

**THE DEVELOPMENT AND ASSESSMENT OF A GENERIC
CARBAMAZEPINE SUSTAINED RELEASE DOSAGE FORM**

A Thesis Submitted to Rhodes University in Fulfilment of the Requirements for the
Degree of

DOCTOR OF PHILOSOPHY

By

Fathima Patel

January 2006

Faculty of Pharmacy
Rhodes University
Grahamstown
South Africa

ABSTRACT

Carbamazepine (CBZ) is a first-line drug used for the treatment of partial and tonic-clonic seizures. It is also the drug of choice for use during pregnancy and recommended for the treatment of seizure disorders in children. CBZ possesses the ability to induce metabolism of drugs that are transformed in the liver and has the unique ability to induce its own metabolism by a phenomenon known as 'auto-induction', where its biological half-life is significantly reduced during chronic administration. Large doses of CBZ are often prescribed as daily divided doses and this often adversely affects patient compliance, with the result that therapy is ineffective.

A sustained-release dosage form containing CBZ is currently marketed as Tegretol[®] CR and the development of a generic product would provide patients with an equivalent product with a similar dosing frequency, at a reduced cost. Therefore, the development of a polymer-based matrix tablet was undertaken to produce a sustained-release dosage form of CBZ, since these dosage forms are relatively simple and cheap to produce when compared to other, more sophisticated forms of sustained-release technology.

Preformulation studies were conducted to assess moisture content of excipients and dosage forms and to identify possible incompatibilities between CBZ and potential formulation excipients. Furthermore, studies were conducted to assess the potential for polymorphic transitions to occur during manufacture. Stability testing was conducted to assess the behaviour of the dosage forms under storage conditions that the product may be exposed to. Dissolution testing was undertaken using USP Apparatus 3, which allowed for a more realistic assessment and prediction of *in vivo* drug release rates. Samples were analysed using a high performance liquid chromatographic method that was developed and validated for the determination of CBZ.

Tablets were manufactured by wet granulation and direct compression techniques, and the resultant drug release profiles were evaluated statistically by means of the f_1 and f_2 difference and similarity factors. The f_2 factor was incorporated as an assessment criterion in the design of an artificial neural network that was used to predict drug release profiles and formulation composition.

A direct compression tablet formulation was successfully adapted from a prototype wet granulation matrix formulation and a number of formulation variables were assessed to establish their effect(s) on the dissolution rate profile of CBZ that resulted from testing of the dosage forms. The particle size grade of CBZ was also investigated and it was ascertained that fine particle size grade CBZ showed improved drug release profiles when compared to the coarse grade CBZ which was desirable, since CBZ is a highly water insoluble compound. Furthermore, the impact of the viscosity grade and proportion of rate-controlling polymer, *viz.*, hydroxypropyl methylcellulose was also investigated for its effect on drug release rates. The lower viscosity grade was found to be more appropriate for use with CBZ. The type of anti-frictional agent used in the formulations did not appear to affect drug release from the polymeric matrix tablets, however specific compounds may have an effect on the physical characteristics of the polymeric tablets.

The resultant formulations did not display zero-order drug release kinetics and a first-order mathematical model was developed to provide an additional resource for mathematical analysis of dissolution profiles. An artificial neural network was designed, developed and applied to predict dissolution rate profiles for formulation. Furthermore, the network was used to predict formulation compositions that would produce drug release profiles comparable to the reference product, Tegretol[®] CR. The formulation composition predicted by the network to match the dissolution profile of the innovator product was manufactured and tested *in vitro*. The formulation was further manipulated, empirically, so as to match the *in vitro* dissolution rate profile of Tegretol[®] CR, more completely.

The test tablets that were produced were tested in two health male volunteers using Tegretol[®] CR 400mg as the reference product. The batch used for this “proof of concept” biostudy was produced in accordance with cGMP guidelines and the protocol in accordance with ICH guidelines. The test matrix tablets revealed *in vivo* bioavailability profiles for CBZ, however, bioequivalence between the test and reference product could not be established. It can be concluded that the polymeric matrix CBZ tablets have the potential to be used as a twice-daily dosage form for the treatment of relevant seizure disorders.

ACKNOWLEDGEMENTS

I would like to express my sincerest thanks and gratitude to the following people:

My supervisor, Prof. R.B. Walker

My co-supervisor, Prof. M.H. Burton

The Dean and Head of the Faculty of Pharmacy, Prof. I. Kanfer

The Head of the Department of Pharmacology, Faculty of Pharmacy, Prof. S. Daya

Dr. M.F. Skinner of the Biopharmaceutics Research Institute

Mrs. S. Emslie and Ms. K. Wentworth of the Biopharmaceutics Research Institute

Mr. and Mrs. Morley of the Faculty of Pharmacy and Mr. T. Samkange, Fezile and Colin Nontyi of the BRG laboratory, for technical support

Post-graduate colleagues of the Biopharmaceutics Research Group, past and present

Fellow colleagues and post-graduates of the Faculty of Pharmacy, Rhodes University

Emmanuel Lamprecht and Mr. Sonneman of the Chemistry department, Rhodes University

Emily Repinz and Noeleen Ferreira for their assistance during the biostudy

Mr. M. Wisch and Mr. P. van der Woude

The National Research Foundation for financial support (2002-2005)

The Mellon Foundation for financial support (2004-2005)

Noveon Pharma (Raubling, Germany) for the donation of carbamazepine

The Dow Chemical Company (Michigan, USA) and Colorcon (Kent, UK) for the donation of excipients

My friends; Wendy Jacobson, Mrs. M. Truscott, Mike Naidoo, JoAnne and Anton Vorster, Jo Kanfer, Susan van den Berg, Jeanette Coupe, Shawn and Gerrie Warren, Lucie and Colm Allan, Mr. I. Kajee and Mr. Ameen of Newcastle, Linda Emslie, Charmaine Johnson.

My parents, Yusuf and Rashida Patel, brothers, Mohammed Ilyas, Imran and Ayub Patel, grandparents, Zuleika and Yunus Patel, aunt Fareeda and uncle Mohammed Patel for their constant love and support.

STUDY OBJECTIVES

Carbamazepine is the drug of choice for the treatment of partial and tonic-clonic seizure disorders and is pharmacologically active within a narrow therapeutic range. Consequently, chronic drug therapy in epileptic patients requires steady-state plasma concentrations with minimal fluctuations below the minimum effective concentration or above the maximum toxic concentration, in order to prevent seizure relapse or the occurrence of adverse side effects. Sustained release delivery systems of carbamazepine with controlled and predictable release kinetics, when compared to conventional dosage forms are likely to result in improved drug therapy. The use of a sustained-release dosage form for the delivery of carbamazepine may therefore be appropriate and desirable to enhance patient adherence with the added advantage of minimal fluctuations in plasma carbamazepine levels. Furthermore, the use of simple controlled release technology may result in the availability of a more cost-effective therapeutic option for epileptic patients stabilized on carbamazepine.

The study objectives were:

1. To develop and validate an isocratic high performance liquid chromatography (HPLC) system for the accurate and precise quantitation of carbamazepine.
2. To develop a generic sustained-release dosage form of carbamazepine (400mg).
3. To develop and assess the feasibility of using a direct compression manufacturing procedure for large-scale production of carbamazepine matrix tablets.
4. To assess the key components that affect drug release from dosage forms produced by direct compression and therefore empirically optimise dosage form performance.
5. To elucidate the mechanisms of drug release using mathematical descriptors.
6. To develop and apply a neural network to the prediction of drug release profiles from general data and to predict formulation compositions for specific profiles.
7. To investigate the *in vivo* performance of an optimised formulation in human subjects.

TABLE OF CONTENTS

ABSTRACTii

ACKNOWLEDGEMENTSiv

STUDY OBJECTIVESv

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE.....	1
CARBAMZEPINE MONOGRAPH.....	1
1.1 Introduction.....	1
1.2 Physicochemical properties.....	2
1.2.1 Description.....	2
1.2.2 Synthesis.....	4
1.2.3 Dissociation constant and solubility data.....	4
1.3 Identification.....	5
1.3.1 X-ray diffraction (XRD).....	5
1.3.2 Infrared spectrum (IR).....	6
1.3.3 Colour test.....	7
1.3.4 Crystal test.....	7
1.3.5 Melting range.....	7
1.3.6 Ultraviolet absorption spectrum.....	7
1.4 Stability.....	8
1.5 Pharmacokinetics.....	9
1.5.1 Absorption.....	9
1.5.2 Distribution.....	9
1.5.3 Metabolism and elimination.....	10
1.5.4 Mode of action (MOA).....	12
1.5.5 Clinical indications.....	13
1.5.6 Therapeutic levels and dosing.....	13
1.5.7 Drug interactions.....	14

1.5.8 Adverse effects and toxicity.....	15
1.6 Conclusion.....	16
CHAPTER TWO.....	18
HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) ANALYSIS OF CBZ....	18
2.1 Method development.....	18
2.1.1 Introduction.....	18
2.1.2 High performance liquid chromatography (HPLC).....	21
2.2 Experimental.....	22
2.2.1 Reagents.....	22
2.2.2 Preparation of stock solutions.....	23
2.2.3 Preparation of buffer solutions.....	23
2.3 HPLC system.....	23
2.3.1 Chromatographic hardware.....	23
2.3.2 Column selection.....	24
2.4 Mobile phase selection.....	25
2.4.1 Factors affecting the choice of mobile phase.....	25
2.4.2 Mobile phase selection.....	26
2.4.3 Buffer molarity.....	27
2.4.4 Buffer pH.....	28
2.4.5 Organic modifier.....	29
2.4.6 Flow rate selection.....	31
2.5 Detection.....	31
2.5.1 Method of detection.....	31
2.5.2 Detection wavelength (λ).....	32
2.5.3 Chromatographic conditions selected.....	32
2.6 Method validation.....	34
2.6.1 Introduction.....	34
2.7 Results and discussion.....	35
2.7.1 Specificity.....	35
2.7.2 Linearity and range.....	35

2.7.3 Precision.....	36
2.7.3.1 Repeatability.....	37
2.7.3.2 Intermediate precision.....	38
2.7.3.3 Reproducibility.....	38
2.7.4 Accuracy and bias.....	39
2.7.5 Limit of quantitation (LOQ) and Limit of Detection (LOD).....	39
2.7.6 Stability of the analyte.....	40
2.8 CONCLUSION.....	43
CHAPTER THREE.....	44
DOSAGE FORM ASSESSMENT.....	44
3.1 Introduction.....	44
3.2 Moisture content analysis.....	45
3.2.1 Methods and equipment.....	45
3.2.2 Results and discussion.....	46
3.2.3 Conclusion.....	47
3.3 Differential scanning calorimetry (DSC).....	47
3.3.1 Method.....	48
3.3.2 Results and discussion.....	49
3.4 X-ray diffraction.....	49
3.4.1 Method.....	49
3.4.2 Results and discussion.....	50
3.5 Physical assessment of tablets.....	52
3.5.1 Methods.....	53
3.5.2 Results and discussion.....	53
3.5.2.1 Tablet diameter, thickness and weight.....	53
3.5.2.2 Tablet hardness and friability.....	54
3.6 Tablet Assay.....	56
3.6.1 Content uniformity assay and residual content analysis.....	56
3.6.2 Method.....	57
3.6.3 Results and discussion.....	58

3.7 Conclusion.....	60
3.8 Dissolution testing.....	61
3.8.1 Variables affecting dissolution rates.....	61
3.8.2 Dissolution apparatus.....	63
3.8.3 Preliminary selection of dissolution testing apparatus.....	64
3.8.4 Method.....	65
3.8.5 Results and discussion.....	66
3.8.6 Sample treatment.....	68
3.8.7 Conclusion.....	69
3.9 Statistical comparison of dissolution rate profiles.....	70
3.10 CONCLUSION.....	75
3.11 Stability assessment of the biostudy test batch.....	77
3.11.1 Introduction.....	77
3.11.2 Stability studies.....	78
3.12 Results of stability testing.....	79
3.12.1 Visual appearance.....	79
3.12.2 Tablet hardness.....	79
3.12.3 Tablet weight.....	80
3.12.4 Tablet friability.....	81
3.12.5 Moisture content.....	82
3.12.6 Assay.....	82
3.12.7 Dissolution testing.....	83
3.13 CONCLUSION.....	85
 CHAPTER FOUR.....	 86
PHARMACEUTICAL APPLICATIONS OF HYDROPHILIC POLYMERIC MATRIX SUSTAINED RELEASE TECHNOLOGY.....	86
4.1 Introduction.....	86
4.2 Sustained-release delivery systems.....	88
4.2.1 Mechanisms of drug release.....	90
4.3 Mathematical modelling of drug release from polymeric matrix tablets.....	92

4.3.1 The ‘power law’	93
4.3.2 Matrix tablets.....	94
4.3.3 Fick’s second law.....	96
4.3.4 The Higuchi model.....	97
4.3.5 An alternative ‘first-order decay model’	98
4.3.6 Application of the ‘first-order decay’ model.....	108
4.3.7 Comparison of the ‘power law’ and the ‘first-order decay’ model.....	109
4.3.8 Elucidation of mechanism of CBZ release from HPMC matrix tablets.....	112
4.4 CONCLUSION.....	116
CHAPTER FIVE.....	119
FORMULATION DEVELOPMENT OF SUSTAINED RELEASE CBZ MATRIX TABLETS.....	119
5.1 Rationale for the sustained delivery of CBZ.....	119
5.2 Formulation development.....	120
5.2.1 Tablet manufacture.....	120
5.2.2 Wet granulation.....	120
5.3 Experimental.....	121
5.3.1 Overview.....	121
5.3.2 Materials.....	122
5.3.2.1 CBZ.....	122
5.3.2.2 Excipients.....	122
5.3.2.3 Hydroxypropylmethylcellulose (HPMC).....	122
5.3.2.4 Dibasic calcium phosphate (DCP).....	124
5.3.2.5 Microcrystalline cellulose (MCC).....	125
5.3.2.6 Lactose.....	125
5.3.2.7 Sodium lauryl sulphate (SLS).....	125
5.3.2.8 Magnesium stearate (Mg stearate).....	126
5.3.2.9 Polyvinyl alcohol (PVA).....	126
5.3.3 Manufacturing methods.....	127
5.3.3.1 Manufacturing procedure.....	128

5.3.3.2 Effect of polymer grade on CBZ dissolution rate.....	130
5.4 Results and discussion.....	131
5.4.1 Comparison of dissolution rates of the four formulations.....	131
5.5 CONCLUSION.....	134
CHAPTER SIX.....	135
THE DEVELOPMENT AND ASSESSMENT OF DIRECT COMPRESSION MATRIX SUSTAINED RELEASE CBZ TABLET FORMULATIONS.....	135
6.1 Introduction.....	135
6.1.1 Direct compression (DC) tableting.....	135
6.2 Experimental.....	136
6.2.1 Overview.....	136
6.2.2 Direct compression excipients.....	137
6.2.2.1 Purified talc.....	137
6.2.2.2 Colloidal silicon dioxide.....	138
6.2.3 Methods.....	138
6.2.3.1 Direct compression tablet composition.....	138
6.2.3.2 Direct compression manufacturing processes.....	139
6.2.3.2.1 Original process (A).....	139
6.2.3.2.2 Modified process (B).....	139
6.3 Investigation of ruggedness of the formulation.....	141
6.3.1 Adaptation of WG to DC method of manufacture.....	141
6.3.2 Effect of CBZ particle size.....	141
6.3.3 Effect of HPMC grade on dissolution rate.....	142
6.3.4 Effect of sodium lauryl sulphate (SLS) on dissolution rate.....	143
6.4 Results and discussion.....	145
6.4.1 Conversion of the WG formula to a DC formula.....	145
6.4.2 Effects of particle size grade of CBZ on <i>in vitro</i> release rates.....	146
6.4.3 Effect of viscosity grade of HPMC on drug release rates.....	149
6.4.4 Effect of SLS on CBZ release rates.....	151
6.4.5 Effects of anti-frictional agents on CBZ release rates.....	153

6.4.5.1 Physical testing of tablets.....	154
6.4.6 Comparison of HPMC DC matrix tablets to Tegretol [®] CR tablets.....	156
6.5 CONCLUSION.....	162
CHAPTER SEVEN.....	163
ARTIFICIAL NEURAL NETWORKS (ANN) IN FORMULATION DEVELOPMENT.....	163
7.1 Introduction.....	163
7.2 Design and construction of a neural network.....	164
7.2.1 The structure of a neural network.....	165
7.3 Applications of ANN for formulation development.....	170
7.3.1 Construction training and deployment of the ANN.....	171
7.4 CONCLUSION.....	182
CHAPTER EIGHT.....	184
IN VIVO ASSESSMENT OF 400MG CBZ SUSTAINED RELEASE POLYMERIC MATRIX TABLETS.....	184
8.1 Introduction.....	184
8.2 Biopharmaceutics.....	187
8.3 Pharmacokinetics.....	189
8.3.1 Volume of distribution.....	190
8.3.2 Biological half-life ($t_{1/2}$).....	191
8.3.3 Clearance.....	192
8.3.4 One-compartment open model.....	193
8.3.5 Zero and first-order kinetics.....	193
8.4 Bioequivalence testing of CBZ matrix tablets.....	194
8.4.1 Ethical approval.....	194
8.4.2 Protocol and study design.....	195
8.4.2.1 Test and reference products.....	195
8.4.2.2 Study design.....	195
8.4.3 Pre-study screening.....	196
8.4.3.1 Inclusion criteria.....	196

8.4.3.2 Exclusion criteria.....	198
8.4.4 Concomitant medication and subject restrictions.....	199
8.5 Pre- and post-study medical screening.....	199
8.6 Sample collection.....	200
8.7 Sample analysis.....	201
8.7.1 Reagents.....	202
8.7.2 Method.....	202
8.8 Statistical analysis.....	203
8.9 Results and discussion.....	205
8.10 CONCLUSION.....	214
 CHAPTER NINE.....	 216
CONCLUSIONS.....	216

LIST OF TABLES

Table 1.1	Properties of the polymorphic forms of CBZ	6
Table 1.2	IR band frequencies for CBZ.....	6
Table 1.3	Drug interactions with CBZ.....	14
Table 2.1	Published HPLC methods used for the analysis of CBZ in biological fluids and dosage forms.....	20
Table 2.2	The effects of altering organic modifier content on retention time, peak shape and resolution.....	31
Table 2.3	Optimal chromatographic conditions for the analysis of CBZ	33
Table 2.4	Repeatability data for CBZ analysis... ..	39
Table 2.5	Intermediate precision data for CBZ analysis.....	39
Table 2.6	Accuracy and percent bias data for the analysis of CBZ.....	40
Table 3.1	Moisture content of raw material used for matrix tablet manufacture.....	47
Table 3.2	Moisture content of selected CBZ tablet blends.....	48
Table 3.3	Parameters and variables used for XRPD analysis.....	51
Table 3.4	Mean tablet weight, thickness and diameter values for selected tablet batches (n=20).....	55
Table 3.5	Hardness and friability values for selected batches.....	56
Table 3.6	Content uniformity and assay results for CBZ matrix tablets.....	60
Table 3.7	Residual content analysis after dissolution of CBZ matrix tablets produced by WG.....	61
Table 3.8	Dissolution test condition for USP apparatus 2 and 3.....	67
Table 3.9	Dissolution test conditions used for all tablet batches.....	70
Table 3.10	Recommended storage conditions for stability in Zones I and II.....	77
Table 3.11	Result of hardness testing.....	80
Table 3.12	Tablet weight uniformity.....	81
Table 3.13	Tablet friability.....	81
Table 3.14	Assay results.....	83
Table 4.1	Statistical and mathematical parameters for the experimental formulations tested.....	108

Table 4.2	R ² values for square root of time plots.....	114
Table 4.3	R ² values for square root of time plots for the first 6 hours.....	115
Table 5.1	Wet granulation tablet formulations.....	127
Table 5.2	Formulation composition for four batches containing CBZ.....	130
Table 5.3	Statistical comparisons of the four formulations.....	131
Table 5.4	Mass balance analysis (n=6).....	133
Table 6.1	DC formula with corresponding WG composition.....	138
Table 6.2	Formulation composition for the formulations used to assess the effect of CBZ particle size on <i>in vitro</i> dissolution rates of CBZ.....	142
Table 6.3	Composition of formulations used to assess the effect of FG and CG CBZ on <i>in vitro</i> dissolution rates of CBZ.....	142
Table 6.4	Formulation composition for the assessment of polymer viscosity grade on CBZ dissolution rates.....	143
Table 6.5	Formulation composition used to evaluate SLS effects on the <i>in vitro</i> dissolution rate of CBZ.....	144
Table 6.6	Formulations used to evaluate the effect of anti-frictional agents on the <i>in vitro</i> dissolution rate.....	144
Table 6.7	Similarity and difference factors for the comparison of formulations with varying proportions of CG and FG CBZ.....	147
Table 6.8	Difference and similarity factors used to assess polymer grade effects on CBZ release.....	150
Table 6.9	Difference and similarity factors used to compare the effect of SLS on CBZ release rates.....	152
Table 6.10	Difference and similarity factors used to compare dissolution profiles of formulations with different anti-frictional agents.....	154
Table 6.11	Physical characteristics of tablets with different anti-frictional agents	155
Table 6.12	Difference and similarity factors used to compare the dissolution profiles of batches C-200603-02 and C-211202-03 to Tegretol [®] CR.....	157
Table 6.13	Difference and similarity factors used to compare the dissolution profiles of Batches C-260303-02 and C-140705-01 to Tegretol [®] CR.....	158

Table 6.14	Similarity factors used to compare the dissolution profiles of batches C-030703-01, C-030703-03, C-190603-01 to Tegretol [®] CR.....	161
Table 7.1	Summary and description of the training algorithms in the MATLAB neural network toolbox.....	169
Table 7.2	Input variable introduced to the ANN	171
Table 7.3	Target vector components.....	172
Table 7.4	First five columns of the data matrix D.....	172
Table 7.5	Difference and similarity factors comparing Tegretol [®] CR to the predicted and actual formulation release profiles.....	180
Table 7.6	Predicted formulation and adjusted formulation.....	180
Table 7.7	Release profile data for the predicted and the adjusted formulations.....	182
Table 8.1	Randomisation schedule.....	196
Table 8.2	Inclusion criteria for the bioequivalent assessment of CBZ matrix tablets	197
Table 8.3	Exclusion criteria for the bioequivalent assessment of CBZ matrix tablets	198
Table 8.4	Pharmacokinetic data reported for CBZ (Mean \pm SD %)......	201
Table 8.5	Definitions of pharmacokinetics and statistical parameters used to analyse CBZ blood level data.....	204
Table 8.6	Point estimate ratios for C _{max} and AUCT using log transformed data.....	205
Table 8.7	Pharmacokinetic and statistical results for the test formulation for both subjects.....	206
Table 8.8	Pharmacokinetic and statistical results for Tegretol [®] CR for both subjects....	207

LIST OF FIGURES

Figure 1.1	The chemical structure of CBZ and the metabolic products of biotransformation.....	3
Figure 1.2	A schematic diagram showing the synthesis of CBZ	4
Figure 1.3	X-ray diffractogram of CBZ (β form).....	5
Figure 1.4	IR absorption spectrum for CBZ.....	6
Figure 1.5	UV absorption spectrum of CBZ in methanol	8
Figure 2.1	The effect of buffer molarity on the retention times of CBZ, PRO, IMP and CYZ.....	28
Figure 2.2	The effect of buffer pH on the retention times of CBZ, PRO, IMP and CYZ.....	29
Figure 2.3	The effect of modifier content on the retention times of CBZ, PRO, IMP and CYZ.....	30
Figure 2.4	Typical chromatogram depicting CBZ and IS (60 μ g/ml).....	34
Figure 2.5	A typical calibration curve obtained for the analysis of CBZ	37
Figure 2.6	Stability data for stock solutions stored in the fridge protected from light	42
Figure 2.7	Stability peak response for samples stored at room temperature (24 $^{\circ}$ C) away from light.....	43
Figure 3.1	X-ray diffractogram of CG CBZ.....	52
Figure 3.2	X-ray diffractogram of CG CBZ, ground for ten minutes, with a mortar and pestle	53
Figure 3.3	Mean (n=6) dissolution rate profiles of CBZ release from Tegretol $^{\circledR}$ CR tablets using either USP Apparatus 2 or Apparatus 3.....	68
Figure 3.4	$f_2(R, T)$ if R differ from T by 5%.....	72
Figure 3.5	$f_2(R, T)$ if R differ from T by 10%.....	73
Figure 3.6	$f_2(R, T)$ if R differ from T by 15%.....	73
Figure 3.7	Plot of $f_2(R, T)$ versus percentage difference between the coordinates of R and T	73

Figure 3.8	Script to produce $f_2(R, T)$ as a function of the percentage coordinate-wise difference between R and T	74
Figure 3.9	f_2 values vs percentage coordinate-wise difference	74
Figure 3.10	$f_2(R, T, w)$ if R differs from T by 15% using a normalised weighting vector	76
Figure 3.11	Dissolution profiles of the test batch C-160905-01 at $T=0$ and after storage under ambient conditions for one month	84
Figure 4.1	Diagrammatic representation of the swelling and erosion processes associated with matrix tablets	91
Figure 4.2	Swelling dimensions for a monolithic matrix tablet.....	92
Figure 4.3	The MATLAB code for the ‘power law’	99
Figure 4.4	A fit depicting the linear relationship between n and r as derived from the MATLAB computational environment.....	101
Figure 4.5	Correlation matrix.....	101
Figure 4.6	A fit depicting the quadratic relationship between n and r as derived from the MATLAB computational environment.....	103
Figure 4.7	A fit depicting the cubic relationship between n and r as derived from the MATLAB computational environment.....	104
Figure 4.8	A fit depicting the quartic relationship between n and r as derived from the MATLAB computational environment.....	105
Figure 4.9	Dissolution profile of Tegretol® CR fitted to the ‘power law’	110
Figure 4.10	Dissolution profile of Tegretol® CR fitted to the ‘first order decay’ model.....	110
Figure 4.11	Batch C-030705-05 fitted to the ‘power law’	111
Figure 4.12	Batch C-030705-05 fitted to the ‘first order decay’ model.....	112
Figure 4.13	Higuchi plots for dissolution profiles of selected formulations over a 22 hour period	113
Figure 4.14	Higuchi plots for dissolution profiles of selected formulations over a 6 hour period	114
Figure 5.1	Flow diagram of the WG manufacturing process	129

Figure 5.2	Dissolution profiles of CBZ from batches C-211202-01 (15.7% w/w Methocel® K4M), C-211202-02 (6.7 Methocel® K4M; 9% w/w Methocel® K100M), C-211202-03 (18.5% w/w Methocel® K4M) and C-211202-04 (3.5% w/w Methocel® K4M; 15% w/w Methocel® K100M)	132
Figure 6.1	Schematic diagram showing the original (a) and modified (b) direct compression process	140
Figure 6.2	Mean dissolution rate profiles (n=6) of CBZ release from a WG formulation (Batch C-211202-02) and a DC formulation (Batch C-280503-01) of similar composition	145
Figure 6.3	Mean dissolution rate profiles (n=6) of CBZ release from formulations containing 40% w/w CG (Batch 030703-04) or FG (Batch C-030703-01) CBZ	146
Figure 6.4	Mean dissolution rate profiles (n=6) of formulations with 50% FG: 50% CG (Batch C-190603-03); 60% FG: 40% CG (Batch C-190603-02) and 80% FG: 20% CG (Batch C-030703-05) and Tegretol® CR	148
Figure 6.5	Mean dissolution rate profiles (n=6) of Batches C-140705-02 (20% w/w K100M), C-180705-01 (32% w/w K100M), C-190705-01 (26% w/w K4M) and C-200705-01 (32% w/w K4M)	149
Figure 6.6	Mean dissolution rate profiles (n=6) of CBZ release from formulations containing FG with SLS (Batch C-280503-01), CG with SLS (Batch C-030703-04), CG without SLS (Batch C-030703-03)	152
Figure 6.7	Mean dissolution rate profiles (n=6) of CBZ release form formulations containing talc (Batch C-200603-02), Cab-o-Sil® (Batch C-200603-03) and Mg stearate (Batch C-200603-01)	153
Figure 6.8	Mean dissolution profiles (n=6) of CBZ release from Batches C-200603-02 and C-211202-03 manufactured by DC and WG, respectively, compared to Tegretol® CR	156
Figure 6.9	Mean dissolution rate profiles (n=6) of Tegretol® CR compared to Batches C-260303-02 and C-140705-01 manufactured by DC	158

Figure 6.10	Mean dissolution rate profiles (n=6) of CBZ release from DC formulations compared to that of Tegretol [®] CR	160
Figure 7.1	Diagrammatic representation of the layers of a neural network	166
Figure 7.2	Graph of MSE versus number of epochs	174
Figure 7.3	Command window display of MSE and gradient during training	175
Figure 7.4	Graph depicting target (actual) and activation (predicted) vectors.....	177
Figure 7.5	Trends in CBZ release profiles when the CG:FG ratios are varied	178
Figure 7.6	Dissolution profiles of CBZ release from Tegretol [®] CR, the network predicted formulation (Batch C-120905-01) and the modified (Batch C-160905-01).....	181
Figure 8.1	Cartesian plot of the mean blood level profiles of both the test and the reference products.....	208
Figure 8.2	Resultant plasma concentration versus time profiles for subject 1 following administration of the test (Phase 1) and reference (Phase 2) products.....	209
Figure 8.3	Resultant plasma concentration versus time profile for subject 2 following administration of the test (Phase 2) and reference (Phase 1) products	210
Figure 8.4	Semi-logarithmic plot of the mean (n=2) plasma concentration of CBZ for both test and reference products... ..	211
Figure 8.5	Plasma CBZ concentration obtained following the administration of the reference product (Tegretol [®] CR) to subjects 1 and 2	212
Figure 8.6	Plasma CBZ concentrations obtained following administration of the test matrix tablets for subjects 1 and 2.....	213

CHAPTER ONE

CARBAMAZEPINE MONOGRAPH

1. Carbamazepine (CBZ)

1.1 Introduction

A seizure can be defined as any interruption of consciousness that may be accompanied by changes in motor, sensory, or behavioral activity [1] or alternately as a recurrent paroxysmal disorder of cerebral function that is characterized by sudden, brief attacks of altered consciousness, motor activity, sensory phenomena, or inappropriate behavior caused by an abnormal excessive discharge of cerebral neurons [2].

Seizures can be classified according to several different criteria and the specific symptom or set of symptoms, reflect the affected part of the cerebral hemisphere in which the seizure originates [2]. Approximately 1 to 2% of the world population is affected by some type of seizure disorder [2, 3], and seizures are the second most common neurological disorder after stroke that occurs in humans [3].

The principal mechanisms of action of 1 have been found to involve voltage-operated ion channels and/or inhibitory and excitatory synaptic function [3]. Following each action potential, voltage-dependant Na^+ channels enter an inactive state, and antiepileptic compounds are thought to prolong the inactive state [3]. CBZ is an effective anti-convulsant agent, used for the control of major motor and psychomotor epilepsy [4] and is used in the treatment of simple and complex partial seizures, tonic-clonic seizures as well as partial with secondarily generalized tonic-clonic seizures [6]. In addition, CBZ is used in the treatment of trigeminal neuralgia and for the treatment of generalized as well as complex partial seizures [7].

CBZ is most frequently prescribed for patients who have failed to respond to first-line anti-convulsant therapy or for those who develop significant side-effects from other anticonvulsant agents [7]. Adult doses of up to 2g per day in divided doses may be prescribed [3,5,6,7] to patients receiving CBZ therapy. Large doses often ensue as treatment progresses and this is principally due to the auto-inducing properties of CBZ [1,3,5,6]. CBZ therefore potentiates its own metabolism through the induction of hepatic cytochrome P450

hepatic enzymes, specifically the CYP3A4 system [1,6]. Up regulation of the CYP3A4 system may also occur in the gut wall epithelial cells resulting in altered absorption in some patients.

Sustained-release formulations allow for the prolonged action of an overall reduced dose and this is seen as beneficial from a patient compliance perspective. Furthermore, the side-effect profile is improved through the constant delivery of drug throughout the gastro-intestinal tract. Currently there are several commercially available sustained-release dosage forms and Tegretol[®] CR is the trade name of the product available in South Africa.

CBZ exhibits anti-diuretic activity through increased levels of the anti-diuretic hormone and is not as effective in the treatment of absence seizures associated with a 3 second spike and wave in electroencephalograms or in myoclonic seizures of childhood or adolescence [5].

1.2 Physico-Chemical Properties

1.2.1 Description

Carbamazepine is an iminostilbene derivative that is structurally related to the tri-cyclic anti-depressants [5, 7, 8] and contains the dibenzazepine ring system of the psychotherapeutic drug, imipramine (4).

CBZ is known as 5-carbamyl-5H-dibenzo[b,f]azepine [1,5], 5H-dibenzo[b,f]azepine-5-carboxamide [1,4,9, 10, 11), 5-carbamoyl-5H-dibenz[b,f]azepine [5, 12] or 2,3:6,7-dibenzazepine-1-carboxylicacid,amide [13].

The chemical structure of CBZ and its metabolites are depicted in Figure 1.1. It has an empirical formula of C₁₅H₁₂N₂O and possesses a molecular weight of 236.26 g/mol [1,5,9, 10,12].

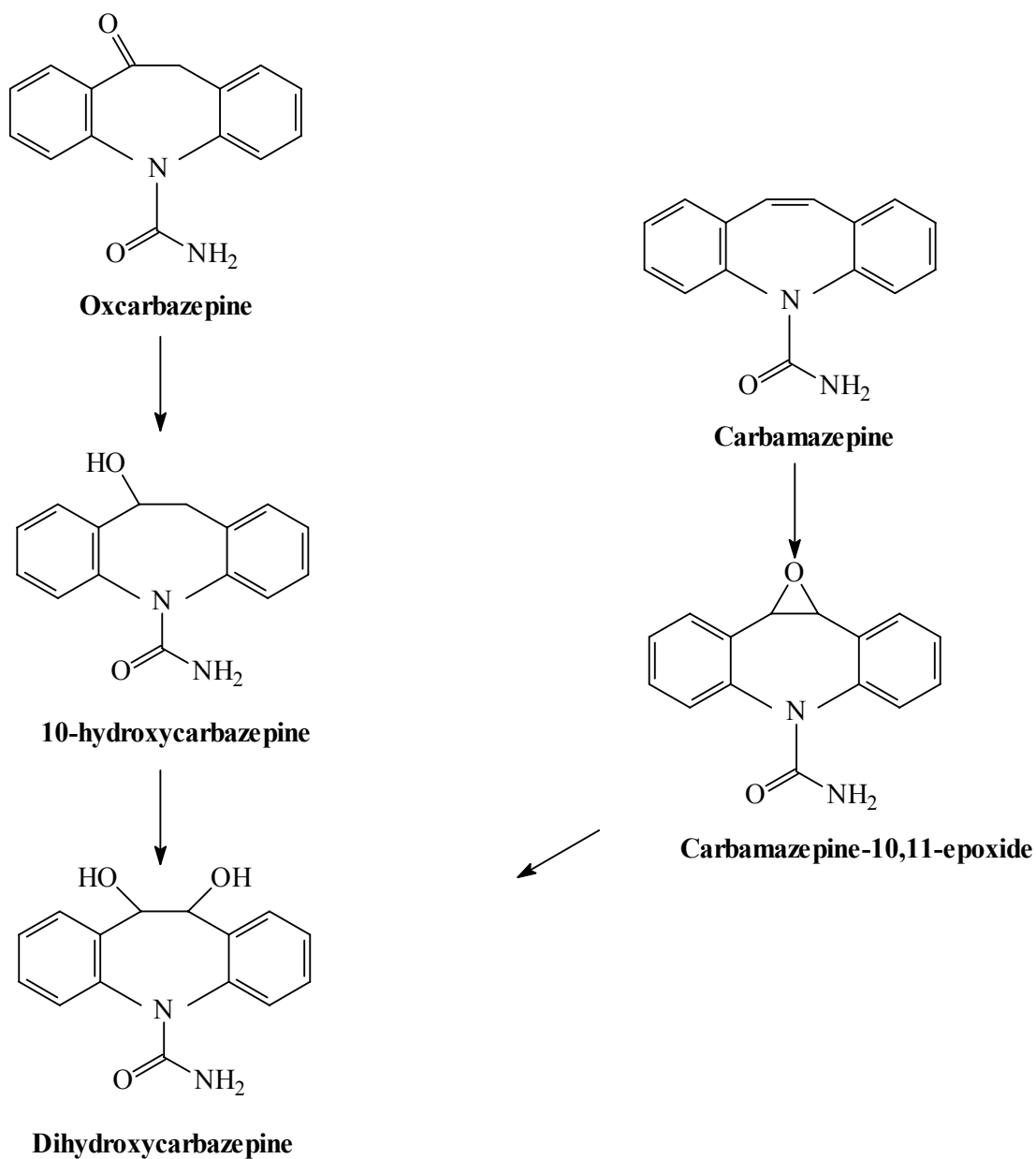


Figure 1.1 The chemical structure of CBZ and the metabolic products of Biotransformation.

The elemental composition of CBZ is approximately 76.25% carbon, 5.12% hydrogen, 11.86% nitrogen and 6.77% oxygen [13]. CBZ is a white, yellowish white or off-white crystalline compound or powder which is odourless and either tasteless or possesses a slightly bitter taste [1,5,9,10,12,13,14]

1.2.2 Synthesis

CBZ can be synthesized by treating iminodibenzyl in toluene with carbonyl chloride (COCl_2) to give 95% of 5-chlorocarbonyl iminodibenzyl, which can be dissolved in carbon tetrachloride (CCl_4). The solution is further treated with 1,3-dibromo-5,5-dimethyl hydantoin and $(\text{PhCO}_2)_2$ to give 90% 5-chloro-carbonyl-10-bromoiminodibenzyl. The latter compound is dissolved in xylene and heated at about 100°C in an autoclave with the introduction of gaseous ammonia (NH_3) to produce 85% 5-carbamoyl-5H-dibenzo[b,f]zepine [1,13]. A schematic representation of this process is shown in Figure 1.2

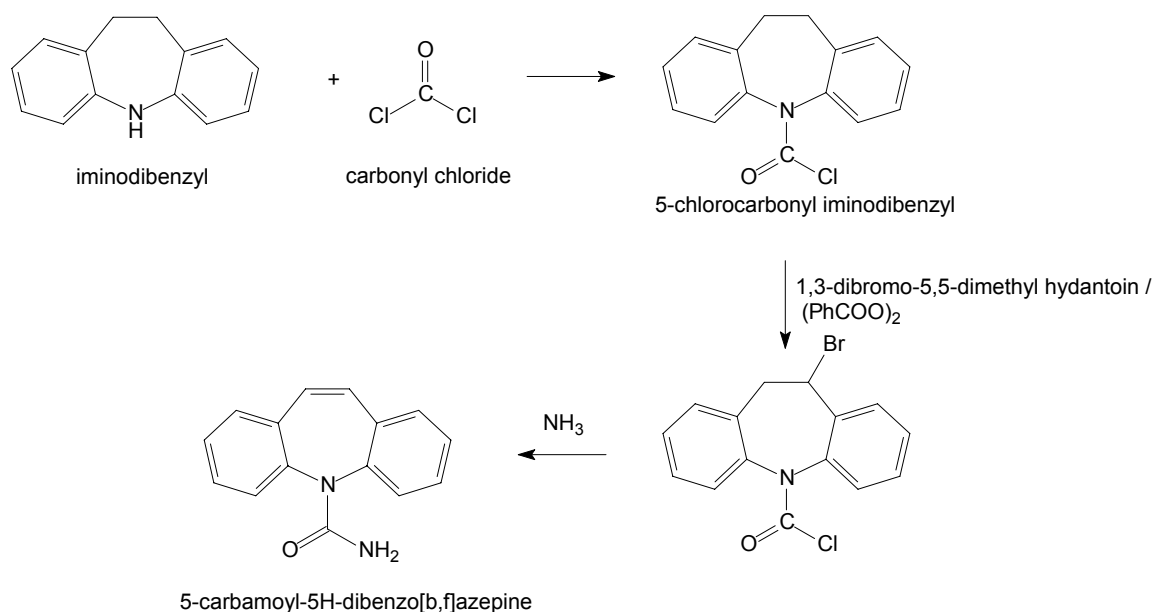


Figure 1.2 A schematic diagram showing the synthesis of CBZ.

1.2.3 Dissociation Constant and Solubility data

CBZ is highly insoluble in water at 25°C [10,12,13,14] with a solubility of 170mg/l. CBZ is a neutral molecule [5] and consequently, does not possess a dissociation constant and therefore is unlikely to be affected by variations in pH during dissolution testing, HPLC analysis and following oral administration in humans. CBZ is readily soluble in non-polar solvents [12] and in propylene glycol [4,12,13] and is sparingly soluble in alcohol and in

acetone [10,12,13,14]. Knowledge of the solubility characteristics of CBZ, become pertinent during formulation development when selecting a granulating fluid for wet granulation (WG), as well as provides alternatives to modify the formulation composition and enables the formulator to assess dissolution profile data appropriately.

1.3 Identification

1.3.1 X-Ray Diffraction (XRD)

XRD reveals that in the three dimensional structure of CBZ, the angle of flexure of α is 53° , the angle of annelation, β is 30° and the angle of torsion γ is 3° [1]. The centres between the benzene rings measured at 4.85\AA is characteristic or typical of the tricyclic psychoactive drugs [1]. CBZ exhibits polymorphism and four polymorphic forms have been isolated with the β form or form III being the polymorph used in commercial pharmaceutical formulations [14-17]. Other polymorphic forms have been reported but appear to be extremely unstable. The properties of two major stable forms of CBZ and the dihydrate form are summarized in Table 1.1. An example of an X-ray diffractogram is depicted in Figure 1.3.

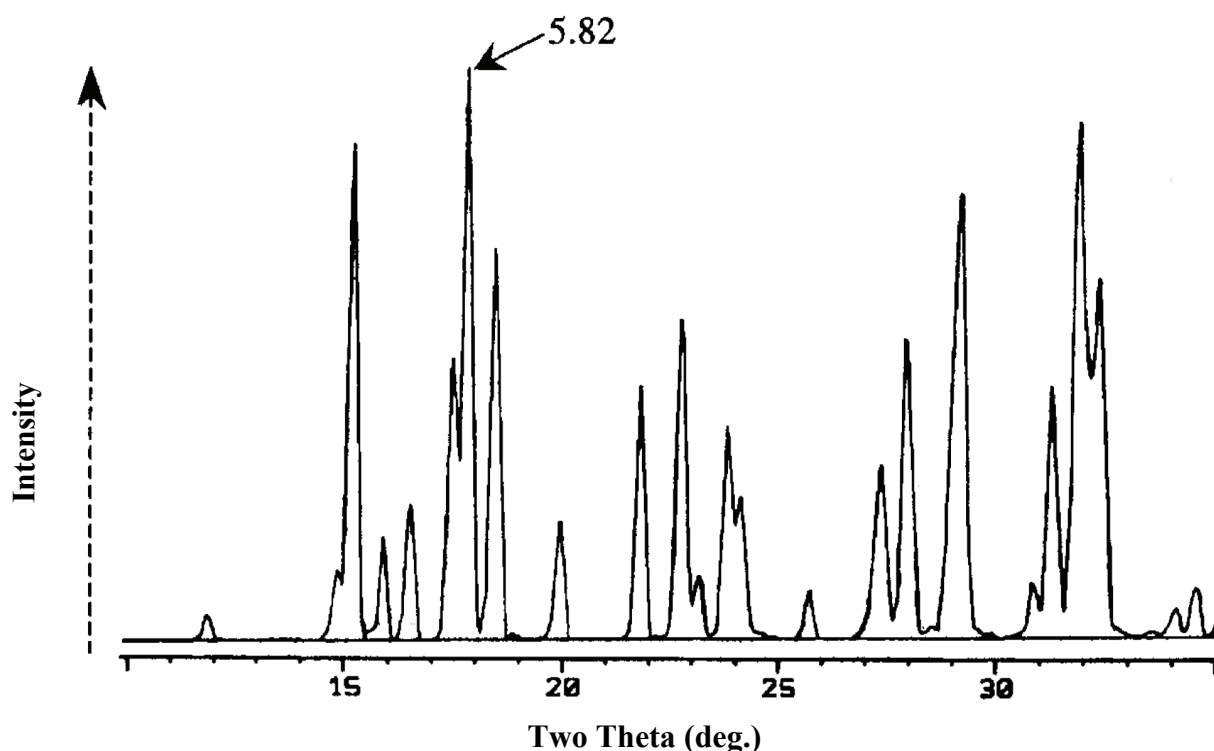


Figure 1.3 X-ray diffractogram of CBZ (β form) [62].

Table 1.1 Properties of the polymorphic forms of CBZ

Property	CBZ α Form	CBZ β Form	CBZ Dihydrate
Behaviour on heating	Melting point 190 - 191°C	Transition at 176°C to alpha form	Dehydration from 30 to 85°C Transition into alpha form
Water Content (Karl Fischer)	<0.20%	<0.20%	13.2%
Shape of particles	Narrow needles	Ground crystals	Needles

1.3.2 Infrared Spectrum (IR)

The characteristic absorption bands in the infrared absorption spectrum of CBZ are shown in Figure 1.4 [10, 13] and a summary of the description of the characteristic IR bands for CBZ are described in Table 1.2 [13]

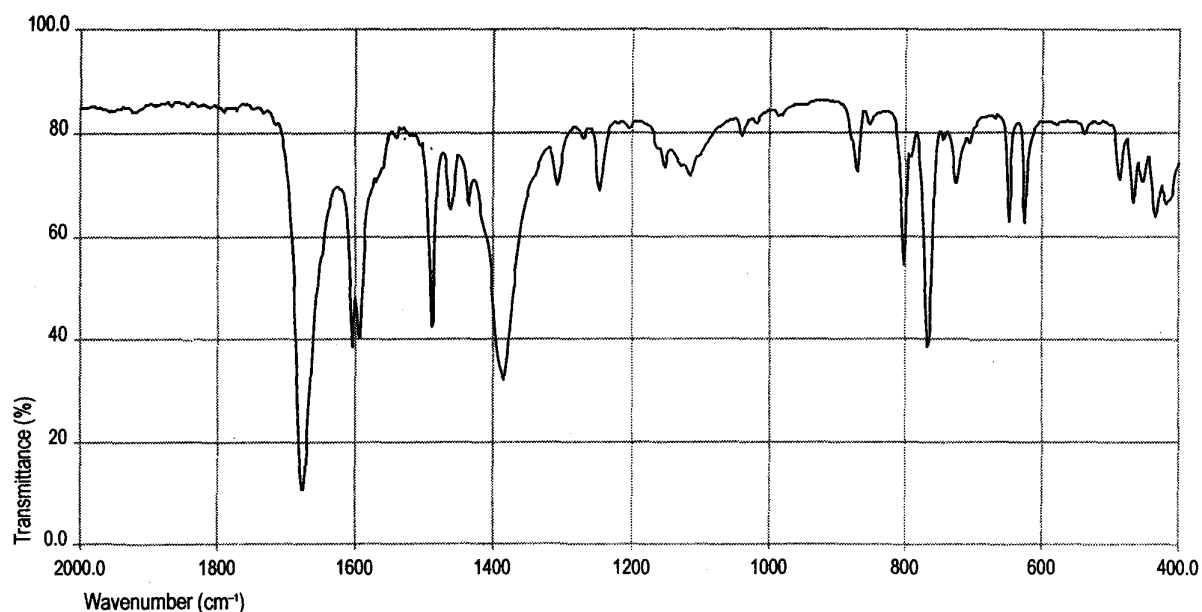


Figure 1.4 IR absorption spectrum for CBZ [9].

Table 1.2 IR band frequencies for CBZ.

Frequency (cm ⁻¹)	Assignments
3470	NH ₂
1680	C = O
1600 shoulder and 1590	Aromatic C = C

Characteristic principal peaks for CBZ when determined in potassium bromide occur at 1678, 1388 and 1594 cm^{-1} .

1.3.3 Colour Test

CBZ can be identified using a colorimetric test in which ammonium molybdate is the reagent of choice and yields a faint blue colour, with an analytical sensitivity of 1.0 μg [13]. An additional colorimetric test procedure has been described, in which 0.1g of the drug is treated with 2mL nitric acid in a water bath for three minutes after which an orange colour becomes apparent [10].

1.3.4 Crystal Test

CBZ can also be identified using a crystallization test, where needle-shaped crystals are formed when CBZ is dissolved in a lead iodide solution [13].

1.3.5 Melting Range

CBZ melts within a range between 187° and 193°C [1,10,13].

1.3.6 Ultraviolet Absorption Spectrum

The wavelength of maximum absorption (λ_{max}) of CBZ in a neutral methanolic solution is 212nm with inflections at 236nm, 283nm and a minimum at 256nm [13]. A typical UV absorption spectrum of CBZ is shown in Figure 1.5. A major peak has been reported to occur at 220nm and a smaller peak at 280-290nm [1]. The solvent in which the UV spectrum is developed may affect the specific UV absorbance of CBZ slightly. For example, in ethanol a λ_{max} of 215nm was observed and a minimum at 257nm, when compared to the UV spectrum developed in a methanolic solution [13].

CBZ also exhibits an intense blue fluorescence when it is radiated with ultraviolet light at 366nm [13].



Figure 1.5 UV absorption spectrum of CBZ in methanol [13].

1.4 Stability

CBZ is a relatively stable compound when stored in the solid state at controlled room temperature [9,13]. CBZ should be stored in airtight containers [9,13,14] and protected from light [9,13].

1.5 Pharmacokinetics

1.5.1 Absorption

The absorption of CBZ following oral administration has been shown to be slow, erratic, unpredictable [1,3,6,12,18] but almost complete [3,20] with approximately 80 - 90% bioavailability [7,12,19]. CBZ is lipid soluble compound, which is slowly and variably absorbed from the GIT [7]. The slow and discontinuous absorption of CBZ from the gastrointestinal tract (GIT), and subsequent variable plasma concentrations, has been attributed in part to the slow dissolution rate of CBZ in GIT fluids [1,20] or through a rapid GIT transit time through the anti-cholinergic effects of CBZ [1]. It is therefore likely, that the absorption of CBZ is primarily dissolution-rate limited [12,18].

It has been reported that concomitant administration of CBZ with food improves the absorption profile of CBZ [1,21] and food has been shown to assist patients to tolerate larger daily doses of CBZ [3]. Peak plasma concentrations are usually observed between 4 to 8 hours following oral administration of CBZ [1,3,6,13]. However, peak levels can usually be reached approximately 3-6 hours after dosing but may be delayed by up to 24 hours after administration [7,12]. The amount of drug administered may also play a role in the bioavailability of CBZ [1]. A consistent delay in the time to reach C_{max} , in addition to an apparent reduced absorption, has been reported when daily doses higher than 20-25 mg/kg were administered to either healthy volunteers or patients [1]. This may be a result of incomplete dissolution of CBZ in the GIT fluids [1]. As a result doses administered 3 or 4 times daily may provide more effective and appropriate therapy as dissolution rates would not be the rate controlling step in absorption when the drug is delivered in divided doses [1].

1.5.2 Distribution

CBZ is rapidly distributed into all tissues and fluids throughout the body [6,14] and is reported to have an apparent volume of distribution of approximately 1 to 2 L/kg [3, 7,12,18].

Plasma protein binding has been reported to be approximately, 70 to 80% [1,3,5-7,12,14,18] when plasma concentrations of CBZ are between 5 – 30 µg/ml [1]. Albumin and α -1-acid-

glycoprotein in plasma are the proteins implicated in protein binding of CBZ [5,18]. No significant displacement interactions of CBZ with other drugs have been reported [3].

The concentration of CBZ in the cerebro-spinal fluid (CSF) appears to correspond to the concentration of unbound drug in plasma (6, 1, 22, 20), which is approximately 17 to 31% of the concentration in plasma [1, 5].

CBZ distributes into amniotic fluid and the concentration has been reported to correspond to the free fraction of the drug in plasma [1,5]. CBZ concentrations in breast milk and saliva were found to be 60% and between 20-30%, respectively, of that found in plasma following oral dosing in healthy subjects [1,12]. A number of studies have shown that a correlation exists between the concentrations of CBZ found in plasma/serum and in saliva, where the reported correlation coefficient, r was 0.84 – 0.99 [1,20,22]. The salivary concentration of CBZ appears to accurately reflect the free fraction of CBZ in plasma/serum [1,22]. CBZ appears to enter red blood cells in only limited amounts [1] where erythrocyte/plasma ratios of 0.15 to 0.38 have been reported [1].

1.5.3 Metabolism and Elimination

The mammalian body does not possess a mechanism for the removal of unchanged exogenous lipophilic compounds, especially those of a neutral character [1]. Consequently biotransformation by hepatic metabolizing enzymes impart a more hydrophilic character to these lipophilic compounds so as to facilitate their excretion from the human body, via either the renal or hepatic-biliary system [1].

The potential sites for metabolic reactions of CBZ are the tricyclic skeleton and the carbamoyl side chain [1]. CBZ is extensively metabolized in the liver with only 1 – 3% of the administered dose excreted unchanged in the urine [1,5,12,24]. The clearance of CBZ is very much affected by changes in hepatic enzyme activity and plasma protein binding where concomitant treatment with compounds such as phenytoin, phenobarbital, primidone, ethosuximide and methsuximide accelerate the elimination of CBZ [1, 22] through hepatic enzyme induction, following prolonged exposure to CBZ. However, changes in hepatic blood flow may not significantly affect CBZ clearance. Four main metabolic pathways that

have been identified for the biotransformation of CBZ have been reported [1,5]. The primary metabolic pathway involves epoxidation of the 10,11 double bond at the azepine ring, which is the predominant pathway of metabolism in humans [6,18] and accounts for approximately 20-40% of the removal of CBZ [11]. The other metabolic pathways include hydroxylation of the six-membered aromatic rings, N-glucuronidation of CBZ at the carbamoyl side-chain and substitution of the six-membered rings with a sulfur-containing group, that account for approximately 25%, 15% and 5% of the metabolic products respectively.

Approximately 98 to 99% of an administered dose of CBZ is metabolized in the liver, mainly to the active metabolite CBZ 10,11-epoxide which possesses pharmacological activity comparable to the parent compound [1,7,12] and the ratio of the of the active metabolite to the parent CBZ remains relatively constant [1].

The elimination half-life of CBZ following a single oral dose of 200mg is reported to be between 30 to 40 hours, whereas after multiple dosing of the half-life is reduced to between 10 to 20 hours [3,5,7,11,13,14,23]. The shorter reported elimination half-life may be attributed to the auto-induction of hepatic enzymes, a phenomenon confirmed for CBZ [6,13,14,18,22-24].

The hepatic clearance of drugs that exhibit auto-inducing properties progressively increases with continued long term or chronic therapy [1,12]. The average clearance values reported for patients receiving CBZ chronically have been reported to be 0.064L/hr/kg [7] and 1.4 ml/min/kg [5]. Single dose studies suggest that CBZ has a clearance value that is one-half to one-third that observed for patients on chronic therapy [7]. The induction of hepatic enzymes occurs within 3 to 5 days after initiation of therapy and continues for up to between 21 to 28 days following the cessation of treatment.

The hepatic cytochrome P450 isoforms primarily responsible for the biotransformation of CBZ are CYP3A4 and CYP2C8 [6,18,24]. [CYP3A4 present in the gut epithelial wall also plays an important role in drug metabolism \[18,24\].](#) In addition CBZ induces the metabolism of other drug molecules, metabolized by this isoform, such as the oral contraceptives [5,6,14]. CBZ is also known to induce the CYP2C, CYP3A isoforms and also UDP-glucuronyl transferases [6]. Consequently CBZ also affects the metabolism of other drugs that utilize these enzymatic pathways, which is especially significant when considering therapy with

other anti-convulsant agents such as phenytoin [7]. The use of a controlled-release dosage form can overcome CYP3A4 heterogeneity issues in the gastro-intestinal tract and this may improve drug therapy.

The metabolism of CBZ in children appears to be faster than in adults [14,22] and therefore larger and more frequent doses are required to achieve comparable therapeutic serum levels of the parent drug [22]. During pregnancy, serum levels of anticonvulsants tend to decrease where clearance may increase two-fold [25], thus necessitating dosage adjustment in order to keep patients seizure free, during gestation.

1.5.4 Mode of Action (MOA)

CBZ has a similar mechanism of action to phenytoin [3,11] and shows maximal activity against electroshock seizures when compared to chemically induced seizures [3,5]. CBZ has been shown to limit the repetitive firing of action potentials evoked by a sustained depolarization of mouse spinal cord or cortical neurons in culture [3,6]. This activity appears to be mediated by a slowing down of the rate of recovery of voltage-activated Na⁺ channels, from inactivation [6]. These effects are observed when CBZ concentrations are within therapeutic levels of between 6 and 12µg/ml in the cerebro-spinal fluid in humans [6].

CBZ is presumed to suppress abnormal neuronal excitability [5], a mode of action postulated for the observed analgesia during the treatment of trigeminal neuralgia [5]. CBZ acts presynaptically to decrease synaptic transmission [3] and these effects probably account for its anti-convulsant activity. CBZ is also thought to block cyclic-AMP mediated calcium influx associated with transmitter release [5] and is known to act as an adenosine receptor antagonist [3,5].

Studies in animals have shown that CBZ has inhibitory effects on hippocampal discharges and inhibits the reticulo-thalamic and thalamo-cortical projections, which are implicated in tonic-clonic seizures [5].

1.5.5 Clinical Indications

CBZ was originally used for the treatment of trigeminal neuralgia [6,26] and is still employed for this purpose [5,14]. CBZ has been shown to be particularly effective for the treatment of complex partial psychomotor or temporal lobeseizures, as well as generalized convulsive or tonic-clonic seizures [5,6,11,26]. CBZ is one of the major anti-convulsant drugs and considered by many clinicians as the drug of first choice in the treatment of focal seizures and has been shown to be as effective as an anti-convulsant in the treatment of partial and grand-mal seizures [5,22].

1.5.6 Therapeutic Levels and Dosing

Divided daily doses of CBZ of 10 – 40 mg/kg/day are effective in children [5,14]. The dose of CBZ should be adjusted to the needs of the individual patient, when treating epilepsy, to achieve adequate control of seizure activity [14]. The effective total plasma concentration of CBZ is approximately 4 - 12µg/ml or 16 – 50 µmol/l for daily doses of 400 to 1200mg [11,12,14,22].

Treatment of patients should begin with an initial low dose, to minimize side effects, after which the dose is gradually titrated upwards in increments of 100 to 200mg, every two weeks to a usual maintenance dose of between 0.8 – 1.2g per day, in divided doses [11,14]. In adults, daily doses of between 1 – 2g may be required to control patients [3,14].

Control of seizures has been demonstrated with CBZ concentrations of between 20 - 40µmol/l or 4.7-9.5µg/ml [5]. However there is a general agreement that the therapeutic level is usually 4 - 8µg/ml [3,5,6,14,26] although the overlap with both sub-therapeutic and toxic levels may be considerable [22]. In such cases some patients may complain of diplopia at drug levels above 7µg/ml whilst others can tolerate levels above 10µg/ml [3].

The frequent difficulty of finding a clear relationship between serum CBZ levels and clinical response is possibly explained by the presence of the active epoxide metabolite, which accumulates in serum to a variable extent depending on whether the patient is receiving single or multiple drug therapy [22].

For the initial treatment of trigeminal neuralgia and pain syndromes administration of 100mg twice daily is recommended and this may be increased by 100mg every 12 hours until the pain is relieved, up to a maximum daily dose of 1.2g [25].

1.5.7 Drug Interactions

CBZ induces its own metabolism as well as that of several other drugs thereby increasing the total body clearance of these compounds. Drug interactions, in which CBZ is implicated, are almost exclusively related to the enzyme-inducing properties of the compound [3,11,12]

Furthermore, CBZ metabolism is affected by the enzyme inducing or inhibitory effects of other drugs. A list of some of the drugs for which clearance is increased as a result of the induction of metabolism due to CBZ administration is shown in Table 1.3 [3,5,6,14,25]

Table 1.3 Drug interactions with CBZ.

Increased Metabolism due to CBZ inducing effect	Drugs that decrease CBZ metabolism
Phenytoin	Valproic acid, Lamotrigine
Ethosuximide	Primidone
Valproate	Phenobarbitone
Clonazepam	Propoxyphene
Clobazam	Cimetidine
Warfarin	Diltiazem
Theophylline	Verapamil
Doxycycline	Isoniazid
Cyclosporin	Erythromycin
Oestrogen	Fluoxetine
Haloperidol	Triacetyloleandomycin

Phenobarbital, phenytoin and valproate may increase the metabolism of CBZ by inducing the enzyme, CYP3A4 [6], whereas propoxyphene is known to inhibit the metabolism and clearance of CBZ [3,5,6]. A list of some of the drugs that induce or inhibit the metabolism of CBZ is listed in Table 1.3 [6,3,5,6,14,25].

Inhibition of CBZ metabolism can lead to increased plasma levels of CBZ such that concentrations rise into the toxic range and thus, these combinations are best avoided. Of primary importance is the fact that therapeutic levels of CBZ may be reduced through enzyme induction into the sub-therapeutic range, which may lead to an increased seizure frequency in patients.

CBZ may also decrease the ability of a patient to tolerate alcohol [5] and the concomitant administration of anti-depressant compounds may antagonize the anti-epileptic activity of CBZ by lowering the convulsive threshold [14].

1.5.8 Adverse Effects and Toxicity

Dose dependant side-effects of CBZ, particularly in the initial stages of therapy are of a neurosensory nature and include drowsiness, general obtundity, ataxia, nystagmus, diplopia, headache [3,7,14,22]. Such side effects tend to become prominent at serum concentrations above 9µg/ml or 38µmol/l [7,22].

Acute intoxication with CBZ may result in stupor or coma, hyperirritability, convulsions and respiratory depression [6]. Symptoms of overdosage with CBZ include erythema of the face, tremor, ataxia, psychomotor restlessness, changes in blood pressure, muscle hypotonia, dilatation of the pupils, unconsciousness and convulsions [3,5,14,25].

Gastro-intestinal side effects are usually mild and transient and include dry mouth, nausea and vomiting, abdominal pain, anorexia, diarrhea or constipation [5,14,25].

Hyponatraemia and water intoxication have been reported [3,5,14] due to an increased circulating anti-diuretic hormone level caused by CBZ [5].

Occasional reports of blood disorders include agranulocytosis, aplastic anaemia, eosinophilia, persistent reversible leucopaenia, leucocytosis, thrombocytopenia, splenomegaly, pneumonitis, abnormalities of liver and kidney function and cholestatic jaundice have been reported and are rare [14].

Serious and even irreversible skin disorders including erythroderma, exfoliative dermatitis, Stevens-Johnson syndrome, systemic lupus erythematosus-like syndrome have been reported [5,6,14].

Severe over-dosage may result in adverse events primarily associated with the CNS and may result in stupor, coma, convulsions (at very high doses), respiratory depression and death.

CBZ should not be prescribed to patients with atrioventricular heart block, blood disorders or bone marrow depression and porphyria [25] and should be used with caution in patients with a history of liver or renal disease and in the elderly [25]. Cross-hypersensitivity may occur in those patients who are allergic to the tricyclic anti-depressants [25].

1.6 CONCLUSION

CBZ is prescribed in large doses especially during chronic therapy as a result of the auto-inducing effect of CBZ on liver enzyme that result in decreased plasma levels, with the subsequent need to dose as much as 1.2g on a daily basis in some patients. The need for large daily doses requires that patients receiving CBZ chronically must use dosage regimens with frequent dosing intervals. From a practical and patient-compliance perspective, this is not desirable since chronic therapy relies on the maintenance of a steady state plasma concentration and the omission of doses may disrupt plasma levels, which may manifest as a seizure episode in patients. The application and use of sustained-release dosage forms, decreases the dosing frequency and has the potential to enhance patient compliance and adherence to the dosage regimen.

