

Development of curcumin containing direct compressible gastro-retentive tablets

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DECLARATION BY RESEARCHER

I, Suzanne Elaine van Niekerk, hereby declare that this dissertation, titled "Development of curcumin containing direct-compressible gastro-retentive tablets", submitted in fulfilment of the requirements for the degree Magister Scientiae in Pharmaceutics at the Potchefstroom Campus of the North-West University, is my own original work and has not been previously submitted for examination to any institution of higher education. I also declare that the resources used in this dissertation have been referenced and acknowledged accordingly.

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ABSTRACT

Curcumin is the main active compound found in turmeric that exhibits many medicinal actions such as anti-inflammatory, anti-oxidative, antimicrobial and anti-cancer effects. However, the systemic delivery of curcumin by means of oral administration is severely limited due to its poor water solubility, instability in neutral and alkaline environments as encountered in certain regions of the gastrointestinal tract and susceptibility to first pass metabolism. The aim of this study was to circumvent these challenges by a formulation approach where functional excipients were incorporated with curcumin in a modified solid oral dosage form namely a gastro-retentive mini-tablet-in-capsule system. The capsule formulation contained both conventional immediate-release mini-tablets (to provide the loading dose) and floating matrix type gastro-retentive mini-tablets (to provide the maintenance dose). A floating matrix type gastro-retentive delivery system prolongs the residence time of the dosage form in the acidic stomach environment where curcumin is expected to be more stable. Incorporation of functional excipients (e.g. hydroxypropyl- β -cyclodextrin) increased its solubility thereby potentially also its bioavailability, while the sustained release formulation was intended to maintain blood plasma levels over an extended period of time. The suitable filler materials and quantities thereof required for the production of the gastro-retentive tablets was determined by using the SeDeM expert diagram system. The solubility of curcumin was noticeably enhanced by forming an inclusion complex with hydroxypropyl- β -cyclodextrin. The efficacy of the formulation approach in terms of curcumin delivery was evaluated across synthetic cellulose nitrate membrane filters in a Sweetana-Grass diffusion apparatus.

This study has shown that the preparation of an inclusion complex of curcumin with hydroxypropyl- β -cyclodextrin markedly increased the solubility of curcumin (30 000-fold). Although the mini-tablet-in-capsule dosage forms showed promise with increased membrane transport of the prepared complex formulations compared to curcumin formulations, overall curcumin delivery was not on an acceptable level. Additional research would therefore be required to further increase the bioavailability of curcumin such as by incorporating drug absorption enhancers in the dosage form.

Keywords:

Curcumin, mini-tablet-in-capsule system, gastro-retentive drug delivery system, SeDeM expert diagram system, functional excipients, inclusion complex, hydroxypropyl- β -cyclodextrin, synthetic cellulose nitrate membrane filters.

TABLE OF CONTENTS

DECLARATION BY RESEARCHER.....	I
ACKNOWLEDGEMENTS	II
ABSTRACT	III
TABLE OF CONTENTS.....	IV
LIST OF TABLES	VIII
LIST OF FIGURES.....	X
LIST OF ABBREVIATIONS	XIV
CHAPTER 1: INTRODUCTION.....	1
1.1 Dissertation layout	1
1.2 Background	1
1.2.1 Curcumin: Origin, uses and physicochemical properties	1
1.2.2 Complexation with cyclodextrin.....	2
1.2.3 Multiple unit dosage forms: The mini-tablet-in-capsule system	3
1.2.4 Gastro-retentive dosage forms.....	3
1.2.5 SeDeM expert diagram.....	3
1.3 Research problem	4
1.4 Aims and objectives.....	5
1.5 Ethical aspects	5
CHAPTER 2: LITERATURE OVERVIEW OF CURCUMA LONGA AND BIOPHARMACEUTICAL ASPECTS OF CURCUMIN.....	6
2.1 Botany and common medicinal uses of Curcuma longa.....	6
2.2 Phytochemicals found in Curcuma longa	8
2.3 Curcumin (the main curcuminoid in Curcuma longa).....	8
2.3.1 Physicochemical properties of curcumin	8
2.3.2 Curcumin bioavailability	11
2.4 Drug absorption process and factors influencing it.....	11
2.5 The Biopharmaceutics Classification System (BCS)	13
2.6 Pharmaceutical strategies to overcome the poor bioavailability of compounds	14
2.6.1 Complex formation with cyclodextrins	15
2.6.2 Multiple-unit dosage forms.....	17
2.7 SeDeM expert diagram system.....	19

