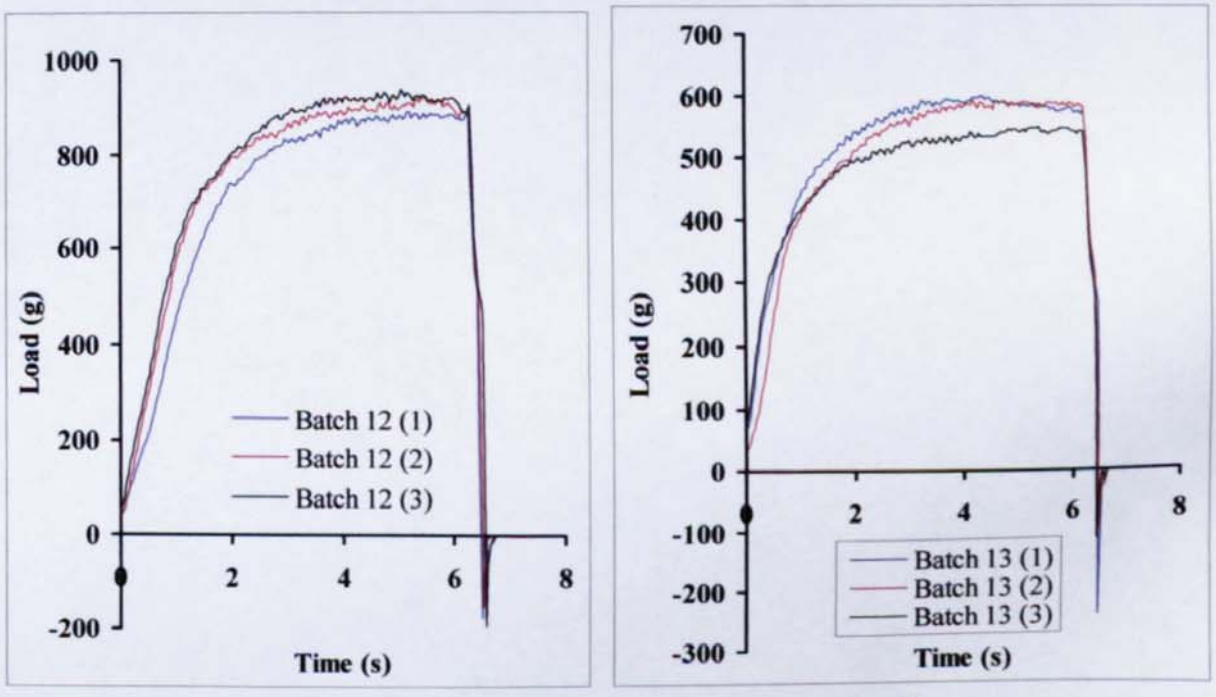


Figure 6.37 pH of the artificial saliva as nicotine from gums containing Nicotine Polacrilex (Bt 5), nicotine Amberlite® IRP69 (Bt 12) and nicotine hydrogen tartrate salt (Bt 13) were chewed *in vitro* (n =3 ± SD).



(a) (b)

Figure 6.38 Texture analysis of batch 12 (a) and batch 13 (b) as a 2 mm diameter stainless probe penetrated the gum to a depth of 3 mm at a rate of 30 mm/min.

	Hardness (N)		Adhesive force (N)		Load *per* unit time (g/s)	
	Mean	SD	Mean	SD	Mean	SD
Bt 5	8.19	0.32	-1.85	0.63	345.59	86.93
Bt 12	8.97	0.19	-1.70	0.17	424.93	38.66
Bt 13	5.69	0.21	-1.52	0.63	300.44	26.83

Table 6.23 Hardness, adhesive forces and the load encountered *per* unit time of nicotine gums Bt 5, gums with incorporated Nicotine Polacrilex, Bt 12, gums with incorporated nicotine Amberlite® IRP69 and Bt 13, gums with incorporated nicotine hydrogen tartrate salt.

Comparison	Hardness P value	Adhesive force P value	Load *per* unit time P value
Bt 5 *vs* Bt 12	< 0.05	> 0.05	> 0.05
Bt 5 *vs* Bt 13	< 0.001	> 0.05	> 0.05
Bt 12 *vs* Bt 13	< 0.001	> 0.05	> 0.05

Table 6.23a Significance levels for the hardness, adhesive force and load *per* unit time of Bt 12, gums with incorporated nicotine Amberlite® IRP69, Bt 13, gums with incorporated nicotine hydrogen tartrate salt and Bt 5, gums with incorporated Nicotine Polacrilex. Output calculated using Instat Tukey-Kramer multiple comparison test.

In Bt 5 and 12, ion-exchange resins were used in the chewing gum formulations. In Bt 12, a stronger ion-exchange resin was used, whilst in Bt 5, a weakly acidic ion exchange resin (Polacrilex) was used. One would expect that the release from Bt 12 (use of a strongly acidic ion exchange resin) would be slower than release from Bt 5 and Bt 13; gums formulated using nicotine salt, in which a faster, complete release was expected due to the high solubility of the nicotine salt.

It can be concluded that the use of the stronger resin (nicotine Amberlite® IRP 69) resulted in a harder gum which released nicotine at a slower, but, steady rate in comparison to gums formulated using Nicotine Polacrilex and nicotine hydrogen tartrate salt. The release profile of Bt 5 suggests that Polacrilex resin had little effect in terms of controlling the release of nicotine from the gum as the release was similar to that observed using the nicotine salt. Also, 100% release was not observed with the use of the nicotine salt which suggests that the incomplete release from the gums could be as a result of the relative impermeability of the gum base and not as a result of nicotine being retained by the ion exchange resin.

6.3.2.7 Effect of time of addition of the active drug

The sequence of the addition of an active drug could increase or delay the release from a chewing gum. The theory is that ingredients added towards the end of the formulation process tend to be released at a faster rate whilst ingredients added at the beginning of the formulation process are generally released at a slower rate.

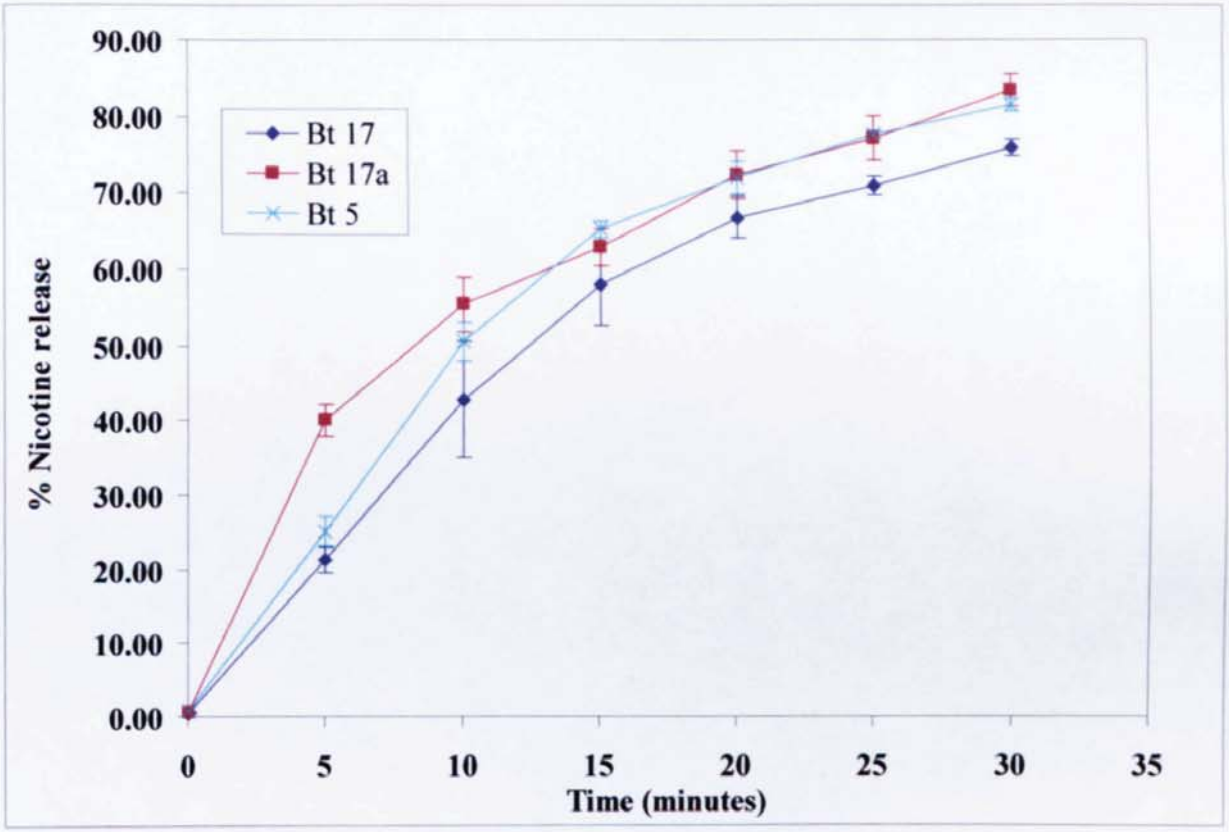


Figure 6.39 Release of nicotine with the effect of time of addition of the active into the gum formulation (n =3 ± SD). Bt 17, nicotine added with the gum base and Bt 17a nicotine added at the end of the formulation process with the buffers.