CBZ can exhibit slow and erratic absorption leading to fluctuating systemic drug levels. The use of a sustained-release delivery dosage form would reduce the peak to trough fluctuations usually associated when conventional immediate release dosage forms are administered

chronically and where sharp increases and decreases in blood levels are observed. Conventional dosage forms deliver large quantities of API immediately in the GIT resulting in the initial spike and decline of blood drug levels that can be observed with conventional therapy.

Furthermore the rapid increase and decrease in blood levels of CBZ can result in concentration levels superceding the maximum therapeutic or minimum toxic concentration of a particular drug. This can result in undesirable adverse drug-related events where the plasma concentrations may fall below the minimum effective concentration, resulting in ineffective therapy.

The clinical use of sustained-release dosage forms can enhance anti-epileptic therapy through a combination of improving patient compliance by reducing the dosing frequency and optimizing drug delivery through controlled and predictable release of CBZ. Therefore, attempts were made to develop and assess a generic CBZ sustained release dosage form that was equivalent to a commercially available sustained release CBZ dosage form, *viz.*, Tegretol[®] CR.

CHAPTER TWO

HIGH PERFORMANCE LIQUID CHROMATOGRAPHIC (HPLC) ANALYSIS OF CBZ

2.1 Method Development

2.1.1 Introduction

High performance liquid chromatography (HPLC) is an analytical tool used for the separation and quantitation of compounds based on their physico-chemical properties [27-30]. HPLC can be used for the analysis of compounds such as macromolecules or thermolabile substances that cannot be analysed using gas chromatography (GC) since HPLC analysis can be conducted at room temperature [31]. Liquid chromatographic techniques that have been developed and that are currently used include reversed-phase, ion-exchange, hydrophilic interaction, hydrophobic-interaction and size-exclusion chromatography [32]. Reversed-phase HPLC (RP-HPLC) allows for the separation of compounds according to their polarity, with relatively non-polar molecules having a high affinity for a non-polar stationary phase. Therefore, these compounds are retained for longer periods on a column, whereas compounds of high polarity will elute more rapidly from the column in the mobile phase [31,50]. HPLC makes use of the fact that a particular solute will display unique retention characteristics under specified conditions. Therefore, an analyte will elute at a specific time depending on the type of column used, the nature of the mobile phase in addition to the flow rate of the mobile phase. In validated systems used for quantitation, these unique characteristics provide a means of identifying a compound of interest under specified conditions. Consequently different HPLC methods exist for the analyses of pharmaceutical compounds and the ultimate method adopted for analysis will depend on the physico-chemical properties of the analyte of interest. RP-HPLC is the most widely used liquid chromatographic procedure for the analysis of drug molecules [33].

During analysis, the sample components partition to differing degrees between a stationary and mobile phase, based on their inherent physico-chemical properties [39]. The nature of the physico-chemical interaction between the mobile and stationary phase allows solute molecules to emerge from the column in individual component zones or bands, which are then monitored as a function of an appropriate detector response versus time [39].

HPLC stationary phases include adsorbent materials such as silica, polymeric substrates, alumina, styrene-divinylbenzene, methacrylate and graphitized carbon, of which silica is the most commonly used for analytes of molecular weight < 1000 g/mol [32]. The physical structure of the stationary phase comprises molecules that are covalently bound to the surface of the silica particles forming a uniform monomolecular layer, which is chemically stable [31-33]. This binding process, called silanisation, results in a variety of stationary bonded phases and produces long chain aliphatic silane groups, of which several derivatives are possible [31,32]. The variety of stationary phases provides the analyst with a range of options to solve potentially challenging analytical problems [31,32].

The quantitative analysis of carbamazepine (CBZ) in plasma has been achieved using a variety of techniques and methods including ultraviolet spectrophotometry following extraction [28,30], visible spectrophotometry, TLC and fluorometry, derivatised and underivatised gas-liquid chromatography, selected ion monitoring as well as HPLC [28,30]. Analytical methods that have been reported for the determination of CBZ in pharmaceutical dosage forms include HPLC with UV detection and UV spectrophotometry [34-37].

Based on the analytical methods described in the literature as well as the availability of equipment in our laboratory, HPLC with UV detection was selected as a potential method of analysis for carbamazepine in dosage forms. Analytical methods reported in the literature for the determination of CBZ, either alone or in combination with related compounds such as other anti-convulsants, metabolites or structural analogues are summarized in Table 2.1.

Table 2.1 Published HPLC methods used for the analysis of carbamazepine (CBZ) in biological fluids and dosage forms

Stationary Phase	Mobile Phase	λ (nm)	Internal Standard	Reference
Spherix [®] C18	MeOH:Water 45:55	254	*	34
Spherisorb [®] ODS 2 C18	MeCN:MeOH:potassium phosphate (0.05M; pH6.9) MeOH:Water 50:50	*	*	41 45
Chrompak [®] column packed with LiChrosorb	*	*	*	46
Partisil [®] C18	MeOH:Water 55:45	254	5-(-p- methylphenyl)-5- Phenylhydantoin	35
Lichrocart [®] C18	MeCN:Water 30:70	*	*	47
Bondsphere [®]	MeCN:Ammonium acetate buffer (0.05M) 20:80	*	*	48
Bondapak [®] C18	MeCN:EtOH:Water 25:25:50	285	*	36
Nucleosil [®] C8	MeOH:Water 75:25	285	*	37
Bondapak [®] C18	MeOH (450ml) diluted to 1L with phosphate buffer (0.002M, pH 7.8), then filtered (0.5 μ m pore size) and degassed under vacuum.	*	*	49
* Not Reported	MeOH: Methanol, EtOH: Ethanol, MeCN: Acetonitrile			

2.1.2 High Performance Liquid Chromatography (HPLC)

HPLC is a technique most commonly used for the quantitation of drugs in pharmaceutical formulations [38]. HPLC involves the simultaneous separation and quantitation of compounds in a sample matrix that has been introduced onto a chromatographic column, packed with a stationary phase [39]. Separation is achieved by the use of a stationary phase and a solvent, termed the mobile phase, that is allowed to flow through the stationary phase [38,39] at a set flow rate, for isocratic chromatography.

During analysis, the sample components partition to differing degrees between a stationary and mobile phase, based on their inherent physico-chemical properties [39]. The nature of the physico-chemical interaction between the mobile and stationary phase allows solute molecules to emerge from the column in individual component zones or bands, which are then monitored as a function of an appropriate detector response versus time [39].

HPLC separations, to a large extent, include liquid-liquid chromatography (LLC), liquid-solid chromatography (LSC), size exclusion chromatography, normal, RP-HPLC, ion exchange and affinity chromatography. In reversed-phase chromatography, the stationary phase is usually a hydrophobic bonded phase, such as an octadecylsilane or octylsilane and the mobile phases are usually polar solvents such as water or mixtures of water and water-miscible organic solvents such as methanol, acetonitrile, THF or isopropanol [40]. Nonionic, ionic and ionisable compounds can be separated using a single column and mobile phase, with or without added buffer salts, using bonded-phase columns that are reproducible and relatively stable [40].

Only one solute was being investigated, thus the use of an isocratic system was deemed appropriate for the development of an HPLC analytical method. For samples in which different solutes are present, it may be advantageous to use gradient elution where the composition of the mobile phase is altered during the separation, usually by blending two or more solvents with different eluting powers in continually changing proportions [41] whereas in isocratic systems, a mobile phase of constant composition is used to effect a separation.

In the case of an HPLC system that may not be accurate or precise, the use of an internal standard improves accuracy, by correcting for variable injection volumes of a test solution. A solution containing a fixed amount of internal standard is added to the sample in a precisely measured volume. Any subsequent losses of the analyte sample are accounted for, since losses of the analyte will be mirrored by losses of the internal standard [50]. A chemical substance may be used as an internal standard if it is related to the analyte of interest, is stable and elutes as close as possible to the analyte of interest whilst is still adequately resolved from the analyte and any possible excipients that may be present in the sample matrix being analysed [50].

2.2 Experimental

2.2.1 Reagents

All chemicals used were at least of analytical reagent grade. Acetonitrile (HPLC grade, distilled in glass) was purchased from Burdick and Jackson (Muskegon, MI, USA) and Romil (Cambridge, IL, USA). Analytical or reagent grade sodium hydroxide pellets (BDH Chemicals, Poole, UK) and ortho-phosphoric acid (85% w/w) (Pal Chemicals, Johannesburg, South Africa and Merck Industries, Johannesburg, South Africa) were used to prepare buffers for the mobile phase preparation.

Two grades of CBZ, a coarse grade (CG) and fine grade (FG) were generously donated by Noveon Pharma (Raubling, Germany). HPLC grade water was used for the preparation of all relevant solutions. Water was purified using a Milli-Ro[®] - 15 Water Purification System (Millipore, Bedford, MA, USA), which consists of a Super-C[®] carbon cartridge, two Ion-X[®] ion-exchange cartridges and an Organex-Q[®] cartridge. The water was filtered through a 0.22µm Millipak[®] stack filter prior to use.

2.2.2 Preparation of Stock Solutions

All stock solutions were prepared using a standard procedure. Approximately 250mg of CBZ was accurately weighed transferred into a 1L A-grade volumetric flask. CBZ was dissolved in approximately 30 to 50 ml of acetonitrile and the solution was made up to volume with HPLC grade water. Calibration standards spanning the concentration range of 10, 20, 30, 40, 60, 80 and 180 µg/mL were prepared by appropriate dilution of the stock solution, where aliquots were withdrawn from the stock solution using A-grade bulb pipettes, according to the concentration required, and made up to volume in a 50 ml volumetric flask. A stock solution of the internal standard, protriptyline (PRO) was prepared by accurately weighing 250mg of the compound. PRO was quantitatively transferred to a 1L A-grade volumetric flask and was made up to volume with HPLC grade water. Calibration and test samples were spiked with 1 ml of PRO at a concentration level of 250µg/ml prior to sample manipulation and analysis.

2.2.3 Preparation of Buffer Solution

0.05 M buffer solutions were prepared by pipetting 3.42ml of 85% v/v phosphoric acid into a 1L A-grade volumetric flask and making up to volume with HPLC grade water. The pH of the solution was adjusted to pH 3 using sodium hydroxide pellets. A model GLP 21 Crison pH meter (Crison, LASEC, South Africa) was used to monitor the pH.

2.3 HPLC System

2.3.1 Chromatographic Hardware

The modular HPLC system comprised of a Waters Model 6000A (Waters Associates, Milford, MA, USA) solvent delivery system module, a Waters Intelligent Sample Processor (WISP) Model 710B autosampler (Waters Associates, Milford, MA, USA), a Spectrochrom UV-100 UV detector (Linear instruments Corporation) and a Perkin Elmer 561 strip chart recorder (Hitachi, Japan).

2.3.2 Column Selection

Silica gel and ODS silica gel are two of the most commonly used stationary phase packing materials used for normal and reversed-phase chromatographic applications, respectively. However, there are a variety of normal and reversed-phase packings available, most of which are based on chemical modification of the silica gel surface, although in recent years stationary phases which have organic polymeric backbones have also become available [50].

For a neutral compound such as CBZ, the balance between the polarity of the mobile phase and lipophilicity of the molecule will determine the retention time on an HPLC column. The pH of the mobile phase will theoretically, have no effect on retention times [50] except, perhaps in the case, where use of an internal standard becomes necessary and where the retention time of the internal standard may be affected by pH, depending on the specific physico-chemical properties of the molecule selected. The reversed-phase retention mechanism is still not completely understood [41] and a possible explanation is that the hydrophobic surface of the bonded phase extracts the less polar constituent of the mobile phase to form a layer at the silica-mobile phase interface [41]. However, it has been proposed that there may be several different mechanisms operating simultaneously [41]. Normally, in reversed-phase chromatography more polar solutes will elute at shorter retention times with lipophilic compounds being retained for longer periods and manipulation of the mobile phase polarity by using modifiers such as methanol and acetonitrile, can allow manipulation of retention times of the analytes of interest, thus effecting a separation.

Silica gel is an adsorbent stationary phase and most frequently used for HPLC applications. It is sold under a variety of brand names such as Lichrosorb[®], LiChrospher[®], Nucleosil[®] and Partisil[®] amongst others. Silica gels with a relatively large specific surface area 950-450m²/g, a large pore volume of 0.7-1.2 ml/g, and a moderate mean pore diameter, of 50-250Å are generally used [42]. Many types of columns are commercially available and factors such as silica particle size, surface area and shape become important in terms of their application for the analysis of APIs.

Reversed-phase C₁₈ columns have been most frequently used for the analysis of CBZ owing to their high degree of lipophilicity. Therefore, a reversed-phase C₁₈ column was chosen as the preferred stationary phase for these analytical studies. Many C₁₈ columns were assessed for their suitability for the analysis of CBZ, and a Prodigy[®] 250 x 4.6mm-i.d column (Phenomenex, Torrance, CA, USA) with 5µm-particle packing was adopted as the stationary of choice phase for analysis. The separation of the analytes of interest on this stationary phase was efficient and resulted in well-resolved peaks.

2.4 Mobile Phase Selection

2.4.1 Factors Affecting the Choice of Mobile Phase

In addition to the stationary phase, the mobile phase composition plays a significant role in the elution or retention of the compounds of interest. HPLC is a multi-faceted process with the appropriate interplay of various parameters being vital during analysis, to produce a desired separation. Parameters such as the physico-chemical properties of an analyte of interest, the type of stationary phase chosen in addition to the mobile phase selected for analysis play a combined role in effecting a suitable separation, and manipulation of one or all of these factors to optimize a separation and improve the chromatographic behaviour of the compound under investigation, may be necessary.

The important characteristics of solvents for use in HPLC analysis include the need for high purity, immiscibility with the stationary phase, absence of reactivity towards an adsorbent, low boiling point and low viscosity [43]. Mobile phases of extreme pH must also be avoided, *i.e.*, pH<3 and pH>9, as these may damage the bonded phase of the silica backbone or lead to dissolution of the silica [41]. However newer stationary phases are reported to be more resilient to extreme pH conditions [41]. The eluents used in reversed-phase chromatography with bonded non-polar stationary phases are generally polar solvents or mixtures of polar solvents, such as acetonitrile, methanol and/or water [42]. Of particular importance, is the fact that the mobile phase should be pure and free from impurities, dust, particulate matter and dissolved air [41,43]. Particulate matter can interfere with the pumping action of the solvent delivery module or pump and this can cause damage to the seals and/or check valves, collect on the top of the column

causing subsequent column blockages thereby promoting chromatographic anomalies such as changes in retention time and poor peak resolution [44].

All potential interference can be successfully avoided by adequate filtration of solutions prior to use in HPLC systems. Dissolved air may collect in the pump or the detector cell and cause an erratic response in the detector or an irregular pumping action, which may result in an unstable flow rate, once again resulting in chromatographic anomalies. The effect of dissolved gases can be prevented by degassing of solvents under vacuum prior to use or by use of an online degassing unit, which use ultrasonic vibrations or helium sparges to remove dissolved air or suspended air bubbles in a mobile phase [43].

Furthermore, an important factor to consider is the UV absorbance and UV cut-off values for solvents to be used in HPLC mobile phases [43], where it becomes unacceptable to use solvents with a similar absorbance as the solute that is to be analysed. Acetonitrile and methanol products have UV cut-off values at 210nm and some acetonitrile products have UV cut-off at 190nm [43] and are commonly used solvents in the mobile phases that have been used for the analysis of CBZ and that are summarized in Table 2.1.

2.4.2 Mobile Phase Analysis

The initial mobile phase selected for evaluation was based on the methods in the literature and summarized in Table 2.1. The mobile phase consisted of acetonitrile and phosphate buffer (pH 3; 0.05M). Since CBZ is a neutral drug, pH and molarity effects of the mobile phase are unlikely to have an effect with respect to its retention time. However, the effect of pH and molarity becomes relevant when selecting an internal standard and optimizing the separation of the internal standard relative to the analyte of interest.

The intended use for the analytical method was to analyse samples collected from dissolution studies conducted during formulation development of a sustained release CBZ dosage form. Consequently, the retention times of CBZ and the IS would have to be fairly short, yet provide

an effective separation of CBZ and the IS with adequate resolution, for purposes of quantitation of CBZ that had been released from the dosage form.

Therefore, the effects of buffer molarity, pH and type as well as amount of organic modifier used in the mobile phase, were assessed for their effects on retention time and resolution of the compounds of interest. The wavelength selected for these studies was 224nm and the detector sensitivity was set at an AUFS setting of 0.1. The mobile phase flow rate was set at 1.0ml/min.

2.4.3 Buffer Molarity

The effect of buffer molarity was investigated at five different molar concentrations ranging in strength from 0.01M to 0.1M. The primary objective of these studies was to investigate the effect of buffer molarity on the retention time (RT) of potential internal standards such as protriptyline HCl (PRO), imipramine HCl (IMP) and cyclizine HCl (CYZ) since the retention time of CBZ is unaffected by the molarity of the buffer as shown in Figure 2.1. In general, an increase in buffer molarity resulted in longer retention times for the internal standards tested, whereas a constant retention time of approximately 4.6 minutes was observed for CBZ.

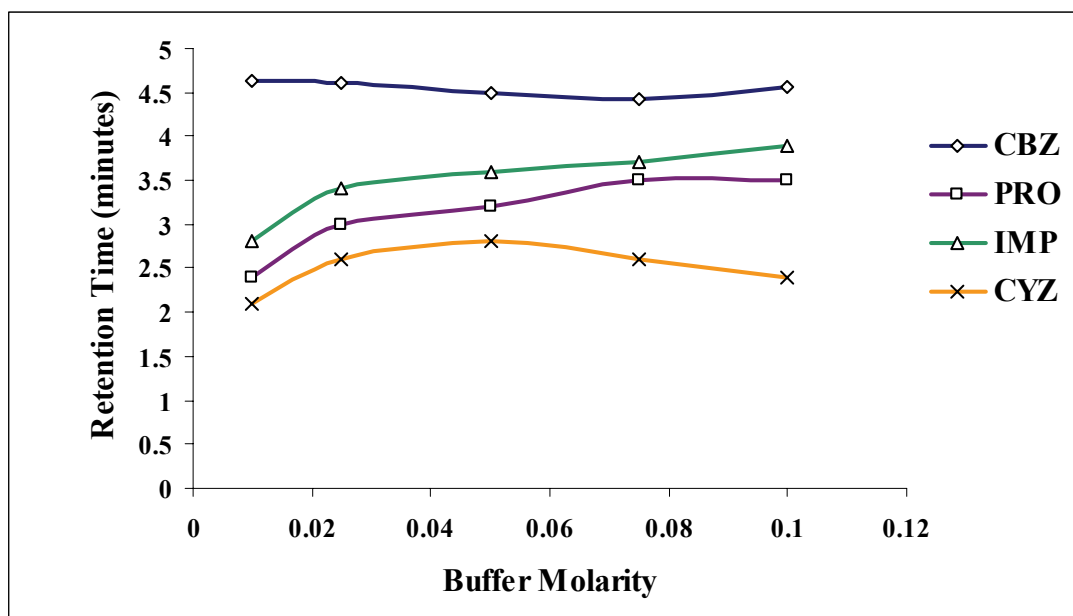


Figure 2.1 The effect of buffer molarity on the retention times of CBZ, PRO, IMP and CYZ.

The peaks of interest were sharp, well-resolved and exhibited no tailing except for CYZ, which due to its basic nature produced broad peaks and thus was considered unacceptable for use as an internal standard for these studies. Both PRO and IMP exhibited excellent peak resolution and PRO was selected for further use. The final buffer concentration selected for the analysis of CBZ was 0.05M due to the favourable retention time of both CBZ and the selected internal standard, PRO, and the resolution between these compounds.

2.4.4 Buffer pH

The effect of buffer pH was investigated empirically over the pH range of 3 – 7 and more specifically at pH values of 3, 4, 5, 6 and 7. The RT of CBZ was unaffected by buffer pH due to its neutral nature, however the RT for the tricyclic compounds considered for use as an IS showed a considerable increase in RT, with increasing pH. Due to their basic nature, these compounds tend to become less ionized as pH increases, therefore they become less polar, thus exhibiting an increased preference for bonding to the stationary phase as shown in Figure 2.2.

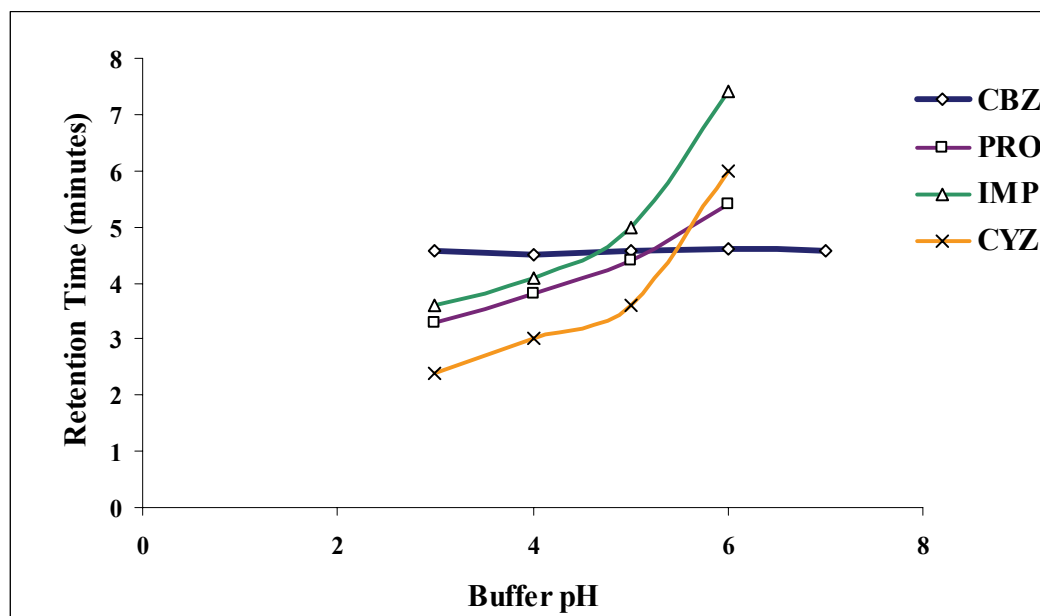


Figure 2.2 The effect of buffer pH on the retention times of CBZ, PRO, IMP and CYZ.

In addition, as the pH of the buffer increased from pH 4 to pH 7, the resolution between CBZ and the internal standards was compromised and considerable overlapping of peaks was observed, making quantitation difficult. In contrast, when a buffer of pH = 3.0 was used, all peaks were well-resolved, sharp and symmetrical except for cyclizine which showed tailing due to its prolonged retention on the column. It has been reported that compounds with a $pK_a > 8$ produce better peak shapes at pH 3.0, and thus this buffer pH was selected for further use in these studies.

2.4.5 Organic Modifier

Mobile phases for reversed-phase chromatography usually consist of an aqueous component, with or without a buffer in combination with an organic modifier such as methanol or acetonitrile. As CBZ is lipophilic, the use of an organic modifier would affect its retention time and thus the effect of varying proportions of organic modifier, in the mobile phase, on retention

times were investigated. The organic modifier used in these studies was acetonitrile and mobile phase compositions of 30% v/v, 35% v/v, 40% v/v and 45% v/v of acetonitrile were evaluated.

An increase in acetonitrile content resulted in a decrease in the retention time for all compounds as shown in Figure 2.3. As expected, mobile phase compositions of 30% v/v and 35% v/v acetonitrile revealed longer retention times than those containing 40% v/v and 45% v/v. In addition, peak resolution was poor and thus quantitation was not effectively possible at mobile phase compositions of 30 and 35% v/v acetonitrile.

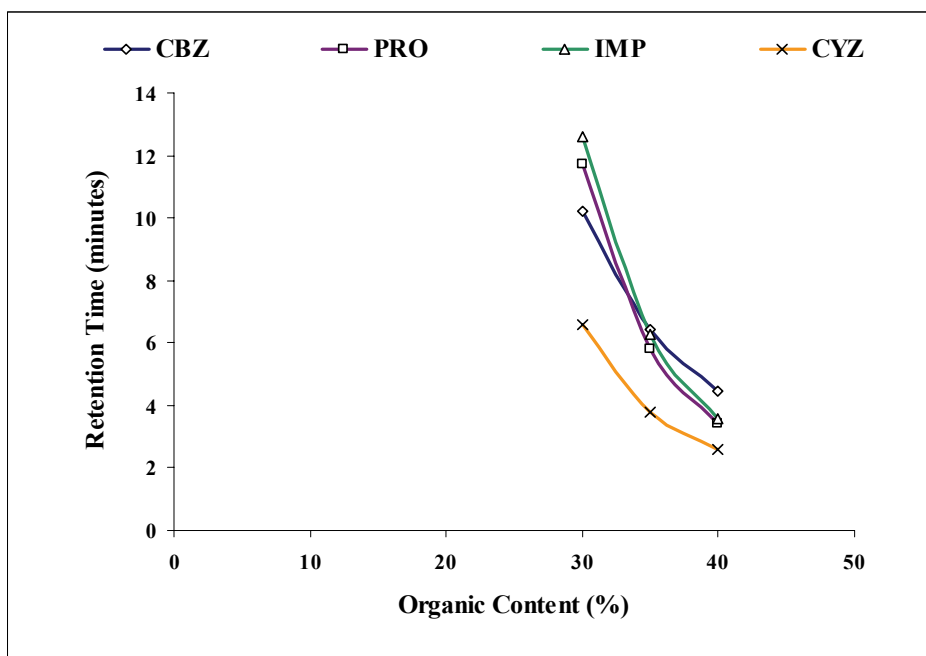


Figure 2.3 The effect of the organic modifier content on the retention times of CBZ, PRO, IMP and CYZ.

A summary of the effects of mobile phase composition on retention time, peak shape and resolution is shown in Table 2.2. A mobile phase composition of 45% v/v: 55% acetonitrile: phosphate buffer (pH = 3; 0.05M) was selected for further studies.

Table 2.2 The effects of altering organic modifier content on retention time, peak shape and resolution.

% Acetonitrile	RT (minutes)				Resolution	Peak Shape
	CBZ	PRO	IMP	CYZ		
30	10.24	1.7	12.6	6.6	Peaks were not distinctly resolved	Peaks overlap. CYZ peak is broad and showed tailing
35	6.44	5.8	6.3	3.8	Peaks were not distinctly resolved	Peaks overlap. CYZ peak is broad and showed tailing
40	4.44	3.4	3.6	2.6	Peaks were well resolved except for Cyclizine HCl	Peaks were sharp, no tailing. CYZ peak broad and showed tailing
45					Peaks were well resolved	Peaks were sharp, no tailing.

2.4.6 Flow Rate Selection

Flow rates ranging from 0.5-1.5 ml/min have been used for the analysis of CBZ a [34,35,47,48]. Lower flow rates minimize any potential deleterious effects on the pump and column and conserve solvents. A flow rate of 1.0ml/min was chosen for use in these studies since adequate peak resolution was observed at this flow rate without the extreme effects of high flow rates and associated high back pressures on equipment. Furthermore, this flow rate enabled a compromise in terms of conserving solvents and retention time, as the slower the flow rate, the longer the resultant retention time.

2.5 Detection

2.5.1 Method of Detection

To monitor the eluent emerging from an analytical column [41], variable wavelength UV detectors offer the best choice for a large range of solutes and are the most popular detectors

used in HPLC [41,44]. The principle of operation of these detectors is that a mobile phase is passed through a small flow cell located in the radiation beam of a UV/visible photometer or spectrophotometer [41] and consequently compounds that have been separated on the column absorb light, at the selected wavelength and will thus be detected. A typical UV detector has a narrow flow cell about 1 mm in diameter with a 10mm path length, with a resultant internal volume of approximately 8 μ l [50]. The linear range of UV detectors is normally between 0.0001 and 2 absorbance units and samples must be diluted sufficiently to fall within this range, to avoid deviations from the Beer-Lambert Law [50].

2.5.2 Detection Wavelength (λ)

The UV absorption maxima (λ_{max}) for CBZ occur at 212nm and 230nm whereas the λ_{max} for PRO is at 256nm. To optimize the detection of both compounds, a wavelength of 224nm was selected for use in these studies.

2.5.3 Chromatographic Conditions Selected

Following an evaluation of the impact of mobile phase composition, flow rate, buffer molarity and pH on the chromatographic separation of CBZ and PRO, the final chromatographic conditions were selected. A summary of these conditions is listed in Table 2.3 and a typical separation achieved, using these conditions is depicted in Figure 2.4

Table 2.3 Optimal chromatographic conditions for the analysis of CBZ.

Mobile Phase	0.05M phosphate buffer (pH 3.0): acetonitrile, 55:45
Flow Rate	1.0 ml.min ⁻¹
Detection Wavelength	224nm
AUFS	0.1
Injection Volume	1µl
CBZ:IS	2:1
Temperature	Ambient
Retention Time	CBZ: 4.2 minutes PRO: 3.4 minutes

A typical chromatogram obtained for the separation of CBZ and PRO is shown in Figure 2.4.

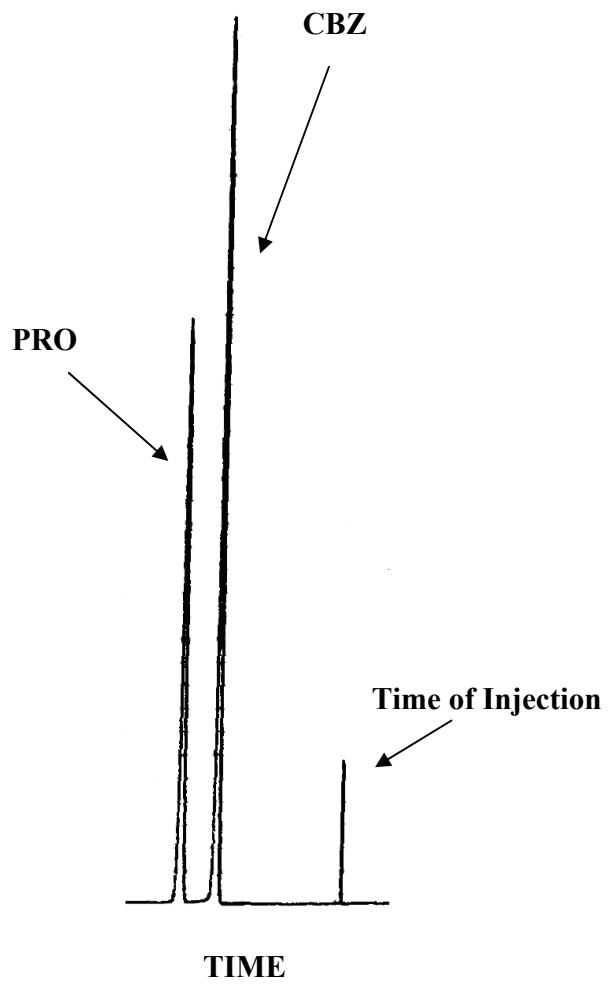


Figure 2.4 Typical chromatogram depicting CBZ and IS (60 μ g/ml).

2.6 Method Validation

2.6.1 Introduction

Following the successful development of an analytical method for the quantitative analysis of an analyte, the method must be validated in order to verify that the results of any subsequent analysis are reliable and valid and that the method will perform in a consistent manner.

Therefore, validation is the process whereby the performance of an analytical method is established as providing reliable and accurate data and proves that the method is suitable for its intended purpose [41,42,50].

It is essential to use well-characterized and fully validated analytical methods in formulation development and assessment studies, in order to yield reliable results which can be satisfactorily interpreted [Shah]. Validation also forms an integral part of any quality-control system in the pharmaceutical industry and therefore HPLC method validation is a vital component of quality assurance [41,42,50].

Method validation includes all procedures that are required to demonstrate that a particular method for the quantitative determination of the concentration of an analyte, or series of analytes, in a particular biological or other matrix are reliable and valid for the intended application of the method [42].

The parameters deemed essential to ensure that the performance of an analytical method is acceptable include linearity, accuracy, precision, sensitivity, specificity, selectivity, response function, reproducibility and stability of the analyte of interest under the study storage conditions [42].

2.7 Results and Discussion

2.7.1 Specificity

The specificity or selectivity of an analytical method implies that the method can separate potential impurities arising from the synthetic process of the API, degradation products and structural analogues or polymorphs that may be present in a sample. More explicitly, the method has the ability to measure accurately and specifically the analyte(s) of interest in the presence of other components that may be present in a sample matrix. [41,50-52]. In these studies, specificity was assessed by comparing the chromatograms produced from a standard aqueous sample to that of a sample mixture obtained by dissolving commercially available tablets or capsules containing 400mg or 200mg of CBZ respectively, in a selected dissolution medium. The commercial products used for this assessment were Tegretol[®] CR (Novartis, Quebec, Canada) tablets and Carbatrol[®] (Shire Laboratories, Rockville, MD, USA) capsules. The resultant peaks were well resolved from the solvent front or void and from each other and no interference was observed at the retention times for CBZ and PRO, therefore, the method was considered specific for the analysis of CBZ in dosage forms.

2.7.2 Linearity and Range

Linearity of a method, is the ability of an analytical method to elicit test results that are proportional, either directly or by a well-defined mathematical transformation, to the concentration of analyte in samples within a specific range [41,51,52].

The ICH guidelines specify that a minimum of five concentration levels must be used [42,52,53] and it is necessary that a minimum of twenty assays be performed for statistical validity [54]. Linearity was assessed by repeated measurements (n=6) of six concentration levels spanning a concentration range of 10 - 180 µg/ml for CBZ. Peak height ratios of CBZ:PRO were measured for the purposes of confirming linearity of response of the data.

The coefficient of determination (R^2) of the regression line for the peak height ratio response versus concentration plot, was used as an indicator of linearity, where an R^2 value of greater than 0.9900 was considered to demonstrate the necessary linearity. The calibration curve was linear over the concentration range studied, with an R^2 value = 0.9998 for CBZ and an

equation for the line of $y = 0.0129x - 0.0279$. A typical calibration curve for the analysis of CBZ is shown in Figure 2.5.

The range was chosen as the upper and lower concentration limits, of the analyte to be quantitated, that showed acceptable accuracy, precision and linearity. The dissolution apparatus to be used and consequently, the expected concentration values are built into this range and the method was shown to be linear over the range 10-180 $\mu\text{g/ml}$.

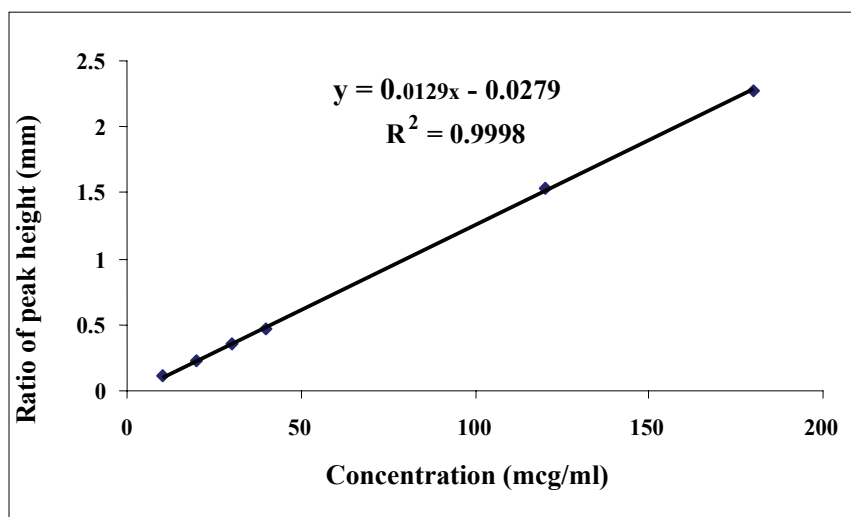


Figure 2.5 A typical calibration curve obtained for the analysis of CBZ.

2.7.3 Precision

The precision of an analytical method is the degree of agreement among individual test results when the procedure is applied repeatedly to multiple aliquots of a homogeneous sample [51]. Precision describes the closeness of replicate determinations of an analyte for an assay and can be further subdivided into intra-day or within-day precision and inter-day or inter-assay precision [42].

According to the ICH guidelines, precision should be performed at a minimum of three different levels, *viz.*, repeatability, intermediate precision, and reproducibility [52,53].

Precision is usually expressed as the percentage relative standard deviation (% RSD) (USP) and the tolerance for % RSD was set at $\pm 5\%$, for these studies.

2.7.3.1 Repeatability

The repeatability or reproducibility of an analytical method refers to inter-assay precision and is expressed as the degree of variation arising from consecutive and non-consecutive injections analysed on the same day [52]. Repeatability should be determined from a minimum of nine replicate determinations covering a specified range for an analytical procedure, for example, at three different concentration levels with three repetitions of each concentration [52].

Consecutive and non-consecutive measurements were made, where consecutive measurements were obtained by repeated injections of the same concentrations taken from different vials placed sequentially in the auto-sampler, whereas non-consecutive measurements were obtained by injecting samples from successive vials that progressed from the lowest to highest concentration of calibration standard within each set of samples.

The repeatability of the analytical method was assessed by repeated measurement (n=5) of calibration solutions containing 20 $\mu\text{g/ml}$, 40 $\mu\text{g/ml}$ and 140 $\mu\text{g/ml}$ of CBZ, respectively. Standard deviations and %RSD values obtained for these assessments were all less than 3.5 % for all solutions tested, indicating that the method demonstrated adequate repeatability for both consecutive, non-consecutive measurements of CBZ. The results of these studies for both consecutive and non-consecutive measurements are summarized in Table 2.4.

Table 2.4 Repeatability data for CBZ analysis

	Concentration (µg/ml)	Standard Deviation	% RSD
Consecutive injections	20	0.21	1.08
	40	0.18	0.44
	140	0.05	2.61
Non-consecutive injections	20	0.23	1.18
	40	0.00	0.00
	140	0.06	3.05

Formatted

Formatted

2.7.3.2 Intermediate Precision

Intermediate precision was used as an indicator of the day-to-day variance that could occur between individual measurements of CBZ. Intermediate precision was determined using calibrators of the same concentrations prepared as described in Section 2.2.2 and samples were analysed daily, over a five-day period. The results of these studies revealed that all standard deviation values were within the acceptable range, resulting in %RSD values \leq to 5%, which was the limit set for these studies in our laboratories. The results of intermediate precision studies are depicted in Table 2.5.

Table 2.5 Intermediate precision data for CBZ analysis

Concentration µg/ml	Mean (n=) measured concentration µg/ml	Standard Deviation	% RSD
20	19.78	0.3310	1.67
40	40.39	0.5511	1.36
140	139.27	2.1640	1.55

2.7.3.3 Reproducibility

Due to the intermediate precision data having been suitably validated, no reproducibility tests were performed. Furthermore, ruggedness was not assessed, as only one analyst using one set of equipment would be using the previously developed and validated HPLC method.

2.7.4 Accuracy and Bias

The accuracy of an analytical method is a measure of the closeness of an individual test result to a true test result value [9,52] and is thus a measure of the exactness of an analytical method. Accuracy serves as a tool to ensure that the values measured are exactly the same as or as close to the theoretical value as possible.

A tolerance of 2% was set for %RSD for accuracy, which complies with the limits set by a number of pharmaceutical industries. The ICH guidelines on method validation recommend collecting data from a minimum of nine determinations over a minimum of three concentration levels covering a specified range for the assessment of accuracy [52,53]. Accuracy studies were performed in triplicate on samples representative of low, medium and high concentrations that were expected during drug dissolution and formulation development studies.

The results of accuracy studies are summarized in Table 2.6. It can be seen that the largest value obtained for % bias was 5.23%, indicating that no value deviated by greater than approximately 6% of the stated value. The resultant % RSD values for these analyses were all less than 1%, indicating that the method can be considered accurate for the determination of CBZ in formulation studies.

Table 2.6 Accuracy and percent bias data for the analysis of CBZ

Theoretical Concentration ($\mu\text{g/ml}$)	Mean Concentration Determined ($\mu\text{g/ml}$)	SD	% RSD	% Bias
112	115.65	0.00	0.00	3.26
140	139.48	0.01	0.01	-0.37
168	159.21	0.01	0.02	-5.23

2.7.5 Limit of Quantitation (LOQ) and Limit of Detection (LOD)

The limit of quantitation (LOQ) of an analytical method is the lowest concentration of an analyte, in a sample, that can be determined precisely and accurately [52]. The limit of detection (LOD) of an analytical method is the lowest amount of an analyte in a sample that can be detected but not quantitated, as an exact value [51].

For chromatographic analysis, the LOD may be defined as that concentration giving a signal to noise ratio of 2:1 or 3:1 [9,51, 52] and the LOQ as that concentration that can be defined as having a signal to noise ratio of 10:1. Chromatographic conditions such as column type and age must be considered, as they affect peak shape and response, which may result in higher LOQ and LOD values being determined with the result that a method may not have the necessary sensitivity for the determination of low concentrations of analyte [52]. Furthermore, changes in detector response due to deterioration of the UV source as a result of prolonged use, may also affect these values [54].

The LOD for these studies was determined at a signal to noise ratio of 3:1 and was found to be 4µg/ml with a resultant % RSD= 8.77. The LOQ of the methods was determined using the method as described in the USP [Ref]. Six blank samples of acetonitrile were injected to establish the baseline noise after which decreasing sample concentrations (n=6) were injected. An LOQ value of 10µg/ml with a resultant % RSD = 6.59 was obtained for CBZ. Although the %RSD values for the lower concentration levels were high, the values were less than 10%, which was considered acceptable for the purpose of these validation studies.

2.7.6 Stability of the Analyte

CBZ is known to exhibit polymorphism and form a water-insoluble dihydrate when stored in aqueous solution. In order to determine suitable storage periods for test and calibrator samples, a stability study was performed.

Stock solutions of CBZ and internal standard were prepared as previously described in Section 2.2.2. The stock solutions were then stored in the refrigerator at 4°C and protected from light using aluminium foil. Samples were injected (n=5) at 0, 1, 2, 4, 8, 12 and 24 hours after preparation, over a twenty-four hour assessment period.

Furthermore, a sample of 50ml withdrawn at time 0.0 hours was stored protected from light at room temperature, in a similar manner to those stored in the refrigerator. Aliquots were removed from this sample and were analyzed daily for one week. The results of these studies are expressed as the peak height ratio of CBZ and internal standard and are shown in Figures

2.6 and Figure 2.7, respectively. Stock samples stored in the fridge showed no significant changes in response and thus indicate the relative stability of samples when stored under these conditions.

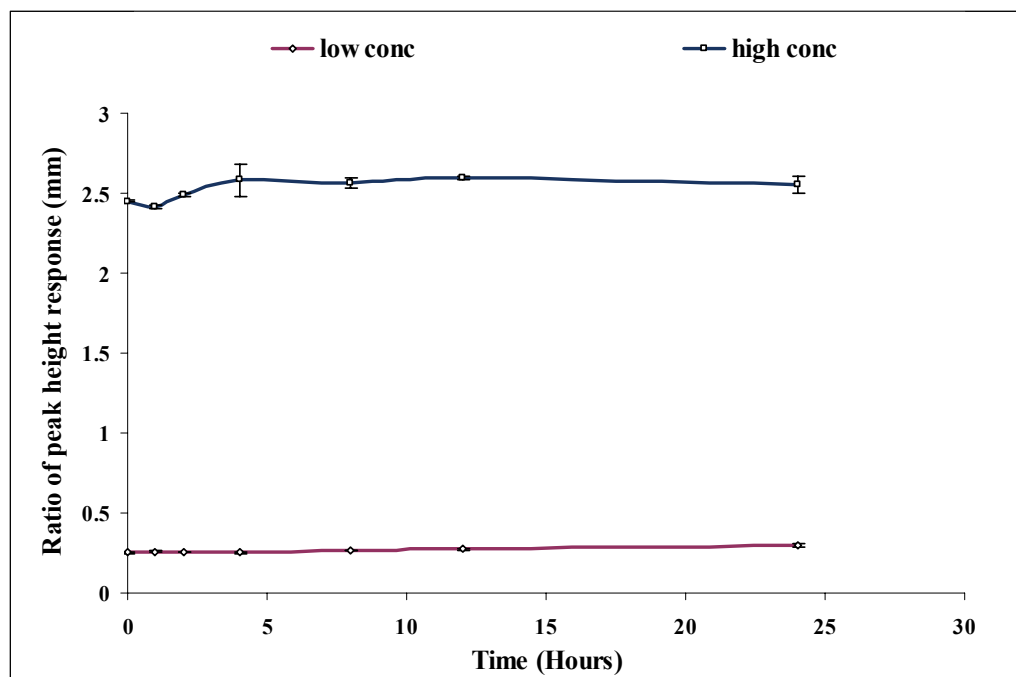


Figure 2.6 Stability data for stock solutions stored in the fridge protected from light.

Samples stored at room temperature away from light revealed significant changes in peak height ratios of CBZ to internal standard. Furthermore, peak distortion and peak tailing became apparent after 48 hours and this was more apparent in samples of the lower concentration of 20 μ g/ml.

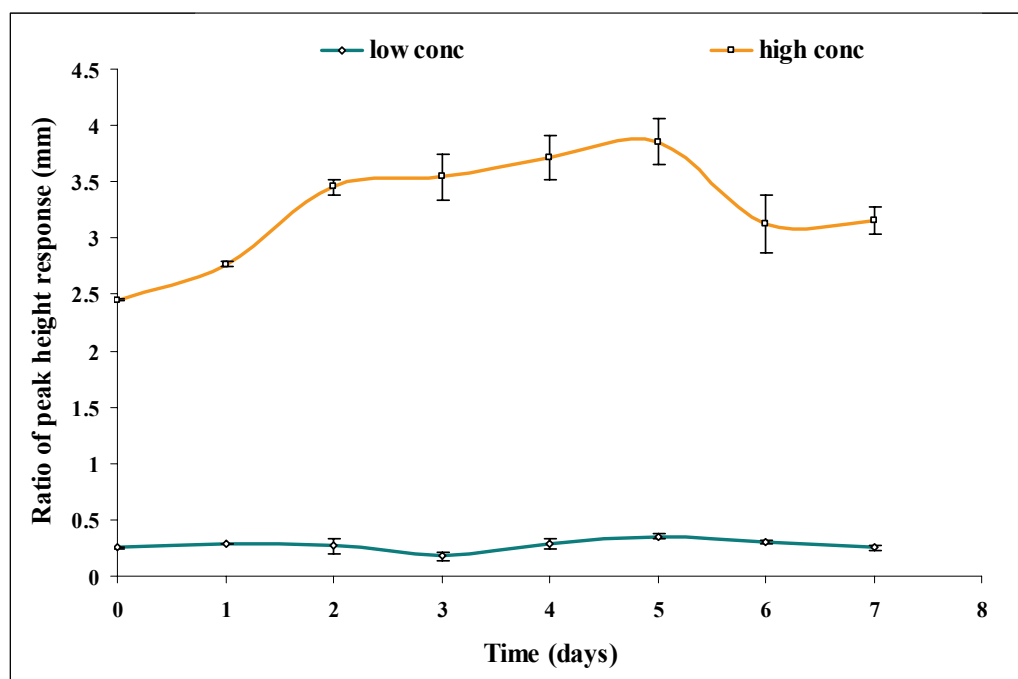


Figure 2.7 Stability peak response for samples stored at room temperature (24°C) away from light.

Although no extra peaks were detected, there were significant changes in peak height ratios and peak shape, which was indicative of a potential degradative process affecting the concentration of the analyte and to a lesser extent, the concentration of the internal standard assessed. It is possible that these degradants could be detected at by using other wavelengths of analysis, higher injection volumes or appropriate manipulation of the sensitivity of the detector.

It was concluded that stock samples should be stored at 4°C protected from light to maximize their shelf life and that calibrator samples should be freshly made and not used after storage for more than 24 hours, after preparation. In addition, storage of stock solutions at 4°C and protected from light would extend their shelf life, thus minimizing the need to prepare fresh stock solutions on a daily basis.

2.8 CONCLUSION

A modular HPLC system was used for the development and validation of an isocratic method for the analysis and quantitation of CBZ in pharmaceutical dosage forms. The analytical method was developed and validated according to scientifically established acceptability criteria.

The method was optimized by the manipulation of mobile phase composition, pH and molarity in addition to the selection of an appropriate analytical column and wavelength for detection. A suitable internal standard was selected and its response optimized to allow for the more accurate quantitation of CBZ during formulation development and dissolution studies.

The method was found to be acceptable for use with respect to linearity, accuracy, precision, specificity, selectivity and stability of the analyte, and by defining the range of the analytical method as well as the LOQ and LOD. These parameters were established in accordance with recognized scientific guidelines, and therefore this analytical method can be applied to the precise and accurate measurement of CBZ during formulation development and assessment studies.

CHAPTER THREE

DOSAGE FORM ASSESSMENT

3.1 Introduction

It is essential that pharmaceutical dosage forms undergo analytical testing to ensure quality assurance of a finished product. Quality control tests are undertaken prior to, during and after production of pharmaceutical dosage forms, as part of the overall manufacturing process to ensure the ultimate quality of pharmaceutical products. Analytical testing of dosage forms was used as a tool during dosage form development, formulation optimisation and for tablet evaluation and assessment, in these studies.

Moisture content analyses were performed using the Karl Fischer titration method, which is a standard well-recognised analytical procedure. Potential solid-solid interactions between drug candidates and tablet excipients were investigated using differential scanning calorimetry (DSC), a thermal analytical technique routinely used for these purposes.

Following manufacture, the physical parameters of tablets such as uniformity of weight, hardness, diameter, thickness and friability were assessed. All of the aforementioned procedures are used for quality control purposes during formulation development and large-scale production in the pharmaceutical industry. Furthermore, content uniformity analyses were performed on selected batches of CBZ sustained release tablets in addition to residual content determination following dissolution testing of particular formulations.

In vitro dissolution rate studies were performed on every batch of tablets manufactured to evaluate the potential *in vivo* performance of tablets and served as a tool to compare batches to each other and to the reference product, Tegretol[®] CR tablets. Dissolution testing provides a useful tool to evaluate the effect of formulation and manufacturing process variables on drug release performance. In addition, dissolution data were used to assess the possible mechanism(s) of drug release and to design an ANN to predict formulation compositions and their respective dissolution profiles. These techniques allow the formulator to modify and optimise formulation parameters so as to produce tablets with the desired physical and release characteristics as rapidly as possible, so as to shorten development times.

3.2 Moisture Content Analysis

The Karl Fischer titration method is a sensitive analytical technique used for measuring the quantity of water present in a solvent or that is adsorbed onto the surface of particulate solids [55,56]. The reagents used in Karl Fischer analysis, consist of I₂, pyridine and SO₂ in a 1:10:3 mole ratio dissolved in methanol or 2-methoxyethanol (CH₃OCH₂CH₂OH) [55,56]. When the analyte or powder to be analysed, is mixed with the reagent, the presence of water results in a sequence of reactions that is driven by the alcoholic solvent. The reagent is initially standardised by titration with the Karl Fischer reagent, prior to dissolving the analyte of interest and commencing the assay [56]. During the reaction process, one molecule of iodine is present in the sample to depolarise the cathode, and therefore ensure that a current will flow [56]. The end point of the titration is reached when the iodine present in solution has been depleted and the current flow decreases to zero. The moisture content is then calculated based on the amount of reagent used during the analytical procedure.

The Karl Fischer titration method was used to determine the moisture content of CBZ and the tablet excipients used for the manufacture of matrix tablets, in these studies. Furthermore, moisture determinations were also performed on selected production and stability batches to assess moisture content prior to and during, stability testing.

3.2.1 Methods and Equipment

The moisture content of the samples to be analysed was determined using a Mettler D18 Karl Fischer titrator (Mettler Toledo, Geneva, Switzerland) with Hydranal[®] Composite 5 (Riedel-de Haen, Seelze, Germany) as the one-component reagent. HPLC grade methanol (Romil, Cambridge, IL, USA) was used as the solvent for all analyses. A stirring time of ten minutes for all powder blends and excipients was used to ensure that the water content of all samples could be accurately determined.

3.2.2 Results and Discussion

The moisture content of the CBZ, excipients and selected batches of tablets are summarised in Table 3.1. Excipients such as HPMC and microcrystalline cellulose are hygroscopic and are able to adsorb atmospheric moisture. Therefore care should be taken during storage of these materials or during the manufacture of dosage forms that contain these compounds, to minimise uptake of water by these excipients. It is clear that these excipients have a higher moisture content than the other excipients used in these studies. A target moisture content of less than 10% was set as the limit for the excipients that were used to produce tablets during these studies. Despite the higher relative humidity moisture content of the HPMC and MCC, when combined in a blend with the API and other excipients, the resultant moisture content of the blend was effectively reduced allowing for the effective manufacture of the relevant batches of tablets.

CBZ possessed a negligible amount of moisture and any filming or sticking of tablets during the manufacture process was attributed to the hygroscopic excipients with an equilibrium moisture content of greater than 5%. The low moisture content of CBZ and other excipients allowed for successful adaptation of the wet-granulation formula to a direct compression formulation which is suitable for direct compression manufacture.

Table 3.1 Moisture content of raw materials used for matrix tablet manufacture.

Raw Material	Moisture Content
CBZ	0.82%
Methocel [®] K4M	6.54%
Methocel [®] K100M	6.11%
Emcompress [®]	0.55%
Emcocel [®] 90M	8.05%
Spray-dried lactose	5.53%

The results of moisture content determination in selected batches of tablets are summarized in Table 3.2. These batches were key batches for formulation decisions during development and also included the batch manufactured for *in vivo* administration. The resultant overall moisture content for these batches and the other production batches was below 5%, which allowed for the production of suitable tablets. Tablets with the low moisture content showed no filming or sticking during tablet manufacture. It is also evident that tablets stored under

accelerated conditions adsorbed a large % w/w of water, whereas those tablets stored under long-term stability conditions did not adsorb water to any great extent.

Table 3.2 Moisture content of selected CBZ tablet blends.

Batch	Moisture Content (%)	Relative Humidity (%)
C-061005-01 (ANN predicted batch)	2.39%	46.0
C-061005-02 (ANN predicted batch)	2.42%	46.0
C-160905-01 (Biostudy batch)	2.46%	74.4
C-160905-01 (Accelerated stability)	12.33%	75.0
C-160905-01 (Ambient stability)	2.52%	60.0

3.2.3 Conclusion

The moisture content of CBZ, excipients and selected batches were accurately determined using a standard Karl Fischer titration method. The determination of moisture content of excipients, powder blends and provides useful information regarding the compression potential of excipients and powder blends prior to compaction and aids the formulator to predict process anomalies that may be related to a high equilibrium moisture content of powder blends. Furthermore, this test was used for the determination of moisture content of batches of tablets that were placed on stability and the results assist in the selection of appropriate packaging materials for batches of tablets susceptible to moisture uptake, as these batches that contain large proportions of MCC and HPMC, are.

3.3 Differential Scanning Calorimetry (DSC)

DSC analysis of materials provides a means of measuring thermal events that are characteristic of a single material and that occur during the application of heat at a constant rate. DSC is a method in which a sample and a control lot are heated by separate energy sources and the difference between the heat that is required to maintain both samples at the same temperature is recorded. In this way, endothermic or exothermic thermal events are reported as deviations from a baseline measurement, with endothermic transitions producing deviations in a positive direction, since more energy is required during an endothermic event, than exothermic events. In comparison, less energy is supplied to the reference sample, to

achieve the desired sample temperature when exothermic events occur with a resultant baseline deviation that moves in a negative direction [57].

DSC may also be used to predict potential incompatibilities between drugs and excipients intended for inclusion into tablets or other pharmaceutical dosage forms [58,59]. Although excipients are considered medicinally inert, physical and chemical interactions with APIs may occur and these should be identified early during preformulation studies so as to prevent any possible future instability or product failure [60]. CBZ was assessed for potential interactions with the excipients to be used for the formulation of sustained release matrix tablets. It was expected that any shifts in the DSC thermograms of the API-excipient mixtures would occur when a potential solid-solid interaction between the drug and excipient in a mixture, may be possible. It is important to note, however, that these deviations do not necessarily indicate an incompatibility [61] but allow the formulator to anticipate potential formulation difficulties that may arise at the later stages of the product development process. In-depth chemical and physical analyses in addition to DSC should be performed during the product development process to eliminate incompatibilities between the intended components of a formulation.

3.3.1 Method

DSC thermograms of CBZ and excipients were generated separately, in order to produce a reference thermogram for the comparison to samples in which CBZ and excipients were combined. Combination samples were prepared for analysis by mixing one part drug to one part excipient (1:1) together, using a pestle and mortar. Due to the requirements of the apparatus used, approximately 2 – 5mg of the powder mixture was accurately weighed and sealed in flat-bottomed aluminium pans prior to analysis. Each sample was analysed under an atmosphere of nitrogen with an empty aluminium pan used as the reference sample. Heating of the sample, from 25°C to a temperature exceeding the melting point of CBZ, *i.e.* 220°C was achieved at a rate of 10°C per minute. All pure excipient samples were analysed in the temperature range from between 25°C to 250°C using a Perkin-Elmer DSC 7[®] Differential Scanning Calorimeter (The Perkin-Elmer Corporation, Norwalk, CT, USA) and Perkin-Elmer Pyris[®] software for the analysis of all sample thermograms.

3.3.2 Results and Discussion

The resultant thermograms generated for the analysis of each of the materials under investigation are presented Appendix 1. No significant thermal shifts were observed for CBZ, when it was assessed in combination with the other excipients intended for use in tablet production. It appears as though CBZ is compatible with all excipients tested and no major interactions were observed between the excipients or between CBZ and the excipients. Therefore, based on thermal analysis data, it was concluded that CBZ could be formulated with combinations of the excipients tested, as potential major incompatibilities were not evident. However, more rigorous, long-term stability testing of manufactured dosage forms should also be conducted to rule out real-time long-term dosage form instabilities.

3.4 X-Ray Diffraction

Many substances have the ability to crystallise in more than one crystalline form and this is true of CBZ where four polymorphic configurations have been identified, the three most often reported forms of which are the alpha, beta and dihydrate form [62]. The existence of different crystal structures which, although chemically identical, can display different physicochemical properties and may influence important pharmaceutical qualities such as tableting characteristic, dissolution profile as well as chemical and physical stability during storage [62,63]. XRD studies were performed to elucidate possible polymorphic transition during powder blending and tablet manufacture. CBZ is available commercially as the beta form and that is the relevant polymorph when anti-epileptic therapy is considered.

3.4.1 Method

The characteristics of the conditions, parameter settings and equipment used for X-ray powder diffraction (XRPD) studies are summarised in Table 3.3. A copper target and nickel filter was used and the radiation obtained was Copper K- α , which generated a radiation of 1.5404-angstrom units. XRPD studies of the coarse grade (CG) CBZ were undertaken on material that had been ground for approximately ten minutes with a mortar and pestle. The use of a mortar and pestle was thought to simulate friction and mechanical stress that may be encountered during manufacturing processes such as blending, granulation and compression.

Although not entirely accurate, this simple procedure was deemed sufficient to detect any possible interference by the manufacturing process on the coarse grade material. Therefore, any potential polymorphic transitions of the drug that may occur during tablet manufacture could potentially be isolated using a XRPD method.

Table 3.3 Parameters and variables used for XRPD analysis.

Generator	Seifert ISO Debyeflex 1001 Seifert, copper target tube Nickel filter
Tube voltage	40 kV
Tube current	50 ma
X-ray diffractometer type	Philips upright goniometer
Detector	Xenon-filled proportional counter
Electronics	Custom-built electronic circuit
Time Constant	1 s
Scan Rate	1 degree per s (files \approx 600kb) 2 degrees per s (files \approx 300kb)

3.4.2 Results and discussion

The x-ray diffractograms that were generated during XRD analysis of the original CG CBZ and the ground CG CBZ are shown in Figures 3.1 and 3.2 respectively. Visual analysis of the XRD patterns showed no significant peak shifts or interactions. A slight decrease in the intensity of response was observed as has been previously reported [63]. Therefore, there is more than likely no potential for polymorphic transformations to occur during the application of physical stress to the CG CBZ during blending, granulation and tablet manufacture.

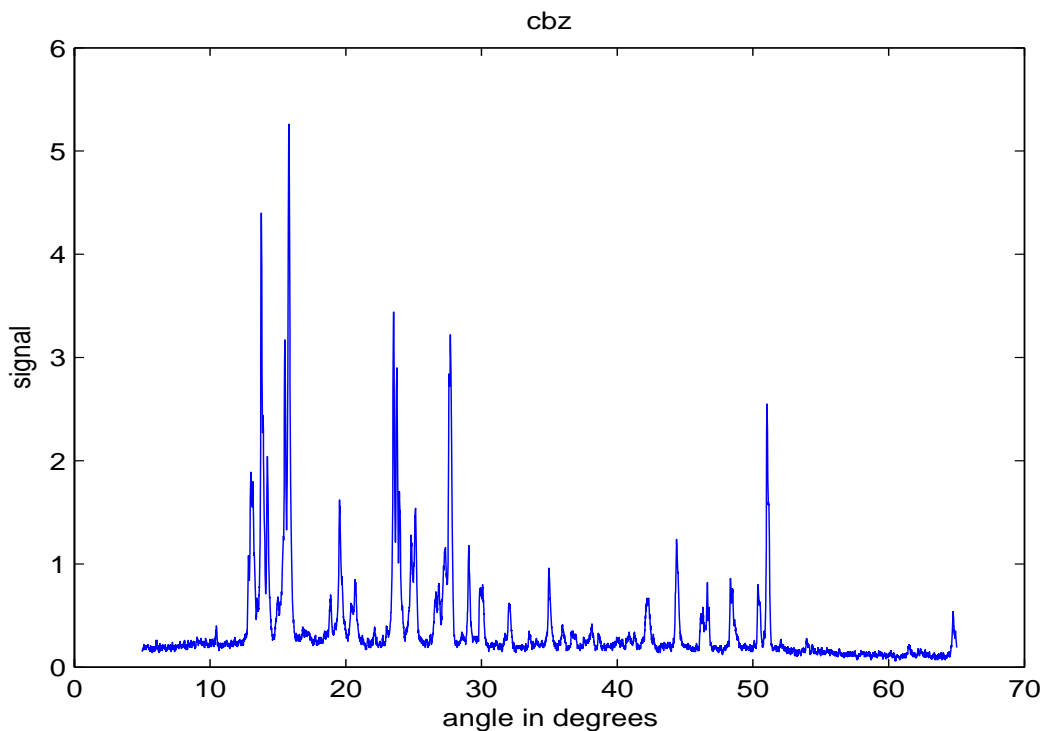


Figure 3.1 X-ray diffractogram of CG CBZ.

The absence of a characteristic and intense peak at $5^{\circ} 2\theta$ in the XRD depicted in Figure 3.2 that was generated for the ground CG CBZ, excluded the possibility of the presence of the α form of CBZ [64]. The same XRD pattern was obtained with the original CG CBZ and is depicted in Figure 3.1 and therefore it was concluded that grinding did not induce any polymorphic transitions of CBZ. Therefore, any changes in the rate and extent of dissolution that may be observed during dissolution testing are more than likely due to the dissolution test conditions and dosage form performance, rather than a consequence of polymorphic transformations that may occur during tablet manufacture.

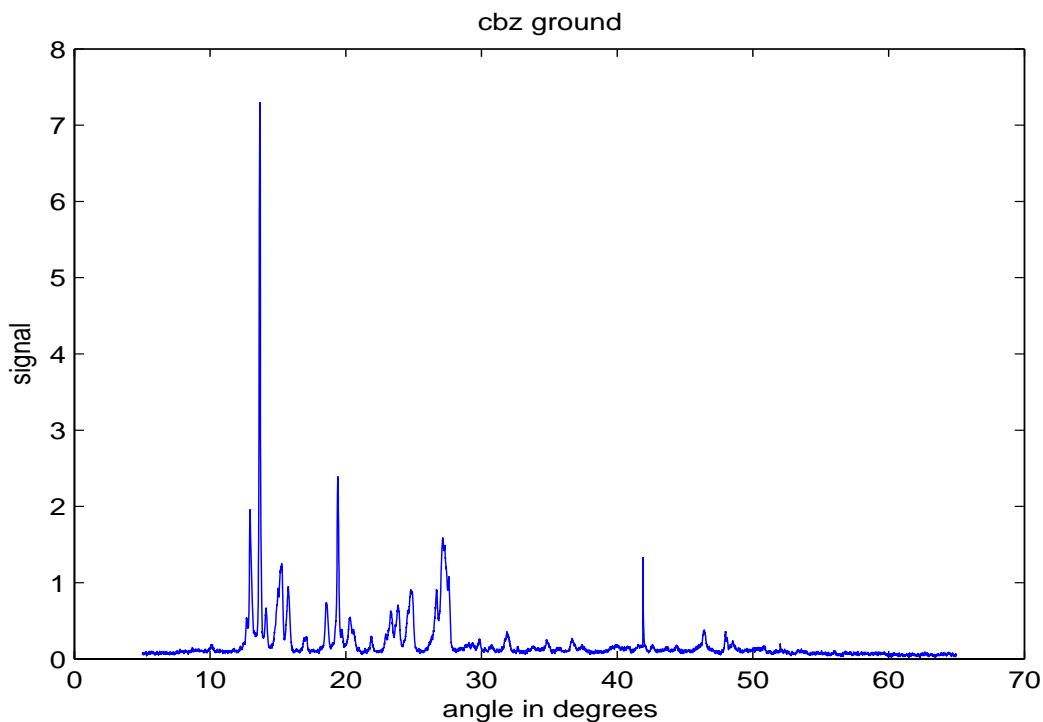


Figure 3.2 X-ray diffractogram of CG CBZ, ground for 10 minutes, with a mortar and pestle.