2.8	Techniques to evaluate drug delivery: permeation studies.....	21
2.9	Summary of literature review on curcumin	22
CHAPTER 3: MATERIALS AND METHODS.....		23
3.1	Introduction to materials and methods	23
3.2	Materials.....	24
3.3	Methods.....	25
3.3.1	Analytical methods for sample analysis	25
3.3.2	Validation of liquid chromatography – mass spectrometry (LC-MS) and fluorescence spectrophotometric (FS) analyses methods	25
3.4	Purity tests of curcumin vs reference standard.....	27
3.5	Preparation of inclusion complexes of curcumin with hydroxypropyl- β -cyclodextrin (HP β CD).....	28
3.6	Characterisation of inclusion complexes with hydroxypropyl- β -cyclodextrin (HP β CD). 28	
3.6.1	Fourier-Transform Infrared spectroscopy (FTIR).....	29
3.6.2	Differential Scanning Calorimetry (DSC).....	29
3.6.3	X-ray Powder Diffraction (XRPD) spectroscopy	29
3.6.4	Visual inspection of hydroxypropyl- β -cyclodextrin (HP β CD) and a physical mixture of curcumin and HP β CD under a microscope	29
3.6.5	Solubility studies.....	29
3.7	Characterisation of powder flow (active ingredient and excipients) according to the SeDeM expert diagram system (EDS)	30
3.7.1	Bulk density (Da) and Tapped density (Dc).....	30
3.7.2	Inter-particle porosity (Ie)	31
3.7.3	Compressibility index (IC%)	31
3.7.4	Hausner ratio (IH)	31
3.7.5	Cohesion index (Icd).....	31
3.7.6	Angle of repose (α)	32
3.7.7	Flowability (t'')	32
3.7.8	Loss on drying (%HR).....	32
3.7.9	Hygroscopicity (%H)	32
3.7.10	Particle size (%Pf)	33
3.7.11	Homogeneity index (I θ).....	33
3.7.12	Conversion of numerical values obtained from the parameters to radius (r) values for diagram construction	34
3.7.13	Parameter index (IP).....	34
3.7.14	Parameter profile index (IPP).....	35

3.8	Preparation of mini-tablet immediate release and gastro-retentive delivery systems...	35
3.9	Evaluation of mini-tablets.....	35
3.9.1	Mass variation	36
3.9.2	Disintegration.....	36
3.9.3	Friability	36
3.9.4	Hardness and diameter	36
3.9.5	Mini-tablet assay.....	36
3.9.6	Buoyancy.....	37
3.9.7	Dissolution studies.....	37
3.10	Permeation studies	37
3.10.1	Preparation of media	38
3.10.2	Preparation of synthetic membranes for permeation studies.....	38
3.11	Evaluation of membrane integrity with Lucifer Yellow (LY)	38
3.12	Summary of materials and methods	41
CHAPTER 4: RESULTS AND DISCUSSIONS		42
4.1	Introduction to results and discussions	42
4.2	Validation of liquid chromatography – mass spectrometry (LC-MS) and fluorescence spectrophotometric (FS) analyses methods.....	42
4.2.1	Validation results for liquid chromatography – mass spectrometry (LC-MS) analytical method	42
4.2.2	Validation results for fluorescence spectrophotometric method.....	44
4.3	Purity tests of curcumin vs reference standard.....	45
4.4	Characterisation of inclusion complexes with hydroxypropyl- β -cyclodextrin (HP β CD). 45	
4.4.1	Fourier-Transform Infrared spectroscopy (FTIR).....	45
4.4.2	Differential scanning calorimetry (DSC)	47
4.4.3	X-ray powder diffraction (XRPD) pattern.....	49
4.4.4	Visual inspection of hydroxypropyl- β -cyclodextrin (HP β CD) and a physical mixture of curcumin and HP β CD under a microscope	50
4.4.5	Solubility studies	50
4.5	Characterisation of powder flow (active ingredient and excipients) according to the SeDeM expert diagram system (EDS)	53
4.5.1	SeDeM diagrams.....	57
4.5.2	Calculated percentage excipient to be included in tablet formulation	58
4.6	Preparation of mini-tablet immediate release and gastro-retentive delivery systems... 60	
4.6.1	Evaluation of mini-tablets	62
4.7	Dissolution studies.....	63