The addition of nicotine earlier (Bt 17) and later (Bt 17a) in the formulation process compared to the standard addition time (Bt 5) altered the release of nicotine from the gum (Figure 6.39). Nicotine Polacrilex added earlier during the formulation process with the gum base (Bt 17) delayed the release of nicotine from the gum. After 5 minutes, compared to Bt 17, twice as much was released from Bt 17a in which Nicotine Polacrilex was added with the buffers, later in the formulation process. However, dissolution curve comparison showed that all dissolution profiles were similar (Table 6.24). The slight difference in the delay of nicotine from Bt 17 was because nicotine, incorporated into the gum base earlier in the formulation process becomes embedded to a greater extent within the matrix of the gum base thus; the release was some what slower in comparison to the nicotine added at towards the

end of the formulation process where the nicotine was not fully incorporated into the gum base therefore resulting in a quicker release.

		Time of addition		
		Bt 17	**Bt 17a**	**Bt 5**
Time of addition	**Bt 17**		50.52	61.86
	Bt 17a	50.52		60.60
	Bt 5	61.86	60.60	

Key

Dissolution curves not similar

Dissolution curves similar

Dissolution curves not compared

Table 6.24 f_2 values highlighting the similar and dissimilar dissolution profiles of gums formulated when nicotine was added earlier in the formulation process (Bt 17) and later in the formulation process (Bt 17a) in comparison to the standard formulation process (Bt 5).

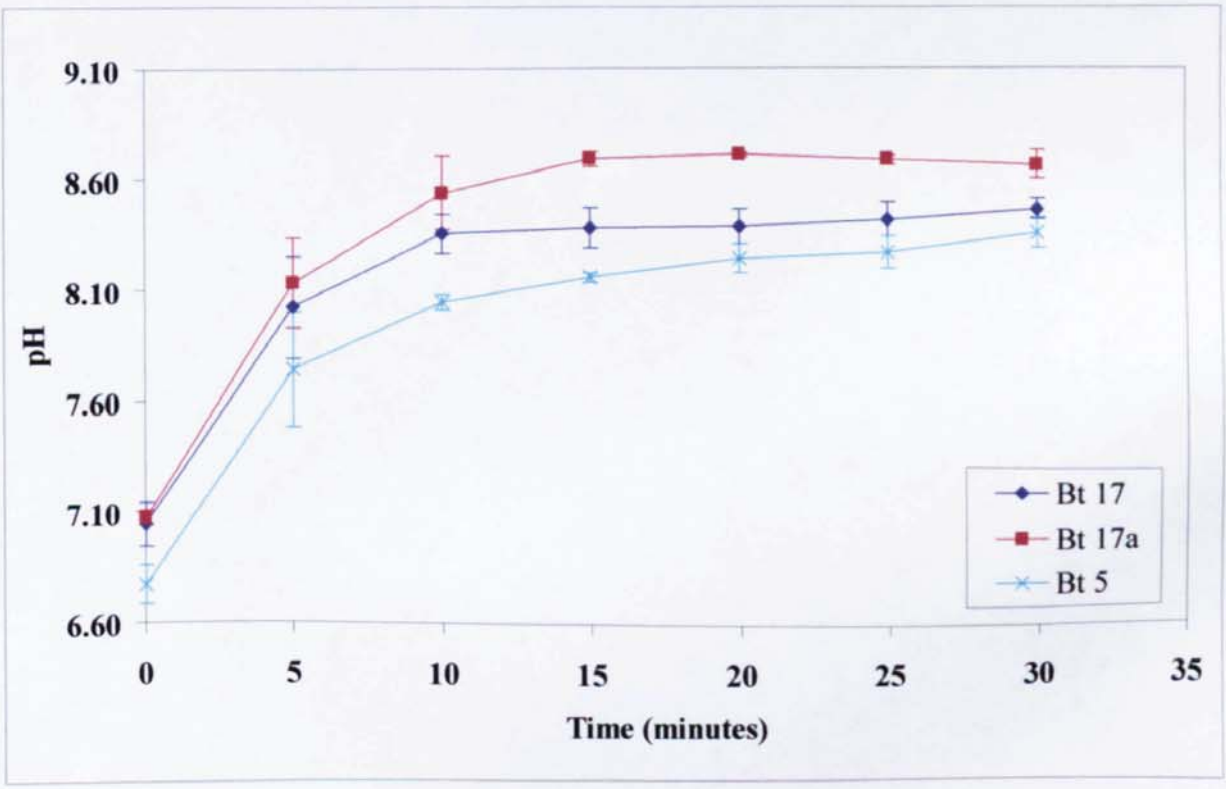
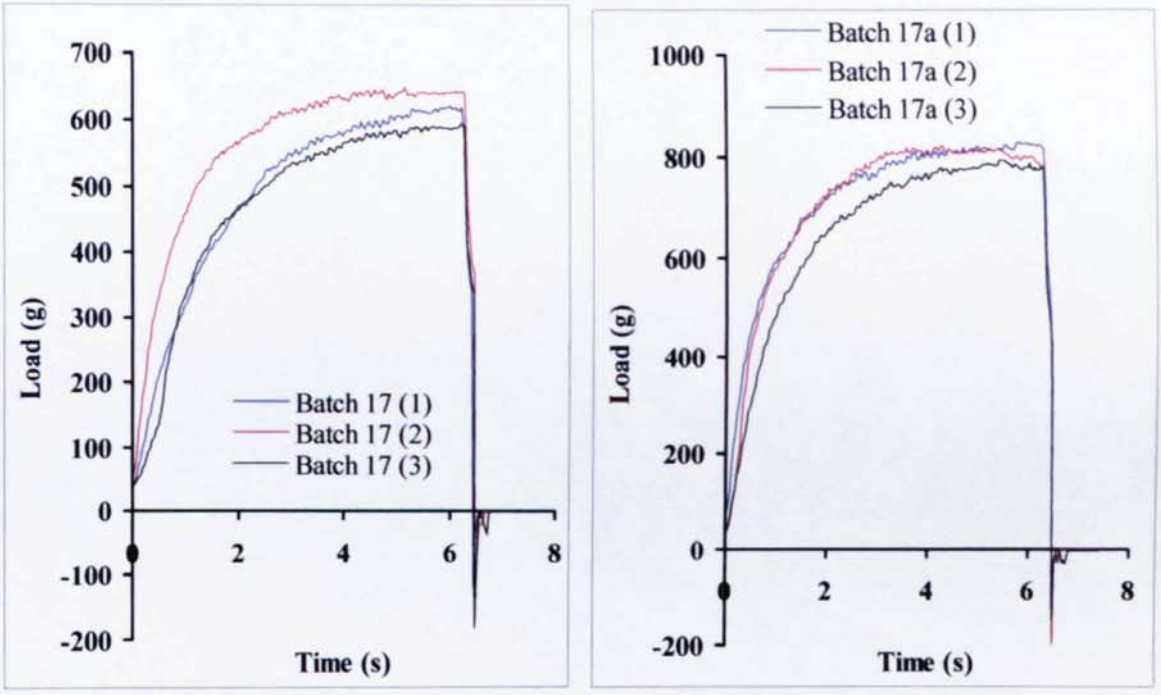


Figure 6.40 pH of artificial saliva as nicotine was released from Bt 17, gum with nicotine added earlier during the formulation process, Bt 17a, gums with nicotine added later in the formulation process and Bt 5, the standard formulation process (n =3 ± SD).

Increases in pH of the artificial saliva highlighted that Bt 5 and Bt 17a were similar (increases of approximately pH 1.6) whilst, after 30 minutes, a lower increase was observed from Bt 17a (increase of pH 1.422). Similarities in Bt 5 and Bt 17a were also highlighted by the texture of the gums (Table 6.25 and Figure 6.41). Values obtained for the hardness, adhesive force and apparent modulus were showed no significant difference between Bt 5 and Bt 17. In comparison, Bt 17 was significantly softer than Bt 17a and the standard formulation (Table 6.25a).



(a) (b)

Figure 6.41 Texture analysis as a 2 mm diameter stainless probe penetrated Bt 17 (nicotine added earlier in the formulation process) (a) and, Bt 17a (nicotine added later in the formulation process) (b) to a depth of 3 mm at a rate of 30 mm/min.