3.5 Physical Assessment of Tablets

The physical characteristics of tablets are regularly tested on random samples to identify potential areas of difficulty that may occur during the manufacturing process. In addition, such testing also forms part of the overall quality assurance of a product intended, to be marketed, for human or animal use. Tablets were subjected to physical testing to provide relevant information that would assist to improve and optimise formulation composition and process variables throughout these studies, as well as to provide an indication of ultimate product quality. All batches that were manufactured in these studies were assessed accordingly however the results for selected batches are depicted here. Summary batch records, in which the results of physical testing of all batches is reported, are contained in the summary sheet in Appendix II.

3.5.1 Methods

Physical testing of all tablets manufactured during formulation development was carried out in accordance with USP specifications where applicable, with tests performed to determine the uniformity of weight, thickness, diameter and hardness (n=20). In addition, friability was determined for each batch of tablets produced during these studies (n=10). Twenty tablets from each batch were individually weighed and the thickness and diameter of each tablet measured using a PharmaTest[®] PTB 311E Automated Tablet Testing Instrument (Pharmatest[®], Hainburg, Germany). Friability was assessed according to the guidelines in the USP [9] on ten tablets from each batch, which are subjected to tumbling at a rotation speed of 33 r.p.m for three minutes using an Erweka[®] friabilator (Erweka, Heusenstamm, Germany).

3.5.2 Result and Discussion

3.5.2.1 Tablet Diameter, Thickness and Weight

Deep concave hardened steel tooling (Chamunde, Ahmedabad, India) was used for the manufacture of all tablets, to ensure that the size and shape of dosage forms remained constant. It was considered important to minimise variations in size and shape of the matrix tablets, as it is well, known that such parameters can affect the rate and extent of drug release from monolithic matrix dosage forms as described in Section 4.2. Different powder blends have varying degrees of elastic and/or plastic deformation of materials after compaction and subsequently behave differently following the release of stress during compaction, resulting in possible variations in tablet thickness, which may impact on release rates.

The bulk densities of formulation blends vary with the inclusion of different excipients, more particularly for formulations prepared for manufacture by blending for direct compression into tablets. Consequently different mass:volume ratios of powder may fill the die cavity, if powder flow is not optimised and therefore tablets of varying weight may be produced. Tablets in these studies were compressed to a constant target hardness therefore any mass variation would be reflected not only in the mean tablet weight but also in the mean thickness of the dosage form. It is evident from the data presented in Table 3.4 that there is a direct relationship between these two tablet features, where heavier tablets produced tablets with a larger mean thickness.

Table 3.4 Mean Tablet Weight, Thickness and Diameter values for selected tablet batches (n=20).

Batch Number	Tablet Weight Mean ± SD mg	Tablet Thickness Mean ± SD mm	Tablet Diameter Mean ± SD mm
C-160905-01 (Biostudy batch)	800.82 ± 4.13	8.56 ± 0.07	11.44 ± 0.02
C-030703-01	506.22 ± 11.60	6.07 ± 0.11	11.22 ± 0.01
C-030703-03	496.37 ± 6.65	6.16 ± 0.08	11.24 ± 0.01
C-030703-04	516.33 ± 11.23	6.18 ± 0.09	11.23 ± 0.01
C-030703-05	532.21 ± 11.71	6.30 ± 0.11	11.22 ± 0.01
C-030703-06	602.18 ± 20.86	6.87 ± 0.15	11.23 ± 0.01
C-190603-01	570.57 ± 8.84	6.53 ± 0.04	11.22 ± 0.01
C-190603-02	622.44 ± 9.60	7.13 ± 0.14	11.22 ± 0.01
C-190603-03	655.75 ± 9.42	7.30 ± 0.09	11.22 ± 0.01
C-200603-01	531.95 ± 4.40	6.34 ± 0.03	11.23 ± 0.01
C-200603-02	527.14 ± 4.06	6.26 ± 0.07	11.22 ± 0.01
C-200603-03	530.56 ± 6.44	6.24 ± 0.05	11.22 ± 0.01
C-250303-00	704.05 ± 10.30	7.54 ± 0.18	11.23 ± 0.01
C-211202-01	674.52 ± 7.32	7.54 ± 0.18	11.23 ± 0.01
C-211202-03	683.65 ± 8.45	7.92 ± 0.26	11.25 ± 0.01
C-091202-01	747.53 ± 6.59	8.02 ± 0.07	11.26 ± 0.01
C-091202-02	728.43 ± 8.52	8.48 ± 0.17	11.29 ± 0.01
C-260303-01	658.32 ± 6.75	7.95 ± 0.10	11.24 ± 0.01
C-260303-02	663.28 ± 10.34	7.68 ± 0.11	11.23 ± 0.01

*All results for all batches can be located in the summary sheets produced for all batches as shown in Appendix II.

The target tablet weight between batches differed depending on the drug-loading requirements for the specific batches, where a heavier tablet was necessary to deliver a larger dose. Twenty tablets were tested for weight uniformity despite the recommendation in the USP [9], which suggests that the use of ten may be sufficient, for certain dosage forms. Direct compression powders do not flow as efficiently as granules and variations in tablet weights, tend to be more significant, therefore, twenty tablets were tested during these studies.

3.5.2.2 Tablet Hardness and Friability

The target hardness for all batches of tablets produced in these studies was set at between 120 and 150 N. For most of the batches manufactured, the resultant values for hardness were between found to be between 40 and 90 N, as summarised in Table 3.5. During the manufacture and compaction of some powder blends, the tablet press was placed under excessive strain, which necessitated a decrease in the compaction force that was applied to

the blends, to ensure that production continued and excessive mechanical damage to the tablet press was avoided. Therefore, a lower actual hardness value was ultimately achieved, when compared with the target hardness. A target hardness of between 120 and 150N was thought to be appropriate for the sustained release matrix tablets so as to maintain their physical integrity, should a protective coating require to be applied to the tablets, at a later stage. Tablets that have a suitable tensile strength and hardness would be resilient to chipping, abrasion and breaking during coating and other transitory abrasive events that may occur during tablet manufacture.

Resistance to abrasion is a significant attribute and takes precedence over tablet hardness since tablets may chip or break during transport of batches between manufacturing procedures or within the final packaging, prior to and during use by patients [65]. The friability results of selected batches as shown in Table 3.5 are acceptable according to USP guidelines [9], since not more than 1% of the tablet mass was lost for all batches tested. This is an indication that the tablets are adequately resistant to any potential abrasion that may be encountered during handling, transport or storage of tablets following manufacture.

Table 3.5 Hardness and friability values for selected batches.

Batch #	Hardness Mean \pm SD (N)	Friability (% Loss)
C-160905-01 (biostudy batch)	132.2 \pm 6.40	0
C-030703-01	101.67 \pm 16.67	0.20
C-030703-03	33.26 \pm 12.16	0.8
C-030703-04	63.62 \pm 7.86	0
C-030703-05	72.46 \pm 17.82	0
C-030703-06	113.62 \pm 12.69	0
C-190603-01	105.77 \pm 17.32	0
C-190603-02	146.91 \pm 13.42	0.16
C-190603-03	142.93 \pm 18.63	0.15
C-200603-01	45.24 \pm 7.61	0.19
C-200603-02	80.39 \pm 7.98	0.19
C-200603-03	89.23 \pm 13.59	0.19
C-250303-00	143.43 \pm 31.48	0.19
C-211202-01	71.04 \pm 7.35	0.31
C-211202-03	57.7 \pm 10.32	0
C-091202-01	63.88 \pm 5.25	30
C-091202-02	59.51 \pm 5.53	50
C-260303-01	87.49 \pm 13.46	0.4
C-260303-02	89.85 \pm 12.29	10

*All results for all batches can be located in the summary sheets produced for all batches as shown in Appendix II

The tablets from Batch C-160905-01 that were stored under accelerated conditions, failed the friability test due to the fact that the tablets broke and crumbled during testing. Friability testing may be used as an indication of the potential for tablets to withstand physical stress and friction during a coating process that may be applied after compaction and where the physical integrity of tablets can be destroyed.

Batches C-091202-01, C-091202-02 and C-260303-02 contained < 5% DCP and this may account for the fact that these batches of tablets were the most friable with friability of 30%, 50% and 10 % respectively. DCP is an excipient that is used to enhance the tensile strength of tablets produced by direct compression.

It is clear that most of the tablets that were produced using the different formulation compositions were found to be suitable based on the results of friability testing, however were not within specification with respect to the achievement of the target hardness. Therefore tablet formulation compositions would require substantial modification to include excipients that would impart the necessary cohesion to powder blends to achieve a desired target hardness of 120N and allow flexibility to the formulator to introduce a coating process to the manufacturing procedure. The development of modified formulations will form part of future development studies, as it was evident from the results of accelerated stability testing, that a protective aesthetic film coating may be necessary to ensure product integrity is retained until use.

3.6 Tablet Assay

3.6.1 Content Uniformity, Assay and Residual Content Analysis

Two methods have been described as being suitable for the demonstration of content uniformity of dosage units in the USP [9]. In certain circumstances, weight uniformity may be used as an indication of content uniformity, however under other conditions, a chemical analysis is considered more appropriate. Content uniformity analysis and bulk assay was performed on all batches during the development process and was also performed on the batch intended for *in vivo* administration. Content uniformity testing is a quality control tool that ensures product quality and marketing integrity of a specific product.

3.6.2 Method

Bulk assay was performed on batches according to USP guidelines, where 20 tablets were weighed and crushed. The mass equivalent containing a theoretical quantity of 100mg was transferred to a 50ml volumetric flask and 40 ml of methanol was added. This was sonicated for fifteen minutes using a Model 8845-30 Ultrasonic Cleaner (Cole-Parmer Instrument Company, Chicago, IL, USA). Methanol was added up to volume and the resultant solution was filtered and the first 5ml of the filtered solution was discarded. A 5ml aliquot was withdrawn from the filtered solution and this was made up to volume in a 50ml volumetric flask with a 1:1 solution of methanol and water. A 2ml aliquot was withdrawn from this solution, spiked with the internal standard and assessed using the validated HPLC method described in Section 2.5.3. All reagents and water used for the assay was HPLC quality reagents. The % values for bulk assay were calculated as mass recovered based on the theoretical value of 100mg.

Content uniformity analysis was performed on ten tablets randomly selected from the specific batch to be analysed. Each tablet was individually weighed and crushed in a mortar and pestle and quantitatively transferred to a 100 ml volumetric flask, with the aid of HPLC grade methanol (Romil, Cambridge, IL, USA). Approximately 70ml of methanol was added to the volumetric flask, which was then mechanically shaken using a Junior Orbit Shaker (Labline Instruments Inc., Melrose Park, IL, USA) for sixty minutes. The solution was sonicated using a Model 8845-30 Ultrasonic Cleaner (Cole-Parmer Instrument Company, Chicago, IL, USA) for fifteen minutes and then made up to volume with HPLC grade methanol. The solution was allowed to stand for approximately ten minutes prior to the removal of a 1ml aliquot, which was further diluted to 100ml with HPLC grade methanol, in an A-grade volumetric flask and then analysed using the validated HPLC method previously described in Section 2.5.3.

Content uniformity was expressed as the mean percent drug recovered for the ten tablets assayed. A similar procedure was used on each tablet core that remained after the twenty-two hour dissolution period, to determine the percent drug retained in the dosage form, for the purposes of mass balance analysis. These studies were considered important to ascertain whether the low % CBZ released from selected batches were a result of low drug loading or

due to the rate retardant formulation composition. The percent content of each formulation, as shown in Table 3.5, was calculated by expressing the amount recovered, as a percent of the total theoretical content of CBZ expected in the tablets for a specific batch.

3.6.3 Results and Discussion

Bulk assay results for all batches are reported in Appendix II. The results of selected content uniformity assays are presented in Table 3.6. Content uniformity assessment of formulations developed during the early stages of these studies, were out of the acceptable range of the specifications that were set according to USP guidelines [9]. These tablets were produced by WG and drug loss was attributed to the granulation and transfer processes that occur during manufacture. During the blending phase, drug and excipient particles become dispersed in the surrounding air and accounts for approximately 15 to 20% drug loss. Furthermore, during the screening of the wet-mass, clumping and retention of some of the wet powder mass occurs, on the metal mesh of the oscillating granulator as previously described in Section 5.3.4.1. Following the drying phase, the granule-powder mass was transferred to the blender for further manipulation that may have accounted for additional drug loss. Furthermore, inadequate blending prior to granulation of CG CBZ as well as potential segregation of the larger particle size CG CBZ may also account for poor blend uniformity.

Table 3.6 Content uniformity and assay results for CBZ matrix tablets.

Batch Number	Content Uniformity	Assay
	% Recovery	% Recovery
C-160905-01 (biostudy batch)	112.44 ± 1.42	104.83
C-030703-01	95.43 ± 4.03	98.35
C-030703-03	90.49 ± 2.63	100.51
C-030703-04	91.10 ± 3.55	103.87
C-030703-05	90.15 ± 5.13	104.32
C-030703-06	93.35 ± 4.15	99.35
C-190603-01	98.53 ± 4.43	101.65
C-190603-02	97.63 ± 3.48	100.06
C-190603-03	96.94 ± 3.51	103.22
C-200603-01	100.23 ± 3.35	107.56
C-200603-02	97.58 ± 4.27	98.95
C-200603-03	99.72 ± 5.24	99.37
C-200704-01	104.42 ± 2.40	98.37
C-250303-00	96.57 ± 4.41	100.15
*C-091202-01	87.52 ± 4.75	99.89
*C-091202-02	88.53 ± 2.61	101.86
*C-171202-00	89.47 ± 4.23	99.09
*C-211202-01	95.44 ± 3.99	100.23
*C-211202-02	101.15 ± 2.30	96.89
*C-211202-03	99.58 ± 2.35	101.59
*C-211202-04	98.12 ± 3.26	104.32

*Batches produced by WG

The percent CBZ recovered for the residual content analyses that were performed on batches where the dosage form was intact following dissolution testing are shown in Table 3.7. This was accomplished by performing assay analysis on the solid remains of the tablet. The tablets that were produced by WG did not completely erode during the twenty-two hour dissolution test period, and were still intact, whereas the batches that were produced by DC were completely eroded. Therefore only the batches of tablets that had been manufactured by WG were subjected to residual content analysis. The results shown in Table 3.7 reveal that not all the CBZ that was in the tablet had been released from these batches during dissolution testing as shown by the mass balance analysis. Consequently the low % drug released from these tablets was more than likely a function of the dosage form design, solubility of the CBZ and/or the formulation composition, rather than poor content uniformity or low CBZ content in the tablets. The final percent of CBZ released from these dosage forms was calculated as a function of the theoretical dose within the dosage form.

Tablets produced by DC completely eroded and no intact tablet remained after the dissolution test period, therefore residual content analysis could not be performed on these batches.

Table 3.7 Residual Content Analysis after dissolution of CBZ matrix tablets produced by WG.

Batch #	Content (%)	Residual Content (%)	Final % Released	Total %
C-211202-01	95.44 ± 3.99	26.35 ± 3.68	63.11 ± 1.68	89.46
C-211202-02	101.15 ± 5.30	29.37 ± 4.52	64.62 ± 4.25	93.99
C-211202-03	99.58 ± 2.35	24.16 ± 3.22	59.20 ± 0.95	83.36
C-211202-04	98.12 ± 3.26	35.15 ± 3.74	52.20 ± 0.64	87.35
C-091202-01	87.52 ± 4.75	16.28 ± 2.77	57.10 ± 0.74	73.38
C-091202-02	88.53 ± 2.61	15.85 ± 3.05	71.62 ± 13.01	87.47
C-171202-00	89.47 ± 4.23	22.80 ± 2.23	59.14 ± 1.23	81.94

3.7 CONCLUSION

The results of content uniformity analysis of selected batches of CBZ matrix tablets are summarised in Table 3.6. It is clearly evident that the results are variable for the tablets manufactured by DC, which is to be expected since blend composition is DC tablets is critical for the maintenance of dosage form quality. Particle size and bulk density differences between API and components of DC formulations may contribute to the variable distribution of CBZ in a batch of tablets produced in this way [66].

The recovery of CBZ was found to be high for the batch intended for *in vivo* testing and was > 110% which is out of the official USP range, of not less than 90% and not more than 110% for such tablets [9]. These results are more than likely due to the use of a 10% overage of CBZ that was included into the biostudy formulation to compensate for potential drug losses that could occur during the weighing, blending and transfer processes involved throughout manufacture. Most of the batches were produced on a small scale and no batch was greater than 800g therefore, any inconsistency in weight uniformity of the tablets as a consequence of poor powder flow or non-uniform die filling as a consequence of the small batch sizes and inconsistent gravitational fill of dies, may be reflected in content uniformity assessment. Furthermore should non-uniform blending or unblending occur during the manufacturing process, content uniformity of the tablets batches might occur. Blend uniformity tests were not conducted on these batches and therefore future studies should investigate blend uniformity and the potential challenges of maintaining content uniformity in direct compression manufacturing processes. The results of the assay on the GMP batch that was used for *in vivo* study revealed that the average amount of CBZ in these tablets was 104.83%

and following investigation, the results of the content uniformity test were evaluated in conjunction with the assay results and the tablets were thus used for *in vivo* testing.

3.8 Dissolution Testing

In order for an API to exert an appropriate pharmacological effect *in vivo*, it must initially be released from a dosage form and the dissolve, to be made available for absorption.

Dissolution testing is an *in vitro* method of assessing the rate and extent of drug release for all batches of a development, new or commercially available products. Dissolution testing is a valuable tool when developing a generic drug product as dissolution profiles of test formulations can be compared to those of the reference product.

In addition to elucidating the drug release characteristics of test products, dissolution testing is a valuable quality control tool for assessing batch-to-batch uniformity and also provides a basis for dosage form improvement. Dissolution testing can also be used to demonstrate dosage form stability and batch-to-batch uniformity, as well as assessing the effects of a change in formulation variables [67]. *In vitro* testing is a preliminary step in predicting the behaviour of the performance of a dosage form *in vivo*, but should not be considered as a substitute for *in vivo* testing.

Dissolution testing was used to compare CBZ release rates from all test formulations to that of the innovator product, Tegretol[®] CR. Furthermore, dissolution rate profiles were used to evaluate the effects of changes of formulation effects on CBZ release rates and elucidate possible mechanisms of drug release from these products.

3.8.1 Variables Affecting Dissolution Rates

There are numerous variables that play a significant role in dissolution testing and the choice of optimal dissolution test conditions will depend, to a large extent, on the properties of the API being tested [43]. It is desirable to select dissolution test conditions, which are relevant to the physiological conditions, *in vivo*, in order to obtain meaningful *in vitro* test results. Therefore for the most part, aqueous dissolution media are preferred and are most frequently used for testing drug release characteristics from solid oral dosage forms.

Active pharmaceutical ingredients have an intrinsic dissolution rate that is dependant on its solubility and particle size [43,68]. The properties of an API may influence the choice of dissolution medium, particularly with respect to pH as many drugs are weak acids or bases, which are more soluble in basic or acidic solutions, respectively [43]. The particle size and size distribution of the drug in a formulation is also an important consideration, as dissolution is a diffusion controlled process that is affected by the area of exposed solid that is available for dissolution to occur [68,69].

HPMC and other hydrophilic matrices have been found to undergo dissolution and therefore drug release rates may be affected by the dissolution rates of the polymeric materials that comprise the matrix. Dissolution rates may also be affected by the test apparatus selected [70] as the hydrodynamic conditions that prevail when using USP Apparatus 3 may increase the erosion rate of the polymeric matrix, and increase the contribution of erosion to the release rate of an API in the dosage form, when compared to those conditions that prevail when using USP Apparatus 1 or 2. Furthermore, high pH, osmotic and ionic strength have been reported to reduce the dissolution rates of API from HPMC matrices, while low pH values are associated with an increase in dissolution rates in media with high osmotic and ionic strength [70].

Although the *in vitro* dissolution profile of an API is useful in postulating the potential *in vivo* behaviour of a dosage form, it does not always provide an accurate prediction of the *in vivo* performance unless an *in vitro-in vivo* correlation has been established, dissolution results should not be assumed to represent the ultimate *in vivo* performance of a product [71].

Differences in behaviour between dosage forms are more pronounced *in vitro* than *in vivo* [72,73], but absorption *in vivo* is frequently slower than release rates *in vitro* [72]. Therefore, the use of an appropriate and discriminatory dissolution test with an established *in vitro-in vivo* correlation would provide the formulation scientist with suitable data to develop a suitable formulation with a shortened development time.

3.8.2 Dissolution Apparatus

The USP lists several official apparatus for the purposes of conducting dissolution testing, some of which have specialised and limited application [9,67]. The two most commonly used apparatus are USP Apparatus 1, commonly known as the Basket apparatus and USP Apparatus 2, commonly referred to as the paddle apparatus. These two apparatus are similar, but when using Apparatus 1 the dosage form to be tested, is placed in a basket attached to a rotating shaft which is lowered into a dissolution vessel containing the appropriate dissolution medium, whereas when using Apparatus 2, the dosage form to be tested is placed at the bottom of a dissolution vessel and a rotating paddle provides the necessary impetus for medium agitation.

The USP Apparatus 3, or the BioDis[®], is specifically designed for the assessment of dissolution rates of extended-release dosage forms [9]. The apparatus consists of six rows and in which each row holds six or seven receptor vessels, depending on the design. The dosage form to be tested is placed in an inner glass tube that has a mesh at each end and which is attached to a drive unit by means of a stainless steel shaft and the six or seven inner tubes correspond to the receptor vessels in a specific row. The drive shaft allows the inner tubes to reciprocate vertically within a corresponding receptor vessel, in a row, at a particular dip rate, for a set period of time.

The inner tubes have two openings, which are closed by use of a mesh of specific dimensions that are selected according to the required specifications for analysis. The mesh permits dissolution fluid to enter the inner tube on the down stroke and exit as the inner tube is withdrawn, thus facilitating dissolution of the drug and excipients within the dissolution medium in the receptor vessels. The time allowed for the dosage form to reciprocate in a row in addition to the dip rate may be changed to suit a specific application. In addition, the pH and molarity of the dissolution media can be varied between vessels and rows, thus allowing for the dissolution test to mimic the physiological conditions in the GIT. The choice of apparatus may influence the results of the dissolution test [74] and thus is important since different dissolution apparatus use varying volumes of dissolution medium and mechanisms of agitation, which promote varying types and degree of flow dynamics. These factors do ultimately affect the *in vitro* performance of dosage forms and therefore the choice of the

correct apparatus to develop a discriminatory dissolution test is vital in the early stages of product development.

The USP also lists several other official apparatus for dissolution testing, and of these only one apparatus, *viz.*, USP Apparatus 4 may have application for the assessment of the dosage forms developed and manufactured in these studies, but has yet to be used in any official monograph for the purposes of dissolution rate testing. Therefore, the aforementioned and other official apparatus are not described in detail, herein.

3.8.3 Preliminary Selection of Dissolution Testing Apparatus

A dissolution test procedure should be designed in such a way such that it is discriminatory and the most appropriate apparatus for dissolution testing of sustained release matrix tablets must be determined. The USP Apparatus 2 is most frequently used for dissolution testing of sustained release dosage forms however, the use of USP Apparatus 3 or the BioDis[®] is becoming more accepted and is the preferred apparatus for dissolution rate studies of sustained release dosage forms [9,75].

The advantages of using Apparatus 3 as compared to Apparatus 2 include better simulation of the gastro-intestinal environment in terms of the pH range and gastric motility that a dosage form may be exposed to during gastric transit, *in vivo*. Furthermore, undesirable effects such as coning that is caused by the laminar flow of dissolution media when using Apparatus 2 are avoided [75].

Dissolution methods used to assess the rate and extent of drug release from sustained release dosage forms using USP Apparatus 3 are usually designed to expose the tablets being tested to dissolution media of six different pH values for various times over periods of between 6 and 24 hours. Dosage forms are therefore exposed to media of different pH and times and therefore exposure is similar to that the dosage form would encounter as it progresses from a low to high pH during GI transit, from the stomach, through the small intestine and ultimately the large intestine. Therefore by selection of an appropriate pH and composition for a series of dissolution media, the test can mimic, in some respects, the transit of dosage forms from an acidic environment in the stomach to a more alkaline environment such as in the small

intestine and colon. In contrast, as dosage forms are exposed to only one dissolution vessel with one dissolution medium when using Apparatus 2, it is more difficult and complex to change the dissolution media during testing over extended periods of time.

3.8.4 Method

In order to determine which dissolution method and apparatus was most appropriate for the purposes of assessing the *in vitro* release rates of CBZ from formulations, the reference product Tegretol[®] CR (Novartis, Quebec, Canada) was subjected to dissolution testing using either USP Apparatus 2 or 3.

The dissolution test conditions for the preliminary dissolution testing of the reference product, using either Apparatus 2 or Apparatus 3 (BioDis[®]) are summarized in Table 3.7. Dissolution testing of the dosage forms using USP Apparatus 2 was achieved using a fully automated Model SR 8 PLUS dissolution apparatus (Hanson Research Corporation, Chartsworth, CA, USA) fitted with an Autoplus[™] Multifill[™] and a Maximizer Syringe Fraction Collector (Hanson Corporation, Chartsworth, CA, USA). The dissolution testing of dosage forms using USP Apparatus 3 was achieved using a Model 25-1100 Vankel BioDis[®] (Varian, Cary, NC, USA) extended release dissolution tester with a Vankel VK750D (Varian, Cary, NC, USA) water circulation heater.

The dissolution media used was phosphate buffer, prepared as described in Section 2.2.3 and was the same molar concentration for both test systems. The pH of the dissolution media used for the evaluation the reference product release rates using Apparatus 2 was maintained at pH=7.2 as it was thought that this would be the pH to which the dosage form would be exposed for the longest period following oral administration, whereas the pH was varied between 1.6 and 7.2 and the dosage form exposed to the different media for different periods of time, for the dissolution method using USP Apparatus 3 as described in Table 3.8.

Table 3.8 Dissolution test conditions for USP Apparatus 2 and 3.

Parameter	USP 2 (Paddle)		USP 3 (BioDis®)		
Temperature	37.5°C		37.5°C		
n	6		6		
Test period	24 hours		22 hours		
Agitation rate	*100 r.p.m		*20 d.p.m.		
Dissolution media	0.1M phosphate buffer pH 7.2 (900ml)		0.1M phosphate buffers of varying pH (185ml)		
Sampling schedule			Vessel	pH	Dissolution time
	0.5 hours				
	1 hour	8 hours	1	1.6	1 hour
	2 hours	10 hours	2	3.4	1 hour
	3 hours	12 hours	6	4.7	4 hours
	4 hours	16 hours	10	6.8	4 hours
	5 hours	20 hours	14	7.2	4 hours
	6 hours	24 hours	22	7.2	8 hours

Test samples were collected automatically, at the pre-determined times throughout the twenty four hour period for the test conducted using Apparatus 2, and at the end of the twenty two hour test period from each receptor vessel for the test conducted using USP Apparatus 3. All samples were analysed using the validated HPLC method previously reported in Chapter 2 and the cumulative percent drug released was plotted as a function of time for the test period under consideration, to generate dissolution rate profiles for the reference product for each apparatus.

3.8.5 Results and Discussion

The dissolution rate profiles generated for CBZ following testing of the Tegretol® CR tablets using both USP Apparatus 2 and 3 are depicted in Figure 3.3.

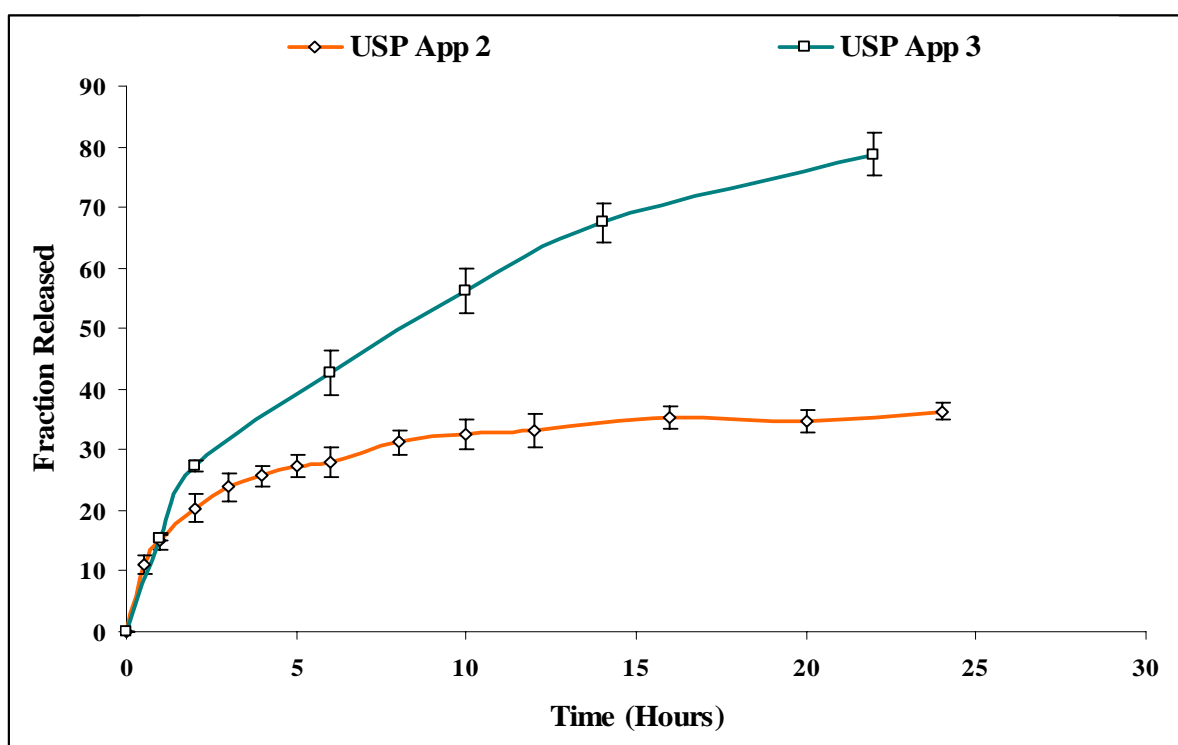


Figure 3.3 Mean (n=6) dissolution rate profiles of CBZ release from Tegretol® CR tablets using either USP Apparatus 2 or Apparatus 3.

The total percent drug recovered or fraction released, at the end of the 24-hour test period using Apparatus 2 was < 50% of the theoretical drug loading and label claim of 400mg. The aqueous solubility of CBZ is 170mg/l and the average amount of CBZ recovered in 900ml of the dissolution media was found to be 147.21mg, which is in excess of the saturation solubility limit of CBZ. Therefore, due to a combination of the high drug loading of 400mg and the poor water solubility of CBZ, the saturation solubility of CBZ is reached after approximately 36% of the CBZ had been released when using Apparatus 2. In contrast, the use of USP Apparatus 3 simulates gastro-intestinal pH conditions more closely and the results of dissolution testing reveal better drug recovery than that observed when testing with USP Apparatus 2. These results are more than likely due to the fact that during extended dissolution testing using USP Apparatus 3, tablets are placed in fresh dissolution media of different pH at predetermined times. Furthermore, the total volume of dissolution media used in Apparatus 3 was 1110ml, subdivided into six equivalent volumes of 185ml and therefore saturation solubility is not reached as early or to as great an extent as that observed when using Apparatus 2.

In addition, tablets tested using USP Apparatus 2 sank to the bottom of the dissolution vessel and therefore the nature of the paddle apparatus and associated fluid dynamics results in a phenomenon of coning which prevents multi-dimensional drug release from dosage forms. Therefore, the surface area of the dosage form exposed to the dissolution medium, was limited to those surfaces exposed to the flow effects that are present during dissolution testing when using this apparatus. In contrast, the vertical reciprocating motion of the BioDis[®] and associated turbulent dissolution medium flow, ensured that the tablets were exposed to dissolution medium on all surfaces, thereby permitting multi-dimensional drug release.

CBZ is a neutral drug and therefore pH is unlikely to affect the rate and extent of dissolution as shown in Section 2.4.4, in which buffer pH did not affect the chromatographic retention times of CBZ. Therefore the relatively high fraction of CBZ that was observed when using USP Apparatus 3 cannot be attributed to pH effects but rather the continual and renewed exposure of the dosage form to sink conditions for the periods described in Table 3.8.

3.8.6 Sample Treatment

Following dissolution testing using Apparatus 3, 1ml samples were removed from the relevant receptor vessels and analysed by the validated HPLC method, described in Chapter 2. The samples were diluted by addition of 1ml of HPLC grade water and internal standard added as previously described in Section 2.2.2. Plots of the cumulative percent drug or fraction released as a function of dissolution time were generated for each batch of tablets manufactured, during these studies. The dissolution profiles of all batches of tablets assessed in these studies are summarized in batch summary records contained in Appendix II.

3.8.7 CONCLUSION

The USP Apparatus 2 was found to be inappropriate for the testing of CBZ sustained-release dosage forms, due to the poor water solubility of CBZ and therefore the resultant low percent drug released. In addition, the associated fluid dynamics and effects related to the use of this apparatus, may have also had an impact on drug release. It is evident that when testing high dose products of sparingly soluble drugs such as CBZ, saturation solubility can occur to a greater extent when using Apparatus 2, than when using USP Apparatus 3.

The USP Apparatus 3 was found to be more applicable for the characterization of dissolution profiles of this matrix type sustained release dosage form. It is likely that the continual movement of tablets into fresh dissolution media, thereby avoiding saturation conditions would enable greater discrimination when testing formulations of different composition. Furthermore, the use of USP Apparatus 3 provides a more stringent test procedure, a higher degree of precision, and a more realistic dissolution environment, through simulation of the GIT pH range and peristalsis [76]. In addition, the exposure of dosage forms to a range of media of different pH was considered valuable and more meaningful for assessing dosage form performance than exposure to a single dissolution medium, as is the case if USP Apparatus 2 were used for testing these products.

Therefore, dissolution testing for all formulation and development batches was undertaken using Apparatus 3, with the conditions as summarized in Table 3.9.

Table 3.9 Dissolution test conditions used for all tablet batches.

Apparatus	USP Apparatus 3 (Vankel)		
Temperature	37.5°C		
Sample size (n)	6		
Test length	22 hours		
Agitation rate	20 dips per minute		
Dissolution media pH (0.1M phosphate buffers, 185ml)	Dissolution medium #	pH	Dissolution time
	1	1.6	1 hour
	2	3.4	1 hour
	3	4.7	4 hours
	4	6.8	4 hours
	5	7.2	4 hours
	6	7.2	8 hours

3.9 Statistical Comparison of Dissolution Rate Profiles

Statistical and mathematical models that have been developed and applied to the assessment and comparison of dissolution rate profiles include the Rescigno Index, ANOVA, model-independent similarity and difference factors and ratio tests [71,77-83]. During *in vitro* dissolution testing when developing new generic formulations, dissolution profiles of test products are compared to that of a selected reference product containing the same drug loading, thus providing valuable information to the formulation scientist during the product development process. Therefore, it is important that these comparisons are made with scientific validity and confidence based on sound statistical models or mathematical relationships.

The Rescigno index provides a different approach, whereby the concentrations of drug at various time points are used to calculate a dimensionless number that falls in the range between 0 and 1, and which is termed the bioequivalence index [84]. This index, when applied to dissolution data as a measure of the dissimilarity between curves, has been shown to have bias when the dissolution curve of a test product produces concentrations that are higher than that of the reference product [81].

Comparisons performed in this study were performed using the f_1 and f_2 difference and similarity factors [81], as these parameters are recommended by the FDA for the comparison of *in vitro* dissolution profiles. Moore and Flanner [81] described two functions that can be used to evaluate the degree of difference and similarity between two release profiles. Each release profile is a vector $x = (x_1, \dots, x_n)$ where each x_i is a non-negative real number.

Usually, one of the profiles, $T = (T_1, \dots, T_n)$ say, is a test profile while the other,

$R = (R_1, \dots, R_n)$ say, is a release profile which is to be compared to T . For such a vector x , the ℓ_1 -norm of x is defined by

$$\|x\|_1 = \sum_{i=1}^n |x_i| = \sum_{i=1}^n x_i \quad \text{Eq 3.1}$$

since each $x_i \geq 0$. The ℓ_2 -norm of x is defined by

$$\|x\|_2 = \sqrt{\sum_{i=1}^n x_i^2}. \quad \text{Eq 3.2}$$

In the case $n = 2$ or $n = 3$, the ℓ_2 -norm is the length of the vector obtained by the theorem of Pythagoras. Both norms are generalisations of the notion of length of a vector and for both norms, $\|x - y\|$ is a generalisation of the notion of distance between the vectors. The norms are equivalent in the sense that $\|x\|_2 \leq \|x\|_1 \leq \sqrt{n} \|x\|_2$. This means that if $\|x - y\|_1 < \varepsilon$ then $\|x - y\|_2 < \varepsilon$ and if $\|x - y\|_2 < \varepsilon$ then $\|x - y\|_1 < \sqrt{n}\varepsilon$. For release profiles R, T the numbers $\|R - T\|_1$ and $\|R - T\|_2$ both give measures of the distances between the vectors. The f_1 difference measure is defined by

$$f_1(R, T) = \frac{100 \|R - T\|_1}{\|R\|_1} \quad \text{Eq 3.3}$$

and $f(R, T)$ represents the relative percentage error between R and T . $f(R, T)$ must fall below 15 for the curves to be considered similar with statistical confidence.

The f_2 similarity measure between R and T is defined by

$$f_2(R, T) = 50 \log_{10} \left(\frac{100}{\sqrt{1 + \frac{\sum_{i=1}^n (R_i - T_i)^2}{n}}} \right) = 50 \log_{10} \left(\frac{100}{\sqrt{1 + \frac{\|R - T\|_2^2}{n}}} \right). \quad \text{Eq 3.4}$$

Since $f(T, T) = 100$ the highest measure of similarity is 100 and the measure decreases logarithmically as the difference, $\|R - T\|_2$, increases. For example if $T = (0, 16, 28, 42, 56, 67, 78)$ and R differs from T by 5% at each coordinate then $f_2(R, T)$ is obtained, using MATLAB, as described in Figure 3.4.

```
T = [ 0    16    28    42    56    67    78]
>> R=1.05*t
R = [0 16.80 29.40 44.10 58.80 70.35 81.90]
>> f2(R, T)
ans = 79.0327
```

Figure 3.4 $f_2(R, T)$ if R differs from T by 5%

By way of further examples, if R differs from T by 10% and 15% at each coordinate then $f_2(R,T)$ is obtained as described in Figures 3.5 and 3.6, respectively.

```
>> R=1.1*T
R =0 17.60 30.80 46.20 61.60 73.70 85.80
>> f2(R,T)
ans =65.2310
```

Figure 3.5 $f_2(R,T)$ if R differs from T by 10%

```
>> R=1.15*T
R =0 18.40 32.20 48.30 64.40 77.05 89.70
>> f2(R,T)
ans =56.6746
```

Figure 3.6 $f_2(R,T)$ if R differs from T by 15%

If $f_2(R,T)$ is plotted against the percentage difference between the coordinates of R and T the resultant plot is as depicted in Figure 3.7.

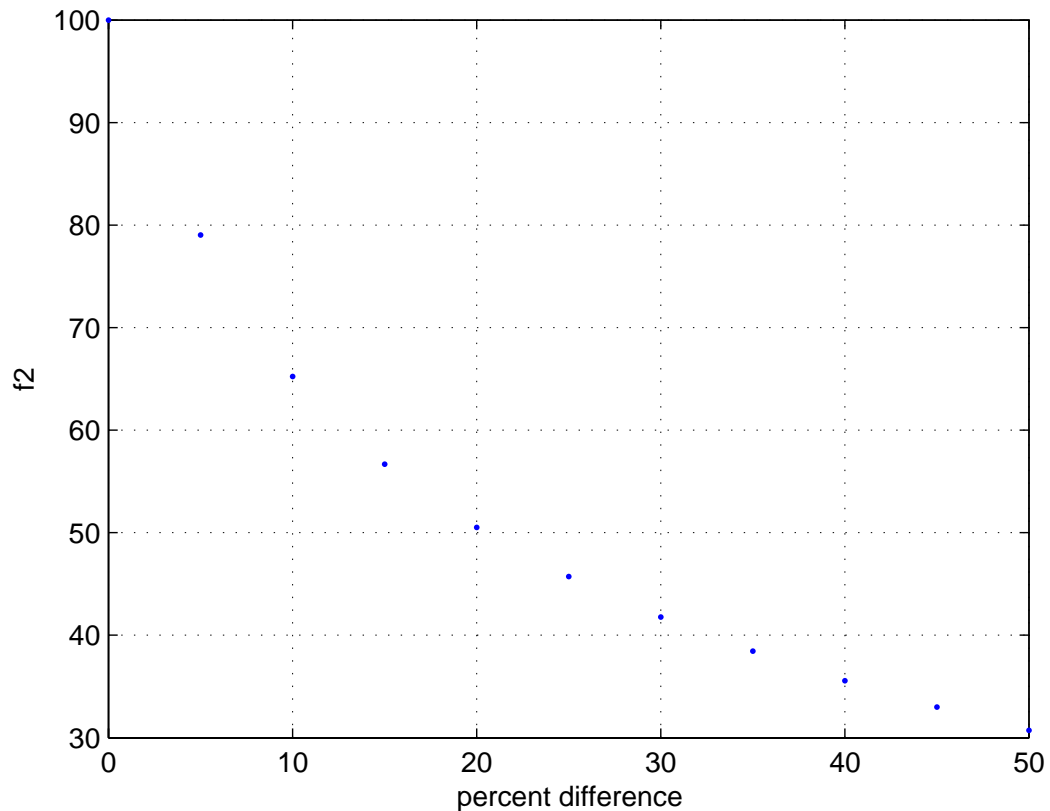


Figure 3.7 Plot of $f_2(R,T)$ versus percentage difference between the coordinates of R and T

This is achieved with the simple MATLAB script given in Figure 3.8

```

%f2comp
clear all; close all
T=[0 16 28 42 56 67 78 ]';
for k=1:11
R=(1+(k-1)*5/100)*T;
F(k)=f2(R,T);
end
F=F(:); P=[0:5:50]';M=[P F]';
fprintf('percent diff: %5.0f \t f2: %5.2f\n',M)
plot([0:5:50],F, '.')
xlabel('percent difference');ylabel('f2');grid

```

Figure 3.8 Script to produce $f_2(R,T)$ as a function of the percentage coordinate-wise difference between R and T

The script also returns the f_2 values given in Figure 3.9

percent diff:	0	f2: 100.00
percent diff:	5	f2: 79.03
percent diff:	10	f2: 65.23
percent diff:	15	f2: 56.67
percent diff:	20	f2: 50.52
percent diff:	25	f2: 45.71
percent diff:	30	f2: 41.77
percent diff:	35	f2: 38.44
percent diff:	40	f2: 35.55
percent diff:	45	f2: 33.00
percent diff:	50	f2: 30.71

Figure 3.9 f_2 values versus percentage coordinate-wise difference

As can be seen from the plot in Figure 3.9, a 20% coordinate-wise difference between the profiles R,T results in $f_2(R,T) = 50.52$. The lower threshold value for statistical similarity is considered to be 50, as defined in the FDA Guidance for Industry [71,85,86].

Although, the FDA accepts this method of comparison, it recommends that the %RSD for dissolution data be less than 20% for early samples and less than 10% for the balance of the data collected during dissolution testing. The f_2 value is reported to be sensitive to measurements in which more than 85% of the API had been released from either the test or reference formulations and it is therefore recommended that only one data point be included for the comparison if several samples reveal that more than 85% API has been released [85]. It has also been suggested that the f_2 similarity factor has complicated statistical properties, which may jeopardise the validity of its use for the comparison of dissolution test results [85].

Currently, the f_1 and f_2 functions developed by Moore and Flanner [81], which appear in the FDA guidance to industry for Scale-Up and Post-Approval Changes (SUPAC) [71],

amongst others, are the most frequently cited parameters used for the comparison of dissolution rate profiles between test and reference products [82,83,85,87]. Furthermore, it has been suggested that most pair wise and model independent statistical test methods give meaningful results for dissolution curve comparisons [82].

Dissolution profile similarity depends on the similarity between the profiles at each time point and the similarity between the overall profiles [85]. The similarity between overall profiles is particularly important for dissolution testing of immediate release formulations [78,85], and has been used in these studies for the comparison of sustained release formulations, with some degree of success. When comparing dissolution profiles it is important to ensure that the test conditions are identical [78,85] and that sample times used are representative of the entire dissolution rate profile being developed [78].

If it is considered more important for the profiles to match at certain coordinates then this is achieved with a weighting vector $w = (w_1, \dots, w_n)$. The weighted similarity measure,

$f_2(R, T, w)$ is given by equation 3.11

$$f_2(R, T, w) = 50 \log_{10} \left(\frac{100}{\sqrt{1 + \frac{\sum_{i=1}^n w_i (R_i - T_i)^2}{n}}} \right). \quad \text{Eq 3.5}$$

If, for example, the second dissolution point is considered very important and the remaining points are of diminishing importance as the index increases, then a weighting vector, $w = (0,8,4,2,1,1,1,)$ could be used to adjust the weighting of the data in the determination of similarity. In order to keep the weighted similarity measures on the same scale, it is necessary to replace the weighting vector by a corresponding normalized vector. Therefore, w would be replaced by $\frac{w}{\|w\|_2}$. In this case, the weighting vector would be $\frac{1}{\sqrt{87}}(0,8,4,2,1,1,1)$ and an example of the application of this weighting vector if the percent difference between R and T were 15% is shown in Figure 3.10.

```
>> T=[0 16 28 42 56 67 78 ];
>> R=1.15*T
```



```

R = 0 18.40 32.20 48.30 64.40 77.05 89.70
>> w=[0,8,4,2,1,1,1];
>> w=w/norm(w)
w = 0 0.8577 0.4288 0.2144 0.1072 0.1072 0.1072
>> f2(R,T,w)
ans = 76.4791

```

Figure 3.10 $f_2(R,T,w)$ if R differs from T by 15% using a normalized weighting vector.

The objective of these studies was to produce a test formulation to match especially the first hour of *in vitro* drug release to that of Tegretol[®] CR. In Chapter 7 an artificial neural network is constructed and then used to simulate on a range of input vectors which represent formulations with gradually changing percentages of two forms of the active ingredient. The corresponding activations represent release profiles which are to be compared with the release profile of Tegretol[®] CR. This can be seen in Figure 7.5. Thus the highest weighting was assigned to the data predicted after the first hour with less emphasis and therefore weighting on subsequent sampling times.

3.10 CONCLUSION

The f_1 and f_2 statistical factors provide a useful and convenient method to establish similarity between two sets of release rate profiles. This allows the formulator to objectively assess release rate profiles and compare them to reference products so that formulation adjustments can be made during dosage form development. The difference and similarity factors adopted for assessment during these studies provided a tool to compare the release profiles of formulations produced in this study to Tegretol[®] CR. Furthermore, the f_2 factor was adopted and incorporated into the neural network application as described in Chapter 7 so that drug release prediction was further tailored with this additional parameter.

The f_1 and f_2 difference and similarity factors is also endorsed by the FDA as a guideline in industry for the comparison of drug release profiles and therefore, concluded the use of this statistical tool.

3.11 Stability Assessment of the Biostudy Test Batch

3.11.1 Introduction

The ultimate stability of a pharmaceutical dosage form may be affected by various factors including the stability of the API, potential physical and chemical interactions between an API and excipients, packaging and storage conditions, length of time between manufacture and use of the product as well as the environmental conditions that the product is exposed to during transport, storage and handling [194,195]. The predominant processes by which drugs degrade, include hydrolysis, oxidation, thermolabile reactions and photo-catalysed reactions in the presence of light or photolysis. Extreme environmental conditions, that include these elements that contribute to product instability, can accelerate degradation processes [194-196]. These factors must be considered and assessed appropriately as this affects both the chemical and physical stability of a drug, the integrity of a dosage form and ultimately, dosage form performance that may be adversely affected.

According to the ICH Guidelines [197,199], stability testing should be conducted on a number of batches of a final product, packaged in the containers and/or devices that will be used during the normal storage, use and distribution of a product. The recommended test conditions include an isothermal and humidity constraint. The recommendations for storage conditions differ between countries, which have been divided into different climatic zones. South Africa falls into climatic Zones I and II and therefore stability testing should be conducted under the conditions described in Table 3.10 [194-199].

Table 3.10 Recommended storage conditions for stability testing in Zones I and II.

	Temperature	Relative Humidity	Test Length	References
Long term	25 ± 2°C	60 ± 5% RH	1-5 years	77-79, 82
Intermediate	30 ± 2°C	60 ± 5% RH	6 months	77-79
Accelerated	40 ± 2°C	75 ± 5% RH	6 months	77-79, 82

Stability testing is important to elucidate information regarding the recommended storage conditions and shelf life or expiry date of a particular product [194-200]. Stability testing is a standard requirement and an essential adjunct to the assurance of quality of all pharmaceutical products. For controlled release formulations, stability testing allows for the identification of any quantitative changes or decline in the content of an active ingredient within a product or a change in release characteristics that may ultimately lead to failure of the dosage form *in vivo*. In addition, stability testing allows for the identification of physico-chemical changes to an active ingredient that may occur and that may induce toxic or adverse effects on administration to patients following long-term storage of these types of products.

3.11.2 Stability Studies

Preliminary stability studies were performed on Batch C-160905-01 to gain insight into the stability profile of the matrix tablets that were to be tested in a bioequivalence study to compare Tegretol[®] CR to the test formulation. The use of accelerated storage conditions may provide insight into the feasibility of using uncoated matrix tablets as a twice daily sustained release dosage form. The use of a protective coating would add complexity to the manufacturing procedure and possibly introduce more variability in product performance and would necessitate the need for additional manufacturing equipment.

The stability study was conducted using humidity chambers prepared in dessicators with saturated salt solutions, to achieve a desired relative humidity condition [139]. The chambers were prepared using sodium chloride which is reported to produce an approximate relative humidity of 75% at 40°C, to simulate the conditions required for accelerated stability testing. Testing was conducted for one month (thirty days) using storage conditions of 25°C/60% RH and 40°C/75% RH.

Two lots of approximately 200 tablets from Batch C-160905-01 were collected and one lot exposed in a dessicator chamber containing a saturated sodium chloride solution. The dessicator chamber was placed in an oven that was maintained at 40°C for one month and the resultant

relative humidity was maintained at 75%. The second lot were stored at room temperature by storing the dessicator chamber in a dark cupboard in the laboratory where the ambient temperature was maintained at $\pm 25^{\circ}\text{C}$ and the humidity of 60%. The tablets were placed in open glass jars and stored for thirty days. Although dosage forms should be tested in their final packaging, the use of exposed tablets in these conditions would give a rapid indication of the ability of the dosage form to resist extreme changes in humidity and temperature.

Thermohygropens (Cole Palmer Instrument Company, Vernon Hill, IL, USA) were used to monitor the temperature and humidity in each chamber on a weekly basis.

At the end of the one month stability test period, the two lots of tablets were assessed in terms of changes in visual appearance, content and weight uniformity, hardness, moisture and friability. Furthermore, dissolution testing of the tablets stored at ambient conditions was conducted. The visual assessment of colour and overall impression of the dosage form provides an early indication of any potential drug or tablet degradation and is therefore a valuable QC tool.

3.12 Results of Stability Testing

3.12.1 Visual Appearance

The tablets exposed to conditions of $40^{\circ}\text{C}/75\%\text{RH}$ showed signs of discolouration, tablet swelling and cracking within twenty four hours of exposure. After one week, the discolouration, and cracking was highly pronounced but did not progress beyond this extent for the remainder of the test period. Tablets that were tested at ambient conditions did not display any discolouration, swelling or cracking behaviour for the duration of the study. These tablets retained their elegant, smooth aesthetic appearance and can therefore be considered for further long term testing.

3.12.2 Tablet Hardness

Tablet hardness was determined using a PharmaTest[®] PTB 311E Automated Tablet Testing Instrument (Pharmatest, Hainburg, Germany). The results of hardness testing for the lots

subjected to different storage conditions as well as the hardness data for the batch determined prior to storage are listed in Table 3.11.

Table 3.11 Result of hardness testing

Batch Type	Average Hardness ± SD	% RSD
(Biostudy Batch # C-160905-01)	n =10	
After Manufacture	132.2 ± 6.41 N	4.85
25°C/60% RH	118.56 ± 17.96 N	15.15
Period of study = 1 month		
40°C/75% RH	12.13 ± 6.49 N	53.54
Period of study = 1 month		

The tablets stored at 40°C/75% RH showed a significant decrease in hardness as can be seen from the data in Table 6.2. The tablets were extremely soft and crumbled upon testing and careless handling. The moisture content increased markedly to approximately 12% under the test conditions with subsequent tablet swelling which caused the tablets to be softer. Tablets stored at ambient conditions showed some loss of hardness and greater variation in hardness between tablets was observed when compared to the control lot. The changes caused by high humidity and temperatures were considered to be unacceptable as these tablets could not be effectively used by a patient and the changes would certainly affect dosage form function.

3.12.3 Tablet Weight

The results of the uniformity of tablet weight determination are listed in Table 3.12. The weight of the tablets stored under accelerated conditions increased markedly, where a mass increase of approximately 37% was observed and the % RSD of 2.92 was higher in this test lot than for the other lots tested. The most significant contributing factor to the weight gain was the moisture content which increased significantly and that is consistent with the swelling behaviour observed with this lot of tablets. The tablets stored under ambient conditions showed an increased weight

variation when compared to the control lot due to its exposure to higher humidity conditions but had far less variation in weight than was observed for the lot stored under accelerated conditions.

Table 3.12 Tablet Weight Uniformity

Batch C-160905-01	Average Weight \pm SD n=10 mg	% RSD
After Manufacture	800.82 \pm 4.13	0.52
25°C/60% RH Period of study = 1 month	807.0 \pm 0.01	0.001
40°C/75% RH Period of study = 1 month	1099.0 \pm 0.03	0.002

3.12.4 Tablet Friability

The determination of friability was undertaken using an Erweka Friabilator (Erweka-Aparatebau-GMBH, Heusenstamm, Germany) set to achieve 33 drops per minute over a 3 minute test period and the results of these determinations are shown in Table 3.13. Tablets stored under accelerated conditions failed the friability test as the tablets crumbled and broke during the test. This corresponds to a complete loss of tensile strength of this test lot. Tablets that were stored under ambient conditions showed a net loss of less than 1% which was within the acceptable limits for friability according to USP guidelines [9]. This is an indication that these tablets would probably maintain their physical integrity during transport and handling of the dosage form if exposed to conditions of 25°C/60% RH for an extended period of time. However, longer test periods must be used to establish real time stability of these dosage forms.

Table 3.13 Tablet friability.

Batch Type C-160905-01	Friability (% mass loss) n=10
T=0	-
25 °C/60% RH T=1 month	0.61
40°C/75% RH T=1 month	-

3.12.5 Moisture Content

The uptake of moisture by tablets during storage was determined using the Karl Fischer procedure as described in Section 3.2.1. The tablets tested prior to storage had an average moisture content of 2.46%. A moisture content limit of 5% was selected as an appropriate limit for these tablets for these studies.

The moisture content of the tablets stored under accelerated conditions increased significantly from 2.6 to 12.33% and these results are consistent with the observed tablet swelling, increased tablet weight and subsequent loss of tensile strength of the tablets that was observed after storage for one month under these conditions.

The tablet batch stored under ambient conditions showed a minor increase in moisture content where an ultimate moisture content of 2.52% was observed for this batch and this increase was considered negligible for these tablets.

Significant moisture increase in formulations, affect the physico-chemical stability of the excipients and drug within the tablets. Thus, dosage form performance becomes affected, which could result in poor or toxic pharmacotherapy due to failure of dosage form function.

Furthermore, physical anomalies in these tablets due to the moisture uptake make the tablets unacceptable for patient use as well as impractical where tablets swell to proportions which are too large for patients to swallow with ease.

3.12.6 Assay

The determination of CBZ in these tablets was performed in accordance with USP guidelines for CBZ extended release tablets that has been previously described in Section 3.7.1. The results obtained for the assay performed for the three test lots of CBZ tablets from batch # C-160905-01 are summarised in Table 3.14. For the purpose of the assay, the theoretical recovery from these

tablets was considered 100mg according to USP method of analysis. The lot stored under accelerated conditions showed an assay determination of approximately 92 mg recovery but this result is within acceptable limits. However, the recovery of 92mg after one month may not be indicative of appropriate stability characteristics and should be further investigated. The lot stored under ambient conditions showed a recovery after of approximately 107mg after one month and that implies the formulation may be stable under these conditions.

Tablet 3.14 Assay results.

Batch C-160905-01	Average Recovery ± SD (n=20) mg	% RSD
T=0	112.44 ± 1.42	1.26
25°C/60% RH T=1 month	106.84 ± 0	0.0
40°C/75% RH T=1 month	92.53 ± 1.42	1.53

3.12.7 Dissolution Testing

The tablets stored under ambient storage conditions were subjected to dissolution testing to assess their *in vitro* performance and the dissolution rate profiles of this lot following storage and that of the control lot are shown in Figure 3.11. The curves appear similar and an assessment for similarity revealed f_1 and f_2 values of 4.0 and 83.0, respectively which indicates that there is no difference between these curves following the period of storage.

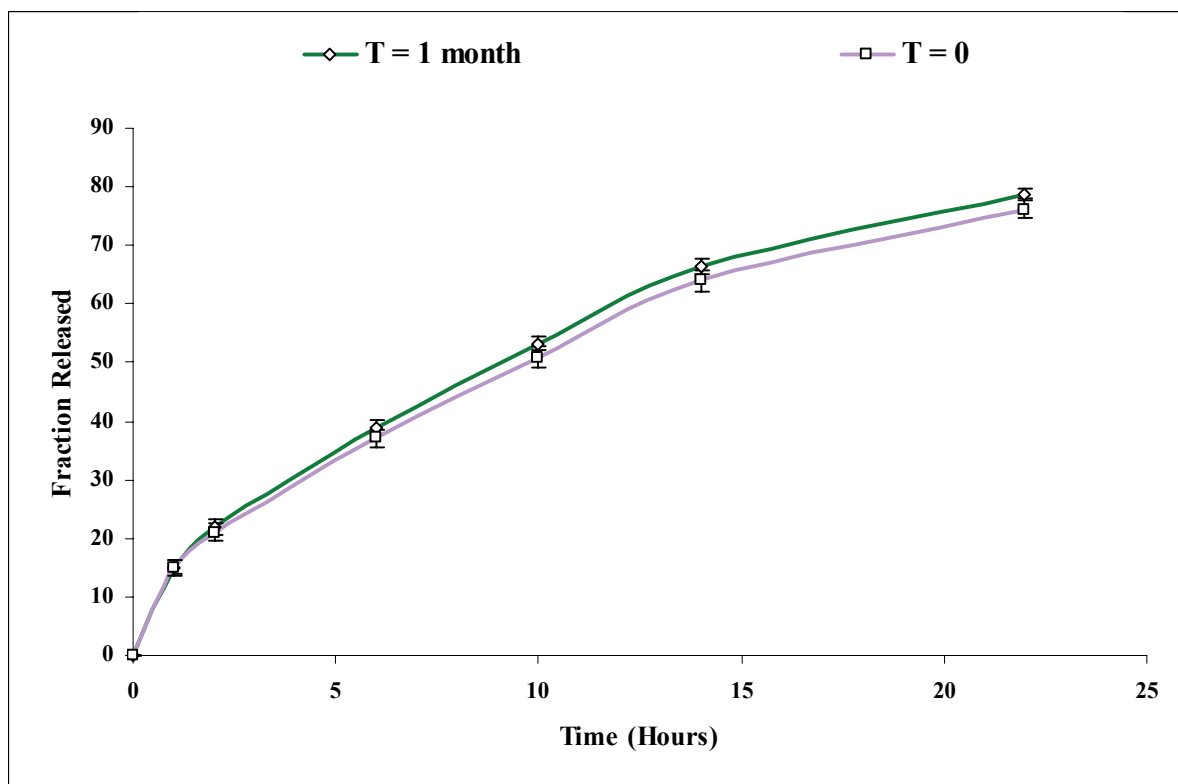


Figure 3.11 Dissolution profiles of the test Batch C-160905-01, at t=0 and after storage under ambient conditions for one month.

In terms of *in vitro* dosage form performance after storage for a month, the tablets tested for stability under ambient conditions show that drug delivery rates are exactly the same when compared to the control lot. Due to the obvious physical unacceptability of the lot stored under accelerated conditions, it was not considered necessary to perform dissolution studies on these tablets.

A key factor in assessing the stability of controlled or sustained release dosage forms is to ascertain whether the dissolution rates of CBZ are altered on storage. In this case, it is apparent that the release of CBZ following a period of storage of one month at 25°C/60% RH is unaltered and therefore the stability of these products must be further evaluated.

3.13 CONCLUSION

The results obtained for the tests conducted on the lots subjected to storage under selected conditions, reveal a definite increase in moisture content was observed. This was evidenced by a corresponding increase in tablet weight and friability and a decrease in tablet hardness. Tablets stored under accelerated conditions showed the greatest increase in moisture content whereas the batch stored at ambient conditions showed a moisture content increase that was well within the acceptable pre-set limits. Temperature and humidity have a considerable effect on the physical properties of a dosage form and environmental limitations should be defined and optimal conditions must be specified for both factors when defining storage conditions for these tablets. The results also indicate that packaging for the tablets would be a critical consideration during further formulation development. Furthermore, the use of a protective non-functional aesthetic coating would be appropriate to maintain the physical stability and acceptability of these tablets for an extended period of time, in particular in high humidity climate.

The tablets stored under ambient conditions displayed reasonably good stability and *in vitro* behaviour despite the lack of a protective coating and the effects of the environmental conditions on this lot were identical to that of the control lot. This indicates the physical robustness of the dosage form where the appropriate packaging and storage instructions given to patients may be adequate to maintain the shelf life as well *in vivo* performance of these dosage forms.

CHAPTER FOUR

PHARMACEUTICAL APPLICATIONS OF HYDROPHILIC POLYMERIC MATRIX SUSTAINED RELEASE TECHNOLOGY

4.1 Introduction

The oral route of drug delivery is a common and convenient means of drug administration [88]. This is due to the inherent simplicity of using this route of administration and the inherent simplicity of oral delivery system formulation [89]. Formulation designs, which aim to tailor drug delivery, have received increasing attention as formulators attempt to deliver drugs more ‘intelligently’ to enhance drug therapy, where the rate of release of a drug is dependent on the delivery device rather than physiological conditions [89-91]. The safety and efficacy profile of drug therapy can be improved significantly, in addition to patient compliance, as a consequence of a reduced dosing frequency with a subsequent improved overall therapeutic benefit for the patient [89,92,93]. Many pharmaceutical companies are now focusing on the development of novel and better methods of drug delivery such that existing and new drug molecules can gain an increased market share or an extended patent life [92].