4.8	Evaluation of membrane integrity with Lucifer Yellow (LY)	65
4.9	Permeation studies across synthetic cellulose nitrate membranes.....	66
		71
CHAPTER 5: CONCLUSION AND RECOMMENDATIONS		72
5.1	Final conclusions	72
5.2	Future recommendations	73
BIBLIOGRAPHY		74
ANNEXURES.....		87
ADDENDUM A: ETHICS APPROVAL LETTER OF STUDY.....		87
		88
ADDENDUM B: CERTIFICATE OF ANALYSIS: CURCUMIN REFERENCE STANDARD.....		89
		89
		90
		92
ADDENDUM C: CERTIFICATE OF ANALYSIS: CURCUMIN RAW MATERIAL.....		93
ADDENDUM D: CERTIFICATE OF ANALYSIS: HYDROXYPROPYL-B-CYCLODEXTRIN....		94
		94
ADDENDUM D: ANCILLARY DATA		95
ANCILLARY DATA FOR THE VALIDATION OF ANALYTICAL METHODS		95
ANCILLARY DATA FOR THE SOLUBILITY STUDIES PERFORMED ON CURCUMIN AND THE PREPARED CURCUMIN-HPBCD COMPLEX		96
ANCILLARY DATA FOR THE DISSOLUTION STUDIES PERFORMED ON CURCUMIN AND THE PREPARED CURCUMIN-HPBCD COMPLEX		96
ANCILLARY DATA FOR THE PERMEATION STUDIES PERFORMED ON LUCIFER YELLOW FOR THE DETERMINATION OF MEMBRANE INTEGRITY OF THE SYNTHETIC CELLULOSE NITRATE FILTER MEMBRANES		97
ANCILLARY DATA FOR THE PERMEATION STUDIES PERFORMED ON CURCUMIN AND THE PREPARED CURCUMIN-HPBCD COMPLEX POWDER SAMPLES AND FINAL MINI-TABLET DOSAGE FORMS		98