	Hardness (N)		Adhesive force (N)		Load *per* unit time (g/s)	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**
Bt 5	8.19	0.32	-1.85	0.63	345.59	86.93
Bt 17	6.00	0.21	-1.40	0.28	235.63	62.11
Bt 17a	7.98	0.14	-1.75	0.27	381.17	52.96

Table 6.25 Hardness, adhesive forces and load *per* unit time of nicotine gum Bt 17, gum with nicotine added earlier during the formulation process, Bt 17a, gums with nicotine added later in the formulation process and Bt 5, the standard formulation process.

Comparison	Hardness P value	Adhesive force P value	Load *per* unit time P value
Bt 5 *vs* Bt 17	< 0.001	> 0.05	> 0.05
Bt 5 *vs* Bt 17a	> 0.05	> 0.05	> 0.05
Bt 17 *vs* Bt 17a	< 0.001	> 0.05	> 0.05

Table 6.25a Significance levels for the hardness, adhesive force and load *per* unit time of Bt 17, gum with nicotine added earlier during the formulation process, Bt 17a, gums with nicotine added later in the formulation process and Bt 5, the standard formulation process. Output calculated using Instat Tukey-Kramer multiple comparison test.

6.3.2.8 Comparison of all dissolution profiles

Release of nicotine from the various formulations showed that there were many factors that affected the release of nicotine from the gum. Experiments highlighted the effect of sodium carbonate, sugar syrups, surfactant, gum base, active drug substance, and the time of addition of the active drug on the release of nicotine from the gum. Further comparisons using Moore and Flanner's f_2 equations highlights the similar and dissimilar release profiles of all the formulated gums (Table 6.26).

To summarise the main findings of the f_2 values, it was found that when comparing the batch reproducibility (Bt 5, 5a and 5b, Figure 6.18), f_2 values of greater than 70 (Table 6.26) suggested that the release profiles were similar and little variation was observed between batches when formulating using the gum kettle. Formulations made using different gum bases (Bt 11, 16 and 18, Figure 6.21) resulted in a different release profiles. Values of f_2 highlighted that greatest difference in release from the gum bases was observed from gums used in Bt 16 (Blacktree gum base) and Bt 18 (Magna T bubble gum base) which gave different release profiles to all other formulation but were similar to each other.

The greatest difference in release was observed from Bt 8, a gum formulation made using a lower concentration of sodium carbonate and sugar syrup (Figures 6.24 and 6.31). After 30 minutes of chewing only 40.16% of nicotine was released from the gum. f_2 dissolution curve comparisons indicate that Bt 8 was dissimilar to all other formulations. f_2 values greater than 60 were obtained when comparing Bt 6 and Bt 10 to the standard formulation showed that altering the concentration of sugar syrup did not affect the release of nicotine from the gum to a great extent; therefore, the delayed released from Bt 8 must predominately be due to the concentration of sodium carbonate within the gum. Similarity values of less than 50 when

comparing Bt 7 to all other formulations also confirms that the differences in release from the gum were due to a reduction in the sodium carbonate concentration as an increase in sodium carbonate concentration (Bt 9) resulted in a similar release profile to the standard nicotine formulation (f_2= 52.06).

Batches 1-4, formulations made using surfactant, also showed similarities in release to the standard gum although Bt 2, 3 and 4 were formulated with a reduced concentration of sugar syrup and sodium carbonate. Results therefore highlight that the use of surfactant within the gum formulation promoted the release from the gum which otherwise would have been delayed (*i.e.* release from Bt 3 and Bt 4 would be similar to Bt 7 and Bt 8 respectively if surfactants were not incorporated into the gum).

The effect of the stronger resin used to control the release of nicotine from the gum also showed some difference in release from the standard formulation (Figure 6.36; f_2 was found to be similar to Bt 5 but dissimilar to Bt 5a and 5b) whilst release using the nicotine salt was similar to that observed from the standard formulation ($f_2 > 50$). The concentration of the nicotine incorporated into the gum also influences the release profile. Bt 14 showed how the use of a 2 mg gum gave dissimilar release to the standard formulation as obtained f_2 values were less than 50. Finally, although the release profile obtained from Bt 17 and Bt 17a appear different (section 6.3.2.7) evaluation using the f_2 equation suggested that the release curves had some similarity (f_2= 50.52), suggesting that the time of addition of the active during the formulation process may have some effect on the release profile.

Batch	1	2	3	4	5	5a	5b	6	7	8	9	10	11	12	13	14	15	16	17	17a	18
1		86.79	63.59	74.92	48.59	54.49	53.69	56.18	29.14	17.96	43.83	61.90	56.41	40.34	48.70	34.07	42.56	44.65	39.50	49.74	39.56
2	86.79		68.31	75.99	48.41	53.69	52.69	56.66	29.06	17.93	45.22	62.39	53.39	41.09	49.01	34.48	42.38	42.38	39.51	48.16	37.81
3	63.59	68.31		73.35	52.73	56.07	54.26	62.66	30.98	19.23	50.78	65.74	45.35	44.85	55.62	37.80	45.95	38.29	42.98	47.47	34.47
4	74.92	75.99	73.35		52.40	57.63	56.39	60.77	30.42	18.83	45.87	65.25	50.74	41.91	53.80	35.87	45.53	42.51	41.92	50.06	37.92
5	48.59	48.41	52.73	52.40		74.51	72.80	69.85	40.08	23.96	52.06	62.08	39.31	52.70	73.82	47.05	71.79	38.32	61.87	60.60	34.39
5a	54.49	53.69	56.07	57.63	74.51		93.77	76.74	37.03	22.35	50.48	71.91	43.42	49.89	65.24	42.88	59.62	41.66	53.62	68.16	37.00
5b	53.69	52.69	54.26	56.39	72.80	93.77		72.05	37.19	22.44	49.17	68.76	43.54	49.20	62.93	42.56	59.57	42.36	53.43	71.83	37.55
6	56.18	56.66	62.66	60.77	69.85	76.74	72.05		36.04	21.86	55.54	82.24	42.68	52.34	66.31	43.56	56.94	39.22	52.21	58.42	34.93
7	29.14	29.06	30.98	30.42	40.08	37.03	37.19	36.04		37.43	36.83	34.16	25.27	41.77	38.23	51.50	44.99	26.45	49.24	37.30	24.42
8	17.96	17.93	19.23	18.83	23.96	22.35	22.44	21.86	37.43		22.74	20.81	15.67	25.14	23.34	30.02	26.27	16.87	27.93	22.58	15.78
9	43.83	45.22	50.78	45.87	52.06	50.48	49.17	55.54	36.83	22.74		53.40	34.60	66.78	52.58	49.57	49.01	31.27	49.64	44.44	28.25
10	61.90	62.39	65.74	65.25	62.08	71.91	68.76	82.24	34.16	20.81	53.40		45.44	49.66	60.04	40.93	52.07	40.64	48.18	57.99	36.06
11	56.41	53.39	45.35	50.74	39.31	43.42	43.54	42.68	25.27	15.67	34.60	45.44		32.79	38.92	28.31	35.46	54.40	33.02	43.38	48.57
12	40.34	41.09	44.85	41.91	52.70	49.89	49.20	52.34	41.77	25.14	66.78	49.66	32.79		50.76	58.20	52.27	30.90	55.28	45.82	27.94
13	48.70	49.01	55.62	53.80	73.82	65.24	62.93	66.31	38.23	23.34	52.58	60.04	38.92	50.76		45.74	62.97	36.99	57.61	53.43	33.56
14	34.07	34.48	37.80	35.87	47.05	42.88	42.56	43.56	51.50	30.02	49.57	40.93	28.31	58.20	45.74		51.28	27.74	57.95	40.51	25.28
15	42.56	42.38	45.95	45.53	71.79	59.62	59.57	56.94	44.99	26.27	49.01	52.07	35.46	52.27	62.97	51.28		35.65	74.89	54.96	32.23
16	44.65	42.38	38.29	42.51	38.32	41.66	42.36	39.22	26.45	16.87	31.27	40.64	54.40	30.90	36.99	27.74	35.65		33.15	44.80	68.01
17	39.50	39.51	42.98	41.92	61.87	53.62	53.43	52.21	49.24	27.93	49.64	48.18	33.02	55.28	57.61	57.95	74.89	33.15		50.52	30.17
17a	49.74	48.16	47.47	50.06	60.60	68.16	71.83	58.42	37.30	22.58	44.44	57.99	43.38	45.82	53.43	40.51	54.96	44.80	50.52		39.53
18	39.56	37.81	34.47	37.92	34.39	37.00	37.55	34.93	24.42	15.78	28.25	36.06	48.57	27.94	33.56	25.28	32.23	68.01	30.17	39.53	

Dissolution curves not compared | Dissolution curves similar | Dissolution curves dis-similar

Table 6.26 f_2 values indicating similarities and differences in the release from the chewing gum formulations

To analyse the release data further, the release of nicotine from the different formulated batches were fitted to a polynomial of the fourth order such that

$$\% \text{ Released} = a_0 + a_1t + a_2t^2 + a_3t^3 + a_4t^4 \qquad \text{equation 6.1}$$

and the best fit coefficient a_0, a_1, a_2, a_3 and a_4 were calculated. The times to 50% release (t½) were then calculated using Newton-Raphson iteration (Table 6.27) using thee values. Newton-Raphson iteration solves a polynomial equation in t using the formula

$$t_{i+1} = t_i - \frac{F(t_i)}{F'(t_i)} \qquad \text{equation 6.2}$$

where, t_i was the current time estimate of this parameter *(i.e.* using release data an estimate of the time to 50% release), $F(t_i)$ was the numerical value obtained by substituting the current time estimate value of t_i into the polynomial (recast in the form $F(t_i) = 0$), $F'(t_i)$ was the numerical value of $t_{(i)}$ into the first derivative of the polynomial and t_{i+1} was the better approximation of the time value required.