Sustained-release dosage forms have been referred to as controlled-, delayed-, extended- or modified-release dosage forms [93-105]. These terms describe a dosage form that controls the drug absorption rate to achieve a desired plasma profile defined by steady-state pharmacology, for a particular compound after pre-determined lag times or intervals [89,106]. However, not all drug candidates are suitable for inclusion in sustained drug delivery dosage forms due to their physicochemical properties, in addition to the physiological constraints associated with their use. Therefore consideration of all interacting factors must be assessed during dosage form development to optimise drug delivery [88]. In general, the development and application of sustained-release delivery systems is suitable for active pharmaceutical ingredients (API) with a high degree of solubility in GIT fluids, rapid and near complete absorption, relatively fast elimination rates and rapid distribution of the drug into body tissues [95].

Conventional dosage forms present challenges relating to optimum drug delivery such as frequency of dosing intervals and extreme plasma drug concentration fluctuations as well as challenges in the achievement of desired clinical effects or a reduction in adverse effects. Modified-release formulations offer solutions to the difficulties associated with conventional drug delivery system development and use [92]. It has been shown that a more stable plasma/serum concentration of CBZ can be achieved through the use of controlled release delivery systems [102].

Some of the advantages of an ideal controlled-release dosage form include the reduction in the high degree of fluctuation of circulating drug levels, the avoidance of night-time dosing, a more uniform therapeutic effect and the reduction of gastro-intestinal irritation and other dose-related side-effects [88,93,101,107-109]. However, controlled-release formulations are often more expensive to manufacture than conventional formulations and cannot be justified unless they offer distinct, measurable, clinical and practical advantages over conventional delivery systems [109].

Several potential disadvantages of controlled release dosage forms include cost, unpredictable and often poor *in vitro-in vivo* correlations and potential dose dumping [109]. Furthermore, there is a decreased potential for dosage adjustment where tablets are required to be administered intact [109]. In general, there is an increased potential for first pass clearance and therefore poor systemic availability of API from these systems [101,102,109]. In addition the effective drug release period for oral dosage forms is influenced and limited by gastro-intestinal transit time which is generally between 8 – 21 hours [101,109].

Therefore the suitability of the drug candidate for inclusion in drug delivery systems in addition to any potential therapeutic benefits must be completely assessed to justify the value of such formulations in drug therapy prior to embarking on expensive formulation development.

4.2 Sustained-Release Delivery Systems

Monolithic polymeric devices are a well-known, well-established and widely used method of sustaining drug delivery [94,110]. Their widespread use is primarily due to their simplicity, relative ease of manufacture and low cost [88,89,111]. Almost all oral solid controlled release products currently marketed are based on matrix, membrane-controlled or osmotic systems [89]. Swelling-controlled release systems are also referred to as hydrogel or polymeric matrices that exhibit moving boundaries or are swellable matrix tablets [89,120]. Monolithic matrix systems generally consist of the API dissolved or dispersed within a skeleton-like structure, which is the matrix, and that is the rate-controlling polymer, with or without the addition of other excipients [95,108].

Both hydrophilic and hydrophobic polymeric matrix systems are widely used to control delivery of drug substances because of their versatility and effectiveness in prolonging drug release during conventional gastric transit times [89,107]. These systems are also suitable for “in-house” development since they are easily manufactured using conventional equipment and processing methods [89,102,105].

A variety of both hydrophilic and hydrophobic polymers are available for matrix device application, of which the most commonly used is hydropropylmethylcellulose (HPMC) [101,106,112,113]. HPMC is highly swellable on contact with aqueous media and the degree of swelling has a considerable effect on the resultant release kinetics of a drug contained in delivery devices that are primarily comprised of HPMC [64,89,103-105,114-116].

There are two major mechanisms by which drug release from these dosage forms is controlled, *viz.*, diffusion or dissolution-control [89,97,102,103]. Controlled drug release from HPMC matrix tablets may be affected by several formulation variables such as polymer content and molecular weight, drug content and solubility, type of excipient(s) as well as tablet shape and size or geometry [116]. Changes or modifications in polymer type, excipients and manufacturing processes can be modified to achieve specific release rates [103,113]. The

HPMC concentration and viscosity grade within a formulation have been most often manipulated to regulate drug release both kinetically and mechanistically from these systems [103].

On exposure to aqueous fluids as water diffuses into the tablet, the matrix swells and a viscous gel layer forms around the exterior of the tablet [116] followed by drug diffusion through the gel layer and into the surrounding medium [88]. Therefore, as the matrix comes into contact with the dissolution medium, the polymer undergoes relaxation and two fronts are established around the core tablet, *viz.*, the penetration and dissolution fronts [103]. At the same time, other soluble excipients or drug substances that comprise the delivery device will also dissolve and diffuse out of the matrix while insoluble excipients or APIs will be retained until the surrounding polymer/excipient/drug complex erodes, dissolves or disintegrates [118].

The penetration front is defined as the interface between the unhydrated or non-relaxed polymer and the gel layer [103], whereas the dissolution front is defined as the interface between the gel layer and the dissolution medium [103]. Hydration, swelling and coalescence of polymer particles occurs at the penetration front whereas polymer chain disentanglement and dissolution of the hydrated matrix occur at the dissolution front [103,115,116]. The diffusional path length available to a drug within a matrix device is represented by the gel layer thickness and corresponds to the distance between the penetration and dissolution fronts [103]. Drug release from swellable and erodible hydrophilic matrices can be attributed to polymer dissolution or a matrix erosion mechanism, drug diffusion through the gel layer, or a combination of both processes [103,104,116]. Diffusion is the principle mechanism governing drug release in the case of water soluble APIs whereas the release of water-insoluble drugs is predominantly dissolution controlled [103,116], and where surface erosion of the matrix polymer may contribute more significantly to overall drug release rates than drug dissolution [116].

The process of formulation development to produce a generic product that matches an innovator or reference product *in vivo*, initially involves matching the dissolution rate profiles based on statistical analysis by use of the difference and similarity, f_1 and f_2 factors. However, during the process these parameters may be of value to assess the impact of modifying formulation parameters and dissolution rate profiles [67]

4.2.1 Mechanisms of Drug Release

Controlled-release systems consist of a drug or solute that is distributed in a polymeric phase that can be referred to as the glassy state [119,120]. Sustained drug release occurs as a result of the use of rate-controlling hydrophilic, swellable polymers such as HPMC, which is a carrier material that is commonly used for oral-controlled drug delivery [123]. Pharmaceutical devices with an initial drug concentration greater than the solubility of the drug in the surrounding fluids are referred to as monolithic dispersions [122]. Dissolution of a polymer within the dissolution medium involves two transport processes, *viz.*, solvent diffusion into the dosage form and subsequent polymer chain disentanglement and relaxation [119,122].

In a thermodynamically compatible solvent the polymer requires an induction time prior to dissolution, after which the polymer swells and dissolves [119,120]. Transitions from a glassy to a rubbery state are accompanied by the simultaneous volume expansion of the device. Swelling of a device and subsequent polymer disentanglement results in the formation of a gel-like pathway, which is adjacent to the solvent-polymer interface due to plasticization of the polymer by the solvent, which is also capable of preventing matrix disintegration and further rapid water penetration [119-121]. Water penetration, polymer swelling, drug dissolution and diffusion and matrix erosion are the phenomena that ultimately determines the gel layer thickness and thus drug release rates [90,118,120].

Diffusion occurs as a result of the relaxation of macromolecular chains taking the polymer from a glassy to rubbery phase and this dynamic, swelling behaviour of the glassy polymer is generally the controlling mechanism for release of an API from polymeric matrices [90,119,120]. A linear relationship exists between the release rates and the reciprocal of the weight of HPMC used in the matrices. Therefore, higher molecular weight HPMC polymers would produce lower drug release rates [121]. Furthermore, upon contact with the receptor medium and by virtue of the excipients used in the formulation, water-filled channels or cavities may be created within the device followed by drug transport from the tablet via diffusion through these domains resulting in a change in the drug release kinetics from these systems [122].

Three moving fronts have been identified to describe drug movement in swellable matrix systems [98]. These fronts are:

- A. a swelling front that describes the boundary between the existing glassy polymer and its rubbery phase;
- B. a diffusion front which describes the boundary between the solid as yet undissolved drug and the dissolved drug in the gel layer; and
- C. an erosion front that describes the boundary between the matrix and the dissolution medium [98,120].

A schematic representation of the processes involved in drug release from polymeric matrix devices is depicted in Figure 4.1

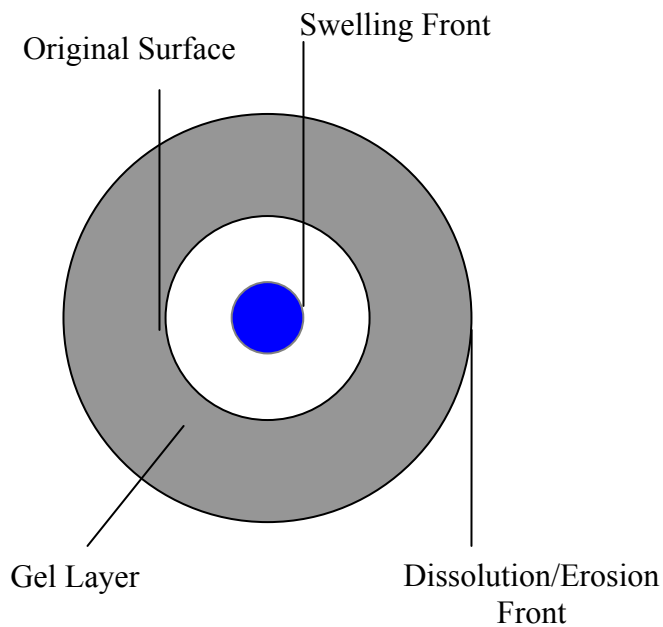


Figure 4.1 Diagrammatic representation of the swelling and erosion processes associated with matrix tablets.

Front synchronisation occurs when the velocity of glass-rubbery interface is in equilibrium with the rubbery-solvent interface. In devices where dissolution-control is predominant, front synchronisation leads to zero-order drug release [119,124]. A schematic representation of the dynamic changes that occur during drug release from polymeric matrix type delivery systems is shown in Figure 4.2.

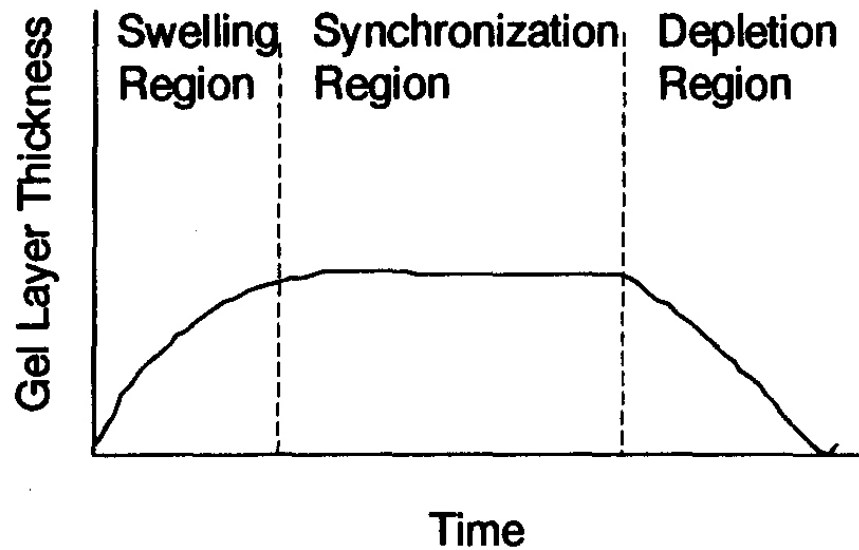


Figure 4.2 Swelling dimensions for a monolithic matrix tablet [111].

There are numerous mathematical models and theories have been developed in an attempt to describe drug release kinetics from controlled-release dispersed systems such as polymeric monolithic matrix tablets [120,123,125,126].

4.3 Mathematical Modelling of Drug Release from Polymeric Matrix Tablets

4.3.1 The ‘power law’

Modelling of drug release from swellable polymeric systems belongs to a category of diffusion problems known as moving-boundary or Stefan-Neumann problems [127]. Throughout drug release from HPMC matrices, synchronization of front movement is difficult to reach, therefore, the kinetics of drug release depends on the relative movement of the erosion and swelling/diffusion fronts [120].

Diffusion is one of the most important mechanisms controlling drug release from pharmaceutical formulations and the “power law” as defined by Peppas *et al* [123] describes more specifically the type of release that occurs in matrix systems with expanding boundaries such as those previously described in Section 4.2. The differential between diffusion and erosion fronts is decisive for describing the release kinetics where drug release rate depends on the diffusion front velocity [120]. A mathematical representation of the “power law” is shown in Equation 4.1

$$\frac{M_t}{M_\infty} = kt^n \quad \text{Eq. 4.1}$$

where,

t = the elapsed time;

M_t = the mass of drug released at time t ;

$M_\infty = \lim_{t \rightarrow \infty} M_t$, the limiting amount of drug released (the amount released in ‘infinite time’);

k = a proportionality constant that incorporates structural and geometric characteristics of a dosage form;

n = the release exponent.

The specific value of n is indicative of the mechanism of drug release from matrix systems. Siepmann and Peppas [123,125] derived different values for specific geometric delivery systems. In the case of a sphere, an n value ≤ 0.45 is indicative of drug release by Fickian diffusion [123,125], with water transport controlled by a concentration gradient [98]. Within a sphere,

values of n between 0.45 and 0.85 are indicative of anomalous transport whereas $n \geq 0.85$ reveals that Case-II type transport is occurring [123,125]. Case-II or time-independent transport represents swelling-controlled drug release with polymer relaxation determining the rate-limiting step [89-91,120] and anomalous transport can be observed in systems where the water uptake mechanism results in drug transport behaviour that displays both Fickian and Case-II type transport [98,123,125]. In Case-II type transport, solvent mobility is much higher than the segmental relaxation rate of the polymer [90,91,117,120,127].

A value of $n = 1$ is indicative of zero-order release from a planar surface but for spheres, such as matrix tablets and cylinders a value of $n \approx 1$ may not correspond directly to zero-order release due to the effect of geometric factors on the mathematical analysis of the data [115]. Thus, a value of 1 merely emphasizes that the release of drugs from these systems is not Fickian diffusion-controlled and may indicate contributions to drug release mechanisms by erosion of the polymeric matrix [115]. In a swellable matrix tablet, the erosion front movement determines the kinetics of the diffusion front and therefore the rate of drug release [90,120].

However, a mathematical description of the entire the drug release process is complex and difficult due to the number of physical characteristics or constraints that must be taken into consideration, including diffusion of water into the HPMC matrix, swelling of the matrix, drug diffusion out of the device, polymer dissolution, axial and radial transport in a three dimensional system and concentration dependant diffusivities of the API or compound(s) of interest [128]. In addition, factors such as the initial drug loading within the device, tortuosity of the water-filled pores following hydration, the partition coefficient of the drug between water and polymer and the particle size distribution of the drug can significantly influence drug release kinetics from these dosage forms [90,120,128]. It has also been shown that a major factor controlling drug release from HPMC matrices is the drug:HPMC ratio [121].

Due to the complexity of the drug release process, certain assumptions, such as the restriction of drug transport to one-dimension or ignoring the effects of polymer swelling and/or polymer dissolution, are made [128]. Consequently, the use of a mathematical model becomes restricted to certain drug-polymer systems which are geometrically specific. Since drug release kinetics

become dependent on the size and shape of a dosage form, a desired release profile can be obtained through the adjustment and manipulation of the geometry and dimensions of a system or by means of adjusting the formulation content and/or the method of manufacture [105,108,116,121].

4.3.3 Matrix Tablets

The type of matrix systems produced in this study, were monolithic polymeric systems. The systems consisted of CBZ dispersed in a hydrophilic polymeric material [122,129]. On exposure to biological or other aqueous fluids, the tablet thickness and volume increases which permits the dissolution of CBZ from the formulation into the surrounding liquid medium at a controlled rate. The retardation of drug release is erosion- controlled in cases where drug diffusion in the gel layer is slow, relative to the rate at which the polymer dissolves [129]. For drugs with low water solubility, such as CBZ, the most important process that determines the rate of drug release is erosion of the polymer used to fabricate the matrix system.

Dissolution-controlled release is achieved when the rate of dissolution of the polymer is slow when compared to the rate of dissolution of the drug. Diffusion-controlled drug release is prevalent when polymer dissolution is slower than drug diffusion [129]. A low viscosity grade HPMC may be more appropriate for use in matrix systems which carry low solubility drugs and a high viscosity grade HPMC may be required in systems in which a water soluble drug is to be formulated.

The aforementioned selection of polymer grade may be considered a guide in the selection of polymeric materials for matrix delivery system development. However, it is likely that a combination of one or more viscosity grade materials may be more appropriate than the use of a single grade of material in order to achieve the necessary control over drug release.

The aim of these studies was to produce a matrix tablet that would sustain CBZ release over a 22-hour period and to elucidate the probable mechanism that controls the release of CBZ from

these systems. In addition, the system should match the *in vitro* dissolution profile of Tegretol[®] CR.

4.3.4 Fick's Second Law

Fick's second law defines mass transport such that a change in concentration occurs at a specific time at a definite location, rather than the mass diffusing across a unit area of a barrier in a unit of time [130]. Fick's second law is depicted in Equation 4.2.

$$\frac{\partial C}{\partial t} = D \frac{\partial^2 C}{\partial x^2} \quad \text{Eq. 4.2}$$

where,

t = the elapsed time;

D = the diffusion coefficient of the drug;

C = the drug concentration in the reservoir;

x = the perpendicular distance travelled by the drug.

Drug transport from the inner core of a matrix type dosage form to the external dissolution medium is facilitated by fluid penetration into the core creating areas of high diffusivity and the API can escape from the release device by diffusion from these high diffusivity 'channels' [91]. The process of diffusion is the principal mechanism that controls drug release from these systems and is more applicable for describing release of water soluble drugs where drug diffusion of the drug from the device occurs before dissolution of the polymer that makes up the device [91].

Deviations from Fick's law have been associated with the rate at which the polymer structure rearranges to accommodate solvent molecules [90]. Case-I type transport corresponds to diffusion-controlled systems where solvent mobility is much slower than the segmental relaxation rate of the relevant polymer system [90]. Diffusion of the drug occurs because of the relaxation of macromolecular chains taking the polymer from a glassy to rubbery phase. Case-I

type transport corresponds to n values ≤ 0.5 derived from the ‘power law’ as described in Section 4.3.1.

The resultant drug release profiles are inherently first-order in nature and show square root of time dependence with continuously diminishing release rates that are a consequence of an increasing diffusion distance and decreasing surface area at the penetrating diffusion front [111].

4.3.4 The Higuchi Model

One of the most common methods of determining whether diffusion is the dominant process controlling drug release is to use the square root of time relationship established by Higuchi for the characterisation of diffusional drug release from non-swelling, non-erodible polymeric slabs [116]. In addition, Higuchi derived a relationship which describes the release of a poorly water soluble drug from the single face of a tablet [115]. The amount of drug released from matrix systems in which the dissolution medium is essentially a perfect sink as defined by Higuchi [126] is shown in Equation 4.3.

$$Q = \sqrt{D(2A - C_s)C_s t} \quad \text{Eq. 4.3}$$

where,

t = the elapsed time;

Q = the amount of drug released after time t per unit exposed area;

D = the diffusivity of the drug in a homogeneous matrix media;

A = the total amount of drug present in the matrix per unit volume (the initial drug concentration);

C_s = the solubility of the drug in the matrix material.

This relationship can be modified to take into account the tortuosity and porosity of a system. For matrix systems in which drug leaches through pores and channels, by diffusion, the previously defined equation is modified to include the effective volume in which diffusion can

occur as well as the effective diffusional path length [126] and the modified equation is depicted as Equation 4.4.

$$Q = \sqrt{\frac{D\varepsilon}{\tau}(2A - \varepsilon C_s)C_s t} \quad \text{Eq. 4.4}$$

where,

t = the elapsed time;

Q = the amount of drug released after time t per unit exposed area;

D = the diffusivity of the drug in the permeating fluid;

τ = a factor representing tortuosity of the capillary system;

C_s = the solubility of the drug in the permeating fluid;

ε = the porosity of the matrix system.

Most matrix-type tablets release drug according to the square root of time kinetic model as described by Higuchi and as shown in Equation 4.4 [105]. For the regions that are a linear function of the square root of time, it has been established that diffusion dominates the overall release mechanism of drug release from such polymeric matrices [105,116,126,130].

4.3.5 An Alternative ‘first-order decay’ model

Several first-order or exponential models have been developed and used for the analysis of dissolution-release data [91,132-136]. These models have attempted to describe the release kinetics of drugs from theoretical laws.

For each formulation, the amount of the drug released at times, $T = [0,1, 2, 6, 10, 14, 22]$ were measured, resulting in the data set $Y = \{Y(t) : t \in T\}$.

A MATLAB script was written which invoked the MATLAB function `nlinfit` to fit the function $y = kt^n$ with parameters k and n to the data sets $\{(t, Y(t) : t \in T)\}$

The relevant lines of code in the script are shown in Figure 4.3

```
s = [1, .6]; %initial estimate of parameters
[S,R,J] = nlinfit(X,Y,@f,s); %optimal parameters
k = S(1);
n = S(2);
```

Figure 4.3 The MATLAB code for the ‘power law’.

The function f referred to is $f(t) = kt^n$. The function `nlinfit` uses a gradient descent method to minimise the sum of squares of the errors between the regression curve and the data points. It returns optimal values of the parameters k and n .

The coefficient of determination R^2 statistic was also determined and it was found that $R^2 \approx 0.97 - 0.99$ in each case, indicating a good fit of the regression curve to the experimental data sets.

Since $\lim_{t \rightarrow \infty} kt^n = \infty$ the model provides a good approximation for only the initial portion of the release. Rinaki *et al* are careful to point out that the value of the “power law” is confined to the first 60% of the release curve [117,132-136,192]. Therefore, an attempt was made to derive a model that is able to describe the entire release profile.

An alternative model was developed and applied to the analysis of CBZ dissolution rate data. This model is not new and can be found in [195] but the details are presented for completeness. To this end, t and M_∞ are as before and $y = M_t$ is the amount of drug released after time t . If dy is the amount of drug released in the time interval from t to $t + dt$ then it is proposed that

$$\frac{\text{amount released}}{\text{amount remaining}} \text{ per unit time is constant} \quad \text{Eq 4.5}$$

In other words, for some constant r :

$$\frac{dy}{M_{\infty} - y} / dt = r \quad \text{Eq 4.6}$$

This is equivalent to

$$\frac{dy}{dt} = r(M_{\infty} - y) \quad \text{Eq 4.7}$$

The solution of this first order ordinary differential equation with the initial condition $y(0) = 0$ is given in Equation 4.8

$$y = M_{\infty}(1 - e^{-rt}) = g(t) \quad \text{Eq 4.8}$$

This model was called the ‘first-order decay’ model for the purposes of these studies. In the case of sustained-release dosage forms, the release data follow apparent first-order kinetics.

A similar MATLAB script as shown in Figure 4.3 was written which fits this new regression curve $g(t)$ to the experimental data points, for the test formulations and stores the parameters M_{∞}, r , in addition to the R^2 statistic.

It was found in all cases that the R^2 values were in the range $\approx 0.97 - 0.99$. The advantage of this natural model over the ‘power law’ is that the parameter r gives the fraction of the amount of the drug remaining to be released per unit time and it models the entire release profile as opposed to 60% described by the ‘Power Law’ [88, 131-136]. Furthermore, the limit of the function $g(t)$ as defined in Equation 4.9 is M_{∞} and is one of the parameters obtained from the regression analysis.

$$\lim_{t \rightarrow \infty} g(t) = M_{\infty} \quad \text{Eq. 4.9}$$

A plot of the linear fit in which r is correlated to n derived from the ‘power law’ is depicted in Figure 4.4, where it is clear from the correlation matrix depicted in Figure 4.5, that there is a strong correlation between the parameters n and r . The parameter r , offers an alternative method to describe release, to the n value derived from the ‘power law’ and due to the correlation can be used to describe the mechanism of drug release throughout the entire drug release profile.

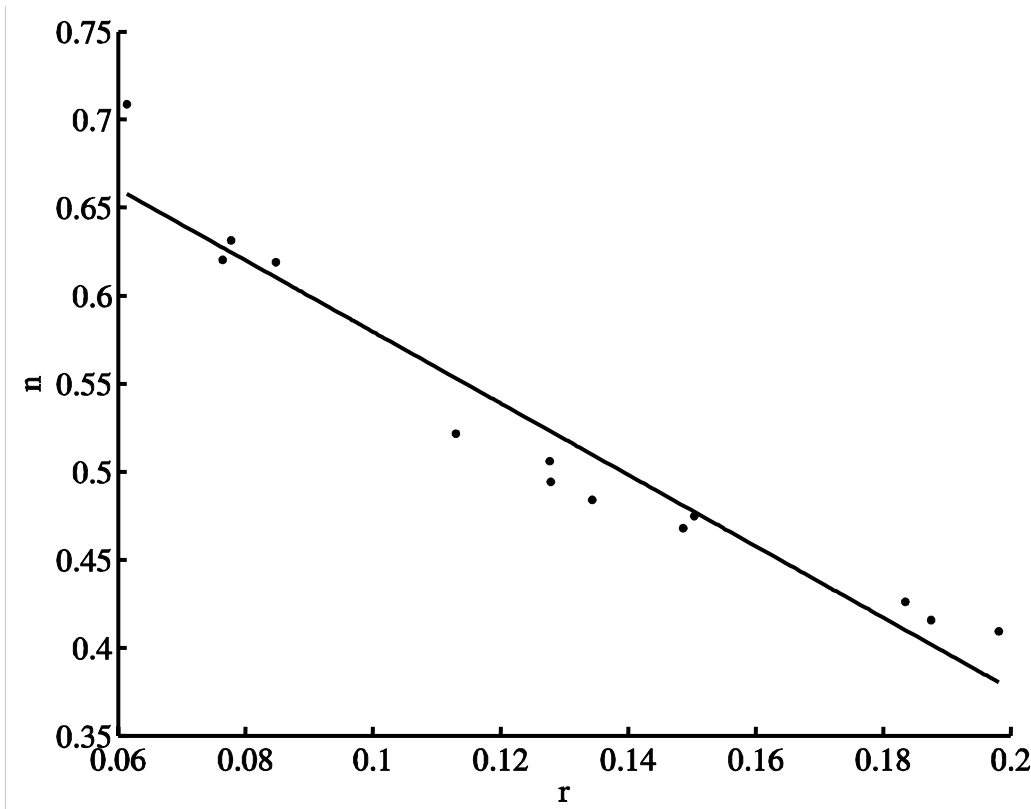


Figure 4.4 Fit depicting the linear relationship between n and r as derived from the MATLAB computational environment.

The correlation matrix between the parameters n and r is shown in Figure 4.5. It is clear from these data that n and r are strongly correlated.

$$cm = \begin{bmatrix} 1.0000 & -0.9554 \\ -0.9554 & 1.0000 \end{bmatrix}$$

Figure 4.5 Correlation matrix

The coefficients of the regression line in Figure 4.4 are $c = [-1.9926, 0.7693]$ and therefore the relationship between n and r can be described by Equation 4.10.

$$n \approx -2r + 0.7693 \quad \text{Eq. 4.10}$$

Using Equation 4.10, a value for r corresponding to $n = 0.5$ can be obtained. Solving Equation 4.10 for $n = 0.5$ yields an r value of approximately 0.35 or 13.5% remaining to be released per unit time.

Rinaki *et al* [91,131-136,192] demonstrated that the ‘power law’, in particular the n value, can describe the mechanism of drug release for a particular drug-release profile. From the above discussion it follows that the alternative first-order model, in particular the r value, can also describe drug-release profiles adequately

An n value of 0.5 represents perfect diffusion of a drug from a matrix and was used as a reference value to determine an equivalent value for the parameter, r . The inverse correlation between n and r as shown in Figure 4.3 means that increments in n represents a decrease in r . The converse is true for decreasing values of n from the ‘power law in relation to the r value from the proposed ‘first-order decay’ model. These parameters can be used as numerical indicators of the probable mechanism of drug release from these systems.

From the correlation established between n and r , an r value of 0.35 or 13.5% is indicative of a diffusion-controlled mechanism of drug release analogous to that described by the $n = 0.5$ exponent from the ‘power law’.

The relationship between n and r was further investigated by regression analysis of a quadratic relationship and the resultant plot of this fit is depicted in Figure 4.6.

Furthermore, the relationship can be mathematically described using Equation 4.11.

$$n \approx 12.3r^2 - 5.23r + 0.9 \quad \text{Eq. 4.11}$$

If we plot n against r for the whole data set of $N = 41$ profiles we obtain:

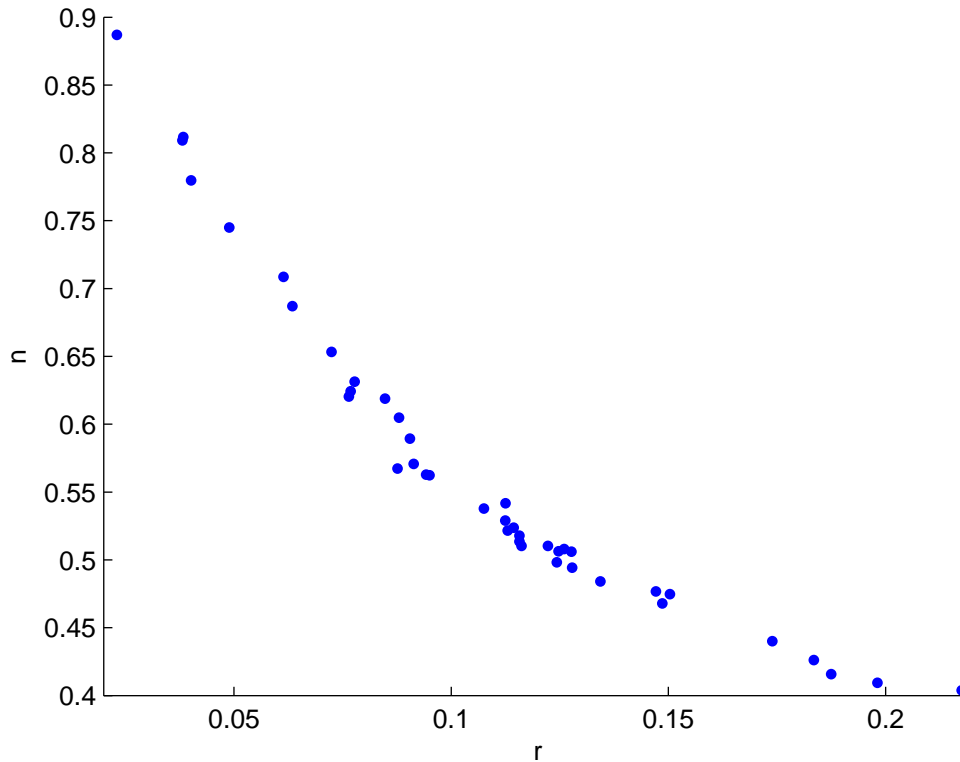


Figure 4.6 Fit depicting the quadratic relationship between n and r as derived from the MATLAB computational environment.

To discover an approximation to the relationship between n and r we can try a regression polynomial $n = p(r)$. The higher the degree the better the fit. But the higher the degree, the greater the number of inflection points and the worse the ability of the polynomial to interpolate.

We assume that the errors, $e_i = p(r_i) - n_i$ are normally distributed about zero. The variance is

then $\frac{1}{N} \sum_{i=1}^N e_i^2$. If the degree of the regression polynomial is k then an unbiased estimate of the

variance is $v = \frac{1}{N-k} \sum_{i=1}^N e_i^2$. One way of deciding on the degree of the regression polynomial is

to increase k until the ν starts to increase. The other way is to simply use common sense and expert knowledge and scientific intuition about the data. The following figures show the regression curves and their R^2 values.

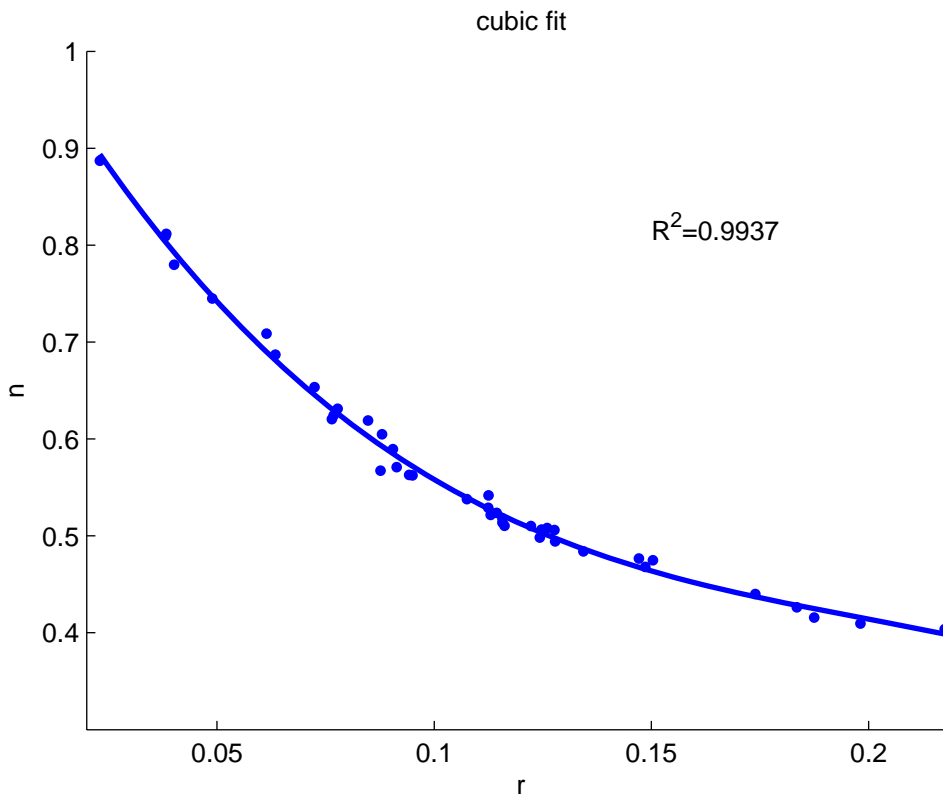


Figure 4.7 Fit depicting the cubic relationship between n and r as derived from the MATLAB computational environment.

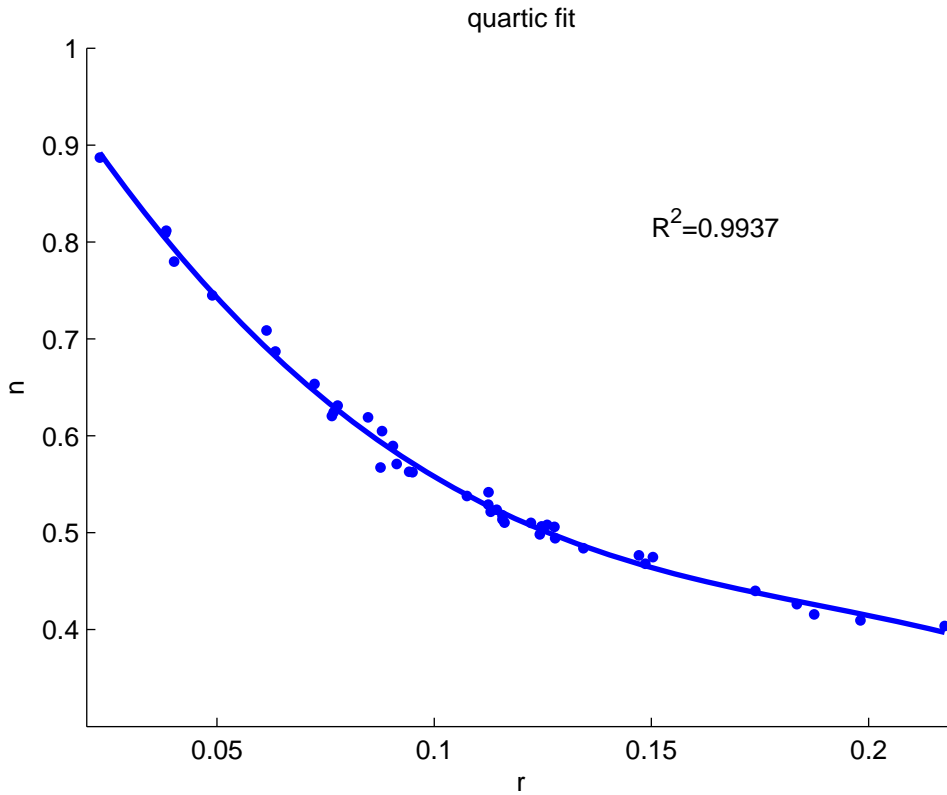


Figure 4.8 Fit depicting the quartic relationship between n and r as derived from the MATLAB computational environment.

The R^2 values and the corresponding estimates of the variance are listed:

degree 1 estimate: 0.00164113 R^2 : 0.86096727

degree 2 estimate: 0.00014643 R^2 : 0.98926734

degree 3 estimate: 0.00008870 R^2 : 0.99369303

degree 4 estimate: 0.00009068 R^2 : 0.99372244

The regression curves are:

$$r = -2.43n + 0.83$$

$$r = 14.62n^2 - 5.83n + 1$$

$$r = -59.58n^3 + 35.72n^2 - 7.99n + 1.06$$

$$r = -90.24n^4 - 16.72n^3 + 28.80n^2 - 7.55n + 1.05$$

The unbiased estimates first decrease and then starts to increase after degree 3. The R^2 value steadily improves. From degree 1 to degree 2 there is a significant improvement. From degree 2 to degree 3 there is a slight improvement at the expense of simplicity. There therefore is a case for choosing the quadratic over the cubic: in regression, simpler is better.

Using the quadratic regression curve, the n, r values are listed:

r	n
0.0500	0.7440
0.0600	0.7018
0.0700	0.6625
0.0800	0.6262
0.0900	0.5928
0.1000	0.5623
0.1100	0.5347
0.1200	0.5101
0.1300	0.4884
0.1400	0.4696
0.1500	0.4537
0.1600	0.4408
0.1700	0.4308
0.1800	0.4237
0.1900	0.4196
0.2000	0.4183

With r as the dependent variable:

n	r
0.4500	0.1527
0.4600	0.1458
0.4700	0.1398
0.4800	0.1343
0.4900	0.1292
0.5000	0.1245
0.5100	0.1200
0.5200	0.1158
0.5300	0.1118
0.5400	0.1080
0.5500	0.1043
0.5600	0.1008
0.5700	0.0974
0.5800	0.0941
0.5900	0.0909
0.6000	0.0878
0.6100	0.0847
0.6200	0.0818
0.6300	0.0789
0.6400	0.0761
0.6500	0.0734
0.6600	0.0707
0.6700	0.0680
0.6800	0.0655
0.6900	0.0629
0.7000	0.0604
0.7100	0.0580
0.7200	0.0556
0.7300	0.0532
0.7400	0.0509
0.7500	0.0486
0.7600	0.0464
0.7700	0.0442
0.7800	0.0420
0.7900	0.0398
0.8000	0.0377

We see that $n = 0.5$ corresponds to $r = 0.1245 = 12.45\%$ and so on.

4.3.6 Application of the ‘first-order decay’ Model.

The dissolution rate profiles of twelve experimental formulations and the innovator product Tegretol[®] CR were generated and fitted to the ‘power law’ and the ‘first-order decay’ model. A summary of the model parameters generated from these data in addition to the f_1 and f_2 difference and similarity factors are shown in Table 4.1.

Table 4.1 Statistical and mathematical parameters for the experimental formulations tested.

Formulation	k	n	f_1	f_2	M_∞	r
Tegretol [®] CR	18.87	0.47	-	-	77.90	0.1486
C-280503-01	25.97	0.42	20.8	48.3	87.86	0.1875
C-190603-02	14.97	0.52	10.7	63	78.43	0.1130
C-190603-03	15.66	0.49	12.5	59.9	72.88	0.1279
C-200603-01	14.95	0.51	13.9	57.5	71.87	0.1277
C-200603-02	19.25	0.43	8	62.6	67.47	0.1835
C-200603-03	21.74	0.41	5.7	72.2	71.59	0.1981
C-030703-01	13.81	0.63	14.7	52.8	114.98	0.0777
C-030703-03	8.78	0.71	17.1	51.0	102.74	0.0614
C-030703-04	13.09	0.62	9.3	62.4	101.04	0.0848
C-030703-05	19.2	0.48	6.2	71.9	85.52	0.1344
C-260303-01	7.26	0.62	45	32.1	59.22	0.0764
C-260303-02	12.00	0.47	35.6	36.6	50.41	0.1504

*Shaded values indicate dissolution profile similarity

From the data summarized in Table 4.1, it is clear, by way of example that Batch C-190603-02 and C-190603-03 exhibit pure diffusion controlled release whereas Tegretol[®] CR with an n value 0.47 and corresponding r value of 14.9% exhibits near pure diffusion controlled release.

The f_1 and f_2 values shown in Table 4.1 were calculated using Tegretol[®] CR dissolution rate data as the reference set. It is clear that the release profiles of several formulations show similarity, using the f_1 and f_2 difference and similarity factors, to the reference product Tegretol[®] CR.

The derived values for n and r were used to determine the mechanism of drug release, as defined by Peppas *et al* [122,123,128], and formulations showing similarity when comparing the f_1 and f_2 difference and similarity values are highlighted in Table 4.2. Batch C-260303-02 has the same n value of 0.47 as Tegretol[®] CR ($r=14.9\%$), and although the amount of drug released may show no similarity to the reference product, the mechanism of release may be simulating similar behavior that of Tegretol[®] CR based on the assumptions and conclusions using these mathematical parameters.

In this manner, it is possible to identify formulations that show *in vitro* mechanistic similarities through the application of statistical and mathematical modelling, to that of the reference formulation. Therefore, it is hoped that these formulations will mimic more closely the *in vivo* behaviour of Tegretol[®] CR which is in clinical use for the prevention of epileptic seizure behaviour. These mathematical models allow for a ‘honing-in’ on the formulation closest to the innovator product.

4.3.7 Comparison of the ‘Power Law’ and the ‘first-order decay’ Model

The dissolution data of Tegretol[®] CR fitted to the “power law” where the k and n values are elucidated is shown in Figure 4.9 The dissolution profile of Tegretol[®] CR fitted to the ‘first-order decay’ model where the r parameter and the total drug release parameter, M_∞ , are elucidated is shown in Figure 4.10. This is an alternative method to describe drug release over the entire drug release profile whereas the ‘power law’ only describes the first 60% of the drug release profile [117].

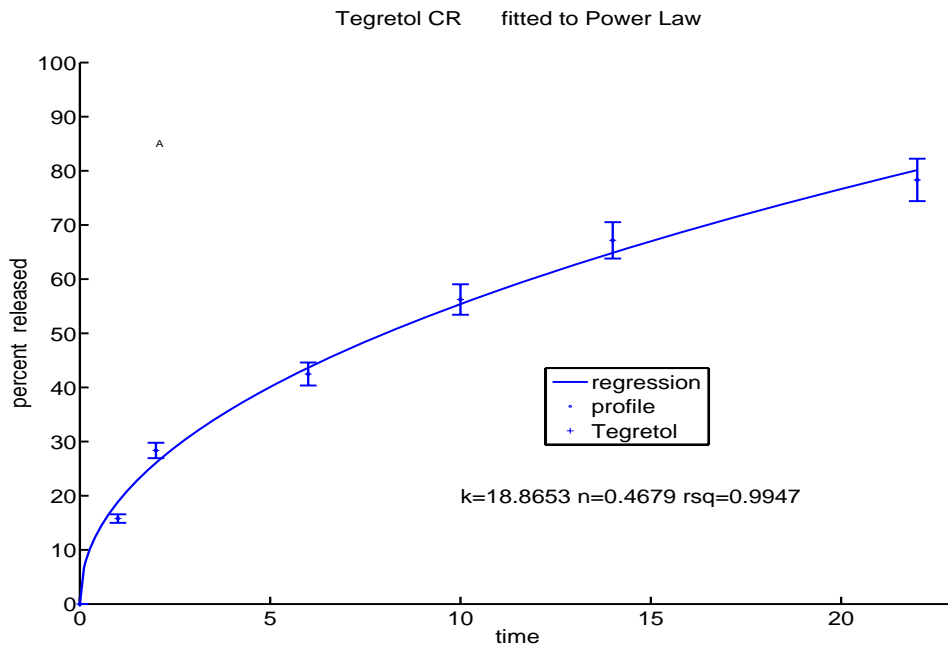


Figure 4.9 Dissolution profile of Tegretol[®] CR fitted to the ‘power law’.

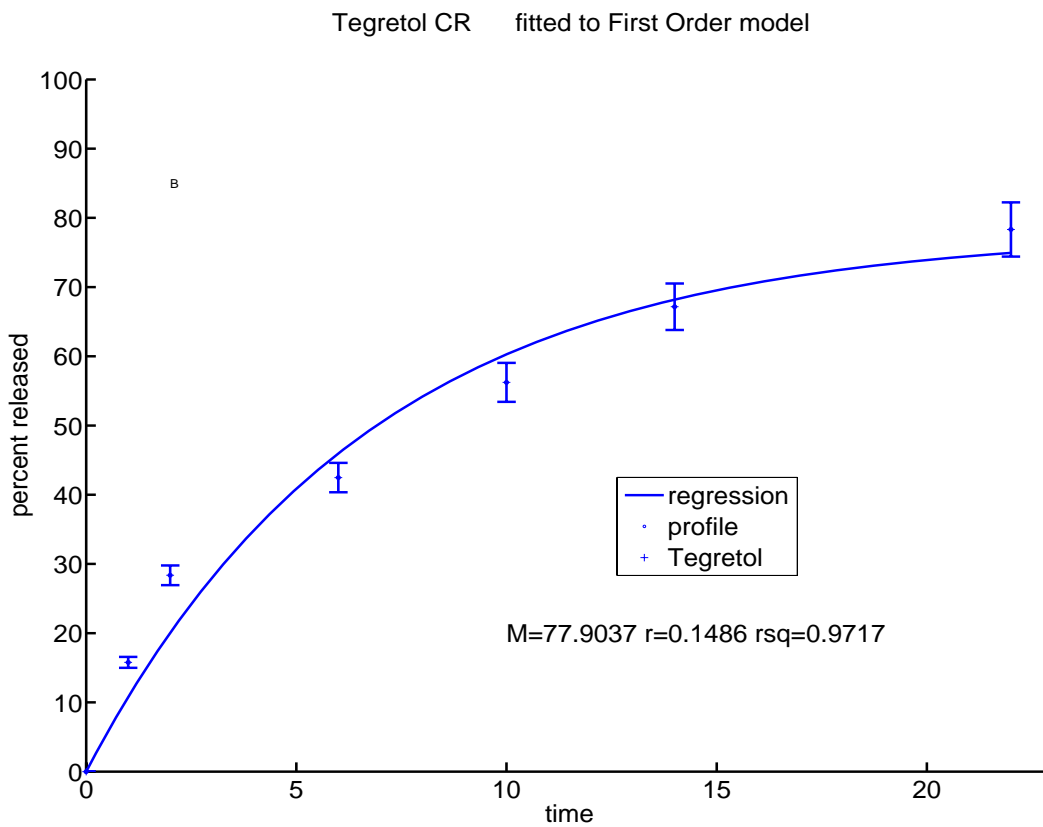


Figure 4.10 Dissolution profile of Tegretol[®] CR fitted to the ‘first order decay’ model.

Drug release from Batch C-030703-05 was similar to Tegretol[®] CR using the f_1 and f_2 difference and similarity factors. In addition the n value was similar to that of Tegretol[®] CR relative to the other formulations produced in these studies. The dissolution profile of batch C-030703-05 was fitted to both models and these data graphically depicted in Figures 4.11 and 4.12.

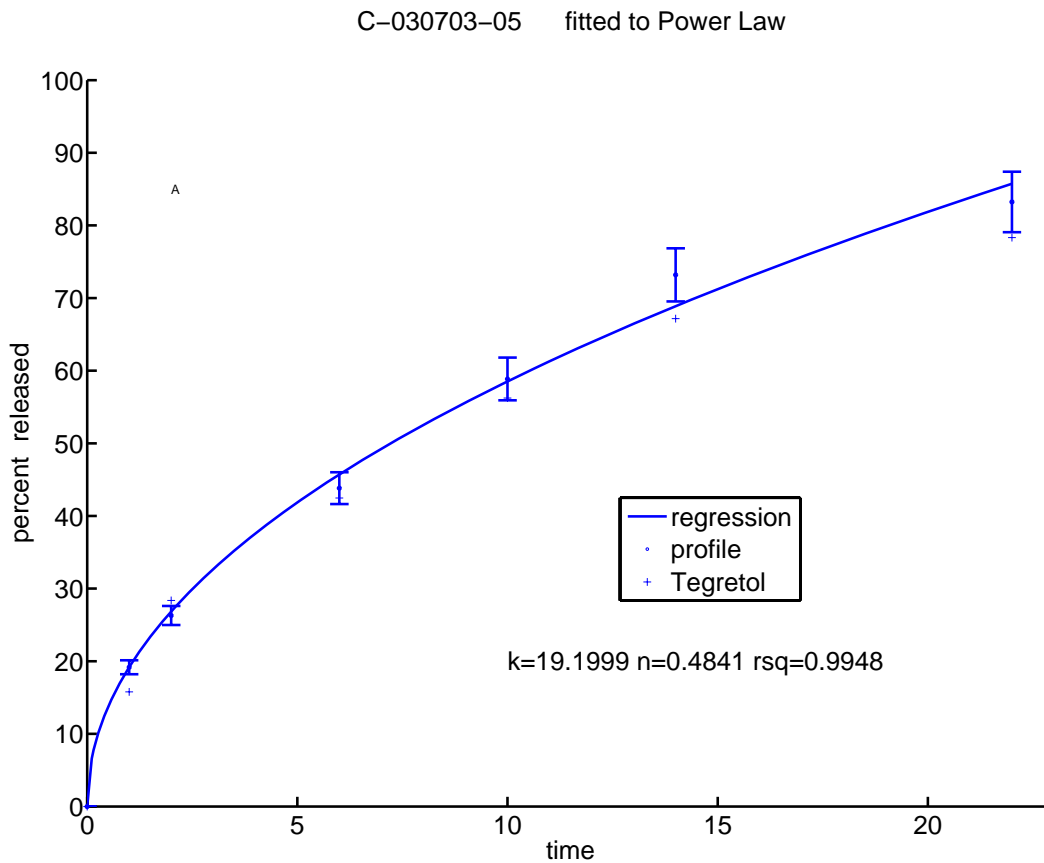


Figure 4.11 Batch C-030703-05 fitted to the 'power law'.

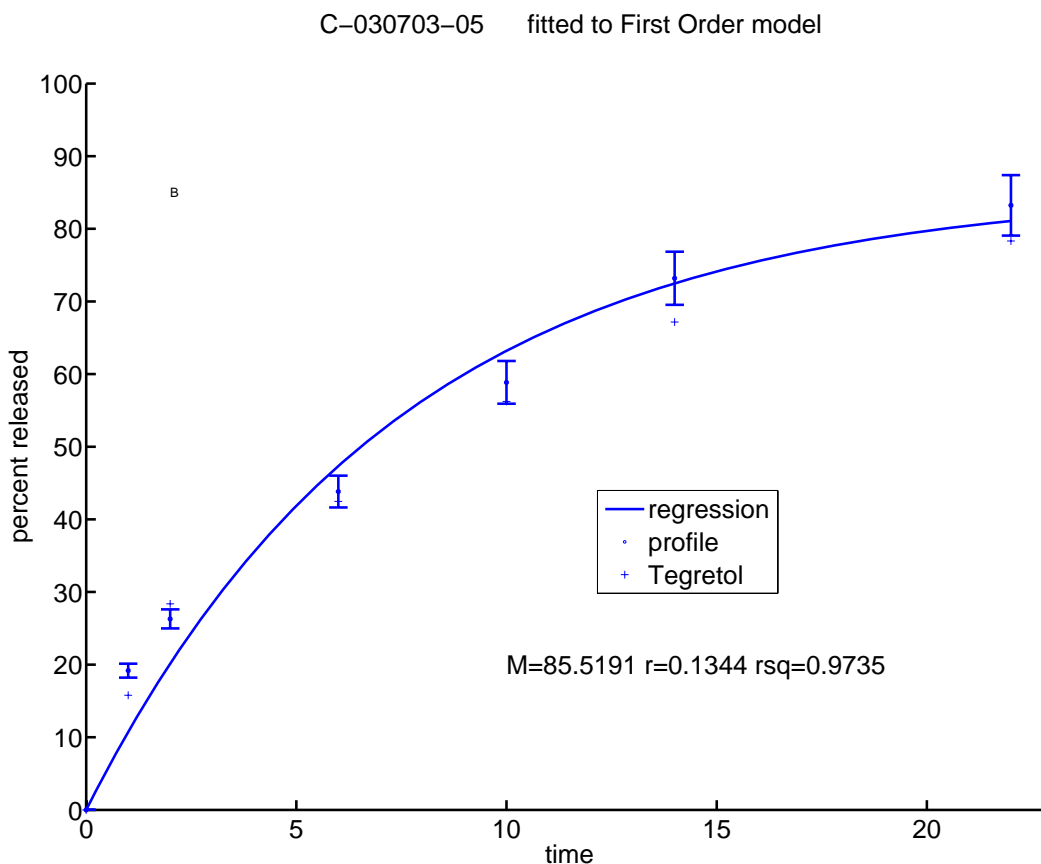


Figure 4.12 Batch C-030703-05 fitted to the ‘first-order decay’ model.

4.3.8 Elucidation of Mechanism of CBZ Release from HPMC Matrix Tablets

The results obtained from fitting experimental dissolution data to the ‘power law’ and to the ‘first-order decay’ model are listed in Table 4.2. In addition, the difference and similarity factors f_1 and f_2 were calculated for these data sets using Tegretol[®] CR as the reference formulation and these data are also summarised in Table 4.2. Formulation profiles that showed similarity to Tegretol[®] CR when comparing f_1 and f_2 difference and similarity factors, did not necessarily show similarity when their respective n exponents and r values were compared to those of Tegretol[®] CR.

The values of n and r obtained for Batches C-190603-02, C-190603-03, C-200603-01 and

C-030703-05 indicated that the most probable mechanism controlling CBZ release from these products is Fickian diffusion with square root of time dependant, drug release.

Square root of time plots are depicted in Figure 4.13 and R^2 values obtained from linear regression analysis of these plots are shown in Table 4.2. Linearity was established using the square root of time relationship and suggests that the predominant mechanism controlling drug release from these specific formulations was diffusion. For highly water-insoluble compounds such as CBZ, erosion of the polymer plays a more significant role controlling drug release from these delivery systems [103].

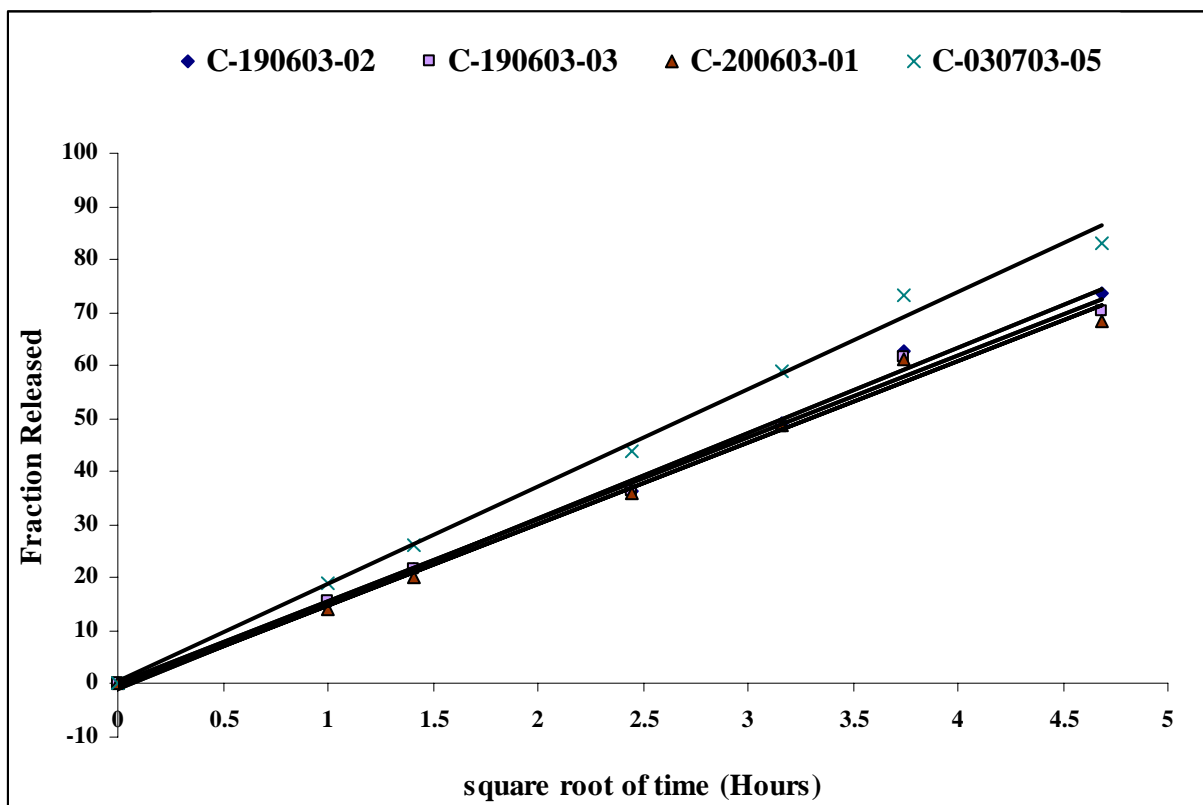


Figure 4.13 Higuchi plots for dissolution profiles of selected formulations over a 22 hour period.

Table 4.2 R² values for square root of time plots.

Formulation	R ²
C-190603-02	0.9954
C-190603-03	0.9948
C-200603-01	0.9921
C-030703-05	0.9945

Diffusion control may be time-specific and square root of time plots, were generated to assess linearity over the first six hours of the dissolution process. Linear regression plots for dissolution data Batches C-190603-02, C-190603-03, C-200603-01 and C-03070305 where the fraction released for the first six hours was assessed according to the Higuchi relationship [126] are depicted in Figure 4.9.

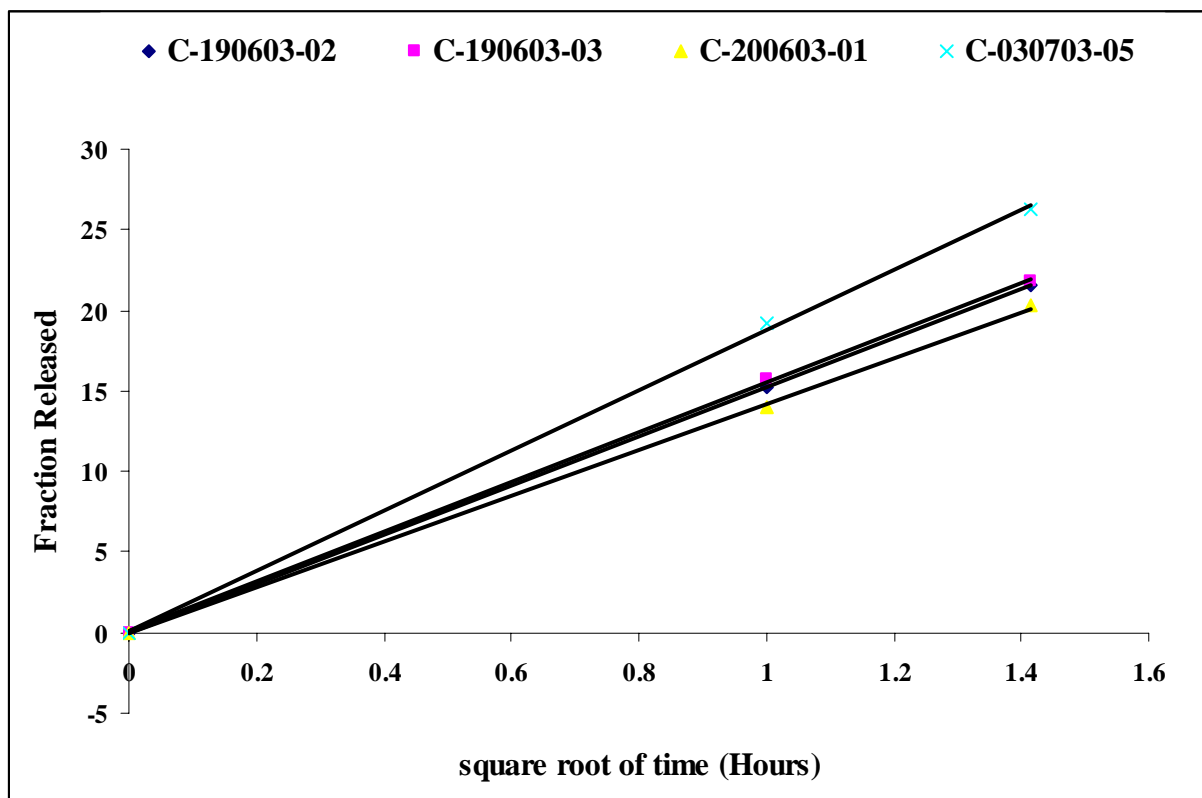


Figure 4.14 Higuchi plots for dissolution profiles of selected formulations over a 6 hour period.

The R^2 statistic for the Higuchi plots shown in Figure 4.14 are depicted in Table 4.3 and from the apparent improved linearity, it may be possible that diffusion is pre-dominant during the early stages of the drug release process from these hydrogel matrix tablets, and as time proceeds, so the control of the release from these dosage forms is affected by the erosion and dissolution of the HPMC in the matrix.

Table 4.3 R^2 values for square root of time plots for the first 6 hours.

Formulation	R^2
C-190603-02	1
C-190603-03	0.998
C-200603-01	0.995
C-030703-05	0.994

The dissolution profiles of CBZ Batches C-200603-02 and C-200603-03 also showed statistical similarity to Tegretol[®] CR but yielded n values < 0.5 which is indicative of pure Fickian diffusion-controlled release. Batches C-030703-01 and C-030703-04 yielded n values > 0.5 , which is a mathematical indicator describing anomalous, Case-II type transport which is complex and confounded by polymer erosion.

According to the mathematical parameters derived for the various formulations tested revealed that, a range of values were obtained where some formulations perform by diffusion control whereas other formulations release the API by anomalous, Case-II type transport where erosion and dissolution rate of the polymer determines the release kinetics of CBZ.

4.4 CONCLUSION

There is no single, overall mathematical model that can be used to describe all the mass transport mechanisms and chemical processes that occur in these disparate systems [90] and thus the availability of a range of mathematical modelling tools provide greater insight into drug release mechanisms from swellable hydrogel matrices.

It is evident that both models, *viz.*, the ‘power law’ and ‘first-order decay’ models fit the data reasonably well and it is not the intention of the author to suggest superiority of one model over another but to highlight that the ‘first-order decay’ model provides mechanistic information effectively over the entire release profile whereas the ‘power law’ predicts and explains drug release mechanisms for only the first 60% of the drug release profile.

The mechanisms by which drug was release from the formulations produced during these studies varied between diffusion control and anomalous transport. Matrix dissolution or matrix erosion, which is an important characteristic of swellable and erodible systems, is not considered in the Higuchi, square root of time model. Therefore mechanistic information obtained by this method should be viewed and assessed with caution [103]. Some of the formulations developed in these studies showed evidence of diffusion controlled release where square root of time plots for the first six hours and the entire dissolution time of twenty two hours showed good linearity.

Changes in the swelling kinetics of the HPMC matrices can lead to changes in drug release through two distinct mechanisms. For a drug that has not completely dissolved in the hydrated matrix, faster matrix dissolution would result in an increased drug release rate by the process of matrix erosion. Secondly, changes in the swelling can lead to changes in the diffusional path length and/or diffusional resistance of the gel layer which becomes pertinent in the case of a drug that completely dissolves at the medium penetration front and released by a diffusional mechanism where changes in the gel layer thickness can affect drug release kinetics [103].