LIST OF TABLES

Table 1-1: Classification of parameters according to factors as evaluated by the SeDeM expert diagram system (Adapted from Suñé-Negre ^b et al., 2011).....	4
Table 2-1: The taxonomic position of the turmeric plant in the scientific classification system (Chattopadhyay et al., 2004).	6
Table 2-2: Amount of residual curcumin that remained after 60 min in solutions with different pH values (Kumavat et al., 2013).	10
Table 2-3: The Biopharmaceutics Classification System for conventional, immediate release tablets as proposed by Amidon (1995).	13
Table 2-4: Types of modified β -cyclodextrins (Rajewski et al., 1996).....	16
Table 2-5: Parameters and equations of the SeDeM expert diagram (Adapted from Suñé-Negre ^b et al., 2011).	20
Table 3-1: Summary of the materials used for this study and the manufacturers/suppliers from which they were purchased.	24
Table 3-2: List of active ingredients and selected excipients for the evaluation of powder flow parameters	30
Table 3-3: Standard lubricant composition for abrasive powders, which were added to the powders where needed (Pérez et al., 2006)	31
Table 3-4: Conversion of powder flow parameter limit values into radius values (r). Adapted from Pérez et al., (2006).....	34
Table 4-1: Validation data obtained for liquid chromatographic mass spectrometric (LC-MS) analytical method for curcumin	43
Table 4-2: Validation data for fluorescence spectrophotometric analytical method for the determination of Lucifer Yellow (LY) samples.....	45
Table 4-3: Comparison of distinct peaks (cm^{-1}) visible in FTIR spectrum for the curcumin in HP β CD complex, curcumin, HP β CD and a physical mixture	46

Table 4-4: Summary of data acquired from solubility studies performed on curcumin and the prepared complex.....	52
Table 4-5: Summary of powder flow properties and the corresponding values for angle of repose, compressibility index (Carr's index) and Hausner ratio (BP, 2020)	53
Table 4-6: Summary of experimentally calculated compressibility (Carr's index), Hausner index and angle of repose values for the active ingredients, curcumin and the curcumin-HP β CD complex	54
Table 4-7: Summary of SeDeM radius/parameter values calculated from experimentally obtained powder flow parameter values for the construction of SeDeM diagrams. Values were determined for the active ingredients, curcumin and the curcumin-HP β CD complex and selected functional excipients.	55
Table 4-8: SeDeM incidence values calculated from parameter values and the determination of incidence deficiencies.....	56
Table 4-9: SeDeM acceptance incidences; parameter index, parameter profile index and good compression index values.	57
Table 4-10: Percentage of each corrective excipient to be included within mini-tablet formulations for curcumin and curcumin HP β CD inclusion complex.....	59
Table 4-11: Composition of the immediate release and gastro-retentive mini-tablet formulations	60
Table 4-12: Summary of mini-tablet evaluation data for the immediate release and gastro-retentive formulations of curcumin and the curcumin-HP β CD complex, respectively	63
Table 4-13: Summary of dissolution data for curcumin and the prepared curcumin-HP β CD complex dosage forms	65
Table 4-14: Summary of the percentage transport, P_{app} value and cumulative transport of marker molecule, Lucifer Yellow, across synthetic cellulose nitrate membranes.....	66
Table 4-15: Summary of the percentage transport (%) values, P_{app} values and cumulative percentage transport values obtained from permeation studies of different forms of curcumin across synthetic cellulose nitrate membranes	68

LIST OF FIGURES

Figure 2-1: Photographic images of <i>Curcuma longa</i> at various stages of its growth cycle. (A) the <i>C. longa</i> plant on the verge of senescence, (B) shows the rhizome cluster with (i), the new season's rhizome and (ii), a mature rhizome from the previous season's growth, (C) the mature rhizome upon harvest and (D) commercially available turmeric powder extract from a dried <i>C. longa</i> rhizome.	7
Figure 2-2: Chemical structures of the main curcuminoids found in <i>Curcuma longa</i> rhizome: (A) curcumin (diferuloylmethane or diferulylmethane), (B) desmethoxycurcumin (demethoxycurcumin, monodemethoxycurcumin) and (C) bisdemethoxycurcumin (didemethoxycurcumin).	8
Figure 2-3: The (A) bis-keto and (B) enolate tautomeric forms of curcumin (Basnet et al., 2011).	9
Figure 2-4: Schematic presentation of the rate processes of drug bioavailability as adapted from Suarez et al., (2016).	12
Figure 2-5: A graphical representation of a drug molecule and a cyclodextrin cone which form an inclusion complex, subsequently 'hiding' the drug molecule within the cyclodextrin cone cavity (adapted from Arun et al., 2008).	15
Figure 2-6: (A), chemical structure of β -cyclodextrin (Arun et al., 2008) and (B), its derivative, hydroxypropyl- β -cyclodextrin (USP). The potential substitution of the hydroxyl group for hydroxypropyl group is represented by the R-group seen in the hydroxypropyl- β -cyclodextrin structure.....	16
Figure 2-7: Graphical representation of the proposed 1:2 complex formed by curcumin and cyclodextrin adapted from Baglolle et al. (2005).	17
Figure 2-8: Example of a SeDeM diagram or polygon based on calculated radius values.	20
Figure 3-1: Photographs of the solid curcumin-HP β CD inclusion complex material obtained after overnight evaporation (A); and the final, ground and sieved curcumin-HP β CD inclusion complex powder obtained (B).	28
Figure 3-2: Photographs from top-left; A) cellulose nitrate membranes were cut and shaped to fit within the spikes of the half-cells. B) An assembled diffusion cell. C) Top-view of	