The calculations were repeated with the new value of time (t_{i+1}) and the process was recycled until consecutive estimates were sufficiently close. Thus, equation 6.1 was rearranged such that

$$F = a_0 + a_1t + a_2t^2 + a_3t^3 + a_4t^4 - R = 0 \qquad \text{equation 6.3}$$

and

$$F' = a_0 + a_1t + a_2t^2 + a_3t^3 + a_4t^4 = 0 \qquad \text{equation 6.4}$$

A value of 50 for R (release) will provide the time for 50% release of nicotine when iteration was complete. An initial estimate of 10 minutes was used in each case and iteration was usually complete within four cycles (Table 6.27)

	Polynomial coefficient						
Batch	a_0	a_1	a_2	a_3	a_4	r^2	**Time to 50% release (min)**
1	0.00015	-0.00571	-0.10480	7.42000	-0.00589	1.000	**7.93**
2	0.00017	-0.00818	-0.01351	6.53000	-0.05420	0.999	**8.44**
3	0.00051	-0.03180	0.51010	2.76400	0.03314	0.999	**9.65**
4	0.00045	-0.02530	0.29810	4.84400	-0.07688	0.998	**8.60**
5	0.00025	-0.01374	0.10930	4.92600	0.44850	0.999	**10.14**
5a	0.00004	0.00025	-0.18680	7.00700	0.64370	1.000	**9.25**
5b	-0.00007	0.00734	-0.33110	7.91400	0.60600	1.000	**9.02**
6	0.00015	-0.00782	0.01832	5.44700	7.85700	0.999	**8.18**
7	-0.00004	0.00476	-0.19520	4.90900	0.35970	1.000	**18.78**
8	0.00013	-0.00858	0.15260	0.99340	0.45830	0.999	**36.23**
9	0.00016	-0.01320	0.28780	1.98300	3.31600	1.000	**12.21**
10	0.00005	-0.00098	-0.12780	6.50100	0.16880	1.000	**9.52**
11	-0.00026	0.02276	-0.77030	12.75000	0.36680	1.000	**5.38**
12	-0.00029	0.01456	-0.23420	4.63200	0.81970	1.000	**14.40**
13	0.00064	-0.03891	0.61890	1.93100	0.18440	0.999	**10.22**
14	0.00002	-0.00532	0.15980	1.97500	6.10200	0.999	**13.64**
15	0.00030	-0.01609	0.14900	4.53700	-0.13650	0.996	**10.79**
16	-0.00059	0.04755	-1.37600	17.22000	0.48400	1.000	**3.67**
17	0.00025	-0.01448	0.17060	3.73400	0.28490	1.000	**12.07**
17a	-0.00038	0.02952	-0.82030	11.18000	0.66060	0.999	**7.62**
18	-0.00059	0.04857	-1.44600	18.31000	1.24000	0.992	**3.54**

Table 6.27 Polynomial coefficients and time to 50% release for each gum batch.

To provide a multivariate approach, a dendrogram was constructed using the variation in composition, and release data after 5 and 15 minutes and time to 50% release as coordinates in multidimensional space (Figure 6.42). These results showed a trend similar to that observed using the f_2 equation. The replicates of the standard formulation, (Bt 5, 5a and 5b) were found to be close indicating good reproducibility between batches. Batches most similar to the standard formulation and to each other were formulation Bt 10 (higher sugar syrup concentration), Bt 15 (4 mg gum formulated using 10 mg sodium bicarbonate and 20 mg sodium carbonate), Bt 3 (addition of surfactant and reduction of sodium carbonate) and Bt 13 (use of the nicotine salt). Bt 1, 2, 4, (added surfactants), Bt 6 (reduced sugar syrup) and 17a (addition of nicotine later during formulation) were also found to be similar to each other as the time required to 50% release was less than that observed from the standard formulation but yet similar. A slower more controlled release was demonstrated by Bt 9 (higher concentration of sodium carbonate), Bt17 (addition of resin to the gum base), Bt 12

(use of stronger resin) and Bt 14 (2 mg formulation) compared to the standard nicotine gum formulation as the time taken to 50% release was greater than that observed from the standard formulation, whilst, the greatest difference in release was observed from Bt 8 which gave the slowest release compared to all other formulations. In the final cluster, we see that the release of nicotine from gums formulated using different gum base (Bt 11, 16 and 18) gave a faster initial release in comparison to all other formulations. Time required to 50% release was less than 5 minutes for Bt 16 and 18 and 5.38 minutes for Bt 11.

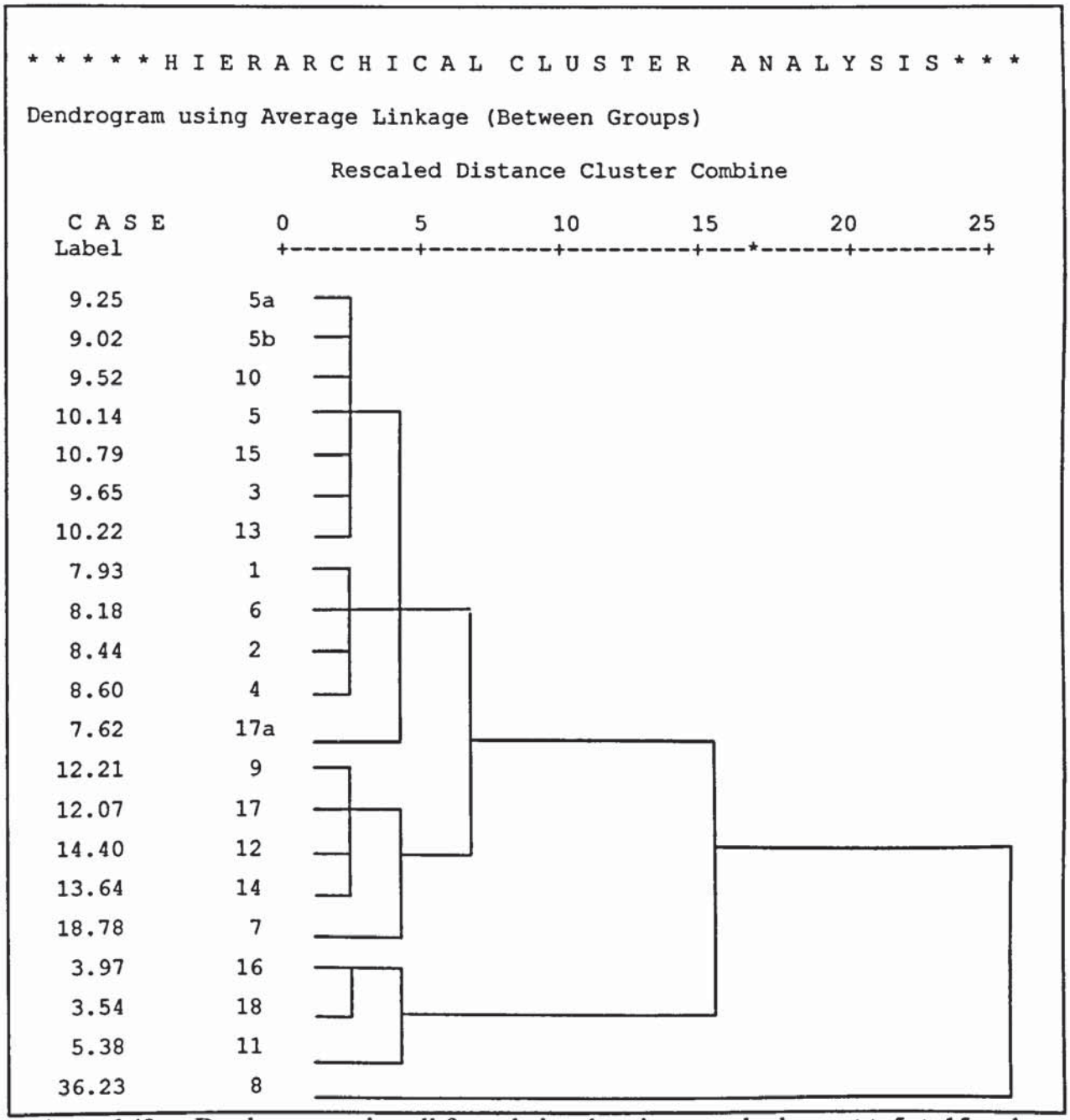


Figure 6.42 Dendrogram using all formulation descriptors and release at t=5, t=15 and t to 50% release. Values shown at the left are times to 50% release