In the cases of the formulations where the n values are suggestive of diffusion-controlled release, it may be possible that polymer erosion is slower than the drug release rate and therefore

diffusion of the API is far more significant at that stage of the release process. These effects become formulation specific since different formulations produced different mathematical parameters following assessment of their dissolution profiles using modelling techniques.

Diffusion is especially significant during the initial stages of tablet behaviour in a dissolution medium as shown in Table 4.4, since as the swelling process proceeds, the gel layer gradually becomes thicker and therefore the drug concentration gradient along the diffusional pathlength is decreased which results in progressively decreased drug release rates [103]. Furthermore, for diffusion-controlled systems, the surface area: volume ratio is a key variable in controlling drug release from HPMC matrices [116]. This becomes especially pertinent with respect to these dosage forms where polymer swelling and erosion affect the surface area: volume ratio. Surface area:volume ratios can be used to duplicate drug release profiles for tablets having different sizes, shapes and dose levels [116], where different physical configurations can be adapted to have the same surface area:volume ratios. Therefore, surface area:volume ratio dependent drug release can be identical for two differently shaped tablets with this parameter the same.

Therefore polymer erosion and dissolution may become more significant in effecting drug release where the concentration and viscosity grade of the polymer become relevant in determining gel layer thickness and degree of erosion during extended dissolution testing.

It is generally believed in the field of oral controlled drug delivery, that zero-order drug release from a delivery system reflects optimised drug delivery [102]. Synchronisation of the swelling and erosion fronts during the release process is associated with zero-order drug release and this is true for drug release from surface erodible systems in the flat sheet geometry [112]. No dosage form produced during these studies showed zero-order drug release ($n = 1$) [105] and this is to be expected given the geometry of the dosage form where due to the shape of the tablets, biconvex or disc-shaped, it is theoretically impossible for these delivery devices to release the API at a zero-order release rate [105]. This is because the system erodes which is the case with most polymers, and the surface area exposed to the dissolution fluid continually decreases, *i.e.*, the diffusion path for water-soluble drugs increases in length [105]. Although values obtained for some formulations assessed during these studies implicated diffusion as a release mechanism,

CBZ is a highly water-insoluble drug therefore, diffusion may be a predominant mechanism for specific formulations. Generally, water soluble drugs follow a diffusion-controlled mechanism of drug release from polymeric matrices. A value $n = 1$ indicates zero-order drug release from a planar surface and not for spheres or cylinders [115]. Thus values of $n = 1$ for the matrix devices assessed during these studies merely emphasize that release from these dosage forms is not Fickian controlled and implies large contributions to drug release kinetics by erosion of the polymer [115].

For the maintenance of zero-order controlled-release, tablet matrices should be as near spherical as possible to produce minimum drug release rates. A linear relationship between release rate and surface area exists where drug release kinetics from hydrogel matrices is controlled by the releasing area increase produced by the swelling phenomenon [104,121].

Mathematical modelling of controlled drug delivery systems can yield information about the mechanisms involved in the release of therapeutic agents from sustained release systems [90]. Furthermore, modelling can provide insight into the effects of varying several dosage form parameters such as geometry or composition, on drug release kinetics. In addition, modelling can be used as a tool for the optimisation of existing drug delivery systems and for the development of more appropriate novel therapeutic drug delivery systems in the future [90].

CHAPTER FIVE
FORMULATION DEVELOPMENT OF SUSTAINED RELEASE CBZ MATRIX
TABLETS

5.1 Rationale for the Sustained Delivery of CBZ

The advantages of a controlled release dosage form over immediate release products includes improved patient compliance due to a reduced dosing frequency, a decreased incidence and/or intensity of side effects, a greater selectivity of pharmacological activity with a prolonged and constant therapeutic effect, in addition to improved cost-effectiveness [101].

CBZ is one of the most important drugs used for the treatment of psychomotor epilepsy and trigeminal neuralgia [102,107]. Physiologically, it displays slow and irregular gastro-intestinal absorption, due to its low water solubility and has a short biological half-life when used for chronic therapy as a consequence of the auto-inductive effects of CBZ on liver enzymes [64,93,97,102,107]. An initial half-life of approximately twenty four to forty hours is reduced to twelve hours with chronic monotherapy and eight hours in patients taking other enzyme-inducing drugs [102,107].

Consequently, CBZ is dosed frequently as three or four doses per day, which reduces patient compliance as well as promoting plasma level fluctuations and therefore, concentration-related side-effects may occur [93,107]. In addition CBZ has a narrow therapeutic window and large diurnal variations in the plasma concentration of CBZ may lead to several side-effects such as headache, sedation, diplopia, blurred vision amongst others [93].

A more stable serum/plasma concentration of CBZ can be achieved through the use of slow-release preparations such as polymeric matrix tablets [102] where the ideal use of such dosage forms would decrease plasma concentration fluctuations through the constant and controlled release of CBZ, thereby reducing dose-related side-effects and improving patient compliance [93].

5.2 Formulation Development

5.2.1 Tablet Manufacture

Tablets are solid oral dosage forms that are a most popular and convenient means of drug delivery. The technology and processes involved in the tableting industry have evolved and advanced to optimise large-scale production in addition to drug delivery techniques. Two methods of tablet manufacture were used during these studies, *viz.*, wet-granulation (WG) and direct compression (DC) methods of manufacture.

5.2.2 Wet Granulation

WG is a well-known and widely used manufacturing technique used for the preparation of powders for the purposes of tablet production. Most powders do not flow readily and for the purposes of tableting, powders are converted into agglomerates or granules that are free-flowing and can be compressed into tablets. The relevant powders, drug and excipients, are weighed and screened through an appropriately sized mesh. The sieved powder bulk is then blended and granulated with the aid of a suitable granulating fluid. The choice of granulating fluid will depend on the properties of the drug candidate to be tableted in addition to the desired release properties of the API from the tablet. The wet mass is then screened through an appropriate mesh and the resultant granules are dried under specific conditions. Dried granules may be re-screened and then further manipulated by the addition of additional drug, excipients and a lubricant which are again blended to promote uniformity of distribution of the API. The blend is then compressed into tablets. A schematic diagram of the WG process used for tablet manufacture, illustrating the processes and variables used in these studies is depicted in Figure 5.1.

Some of the factors and process variables that affect tablet manufacture in addition to dosage form performance include the amount and method of binder added during granulation, the method of granulation, *e.g.*, high or low shear, granulate size distribution and compression force [137].

Changes or modifications in polymer type and excipients and manufacturing processes can modify and obtain specific drug release rates [113]. The presence of low percent w/w quantities of HPMC and the high water mobility during WG allows for greater chain mobility, swelling and spreading which results in slower tablet erosion and thereby increasing the lag-time of drug release from time-delayed HPMC dosage forms [106]. This may contribute to low drug release rates from controlled release dosage forms and becomes important when using a highly water-insoluble drug such as CBZ in such systems.

5.3 Experimental

5.3.1 Overview

A wet granulation formulation was adopted as a prototype formulation and further modified to investigate the effects of selected formulation variables on CBZ release from these products [138].

The performance of the manufactured matrix formulations and in relation to formulation modifications was assessed by means of *in vitro* dissolution testing and by assessment of the physical properties of the tablets produced. Dissolution studies were conducted as described in Section 3.8.4 and the CBZ release profiles and data generated were assessed statistically as described in Section 3.9 to elucidate the performance of the batches relative to each other and relative to the innovator product Tegreto[®] CR (Novartis, Quebec, Canada).

Furthermore, two particle size grades of CBZ were assessed for their effect on dosage form performance, either alone or in varying proportions and combinations. Other formulation variables were assessed for their impact on dosage form performance as discussed in Chapter 6 where formulations were assessed and effectively optimised to produce tablets with specific drug release and physical characteristics.

5.3.2 Materials

5.3.2.1 CBZ

Two particle size grades of CBZ were donated by Noveon Pharma (Raubling, Germany). The fine grade particle size FG (approximately 7 μm tested diameter) and course grade particle size CG (approximately 13 μm tested diameter) were used without further modification.

Properties of the drug substance that influence the dissolution rate and its subsequent bioavailability include its solubility, particle size distribution as well as crystalline state such as polymorphism, state of hydration or complexation [118]. These factors were considered and some were investigated for their impact on drug release rates.

5.3.2.2 Excipients

All materials used in this study are Generally Regarded as Safe (GRAS) listed, and appear in the Food and Drug Administration Inactive Ingredients Guide for inclusion in oral formulations [139].

5.3.2.3 Hydroxypropylmethylcellulose (HPMC)

Cellulose derivatives are widely used in the formulation of hydrogel matrices for controlled drug delivery [104]. HPMC is a non-ionic, water-soluble, semi-synthetic ether derivative of cellulose made by The Dow Chemical Company (Midland, MI, USA) and marketed as Methocel[®] by Colorcon (Orpington, Kent, UK) [101,112]. It is soluble in water and forms a viscous colloidal solution on hydration [34]. HPMC is frequently used due to its low toxicity, ease of use and availability, minimal influence of processing variables on drug release, its ease of compression and its capability to accommodate high levels of drug [103-105,112]. Some additional advantages, especially pertinent to this study, is their innate flexibility and ability to achieve specific drug release profiles, their application in matrix tablets which is fairly extensive, in addition to their cost-effectiveness and broad FDA acceptance [118].

The concentration and viscosity grade of HPMC are the most often variables that are changed to regulate drug release from dosage forms and these variables may affect the kinetics and mechanisms of drug release [103-104]. Hydrogel polymers such as HPMC form the basis for implantable, transdermal and oral-controlled release systems [115], where it could be used either as a binder, a film-coating agent or as a sustained release matrix-forming excipient [139].

It is available in a variety of different grades, depending on the degree of substitution on the polymer chain backbone and the average molecular weight of the polymer [139]. One of the most important characteristics of HPMC is its high swellability on contact with aqueous media, which has a considerable effect on the release kinetics of drugs in HPMC matrices [114].

The manufacturing process has a significant impact in determining the dissolution characteristics of HPMC matrix tablets [137]. HPMC displays good compression characteristics and has adequate swelling properties and can accommodate a high level of drug loading [140]. In addition, HPMC is unaltered by shear forces that are applied in the granulation step where the presence of water mobility during wet granulation at low concentrations of HPMC allows greater chain mobility/swelling and spreading [106].

High viscosity grades may be used to retard the release of drugs from matrices at levels of 10% w/w to 80% w/w in tablets and capsules [139]. It appears likely that different combinations of polymers will be applicable to different drugs in order to match the rate of the advancement of swelling into the glassy core and the rate of attrition of the polymer [105]. The drug candidate used in this study was highly water insoluble and both high and low viscosity grades of HPMC were assessed for their effects on drug release rates. For hydrophilic matrix tablets that contain water-soluble, swellable polymers such as HPMC, the release kinetics are described by a combination of drug diffusion and polymer dissolution or surface erosion as discussed in Section 4.2 [116].

Some approaches to overcoming the dissolution limitation of poorly water-soluble drugs is the formulation of the drug in an amorphous form [141]. HPMC is known to promote the stability of this form through the inhibition of the transformation of CBZ to CBZ dihydrate in the gel layer

and participates in the crystallisation process to induce amorphism, which may aid the rate and extent of drug dissolution [142,143].

For a highly water-insoluble drug such as CBZ, the lower viscosity grades of HPMC become more suitable for controlling drug release since surface erosion of the polymer may be the predominant factor, controlling drug release from these systems [116]. Polymer grade, proportion and polymer grade combinations were assessed and optimised to effectively deliver CBZ from a matrix formulation produced by either wet granulation or direct compression. Methocel[®] K4M and K100M were donated by Colorcon Limited (Orpington, Kent, UK) and used in varying proportions in an attempt to sustain drug release.

In addition, the addition of a partial amount of HPMC intergranularly in the dry blend step of the wet granulation process has been shown to desensitize drug release profiles to the effects of the manufacturing process [137]. This was the case for these formulations where additional HPMC (grade specific) was added during the blending step of dried granules with additional excipients.

5.3.2.4 Dibasic Calcium Phosphate (DCP)

DCP is available in an anhydrous or dihydrate form and both forms are used as an excipient and a source of calcium in nutritional supplements [139]. Two particle-size grades of both anhydrous and dihydrate DCP are used in the pharmaceutical industry, *i.e.*, a milled version and unmilled or coarse grade material which is typically used in direct-compression formulations [34]. DCP imparts tensile strength to tablets due to its abrasive properties and a lubricant is usually required for tableting, *e.g.*, 1% magnesium stearate or 1% sodium stearyl fumarate [139]. In these studies, Emcompress[®] (Edward Mendell Co. Inc, NY, USA) was used as the DCP source. DCP has been shown to yield lower drug release, and to a lesser extent, the soluble excipients within a formulation due to its water insolubility [118].

5.3.2.5 Microcrystalline Cellulose (MCC)

MCC is widely and commonly used in pharmaceutical manufacturing [144], primarily as a binder/diluent in oral tablet formulations where it is used in both wet-granulation and direct compression formulations [139,144]. In addition MCC has some lubricant and disintegrant properties that make it useful in tableting [139]. It is available in different particle size and moisture grades [144], where larger particle size grades afford better flow properties to formulations and low moisture grades are used with moisture-sensitive materials [139]. Emcocel[®] 90M (Edward Mendell Co. Inc, NY, USA) was used for the formulations in these studies. This grade of MCC has a mean particle size of 91 μm , and moisture content of less than 5.0%. MCC is a hygroscopic material and should be stored appropriately [139].

5.3.2.6 Lactose

Lactose is widely used as a filler or diluent in tablets with various grades that have different physical properties such as particle size distribution and flow characteristics being available commercially [139]. The grade of lactose chosen for inclusion in formulations is dependant on the type of dosage form to be produced, where direct-compression grades of lactose are more fluid and compressible than crystalline or powdered lactose. DC grades of lactose are generally comprised of spray-dried lactose that contains specially prepared pure α -lactose monohydrate along with a small amount of amorphous lactose [139]. Lactose is water soluble [139] and also adds to the wetting capabilities of matrix tablets which become especially useful for application to insoluble drug candidates such as CBZ. Spray dried lactose monohydrate (The Lactose Company, Wellington, New Zealand) was used in these studies. The combination of HPMC and lactose may be used to control drug delivery from extended release formulations [106].

5.3.2.7 Sodium Lauryl Sulphate (SLS)

Surfactants are commonly used in pharmaceutical dosage forms as wetting agents [146]. SLS is an anionic and water-soluble surface active lubricant [139,145]. It can be used effectively as a wetting agent in both alkaline and acidic conditions [139]. SLS is stable under normal storage

conditions but undergoes hydrolysis to lauryl alcohol and sodium bisulphate at pH values of ≤ 2.5 [139]. SLS has been used in quantities of 1% w/w for its surface tension lowering capability and to improve dissolution of CBZ [145]. When used in formulations in which magnesium stearate is included, in the solid state at a ratio of 1:5, SLS can suppress any retardation of *in vitro* dissolution caused by prolonged mixing of magnesium stearate [145]. SLS has been shown to improve the water solubility of CBZ even at a concentration of 0.1% w/w [146], which is considerably below the critical micelle concentration (CMC) of SLS [146]. Surfactants can also modify the rate of crystal form transformation and the rate of growth of the stable crystal form as well as the crystal habit and therefore affect stability and bioavailability of CBZ [146]. SLS was purchased from Sigma Aldrich (Johannesburg, Gauteng, South Africa).

5.3.2.8 Magnesium Stearate (Mg stearate)

Mg stearate is widely used as a lubricant in tablet manufacture for pharmaceutical formulations [139]. Mg stearate is hydrophobic and when used in quantities $> 5\%$ w/w may retard drug release from solid dosage forms [139]. Blending times with Mg stearate have to be monitored and controlled when using Mg stearate as this material when over-blended results in a decrease in tablet dissolution rates and crushing strength of tablets, as well as increased tablet friability [139]. During these studies, Mg stearate was used at concentrations of $\leq 1\%$ w/w. Mg stearate was donated by Aspen-Pharmacare (Port Elizabeth, Eastern Cape Province, South Africa).

5.3.2.9 Polyvinyl Alcohol (PVA)

PVA is a water-soluble synthetic polymer used in sustained-release formulations for oral administration [139]. It is useful for application as a granulating fluid, for both hydrophilic and hydrophobic drugs despite their high water content [147]. Solutions of 2% w/v were used to granulate powder blends in these studies, where it was intended that the wetting potential of PVA, due to its solubility, would enhance fluid penetration into the dosage form and thus enhance drug release from the tablet. PVA was purchased from Sigma Aldrich (Johannesburg, Gauteng, South Africa).

5.3.3 Manufacturing Methods

Matrix tablets were manufactured using the formula listed in Table 5.1 and the WG method outlined in section 3.6.3.2. Tablets were manufactured using the principles of Good Manufacturing Practice (GMP) with the maintenance of appropriate documentation of any observations and irregular events that were encountered during the manufacturing process, *viz.*, granulation, blending and compaction. The relevant documentation for selected batches of tablets, manufactured both by WG and by DC are shown in Appendix II. A summary of the pertinent characteristics of all batches produced for these studies is shown in Appendix II.

Table 5.1 Wet Granulation Tablet Formulations

1. Granules		C-211202-01	C-211202-02	C-211202-03	C-211202-04
		% w/w	% w/w	% w/w	% w/w
	Active	50	50	50	50
	Methocel®	10	10	10	10
	K4M				
	Emcocel® 90M	20	20	20	20
	Emcompress®	12	12	12	12
	Lactose	8	8	8	8
		100	100	100	100
2. Polyvinyl alcohol	≈ 100 mL per				
(2% w/v)	1000g (10%)				
3. Final Formulation					
	Active	16	16	32	32
	Granules	67	67	35	35
	Methocel®	-	9	-	15
	K100M				
	Methocel®	9	-	15	-
	K4M				
	Emcocel® 90M	4	4	8	8
	Emcompress®	4	4	10	10
	SLS	1	1	1	1
4. Mg stearate		1	1	1	1
	TOTAL	102	102	102	102

5.3.3.1 Manufacturing Procedure

A schematic diagram of the manufacturing procedure used to manufacture tablets, is depicted in Fig 5.1. All powders in section 1 (Table 5.1), were separately weighed, screened through a size 20 mesh and granulated with water or PVA solution (2, Table 5.1) using approximately 100mL fluid per 1000g of powder. A Kenwood[®] planetary mixer (Kenwood Limited, Havant, UK) was used for blending and the granulating fluid was introduced using a Masterflex Easyload peristaltic pump (Cole-Palmer Instrument Company, Vernon Hill, IL, USA) at a constant rate of 1mL/minute. The wetted powder mass was then screened through a size 10 sieve using an oscillating granulator (Erweka, Heusenstamm, Germany) set at 50 r.p.m.

The resultant granules were dried in an oven at 60°C (Gallenkamp, Loughborough, UK) for twelve hours. The dry granule mass was re-screened through a size 10 mesh and the granule mass was recorded. The final formulation composition, granules and excipients (3, Table 5.1) were then individually weighed, screened through a size 20 mesh and blended in a 1 kg capacity cube blender (Erweka, Heusenstamm, Germany) set at a horizontal angle, and rotated for twenty minutes at 100 r.p.m.

The magnesium stearate (4, Table 5.1) was then weighed, sieved through a size 44 mesh and added to the blend and blending was continued for a further three minutes. The blend was compressed into tablets on a Manesty B3B Rotary Press (Manesty, Liverpool, UK), tooled with two sets of biconcave punches to a target weight of between 700 and 850mg and a target hardness of between 120 and 150 Newtons (12 – 15 Kp). Tablets were dedusted using a vacuum through a sieve, and stored away from light until analysed.

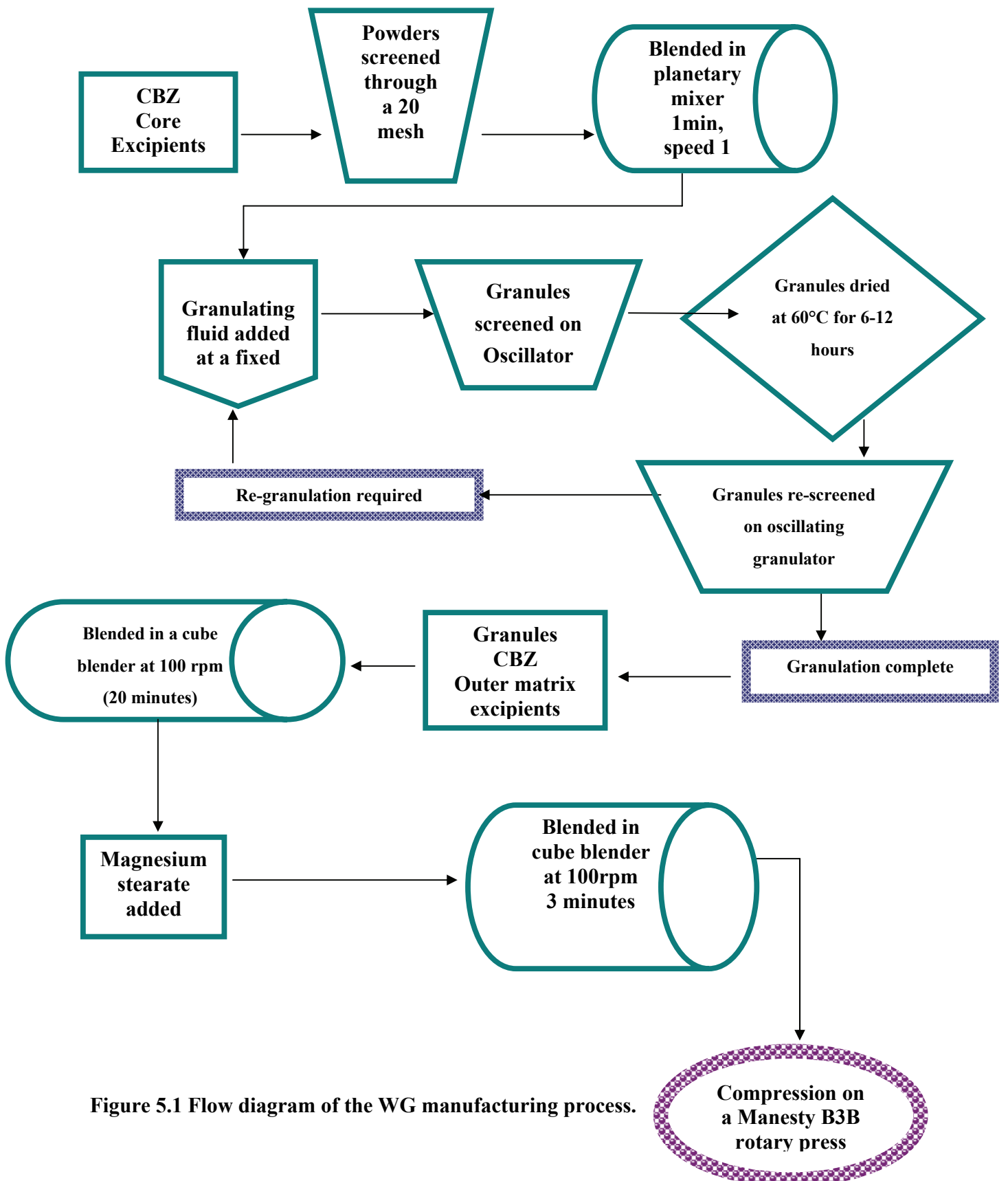


Figure 5.1 Flow diagram of the WG manufacturing process.

5.3.3.2 Effect of polymer grade on CBZ Dissolution Rate.

To assess the effect of the polymer grade, *viz.*, Methocel[®] K4M and K100M, on drug release rate from these tablets, four formulations were developed for tablet production. The core granulate of the formulation (1, Table 5.1) remained the same and adjustments were made to the outer matrix composition (3, Table 5.1) to produce four different products where the HPMC and granule composition was varied for these studies.

Two formulations containing 15.7% w/w and 18.5% w/w Methocel[®] K4M and two formulations containing 9% w/w and 15% w/w Methocel[®] K100M were produced. Formulations with Methocel[®] K100M also contained an additional 6.7% w/w and 3.5% w/w Methocel[®] K4M from the due to the K4M present in the granules (1, Table 5.1) within the formulation. Therefore, formulations with the same polymer were compared to each other to determine the effects of HPMC content and further compared to the other formulations with a different grade of polymer to assess the effects of both quantity and type of polymer on drug release rates.

The composition of the test formulations are listed in Table 5.2 and the resultant dissolution profiles were compared using the f_1 and f_2 difference and similarity factors [81].

Table 5.2 Formulation composition for four batches containing CBZ.

	Batch C-211202-01	Batch C-211202-02	Batch C-211202-03	Batch C-211202-04
	% (w/w)	% (w/w)	% (w/w)	% (w/w)
CBZ (coarse grade)	16	16	32	32
core granules	67	67	35	35
Methocel [®] K4M	9	-	15	-
Methocel [®] K100M	-	9	-	15
Emcocel [®] 90M	4	4	8	8
Emcompress [®]	4	4	10	10
SLS	1	1	1	1
Mg stearate	1	1	1	1
<hr/>				
% K4M	15.7	6.7	18.5	3.5
% K100M	-	9	-	15
Total HPMC %	15.7	15.7	18.5	18.5

These four formulations formed the empirical basis for the selection of components for all subsequent formulations where all further formulations developed were adapted predominantly from the compositions of Batch # C-211202-02.

5.4 Results and Discussion

5.4.1 Comparison of Dissolution Rates of the Four Formulations

Batches C-211202-01 and C-211202-02 were manufactured using the procedure described in Section 3.6.3.2 and were the same composition except that in batch C-211202-02, Methocel[®] K100M (9% w/w) was substituted for Methocel[®] K4M. Similarly, batches C-211202-03 and C-211202-02 differed in that batch C-211202-04 contained 15% w/w Methocel[®] K100M whereas batch C-211202-03 contained 18.5% w/w Methocel[®] K4M only.

Following dissolution testing using the method described in Section 3.8.4, the difference and similarity factors [81] were calculated and the resultant f_1 and f_2 values are listed in Table 5.3 and the dissolution profiles are depicted in Figure 5.2. Batches C-211202-01 and C-211202-04 were found to be different and this may be attributed to the fact that batch C-211202-04 contained 15% w/w HPMC K100M whereas C-211202-01 contained K4M which is the lower grade HPMC. It is evident that batches C-211202-01 and C-211202-02 are similar as are batches C-211202-01 and C-211202-03 as well as batches C-211202-02 and C-211202-03.

Table 5.3 Statistical comparisons of the four formulations

Formulation	f_1	f_2
C-211202-01 vs C-211202-02	4.5	81.3
C-211202-01 vs C-211202-03	10.6	67.7
C-211202-01 vs C-211202-04	27.4	47.4
C-211202-03 vs C-211202-04	18.8	57.7
C-211202-02 vs C-211202-03	7.5	74.0
C-211202-02 vs C-211202-04	24.9	50.0
Tegretol [®] CR vs C-211202-01	18.8	50.3

*Shaded areas represent out-of-specification results

The release rate profiles of the four test formulations. The slowest release was recorded from the formulation with the highest proportion of HPMC and also containing Methocel® K100M. This result for Batch C-211202-04, is expected, since Methocel® K100M is the higher viscosity grade and in combination with a higher quantity of HPMC, forms a thicker gel layer after hydration, which is more effective in prolonging drug release from these products.

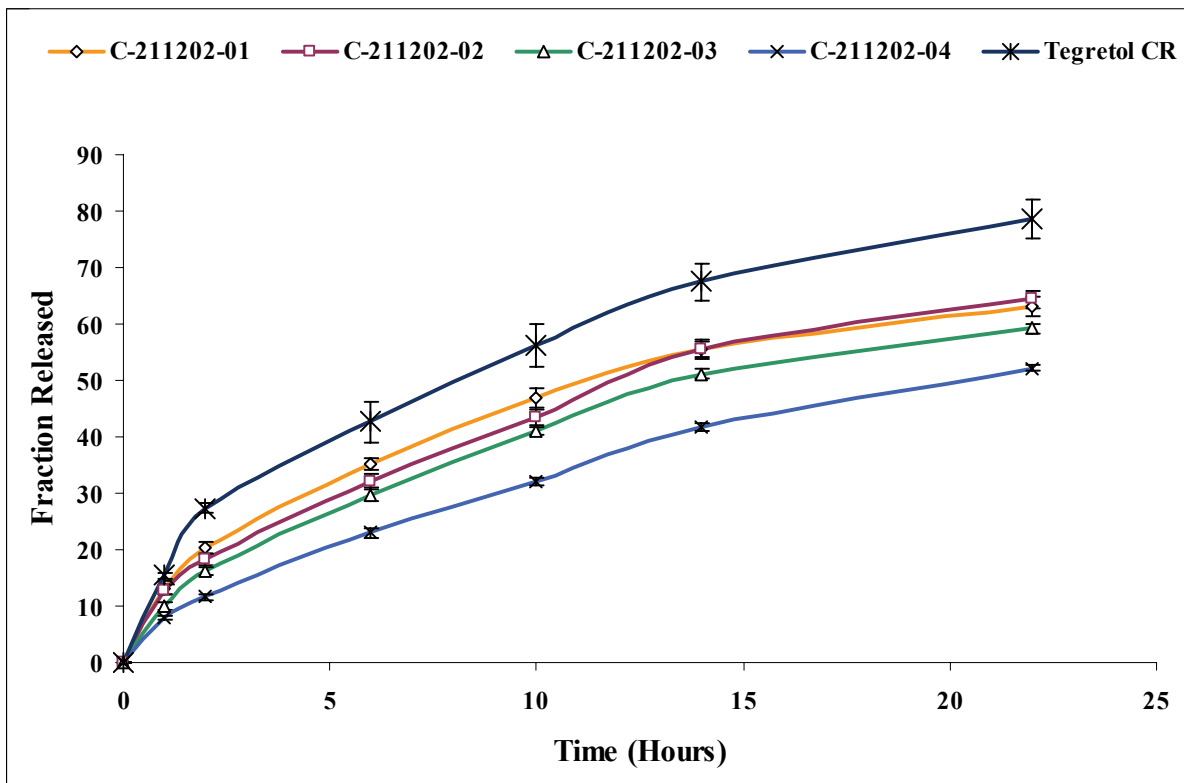


Figure 5.2 Dissolution profiles of CBZ from batches C-211202-01 (15.7% w/w Methocel® K4M), C-211202-02 (6.7 Methocel® K4M; 9% w/w Methocel® K100M), C-211202-03 (18.5% w/w Methocel® K4M) and C-211202-04 (3.5 % w/w Methocel® K4M; 15% w/w Methocel® K100M)).

All formulations contained Methocel® K4M within the granule component (1, Table 5.1) of the final formulation, therefore drug release behaviour from batches C-211202-02 and C-211202-04 may be attributed to the combined effects of both grades of HPMC. Batches C-211202-02 and C-211202-04 contain Methocel® K100M and K4M. It becomes apparent that the formulation with a combination of the two different grades, viz., Batches C-211202-01 and C-211202-02 displayed a higher drug release than the formulations Batches C-211202-03 and C-211202-04.

This suggests within certain limits that the concentration of the polymer content plays a more significant role in drug release rather than the actual viscosity grade of HPMC that is used.

HPMC polymers are usually used as extended release-matrix forming agents at concentrations of between 15 and 35% w/w [139]. The formulations with greater percent of drug release had a total polymer concentration of 15.7% w/w whereas the batches showing lower drug release contained 18.5% w/w of the polymer. This is true when using HPMC polymers since a higher polymer concentration or the use of a higher viscosity grade polymer would be more effective to delay drug release from matrix formulations.

It has been reported that the use of an anionic surfactant, such as SLS, may increase the viscosity of HPMC and hence decrease the release rate of a drug from HPMC matrix delivery systems [139]. Therefore, the additive effects of surfactant and higher HPMC concentration may contribute to effective retardation of drug release whereas the lower concentrations, irrespective of the viscosity grade, show more rapid and complete drug release. These data sets were used to assist in selection of suitable composition for future formulations.

The total apparent drug released from these test formulations was in the region of approximately 60%. In order to determine whether this amount released was a function of drug loading, a mass balance analysis was performed. The result of the mass balance analysis are shown in Table 5.4 and reveal that the apparent amount of drug released was low as a result of dosage form performance and not due to a low content of CBZ within the tablets.

Table 5.4 Mass balance analysis (n=6)

Batch #	% Recovered (Mean ± SD) n=6	% RSD
C-211202-01	89.46 ± 5.68	6.35
C-211202-02	93.99 ± 4.08	4.34
C-211202-03	83.36 ± 6.04	7.25
C-211202-04	87.35 ± 6.17	7.06

The particle size grade of CBZ used in these studies was the coarse grade and in the direct compression adaptation of the wet granulation formulation, a fine grade particle size was obtained for tableting purposes as particle size was viewed as a causative factor, since CBZ is a highly water insoluble drug and this formed part of subsequent experiments to produce a dosage form that matched the dissolution rate of Tegretol[®] CR (Novartis, Quebec, Canada). A smaller particle size of CBZ would improve the surface area available for CBZ diffusion out of the tablets and thus improve the rate of solution of CBZ during *in vitro* analysis.

Subsequently, a direct compression method of manufacture was chosen and Batch C-211202-02 served as a reference formulation that was adapted for future formulation development.

5.5 CONCLUSION

The formulations produced in this section of the study did not match the release profile of Tegretol[®] CR. The CG was used in these formulations and CBZ is highly water insoluble, therefore particle size may be a factor contributing to lower drug release. In addition these tablets were produced by the WG process where PVA was used as the granulating fluid and this aids binding during granulation which aids prolonged drug release.

Furthermore, these tablets were still intact at the end of dissolution testing and residual content assay as shown in Section 3.7.1 confirmed that there was undissolved drug present within the remaining matrix skeleton at the end of the dissolution period.

In addition, HPMC effectively functions to retard drug release. The combination of all these factors may serve to explain the low drug release rate from the four formulations tested. The DC method of manufacture was considered for further formulation development to avoid the effects of the WG method of manufacture as well as granulating binding fluid effects. The FG was used for subsequent formulations to aid drug release and dissolution rate of CBZ.

CHAPTER SIX
THE DEVELOPMENT AND ASSESSMENT OF DIRECT COMPRESSION MATRIX
SUSTAINED RELEASE CARBAMAZEPINE TABLET FORMULATIONS

6.1 Introduction

6.1.1 Direct Compression (DC) Tableting

Wet granulation (WG) is a convenient means of creating flowable blends from non-flowing powders and excipients. The WG process is time-consuming and many process variables affect dosage form production and may subsequently affect dosage form performance *in vitro* and *in vivo*. Furthermore, variability may be enhanced during WG when compared to DC due to the increased number of unit processes in WG procedures and associated variables. However, DC has several limitations which must be overcome during manufacture to ensure uniform dosage form production.

It has been suggested in Section 3.5.2 that the WG process may contribute further to lag times and sustained-drug release from tablets than other manufacturing methods. The formulations produced by WG yielded a low fraction of CBZ released and therefore it was decided to investigate the DC method of manufacture to determine whether the manufacturing process contributes significantly to the low rate and extent of CBZ release observed from tablets produced by WG

Advances in pharmaceutical excipient technology, have led to the development of powders that compress and flow better than those traditionally used for WG and excipients such as spray-dried lactose and microcrystalline cellulose have been adapted for use in direct compression manufacture. The method of DC tablet manufacture is a less time and energy consuming process with a reduced number of unit processes, thus minimising processing errors that can occur during tablet manufacture. In addition, modern tablet presses may use forced or induced powder feeders that ensure adequate and constant filling of die cavities with powders, that do not exhibit optimum gravitational flow characteristics, to facilitate the tableting process [84,148].

For drug loads in tablets of lower than 25% w/w, suitable diluents may be used in a formulation to provide adequate flow and compaction properties to the powder blend,

whereas for formulations containing higher drug loads, the drug itself should be sufficiently compressible and have a minimal effect on the flow properties of a powder blend [148].

Although the DC process contributes to the efficiency of a manufacturing process, this method produces tablets that show a high degree of variability in terms of tablet weight, hardness and thickness, which is more than likely due to the sub-optimum flow and compression properties of powder blends, when compared to wet granulation blends. Sticking of tablets to die walls and punch face surfaces is also commonly observed with DC formulations and the use of anti-frictional agents is usually required to enhance the flow properties of a blend and to minimise or prevent sticking, picking or other difficulties during the compression or ejection phases of tablet manufacture.

6.2 Experimental

6.2.1 Overview

The objective of these studies was to use the composition of a reference wet granulation formulation and adapt it to develop a DC formulation with an *in vitro* release comparable to that of the previously developed wet granulation product. Comparisons between DC and WG dosage forms were also made with respect to the physical characteristics of the resultant tablets such as uniformity of weight, thickness, diameter, hardness and friability.

Furthermore, the DC formulations were optimised in an attempt to produce *in vitro* release profiles comparable to a commercially available reference product, *viz.*, Tegretol[®] CR (Novartis, Quebec, Canada). Dissolution profiles were compared statistically for similarity using the f_1 and f_2 difference and similarity factors [81].

To further understand the behaviour of these dosage forms *in vitro*, the role of selected excipients were investigated for their effects on matrix tablet performance. The viscosity grade of HPMC was varied and assessed for its impact on *in vitro* drug release rates. As CBZ is poorly water soluble, the use of a surfactant (SAA) such as SLS in the formulation may improve the rate and extent of drug release from these tablets. Therefore, the impact of SLS use in the formulation composition, in addition to the type of anti-frictional agent included, was also investigated during early formulation studies.

Drug particle size distribution may also affect drug release rates from sustained release dosage forms and two particle size grades of CBZ were used and assessed to determine their effect on CBZ release rates. Furthermore various proportions of the two particle size grades, used in combination, were assessed to determine their effect on CBZ *in vitro* release rates. The advantage of combination formulations is that release rates may be modified by either enhancing or limiting drug release by the selective use of a specific particle size grade of CBZ.

Finally, a database of *in vitro* release profiles of 36 selected formulations was established and used to develop an artificial neural network (ANN). The *in vitro* release data was matched to the respective formulation composition and these formed the basis of the data sets/pairs for the training of the ANN. The ANN was then used to predict a formulation composition that would provide an *in vitro* release profile that matched the innovator reference product, Tegretol[®] CR (Novartis, Quebec, Canada).

6.2.2 Direct Compression Excipients

All the excipients except for talc and colloidal silica included in the direct compression formulae had been used in the wet granulation method of tablet manufacture. A summary of the relevant properties of these excipients are described in Section 5.3.2. Talc and colloidal silica were assessed only in DC formulations. All of the materials are included in the FDA Inactive Ingredient Guide and all except purified talc are GRAS listed.

6.2.2.1 Purified Talc

Purified talc is a native, anhydrous magnesium silicate [139,143], mainly used as a dusting powder in topical preparations and in solid oral dosage forms, as a lubricant at loads of between 1 and 10% w/w or as a diluent at loads of between 5 and 30% w/w. Talc is not absorbed following oral ingestion and is therefore regarded as non-toxic and is included in the FDA Inactive Ingredient Guide [139], however in South Africa, the Medicines Control Council (MCC) requires that talc used for oral preparations be shown to be asbestos free [149].

6.2.2.2 Colloidal Silicon Dioxide

Colloidal silicon dioxide is submicroscopic fumed silica, which has a small particle size and an associated large specific surface area, resulting in excellent flow properties. It can therefore be included into tablet formulations as a glidant [139]. Other uses for colloidal silicon dioxide are in aerosols that are not intended for inhalation and in semi-solid preparations, where it used as a stabilising or suspending agent [139]. Different grades of colloidal silica with different particle sizes are available and the grade used in this study, Cab-O-Sil[®] M5 (Cabot Corp, Madras, India) has a specific surface area of approximately 150 m²/g and a mean particle diameter of approximately 15 nm [139]. All grades of colloidal silica are hygroscopic, however large quantities of water may be absorbed without the powder liquefying or the flow properties being affected [139]. Consequently, colloidal silica can also be employed as a desiccant in tablet formulations in which this function may be required. Colloidal silica was used an anti-frictional agent in these studies.

6.2.3 Methods

6.2.3.1 Direct Compression Tablet Composition

A prototype DC formulation, *viz.*, Batch C-250303-01, was adapted from the WG formulation that was used to manufacture Batch C-211202-02 and the compositions are listed in Table 6.1 and the percentage w/w of each excipient incorporated in the wet granulation formula has been listed for comparative purposes. CG CBZ was used for the production of the DC and WG formulation.

Table 6.1 DC Formula with corresponding WG composition

Composition	Batch	Batch
	C-250303-01	C-211202-02
	% w/w	% w/w
CBZ (CG)	49.5	49.5
Methocel [®] K100M	9	9
Methocel [®] K4M	6.7	6.7
Emcocel [®] 90M	17.4	17.4
Emcompress [®]	12.04	12.04
Spray-dried lactose	5.36	5.36
SLS	0.4	1
Mg stearate	0.2	1

6.2.3.2 Direct Compression Manufacturing Process

6.2.3.2.1 Original Process (A)

A schematic diagram depicting the DC manufacturing process is shown in Figure 6.1. All powders, except for the magnesium stearate were individually weighed, screened through a size 20 mesh and blended in a cube blender, set at a horizontal angle, at 100 r.p.m for twenty minutes. The magnesium stearate was weighed, screened through a size 44 mesh and added to the powder mix and blending was continued for a further three minutes. The powder blend was subsequently compressed on a Manesty[®] B3B (Manesty, Liverpool, UK) rotary press set at between 40 and 60 r.p.m and that was tooled with one or two sets of deep biconcave punches. The target weight was set at between 700 to 750mg and target hardness was set at between 120 and 150 Newtons (12 – 16 Kp). Tablets were dedusted by vacuum through a screen and stored away from light in airtight bags prior to further testing.

6.2.3.2.2 Modified Process (B)

During the latter part of these studies, the DC method of manufacture was further modified where the powder for compression was compacted using deep biconcave punches. The target hardness of pre-compressed tablets was set at between 100 and 120N. Thereafter, the tablets were crushed and granulated by passing the pre-compressed tablets through a Model TG 2000 dry granulator (Erweka, Heusenstamm, Germany) set at between 170 and 180 r.p.m. This process was repeated twice to ensure that the ‘slugs’ had been completely granulated. The resultant granules were compressed again to the pre-set requirements using a Manesty[®] B3B rotary press that was tooled with the same deep biconcave punches. The resultant tablets were stored away from light in airtight containers prior to further testing.

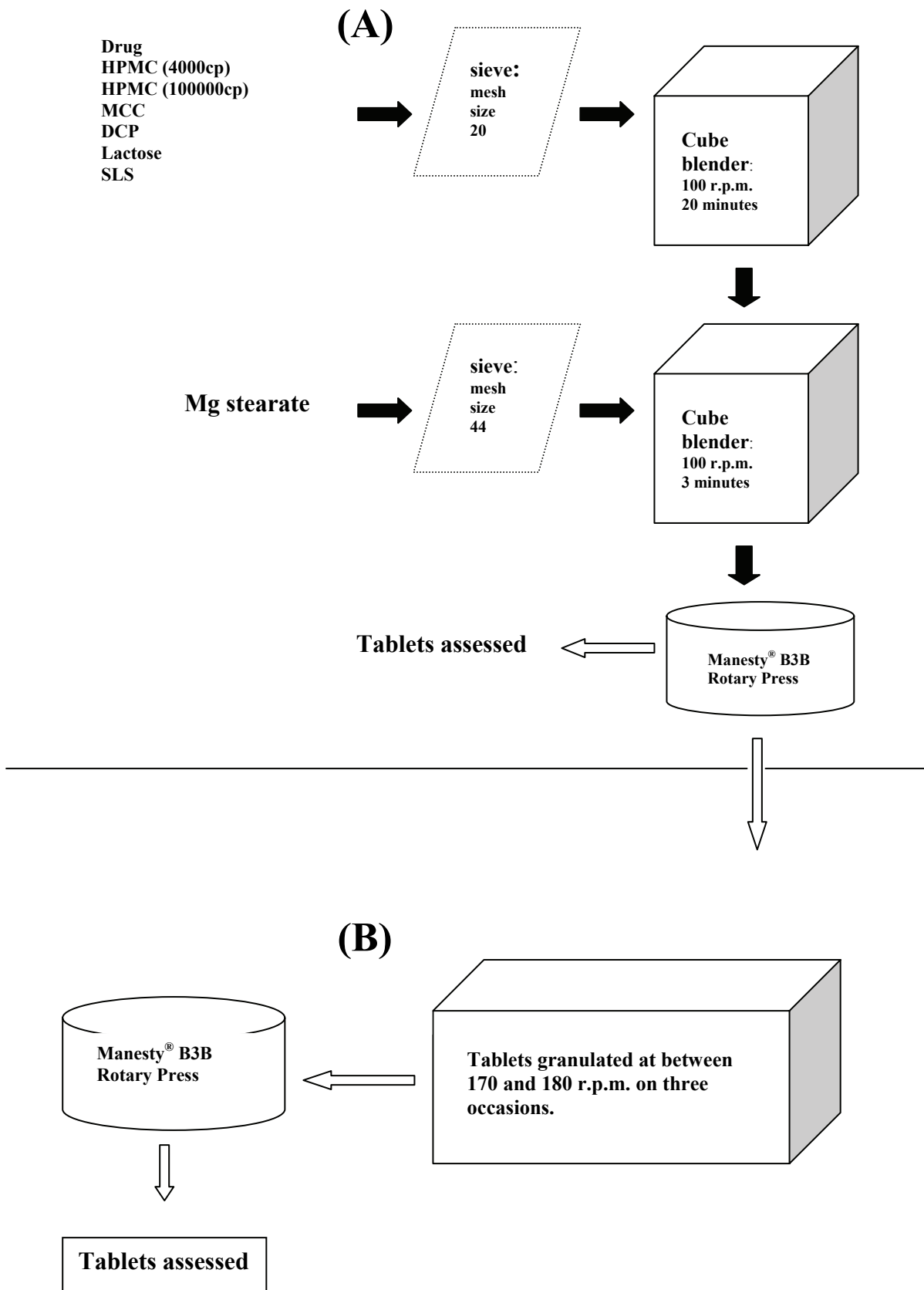


Figure 6.1 Schematic diagram showing the original (A) and modified (B) direct compression process.

6.3 Investigation of Ruggedness of the Formulation

6.3.1 Adaptation of WG to DC method of Manufacture

The WG formula used for Batch C-211202-01 was adapted for the manufacture of a DC Batch C-250303-01 and the two compositions are shown in Table 6.1. The proportions of the formulation components of the WG batch were retained and tablets were manufactured as described in Section 6.2.3.2. The particle size grade of CBZ used for both formulations remained the same. The profiles were compared for similarity using the f_1 and f_2 difference and similarity factors [81].

6.3.2 Effect of CBZ Particle Size

Anhydrous CBZ is practically insoluble in water as described in Section 1.2.3 and the particle size distribution of CBZ can influence the dissolution rate [118,142]. CBZ was generously donated by Noveon Pharma (Raubling, Germany) and was made available in two particle size grades, *viz.*, a coarse grade (CG) particle size and a fine grade (FG) particle size. The initial WG formulations, *viz.*, Batches C-211202-01, -02, -03, -04 were manufactured using the CG CBZ. The total percent drug released for these tablets was between 50% and 60%, and therefore it was decided to investigate the use of FG CBZ in the test formulations. Two formulations with the same amount of a specific grade of CBZ were manufactured and assessed to investigate the effect of particle size on CBZ release from DC matrix tablets. The two formulation compositions for the batches in which particle size effects were assessed, are listed in Table 6.2. Batches C-030703-04 and C-030703-01 contained the CG and FG CBZ respectively and the tablets were manufactured using the procedure outlined in Section 6.2.3.2.

Table 6.2 Formulation composition for the formulations used to assess the effect of CBZ particle size on *in vitro* dissolution rates of CBZ

Composition	Batch C-030703-04	Batch C-030703-01
	% w/w	% w/w
CBZ (FG)	-	40
CBZ (CG)	40	-
Methocel [®] K100M	9	9
Methocel [®] K4M	13	13
Emcocel [®] 90M	17	17
Emcompress [®]	15	15
Spray-dried lactose	5	5
SLS	0.67	0.67
Mg Stearate	0.33	0.33

In addition, various combinations of the two particle size grades were investigated in an attempt to modify drug release rates to achieve dissolution profiles that were equivalent to that of the innovator product Tegretol[®] CR (Novartis, Quebec, Canada). The compositions of the formulations in which different combinations of FG and CG were used are summarised in Table 6.3.

Table 6.3 Composition of formulations used to assess the effect of FG and CG CBZ on *in vitro* dissolution rates of CBZ.

Composition	Batch C-030703-06	Batch C-190603-02	Batch C-190603-03
	% w/w	% w/w	% w/w
CBZ (FG)	39.6	30	24.75
CBZ (CG)	9.9	19.5	24.75
Methocel [®] K100M	9	9	9
Methocel [®] K4M	6.7	6.7	6.7
Emcocel [®] 90M	17.4	17.4	17.4
Emcompress [®]	12.04	12.04	12.04
Spray-dried lactose	5.36	5.36	5.36
SLS	0.4	0.4	0.4
Mg stearate	0.2	0.2	0.2

6.3.3 Effect of HPMC Grade on Dissolution Rate

The rate retarding effects of two viscosity grades of HPMC, *viz.*, Methocel[®] K100M and K4M were investigated to determine the effects and degree of control over CBZ release rates that can be achieved by use of HPMC in these formulations. The composition of the four formulations adopted for the purposes of these studies are summarised in Table 4.4.

Table 6.4 Formulation composition for the assessment of polymer viscosity grade on CBZ dissolution rates.

Composition	Batch	Batch	Batch	Batch
	C-140705-02	C-180705-01	C-190705-01	C-200705-01
	% w/w	% w/w	% w/w	% w/w
CBZ (FG)	59	59	59	59
Methocel [®] K100M	26	32	-	-
Methocel [®] K4M	-	-	26	32
Emcompress [®]	4	4	4	4
Spray-dried lactose	10	4	10	4
Mg stearate	1	1	1	1

Two of the formulations contained Methocel[®] K100M at levels of 26% and 32% w/w whereas two different formulations were manufactured with Methocel[®] K4M at 26% and 32% w/w. These formulations were adapted from the initial test batches, *viz.*, the C-211202-series and thus the compositions were selected based on the compositions of those formulations.

Since these were preliminary studies intended to elucidate the potential effects of HPMC content on drug release, only a six percent increase in HPMC content was used. Any larger increase would necessitate major adjustments to the formulation composition in order to manufacture suitable tablets. Furthermore, to maintain the target tablet weight, the 6% w/w increase of HPMC content was compensated for by decreasing the content of spray-dried lactose by an equivalent amount.

6.3.4 Effect of Sodium Lauryl Sulphate (SLS) on Dissolution Rate

In order to determine whether the inclusion of a SAA would enhance the rate and extent of CBZ release from these matrix tablets, SLS was either included or excluded from the formulation. The formulation composition was kept constant and was similar to that used for the previous batches studied, except that SLS only was included or excluded to determine the effect of its use on dissolution rates. SLS was included at 0.67% w/w in the formulation since it is effective even at concentrations < 1%. Furthermore, large quantities were avoided so that the delivery device could still maintain its sustained-delivery mechanism. The formulation compositions for these studies are listed in Table 6.5.

Table 6.5 Formulation composition used to evaluate SLS effects on the *in vitro* dissolution rate of CBZ.

Composition	Batch	Batch
	C-030703-03	C-030703-04
	% w/w	% w/w
CBZ (CG)	40	40
Methocel [®] K100M	9	9
Methocel [®] K4M	13	13
Emcocel [®] 90M	17	17
Emcompress [®]	15	15
Spray-dried lactose	5	5
SLS	-	0.67
Mg stearate	1	0.33

6.3.5 Effect of Anti-Frictional Agents on Dissolution Rate

The lubricant of choice for these studies was the anti-frictional agent, magnesium stearate. Two additional anti-frictional agents were also assessed for their impact on drug release as well as the physical characteristics of the resultant tablets. Purified talc (Aspen-Pharmacare, Port Elizabeth, South Africa) and fumed silica (Cab-O-Sil[®] M5, Cabot Corp, Madras, India) were assessed as glidants. A summary of the formulation compositions for the batches tested to determine the effect of anti-frictional agent used, on CBZ dissolution rates, are shown in Table 6.6.

Table 6.6 Formulations used to evaluate the effect of anti-frictional agents on the *in vitro* dissolution rate of CBZ.

Composition	Batch	Batch	Batch
	C-200603-01	C-200603-02	C-200603-03
	% w/w	% w/w	% w/w
CBZ (CG)	49.5	49.5	49.5
Methocel [®] K100M	9	9	9
Methocel [®] K4M	6.7	6.7	6.7
Emcocel [®] 90M	17.4	17.4	17.4
Emcompress [®]	12.04	12.04	12.04
Spray-dried lactose	5.36	5.36	5.36
SLS	0.67	0.67	0.67
Mg stearate	0.33	-	-
Talc	-	0.33	-
Cab-O-Sil [®]	-	-	0.33

6.4 Results and Discussion

6.4.1 Conversion of the WG Formula to a DC Formula

The resultant dissolution rate profile for the DC formulation Batch C-250303-01 adapted from the WG Batch C-211202-02 prototype was almost super-imposable as can be seen in the graphical representation of the release profiles in Figure 6.2. The dissolution rate profiles were assessed for similarity using the difference and similarity factors, and the resultant f_1 and f_2 factors were 1.6 and 92.5, respectively, indicating that the profiles are indeed similar.

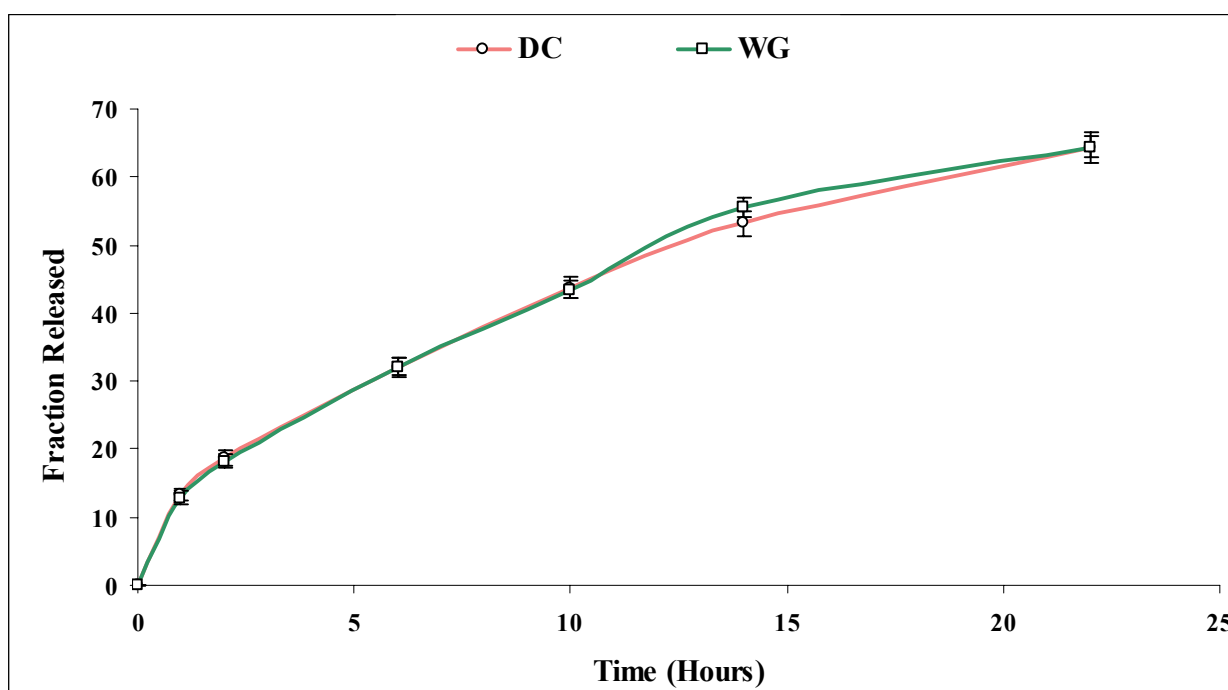


Figure 6.2 Mean dissolution rate profiles (n=6) of CBZ release from a WG formulation (Batch C-211202-02) and a DC formulation (Batch C-280503-01) of similar composition.

The performance of HPMC is unaltered by shear forces that are present during granulation and therefore this can be eliminated as a contributing factor that may affect drug release rates from these dosage forms [106]. The similarity of the two profiles confirms that the manufacturing process has little or no impact on drug release rates, in addition to the significance of the contribution of HPMC with respect to controlled release. As the resultant DC dissolution profile was similar to that of the WG profile, the DC method of manufacture was adopted for the remainder of these studies, as it is simple and more cost-effective when

compared to WG. In addition, the potential effects of WG on drug release rate are therefore eliminated.

6.4.2 Effects of Particle Size Grade of CBZ on *in vitro* Release Rates

The dissolution rate profiles of two formulations with identical compositions but that differ in the particle size grade of CBZ only are depicted in Figure 6.3. The challenge when formulating a poorly water-soluble drug is to control the rate of dissolution so as to minimise variations in the *in vitro* release rate profiles, which may minimise plasma level variations *in vivo*. Furthermore, the maintenance of a well-dispersed system is vital to ensure optimal absorption of the drug, so as to ensure constant therapeutic levels are achieved *in vivo* [141].

Formulations developed with CG CBZ generally showed incomplete drug release, with a total fraction of CBZ released ranging between 60 and 65%. Therefore, the concept of decreasing the particle size and thus increasing the available surface area of CBZ, for dissolution was considered as a potential solution to increase the amount of CBZ released from these tablets. Therefore the use of FG CBZ was evaluated for its potential to enhance the rate and extent of CBZ release from these formulations during *in vitro* release testing.

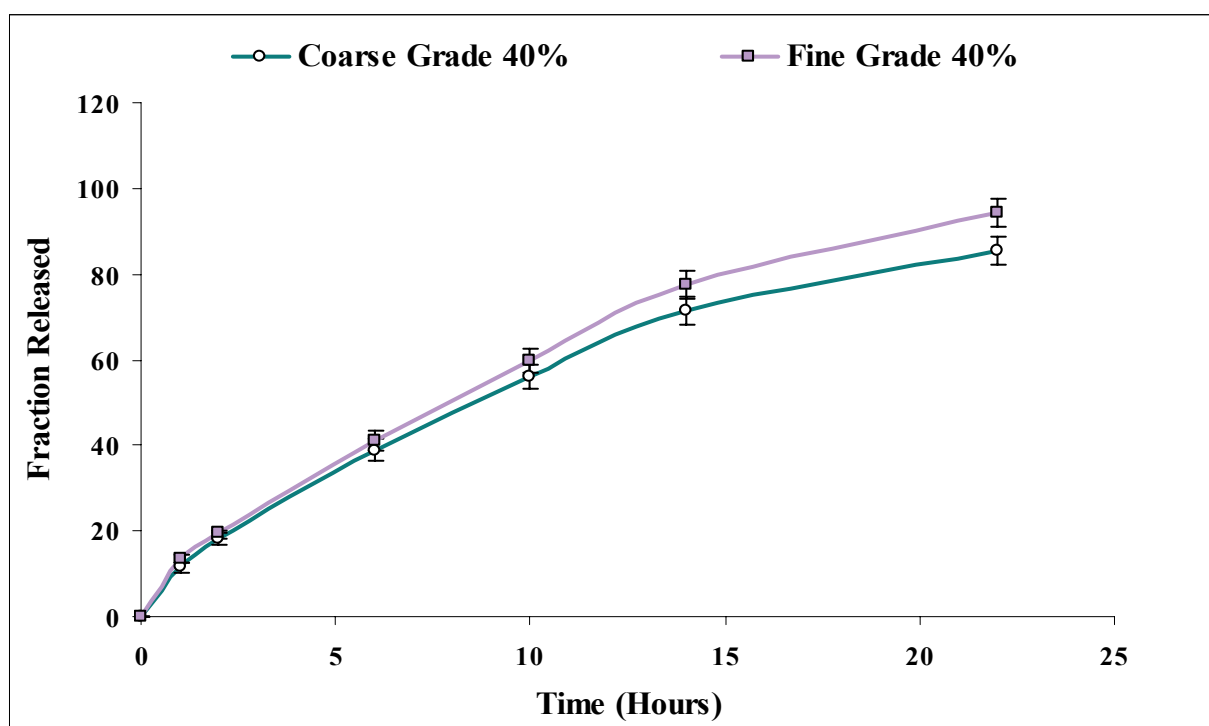


Figure 6.3 Mean dissolution rate profiles (n=6) of CBZ release from formulations containing 40% w/wCG (Batch C-030703-04) or FG (Batch C-030703-01) CBZ.

It is evident from the f_1 and f_2 values of 7.7 and 65.4, obtained for the comparison of CBZ release from formulations manufactured using either fine or coarse grade raw material that the two profiles are similar. However, the effect may be process or formulation specific and warrants further investigation.

The release rate profiles shown in Figure 6.3 represent those of two formulations that were manufactured by DC with exactly the same formulation excipient composition but differ with regard to the particle size of CBZ. However, the drug release is still comparable between these two formulations as well as showing an average drug release higher than that displayed by earlier formulations that were manufactured by wet granulation. Thus the specific manufacturing method such as wet granulation method may also affect drug release and account for any lag time or incomplete drug release observed from these matrix tablets. The FG CBZ formulation however, revealed a greater extent of CBZ release from the matrix structure. In addition, visual inspection of the two profiles depicted in Figure 6.3 reveal that drug release is more complete for the formulation in which fine grade CBZ was used.

The effect of different combinations of the two particle size grades of CBZ on *in vitro* release rates were assessed at proportions of 50% FG: 50% CG; 60% FG: 40% CG as well as 80% FG: 20% CG for their impact on the rate and extent of drug release. The dissolution rate profiles of these ‘combination’ formulations are shown in Figure 6.4. The resultant f_1 and f_2 fit factors calculated for the comparison of all formulations to Tegretol[®] CR and each other are listed in Table 6.7.

It is clear that only the 50% FG and 80% FG formulations are different as both the f_1 and f_2 values for this comparison did not meet the required specification.

Table 6.7 Similarity and difference factors for the comparison of formulations with varying proportions of CG and FG CBZ.

Comparison	f_1	f_2
FG 80% vs FG 50%	16.3	51.4
FG 80% vs FG 60%	14.6	54.5
FG 50% vs FG 60%	2.3	87.4
Tegretol [®] CR vs FG 80%	6	69.8
Tegretol [®] CR vs FG 60%	10.6	63.3

Therefore, it is clear that drug release rates can be modified and optimised by manipulation of the proportion of FG:CG ratio in a formulation.