diffusion cells placed between heating blocks and connected to the carbogen gas. D)	
Front-view of diffusion cells containing marker molecule, LY.	40
Figure 4-1: Standard curve for curcumin obtained by a liquid chromatographic mass spectrometric (LC-MS) analytical method where peak area is plotted as a function of concentration	
Figure 4-2: Standard curve for the determination of the linear range for marker molecule, Lucifer Yellow (LY), on a Spectramax® paradigm plate reader where fluorescence value is plotted as a function of concentration	44
Figure 4-3: Superimposed FTIR spectra for the prepared curcumin-hydroxypropyl-β-cyclodextrin (HPβCD) complex (red curve), curcumin raw material (black curve), HPβCD (grey curve) and a physical mixture of curcumin and HPβCD (green curve)	46
Figure 4-4: DSC thermogram overlay of melting points (°C) for hydroxypropyl-β-cyclodextrin (grey curve), curcumin (black curve), curcumin-HPBCD complex (blue curve) and a physical mixture (red curve).....	47
Figure 4-5: DSC thermograms of the melting point and enthalpy required for transition of the curcumin-HPβCD complex from solid state to liquid state.....	48
Figure 4-6: DSC analysis of the melting point and enthalpy required to transition a physical mixture of curcumin and HPβCD from solid state to liquid state	48
Figure 4-7: X-ray powder diffraction (XRPD) pattern of curcumin-HPβCD complex, curcumin hydroxypropyl-β-cyclodextrin (HPβCD) and a physical mixture of curcumin and HPβCD	49
Figure 4-8: Microscopic image of a simple physical mixture of curcumin and HPβCD (A) and the formulated curcumin-HPβCD complex (B)	50
Figure 4-9: Photographic images of (A) the visible yellow staining caused by the curcumin-HPβCD complex compared to the absence of staining of the solubility tube containing curcumin. (B) Solubility tubes loaded onto a rotating aluminium axis within a water bath (37°C ± 2°C). From the left, three tubes containing curcumin-HPβCD complex and three tubes containing curcumin followed by three tubes containing curcumin-HPβCD complex. The white arrows indicate the powder agglomerates present within the curcumin-simulated gastric fluid (SGF) solution.	51