6.4 Conclusion

The various formulations of nicotine gum resulted in an array of release profiles and textures. The use of a directly compressible gum base was found to be a more convenient, speedy and cost-effective way of producing gums. The gums formed were harder, more rigid and less adhesive and gave a faster release profile compared to Nicorette® gum. However, due to the nature of the gum formed, during chewing, the gum crumbled resulting in an undesirable drug delivery system. For gums produced using the conventional melt method, there were various formulation factors and excipients that affected the release from the gum. A reduction in sodium carbonate and sugar syrup resulted in very slow release with the most important contributing component being sodium carbonate rather than the syrup. In contrast, altering the gum base used in the formulation resulted in a faster release, harder gum compared to the standard nicotine formulation. In terms of the formulation process, it was found that the order of mixing and the time at which the active was incorporated into the gum mix will also impact the release from the gum thus, careful consideration when formulating medical gums is needed to ensure that the formulation process results in the desired release profile.

CHAPTER SEVEN
GENERAL SUMMARY

With more than three and half million deaths each year caused by cigarette smoking, it was recently estimated that two-thirds of the smokers would like to give up smoking (GSK data on file). This creates a growing market for products that will help smokers overcome tobacco dependences. Historically a problem with smoking control therapies were their relative lack of efficacy, however, this situation has evolved dramatically with the introduction of nicotine replacement therapies (NRT). NRTs can approximately double the rate of success of people who attempt smoking cessation compared to those using willpower alone (Henningfield, 1995).

The first clinically proven nicotine replacement product to obtain regulatory approval was Nicorette® gum, currently available in two different strengths a 2 mg and 4 mg gum. Since launch, Nicorette® gum has been investigated in numerous clinical studies demonstrating that the release of nicotine from the chewing gum was less than 100% (section 1.5.2). However, due to the large variations in study design and degree of sophistication, it was often difficult to compare and contrast trials of clinical efficacy or integrate data from previously published studies, thus, the need for and value of *in vitro* drug release testing for chewing gum to enable standardised conditions and tests so that reliable comparisons can be made.

Dissolution testing is well established for a range of dosage forms, however standard dissolution apparatus are not suitable for monitoring release from chewing gums. Preliminary studies conducted using Nicorette® gum in artificial saliva without mastication showed that release of nicotine due to diffusion of nicotine through the gums was low. After 60 minutes, less than 4% of the nicotine contained within the 2 and 4 mg gum was released, thus, highlighting the need for a mastication device to test the release of drug substances from chewing gums.

In 2000, the European Pharmacopoeia produced a monograph describing a suitable apparatus for studying the *in vitro* release of drug substances from chewing gum. With use of the chewing machine, the main aims of this project were to:-

- determine factors that could affect release from Nicorette® chewing gum *in vitro*,
- to develop an *in vitro in vivo* correlation and
- to investigate formulation variable on the release of nicotine from gums

Firstly, in order to promote reproducible data and to minimise variation, the rate at which the gum was chewed *in vitro* and the use of artificial saliva as a dissolution medium was investigated. A major concern regarding the use of the EP chewing machine within this study was the performance of the interval timer, as this component would control the rate at which the gum was chewed. Therefore, in order to ensure reproducible chew rates, the chewing machine was validated to determine any variation in the number of chews *per* minute and any inter-and intra-day variation in the chew rates. Nicorette® gum was placed in the chewing chamber with 40 mL of buffer and the number of chews *per* minute on each interval timer setting recorded. The same procedure was applied for testing inter-and intra-day variation. The gum was chewed for 10 minutes, twice a day for 4 consecutive days and the number of chews *per* minute on each interval timer setting noted. Analysis of the results showed that the variation in the chew rate on a continuous run of 30 minutes for each interval timer setting was small (SD < 2.0). Also, when assessing the inter-day and intra-day variations, within the same day, there was no difference in the chew rate however, some variation in the chew rate existed inter-day. Therefore, on each day of release testing, the chewing machine was calibrated prior to use to ensure the correct chew rate setting was attained.

When selecting an appropriate dissolution buffer, it was not appropriate to use human saliva so an, artificial saliva was considered to be suitable alternative for *in vitro* testing. A comparisons study was conducted to establish the suitability of artificial saliva as an alternative to human saliva. 4 mg Nicorette® was chewed in both real and artificial saliva and the release profiles compared using the f_2 equation. It was found that the release of nicotine from the gum was similar in both real and artificial saliva ($f_2 > 50$). Similar pH measurements at the different time increments of the dissolution profile also suggested that the buffering capacities of the real and artificial saliva were similar therefore; artificial saliva was accepted for use as an appropriate dissolution medium for the *in vitro* testing of chewing gums.

7.1 Factors affecting release from chewing gum

Specifications set by the European Pharmacopoeia for the *in vitro* dissolution testing of chewing gum are brief and non-specific. However, to characterise a drug product successfully and to ensure minimal variations between results, a standard test method is required. To investigate the affects of altering the *in vitro* dissolution conditions on the release of nicotine from Nicorette® gum, numerous studies were conducted altering the chew rate, the type of dissolution media used, the pH, volume, temperature and the ionic strength of the dissolution media.

Studies have been conducted investigating the effect of chew rate on the release of nicotine *in vivo* (section 3.6.2.1). It has also been reported that the normal physiological chewing rate ranges from 40-80 chews *per* minute (Kerr, 1961, Neil, 1967, Louridis, *et al.*, 1970). When examining the *in vitro* release of nicotine from Nicorette® gum chewed at 82, 60, 42, 22, 12, 6 and 4 chews *per* minute, at the higher chew rates (> 42 chews *per* minute), a greater release of nicotine was observed in comparison to gum chewed at the lower chew rate settings of (< 22 chews *per* minute).

In a previous study conducted by Kvist, *et al.* (2000), approximately 90% drug release was reported from Nicorette® 2 mg gum when using 0.1% w/v SLS as the release medium. In comparison, approximately 50% release was observed during this study. It was therefore postulated that the inclusion of surfactants increased the release and it was confirmed that, the addition of surfactant (0.1% w/v sodium lauryl sulphate) to the artificial saliva doubled the release of nicotine from the gums, whilst, release in water was similar to artificial saliva ($f_2 = 68.25$).

To mimic the effect of having hot or cold beverage whilst chewing Nicorette® gum and to appreciate the effect of environment on release rate, the temperature and the initial starting pH of the dissolution medium were altered. It was found that at 53°C, the gum became softer, more adhesive and less rigid which, resulted in a greater release of nicotine. However, at lower temperature (23°C), the gums were more resistant to deformation, harder and less adhesive and resulted in a considerably lower release compared to gums chewed at 53°C, but, were found to be similar to gums chewed at the physiological temperature of 37°C. When altering the pH of the artificial saliva to obtain a range of pH 3.0-9.0, sodium hydroxide or hydrochloric acid was used as appropriate. Dissolution studies conducted at the

different pH values showed that there was no difference in the release of nicotine from Nicorette® ($f_2 > 50$). However, at the lower initial pH values (3.0 and 5.0), the increases would suggest that the released nicotine was predominately in the mono-protonated state at the end of 40 minutes *in vitro* chewing. In experiments conducted to determine the effect of pH on buccal diffusion of nicotine, it was found that diffusion of nicotine across the buccal membrane was more favourable at higher pH values when nicotine was predominately present in the unionised form. Therefore, at the lower starting pH (pH 3.0 and 5.0) the increase in pH due to the released buffers would be insufficient to allow optimised nicotine delivery.