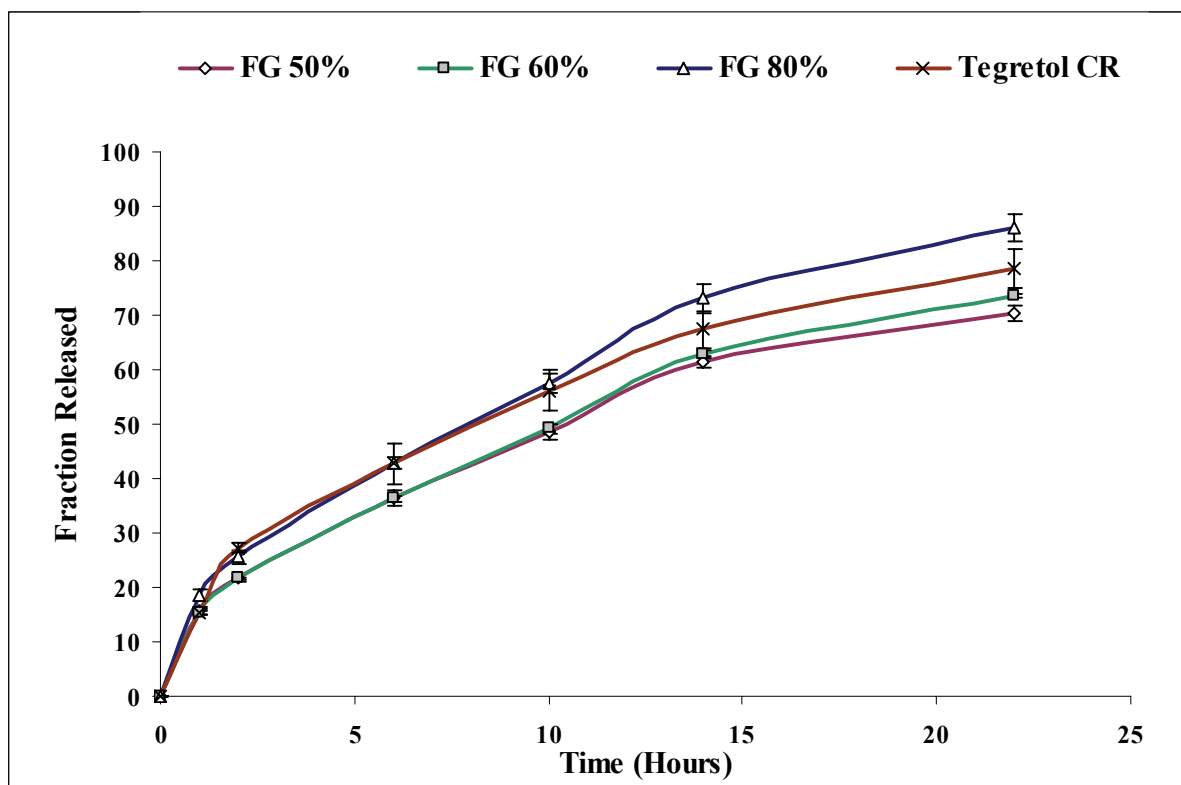


Figure 6.4 Mean dissolution rate profiles (n=6) of formulations with 50% FG: 50% CG (Batch C-190603-03); 60% FG: 40% CG (Batch C-190603-02) and 80% FG: 20% CG (Batch C-030703-05) and Tegretol[®] CR.

Formulations with a 10% difference in the FG:CG ratio showed better similarity when their dissolution profiles were compared, whereas at higher concentrations of the FG CBZ, marked drug release rate differences are observed. This is probably due to the higher FG content contributing to higher drug release rates due to a greater surface area, which becomes available for drug dissolution.

6.4.3 Effect of Viscosity Grade of HPMC on Drug Release Rates

The dissolution rate profiles of the formulations manufactured to assess the effects of different viscosity grades of HPMC on drug release are shown in Figure 6.5. Visual inspection of these profiles reveals a distinct difference in CBZ release rates for the two viscosity grade polymers and furthermore, there is a difference in release rate, depending on the amount of the respective polymer used in a specific formulation. It also seems likely that different combinations of polymers would be appropriate to alter the release rate, mechanism and kinetics of release of different APIs from HPMC hydrogel matrices [105].

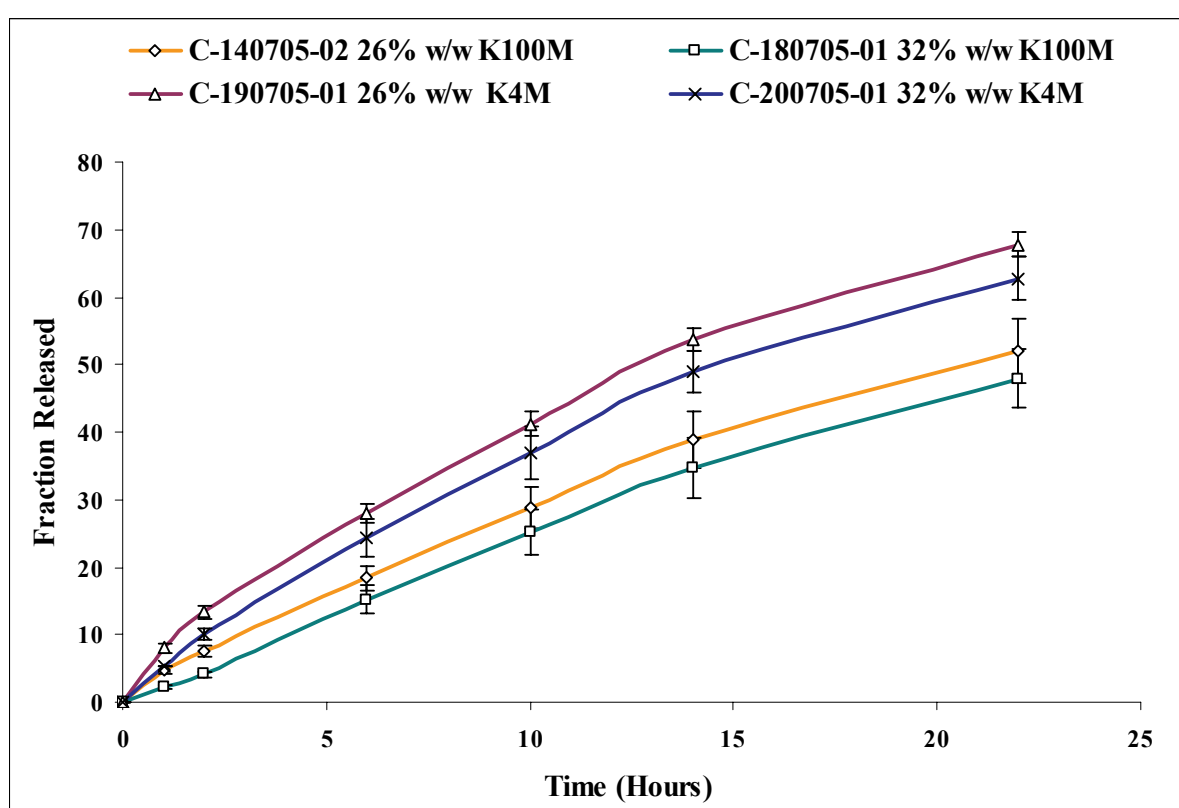


Figure 6.5 Mean dissolution rate profiles (n=6) of Batches C-140705-02 (26% w/w K100M) C- 180705-01 (32% w/w K100M), C-190705-01 (26% w/w K4M) and C-200705-01 (32% w/w K4M)

Batches C-190705-01 and C-200705-01 were manufactured with the lower viscosity grade polymer, *i.e.*, Methocel[®] K4M and as shown in Figure 6.5, drug release from these formulations was faster and more complete than from those batches that were manufactured using Methocel[®] K100M, *viz.*, Batches C-140705-01 and C-180705-01. In addition, two batches were manufactured with Methocel[®] K4M and a further two batches with Methocel[®]

K100M in which the level of HPMC content differed by 6% w/w of the respective polymer grade.

The f_1 and f_2 difference and similarity factors calculated to determine differences or similarity in the release rates of CBZ from formulations manufactured to assess the impact of HPMC are summarised in Table 6.8.

Table 6.8 Difference and similarity factors used to assess polymer grade effects on CBZ release.

Comparison	f_1	f_2
K100M (26% w/w) vs K100M (32% w/w)	12.9	72.2
K100M (26% w/w) vs K4M (26% w/w)	39.9	47.6
K100M (32% w/w) vs K4M (26% w/w)	60.6	41.9
K100M (26% w/w) vs K4M (32% w/w)	25.4	56.6
K100M (32% w/w) vs K4M (32% w/w)	44	48.6
K4M (26% w/w) vs K4M (32% w/w)	10.4	69.7

*Shaded areas denote out-of-specification values

The difference and similarity factors used to compare the dissolution rate profiles of formulations manufactured with the same polymer but a different % w/w composition, confirmed similarity in drug release from these formulations for both the polymer grades tested.

When comparing the formulations with different grades of polymers, drug release was shown to be different and this effect was shown to be more relevant when comparing formulations that had a high proportion of each polymer, *i.e.*, the formulations with 32% w/w of each grade of HPMC.

It is possible to deduce from these results, that the grade of HPMC used in the formulation may impacts on the rate and extent of drug release. The extent of CBZ release from the formulation in which Methocel[®] K4M was included at 26% w/w level was higher than that from the formulation in which Methocel[®] K4M had been included at a 32% w/w level. The f_1 and f_2 values of 10.4 and 69.7 respectively, confirm that release was similar between the formulations with Methocel[®] K4M with a concentration difference of 6%. As would be

expected, the higher viscosity grade of HPMC sustained drug release more effectively and this becomes more significant at the 32% concentration level of Methocel[®] K100M which yielded the lowest CBZ release rates. Statistical comparisons between formulations with the same concentration w/w but different grades of HPMC confirm dissimilar release rates and these are depicted in Table 6.8.

It is apparent that the higher viscosity grade HPMC controls drug release more effectively than the lower viscosity grade as can be seen by the lower rate and extent of drug release from formulations in which it was included, and this was confirmed by the difference and similarity factors listed in Table 6.10. The opposite, however is true, where dissolution profiles of formulations with equivalent amounts of different grades of HPMC showed higher rates and extent of CBZ release to those formulations with the lower viscosity grade HPMC.

6.4.4 Effect of SLS on CBZ Release Rates

Surfactants are commonly used in pharmaceutical dosage forms as wetting agents and SLS can improve the water solubility of CBZ even at concentrations of 0.1% w/w which is considerably below the critical micelle concentration (CMC) of SLS [146]. SLS has lubricant properties and was incorporated at a concentration of 0.67% w/w in the tablets assessed in these studies. This was the same concentration as that of the lubricant incorporated in the development formulations used to manufacture WG dosage forms where SLS was included in the formulation during the blending of granules with additional excipients.

The resultant dissolution rate profiles generated following testing of formulations in which the impact of SLS inclusion on either CG or FG CBZ release from matrix, tablets are depicted in Figure 6.6. The formulation in which CG CBZ was used in conjunction with SLS as compared to that without SLS showed poor similarity to each other as seen from the f_1 and f_2 values listed Table 6.9, and where drug release was considerably higher from the formulations containing SLS.

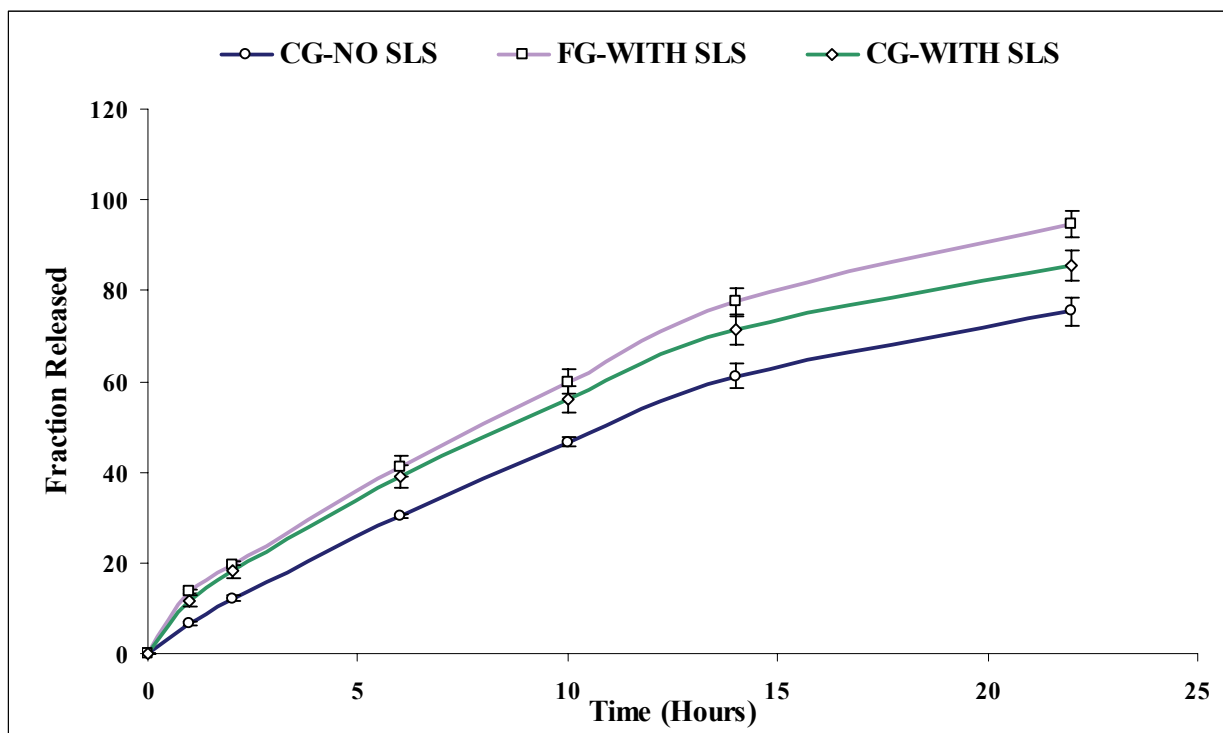


Figure 6.6 Mean dissolution rate profiles (n=6) of CBZ release from formulations Containing FG with SLS (Batch C-280503-01), CG with SLS (Batch C- 030703-04), CG without SLS (Batch C-030703-03).

Table 6.9 Difference and similarity factors used to compare the effect of SLS on CBZ release rates.

Comparison	f_1	f_2
Coarse grade with SLS vs coarse grade without SLS	16.4	54.7
Coarse grade with SLS vs fine grade with SLS	8.4	65.4
Fine grade with SLS vs coarse grade without SLS	22.8	44.7

*Shaded areas represent out-of-specification results

The increased rate and extent of CBZ release from formulations in which SLS was included, may be due to molecules of the solubiliser, CBZ, aligning between surfactant molecules, therefore decreasing the repulsive forces between the molecules and inducing a lowering of the CMC [146]. The limited association between the solubiliser and SLS, even at this low concentration may be responsible for the apparent increased solubility, as is evidenced by the dissolution rate profiles [146].

Any rate-retarding or rate-accelerating effect of surfactant inclusion may be linked to the solubilizing capacity of SLS [146] which becomes significant for a drug such as CBZ which has inherently poor aqueous solubility. SLS may also inhibit the transformation of CBZ to the stable CBZ dihydrate which is a more crystalline less soluble form of CBZ [146]. This may also contribute to any rate-enhancing effects observed with the use of SLS in these products.

6.4.5 Effects of Anti-frictional Agents on CBZ Release Rates

The dissolution rate profiles of CBZ from formulations in which the effects of anti-frictional agent used, are depicted in Figure 6.7. Statistical evaluation of these dissolution profiles revealed that in most cases no significant differences existed between the formulations tested and it was thus concluded that the anti-frictional agent used in the formulations did not significantly affect the *in vitro* release rates of CBZ from these tablets. The resultant f_1 and f_2 values for all comparisons are listed in Table 6.10.

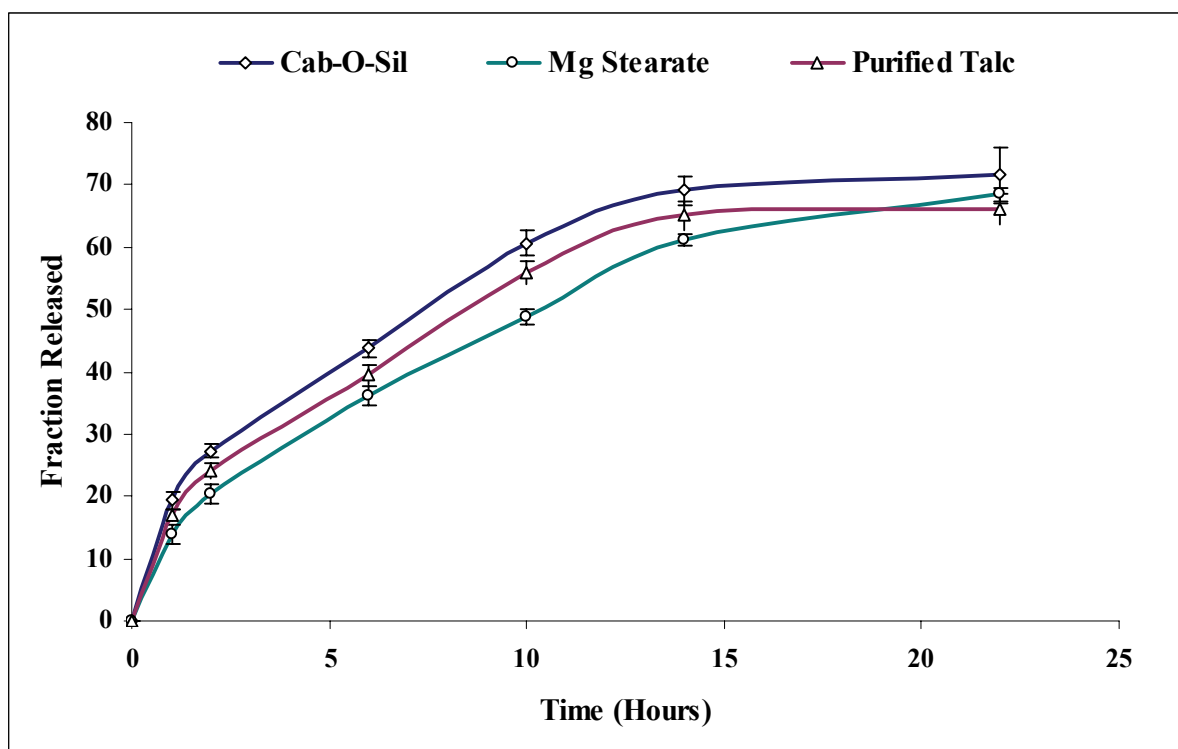


Figure 6.7 Mean dissolution rate profiles (n=6) of CBZ release from formulations containing talc (C-200603-02), Cab-o-Sil® (Batch C-200603-03) and Mg stearate (Batch C-200603-01)

Table 6.10 Difference and similarity factors used to compare dissolution profiles of formulations with different anti-frictional agents.

Comparison	f_1	f_2
Mg stearate vs purified talc	8.8	69.1
Mg stearate vs Cab-O-Sil [®]	16	56.6
Cab-O-Sil [®] vs purified talc	8.6	69.2

*Shaded values represent out-of-specification results

The f_1 value of 16 calculated when comparing the formulations manufactured with Cab-O-Sil[®], Batch C-200603-03 and Mg stearate, Batch C-200603-01, indicate the potential for some alteration in drug release rates, due to the type of anti-frictional agent incorporated in the formulation. The f_2 value of 56.6 indicates some similarity but for the purposes of these studies, agreement between both f_1 and f_2 values were required to confirm similarity.

Therefore, these batches were considered different and the effect of anti-frictional agents on CBZ release warrants further investigation

6.4.5.1 Physical testing of tablets

A summary of the results of physical testing of formulations manufactured to assess the impact of anti-frictional agents on tablet performance are listed in Table 6.11. Tablets of the lowest hardness and thus tensile strength were produced when Mg stearate was used as the anti-frictional agent. All three formulations produced tablets with hardness values that did not meet our pre-determined specifications. The hardness of these formulations may be a consequence of the other excipients within the formulation and not significantly affected by the anti-frictional agent used, since the hardness values were similar to values obtained for all batches manufactured previously. However, as can be deduced from the results of this specific series of formulations, Mg stearate produced softer tablets and it has been reported to reduce radial tensile strength of tablets [150]. This is explained by the formation of weaker bonds between lubricant/lubricant particles rather than strong excipient/excipient bonds [150]. Mg stearate was adopted for these formulation studies due to its availability and widespread application and its value in improving tablet manufacturability, despite the well-known disadvantages associated with the use of this compound.

Table 6.11 Physical characteristics of tablets with different anti-frictional agents.

	Thickness:Diameter (Mean ± SD) n = 10 mm	Hardness (Mean ± SD) n = 10 N	Weight (Mean ± SD) n = 10 mg	Friability % n = 10
Mg stearate C-200603-01	0.57 ± 0	45.24 ± 7.61	531.95 ± 4.4	0.19
Talc C-200603-02	0.57 ± 0	80.39 ± 7.98	527.14 ± 4.06	0.19
Cab-O-Sil® C-200603-03	0.56 ± 0	89.23 ± 13.59	530.56 ± 6.44	0.19
TARGET	-	120 – 150 N	500 – 550mg	< 1%

In terms of tablet weight, there was minimal variation between the tablets and friability was found to be 0.19% for all batches, which was well below the acceptance limit of 1% for this test. The thickness to diameter ratios were consistent between these batches, thus the anti-frictional agent does not contribute significantly to fill volume as confirmed by the uniformity of weight determinations and the obvious similarity in tablet weights.

To thoroughly elucidate anti-frictional agent effects, further in depth studies would have to be performed. These studies would involve testing new formulations with varying concentrations of the anti-frictional agents, alone and in combination in addition to evaluating process effects. The studies reported here were conducted to determine if there was any potential for different anti-frictional agents to affect tablet performance and function.

It is evident that the choice of anti-frictional agent has little or no effect on CBZ release rates and this can be attributed, in part, to the poor solubility characteristics of the API, such that CBZ release is more than likely independent of any potential effects that small amounts of the anti-frictional agent used can influence. It was concluded that the low hardness values were a consequence of the specific excipient combinations rather than solely due to the choice of anti-frictional agent and Mg stearate was used for further formulation development, due to its availability and a knowledge of its effects on drug release rates and dosage form characteristics. Furthermore, Mg stearate shows the ability to reduce the residual and ejection forces that are prevalent during tableting [150].

6.4.6 Comparison of HPMC DC Matrix Tablets to Tegretol[®] CR Tablets

The ultimate purpose of formulation development in this project was to produce sustained-release matrix tablets for which the *in vitro* dissolution profile matched that of a commercially available dosage form, *viz.*, Tegretol[®] CR (400mg) (Novartis, Quebec, Canada) that was used as the reference product for all *in vitro* and *in vivo* studies. All formulation profiles produced during these studies were compared to those of Tegretol[®] CR and these profiles were generated using the same dissolution and analytical conditions as described previously in Sections 3.8.4 and 2.5.3 in Chapters 2 and 3, respectively.

A number of formulations were produced during the initial development stages of the study where batches differed primarily with respect to polymer content and ratio as well as drug content, CBZ particle size grade and ratios that were included in the formulation. An example of two dissolution profiles compared to that of Tegretol[®] CR (400 mg) is shown in Figure 6.8. Batch C-200603-02 showed similarity to Tegretol[®] CR whereas Batch C-211202-03 showed poor similarity when using the difference and similarity factors that are summarised in Table 6.12.

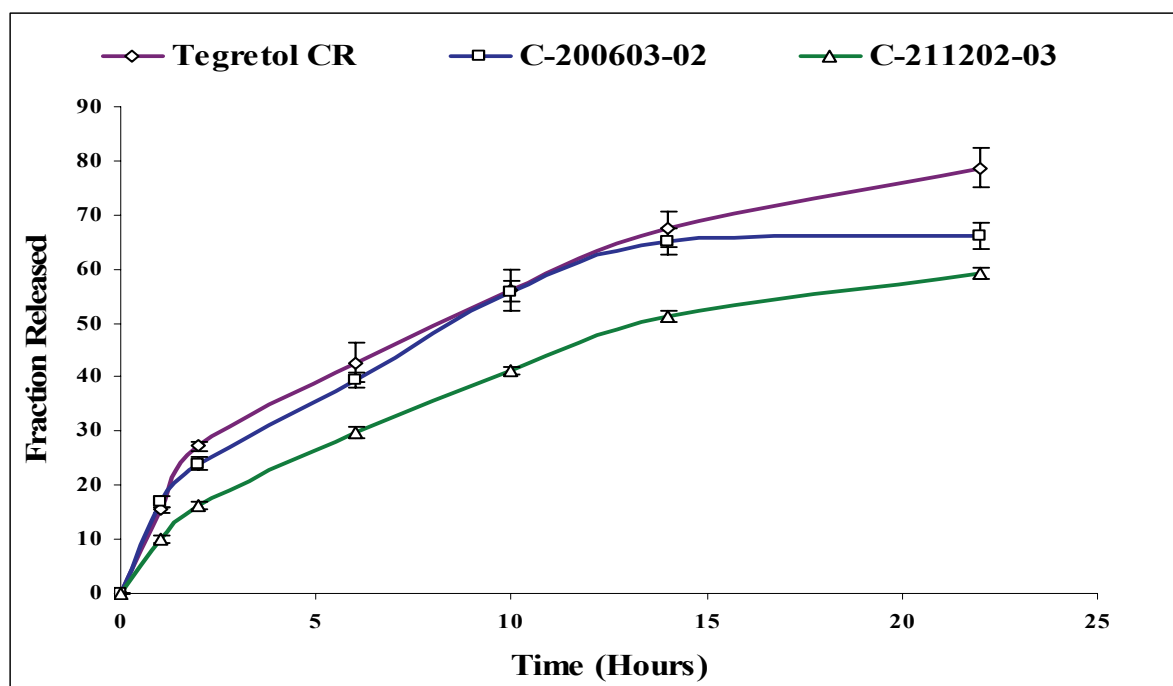


Figure 6.8 Mean dissolution profiles (n=6) of CBZ release from Batches C-200603-02

and C-211202-03 manufactured by DC and WG, respectively for Tegretol[®] CR.

Batch C-200603-02 was a DC formulation that contained talc whereas Batch C-211202-03 was a wet granulation batch in which PVA was used as the granulating fluid. As previously mentioned in Section 3.5.2, the wet granulation process may contribute to any lag time observed in the dissolution profiles, which can be seen in Figure 6.8.

Table 6.12 Difference and similarity factors used to compare the dissolution profiles of batches C-20603-02 and C-211202-03 to Tegretol[®] CR.

	f_1	f_2
Tegretol [®] CR vs C-200603-02	7.9	62.4
Tegretol [®] CR vs C-211202-03	27.4	42.8

*Shaded areas represent out-of-specification results.

The dissolution profiles of two further formulations produced during the initial stages of development are depicted in Figure 6.9. These formulations formed part of initial development studies and served as reference formulations upon which further development was based. Tablets were manufactured using the composition of the initial formulations listed in Tables 6.1 and 6.6. Dosage forms were manufactured in order to optimise CBZ release so as to produce release rate profiles similar to that of Tegretol[®] CR.

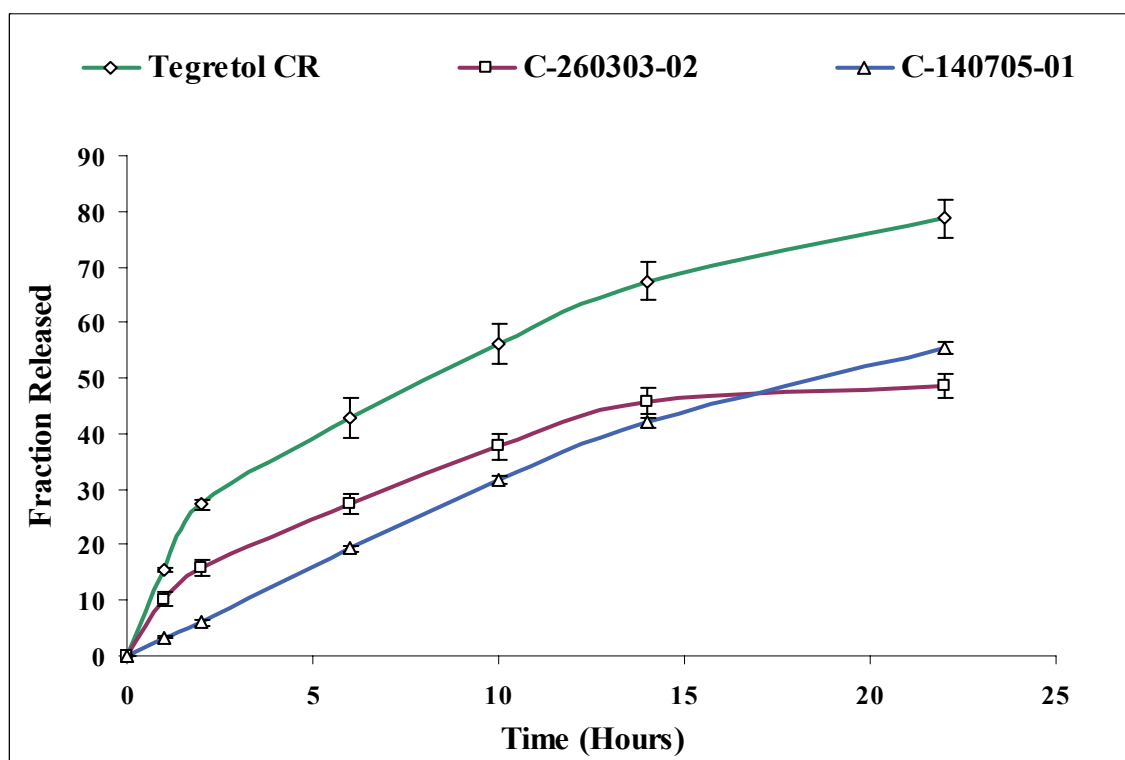


Figure 6.9 Mean dissolution rate profiles (n=6) of Tegretol[®] CR compared to Batches C-260303-02 and C-140705-01 manufactured by DC.

The similarity and difference factors for the dissolution profiles of the formulations depicted in Figure 6.9 are shown in Table 6.13, and it is clearly evident that the profiles are not similar.

Table 6.13 Difference and similarity factors used to compare the dissolution profiles of batches C-260303-02 and C-140705-01 to Tegretol[®] CR.

Comparison	f_1	f_2
Tegretol [®] CR vs C-260303-02	35.5	36.5
Tegretol [®] CR vs C-140705-01	43.3	33.3
C-140705-01 vs C-260303-02	22.3	58.9

*Shaded values represent out-of-specification results

Batch C-260303-02 contained CG CBZ at 60% w/w drug loading, whereas Batch C-140705-01 contained FG CBZ at 49% w/w drug loading. Furthermore, Batch C-140705-01 contained a total of 29.7% w/w HPMC (K4M 13.7% w/w and K100M 16% w/w) whereas Batch C-260303-02 contained only Methocel[®] K4M at 18% w/w loading. The f_1 and f_2 values used to compare these two batches indicated that the formulations were different in terms of their release profiles to Tegretol[®] CR and to each other.

It is probable that within formulations, compensations to facilitate drug release are accommodated in terms of drug content and HPMC loading where the drug release from the Batch C-260303-02 which has a higher CG drug loading is compensated for by the presence of the lower viscosity grade HPMC at a lower loading. Batch C-140705-01 contained the FG CBZ which facilitates drug release rates but release is further controlled by the presence of high concentrations of both the high and low viscosity grades of HPMC, as well as a lower drug loading when compared to Batch C-260303-02.

CBZ release rate profiles of several other batches of tablets manufactured during initial formulation development studies are shown in Figure 6.10. It is clear that the extent of drug release from some formulations were better and exceeded that of Tegretol[®] CR, and were thus not similar to Tegretol[®] CR according to the f_1 and f_2 statistical factors listed in Table 6.13.

Many formulations that were developed and manufactured showed similarity to the *in vitro* dissolution profile of Tegretol[®] CR using the f_1 and f_2 factors and two such formulations are shown in Figure 6.10.

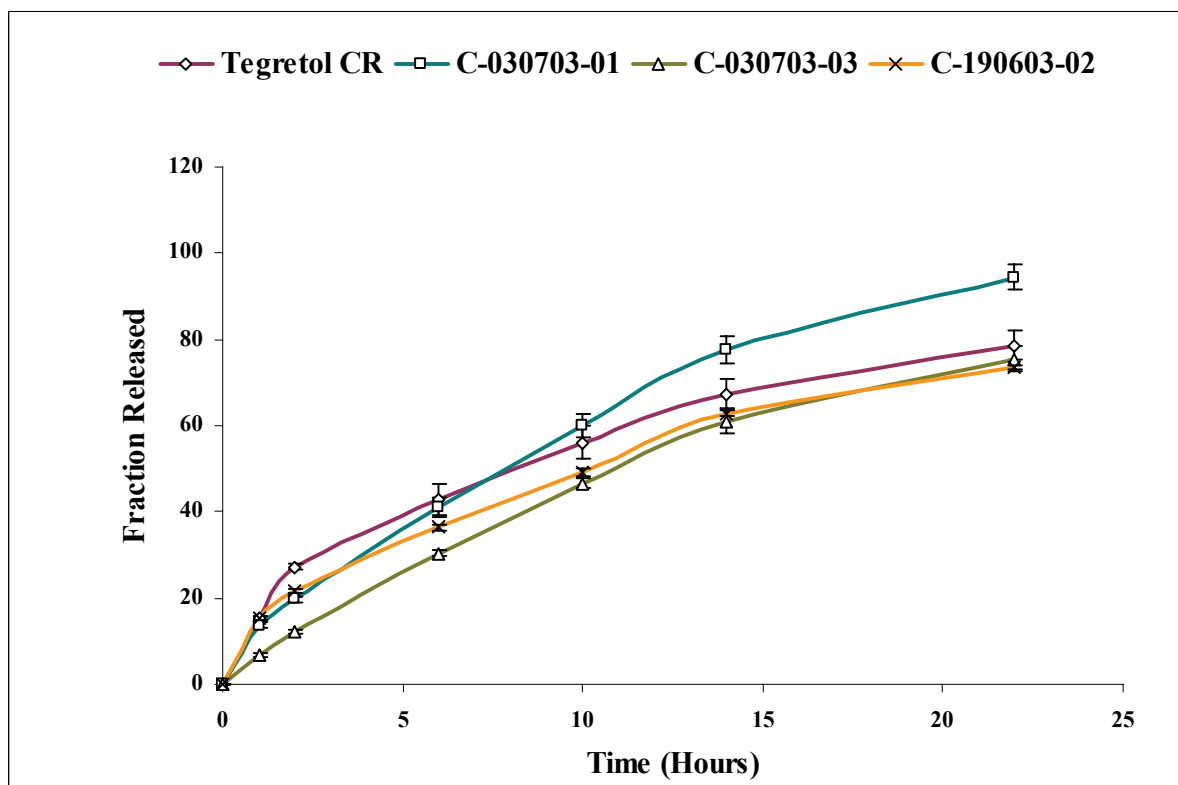


Figure 6.10 Mean dissolution rate profiles (n=6) of CBZ release from DC formulations compared to that of Tegretol[®] CR.

Batches C-190603-02 and C-030703-01 showed similar dissolution profiles to Tegretol[®] CR which was confirmed by the f_1 and f_2 difference and similarity factors shown in Table 6.16. Batch C-190603-02 contained both CG and FG CBZ at a loading of 49.5% w/w whereas Batch C-030703-01 contained only the FG CBZ at a lower loading of 40% w/w. Batch C-190603-02 showed better similarity to Tegretol[®] CR from the f_1 and f_2 values listed in Table 6.14 and contained 6.3% w/w less HPMC than C-030703-01. Therefore, the combination of a lower drug loading and high HPMC concentration may be factors that account for the difference in the drug release rates observed.

Batch C-030703-03 showed differences based on f_1 and f_2 values to Tegretol[®] CR release rates and this formulation was similar to that of Batch C-030703-01 except that the formulation did not contain any SLS. In addition, this formulation contained CG CBZ at a drug loading of 40% w/w with a high HPMC concentration when compared to Batch C-190603-02 and the combination of these variables contributes to lower drug release rates such

that the dissolution profiles are not comparable to that of the innovator product, Tegretol[®] CR. Summaries of all dissolution profiles can be seen in the Appendices.

Table 6.14 Similarity factors used to compare the dissolution profiles of batches C-030703-01, C-030703-03, C-190603-01 to Tegretol[®] CR.

	<i>f</i> ₁	<i>f</i> ₂
Tegretol [®] CR vs C-030703-01	14.2	53.6
Tegretol [®] CR vs C-030703-03	17.1	51.4
Tegretol [®] CR vs C-190603-02	910.6	63.3

*Shaded values represent out-of-specification results

6.5 CONCLUSION

Various components of potential formulations were assessed for their impact on CBZ release rate and adjustments were made to the formulations based on the resultant dissolution profiles, where drug release was either enhanced or prolonged through manipulation of the components used. The effects of selected factors served as a quick reference to determine whether these variables significantly affected CBZ release rates. Studies to further elucidate the contribution of individual components to drug release from these matrix formulations should be considered in future research projects.

For the purposes of these studies, the information obtained during early experiments was sufficient to make formulation decisions during the development and optimisation process. It was also clearly evident that the DC method of manufacture produced results comparable to the same formulation produced by WG manufacture. Therefore, the DC method of manufacture was adopted for the production of all subsequent formulations, as this method of manufacture simplifies the tablet production process.

The release rate profiles of CBZ from these formulations were compared to that of the innovator product Tegretol[®] CR, which was chosen as the target release profile and reference formulation for these studies. It is apparent that the variables in a formulation function as a complete system where individual components contribute to the overall observed release rate and effects and in which drug release rate profiles are affected by the concentration and type of excipients used. In particular

Several formulations showed similar drug release profiles to Tegretol[®] CR and the formulations served, in particular to identify the parameters such as SLS, HPMC content and CBZ grade effects as key components of the formulation that were found to be causative and therefore used for ANN training and subsequently, the production of a large-scale batch of matrix tablets for *in vitro*, *in vivo* and stability testing.

CHAPTER SEVEN

ARTIFICIAL NEURAL NETWORKS (ANN) IN FORMULATION DEVELOPMENT

7.1 Introduction

A biological neural network possesses the ability to recognize features of information presented to it and classify this information based on previous learning or cognitive experiences. Artificial neural networks (ANNs) represent an area of computational mathematical modelling that aims to mimic or simulate the information processing ability of the human brain [151-156] by using the basic learning processes of the human brain to learn and solve pattern recognition types of problems [154]. Another manner of describing ANNs is that the networks are machine-based computational tools, which attempt to simulate some of the neurological processing ability of the human brain [151-155,157-160].

The simplest processing element of the human brain is the neuron which consists of a cell body and axon along which a nerve impulse, that is generated, travels. Synaptic connections allow for the interconnection of adjacent neurons. A similar concept is adopted for ANNs where the artificial neuron may be referred to as a 'node' and is used to denote the basic processing elements (PE) of this computing network [151-155,158,161].

Neural networks are trained to solve problems involving patterns, pattern-mapping, pattern classification and pattern completion [160]. ANNs simulate the learning behaviour of the brain through data modelling and pattern recognition for complicated, multi-dimensional problems [162]. ANNs can identify and learn correlative patterns between input and output data pairs and, once trained on a well-established set of data records, may be used to predict, forecast or estimate outputs from new sets of input conditions [153].

The potential predictive ability of neural networks is of value in the pharmaceutical formulation development process, where they may be used to optimise formulation variables and processes involved in the design and engineering of successful formulations [153]. The use of *in silico* methods such as ANNs, substantially reduce the need for empirical data production and allows

for a more rational formulation development process. Resources required for formulation research may be significantly reduced, thereby reducing costs and overall development time(s). Furthermore, ANNs have gained increasing interest in pharmaceutical applications due to their model-independence, non-linearity, flexibility and superior data fitting and prediction ability [163]. This study provides an example of this in that a relatively small, but well-chosen, data set is used to train a neural network which was then used to find an optimal formulation.

7.2 Design and Construction of a Neural Network

The multi-layer perceptron (MLP) configuration is the most common architecture employed in the application of neural networks [151]. The network consists of a series of highly interconnected layers of neurons, which are a mathematical analogue of biological neurons [153,164].

The neurons in an ANN are arranged into layers. The basic arrangement of these layers is that of an input layer which itself does not process information but serves merely as a distribution point of information to the subsequent layer, which is the first 'hidden' layer. There may be more than a single 'hidden' layer and the outputs from the last single layer are conveyed to the output layer, which is the final layer of information processing and data output. Thus, the network has one input layer, one or more 'hidden' layer/s and a single output layer and each layer has some units corresponding to neurons [159,165].

The number of neurons in the input layer is determined by the number of variables within the data record set and the neurons in neighbouring layers are fully inter-connected with links corresponding to biological synapses [159]. The most popular network design employed and reported in the literature is the Delta Back-Propagation Network (DBPN) [159,166,167] architectural design where input layers provide input and output layers produce an output where the 'hidden' layers provide the inter-connections between input and output layers [159]. The delta rule is based on reducing the error between the actual output of a node and its desired target through the modification of the connection weights and biases [167]. The term 'back-propagation' is derived from the process of propagating the error information backward from the

output nodes to the hidden nodes [168] to allow modifications of the network weights and biases [166].

The arrangements of neurons in a network is referred to as the network topology or architecture. The most frequently used ANNs possess more than one hidden layer, but one layer is usually sufficient for most classification problems [154,159].

7.2.1 The Structure of a Neural Network.

Let p be an $r \times 1$ input pattern vector, b^1 the $s_1 \times 1$ vector of biases in the first layer and f^1 the transfer function in each neuron of the first layer. The weights are on the interconnections between the r input nodes and the s neurons in the first hidden layer. Let the weight, W_{ij}^1 be the strength of the connection between p_j and neuron i . The weight matrix, W^1 , has size $s_1 \times r$ and so the vector $n^1 = W^1 p + b^1$ is the input into the first layer. The activation output from the first layer is the vector $a^1 = f^1(n^1)$. Therefore $a^1 = f^1(W^1 p + b^1)$ is the activation output from the first layer of the network.

If a^i is the activation output from layer i , b^i is the bias vector in layer i , f^i is the transfer function in each neuron of layer i and W^i is the weight matrix for the connections between layer $i-1$ and layer i then $a^i = f^i(W^i a^{i-1} + b^i)$.

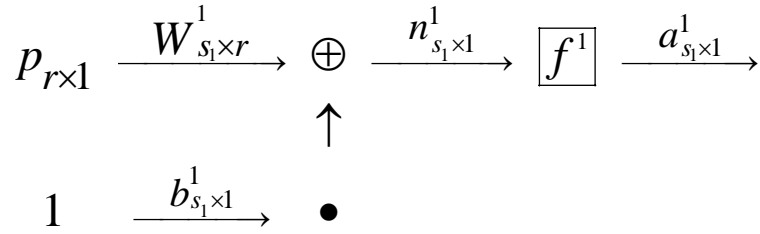
If there are m layers then an input pattern, p , is fed through the network we have:

$$a^1 = f^1(W^1 p + b^1), a^2 = f^2(W^2 a^1 + b^2), \dots, a^m = f^m(W^m a^{m-1} + b^m)$$

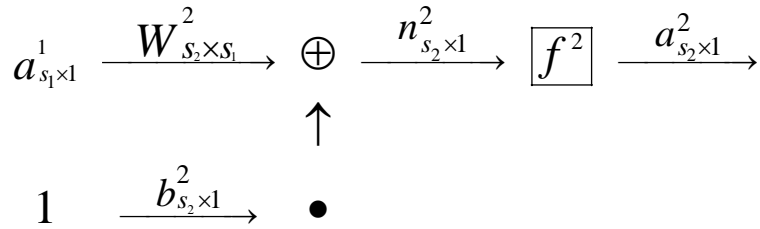
The final activation, $a = a^m$, is then compared with the corresponding target vector t .

A network that receives an r -vector and sends it through m layers of neurons with transfer functions f^i in layer i is given a diagrammatic representation as shown in Figure 7.1.

First Layer:



Second layer receives the output from the first layer:



This is propagated forward through the third layer and beyond until the m^{th} layer is reached:

m^{th} layer:

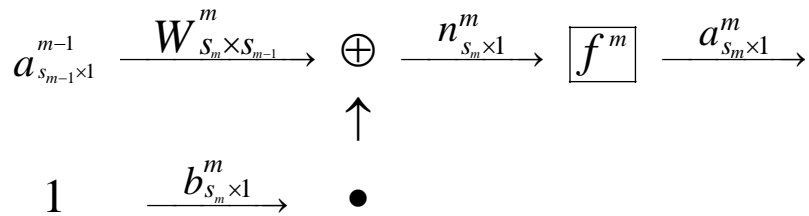


Figure 7.1 Diagrammatic representation of the layers of a neural network.

The network is described as a (r, s_1, \dots, s_m) -feed-forward network. For example if the inputs are 3-vectors and the network has 2 neurons in layer 1, 2 neurons in layer 2 and 1 neuron in layer 3 then it is described as a 3-layer network and more specifically as a (3, 2, 2, 1)-feed-forward network. In the literature there is some confusion about this notation. The first number in the list

is the dimension of the input pattern space. Since this is determined by the dimension of the input space and the nodes which receive these vectors do no computation, some authors omit them from the description, listing only the sizes of the hidden layers and the output layers. In particular, the MATLAB Neural Network toolbox function, `newff`, uses only (s_1, \dots, s_m) to specify the layer sizes.

The sum of squares error (*SSE* or simply *E*), which is the square of the Euclidean norm of the error vector, $e = a - t$, is then a function of the weights and biases in the network. It is given by

$$E = \|a - t\|^2 = \sum_i (a_i - t_i)^2 \quad \text{Eq 7.1}$$

If the weights and biases of the network are written as a vector $x = (x_1, \dots, x_n)$ then $E = E(x)$.

Therefore *E* is a function of many variables and its global minimum, if it exists, can be sought by means of the steepest descent algorithm. This is an iterative procedure which produces a sequence, (x^1, x^2, \dots) , of weights and biases such that $E(x^k)$ decreases as *k* increases. This requires that the transfer functions are continuously differentiable since the gradient vector, ∇E , is used at each iteration to determine the direction of the next step.

The log-sigmoid function, `logsig`, the tan-sigmoid (hyperbolic tangent) function, `tansig`, and of course, the pure linear function, `purelin`, all have this property and are common choices for transfer functions. In neural network research, the investigation of this optimisation procedure led to the discovery of the back-propagation algorithm [165,170]. This algorithm allows the weights and biases to be adjusted in a *pro rata* fashion starting at the last layer and working back through the network. The weights are initially assigned randomly and then adjusted after each epoch using back-propagation. This process of adjusting the weights and biases in such a way that *E* decreases is called training of the network.

The usual procedure for the training of a neural network is to partition the data set into three subsets: a training set, a test set and a validation set. The input patterns in the training set are presented to the network and each pass of the input patterns in the training set is called an epoch.

By virtue of the gradient-descent algorithm, the sum of squares error on the training set, E_{train} , diminishes with each epoch.

A crucial question to be answered prior to the training of the network is the number epochs to present. If too few are presented, the net will be inadequately trained and, conversely, if too many are presented the net may become overtrained. Overtraining occurs when the weights and biases have been adjusted to minimise the sum of squares error, E_{train} , bringing the activations ever closer to the targets at the expense of the ability of the net to interpolate. To prevent overtraining, the sum of squares error, E_{val} , on the validation set, is monitored while the net is being trained. E_{val} will generally decrease during the early stages of training but will start to increase when overtraining begins to occur. At this stage training must be stopped and the performance of the net can then be assessed on the test set. It is the sum of squares error, E_{test} , on the test set that determines the ability of the network to interpolate. If E_{test} is low then there is a good chance that the network will be able to produce sensible activations (simulations) on input patterns that are similar to the ones on which it was trained. However, neural networks are not able to produce sensible activations on input patterns which are dissimilar to those of the training set. In other words, an artificial neural network can be used for interpolation but usually not for generalisation on input patterns which are outside of its experience.

The number of neurons in each layer also affects the ability of the net to interpolate. If a hidden layer has too many neurons, patterns will be memorised which handicaps the network's ability and speed to discern relationships [154,168]. This 'over-fitting' or 'memorisation' will result in poor generalisation ability of the network [154,167]. Complete memorisation tends to occur when the number of facts used to train the network is equivalent to the number of hidden nodes [154] or if it is trained over too many epochs. However, too few nodes may mean that the network might not have enough power to classify patterns in the data [154] and thus display poor mapping [167].

The neural networks in this study were constructed and deployed in the MATLAB computational environment. In particular the MATLAB Neural Network Toolbox was used. In this environment there are many training algorithms available and they are summarised in Table 7.1.

Table 7.1 Summary and description of the training algorithms in the MATLAB Neural Network Toolbox.

Training Algorithm	Description
<code>trainb</code>	Batch training with weight and bias learning rules
<code>trainbfg</code>	BFGS quasi-Newton backpropagation
<code>trainbr</code>	Bayesian regularization
<code>trainc</code>	Cyclical order incremental update
<code>traincg</code>	Powell-Beale conjugate gradient backpropagation
<code>traincgf</code>	Fletcher-Powell conjugate gradient backpropagation
<code>traincgp</code>	Polak-Ribiere conjugate gradient backpropagation
<code>traingd</code>	Gradient descent backpropagation
<code>traingda</code>	Gradient descent with adaptive lr backpropagation
<code>traingdm</code>	Gradient descent with momentum backpropagation
<code>traingdx</code>	Gradient descent with momentum and adaptive lr backprop
<code>trainlm</code>	Levenberg-Marquardt backpropagation
<code>trainoss</code>	One step secant backpropagation
<code>trainr</code>	Random order incremental update
<code>trainrprop</code>	Resilient backpropagation (Rprop)
<code>trains</code>	Sequential order incremental update.
<code>trainscg</code>	Scaled conjugate gradient backpropagation.

Of the algorithms available for use, the scaled conjugate gradient training algorithm (`trainscg`) has been found to be useful and was used in this study. In [169] it is reported that among the MATLAB training algorithms the Levenberg-Marquardt (`trainlm`) algorithm seems to produce a network with the best overall predictive capability. This algorithm requires sufficient memory to deal with large Jacobian matrices and their inverses. However, there is a memory reduction feature for large training sets. Furthermore, it converges to a solution in far fewer epochs than `trainscg` does. The `trainlm` algorithm was used as an alternative training method but no improvement was noticed. Once a neural network is successfully trained, it does not matter how the training was achieved.

7.3 Applications of ANN for Formulation Development

The development of controlled-release drug delivery devices with the desired *in vitro* dissolution and *in vivo* bioavailability characteristics is often challenging and complex since non-linear relationships usually exist between independent formulation variables and the resultant, dependent drug release characteristics [162]. The ANN that was developed and used in this study was designed for the purpose of predicting formulation properties, characteristics and compositions that were required to match CBZ release from a commercially available CBZ-containing dosage form, *viz.*, Tegretol[®] CR (Novartis, Quebec, Canada). A data set consisting of 36 formulations matched according to their respective *in vitro* dissolution profiles generated using USP Apparatus 3 and the method described in Section 3.8.4 and was used as the training set for the network. The trained network attempts to predict the percent drug released from a dosage form at specific time intervals. A list of the formulation components used in these studies is summarized in Table 7.2. The individual formulation components were assigned as input vectors and formulation composition or drug and excipient properties were matched to the relevant dissolution profile generated over a 22-hour period, for the purposes of training the network. The quantitative effects of the excipients and formulation composition on *in vitro* performance of the matrix tablets were then evaluated. The output data was the percent dissolved drug at pre-determined time intervals *i.e.* at 0, 1, 2, 6, 10, 14 and 22 hours, respectively. Following the completion of the training phase, the ANN was used to predict the percent drug release from a dosage form of different composition at the specified pre-determined time intervals.

Table 7.2 Input variables introduced to the ANN.

Component	Component Description
p_{i_1}	carbamazepine, course Grade
p_{i_2}	carbamazepine, fine Grade
p_{i_3}	microcrystalline cellulose (grade specific)
p_{i_4}	dibasic calcium phosphate
p_{i_5}	hydroxypropylmethylcellulose (grade specific)
p_{i_6}	hydroxypropylmethylcellulose (grade specific)
p_{i_7}	hydroxypropylmethylcellulose (grade specific)
p_{i_8}	lactose (hydrous)
p_{i_9}	sodium lauryl sulphate
$p_{i_{10}}$	magnesium stearate

7.3.1 Construction, Training and Deployment of the ANN

Thirty six tablet formulations were manufactured and their dissolution profiles generated and recorded. For each $i \in \{1, \dots, 36\}$ the vectors p_1, \dots, p_{36} constituted input patterns for the neural network. Each input vector represented a formulation. For each $i \in \{1, \dots, 36\}$, the percentage CBZ released at times: 1,2,6,10,14 and 22 hours was measured and the associated target vector, t_i , was obtained. Each target vector represented a release profile. The target vector components are described in Table 7.3

Table 7.3 Target Vector Components

Component	Component Description
t_{i_1}	percentage CBZ released at time 1 hour by formulation i
t_{i_2}	percentage CBZ released at time 2 hours by formulation i
t_{i_3}	percentage CBZ released at time 6 hours by formulation i
t_{i_4}	percentage CBZ released at time 10 hours by formulation i
t_{i_5}	percentage CBZ released at time 14 hours by formulation i
t_{i_6}	percentage CBZ released at time 22 hours by formulation i

Since the percentage CBZ released at time 0 is always 0 this was not included as a component of the target vectors. The 10 x 36 matrix, p and the 6 x 36 matrix, t , were collected together in the

data matrix, $D = \begin{bmatrix} p_1, \dots, p_{36} \\ t_1, \dots, t_{36} \end{bmatrix}$, the first five columns of which are depicted in Table 7.4.

Table 7.4 First Five Columns of the Data Matrix D

60.00	60.00	0.00	19.50	24.75
0.00	0.00	49.50	30.00	24.75
0.00	0.00	17.40	17.40	17.40
4.00	4.00	12.04	12.04	12.04
18.00	0.00	6.70	6.70	6.70
0.00	18.00	9.00	9.00	9.00
0.00	0.00	0.00	0.00	0.00
18.00	18.00	5.36	5.36	5.36
0.00	0.00	0.40	0.40	0.50
0.33	0.33	0.20	0.20	0.25
10.22	7.65	24.88	15.27	15.27
15.80	11.30	33.63	21.61	21.83
27.36	20.86	53.72	36.35	36.46
37.78	30.28	69.27	49.12	48.52
45.79	38.74	82.30	62.80	61.46
48.74	48.85	90.29	73.57	70.26

For example, the first column contains the input vector

$$p_1 = \begin{bmatrix} 60 \\ \vdots \\ 0.33 \end{bmatrix}$$

and an associated target vector

$$t_1 = \begin{bmatrix} 10.22 \\ \vdots \\ 48.74 \end{bmatrix}.$$

This data set was too small to have a validation set and therefore it was decided to adopt the ‘leave one out’ training strategy. This involves selecting just one input and corresponding target vector as a test set. The number of epochs over which to train the network was decided by monitoring the training window, in which the performance, SSE (or MSE: mean square error) is displayed as a function of the number of epochs. An example is shown in Figure 7.2.

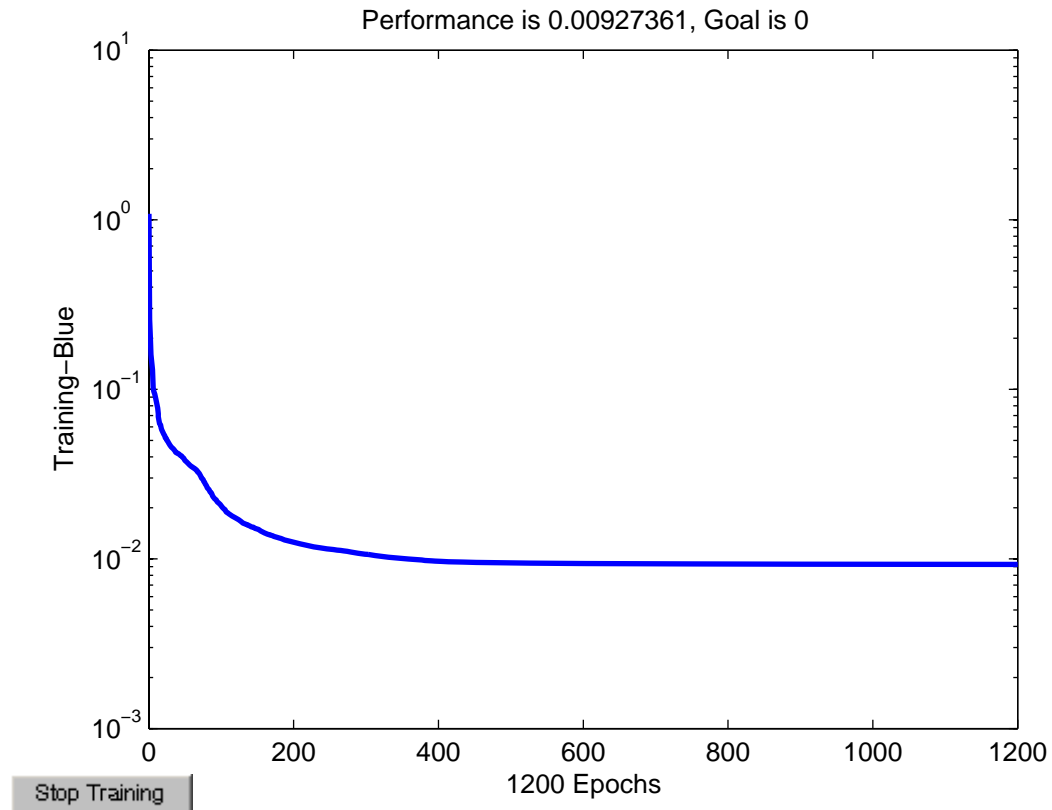


Figure 7.2 Graph of MSE versus number of epochs.

When the graph of MSE versus number of epochs flattens and the norm of the gradient becomes low it is likely that further training will produce marginal improvement at the expense of the ability of the net to interpolate.

The MSE and the gradient is also displayed in the command window as training proceeds and an example is shown in Figure 7.3.

TRAINSCG, Epoch 900/1200, MSE 0.00932127/0, Gradient 0.000519778/1e-006
 TRAINSCG, Epoch 950/1200, MSE 0.00931476/0, Gradient 0.000477872/1e-006
 TRAINSCG, Epoch 1000/1200, MSE 0.00930597/0, Gradient 0.000705202/1e-006
 TRAINSCG, Epoch 1050/1200, MSE 0.00929421/0, Gradient 0.000473016/1e-006
 TRAINSCG, Epoch 1100/1200, MSE 0.0092854/0, Gradient 0.000482811/1e-006
 TRAINSCG, Epoch 1150/1200, MSE 0.00927919/0, Gradient 0.000334451/1e-006
 TRAINSCG, Epoch 1200/1200, MSE 0.00927361/0, Gradient 0.000403976/1e-006

Figure 7.3 Command Window Display of MSE and Gradient During Training.

From the information depicted in Figure 7.3 it can be seen that changes in the MSE are minimal for epochs beyond 900 and this indicates that 900 epochs may be sufficient for training the ANN. Therefore, it was decided to use 900 epochs to train the ANN.

The formulation vector and corresponding release profile for a tablet with 19.5% CBZ CG and 30% CBZ FG, column 4 in Table 7.4, was hidden from the network which was trained on the remaining data set.

The network structure was of the form $[s_1, s_2, s_3] = [16, 10, 6]$, where s_i , denotes the number of neurons in layer i . The input layer had 10 nodes to receive the 10-dimensional input vectors, p_i , the two hidden layers had 16 and 10 neurons, respectively and the output layer had to have 6 neurons in order to produce a 6-dimensional activation vector, a_i , the same size as the target vector t_i . The numbers s_2 and s_3 were obtained by experimentation and there is some flexibility here. There is no deterministic method of obtaining the layer sizes. A neural network with $s_1 = 17, s_2 = 9$, for example, produces very similar results. The layer sizes, s_1, s_2 were decided by adjusting these numbers and then observing E_{test} and E_{train} as well as visually inspecting the activation vectors compared to the target vectors.

Since the input vectors were 10-dimensional it would be necessary to have $2^{10} = 1024$ input vectors if each component were to have just two different values. Since there were only 36 input (and 36 corresponding target vectors) and a neural network cannot generalise outside its

experience, it was essential that the data set was such that the network could find the required formulation by interpolation. Formulation expertise and human experience were necessary to provide suitable training experience, in the form of input patterns and targets, for the neural network.

The transfer functions in the layers were `tansig`, `logsig` and `purelin` respectively. Again, there is some flexibility in the choice of transfer functions and no deterministic method of deciding on them. The `purelin` transfer functions in the last layer allow the output vector components to attain arbitrary size. The scaled conjugate gradient method, `trainscg`, was used to train in the network over 900 epochs.

Prior to training the network, the data was normalized in such a way that each component of the input vectors was between -1 and 1. This is achieved by a simple transformation given in Equation 7.2.

$$p_n = 2 \frac{(p - \min p)}{(\max p - \min p)} - 1 \quad \text{Eq 7.2}$$

MATLAB has the function `premnmx` which accepts the matrix, p , of all input vectors and the matrix, t , of all corresponding output vectors as input and returns p_n and t_n , the normalized matrices, as well as vectors $\min p$, $\max p$, $\min t$, $\max t$ whose components are the minima and maxima of each row of p and t . After training the activations need to be rescaled using the inverse of the transformation, `postmnmx`. For simulation, the function, `tramnmx` is used to perform the appropriate scaling of inputs.

The activation on the test set, which consisted of a single vector, was then compared with the corresponding target. The graphical output is shown in Figure 7.4, in which the components of the target vector are indicated with the \circ marker while the components of the activation vector are indicated with the $*$ marker.

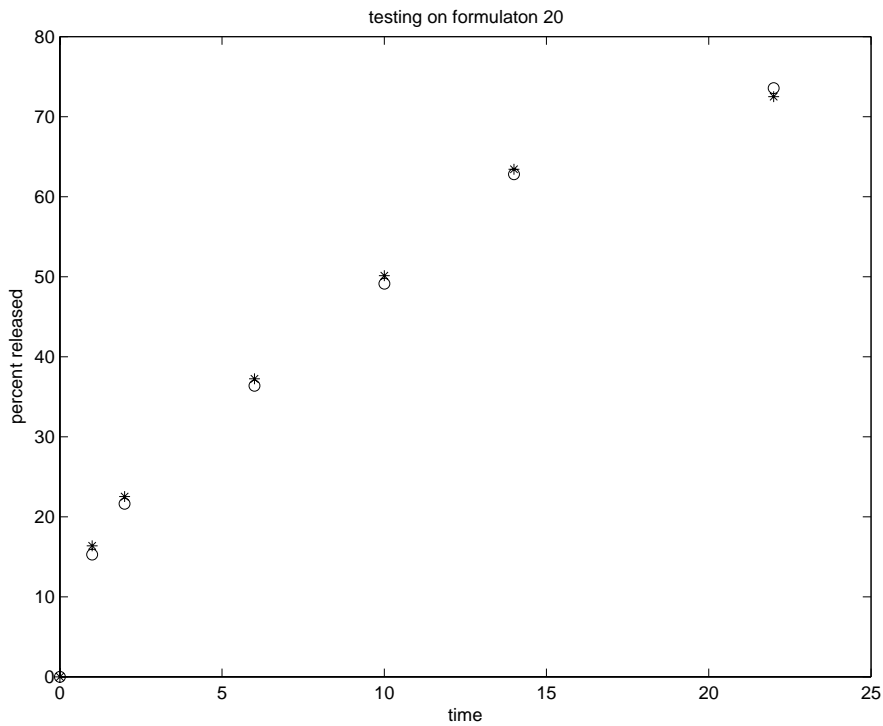


Figure 7.4 Graph depicting target and activation vectors as percent released vs. time (hours).

Having established that the neural network was not overtrained and that it was able to interpolate, it was then trained on the entire data set and the resulting neural network object was saved in order to be used for simulation. A simulation set, p_{sim} , consisting of 21 input patterns was generated. These vectors were such that the composition of CBZ CG varied from 9.5 to 29.5 % w/w, while the CBZ FG composition was correspondingly varied from 40 to 20% w/w. The regression curves $y = M_{\infty} (1 - e^{-rt})$ fitted to the corresponding activations indicated a clear trend as can be seen in Figure 7.5

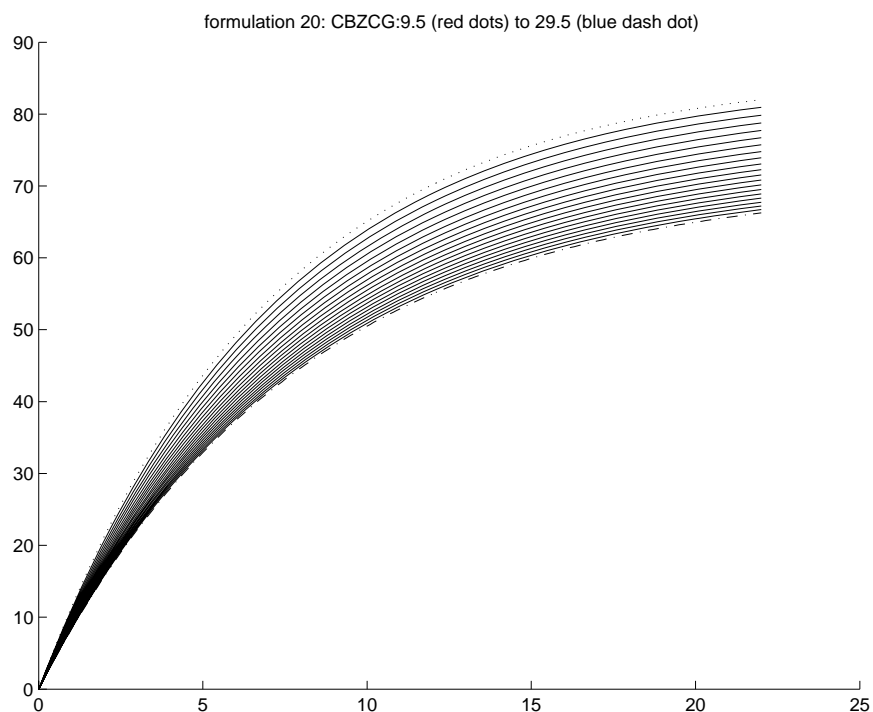


Figure 7.5 Trend in CBZ release profiles when CG:FG ratios are varied. This is presented as percent of drug released vs. time (hours).

As the percentage composition of CBZ CG was increased, and the percentage composition of CBZ FG correspondingly decreased, the profiles flattened, showing a corresponding decrease in the release rate of CBZ from these dosage forms. This observation suggested that it might be possible to match the release profile of Tegretol[®] CR by finding the optimal balance between the CG and FG CBZ content in the test formulations. The formulation vector for which the resultant dissolution profile was most similar to that of Tegretol[®] CR was then found. This was achieved by comparing f_2 values of the activations and that of the reference formulation, Tegretol[®] CR. This is the f_2 similarity measure of Moore and Flanner introduced earlier in Section 3.9. It was considered vitally important to match the release of CBZ from the Tegretol[®] CR dosage form in the early stages of the dissolution test since most previous formulation release

profiles failed to match that of Tegretol[®] CR especially during the first hour dissolution testing.

The release profile vector, T , for Tegretol[®] CR had been previously obtained. A normalised weighting vector, $w = \frac{v}{\|v\|}$, where $v = [8,4,2,1,1,1]$ was generated and for each activation, a , from the simulation set, the weighted f_2 value, as defined in Equation 7.3 was recorded.

$$E \quad f_2(a, T) = 50 \log \left(\frac{100}{\sqrt{1 + \frac{\sum_{i=1}^6 w_i (a_i - T_i)^2}{6}}} \right) \quad \text{Eq 7.3}$$

The formulation vector, p , whose activation vector, a , was most similar (largest) in this sense to T was then found using the MATLAB functions `max` and `find`. The formulation predicted by the network (Batch C-120905-01) was manufactured and assessed using the USP Apparatus 3 (Vankel, USA).

Comparison of the dissolution profiles was done by visual inspection and regression analysis of the ANN predicted profiles versus the experimentally observed profile for the manufactured formulation as well as comparing the f_1 and f_2 difference and similarity factors where an f_1 value less than 15 and an f_2 value greater than 50 showed similarity between two profiles. Tegretol[®] CR served as the reference data set. The release profiles are shown in shown in Figure 7.6 and the difference and similarity factors are given in Table 7.5.

Table 7.5 Difference and similarity factors for Tegretol[®], predicted and actual formulations.

Formulation	f_1	f_2
Tegretol [®] CR	-	-
C-120905-01 (Network-predicted)	15.7	54.8
C-160905-01 (Actual Test)	8.5	66.6

*Shaded value represents out-of-specification result.

In an attempt to ensure that the similarity of the dissolution profile of the test formulation better matched that of the reference formulation, the proportions of the two particle size grades of CBZ were retained and the proportions of the two grades of HPMC used were varied. The differences between the predicted and actual formulations that were manufactured, where adjustments of 2% were made to the percentage composition of Emcompress[®], Methocel[®] K4M and Methocel[®] K100M are summarized in Table 7.6.

Table 7.6 Predicted Formulation and Adjusted Formulation

Formulation Component	C-120905-01 (%)	C-160905-01 (%)
CBZ CG	10.7	10.7
CBZ FG	38.8	38.8
Emcocel [®] 90M	17.4	17.4
Emcompress [®]	12.04	10.04
Methocel [®] K4M	6.7	8.7
Methocel [®] K100M	9	7
lactose	5.36	5.36
sodium lauryl sulphate	0.4	1.5
Mg stearate	0.2	0.5

The rationale behind attempting these adjustments was to decrease the higher viscosity polymer so as to facilitate greater drug release early in the dissolution test. Emcompress[®] contributes to tablet hardness and therefore the amount used in the formulation was reduced to enhance matrix dissolution and erosion, which in turn would facilitate or promote drug release. The modified formulation was manufactured (Batch C-160905-01) and assessed and the resultant dissolution profile is also depicted in Figure 7.6. The release data for the formulations shown in Figure 7.6 is presented in Table 7.7.

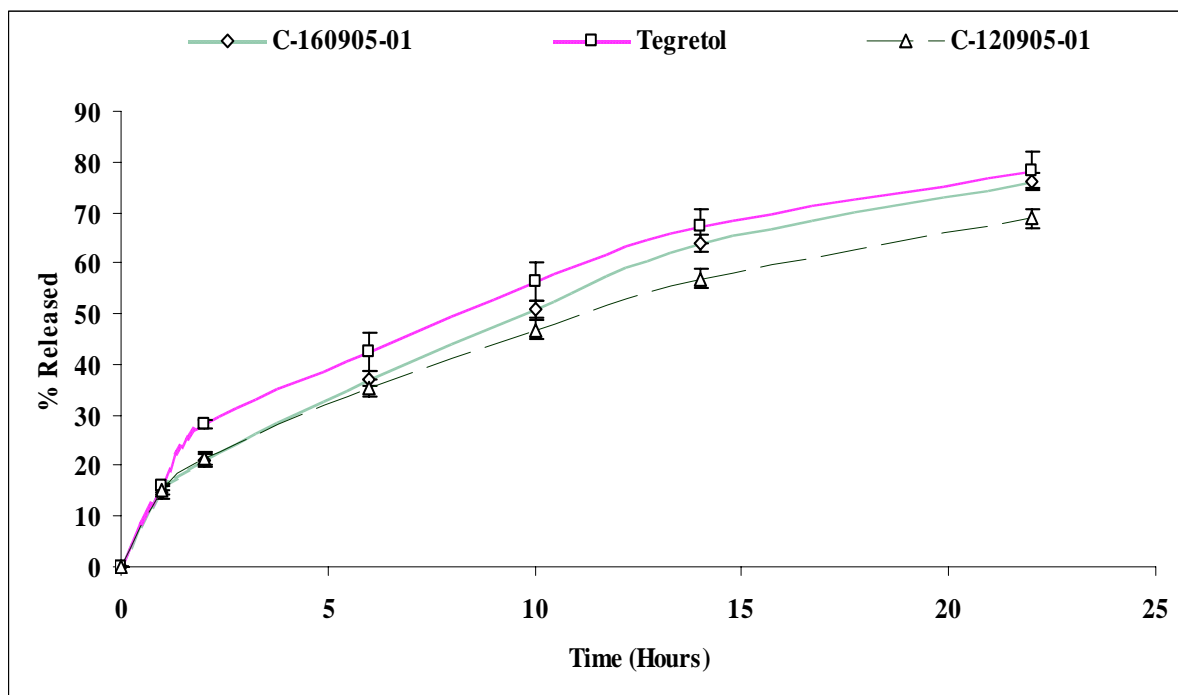


Figure 7.6 Dissolution profiles of CBZ release from Tegretol[®] CR, the network-predicted formulation (Batch C-120905-01) and the modified formulation (Batch C-160905-01)

Table 7.7 Release data for the predicted and adjusted formulations.

Time (Hours)	C-120905-01 (Predicted)	C-160905-01 (Adjusted)	Tegretol® CR
t_{i_1} =1	15.065	14.917	15.779
t_{i_2} =2	21.265	21.087	28.361
t_{i_3} =6	35.354	37.128	42.475
t_{i_4} =10	46.872	50.930	56.237
t_{i_5} =14	56.973	63.984	67.158
t_{i_6} =22	68.928	76.162	78.320

7.4 CONCLUSION

ANN analysis is reasonably flexible as to the amount and form of the data required for training of a network, which makes it possible to use more informal experimental designs than those required with other statistical approaches, such as RSM [165] for formulation development. The application of ANNs is a totally different approach in which all possible data are used for developing accurate models [165]. There is a need for historical and empirical data when using computing networks but this is an additional tool that can be used in the formulation development process, which can substantially reduce the time and the cost of formulation design and development.

It is clearly evident, based on the difference and similarity factors as listed in Table 7.3 that the empirically modified formulation matched Tegretol® CR better than the composition predicted by the ANN. The formulation parameters introduced to the ANN in this study did not include manufacturing process effects such as for example, direct compression or wet granulation methods manufacture and especially did not account for the effects of tablet hardness on drug release rates. More comprehensive and tailored experimental studies would have to be conducted to create a network that would predict formulations for which consideration of these factors have been included. The lack of inclusion of these parameters in the ANN could account for the small differences observed in drug release rates predicted by the formulation composition

predicted by the ANN and the actual *in vitro* dissolution profile generated following manufacture of the formulation.

In addition, the learning nature of ANNs determines that its ability to interpolate would be better with a large training data set and extensive internal mapping of the design space [165] and this would contribute to more accurate data prediction. The size of the network training data set of 36 input and output data pairs, can be considered small and this may account for the discrepancies observed between the ANN predicted and actual target results. However, there is no definite rule to regulate how many data sets are sufficient for ANN training. Furthermore, ANNs with a general structure may occasionally produce a poor estimation of some responses, which will lead to unreasonable predictions of results at some dissolution time points in the case of predicting drug release kinetics [165].

However, this ANN provided a rational basis from which a modification to a formulation composition could be made, to alter the drug release profile based on the knowledge of the physico-chemical properties of the formulation components. The formulator is thus able to make rational decisions regarding formulation improvements based on ‘science’ rather than the ‘art’ of formulation. Further studies with appropriate modifications to the network training data set may still yield better estimations of compositions and dissolution profiles. Ultimately, ANNs provide a useful tool for the prediction of the *in vitro* performance of drug delivery systems [165].

CHAPTER EIGHT
***IN VIVO* ASSESSMENT OF 400MG CBZ SUSTAINED RELEASE POLYMERIC**
MATRIX TABLETS

8.1 Introduction

The Medicines Control Council (MCC) in South Africa is a statutory body that was established in terms of the Medicines and Related Substances Control Act (MRSCA), 101 of 1965, to oversee the regulation of medicines in South Africa. The registration of generic drug products requires that an application be submitted to the MCC and guidelines have been prepared to serve as a recommendation to applicants wishing to submit data in support of the registration of such products [170].

Recent legislation requires that generic medicines registered in South Africa must be offered to all patients when a physician prescribes an innovator product. More specifically, the amendment to the MRSCA [171] states the following:

Subject to subsections (2), (3), and (4) of the Act, a pharmacist or a person licensed in terms of section 22C shall:

- a) Inform all members of the public who visit the pharmacy or any other place where dispensing takes place, as the case may be, with a prescription for dispensing, of the benefits of the substitution for a branded medicine by an interchangeable multi-source medicine; and
- b) Dispense an interchangeable multi-source medicine prescribed by a medical practitioner, dentist, practitioner, nurse or other person registered under the Health Professions Act, 1974, unless expressly forbidden by the patient to do so.

Furthermore, subsection (4) states that “A pharmacist shall not sell an interchangeable multi-source medicine

- if the retail price of the interchangeable multi-source medicine is higher than that of the prescribed medicine; or
- where the product has been declared not substitutable by the council”

Therefore in the South African context the use of generic medicines is mandated, wherever possible.

The main purpose of the MCC is to safeguard and protect the public through ensuring that all medicines that are sold and used in South Africa are safe, therapeutically effective, and consistently meet acceptable standards of quality. In this respect, all submissions must provide the necessary data for Quality, Safety, and Efficacy to register an interchangeable multi-source or generic pharmaceutical product (medicine) and thereby infer that it is therapeutically equivalent. Furthermore, the intention of the new legislation is to make medicines more affordable and available to the wider South African public.

In order to understand the implications for generic substitution, several important definitions and specific terms have been described in the relevant Act [171] as well as in the guidelines [170]. These considerations parallel the requirement for multisource interchangeability defined within the US and the European Union (EU).

As defined in the Act, an interchangeable multi-source medicine means a medicine that contains the same active substance(s) as an approved drug product, and is identical in strength or concentration, dosage form and route of administration. An interchangeable multisource medication must meet the same or comparable standards that will confirm that the generic product complies with the requirements for therapeutic equivalence. Such demonstration may be accomplished via appropriate bioequivalence studies, pharmacodynamic studies, clinical endpoint studies or *in vitro* studies.

Consistent with conventional thinking [C,D,E and with the regulatory bodies in other jurisdictions, the Act [171] defines the term bioequivalence as follows:

“Bioequivalence” means the absence of a significant difference in the rate and extent to which the active ingredient or active moiety in two pharmaceutically equivalent products becomes available at the site of drug action when administered at the same molar dose under similar conditions in an appropriately designed study.

Regulation 2 of the Act [171] describes the requirements for therapeutic equivalence wherein “a medicine is regarded as therapeutically equivalent to another medicine if both medicines are pharmaceutically equivalent, *i.e.*, contain the same amount of active substances in the same dosage form, meet the same or comparable standards and are intended to be administered by the same route, and that have essentially the same safety and efficacy profile.” Consequently, a generic product is one that contains the same amount of and active compound delivered via the same route of administration as a specific type of dosage form, *i.e.*, solid-oral or liquid-oral, as the innovator or marketed brand product.

As part of the process of formulation development and testing, *in vitro* dissolution studies are conducted to assess the rate and extent of the release of and API in selected dissolution media [172]. Comparisons are made between the dissolution profile of the test product to that of an innovator or reference product and formulation adjustments are made to achieve a dissolution profile that is comparable to that of a reference product. However, in order to prove bioequivalence, the evaluation of the *in vivo* behaviour of a generic product in comparison to that of an innovator product is a requirement for the registration of the generic formulation.

Bioequivalence requirements may be imposed by Regulatory Agencies such as the MCC, FDA or EMEA on the basis of the physicochemical properties of an API, where such properties can have a significant impact on the *in vivo* availability of the API, from solid oral dosage forms. Compounds that have low water solubility and where particle size and polymorphism play a critical role in determining bioavailability are required to show bioequivalence to an appropriate innovator product [173]. Drug products in which there is a high ratio of excipients to API and

where dose-dependant kinetics in or near the therapeutic range is evident and the rate and extent of absorption are important to bioequivalence, must undergo *in vivo* testing [173].

CBZ is a well-known and documented API, which is the drug of choice for all partial and tonic-clonic seizures [174,175]. Therapeutic regimens in which conventional immediate-release CBZ tablets are used, often necessitates dosing three to four times daily to achieve optimum and sustained pharmacological responses with minimal variability [174]. Plasma concentration variability and dosing frequency can be reduced by the introduction of a twice-daily dosing regimen through the use of extended release products [174]. The advantages of a controlled release dosage form have been previously discussed in Section 5.1. Controlled release CBZ products do not potentiate the anti-epileptic activity of CBZ, however can dramatically improve patient compliance and convenience, and therefore effect a reduction in the concentration-related side-effects, which can lead to improved anti-epileptic activity and improved patient adherence [176].

All FDA approved generic CBZ tablet products are required to demonstrate bioequivalence to an innovator product in a single-dose crossover study conducted in healthy human volunteers [177]. In addition, bioequivalence-testing may also become important when comparing the *in vivo* behaviour between generic products. The most important objective of bioequivalence testing is to guarantee that generic products are safe and clinically effective for patients, within the well-established limits for bioequivalence testing [178].

8.2 Biopharmaceutics

Biopharmaceutics is the study of the inter-relationship of the physicochemical properties of the drug, dosage form, route of administration and the rate and extent of systemic drug availability, *i.e.*, bioavailability [173]. Pharmaceutical dosage forms can be precisely evaluated, controlled and corrected where necessary, whereas physiological factors cannot be controlled to the same extent. However, an awareness of impact of physiological factors on dosage form performance is important in determining a suitable and appropriate dosage regimen for a specific API [179].

Knowledge of the physicochemical properties of a drug and the associated pharmacokinetic behaviour is essential and necessary when evaluating the potential bioavailability of an API from a particular dosage form [179]. Furthermore, the use of several excipients in combination in a formulation, to produce a specific delivery system may influence the *in vivo* performance of a drug [179].

The chemical structure of a drug will influence the rate and extent of dissolution and ultimately the bioavailability of the API [173,179]. It is important that an API has some degree of aqueous solubility to be effectively absorbed, when administered via the oral route [179]. Furthermore specific crystalline states, such as amorphicity, polymorphic structure and hydration, in addition to particle size may also influence the *in vivo* behaviour of an API [173,179].

As a general rule, a drug substance with an aqueous solubility < 1mg/ml may present a potential bioavailability challenge and therefore, methods to improve the aqueous solubility of such a compound, such as micronisation of the API, complexation with cyclodextrins, salt formation and esterification should be explored during preformulation and formulation development studies [141,173,179]. The API should also have the potential to cross the lipid barriers of cell membranes in the region of the site of absorption in the GIT, in order to enter the systemic circulation [173,179]. The dissolution rate of a solid oral dosage form also plays a major role in controlling the rate and extent of absorption of an API from the GIT and is an important consideration when formulating sparingly or highly insoluble compounds such as CBZ [173,179].

Other biopharmaceutical factors that can influence bioavailability are related to dosage form design and the effects of various adjustments to a formulation during development [173,179]. Excipients may be used as binders, diluents, disintegrants and lubricants, which are necessary components for tablet production and the type and amount of each of these adjuvants may affect the dissolution rate of a drug from such dosage forms and adversely impact its bioavailability [173,179]. In addition, the method of manufacture, *viz.*, direct-compression, dry or wet granulation and processing controls such as humidity, temperature, storage time of in-process

material, may also adversely influence the *in vitro* and *in vivo* performance of a dosage form [179].

The physiology of the biological system also poses several challenges with respect to drug absorption and ultimately the pharmacokinetics of a specific drug compound. In humans, the average pH range of GIT fluids extends from approximately a pH = 1 to pH = 2.5 in the stomach to between pH = 7 and up to pH = 8 in the lower ileum [183]. Drugs that show pH dependant dissolution and therefore, absorption, will be affected by the changing pH of the GIT fluids. Certain disease states or injury to the gut wall, such as duodenal ulcers as well as the presence of food or other substances in the GIT will also affect drug absorption.

The role of the stomach mucosa is a relatively minor consideration in drug absorption due to its small surface area [179] and drugs such as CBZ are absorbed erratically whereas other compounds are not absorbed at all, from this organ [7,173,179]. Controlled-release dosage forms contain a greater quantity of drug than conventional immediate release formulations and are also dosed less frequently, therefore, a pronounced food effect food on bioavailability may result in a serious and/or long-lasting influence on plasma levels [180].

The aforementioned factors were taken into account when designing the controlled-release matrix delivery systems that were intended for administration of CBZ *in vivo*, where complex physiological processes affect dosage form performance and therefore, the therapeutic effectiveness of the API intended for pharmacological action.

8.3 Pharmacokinetics

The study of the kinetics of drug absorption, distribution and elimination is termed pharmacokinetics, whereas drug disposition is a general term that covers drug distribution controlled by the ability of the drug to partition into and between various biological compartments [173,179]. Drug disposition also describes any alteration in the chemical structure of the drug inside the body, *viz.*, biotransformation as well as all the processes leading to the transfer or removal of a xenobiotic from the body, *viz.*, drug excretion/elimination [173,179]. It

is important to have an understanding of pharmacokinetic principles in order to be able to interpret *in vivo* blood level data.

In pharmacokinetics, the most common approach is to consider the body as comprising several hypothetical compartments, through which the drug can enter and exit according to first-order rate processes or kinetics [179]. The compartment itself is a mathematical concept, which is not easily visualised in anatomical or physiological terms [179]. The kinetics of most of the pharmacokinetic and drug disposition processes in the body can be described by hypothetical models that consist of one, two or at most three functional compartments arranged either in parallel or in series [179].

The pharmacokinetic parameters of CBZ, *i.e.*, absorption, distribution, metabolism and excretion are comprehensively described in Chapter 1. CBZ is a lipid soluble compound, which is slowly and variably absorbed from the GIT [7,181]. The bioavailability of CBZ has not been directly determined but it is estimated to be in excess of 70% and may even approach 100% [7].

The design of a dosage form and its subsequent composition may also significantly affect drug dissolution and therefore absorption [178,180]. Differences in the rate of absorption from different products may be responsible for the occurrence of side effects when patients switch from one product to another [178,182]. Studies have shown that there is a lack of food interaction for CBZ controlled-release formulations and patients may take the dosage form before or after a meal [180]. However, for long-term treatment a consistent dosing regimen is advocated and it has been suggested that CBZ generally taken after meals may minimise any possible GIT effects such as gastric irritation or nausea [180].

8.3.1 Volume of Distribution

The apparent volume of distribution of a drug, V_d , is a guide to the extent of distribution of that drug in the body and is a mathematical and not a physiological constant. Consequently the calculated V_d of a drug may exceed the blood and body volume extensively [179]. The V_d of a drug following an extravascular dose may be estimated using Equation 8.1.

$$V_d = \frac{FD}{kAUC} \quad \text{Eq. 8.1}$$

where,

V_d = the volume obtained by extrapolation of the drug concentration

F = fraction of drug available to the systemic circulation

K = elimination rate constant

AUC = area under the curve represented by concentration as a function of time

On average the volume of distribution of CBZ is approximately 1.4L/kg but a wide range of values have been reported as previously described in Chapter 1 [7]. CBZ is primarily bound to albumin with a free fraction of 20 to 30% [7].

8.3.2 Biological Half-Life ($t_{1/2}$)

The biological half-life of a drug can be expressed as the period of time required for the amount or concentration of unchanged drug in the plasma to reduce to one-half of the initial concentration under equilibrium conditions [173,179]. The biological half-life of a drug can be calculated as shown by Equation 8.2

$$t_{1/2} = \frac{0.693}{k} \quad \text{Eq. 8.2}$$

where,

k = elimination rate constant of a drug

Clinically, the elimination rate constant (k) and the biological half-life ($t_{1/2}$) can be used to estimate the time to reach steady-state plasma concentration after initiation of change in maintenance dose [7]. Furthermore these parameters can be used to estimate the time required to

eliminate all or a fraction of an administered drug from the body once therapy has been discontinued [7]. These parameters are a significant consideration, when designing bioequivalence studies since it will allow the investigator the opportunity to determine the necessary sampling schedule and requisite washout period between each of the phases of that bioequivalence study.

Although single dose studies suggest that the half-life of CBZ is approximately 30 to 35 hours, steady-state data suggest that the half-life is reduced to approximately 15 hours [7]. The decrease in the half-life observed on multiple dose therapy is more than likely due to the auto-inductive effect of CBZ on its metabolism and which has been described in Chapter 1. Therefore, pharmacokinetic data derived from single-dose studies should not be used to calculate maintenance dose regimens for patients that have been prescribed CBZ for chronic conditions and patients must be monitored carefully when dose adjustments are made [7].

8.3.3. Clearance

Total body clearance (Cl_T) is a pharmacokinetic parameter that is used to describe the intrinsic ability of the body or its organs of elimination, *i.e.*, kidneys and liver, to remove drug from blood or plasma and clearance is usually expressed as a volume cleared of drug per unit time [7]. The mathematical prediction of clearance is achieved using Equation 8.3.

$$Cl_T = \frac{FD}{AUC} \quad \text{Eq. 8.3}$$

where,

F = fraction of drug available in the systemic circulation

D = Administered dose

AUC = area calculated under the curve and denoted by concentration as a function of time

8.3.4 One-Compartment Open Model

The open-one compartment model is the simplest model that can be used in pharmacokinetics to describe the body [179]. The open-one compartment model is used to represent the body as a single compartment with no barriers to drug transfer [179]. The one compartment open model assumes that any changes that occur in the plasma levels of a drug reflect proportional changes in tissue drug levels, however this model does not assume that drug concentrations in each tissue are the same at any given point in time [173]. The pharmacokinetics of CBZ have been appropriately described by an open one-compartment linear model [1,175] and therefore the use of mathematical relationships that reflect the simple model to calculate pharmacokinetic parameters for CBZ can be considered appropriate.

8.3.5 Zero and First-Order Kinetics

The rate of drug absorption is constant and continues until the amount of the drug in the GIT is depleted, if the absorption process is best described by zero-order kinetics [179]. Once the amount of drug available for absorption has been depleted and no drug is available for further absorption from the GIT, first order rate processes then dominate the pharmacokinetics of the drug [179].

First-order elimination kinetics refers to a process in which the amount or concentration of drug in the body diminishes logarithmically over time and where total clearance and volume of distribution remain constant and do not vary with dose or concentration [7]. A mathematical equation to describe the plasma concentration versus time profile for a drug in which first-order elimination is dominant is shown in Equation 8.4.

$$C_p = C_p^0 e^{-kt} \quad \text{Eq. 8.4}$$

where,

C_p = plasma concentration observed at time t

C_p^0 = initial plasma concentration observed

k = elimination rate constant

t = time

8.4 Bioequivalence Testing of CBZ Matrix Tablets

8.4.1 Ethical Approval

The bioequivalence study undertaken to evaluate the *in vivo* performance of the test formulation was conducted in accordance with the recommendations and guidelines as set out in the Declaration of Helsinki (1964) and all its subsequent amendments of Tokyo (1975), Venice (1983), Hong Kong (1989), Somerset West (1996) and Edinburgh (2000) [187]. Furthermore, the study was conducted in accordance with the ICH Good Clinical Practice (GCP) guidelines [184] and in compliance with Standard Operating Procedures and requirements of the MCC [185].

Ethical approval for the study was obtained from the Rhodes University Ethical Standards Committee (RUESC) before the study commenced. The Faculty of Pharmacy retained the original signed copy of the letter indicating that ethical approval has been granted to conduct the study.

Prior to the commencement of the study, the nature, purpose and risk of participating in the study was explained to all volunteers. If volunteers so desired, they were given time to consider the information or express any concerns regarding their participation in the study. Volunteers were also informed that they could withdraw from the study at any time without penalty to themselves, other than reduced remuneration, but that they should be committed to completing the study prior to their enrolment. A signed consent form obtained in the presence of the person conducting the informed consent discussion was collected from each potential participant and all subjects received written, detailed instructions concerning the study performance and relevant restrictions.

8.4.2 Protocol and Study Design

Carbamazepine exhibits simple simultaneous first-order and zero-order absorption kinetics and absorption is not significantly affected by the presence of food. A pilot single-dose bioequivalence study in which the CBZ matrix formulation developed in these studies was compared to Tegretol[®] CR (Novartis, Quebec, Canada) in the fasted state in healthy male subjects, was undertaken.

8.4.2.1 Test and Reference Products

The batch of CBZ tablets that was used for *in vivo* bioequivalence testing was manufactured as described in Section 6.2.3.2.2, in accordance with GMP conditions and the batch manufacturing record has been included as Appendix III. The test batch was also subjected to the necessary quality control tests such as, for example, dissolution testing, prior to administration as previously described in Chapter 3.

The reference product Tegretol[®] was stored in a temperature controlled environment on receipt and for the duration of all the formulation and development studies. The test and reference products were dispensed twenty-four hours prior to each phase of the bioequivalence study.

8.4.2.2 Study design

A single oral dose of either Tegretol[®] CR (Novartis, Quebec, Canada) 400mg or carbamazepine 400mg sustained-release tablet was administered during each phase of the clinical studies. Each phase was separated by a minimum washout period of 10 days. The randomisation schedule for administration of the test and reference products to healthy volunteers is shown in Table 8.1.

Table 8.1 Randomisation schedule

Sequence Number	Phase 1	Phase 2
1	Test	Reference
2	Reference	Test

The intake of food and fluid was standardized from check-in until the 24-hour post-dose sample had been taken. Subjects fasted for at least 10 hours prior to dosing and standard meals or snacks were served at 4, 8, 12 and 16 hours after dosing. The intake of fluids was restricted from two hours prior to dosing until 4 hours after dosing. Water (240 mL) was administered at the time of dosing and subjects received an additional 240 mL of water 2 hours after the dose. Apart from the study restrictions the subjects were allowed to continue with their normal daily routine between phases 1 and 2.

The ultimate objective of this bioequivalence study was to prove that the concept of administration of CBZ twice-daily in the form of HPMC matrix tablets would provide plasma drug levels equivalent to that of a commercially available product, *viz.*, Tegretol[®] CR. Therefore, it was decided to conduct the study with only two subjects as a ‘proof of concept’ pilot study to assess the *in vivo* potential of the sustained-release matrix tablets developed, assessed and manufactured during these studies. Furthermore, it was decided to use only two subjects to reduce the costs associated with conducting a full-scale bioequivalence assessment of the test products.

8.4.3 Pre-Study Screening

8.4.3.1 Inclusion Criteria

Only those subjects meeting the relevant inclusion criteria were included in the study. The inclusion criteria for the bioequivalence assessment of the CBZ tablets are summarized in Table 8.2.

Table 8.2 Inclusion criteria for the bioequivalence assessment of CBZ matrix tablets

Criterion	Description
1	Mentally competent subjects who are available for the entire study period and willing to adhere to the protocol requirements and able to give informed consent by signing the informed consent form, which is also signed by the person performing the informed consent discussion.
2	Males between the ages of 18 and 55 years of age.
3	Volunteers with a body mass index (BMI) of between 19 and 30.
4	Non-smoking volunteers who have not smoked for at least two months.
5	Normal in terms of medical history at the pre-study screening medical, or in the case of an abnormality, the medical practitioner considers the abnormality to be clinically insignificant.
6	Normal in terms of physical examination at the pre-study screening medical, or in the case of an abnormality, the medical practitioner considers the abnormality to be clinically insignificant.
7	Normal in terms of laboratory test values for the pre-study screening medical within the laboratory's stated normal range, or in the case of an abnormality, the medical practitioner considers the abnormality to be clinically insignificant

8.4.3.2 Exclusion Criteria

The exclusion criteria used to eliminate subject participation in the bioequivalence assessment of the CBZ tablets are summarized in Table 8.3.

Table 8.3 Exclusion criteria for the bioequivalence assessment of CBZ matrix tablets

Criterion	Description
1	Any history of hypersensitivity to carbamazepine or any other related anti-epileptic.
2	Any history of cardiac, liver or renal dysfunction.
3	Any history of adrenal or pituitary insufficiency.
4	Any history of chronic asthma, bronchitis or other bronchospastic conditions.
5	Any history of epilepsy or other convulsive disorders or of mania.
6	Any history or other condition which the study physician regards as clinically significant to the study (including fainting upon blood sampling).
7	Any history of drug or alcohol abuse, or the use of tobacco, within two months of the study start date.
8	Treatment with any drug known to have a well-defined potential for toxicity to one of the major organs, particularly renal and hepatic toxicity, within 3 months of the study start date.
9	Treatment with any drug, which could modify renal excretion of drugs (eg. probenecid) within 1 month of the study start date.
10	The intake of a restricted or abnormal diet for longer than 1 week within 4 weeks of the study start date.
11	Maintenance therapy with any drug or regular use of chronic medication.
12	A major illness considered to be clinically significant by the study physician within 3 months of the study start date.
13	Participation in another study or the donation of one pint or more of blood within 1 month of the study start date.
14	A positive test for Hepatitis B surface antigen, Hepatitis C or HIV.
15	Treatment with any prescription drug within one week of the study start date, unless the drug is considered to be clinically insignificant by the study physician.
16	Treatment with any OTC drug within one week of the study start date, unless the drug is considered to be clinically insignificant by the study physician.
17	The consumption of alcohol or other enzyme inducing agents within 96 hours of the start of the study (all barbiturates, corticosteroids, phenylhydantoins etc).

8.4.4 Concomitant Medication and Subject Restrictions

No prescription or OTC medication such as cold remedies, vitamins and natural products used for therapeutic benefits or antacids were allowed for at least one week prior to the commencement of the study. Any medication taken between the screening medical and dosing was assessed for its potential effects on the study and subjects were excluded if necessary. With the exception of the study drugs, the subjects took no medication for the entire duration of the study.

The consumption of alcohol was prohibited from 96 hours prior to dosing until the last sample of each phase had been taken and following phase two, until the post-study clinical investigations had been completed.

Caffeine-containing beverages and foods such as tea, coffee, cola drinks and chocolate were restricted by the subjects from 48 hours before dosing until the last sample of each phase had been taken.

Strenuous physical activity was not undertaken by the subjects from 24 hours prior to the commencement of each phase and was prohibited until the last sample of phase 1 had been taken, and until the post-study clinical investigations after phase two had been completed. Subjects were to have ceased smoking for at least 2 months prior to the commencement of the study and were to refrain from smoking until the end of the second phase after the last sample has been withdrawn.

8.5 Pre- and Post-Study Medical Screening

Pre-study screening was conducted not more than 30 days prior to the start of Phase 1 and post-study screening was conducted within 48 hours of the final sample of Phase 2 having been drawn or at the termination of the study.

A full physical medical examination was conducted for each subject and the demographic

data documented. The physical examination assessed height and weight to determine the Body Mass Index of each subject, vital signs such as BP, pulse and oral temperature, skin, head-neck, thyroid, eyes, ears-nose-throat, chest, lungs, heart, 10-lead ECG, neurological, musculoskeletal, abdomen, nutritional status and jaundice, anaemia, cyanosis, clubbing, oedema and lymphadenopathy (JACCOL).

Haematological assessment included tests for haemoglobin, total and differential red cell count, haematocrit, total and differential white cell count, platelet count and sedimentation rate. Blood chemistry assessments were also performed and included tests for sodium, potassium, chloride, urea, creatinine, cholesterol, random glucose, total protein, albumin, total and conjugated bilirubin, alkaline phosphatase, GGT (glutamyl transpeptidase), ALT (alanine transaminase), AST (aspartate transaminase) and urate concentrations.

Urine analysis was also undertaken and included checking the appearance of the urine and testing for glucose, ketones, blood, protein, nitrite, specific gravity, pH, leucocyte esterase, bilirubin, urobilinogen and microscopic examination, if positive, for sediment.

The subjects received counselling and provided informed consent documents for the purposes of HIV testing. Blood analysis to detect the presence of antigens for Hepatitis B and C were also undertaken.

8.6 Sample Collection

Sample collection times were defined according to published pharmacokinetic data for CBZ in human subjects. A summary of selected pharmacokinetic parameters that have been reported for CBZ sustained-release dosage forms and that was used to define the sampling protocol following drug administration for this study are listed in Table 8.4.

Table 8.4 Pharmacokinetic data reported for CBZ (Mean \pm SD %).

Reference	Subjects	n	Dose mg	C _{max} $\mu\text{g/mL}$	T _{max} hr	AUC _{0-t} $\mu\text{g/mL/hr}$	t _{1/2} hr
176	Healthy male	30	Test 400	2.9 \pm 0.4	23 \pm 5.8	236 \pm 53	38 \pm 7
			Ref 400	3.1 \pm 0.5	24 \pm 6.7	256 \pm 59	38 \pm 8
187	Healthy male and female	16	Test 400	2.32	13.8	157.4	
		4	Ref 400		7.1	163.3	
180	Healthy male	24	Test 400	2.0 \pm 0.3	23 \pm 8.4	2.9 \pm 0.4	31.1 \pm 4.2
			Ref 400	2.3 \pm 0.2	15.8 \pm 7.3	3.1 \pm 0.5	30.8 \pm 4.4

Based on the information described in Table 8.4, blood samples were withdrawn and collected into Vacutainer[®] tubes (BD Vacutainer Systems, Blliver Industrial Estate, Plymouth, UK) containing EDTA as an anticoagulant, prior to dosing and subsequently at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 16, 24, 36, 48 and 72 hours after dosing. A total of 15 samples were collected from each subject for each phase, where the total volume of blood withdrawn from each subject, including pre- and post-study blood screening, did not exceed 340ml for the duration of the study.

Blood samples were stored in an ice-bath immediately after phlebotomy until centrifugation commenced. Centrifugation commenced within 30 minutes of sample withdrawal. Duplicate aliquots of plasma were placed in polypropylene tubes (Simport, Beloell, Canada), which were stored at -80°C \bigcirc 10°C until analysis. At the end of each phase, samples were stored on ice and couriered to the analytical facility within four hours of packing.

8.7 Sample Analysis

All CBZ concentrations were determined in plasma samples using a suitably sensitive and validated analytical method by Dr. Du Buisson and partners (East London, Eastern Cape, South Africa).

8.7.1 Reagents

1% CBZ polyclonal sheep antiserum in phosphate buffer with protein stabilisers preserved with sodium azide was used for the analysis. The sample pre-treatment solution consisted of surfactant in phosphate buffer preserved with sodium azide. A 0.01% CBZ fluorescein tracer in phosphate buffer containing surfactant and preserved with sodium azide was used as the competing agent on the protein binding sites.

8.7.2 Method

CBZ plasma concentrations were measured according to a suitably sensitive, assay procedure using an Abbott AxSYM[®] (Abbott Diagnostics, Abbott Park, IL, USA) system.

The AxSYM[®] CBZ assay is a reagent system for the quantitative measurement of CBZ in serum or plasma. The method is based on Fluorescence Polarisation Immunoassay (FPIA) technology. The sample for analysis and all AxSYM[®] CBZ reagents required for one test were pipetted by sampling probe into various wells of a reaction vessel. The sample and Solution 4, the line diluent, were pipetted into one well of the reaction vessel. Thereafter, an aliquot of the predilution mixture, pretreatment solution and Solution 4 were transferred to the cuvette of the reaction vessel. The reaction vessel was immediately transferred to the processing centre where further pipetting was performed with the processing probe.

A second aliquot of the predilution mixture was transferred to the cuvette along with the CBZ antiserum antibody and the CBZ fluorescein tracer, in the Processing Centre. CBZ from the sample and the CBZ fluorescein tracer compete for binding sites on the antibody molecule and the FPIA optical assembly measured the resultant intensity of the polarized fluorescent light.

All plasma samples collected in these studies were analysed using this method of quantitation.

CBZ is metabolized to an active metabolite, the 10,11-epoxide, which was not measured for the purposes of these studies and the resultant concentrations reflect blood levels of the parent molecule only.

8.8 Statistical Analysis

Carbamazepine plasma concentration time profiles were constructed from the blood level data received from the analytical laboratory. Pharmacokinetic and statistical parameters were calculated using the SAS (SAS Institute Inc., Cary, NC, USA) statistical package and the relevant parameters that were calculated are defined in Table 8.5.

It was not possible to conduct an Analysis of variance (ANOVA) test for these data, as the power of the study was too low.

Table 8.5 Definitions of pharmacokinetic and statistical parameters used to analyse CBZ blood level data

Abbreviation	Definition
C_{\max}	The maximum concentration of carbamazepine in a subject's plasma
C_{last}	The last measurable concentration
T_{\max}	The sample time at which the C_{\max} was attained. If this occurs at more than one time point, it is defined as the first time point with this value (hour).
T_{last}	The time of last quantifiable concentration (hour).
T_{Lin}	The time point where log linear elimination begins (hour).
AUC_{0-T}	The cumulative area under the plasma concentration vs. time curve from time zero to the last measured or measurable concentration, determined using the trapezoidal rule.
$AUC_{0-\infty}$	The area under the extrapolated plasma concentration vs. time curve from time zero to infinity calculated as the sum of AUC_{0-T} and $C_{\text{last}}/k_{\text{el}}$.
$AUC_{0-T/0-\infty}$	The percent ratio of AUC_{0-T} to $AUC_{0-\infty}$ as a measure of the extent to which the elimination of the drug was followed.
k_{el}	The apparent first-order terminal elimination rate constant calculated by linear regression of the terminal linear portion of a semi-logarithmic plot of plasma concentration vs. time using at least the last three measurable concentrations.
$T_{1/2\text{el}}$	Mean elimination half-life (hour).

8.9 Results and Discussion

Both subjects successfully completed both phases of the study. The resultant mean plasma concentration versus time profiles following analysis of blood samples are depicted in Figure 8.1 and a summary of the resultant plasma concentrations used to plot these curves are summarized in Appendix III. During the first phase, subject one, expressed signs of significant drowsiness at approximately twelve hours post-dose. Similarly, subject two when administered the test product also reported sleepiness, however, at approximately fifteen hours post-dose. The volunteers reported no other side effects and no adverse event occurred that, may or may not have been related to the conduct of the study. *In vivo*, differences in absorption rates may be reflected by the occurrence of side effects, where central nervous system effects have been reported to be significantly more common when immediate release tablets that promote rapid and fast absorption are used [176,178,172].

As is the norm for bioequivalence testing for the purposes of registration of generic drug products, the rate of absorption is reflected by evaluation of C_{\max} and the t_{\max} [178]. The mean C_{\max} obtained for the test tablet formulation and Tegretol[®] CR was to be 13.10 $\mu\text{mol/l}$ and 9.45 $\mu\text{mol/l}$, respectively. The average time to reach maximum plasma levels was found to be 35.00 and 29.50 hours for the test and reference formulations, respectively. The time taken of approximately 35 hours to reach maximum concentration for CBZ from the test matrix tablet was found to be similar for both subjects. Point estimate ratios for the log-transformed data are shown in Table 8.6 and both values fall between the margin of 0.8 and 1.25. The pharmacokinetic parameters determined for both subjects for Phases one and two, are summarised in Tables 8.7 and 8.8 respectively.

Table 8.6 Point estimate ratios for C_{\max} and AUCT using log transformed data

Mean AUCT Ratio	Mean C_{\max} Ratio
1.23	1.16

In order for a generic product to be approved by the FDA or other regulatory agency and rated therapeutically equivalent, the 90% confidence limits for the ratio of the mean of the log-transformed data for AUC_{last} or AUCT and C_{max} of the generic and reference product must usually fall within 80 – 120% or 0.8-1.25 [178,186-188]. The three classic pharmacokinetic parameters used to assess bioequivalence are AUCT or area under the curve to the last measurable concentration, C_{max} and T_{max} [8,176,178,180,182,189] and these were the parameters that were calculated to compare the test and reference formulations in these studies. It has been reported that C_{max} should be the characteristic of choice for the rate of absorption in single-dose studies with CBZ products [178]. Consequently evaluation of the point estimate ratio for C_{max} would be of importance in assessing equivalence. Notwithstanding the small sample and low power of this study, it is evident that the point estimates of the ratio of AUCT and C_{max} fall within the conventional 0.8 – 1.25 90% confidence interval. These results provide evidence that the test formulation has the potential to be developed further to produce a generic sustained release CBZ product. Further, it must be noted that C_{max} and T_{max} are single-point observed parameters, and may be misleading when comparing flat, plateau like or multiple-peak concentration-time curves obtained after dosage of sustained-release formulations [176,190]. Furthermore, the values obtained for these parameters are also dependant on the sampling schedule used during a study and that may introduce significant experimental error if the maximum plasma concentrations and/or time to reach that concentration are not appropriately characterized [176].

Table 8.7 Pharmacokinetic and statistical results for the Test formulation for both subjects.

ID	Seq	Period	C_{max}	T_{max}	AUCT	AUCI	k_{el}	T_{Lin}	$T_{1/2el}$
1	BA	2	14.60	35.00	764.75	1364.80	0.0136	24.0	50.94
2	AB	1	11.60	35.00	591.45	925.64	0.0168	24.0	41.33
Mean	-	-	13.10	35.0	678.10	1145.20	0.015	24.00	46.14
SD	-	-	2.12	-	12.54	310.51	0.02	-	6.8
CV	-	-	16.19	-	18.07	27.11	14.74	-	14.74

Table 8.8 Pharmacokinetic and statistical results for Tegretol[®] CR for both subjects.

ID	Seq	Period	C _{max}	T _{max}	AUCT	AUCI	k _{el}	T _{Lin}	T _{1/2el}
1	BA	1	9.80	35.00	573.00	3321.90	0.03	24.00	238.2
2	BA	2	9.10	24.00	526.35	996.75	0.01	24.00	59.86
Mean	-	-	9.45	29.50	549.68	2159.30	0.01	24.00	149.00
SD	-	-	0.50	7.78	32.99	1644.10	0.01	-	126.10
CV	-	-	5.24	26.37	6.00	76.14	84.61	-	84.61

The coefficient of variability (CV) of CBZ for AUCT was approximately 12% higher for the test tablets than that observed for the Tegretol[®] CR tablets. Notwithstanding the small sample size, these results suggest that CBZ may be delivered more consistently and predictably from the commercially available Tegretol[®] CR tablets than the test matrix tablets [174]. These results may be a consequence of the differences in the technology used to deliver CBZ or an artefact of the small population used in the bioequivalence study. Consistency of drug release and subsequent absorption is highly desirable for sustained-release dosage forms to ensure consistent blood levels and this is particularly critical for conditions where clinically important blood level concentration ranges have been established such as for CBZ, which is known to function within a narrow therapeutic index [174]. However, the results of these findings provide the formulation scientist with insight into the predictability of the *in vivo* performance of these dosage forms.

The average blood level profiles for both products are depicted on a Cartesian plot in Figure 8.1. Visual assessment of these profiles reveals that both the test and reference products released CBZ at equivalent rates as indicated by the similar blood levels achieved, for the first twelve hours after dosing. A key feature of this assessment is the attainment of measurable blood levels for the test formulation, which provides confidence in the ability and potential of these dosage forms to deliver the API in controlled and sustained manner.

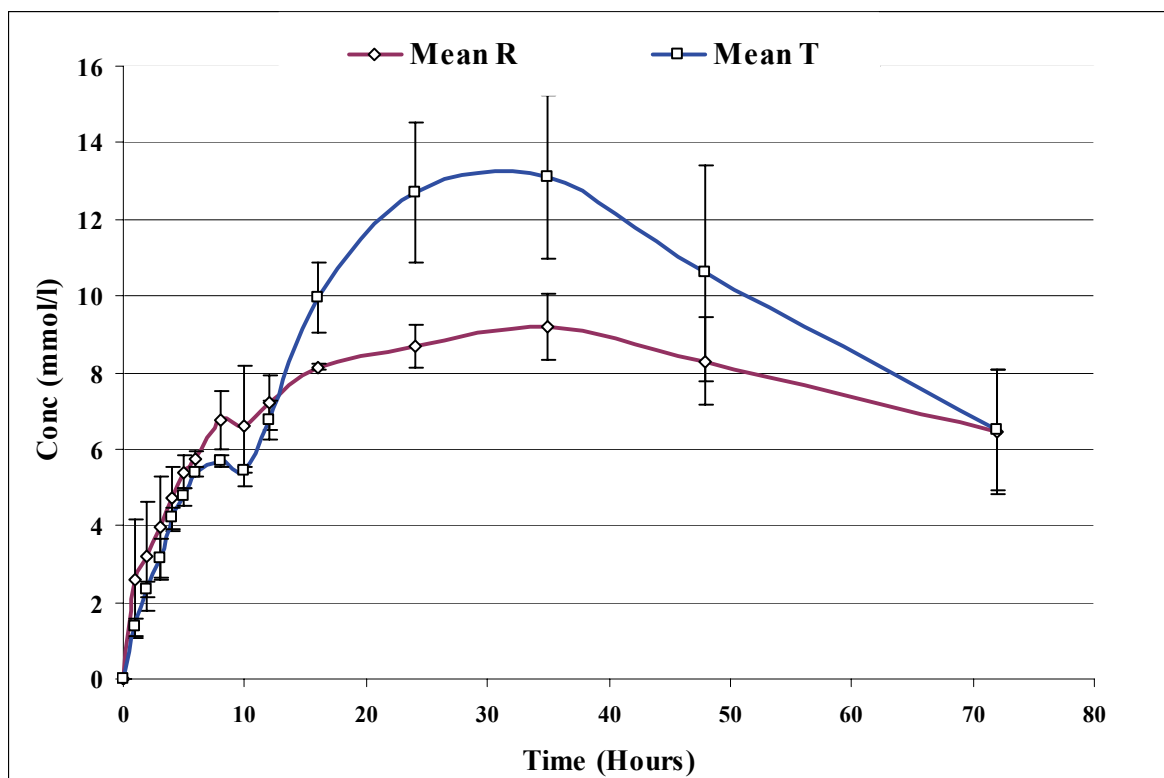


Figure 8.1 Cartesian plot of the mean blood level profiles of both the test and the reference products

Low peak to trough variability is also important for sustained-release dosage forms to ensure that the lowest attained levels are in the therapeutic range while the peak levels do not exceed the limits of toxic drug concentration [174]. The effective therapeutic range as previously reported in Section 1.5.6 is 16 to 50 $\mu\text{mol/l}$ or 4 to 12 $\mu\text{g/ml}$ on chronic therapy to maintain seizure control. Peak to trough levels ratios of 50% or more are less desirable [174]. The mean C_{max} for the matrix test formulation and Tegretol[®] CR were 13.1 and 9.7 $\mu\text{mol/l}$, respectively (Appendix III), and the mean C_{min} values for the test matrix tablets and Tegretol[®] CR were 1.35 and 2.6 $\mu\text{mol/l}$. The variation between peak and trough levels is higher for the matrix tablets and this corresponds with the higher CV for matrix tablets. However, these variations are based on a single dose and further investigative studies must be conducted to determine the extent of peak to trough fluctuations following chronic dosing.

The Cartesian plasma drug level concentration profiles for subjects 1 and 2, for both test and reference products are depicted in Figures 8.2 and 8.3, respectively. Both subjects showed symptoms of extreme drowsiness approximately 12 - 14 hours following administration. The subjective assessment by the subjects are substantiated by the plasma profiles where drug levels show a dramatic increase in concentration after twelve hours and this effect appears to be consistent for this product in both subjects.

CBZ has significant CNS effects and neuron activity is decreased as a result of its physiological effects as has been mentioned in Chapter 1. The decreased neuronal activity could account for the onset of decreased mental alertness in both subjects as the plasma levels of CBZ increased dramatically, twelve hours following administration.

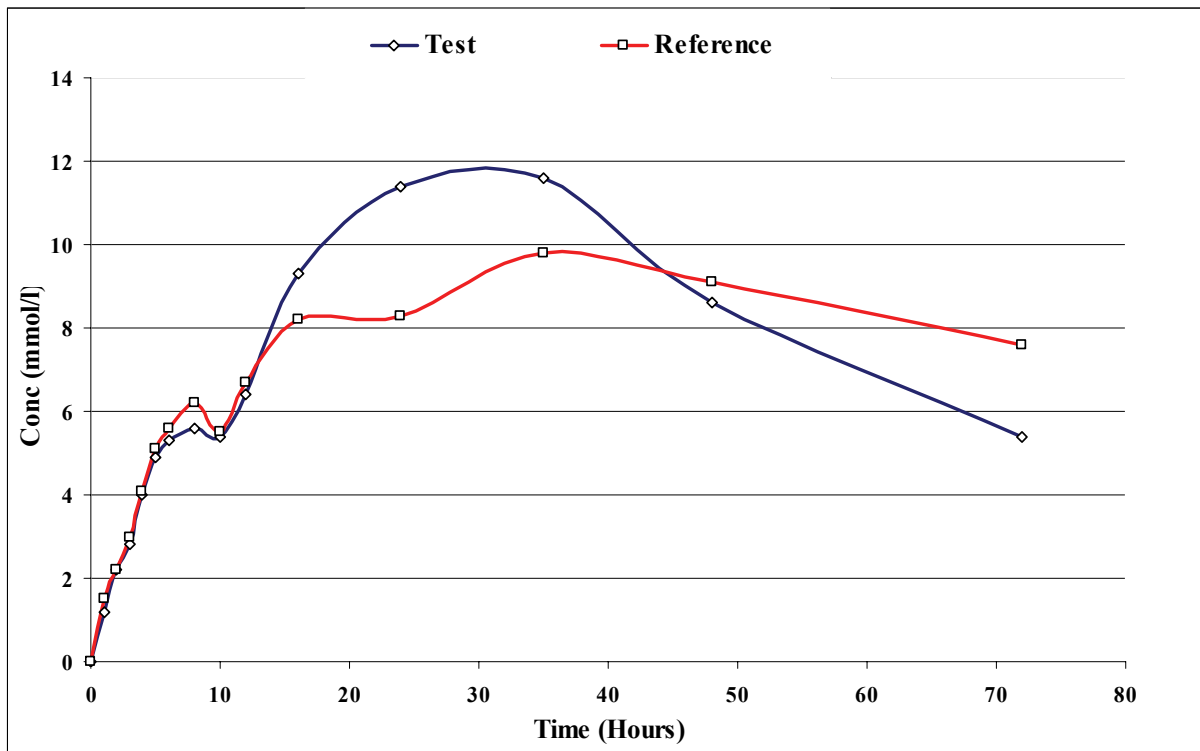


Figure 8.2 Resultant plasma concentration versus time profiles for subject 1 following administration of the test (Phase 1) and reference (Phase 2) products

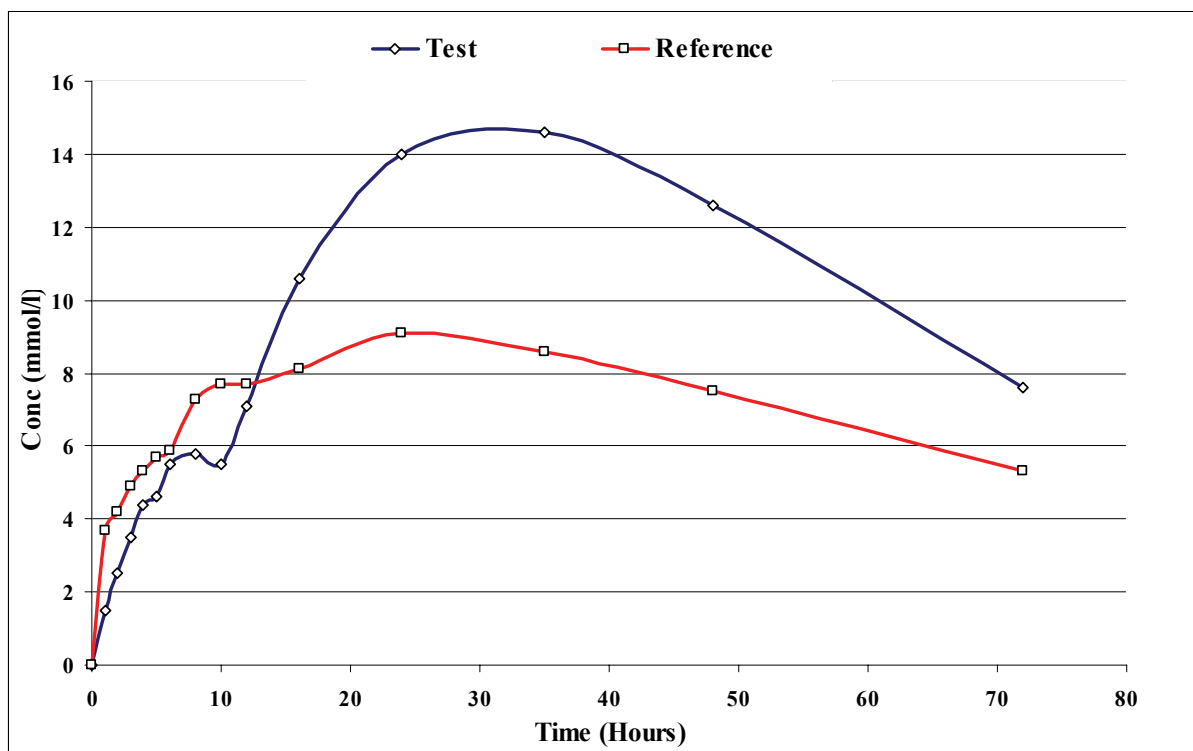


Figure 8.3 Resultant plasma concentration versus time profiles for subject 2 following administration of the test (Phase 2) and reference (Phase 1) products

A mean semi logarithmic plot of CBZ plasma concentration versus time profiles for both the test and reference products is depicted in Figure 8.4. It is clearly evident from a visual assessment of these profiles that the two products appear to perform similarly *in vivo*. For the purposes of comparison, point estimates of the mean log transformed data for C_{max} and AUCT were calculated and are listed in Table 8.6. However, as the study population was small such comparisons must be made with caution.

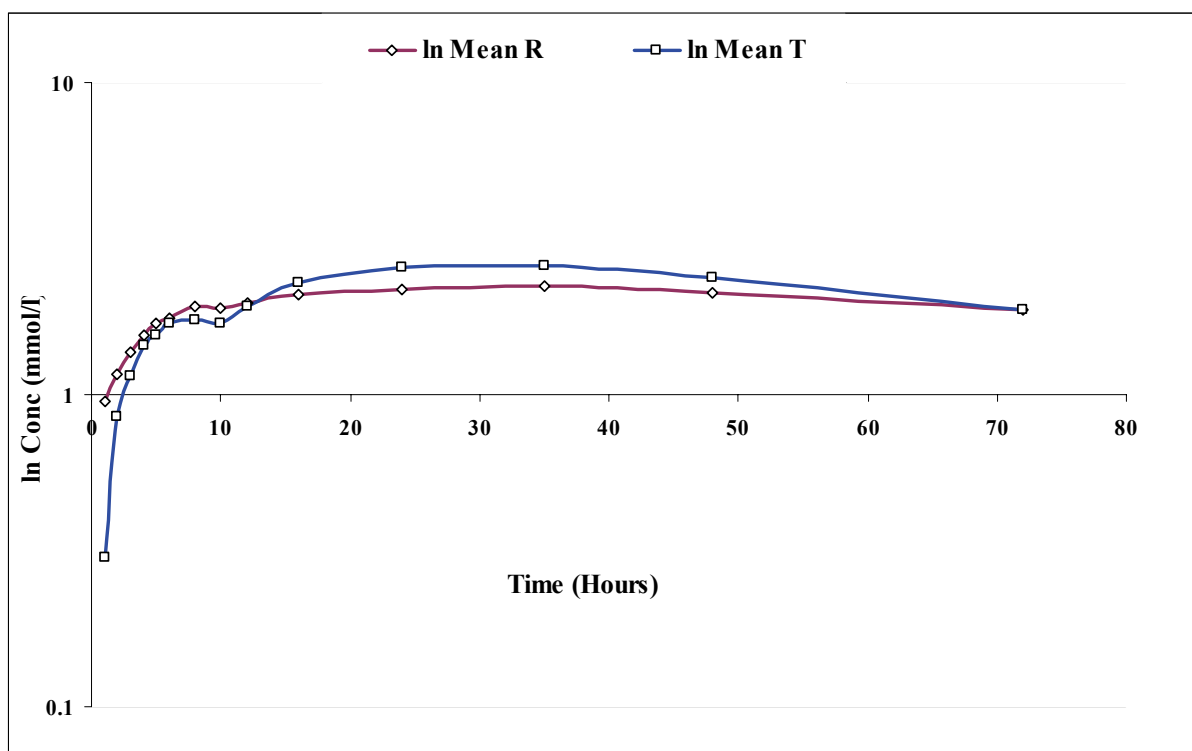


Figure 8.4 Semi-logarithmic plot of the mean (n=2) plasma concentration of CBZ for both test and reference products.

CBZ is very sensitive to moisture and the degree of hydration will affect *in vitro* and *in vivo* dissolution rates and the altered dissolution rate may result in the incidence of adverse side-effects such as, for example, sedation. The altered dissolution rates could account for the seemingly erratic plasma profile observed for the reference product for both subjects depicted in Figure 8.5, where the sustained-release beads in the dosage form may release CBZ at varying rates which, may account for the erratic plasma profile seen for subject one at twelve hours following administration of Tegretol[®] CR. Tegretol[®] CR uses coated beads compressed into a tablet and this may pass through the pyloric sphincter in a random manner further complicating the *in vivo* release and absorption patterns of CBZ. The use of different controlled-drug release technologies between the test and reference products may account for individual differences observed between the two subjects, for both products.

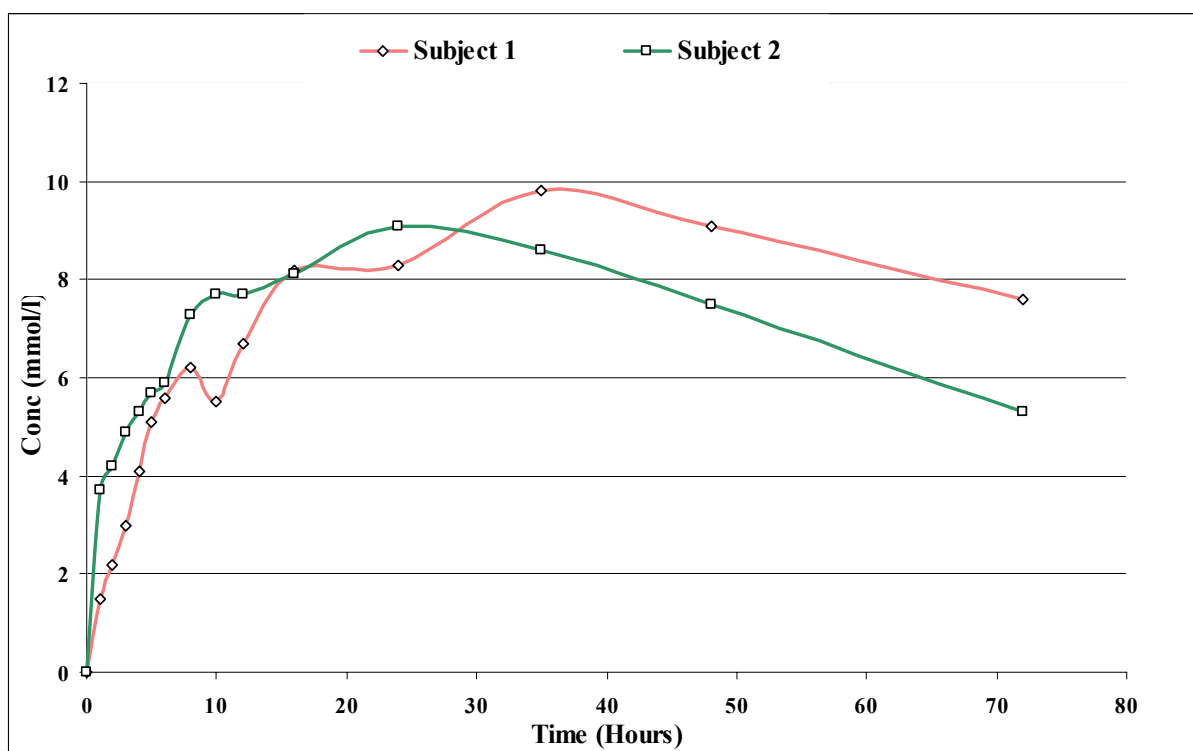


Figure 8.5 Plasma CBZ concentrations obtained following administration of the reference product, Tegretol[®] CR to subjects 1 and 2.

The blood level profiles for the test formulations in both subjects are depicted in Figure 8.6. It is obvious that there is a significant increase in the amount of CBZ that is absorbed approximately 12 hours after dosing. The steep rise in drug levels may be a consequence of the physical collapse of the dosage form due to peristaltic forces present within the GIT. Therefore, a large dose of the API becomes readily available for absorption. Since this phenomenon seems to have occurred in both subjects, it is highly likely to be a function of the dosage form design rather than due to physiological factors that can affect dosage form performance. Furthermore this increased release of CBZ corresponds closely, with the onset and report of drowsiness experienced by the subjects during these studies.