When altering the ionic strength of the artificial saliva using sodium chloride, high ionic strengths was found to decrease release of nicotine from Nicorette® gum. f_2 comparison of the dissolution profiles using different volumes of dissolution medium showed that irrespective of the volume in the chewing chamber, the release from the gums was similar ($f_2 > 50$). However, pH measurements as the gums were chewed highlighted that at decreased volumes, due to the higher concentration of carbonates and bicarbonates released, the pH of the artificial saliva was elevated. After 10 minutes of chewing in decreased volumes, the pH of the dissolution medium was greater then pH 8.5. At this pH, nicotine is predominantly unionised therefore presenting optimised condition for nicotine diffusion.

Nicotine within a Nicorette® formulation is in the form of Nicotine Polacrilex resin, nicotine bound to a weakly acidic ion exchange resin, Amberlite® IRP 64. It has been reported (Pharmacia, 1999) that the role of the Nicotine Polacrilex is to control the release of nicotine from the gum. In parallel with experiments conducted on the factors affecting release from chewing gum, a number of experiments were conducted to study release of nicotine from the Polacrilex resin. With increased agitation, as with increased chew rate, a greater release of nicotine was observed from the resin. Since a greater release of nicotine was observed from Nicorette® gum with the addition of 0.1% w/v SLS to the dissolution medium, dissolution of resins in SLS, Tween 20, water, AS and TTAB were compared to determine if a similar effect was observed. Results suggest, since that the incorporation of SLS doubled the release of nicotine from Nicorette® but, resulted in decreased release from resin, SLS exerts an effect on the chewing gum formulation. Minimal release of nicotine was observed from resins in water so; it can be proposed that the sodium carbonate and bicarbonate within the formulated gum product provide a source of cations to facilitate release from the ion

exchange resins. Changing temperature had no effect on release from the exchange resins but increased volumes promoted release due to the presence of more ions in solution. Finally, greatest differences in release from resins were observed when the ionic strength of the dissolution media was altered. At the higher ionic strengths a greater release of nicotine was observed in comparison to the lower ionic strength solution as exchange was more efficient

From these experiments it was found that there were many contributing factors that altered the release of nicotine from Nicorette® gum and Nicotine Polacrilex resin. In terms of release from Nicorette® gum, the main contributing factors that affected the release were found to be the temperature of the dissolution medium, the rate at which the gum was chewed, the ionic strength and, the type of dissolution media used. In contrast, the main factors governing the *in vitro* release of nicotine from Polacrilex resin was the degree of agitation of the resin and the type as well as the ionic strength of the dissolution medium. Since a large portion of nicotine was released from the resin within the first minute, the resin does not have a major role in controlling release. Another reason for the use of the resin could be to provide a means of stabilising the nicotine, which in the free base form is a volatile oil, prone to migration and oxidation.

Although, the volume and the starting pH of the dissolution medium did not affect the release of nicotine from Nicorette®, the pH profiles showed how increases in the pH may be insufficient to provide optimal conditions for nicotine absorption *in vivo*. Since many popular hot and cold beverages are acidic in nature, the pH of the oral cavity could be reduced such that after chewing Nicorette® gum a sufficient increase in pH is not obtained thus hindering nicotine absorption. Therefore, when using Nicorette® gum *in vivo,* precautions should be taken to refrain from drinking hot or cold beverages prior, during or immediately after chewing Nicorette® gum.

Since a considerable number of factors affect the release of nicotine from gums and resins consideration should be taken when selecting conditions for the *in vitro* testing of drugs. Where possible, conditions should mimic those which are expected to be found *in vivo* and should remain consistent to minimise variation.

7.2 *In vivo in vitro* correlation

One of the main aims of this study was to establish a level A correlation for *in vitro* and *in vivo* release of nicotine from 4 mg Nicorette®. *In vivo* work was conducted at the University of Sheffield and, all *in vitro* work conducted using the EP chewing apparatus. Using a time mapping function, all chew rates used in the *in vitro* study could be used successfully for IVIVC purposes, however, statistically, chew rates of 10 and 20 chews/minute performed better than all other chew rates. On this basis, this technique can be used in future studies on experimental gum formulations to predict their *in vivo* nicotine release profiles.

7.3 Investigation of formulation variables on release from gums

A series of nicotine gum formulations were made using the traditional chewing gum manufacturing method to determine if, altering the formulation would result in different release profiles. Also, with the introduction of a directly compressible gum base, produced by SPI Pharma, namely Pharmagum® M and S, a series of nicotine gums were made using the compressible gum and compared to the standard Nicorette® gum.

Attempts to reduce crumbling of gums by increasing compression and further granulating the formulation were not successful. It was proposed that the crumbling of the gum was a result of the formulation method used to produce Pharmagum® M and S. In order to provide a free-flowing gum powder, the individual gum particles were coated in soluble and insoluble excipients. On compression, in between each gum particle, a small layer of excipient is entrapped within the gum piece, thus on chewing the gum crumbles into the individual gum particles.

A number of formulations were made using the traditional manufacturing methods with the aim of obtaining an array of differing release profiles. Gums were formulated using:-

- different gum bases,
- different concentrations of sodium carbonate and bicarbonate,
- differing concentrations of sugar syrup,
- surfactant,
- different forms of active drug and
- different addition sequence.

Release of nicotine from the gums was then testing using the EP chewing apparatus and the texture of each batch of gums determined using the texture analyser.

f_2 similarity test and hierarchical cluster analysis showed that the release from the standard gum batch and two replicate formulations was good (f_2.> 70). Therefore, the method was deemed to be reproducible. Gum formulated using different gum base, namely, Blacktree, Magna T bubble gum base and Dreyco base, resulted in harder, more rigid gums with faster release of nicotine and dissimilar to the standard formulation ($f_2 < 50$).

Using a stronger ion exchange resin gave a slower initial release of nicotine in comparison to the standard formulation. In contrast, the use of nicotine hydrogen tartrate salt gave a similar release profile. f_2 comparisons of the dissolution profiles deemed all the profiles similar ($f_2 >$ 50), however, hierarchical cluster analysis constructed using the variation in composition and release data after 5 and 15 minutes and time to 50% release as coordinates, showed how the use of the different active forms produced dissimilar release profiles. This highlights that with use of the stronger resin, the release of nicotine from the gum was delayed.

Another factor affecting release profiles was the time at which the active drug component was added to the formulation. When Nicotine Polacrilex was added with the buffers during the latter stages of the formulation process, the initial release of nicotine from the gums was increased. Although f_2 comparison showed that the release was similar ($f_2 = 50.25$), cluster analysis results highlighted differences in the release profile. Time to 50% release calculated using the polynomial was estimated to be 12.07 min in comparison to 7.62 min observed when nicotine was added earlier in the formulation process.

Altering the concentration of the sugar syrup added to the formulation had little effect on the release of nicotine. Cluster analysis results showed that the release profiles were fairly similar as time to 50% release were 9.52 min and 8.18 min for increased and decreased sugar syrup formulated gums respectively. The main difference observed with the effect of altering the sugar syrup concentration was the texture of the gums. At a higher sugar syrup concentration the gums were softer and less rigid than gums formulated with a lower concentration of sugar syrup.

When a 2 mg gum was formulated with 30 mg of sodium carbonate, a buffer normally added to a 4 mg formulation, release was affected. In contrast, a 4 mg gum formulated with the combination of buffers normally incorporated within a 2 mg gum gave a similar release profile to the standard gum formulation. In an earlier experiment conducted comparing the

in vitro release from a 2 mg and 4 mg Nicorette® gum, the release was also found to be different. It was previously speculated that the release from the gums was affected by the different buffers incorporated into the gums. However, when the buffers normally incorporated into a 4 mg gum were used in a 2 mg gum and vice-versa, a difference in release was observed. This can be explained by following the pH profile during release. As the formulated 2 mg gum was chewed *in vitro*, the pH increased rapidly in comparison to the formulated 4 mg gum. This difference in pH profile observed by the formulated gums was not observed when 2 mg and 4 mg Nicorette® were chewed *in vitro*. It can be speculated that the incorporation of the different buffers is so that increases in pH from both gums are equal. At the higher nicotine concentration (4 mg gum), a greater concentration of sodium salt was required to increase the pH to provide optimal absorption conditions. However, the use of the same buffer in a 2 mg formulation resulted in large increases in pH, therefore, to maintain equal pH increase from both gums, 10 mg of sodium carbonate is replaced with 10 mg of a lower sodium salt (sodium bicarbonate).

Altering the concentration of sodium carbonate within the gum had a major impact on the release of nicotine from the gums. Increasing the concentration produced a similar release profile to the standard gum formulation. In contrast, reducing the concentration of sodium carbonate within the gum resulted not only in a smaller pH increase but, also a reduction in the release of nicotine from the gum. Calculated time to 50% release was 18.78 min appose to 10.14 min observed from standard formulation. The greatest difference in release was observed from gums formulated with a reduction in both sugar syrup and sodium carbonate. Results from the formulations suggested that the main contributing factor was the reduction of sodium carbonate rather than the sugar syrup.

Finally, *in vitro* release experiments showed how the addition of surfactant to the dissolution medium resulted in an increase of release from the gums. When surfactants were added to the gum formulation, a similar release to that of the standard formulation was observed, ($f_2 >$ 50). The most interesting result was observed when surfactants were incorporated into the gum formulation with reduced quantities of sodium carbonate and sugar syrup. Without the surfactant, the formulation resulted in a reduced release of nicotine from the gum, but with the addition of the surfactant, a greater release was observed (t 50% = 36.23 and 8.60 with addition of surfactant).

From the series of formulations made there were many factors which altered the release of nicotine from the gum. This highlights possible variables that can be controlled to obtain a desired release profile. Factors such as the time of addition of the active, the use of surfactants and changing the gum base can all increase the release of nicotine from the gum. In contrast, reducing the concentration of sodium could result in a delay in release, whilst altering the sugar syrup will affect the overall texture of the gum whereby, an increasing the sugar concentration would result in a more softer, less rigid formulation.

In terms of producing an ideal nicotine formulation, it is often difficult to choose one release profile over another as the need for nicotine replacement is different for each individual. Highly-dependent smokers may require a rapid initial intake of nicotine to reduce the nicotine cravings, whilst, others may prefer a gradual controlled release formulation in which nicotine is delivered over a period of time. It can be postulated that during the early stages of smoking cessation a product which provides a rapid release of nicotine may be required to overcome the high dependency, whilst during the latter stages of the smoking cessation program, a mild and gradual increase may be desirable. In such situations, the formulation of the gum can be adapted to obtain the required response. In order to obtain a rapid initial release, nicotine could be incorporated into the gum during the latter stages of formulation. Altering the gum base could also result in a increase initial release, whilst in contrast, to delay the release of nicotine in order to provide a controlled, gradual release, a stronger ion exchange resin or the addition of nicotine earlier during the formulation process could delay the release of nicotine from the gum. Although decreasing the concentration of sodium carbonate within the gum would also result in a delayed release, the incorporation of sodium carbonate is important in terms of delivering nicotine. Not only does the sodium bicarbonate and carbonate supply a source of cations to facilitate the release of nicotine from the resinate, but also ensures an alkaline environment in the buccal cavity to optimise nicotine absorption, thus reducing the concentration of sodium carbonate and bicarbonate would be undesirable.

7.4 General conclusion

Although chewing gum as a drug delivery system has gained wide acceptance within smoking cessation, interest has been shown in this method as a drug delivery system. Since chewing gum is a unique way of delivery drug substances, in the coming years, it is very likely that other medicinal chewing gum formulations will be marketed. However, very limited systematic technical information about chewing gums is available in the public

domain. Although patents exist that cover specific gum compositions and processes, there is no literature outlining a systematic approach to chewing gum formulations and release studies.

With the introduction of the EP chewing apparatus, it has now become possible to test the release of active drug substances from the chewing gum *in vitro*, thus minimising the need for *in vivo* chew-out studies and aiding further development of medicinal chewing gums. Since official monographs and specifications are vague and ambiguous, as with other pharmaceutical products, standardised test methods and specifications are required in order to minimise variations during testing.

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APPENDIX 1

HPLC METHOD VALIDATION

A1.1 Linearity

The linear range for nicotine was found to be 0.105-211.1 µg/mL (Figure A1.1, Table A1.1)

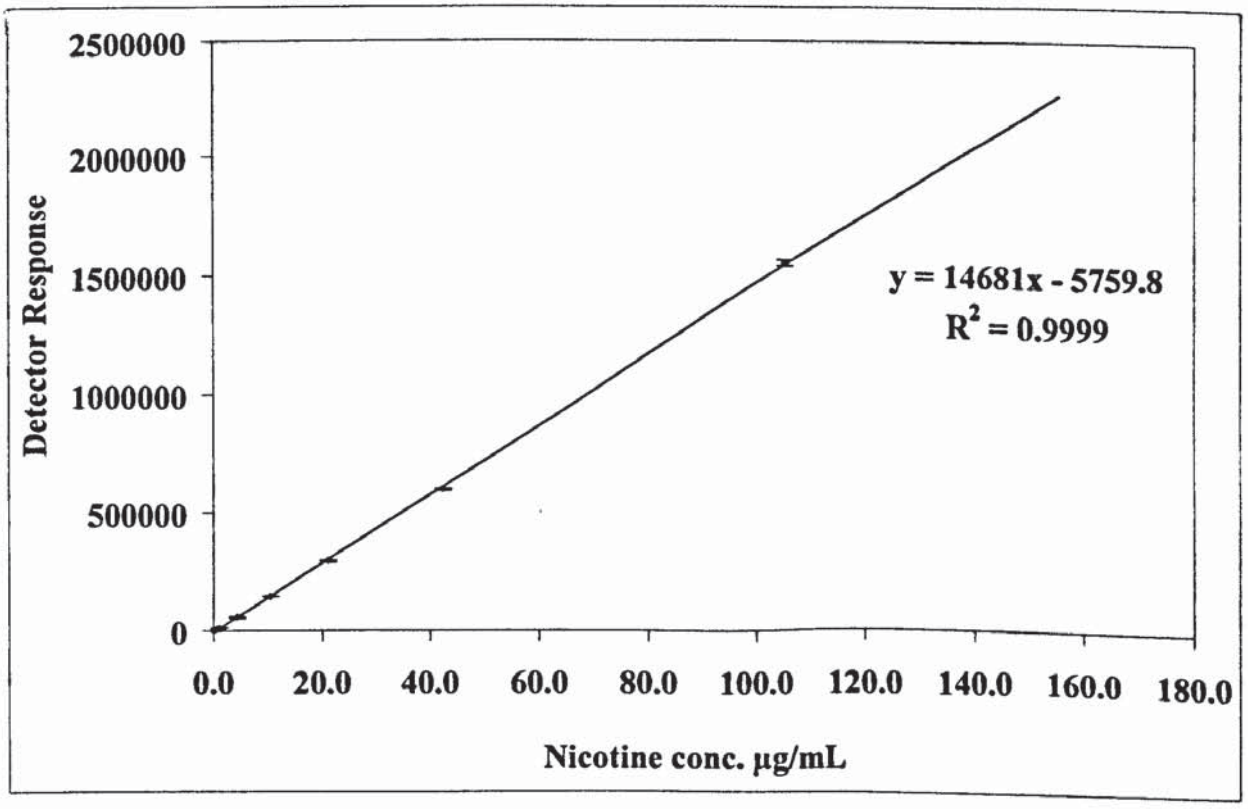

Figure A1.1 Nicotine calibration curve (n=5± SD)

Nicotine hydrogen tartrate µg/mL	Nicotine µg/mL	Mean response	SD
0.120	0.042	0.00	0
0.300	0.105	587.6	79.23
1.200	0.421	3159.6	73.78
3.000	1.053	10649.8	408.87
12.000	4.210	56134.0	2828.93
30.000	10.525	146185.4	1564.43
60.000	21.051	296567.8	2219.96
120.000	42.101	602319.2	3542.98
300.000	105.253	1545026.4	10203.28

Table A1.1 Nicotine Linear range (n=5)

A1.2 Method precision

% RSD =1.03% hence within the acceptance limit of < 2% RSD (Table A1.2)

Day	Injection	Area	Nicotine (µg/mL)	% Recovery
1	21.08	297033	20.56	97.53
2	21.09	297033	20.58	97.58
3	21.07	293271	20.46	97.10
4	21.04	299426	20.82	98.95
5	21.10	296076	20.67	97.96
6	21.06	282214	21.01	99.76
Mean				98.15
SD				1.01
%RSD				1.03

Table A1.2 Method Precision

A1.3 Instrument precision

% RSD of < 1.202 % this was below the acceptance limit of % RSD < 2% hence the result accepted (Table A1.3).

Injection	Area	Nicotine (µg/mL)
4.222	57646	4.129
4.222	57433	4.114
4.222	57080	4.089
4.222	56777	4.068
4.222	55679	3.991
4.222	57180	4.096
Mean		4.081
SD		0.049
%RSD		1.202

Table A1.3 Instrument precision

A1.4 Accuracy

Recovery of nicotine from the artificial saliva at three levels (5.32, 53.21 and 106.41 µg/mL) were all within the acceptance limit on the recovery of ± 2% recovery with < 2% RSD (Table A1.4).

Solution µg/mL	Area	Actual conc^n	Mean	SD	%RSD	% Recovered
5.32	72065	5.32				99.92
	72549	5.28				99.25
	73068	5.25	5.28	0.035	0.65	98.63
53.21	779677	53.76				101.04
	776121	53.68				100.89
	777280	53.93	53.79	0.125	0.23	101.35
106.41	1560915	107.29				100.83
	1553335	107.15				100.70
	1555399	107.67	107.37	0.70	0.25	101.19

Table A1.4 Recovery

A1.5 Limit of detection

The calculated slope of the calibration curve was 1 (Figure A1.2), thus the limit of detection using the Shimadzu HPLC system was determined as 0.0112 µg/mL (Table A1.5).