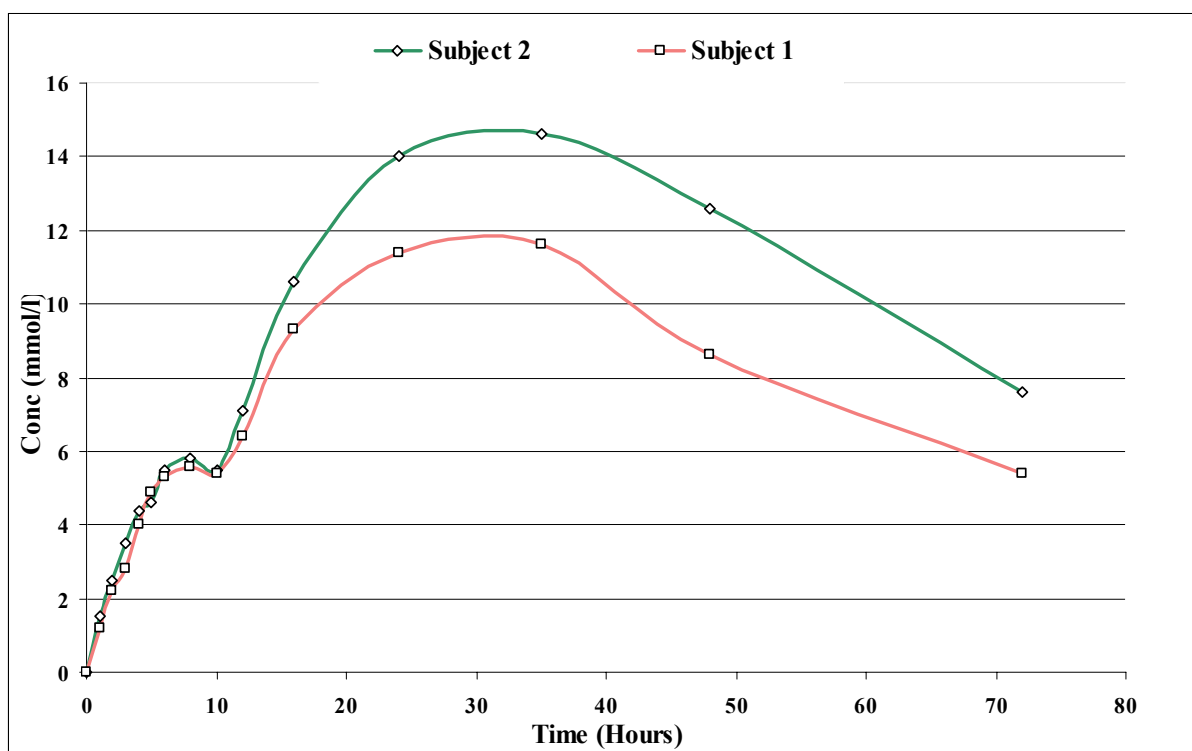


Figure 8.6 Plasma CBZ concentrations obtained following administration of the test matrix tablets for subjects 1 and 2

The reference product, Tegretol[®] CR disintegrates within fifteen minutes during *in vitro* dissolution testing, to release beads that deliver CBZ in a controlled manner thereafter. Therefore, the transport and integrity of the beads may not be significantly affected when passing through the pyloric sphincter and thus the sharp rise in CBZ levels is not observed in the plasma profiles obtained following the oral administration of the reference product, Tegretol[®] CR. Furthermore the dramatic increase in CBZ blood levels observed at approximately 12 hours after oral administration of the test matrix tablets corresponds closely, with the onset and report of drowsiness experienced by the subjects during these studies.

8.10 CONCLUSION

The primary purpose of this bioequivalence study was to show via ‘proof of concept’ that the sustained release test formulations, *viz.*, the HPMC matrix tablets administered to two subjects clearly released sufficient CBZ, such that it was bioavailable and blood levels could be monitored. The results revealed that the formulation has the potential for further testing in a larger study population and clearly has commercial potential and ability of these dosage forms to effectively deliver CBZ in a sustained manner.

It was not possible to conduct an ANOVA assessment of the blood level data since only two subjects were used in this study and in order to conduct an appropriate statistical analysis with sufficient power a larger study population would be required.

The ultimate purpose of these studies was to provide a dosage form that could effectively control the release of CBZ at a rate comparable to that of Tegretol[®] CR. The matrix tablets tested in the two subjects showed similar release rates to that of Tegretol[®] CR especially over the first twelve hours of after dosing.

The use of different drug release technologies for the same route and form of delivery can show variations between test and reference products. The ultimate objective is to achieve an overall release rate comparable to that of the reference product and to ensure that the extent of absorption as reported by AUCT become useful for the comparison of two seemingly dissimilar plasma profiles. The dramatic increase in the plasma levels observed at approximately twelve hours after administration was observed for both subjects and this is more than likely a function of dosage form design and *in vivo* performance than to the influence of physiological conditions.

The tablets produced for *in vivo* testing were large biconvex tablets weighing approximately 800mg. To avoid the sharp increase in drug levels that was observed, it may be possible to reduce the size of the dosage form or alter the shape of the dosage form to allow for easier transit through the GIT and thus avoid disruption to the integrity of the dosage form where dose dumping may become an issue during the latter stages of a dosing interval during drug therapy.

These tablets have shown release rates similar to that of Tegretol[®] CR during the first twelve hours which strongly suggests that these tablets may be a potential generic equivalent to Tegretol[®] CR for the treatment of epileptic patients. However, further comprehensive investigations must be undertaken to establish such equivalence.

The formulation components of the matrix tablets produced by the direct compression method of manufacture as described in Section 6.2.3.2.2, effectively release CBZ in a controlled and sustained manner. As mentioned previously, adjustments to the dosage form configuration may reduce the possibility of dose dumping from these matrix tablets but may affect the rate and mechanism of drug release and therefore, further assessments would be necessary.

Ultimately, the availability of generic equivalent products for patients requiring anti-epileptic therapy will reduce the cost of chronic therapy and may in some cases, enhance therapy through the availability of dosage forms that perform better than the innovator product.

CHAPTER 9

CONCLUSIONS

The objectives of these studies were to develop and assess sustained-release formulations of a hydrophobic API, *viz.*, CBZ. Sustained-release drug delivery systems are designed to deliver a constant amount of drug in the GIT, which in turn allows for the controlled absorption and subsequent maintenance of relatively constant plasma concentrations, which is vital for a drug such as CBZ that has a narrow therapeutic range. In the case of CBZ, a reduction of plasma level fluctuations will decrease the incidence and severity of associated side effects such as drowsiness, general obtundity, ataxia, nystagmus, diplopia and headache, in particular, since these side effects are dose-dependent.

The clinical use of sustained-release dosage forms can enhance anti-epileptic therapy through a combination of improving patient compliance by reducing the dosing frequency and optimizing drug delivery through the controlled and predictable release of CBZ. Therefore the development of a generic CBZ sustained release dosage form that would be equivalent to a commercially available sustained release CBZ dosage form, *viz.*, Tegretol[®] CR, was undertaken.

A modular HPLC system was used for the development and validation of an isocratic method for the analysis and quantitation of CBZ in pharmaceutical dosage forms and was applied to the assessment of dosage form performance. The analytical method was developed and validated according to scientifically established acceptability criteria and was found to be selective, sensitive, precise and accurate for the evaluation of the *in vitro* performance of test and reference products.

The moisture content of the CBZ, excipients and selected batches was accurately determined using a standard Karl Fischer titration method. DSC thermograms revealed no significant thermal shifts for CBZ, when it was assessed in combination with the excipients intended for use in tablet production. Furthermore, XRPD studies revealed that CBZ did not show polymorphic transitions during blending, granulation and

and tableting and therefore, it was concluded that CBZ release rates were a function of dosage form design and composition rather than transformation to a low solubility polymorph during production.

Dissolution testing using USP Apparatus 3 was found to be more applicable than USP Apparatus 2, for the characterization of *in vitro* dissolution rate profiles of the hydrophilic matrix sustained release dosage forms. It is likely that exposure of test tablets to fresh dissolution media, at predetermined times, prevented the formation of saturation conditions and enabled greater discrimination when testing formulations of different composition, than that achieved using USP Apparatus 2. Furthermore, the use of USP Apparatus 3 is a more stringent test procedure that has a high degree of precision, and facilitates the use of a more realistic dissolution environment, through simulation of the GIT pH range and peristalsis. In addition, the exposure of dosage forms to a range of media of different pH was considered valuable and more meaningful for assessing dosage form performance than exposure to a single dissolution medium, as is the case if USP Apparatus 2 were used to assess the dissolution rates of CBZ from the test and reference products, used in these studies.

Dissolution rate profiles were compared using the f_1 and f_2 difference and similarity factors and the use of these factors allowed for effective and objective assessment of the release rate profiles. In addition, the fit factors were used as a key criterion when designing and developing the ANN and were integral when attempting to find appropriate solutions during the application of the ANN to optimise formulation compositions.

Matrix tablets were initially manufactured by wet granulation and the resultant percent CBZ released *in vitro* was found to be less than 65% of the total content. Residual content analysis undertaken on tablet cores that remained following dissolution testing revealed that the low percent CBZ released was more than likely due to the design and composition of the formulation and not due to low CBZ content. Therefore, direct compression manufacture was considered as an alternate method of production and was investigated for its potential to produce batches of these tablets.

The conversion of a wet granulation formulation to a direct compression composition proved successful and the direct compression method of manufacture was adopted for all future manufacture. Several excipients and associated factors of potential formulations were assessed for their impact on CBZ release rates and adjustments were made to the formulations based on the resultant dissolution profiles, where drug release was either enhanced or prolonged through manipulation of the specific variable being assessed. The effects of the factors evaluated, served as a reference to determine whether these variables significantly affected CBZ release rates from the early test formulations. The effect of using fine particle size grade CBZ on release performance from these dosage forms was also ascertained and further optimisation of formulations was achieved by use of the fine and coarse grade CBZ in different combinations. Variation of the proportions of the different particle size grades of CBZ, yielded more defined drug release rate profiles that could be modified depending on the content of the respective particle size grade of CBZ used.

The effects of tablet components such as the type, grade and proportion of the HPMC polymer used during formulation, significantly influenced drug release rates from these matrix tablets where the use of a lower viscosity grade HPMC revealed a greater rate and extent of CBZ release from tablets. The incorporation of a surfactant in the formulation composition even at low concentrations further enhanced the rate and extent of drug release from these dosage forms.

Test tablets from the batch produced for *in vivo* testing and stored under accelerated conditions showed the greatest increase in moisture content compared to tablets from the same the batch stored under ambient conditions, had a moisture content well within specification. The results of preliminary stability testing provided insight into the potential long term viability of these tablets and revealed that the packaging would be a critical consideration during future formulation development studies and where the use of a protective non-functional aesthetic coating may also be required to maintain the physical integrity and acceptability of tablets for an extended period of time, in particular in high humidity climates.

The mechanisms by which drug was released from the formulations produced during these studies was found to be diffusion controlled or to occur via a process termed anomalous transport, when evaluating the release exponent n , derived from the Korsmeyer-Peppas model. Several of the formulations studied showed evidence of diffusion controlled release as square root of time plots for the first six hours and in a number of cases over the entire 22-hour dissolution test period were found to be linear following least squares linear regression analysis. However matrix dissolution or erosion, which is an important feature of swellable and erodible systems, is not considered in the Higuchi model and therefore mechanistic information about drug release that is derived using this model should be viewed and assessed with caution [103].

Values of n , derived following modelling of drug release data, that are suggestive of diffusion-controlled release are more than likely reflective of polymeric erosion rates that are slower than the rate of release of CBZ from the tablets and therefore diffusion of CBZ within the dosage form is a far more significant than the effects of polymeric dissolution, at that stage of the release process. These effects become formulation specific since different formulations produced different results following assessment of their dissolution profiles using modelling techniques.

A 'first-order decay' model was developed and applied to the assessment of the resultant dissolution data. The model is able to describe drug release for the entire dissolution profile whereas the 'power law' as described by Peppas *et. al.* is only applicable to the first 60% of drug release. The parameters of the 'first-order decay' model were also related to the 'power law' and strong correlations were found to exist between the n values derived from the 'power law' and the r values derived from the 'first-order decay' model. The correlations that have been derived, suggest that the r values can be used to gain insight into mechanistic information pertaining to CBZ release when using model. Consequently the 'first-order decay' model provides another mathematical tool to predict and describe drug release from the HPMC based matrix tablets.

Polymer erosion and dissolution may become more significant in affecting drug release where the concentration and viscosity grade of the polymer become relevant in determining gel layer thickness and degree of erosion during extended dissolution testing.

Synchronisation of the swelling and erosion fronts during the release process is associated with zero-order drug release and this is true for drug release from surface erodible systems with flat sheet geometry. CBZ release from tablets produced during these studies did not show zero-order drug release ($n = 1$), more than likely as a result of the geometry of these dosage forms. The biconvex shape of the tablets produced during these studies makes it theoretically impossible for CBZ to be release via a zero-order release process, since as the system erodes, which is the case with most polymers the surface area exposed to the dissolution fluid continually decreases, *i.e.*, the diffusion path for drugs increases in length.

The resultant values of $n = 1$ for the matrix devices developed and assessed during these studies emphasize that release from these dosage forms is not Fickian controlled and implies large contributions to drug release kinetics by erosion of the polymer.

An ANN was designed and developed based on the existing CBZ release rate data that had been obtained during the early phases of these studies. The ANN was trained and applied to the prediction of formulation compositions and associated dissolution rate profiles. The ANN that as developed added to the versatility of dosage form design and provided another tool to predict formulation composition to match specific drug release rate profiles, such as for example that of the reference product, Tegretol[®] CR. The parameters introduced to the ANN and subsequently used to train the network did not include manufacturing process effects, such as for example, method of manufacture and the effects of tablet hardness on drug release rates. Additional comprehensive and tailored experimental studies would have to be conducted to create a network that would predict formulations for which consideration of these factors have been included. The formulation that was ultimately adopted for *in vivo* analysis, was an empirically modified version of a formulation predicted by the ANN. The formulations developed prior to the

application of the ANN were not comparable to that of the Tegretol[®] CR tablets, during the first hour of dissolution testing. However, although the neural network predicted formulation had to be further adjusted, the resultant *in vitro* dissolution profile of CBZ from the tablets produced from the predicted formulation, matched that of CBZ from Tegretol[®] CR, especially during the first hour of the test. The ANN was designed such that the highest weighting for the f_1 and f_2 fit factors were applied to the release rate data for the first hour of dissolution testing, so as to match more precisely the release rate of CBZ from the Tegretol[®] CR tablets during this time.

The size of the network training data set of 36 input and output data pairs, can be considered small and in conjunction with insufficient weight allocation may account for the discrepancies observed between the ANN predicted and actual target results.

The resultant sustained-release matrix tablets were designed with the intention for use in human subjects. Therefore, since the test and reference product dissolution rate profiles were similar, additional tablets were produced and assessed *in vivo*. The primary purpose of the *in vivo* study was to show that the sustained release test formulation, *viz.*, the HPMC matrix tablets released sufficient CBZ, such that it was bioavailable and that blood levels could be monitored. Furthermore, the same subjects were administered Tegretol[®] CR tablets for comparative purposes. The results following analysis of plasma samples revealed that the test formulation has potential for further testing in a larger study population and is clearly a commercial prospect. Furthermore the test product can effectively deliver CBZ in a sustained manner over a 24-hour period and may be further assessed for its potential to be used as a once-daily delivery system.

Although bioequivalence could not be established due to the small study population and low power of the study, the results of the *in vivo* testing in two human subjects were considered adequate to show that these dosage forms produced plasma concentration versus time profiles for CBZ that are comparable to that obtained when Tegretol[®] CR tablets were administered. The ultimate purpose of these studies was to provide a dosage form that could effectively control the release of CBZ at a rate comparable to that of

Tegretol[®] CR. The matrix tablets tested in the two subjects showed similar release rates to that of Tegretol[®] CR especially over the first twelve hours following oral administration. It is clear that the potential exists for these dosage forms to release their drug load in a sustained and controlled fashion without ‘dose-dumping’ immediately following administration and that would have become evident by subjects presenting with severe and adverse side effects.

The availability of generic products for patients requiring anti-epileptic therapy will reduce the cost of chronic therapy and may in some cases, enhance therapy through the availability of dosage forms that perform *in vivo* such that they are equivalent to the innovator product.

Further development and assessment of the aforementioned dosage forms are required to ascertain whether improvements to the formulation can be made such that stability, *in vitro* and *in vivo* performance can be enhanced. In addition, the results of the *in vivo* study revealed that the HPMC based polymeric tablets produced in these studies have the potential for being developed into a product that performs better than the innovator product. Furthermore, it may be possible to achieve similar plasma concentration time profiles to that obtained for CBZ from the Tegretol[®] CR tablets using a lower dose of CBZ, which would reduce the possibility and incidence of unwanted side effects that may reduce patient compliance.

REFERENCES

1. *Antiepileptic Drugs*. Woodbury, D.M., Penry, J.K., Pippenger, C.E. (eds.). Second Ed, 1982, Raven Press, New York, pp. 453 – 547.
2. *The Merck Manual*, de Korte D.F. (ed.), Merck and Co., 1992, pp 1436-1444.
3. *Basic and Clinical Pharmacology*, Katzung B.G. (ed.), Appleton and Lange, 7th Edition, 1998. pp 386-393.
4. Isaacson, E.I. Central Nervous System Depressants. In *Textbook of Organic Medicinal and Pharmaceutical Chemistry*. Block, J.H., Beale Jr., J.M. (eds). Lippincott, Williams, Wilkins, 2004, pp.506.
5. *Therapeutic Drugs*. Boobis, A.R., Burley, D., Davies, D.M., Davies, D.S., Harrison, P.I., Orme, M.L'.E., Park, B.K., Goldberg, L.I. Vol. 1, Churchill Livingstone, New York, 1991, pp. 49-53.
6. *Goodman and Gilman's The Pharmacological Basis of Therapeutics*. McNamara, J.O., Hardman, J.G., Limbird, L.E. (eds.). Tenth Ed. McGraw-Hill, 2001, pp. 521-535.
7. Winter, M.E. Carbamazepine. In *Basic Clinical Pharmacokinetics*. Koda-Kimble, M.A, Young, L.Y. (eds.), 2nd Ed. Applied Therapeutics, Inc. 1990, pp. 7-78,139-145.
8. Wangemann, M., Retzow, A., Evers, G., Mazur, D., Schug, B., Blume, H. *Bioavailability Study of Two Carbamazepine Containing Sustained Release Formulations after Multiple Oral Dose Administration*. *Arzneim.-forsch./Drug Res*. Vol. **48**(12), 1998, pp.1131-1137.
9. *United States Pharmacopoeia incorporating 'The National Formulary'*, United States Pharmacopoeial Convention, Washington, 28th Edition, 2005, pp. 341-344, 2411-2420, 2501-2510, 2670.
10. *The British Pharmacopoeia Incorporating the requirements of the 5th Edition of the European Pharmacopoeia 2004 as amended by Supplements 5.1 and 5.2*, The Stationery Office, London, 2005, pp. 348-350.

11. *Clinical Neuropharmacology*. Kutt, H., McDowell, F. Churchill Livingstone, New York, Vol. 4, 1979, pp. 18-20, 40-45.
12. MacKichan, J.J., Kutt, H. Carbamazepine Therapeutic Use and Serum Concentration Monitoring. In *Individualising Drug Therapy, Practical Applications of Drug Monitoring*, Vol. 2. Taylor, W.J., Finn, A.L. (eds.), Gross, Townsend, Frank, Inc., New York, 1981, pp. 2-22.
13. *Analytical Profiles of Drug Substances*. Aboul-Enein, H.Y., Al-Badr, A.A. Florey, K. (ed.). Vol. 9, Academic Press, 1980, pp. 87-103.
14. *Martindale: The Extra Pharmacopoeia*. Pharmaceutical Press, London, 33rd Ed., 2002, pp. 342-347.
15. Lefebvre, C., Guyot-Hermann, A.M. *Polymorphic Transitions of CBZ during Grinding and Compression*. Drug Dev. Ind. Pharm., Vol. 12(11-13), 1986, pp. 1913-1927.
16. Tanninen, V.P., Luhtala, S., Yliruusi, J. *X-ray Powder Diffraction Line Profile Analysis in the study on the effect of Surface Active Agent on the Cristal Properties of Carbamazepine*. Acta Pharm. Nord. Vol. 4(4), 1992, pp. 277-282.
17. Davis, T.D., Peck, G.E., Stowell, J.G., Morris, K.R., Byrn, S.R. *Modeling and Monitoring of Polymorphic Transformations During the Drying Phase of Wet Granulation*. Pharm. Res. Vol. 21(5), 2004, pp. 860-866.
18. Spina, E., Pisanti, F., Perucca. *Clinically Significant Pharmacokinetic Drug Interactions with Carbamazepine*. Clin. Pharmacokinet. Vol. 31(3), 1996, pp. 198-214.
19. Nimmo, W.S. *Drugs Affecting the Autonomic and Central Nervous Systems*. In *Clinical Pharmacology*, 24th ed. Girdwood, R.H. (ed.), Cassell Ltd., New York, 1979, pp. 290, 401.
20. *Therapeutic Drug Monitoring*, Widdop, B. (ed.), Churchill Livingstone, New York, 1985 pp.11,67, 93, 135-147.
21. Retzow, A., Schurer, M., Schulz, H.-U. *Influence of Food on the Bioavailability of a Carbamazepine Slow-Release Formulation*. Int. J. Clin. Pharmacol. Ther., Vol. 35(12), 1997, pp.

557-560.

22. Perrucca, E., Richens, A. Antiepileptic Drugs: Clinical Aspects. In *Therapeutic Drug Monitoring*. Richens, A., Marks, V. (eds.), Churchill Livingstone, London, 1981, pp. 322, 329, 331-335, 357, 350.
23. *Drug Level Monitoring, Analytical Techniques, Metabolism and Pharmacokinetics*. Sadee`, W., Beelen, G.C. John Wiley and Sons Inc. 1980, pp. 162.
24. Parant, F., Bossu, H., Gagnieu, M.C., Lardet, G., Moulisma, M. *Cross-Reactivity Assessment of Carbamazepine-10,11-epoxide, Oxcarbazepine, and 10-Hydroxy-Carbamazepine in Two Automated Carbamazepine Immunoassays: PETINIA and EMIT 2000*. *Therap Drug Monit.* Vol. **25**, 2003, pp. 41-55.
25. *South African Medicines Formulary*, C.J. Gibbon (ed.), South African Medical Association, health and Medical Publishing Group, 2000, pp375, 380-381.
26. Baer, D.M., Key Points for Interpretation of Drug Concentrations. In *Therapeutic Drug Monitoring*. Baer, D.M., Dito, W.R. (eds.), American Society of Clinical Pathologists, 1981, pp. 350-355.
27. *Pharmaceutical Analysis*. Watson, D.G. Churchill Livingstone, London, 1999, pp. 239-276.
28. *High Performance Liquid Chromatography*. Lindsay, S., Kealey, D. (ed). Wiley & sons, London, 1987.
29. Engelhardt, H., Elgass, H. *High Performance Liquid Chromatography: Advances and Perspectives*. Vol. **2**. Horvarth, C.(ed). Academic Press Inc., New York, 1980.
30. R.J. Hamilton, R.J., Sewell, P.A. *Introduction to High Performance Liquid Chromatography*. 2nd Ed, Chapman and Hall, New York, 1982.
31. *Encyclopedia of Pharmaceutical Technology*, Vol. **2**. Swarbrick, J., Boylan, J.C. Marcell Dekker Inc., New York and Basel, 1990, pp. 437-445.

32. *Waters Chromatography Columns and Supplies Catalogue 1999-2000*. Waters Corporation, USA, pp. 44-55.
33. *Instrumental Methods of Analysis* 7th Ed. Willard, H.W., Merritt Jr. L.L., Dean, J.A., Settle Jr. F.A. Wadsworth Publishing Company, Belmont, California, 1988, pp. 626-644.
34. Al-Zein, H., Riad, L.E., Abd-Elbary, A. *Effect of Packaging and Storage on the Stability of Carbamazepine Tablets*. *Drug Dev. Ind. Pharm.* Vol. **25**(2), 1999, pp. 223-227.
35. Etman, M.A. *Effect of a Bulk Forming Laxative on the Bioavailability of Carbamazepine in Man*. *Drug Dev. Ind. Pharm.* Vol. **21**(26), 1995, pp. 1901-1906.
36. Choudhury, S., Nelson, K.F. *Improvement of Oral Bioavailability of Carbamazepine by Inclusion in 2-hydroxypropyl- β -cyclodextrin*. *Int. J. Pharm.* Vol. **85**, 1992, pp. 175-180.
37. Matthews, G.P., Lowther, N., Shott, M.J. *Crystallisation of Carbamazepine in Tablets stored at Elevated Temperatures*. *Int. J. Pharm.* Vol. **50**, 1989, pp. 111-115.
38. Dumortier, G., Pons, D., Zerrouk, A., Januel, D., Saba, G., Degrasat, K. *Concomitant HPLC Method for Determination of Lamotrigine, Carbamazepine and 10,11-carbamazepine epoxide in Plasma using dual 240-220nm Wavelength Detection*. *J. Liq. Chrom. Rel. Tech.* Vol. **24**(20), 2001, pp. 3171-3180.
39. Meyer, V.R., Majors, R.E. *Minimising the Effects of Sample Preparation on Measurement Uncertainty*. *Liq. Chrom. Gas Chrom.* Vol. **20**(2), 2002, pp. 106-112.
40. Wysocki, J. *What drives a separation – the stationary phase or the mobile phase?* *Liq. Chrom. Gas. Chrom.* Vol. **19**(11), 2001, pp. 1150-1159.
41. Paul, W.L. *USP Perspectives on Analytical Methods Validation*. *Pharm. Tech.*, Vol. **15** 1991, pp. 130-141.

42. Shah, V.P., Midha, K.K., Dighe, S., McGilveray, I.J., et. al. *Analytical Methods Validation: Bioavailability, Bioequivalence and Pharmacokinetic Studies*. Int. J. Pharm. Vol. **82**, 1992, pp.-7.
43. Skoug, J.W., Halstead, G.W., Theis, D.L., Freeman, J.E., Fagan, D.T. *Strategy for the Development and Validation of Dissolution Tests for Solid Oral Dosage Forms*. Pharm. Tech., Vol. **20**. 1996, pp. 58-68.
44. Gent, C.L. *A System Developers Approach to Validation*. Pharm. Tech. Vol. **26**(7), 2002, pp. 62-66.
45. El-Zein, H., Riad, L., Abd El-Bary, A. *Enhancement of Carbamazepine Dissolution: In Vitro and In Vivo Evaluation*. Int. J. Pharm. Vol. **168**, 1998, pp. 209-220.
46. Bundgaard, H., Johansen, M., Stella, V., Cortese, M. *Pro-drugs as drug delivery systems XXI. Preparation, physicochemical properties and bioavailability of a novel water-soluble pro-drug type for Carbamazepine*. Int. J. Pharm. Vol. **10**, 1982, pp. 181-192.
47. Lanchote, V.L., Bonato, P.S., Campos, G.M., Rodrigues, I. *Factors Influencing Plasma Concentrations of Carbamazepine and Carbamazepine-10,11-Epoxyde in Epileptic Children and Adults*. Ther. Drug. Monit. Vol. **17**, 1995, pp. 47-52.
48. Matsuda, Y., Akazawa, R., Teraoka, R., Otsuka, M. *Pharmaceutical Evaluation of Carbamazepine Modifications: Comparative Study for photostability of Carbamazepine Polymorphs by using Fourier-transformed Reflection-absorption Infrared Spectroscopy and Colorimetric Measurement*. J. Pharm. Pharmacol. Vol. **46**, 1994, pp. 162-167.
49. Reith, D.M., Hooper, W.D., Parke, J., Charles, B. *Population pharmacokinetic modeling of steady state Carbamazepine clearance in children, adolescents, and adults*. J. Pharmacokinet. Pharmacodynam. Vol. **28**(1), 2001, pp. 79-92.
50. Wilson, T. *Liquid Chromatographic Methods Validation for Pharmaceutical Products*. J. Pharm. Biomed. Anal. Vol. **8**(5), 1990, pp. 389-400.
51. Hokanson, G.C. *A life cycle approach to the validation of analytical methods during pharmaceutical development. Part I: the initial method validation process*. Pharm. Tech. Vol. **18**,

- 1994, pp. 118-15.
52. *Analytical Method Development and Validation*. Swartz, M.E, Krull, I.S. Marcell Dekker Inc., 1997, pp. 17-83.
53. <http://www.nihs.go.jp/dig/ich/quality/q2a/q2astep4.pdf> Retrieved 20/05/02.
54. <http://www.fda.gov/cder/guidance/2396dft.htm> Retrieved 20/05/02.
55. *Quantitative Chemical Analysis*. Harris, D.C. Freeman and Company, San Francisco, 1982, pp. 459 – 461.
56. *Vogel's Textbook of Quantitative Chemical Analysis* 5th Ed. Jeffery, G.H., Bassett, J., Mendham, J., Denney, R.C. Longman Scientific and Technical. John Wiley and Sons, Inc., New York, 1989, pp. 637 – 638.
57. *Introduction to Thermal Analysis, Techniques and Applications* 2nd Ed. Brown, M.E. (ed.), Kuwer Academic Publishers, Netherlands, 2001, pp. 55 – 90.
58. Vueba, M.L., Veiga, F., Sousa, J.J., Pina, M.E. *Compatibility studies between ibuprofen or ketoprofen with cellulose ether polymer mixtures using thermal analysis*. Drug Dev. Ind. Pharm. Vol. **31**, 2005, pp. 943-949.
59. Otsuka, M., Ishii, M., Matsuda, Y. *Effect of Surface modification on hydration kinetics of carbamazepine anhydrate using isothermal microcalorimetry*. AAPS PharmSciTech, Vol. **4**(1), 2003, pp. 33-41.
60. Brown, M.E., Antunes, E.M., Glass, B.D., Labete, M., Walker, R.B. *DSC Screening of Potential Prochlorperazine-Excipient Interactions in Preformulation Studies*. J. Therm. Anal. Calorimetry. Vol. **56**, 1999, pp. 1317 – 1322.
61. Botha, S.A., Lotter, A.P. *Compatibility Study between Oxprenolol Hydrochloride, Temazepam and Tablet Excipients using Differential Scanning Calorimetry*. Drug Dev. Ind. Pharm. **16**(2), 1990, pp. 331 – 345.

62. Gosselin, P.M., Thibert, R., Preda, M., McMullen, J.N. *Polymorphic Properties of micronized carbamazepine produced by RESS*. Int. J. Pharm. Vol. **252**, 2003, pp. 225-233.
63. Lefebvre, C., Guyot-Hermann, A.M. *Polymorphic Transitions of Carbamazepine during Grinding and Compression*. Drug Dev. Ind. Pharm. Vol.**12**(11-13), 1986, pp. 1913 – 1927.
64. Koester L.S., Xavier, C.R., Mayorga, P., Bassani, V.L. *Influence of β -cyclodextrin complexation on carbamazepine release from hydroxypropyl methylcellulose matrix tablets*. Eur. J. Pharm. Biopharm. Vol. **55**, 2003, pp. 85-91.
65. Wells, J.I. *Tablet Testing*. In *Encyclopedia of Pharmaceutical Technology*. Swarbrick, J., Boylan, J.C. (eds), Marcel Dekker Inc., New York, Vol. **14**, 1996, pp. 401-418.
66. Augsberger, L.L., Zellhofer, M.J. *Tablet Formulation*. In *Encyclopedia of Pharmaceutical Technology*. Swarbrick, J., Boylan, J.C. (Eds), Marcel Dekker Inc., New York, Vol. **14**, 1996, pp. 385-400.
67. I. Kanfer, R.B. Walker and P. Persicaner. *Experimental Formulation Development*. In *Generic Drug Product Development: Solid Oral Dosage Forms*, Shargel L and Kanfer I (eds), Drugs and the Pharmaceutical Sciences Series, Volume **143**, Marcel Dekker, New York, 2005.
68. Dressman, J. *Future Directions for academic research in dissolution testing*. Dissolution Technology August:9, 1999.
69. Guo, J.H., Harcum, W.W., Skinner, G.W., Dluzenski, P.R., Trumbull, D.E. *Validation of tablet dissolution method by high performance liquid chromatography*. Drug Dev.. Ind. Pharm. Vol. **26**(3), 2000, pp. 337-342.
70. Madden, H., Butler, J., Polli, J., Madden, S., Corrigan, O., Devane, J. *Impact of dissolution medium on the release of a highly soluble drug from a hydrophilic matrix tablet*. PharmSci AAPS, Virginia, Vol. **1**, 1998, pp 643.
71. *Guidance for Industry: Dissolution Testing of Immediate Release Solid Oral Dosage Forms*. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug

Evaluation and Research (CDER), August 1997. <http://www.fda.gov/cder/guidance/1713bp1.pdf>

72. Yuen, K.H. Peh, K.K., Tan, B.L. *Relating in vitro-in vivo data of two controlled release metformin formulations*. Drug Dev. Ind. Pharm. Vol. **25**(5), 1999, pp. 613-618.
73. Siewart, M. *Role of dissolution testing in the pharmaceutical industry*. Dissolution Technology, Vol. **8**, 1999.
74. Banaker, U. *Factors that influence dissolution testing*. In Pharmaceutical Dissolution Testing, Swarbrick, J. (ed.), Marcel Dekker, New York, 1992, pp. 133-187.
75. Borst, I., Ugwu, S., Beckett, A. *New and Extended Applications for USP Drug Release Apparatus 3*. Dissolution Technologies, Vol. **4**(1), 1997.
76. Kieser, L.F., Walker, R.B. *Dissolution testing of Metoprolol Tartrate sustained release matrix tablets: Comparison of USP Apparatus 2 (paddles) and 3(BIO-DIS)*. Presented at the Annual Meeting of the Controlled Release Society, Seoul, Korea, July, 2002.
77. Rescigno, A. *Bioequivalence*. Pharm. Res. Vol. **9**(7), 1992, pp. 925-928.
78. Tsong, Y., Hammerstrom, T., Sathe, P., Shah, V.P. *Statistical assessment of mean differences between two dissolution data sets*. Drug Inf. Journ. Vol. **30**, 1996, pp. 1105-1112.
79. Wang, M-T., Tsa, F-H., Wang, D-P. *Formulation optimization of controlled release pellets of metaclopramide hydrochloride using dissolution fit factor approach*. Drug Dev. Ind. Pharm. Vol. **26**(5), 2000, pp.577-581.
80. Shah, V.P., Tsong, Y., Sathe, P., Williams, R.L. *Dissolution comparison using similarity factor f_2* . Dissolution Technology, Vol.**15**, 1999.
81. Moore, J.W., Flanner, H.H. *Mathematical comparison of dissolution profiles*. Pharm. Tech. Vol. **20**(6), 1996, pp. 64-70.

82. Polli, J.E., Rekhi, G.S., Shah, V.P. *Methods to compare dissolution profiles*. Drug Info. Journ. Vol. **30**, 1996, pp.1113-1120.
83. Langenbucher, F. *IVIVC: Indices for comparing release and response profiles*. Drug Dev. Ind. Pharm. Vol. **25**(11), 1999, pp. 1223-1225.
84. *Introduction to Pharmaceutical Dosage Forms*. 3rd Ed. Ansel H.C. Lea & Febiger, Philadelphia, USA, 1981, pp. 189-219.
85. Shah, V.P. *et al. In Vitro Dissolution Profile Comparison – Statistics and Analysis of the similarity factor f_2* . Pharm. Res. Vol. **15**(6), 1998, pp. 889-996.
86. *Guidance for Industry: Immediate release solid oral dosage forms. Scale-Up and Post-Approval Changes: Chemistry, Manufacturing and Controls, In Vitro Dissolution Testing, and In Vivo Bioequivalence Documentation*. U.S. Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and research (CDER), November 1995, <http://www.fda.gov/cder/guidance/cmc5.pdf>
87. Gohel, M.C., Panchel, M.K. *Comparison of in vitro dissolution profiles*. Pharm. Tech. Vol. **24**(3), 2000, pp. 92-102.
88. Charman S.A, Charman, W.N. *Oral Modified-Release Delivery Systems*. Rathbone, M.J., Hadgraft, J., Roberts (eds.), M.S. Modified-Release Drug Delivery Technology. Marcel Dekker Inc., NY, Vol. **126**, 2003, pp 1 – 8.
89. Vendatraman, S., Dava, N., Chester, A., Kleiner, L. An Overview of Controlled Release Systems. In *Handbook of Pharmaceutical Controlled Release Technology*. Wise, D.L. (ed). Marcel Dekker, New York, 2000, pp. 435-445.
90. Kanjickal, D.G., Lopina, S.T. *Modeling of Drug Release from Polymeric Delivery Systems – A Review*. Critical Reviews in Therapeutic Drug Carrier Systems, Vol. **21**(5), 2004, pp. 345-386.
91. Kosmidis, K., Argyrakis, P., Macheras, P. *Fractal Kinetics in Drug Release from Finite Fractal Matrices*. J. Chem. Phys. Vol. **119**(12), 2003, pp. 6373-6377.

92. Shah, A.C. Design of Oral Sustained Release Drug Delivery Systems: In-Vitro/In-Vivo Considerations. Yacobi, A., Halperin-Walega, E. (eds). In *Oral Sustained Release Formulations: Design and Evaluation*. Pergamon Press, NY, pp 35 – 40.
93. Bhalla, H.L., Handa, A.K. *Development and Evaluation of Controlled Release Tablets of CBZ. Indian Drugs*, Vol. **36**(2), 1999, pp. 100 – 105.
94. *Sustained Release Medications*. Johnson, J.C. (ed.), Chemical Technology Review. Noyes Data Corporation, No. 177, 1980, pp 109 – 110.
95. *Drug Formulation*. Racz, I. John Wiley and Sons, NY, 1989, pp 359 – 381.
96. Grass IV, G.M., Robinson, J.R. Sustained- and controlled release drug delivery systems. In *Modern Pharmaceutics*, Banker, G.S., Rhodes, C.T (eds.), 1990, pp. 657-658.
97. Rodriguez, C.F., Bruneau, N., Barra, J., Alfonso, D., Doelker, E. Hydrophilic Cellulose Derivatives as Drug Delivery Carriers: Influence of Substitution Type on the Properties of Compressed Matrix Tablets. Wise, D.L. (ed.). In *Handbook of Pharmaceutical Controlled Release Technology*. Marcel Dekker Inc., NY, 2000, pp 1 – 30.
98. Colombo, P., Santi, P., Bettini, R., Brazel, C.S., Peppas, N.A. Drug Release from Swelling-Controlled Systems. Wise, D.L (ed.). In *Handbook of Pharmaceutical Controlled Release Technology*. Marcel Dekker, Inc., NY, 2000, pp 183 – 205.
99. Narasimhan, B Accurate Models in Controlled Drug Delivery Systems. Wise, D.L. (ed.). In *Handbook of Pharmaceutical Controlled Release Technology*. Marcel Dekker Inc., NY, 2000, pp 155 – 178.
100. Venkatraman, S., Davar, N., Chester, A., Kleiner, L. An Overview of Controlled Release Systems. Wise D.L. (ed). In *Handbook of Pharmaceutical Controlled Release Technology*. Marcel Dekker Inc., NY, 2000, pp 435 – 445.
101. Qiu, Y., Zhang, G. Research and Development Aspects of Oral Controlled-Release Dosage Forms Wise, D.L. (ed.). In *Handbook of pharmaceutical Controlled Release Technology*. Marcel

- Dekker Inc., NY, 2000, pp 465 – 499.
102. Giunchedi, P., Maggi, L., Conte, U., La Manna, A. *Linear Extended Release of a Water-Insoluble drug, Carbamazepine, from Erodible Matrices*. Int. J. Pharm., Vol. **94**, 1993, pp. 15 – 22.
 103. Sung, K.C., Nixon, P.R., Skoug, J.W., Ju, T.R., Gao, P., Topp, E.M., Patel M.V. *Effect of Formulation Variables on Drug and Polymer Release from HPMC-based matrix tablets*. Int. J. Pharm. Vol.**142**, 1996, pp. 53 – 60.
 104. Sung, K.C., Nixon, P.R., Skoug, J.W., Ju, T.R., Gao, P., Topp, E.M., Patel M.V. *Effect of Formulation Variables on Drug and Polymer Release from HPMC-based matrix tablets*. Int. J. Pharm. Vol.**142**, 1996, pp. 53 – 60.
 105. Danckwert, M.P. *Development of a zero-order release oral compressed tablet with potential for commercial tableting production*. Int. J. Pharm. Vol. **112**, 1994, pp. 37 – 45.
 106. McConville, J.T., Ross, A.C., Chambers, A.R., Smith, G., Florence, A.J., Stevens, H.N.E. *The effect of wet granulation on the erosion behaviour of an HPMC-lactose, used as a rate-controlling component in a pulsatile drug delivery capsule formulation*. Eur. J. Pharm. Biopharm., Vol. **57**, 2004, pp. 541-549.
 107. Giunchedi, P., Conte, U., La Manna, A. *Carbamazepine Modified Release Dosage Forms*. Drug Dev. Ind. Pharm. Vol. **17**(13), 1991, pp. 1753-1764.
 108. Kannan, V., Kandarapu, R., Garg, S. *Novel Optimisation Techniques for the Design and Development of Novel Drug Delivery Systems, Part I*. Pharm. Tech. Feb. 2003, pp 74 – 90.
 109. *Controlled Drug Delivery, Fundamental and Applications*. Robison, J.R., Lee, V.H.L. Marcel Dekker, Inc., New Cork, 1987, pp. 11-42, 180-205.
 110. Gu, X., Fediuk, D.J., Estelle, F., Simons, R., Simons, K.J. *Evaluation and comparison of five matrix excipients for the controlled release of acrivastine and pseudoephedrine*. Drug Dev. Ind. Pharm. Vol. **30**(10), 2004, pp. 1009-1017.

111. Lee, P.I. Oral ER Technology: Mechanism of Drug Release. In *Scientific Foundations for Regulating Drug Product Quality*. Amidon, G.L., Robinson, J.R., Williams, R.L. (eds.) AAPS Press, USA, 1997, pp. 221-230.
112. Pham, A.T., Lee, P.I. *Probing the Mechanism of drug release from HPMC matrices*. Pharm. Res. Vol. **11**(10), 1994, pp. 1379-1384.
113. Roshdy, M.N., Schnaare, R.L., Sugita, E.T., Schwartz, J.B. *The effect of controlled release tablet performance and hydrogel strength on in vitro/in vivo correlation*. Pharm. Dev. Tech. Vol. **7**(2), 2002, pp. 155-168.
114. Samani, S.M., Montaseri, H., Kazemi, A. *The effect of polymer blends on release profiles of diclofenac sodium*. Eur. J. Pharm. Biopharm. Vol.**55**, 2003, pp. 351-355.
115. Hogan, J.E. *Hydroxypropylmethylcellulose. Sustained Release Technology*. Vol. 15 (6 and 7), 1989, pp. 975 – 999.
116. Reynolds, T.D., Mitchell, S.A., Balwinski, K.M. *Investigation of the effect of tablet surface area/volume on drug release from HPMC controlled-release matrix tablets*. Drug. Dev. Ind. Pharm. Vol. **28** (4), 2002, pp. 457 – 466.
117. Ritger, P.L., Peppas, N.A. *A simple equation for description of solute release I. Fickian and Non-Fickian Release from non-swellable devices in the form of slabs, spheres, cylinders or discs*. J. Control. Rel. Vol. **5**, 1987, pp. 23-36.
118. Williams III, R.O., Reynolds, T.D., Cabelka, T.D., Sykora, M.A., Mahaguna, V. *Investigation of Excipient Type and Level on Drug Release from Controlled Release Tablets Containing HPMC*. Pharm. Dev. Tech. Vol. **7**(2), 2002, pp. 181 – 193.
119. Narasimhan, B. *Mathematical models describing polymer dissolution: consequences for drug delivery*. In *Advanced Drug Delivery Reviews*, Vol. **48**, 2000, pp. 195-210.
120. Colombo, P. Bettini, R., Santi, P., De Ascentiis, A., Peppas, N.A. *Analysis of the swelling and release mechanisms from drug delivery systems with emphasis on drug solubility and water*

- transport*. J. Control. Rel. Vol. **39**, 1996, pp. 231-237.
121. Ford, J.L., Rubinstein, M.H., McCaul, F, Hogan, J.E., Edgar, P.J. *Importance of Drug Type, Tablet Shape and Added Diluents on Drug Release Kinetics from HPMC Matrix Tablets*. Int. J. Pharm., Vol. **40**, 1987, pp. 223 – 234.
 122. Siepmann, J., Lecomte, F., Bodmeier, R.D. *Diffusion controlled drug delivery systems: calculations of the required composition to achieve desired release profiles*. J. Control. Rel. Vol. **60**, 1990, pp. 379-389.
 123. Siepmann, J. Peppas, N.A. *Modeling of drug release from delivery systems based on hydroxypropylmethylcellulose (HPMC)*. In Advanced Drug Delivery Reviews, Vol. **48**, 2001, pp. 139-157.
 124. Bettini, R., Catellani, P.L, Santi, P., Massimo, G., Peppas, N.A., Colombo, P. *Translocation of Drug Particles in HPMC Matrix Gel Layer: Effect of Drug Solubility and Influence on Release Rate*. J. Control. Rel., Vol. **70**, 2001, pp. 383 391.
 125. Ranga Rao, R.V., Padmalatha Devi, K., Buri, P. *Influence of molecular size and water solubility of the solute on its release from swelling and erosion controlled polymeric matrices*. J. Control. Rel. Vol..**12**, 1990, pp. 133-141.
 126. Higuchi, T. *Mechanism of sustained-action medication; Theoretical Analysis of rate of release of solid drugs dispersed in solid matrices*. J. Pharm. Sci., Vol. **52**(12), 1963, pp. 1145-1149.
 127. Ritger, P.L., Peppas, N.A. *A simple equation for description of solute release II. Fickian and anomalous release from swellable devices*. J. Control. Rel. Vol. **5**, 1987, pp. 37-42.
 128. Siepmann, J., Kranz, H., Peppas, N.A., Bodmeier, R. *Calculation of the required size and shape of hydroxypropylmethylcellulose matrices to achieve desired drug release profiles*. Int. J. Pharm. Vol. **201**, 2000, pp. 151-164.

129. Katzhendler, I., Hoffman, A., Goldberger, A., Friedman, M. *Modeling of drug release from erodible tablets*. J. Pharm. Sci. Vol. **86**(1), 1997, pp. 110-115.
130. *Physical Pharmacy*, Martin, A.N. Lea & Febiger, 1993, pp. 325-326, 353-354.
131. E. Rinaki, G. Valsami, P. Macheras, *The power law can describe the drug release curve from HPMC-based matrix tablets: a hypothesis*. Int. J. Pharm., Vol. **255**, 2003, pp.199-207.
132. Lansky, P., Weiss, M. *Does the Dose-Solubility Ratio Affect the Mean Dissolution Time of Drugs?* Pharm. Res. Vol. **16**(9), 1999, pp.1470-1476.
133. Macheras, P., Dokoumetzidis, A. *On the Heterogeneity of Drug Dissolution and Release*. Pharm. Res. Vol. **17**(2), 2000, pp. 108-112.
134. Rinaki, E., Dokoumetzidis, A., Macheras, P. *The Mean Dissolution Time Depends on the Dose/Solubility Ratio*. Pharm. Res. Vol. **20**(3), 2003, pp. 406-408.
135. Kosmidis, K., Argyrakis, P., Macheras, P. *A Reappraisal of Drug Release Laws Using Monte Carlo Simulations: The Prevalence of the Weibull Function*. Pharm. Res. Vol. **20**(7), 2003, pp. 988-995.
136. Lansky, P., Weiss, M. *Classification of Dissolution Profiles in Terms of Fractional Dissolution Rate and a Novel Measure of Heterogeneity*. J. Pharm. Sci. Vol. **92**(8), 2003, pp.1632-1647.
137. Huang, Y., Khanvilkar K.H., Moore A.D., Hilliard-Lott, M. *Effects of Manufacturing Process Variables on In Vitro Dissolution Characteristics of Extended-Release Tablets Formulated with Hydroxypropylmethylcellulose*. Drug Dev. Ind. Pharm. **29**(1), 2003, pp. 79 – 88.
138. *The Development and Assessment of Both a Separate, Once-Daily Modified Release Matrix Formulation of Metoprolol Tartrate and a Combination Formulation with Hydrochlorothiazide*. Arjun, J. MSc. Thesis, 2001.
139. *The Handbook of Pharmaceutical Excipients 2nd Ed.* Wade, A., Weller, P.J. (eds).. London Pharmaceutical press, 2000, pp. 252-255,305-308, 424-425,487, 555-557, 143-145,109-111,60-

62.

140. Kiil, S., Dam-Johansen, K. *Controlled Drug Delivery from Swellable HPMC Matrices: Model-Based Analysis of observed Radial Front Movements*. J. Control. Rel. Vol. **90**, 2003, pp. 1-21.
141. Charman, S.A., Charman, W.N. *Modified-Release Drug Delivery Technology Part I*. Rathbone, M.J., Hadgraft, J., Roberts, M.S. (Eds.), Marcel Dekker Inc. New York, 2003, pp. 1-8.
142. Katzhendler, I., Azoury, R., Friedman, M. *Crystalline Properties of CBZ in Sustained Release Hydrophilic Matrix Tablets Based on HPMC*. J. Control. Rel. Vol. **54**, 1998, pp. 69-85.
143. Moneghini, M., Voinavich, D., Perisutti, B., Princivalle, F. *Action of Carriers on Carbamazepine Dissolution*. Pharm. Dev. Tech. Vol. **7**(3), 2002, pp. 289-296.
144. Kumar, V., de la Ruz, M., Yang, D. *Preparation, characterisation and tableting properties of a new cellulose-based pharmaceutical aid*. Int. J. Pharm. Vol. **235**, 2002, pp. 129-140.
145. Wang, L-H., Chowhan, Z.T. *Drug Excipient Interactions resulting from powder Mixing*. Int. J. Pharm. Sci. Vol. **60**, 1990, pp. 61-78.
146. Luhtala, S. *Effect of Sodium Lauryl Sulphate and Polysorbate 80 on Crystal Growth and Aqueous Solubility of Carbamazepine*. Acta. Pharm. Nord. Vol. **4**(2), 1992, pp. 85-90.
147. Thanoo, B.C., Sunny, M.C., Jayakrishnan, A. *Controlled Release of Oral Drugs from Cross-Linked Polyvinyl Alcohol Microspheres*. J. Pharm. Pharmacol. Vol. **45**, 1993, pp. 16-20.
148. *Remington: The Science and Practice of Pharmacy* 9th Ed. Gennaro, A.R. (Ed.) Mack Publishing Company, Easton, Pennsylvania, 1995, pp. 1615-1649.
149. *Code of Federal Regulations, Food and Drug Administration, Current Good Manufacturing Practice for the Manufacture, Processing, Packing and Holding of Drugs*, 21 CFR – parts 210 and 211, March 1979 revised April 1997.
150. Jonat, S., Hasenzahl, S., Gray, A., Schmidt, P.C. *Influence of Compacted Hydrophobic and Hydrophilic Colloidal Silicon Dioxide on Tableting Properties of Pharmaceutical Excipients*.

- Drug. Dev. Ind. Pharm. Vol. **31**, 2005, pp. 687-696.
151. Plumb, A.P., Rowe, R.C., York, P., Brown, M. *Optimisation of the Predictive Ability of ANN Models: A Comparison of Three ANN Programs and Four Classes of Training Algorithm*. Eur. J. Pharm. Sci. Vol. **25** (2005) pp 395-405.
 152. Gobburu, J.V.S., Shelver, W.H. *Quantitative Structure-Pharmacokinetic Relationships (QSPR) of Beta Blockers Derived Using Neural Networks*. J. Pharm. Sci. Vol. **84**(7), July 1995, pp 862 – 865.
 153. Hussain, A.S., Yu, X., Johnson, R.D. *Application of Neural Computing in Pharmaceutical Product Development*. Pharm. Res. Vol. **8**(10), 1991 pp 1248 – 1252.
 154. Erb, R.J. *Introduction to Backpropagation Neural Network Computation*. Pharm. Res. Vol. **10**(2), 1993, pp 165 – 170.
 155. Plumb, A.P., Rowe, R.C., York, P., Doherty, C. *The Effect of Experimental Design on the Modeling of a Tablet Coating Formulation using ANNs*. Eur. J. Pharm. Sci. Vol. **16**, 2002 pp 281 – 288.
 156. Bourquin, J., Schmidli, H., van Hoogevest, P., Leuenberger, H. *Application of Artificial Neural Networks (ANN) in the Development of Solid Dosage Forms*. Pharm. Dev. Tech. Vol. **2**(2), 1997, pp. 111 – 121.
 157. Turkoglu, M., Ozarslan, R., Sakr, A. *Artificial Neural Network Analysis of a Direct Compression Tableting Study*. J. Pharm. Biopharm. Vol. **41**(5) 1995 pp 315 – 322.
 158. Hussain, A.S., Johnson, R.D., Vachharajani, N.N., Ritschel, W.A. *Feasibility of Developing a Neural Network for Prediction of Human Pharmacokinetic Parameters from Animal Data*. Pharm. Res. Vol. **10**(3), 1993, pp 466 – 469.
 159. Takayama, K., Fujikawa, M., Obata, Y., Morishita, M. *Neural Network Based Optimization of Drug Formulations*. Advanced Drug Delivery Reviews, Vol. **55**, 2003, pp 1217 – 1231.

160. Achanta, A.S., Kowalski, J.G., Rhodes, C.T. *Artificial Neural Networks: Implications For Pharmaceutical Sciences*. Drug Dev. Ind. Pharm. Vol. **21**(1), 1995, pp 119 – 155.
161. Bourquin, J., Schmidli, H., van Hoogevest, P., Leuenberger, H. *Basic Concepts of Artificial Neural Networks (ANN) Modeling in the Application to Pharmaceutical Development*. Pharm. Dev. Tech. Vol. **2**(2), 1997, pp. 95 – 109.
162. Chen, Y., McCall, T.W., Baichwal, A.R., Meyer, M.C. *The Application of an Artificial Neural Network and Pharmacokinetic Simulations in the Design of Controlled-Release Dosage Forms*. J. Cont. Rel. Vol. **59**, 1999, pp 33 – 41.
163. Li, Y., Rauth, A.M., Wu, X.Y. *Prediction of Kinetics of Doxorubicin Release from Sulfopropyl Dextran Ion-Exchange Microspheres using Artificial Neural Networks*. Eur. J. Pharm. Sci. Vol. **24**, 2005, pp. 401 – 410.
164. *Neural Networks in Chemistry and Drug Design*, 2nd Ed., Zupan, J., Gasteiger, J., Wiley-VCH, 1999, pp 29.
165. Murtoniemi, E., Yliruusi, J., Kinnunen, P., Merkkü, P., Leiviska, K. *The Advantages by the Use of Neural Networks in Modelling the Fluidized Bed Granulation Process*. Int. J. Pharm. Vol.**108**, 1994, pp 155 – 164.
166. Richardson, C.J., Barlow, D.J. *Neural Network Computer Simulation of Medical Aerosols*. J. Pharm. Pharmacol. Vol. **48**, 1996, pp 581 – 591.
167. Hussain, A.S., Shivanand, P., Johnson, J.D. *Application of Neural Computing in Pharmaceutical Product Development: Computer Aided Formulation Design*. Drug. Dev. Ind. Pharm. Vol. **20**(10), 1994, pp 1739 – 1752.
168. *Neural Computing: An Introduction*. Beale, R., Jackson, T., J W Arrowsmith Ltd., 1990, pp 15 – 37.
169. Plumb, A.P., Rowe, R.C., York, P., Brown, M. *Optimisation of the predictive ability of Artificial Neural Network (ANN) models: A comparison of three ANN programs and four classes of*

- training algorithm*. Eur. J. Pharm. Sci. Vol. **25**, 2005, pp. 395-405.
170. Bioavailability and Bioequivalence Data Required as Proof of Efficacy. Regulation Gazette Number 7659, Volume 455, No 24785, 168-196, Pretoria 2 May 2003.
171. Medicines and Related Substances Control Act, 1965 (Act No. 101 of 1965) as amended by Act. No. 90 of 1997 and Act. No. 59 of 2002.
172. P.M. Sathe, A.S. Raw, L.A. Ouderkerk, L.Z. Yu and A.S. Hussain. Drug Product Performance, *In Vitro*, L Shargel and I Kanfer (eds). In *Generic Drug Product Development: Solid Oral Dosage Forms, Drugs and the Pharmaceutical Sciences*. Vol. **143**, Marcel Dekker, New York, 2005.
173. *Applied Biopharmaceutics and Pharmacokinetics* 3rd Ed. Shargel, L., Yu, A.B.C. Appleton & Lange, 1993, pp. 146-162, 33-44, 193-205, 231-249.
174. Stevens, R.E., Limsakun, T., Evans, G., Mason Jr., D.H. *Controlled Multidose, Pharmacokinetic Evaluation of Two Extended-Release Carbamazepine Formulations (Carbatrol and Tegretol-XR)*. J. Pharm. Sci. Vol. **87**(12), 1998, pp. 1531-1534.
175. Alexishvili, M.M., Rukhadze, M.D., Okujava, V.M. *Simultaneous Determination of Carbamazepine and Carbamazepine-10,11-Epoxyde by Using Microcolumn HPLC: Study of Pharmacokinetics of Carbamazepine in a Volunteer*. Biomed. Chrom. Vol. **11**, 1997, pp. 36-41.
176. Bialer, M., Yacobi, A., Moros, D., Levitt, B., Houle, J-M., Munsaka, M.S. *Criteria to Assess In Vivo Performance and Bioequivalence of Generis Controlled-Release Formulations of Carbamazepine*. Epilepsia, Vol. **39**(5), 1998, pp. 513-519.
177. Meyer, M.C., Straughn, A.B., Mhatre, R.M., Shah, V.P., Williams, R.L., Lesko, L.J. *The Relative Bioavailability and In Vivo-In Vitro Correlations for Four Marketed Carbamazepine Tablets*. Pharm. Res. Vol. **15**(11), 1998, pp. 1787-1791.
178. Olling, M., Mensinga, T.T., Barends, D.M., Groen, C., Lake, O.A., Meulenbelt, J. *Bioavailability of Carbamazepine from Four Different Products and the Occurrence of Side Effects*. Biopharm.

- Drug Disposition. Vol. **20**, 1999, pp. 19-28.
179. *Dissolution, Bioavailability and Bioequivalence*. Abdou, H.M. Mack Printing Company, 1989, pp. 297-300, 304, 307-308, 336-341, 353, 367-381, 401, 416.
180. Retzow, A, Schurer, M., Schulz, H.-U. *Influence of food on the bioavailability of a carbamazepine slow-release formulation*. Int. J. Clinical Pharmacol. Therap. Vol. **35**(12), 1997, pp. 557-560.
181. Bertilsson, L., Tomson, T. *Clinical Pharmacokinetics and Pharmacological Effects of Carbamazepine and Carbamazepine-10,11-Epoxyde*. Clinical Pharmacokinet. Vol. **11**, 1986, pp. 177-198.
182. Lake, O.A., Olling, M., Barends, D.M. *In vitro/in vivo correlations of dissolution data of carbamazepine immediate release tablets with pharmacokinetic data obtained in healthy volunteers*. Eur. J. Pharm. Biopharm. Vol. **48**, 1999, pp. 13-19.
183. World Medical Association 2000 Declaration of Helsinki Ethical Principles for Medical Research Involving Human Subjects, Adopted by the 18th WMA General Assembly, Helsinki, Finland, June, 1964. Amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975; 35th WMA General Assembly, Venice, Italy, October 1989; 41st WMA General Assembly, Hong Kong, September 1989; 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000.
184. *International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use*. (1996). ICH Tripartite Guideline. Guidelines for Good Clinical Practice. Recommended for Adoption at Step 4 of the ICH Process on 1 May 1996 by the ICH Steering Committee.
185. http://www.doh.gov.za/docs/policy/trials/trials_contents.html Retrieved 24/11/2005.
186. Meyer, M.C., Straughn, A.B., Jarvi, E. J., Wood, G.C., Pelsor, F.R., Shah, V.P. *The Bioequivalence of Carbamazepine Tablets with a History of Clinical Failures*. Pharm. Res. Vol.

- 9(12), 1992, pp. 1612-1616.
187. US Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research, *Guidance for Industry: Bioavailability and Bioequivalence Studies for Orally Administered Drug Products – General Considerations*. March 19, 2003.
 188. US Department of Health and Human Services, Center for Drug Evaluation and Research. *Guidance for Industry: Statistical Approaches to Establishing Bioequivalence*. January, 2003.
 189. Yacobi et. al. *A Multiple-Dose Safety and Bioequivalence Study of a Narrow Therapeutic Index Drug: A Case for Carbamazepine*. Clin. Pharmacol. Ther. April, 1999, pp. 389-394.
 190. Bialer, M., Arcavi, L., Sussan, S., Volosov, A., Yacobi. A., Moros, D., Levitt, B., Laor, A. *Existing and new criteria for bioequivalence evaluation of new controlled release (CR) products of carbamazepine*. Epilepsy Research. Vol. **32**, 1998, pp. 371-378.
 191. O'Neill, M.H. *The Polymorph Life Cycle From Screening to Developing a Robust Crystallisation Process*. Pharm. Tech., Outsourcing Resources, 2003, pp. 44-56.
 192. E. Rinaki, G. Valsami, P. Macheras, *The power law can describe the drug release curve from HPMC-based matrix tablets: a hypothesis*. Int. J. Pharm. Vol. **255**, 2003, pp.199-207.
 193. P. Costa, J. M. S. Lobo, *Modeling and comparison of dissolution profiles*. Eur. J. Pharm. Sci. Vol. **13** (2001), pp. 123-133.
 194. Matthews, B.R. *Regulatory aspects of stability testing in Europe*. Drug Dev. Ind. Pharm. Vol. **25**(7), pp. 831-856.
 195. Kommanaboyina, B., Rhodes, C.T. *Trends in stability testing, with emphasis on stability during distribution and storage*. Drug Dev. Ind. Pharm. Vol. **25**(7), 1999, pp. 857-868.
 196. <http://www.australianprescriber.com/magazines/vol17no2/expiry.htm> Retrieved 24/11/05.
 197. ICH Harmonised Tripartite Guideline: Stability Testing of New Drug Substances and Products (Revised). International Conference on Harmonisation of Technical Requirements for Registration

of Pharmaceuticals for Human Use, November 2000.

<http://www.ich.org/pdf/ICH/q1a1step4.pdf>

198. Singh, S. *Drug stability testing and shelf-life determination according to international guidelines*. Pharm. Tech. Vol. **18**(6), 1989, pp. 69-88.
199. Singh, S. *Stability test storage conditions for Zones III and IV – some unresolved issues*. Pharm. Tech. Vol. **23**(10), 1999, pp. 130-142.
200. Some, I.T., Bogaerts, P., Hanus, P., Hanocq, M., Dubois, J. *Improved kinetic parameter estimation in pH-profile data treatment*. Int. J. Pharm. Vol. **198**, 2000, pp. 39-49.
201. Mosquera, M.J., Cal, S., Souto, C., Concheiro, A., Martinez-Pancheco, R. Gomez-Amoza, J.L. *Effects of storage humidity on the mechanical, microstructural and drug release properties of hydroxypropylmethylcellulose-based hydrophilic matrix tablets*. Drug Dev. Ind. Pharm. Vol. **23**(4), 1997, pp. 403-406.
202. Luukkonen, P., Shaefer, T., Podczek, F., Newton, M., Hellen, L., Ylliruusi, J. *Characterisation of microcrystalline cellulose and silicified microcrystalline cellulose wet masses using a powder rheometer*. Eur. J. Pharm. Sci. Vol. **13**, 2001, pp. 143-149.
203. Taborsky, C.J., Mehta, U., Kusz, M., Caspersen, G. *Multiple-unit packaging for solid oral dosage forms*. Pharm. Tech. Vol. 24(3), 2000, pp. 44-51.
204. <http://www.devicelink.com/pmpn/archive/99/04/002.html> Retrieved 05/12/05
205. Guidance for Industry: Q1A Stability Testing of New Drug Substances and Drug Products. United States Department of Health and Human Services, Food and Drug Administration, Center for Drug Evaluation and Research (CDER), Center for Biologics Evaluation and Research (CBER), ICH, August 2001. <http://www.fda.gov/cder/guidance/4282fnl.pdf>.
206. Johnson, C.M., Thummel, K.E., Kroetz, D.L., et. Al. Human Liver Carbamazepine Metabolism: role of CYP3A4, 2C8 and 1A2. Pharm. Res., 1992, 9 Suppl.: 301

APPENDICES

APPENDIX I

APPENDIX II

APPENDIX III