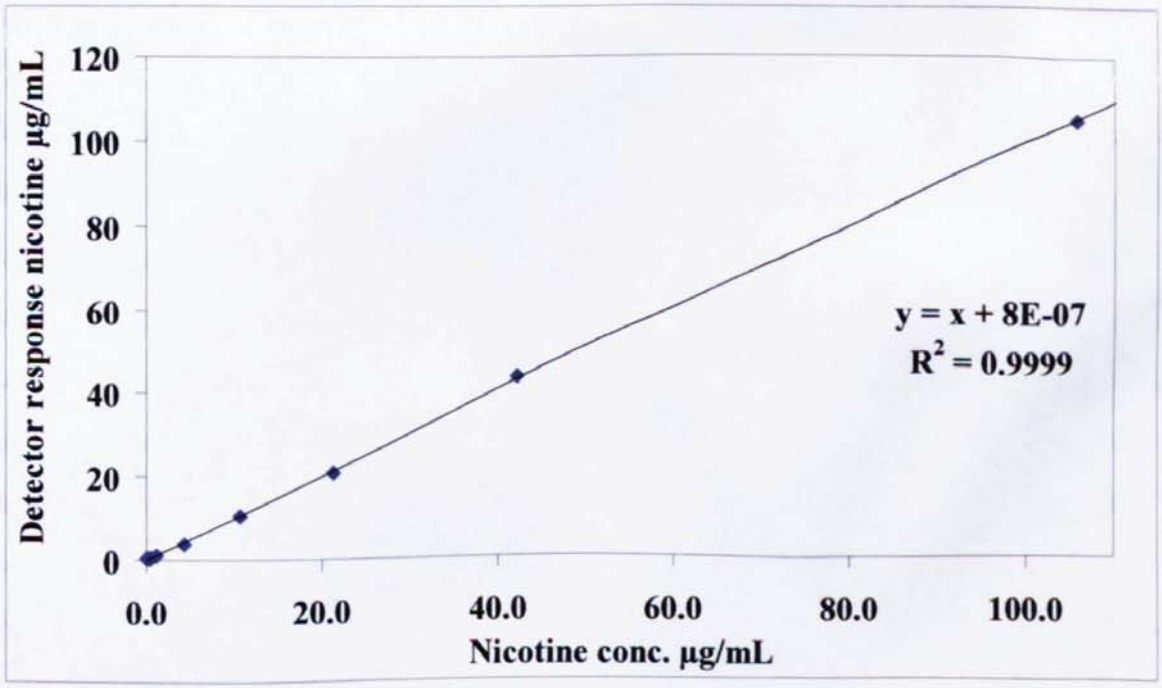

Figure A1.2 Calibration curve of nicotine

Injection	Area	Nicotine (µg/mL)
0.422.	2629	0.434
0.422	2676	0.438
0.422	2594	0.432
0.422	2639	0.436
0.422	2697	0.440
0.422	2569	0.430
Mean		0.435
SD		0.0034
%RSD		0.78
LOD		0.0112
LOQ		0.0340

Table A1.5 Limit of detection and quantification

A1.6 Limit of quantification

Replicate injections at the 0.338 µg/mL (LOQ calculated from Table A1.5) showed a % RSD of 2.46% hence < 5% therefore the value of 0.340 was accepted as the limit of quantification (Table A1.6)

Injection	Area	Nicotine conc^n (µg/mL)
0.338	3997	0.356
0.338	3708	0.336
0.338	3784	0.341
0.338	3682	0.334
0.338	3695	0.335
0.338	3730	0.337
Mean		0.340
SD		0.008
%RSD		2.458

Table A1.6 Limit of quantification

A1.7 Selectivity and specificity

A typical HPLC column well packed with 5 μm particles should show a height equivalent of the theoretical plate (HETP) within the range of 0.01-0.03. Column capacity should be in the range 1 to 10 where, values less than 1 indicate inadequate separation from the solvent front and values greater then 10 are associated will long retention time and broadened peaks and values greater than 2000 for the calculated number of theoretical plates show adequate column efficiency (Bradshaw, 1998).

Using the water xterra column, the calculated number of theoretical plate for the column used was found to be 6059, 0.025 HETP and the column capacity was found to be 1.57 indicating a good separation from the solvent front and good column efficiency (Table A1.7).

Nicotine (μg/mL)	Column length	Retention Time	Solvent front	Peak Width	N	HETP	K'
105.46	150 mm	3.425 min	1.334 min	0.176 min	6059	0.025	1.57

Table A1.7 Number of theoretical plates (N), the height equivalent of the theoretical plate (HETP) and column capacity (K') of a nicotine peak

APPENDIX 2

NICOTINE CONTENT OF RESINS

A2.1 Nicotine Polacrilex

Sample	Absorbance	Concentration of sample µg/mL	Dilution factor.	Concentration mg/mL	Nicotine released (mg)
1	140738	10.71	333	3.57	17.84
2	154141	11.69	333	3.90	19.48
3	130776	9.97	333	3.32	16.62
Mean				3.60	17.99
SD				0.286	1.44

Table A2.1 Nicotine content of Nicotine Polacrilex resin

18 mg of nicotine was released from 100 mg of Nicotine Polacrilex thus showing 18% loading of the resin.

A2.2 Nicotine Amberlite® IRP69

Sample	Absorbance	Concentration of sample µg/mL	Dilution factor.	Concentration mg/mL	Nicotine released (mg)
1	153996	11.68	333	3.89	19.47
2	162369	12.30	333	4.10	20.49
3	156988	11.90	333	3.97	19.84
Mean				3.99	19.94
SD				0.104	0.520

Table A2.2 Nicotine content of nicotine Amberlite® IRP69 resin

19.84 mg is released from 100 mg of nicotine Amberlite® IRP69 thus showing 19.84% loading of resin.

APPENDIX 3

IN VIVO STUDY POPULATION

A3.1 Inclusion Criteria for subjects

1. Age

   Aged from 18-55 years

2. Sex

   Male or female, females must not be pregnant, breast-feeding or planning to become pregnant during the course of the study, and had a negative serum pregnancy test as screening.

3. Weight

   50-90 kg

4. Smoking status

   Subjects smoke 10 or more cigarettes per day and have a plasma cotinine level >100 ng/mL at screening

5. Health

   Subjects must not have any clinically significant abnormal findings at screening physical examination or medical history which may influence the outcome of the study

6. Clinical laboratory safety tests

   All clinical laboratory safety tests must be within normal range

7. Drug screen

   Subjects must have a negative urine drug/alcohol screen for cannabinoids, cocaine, opiates and alcohol

8. Compliance

   Subjects must understand and are willing to comply with all study procedures and restrictions

9. Consent

   Subjects must understand the study and be willing to participate as demonstrated by voluntary written information

A3.2 Exclusion criteria

1. Disease

   a) A medical history, which in the opinion of the investigator would jeopardise the safety of the subject or interfere with nicotine absorption *e.g.* coronary heart disease or history of severe gastritis, peptic ulcers or malaborption syndrome.

   b) Dentures or any dental work that could affect the conduct of the study

   c) Temporomandibular joint disease or oral pathology including lesions, sores or inflammation

2. Pregnancy

   a) Females who are pregnant or lactating

   b) Positive serum pregnancy test

3. Smoking and NRT

   a) Use of less than 10 cigarettes per day

   b) The inability to abstain from smoking, or use of any other nicotine delivery product

4. Alcohol

   Current or recent history of excessive use within 12 months of screening visits at the start of the study (averaging greater than two drink/day) (one drink equals one 240 mL bottle beer, one 125 mL glass of wine; or 42 mL of 80 proof distilled spirits) or other substances abuse which will be detected by urine screen.

5. Allergy/Intolerance

   a) Subjects who have a history of an adverse event associated with use of nicotine gum or other nicotine replacement product.

   b) Subjects who have a history of allergic response to nicotine

6. Clinical Trials/Experimental medication

   Participation in a previous clinical trial within 30 days prior to the first study session

7. Blood

a) Blood donation of ≥ 1 unit, 500 mL within 90 days before the first study session

b) Plasma donation within the 90 days before the first study session

8. Medications

a) Treatment with any known enzyme altering agents (barbiturates, phenothiazines, cimetidine, theophyllines) within 30 days prior to the first study session

b) Use of any prescription medication within 14 days prior to the first study session excluding hormonal contraceptive or hormone replacement therapy

c) Use of any over-the-counter medication with 24 hours of drug dosing