Figure 4-10: Photograph of curcumin (transparent samples) and inclusion complex (yellow toned samples) solubility samples for each time point over 48 hours. From left, samples withdrawn after 2, 4, 8, 24 and 48-h time periods.	51
Figure 4-11: Solubility curves for the curcumin-HP β CD complex (red curve) and curcumin (blue curve) in simulated gastric fluid (pH 1.2) over a 48 hour period. (*The curcumin-HP β CD complex graph is expressed as μ g/ml while the **curcumin graph is expressed as ng/ml). (Error bars omitted for reasons of clarity, data available in appendices).....	53
Figure 4-12: The constructed SeDeM diagrams for (A) curcumin and (B) the curcumin-HP β CD complex followed by the diagrams for the excipients; (C) microcrystalline cellulose, (D) hydroxypropyl methyl cellulose, (E) vinylpyrrolidone acetate copolymer and (F) styrene-divinylbenzene copolymer	58
Figure 4-13: Superimposed SeDeM diagram areas to indicate the suitability of selected excipients, microcrystalline cellulose (MCC) and hydroxypropyl methyl cellulose (HPMC) for the correction of poor compressibility characteristics of curcumin and the curcumin-HP β CD complex. (A) curcumin and MCC, (B) curcumin and HPMC, (C) curcumin-HP β CD complex and MCC and (D) curcumin-HP β CD complex and HPMC.	59
Figure 4-14: (A) A representation of the final mini-tablet in capsule system formulated for this study (B) From left, curcumin-HP β CD complex and curcumin immediate release mini-tablets and curcumin- HP β CD complex and curcumin gastro-retentive mini-tablets (C) Complex (left) and curcumin (right) gastro-retentive mini-tablets floating in acidic buffer (pH 1.2) during the buoyancy test.....	61
Figure 4-15: Superimposed polygon areas for the major excipients in the four final formulations. Diagrams (A) and (B) represent the gastro-retentive mini-tablet formulations for curcumin and the curcumin-HPBCD complex, respectively. Diagrams (C) and (D) represent the direct-compressible mini-tablet formulations for curcumin and the curcumin-HPBCD complex, respectively..	62
Figure 4-16: Visual comparison of curcumin mini-tablet dosage form (A) and complex mini-tablet dosage form (B), minutes after being placed in the respective dissolution chambers.	64

Figure 4-17: Dissolution profile for the curcumin-HP β CD complex (red curve) and curcumin (blue curve) dosage forms plotted as function of time. (*The curcumin-HP β CD complex graph is expressed as μ g/ml while the **curcumin graph is expressed as ng/ml). (Error bars omitted for reasons of clarity, data available in appendices)..	64
Figure 4-18: Percentage transport of marker molecule, Lucifer Yellow (LY), as a function of time, across synthetic cellulose nitrate membranes for the determination of membrane integrity.....	66
Figure 4-19: Average percentage transport comparison of curcumin powder samples (blue curve) and curcumin-HP β CD complex powder samples (red curve). (Error bars omitted for reasons of clarity, data available in appendices).	67
Figure 4-20: Average percentage transport comparison of curcumin mini-tablet dosage forms (blue curve) and curcumin-HP β CD complex mini-tablet dosage forms (red curve). (Error bars omitted for reasons of clarity, data available in appendices).	67
Figure 4-21: Photographic images of each of the four formulations, powder or mini-tablets and their corresponding synthetic membranes following a five-hour permeation study. From top left; (A) curcumin powder, (B) curcumin powder membrane, (C) complex powder, (D) complex powder membrane, (E) curcumin mini-tablets, (F) curcumin mini-tablet membrane, (G) complex mini-tablets, (H) complex mini-tablets membrane..	69
Figure 4-22: Side-view photographic images of (A) curcumin mini- tablets and (B) complex mini-tablets in modified Sweetana-grass diffusion chambers illustrating the yellowing of the simulated gastric fluid (pH1.2) solution caused by the mini-tablets containing the curcumin-HP β CD complex.	69
Figure 4-23: Diffusion chambers containing (A) a curcumin-HP β CD complex powder sample equivalent to 50 mg curcumin and (B) the curcumin-HP β CD complex mini-tablet dosage (50 mg)	71

LIST OF ABBREVIATIONS

% RSD	Percentage relative standard deviation
%H	Hygroscopicity
%HR	Loss on drying
%Pf	Particles < 50 μm
(I θ)	Homogeneity index
(α)	Angle of repose
AUC	Area under the plasma concentration time curves
BCS	Biopharmaceutics Classification System
Caco-2	Colonic adenocarcinoma cell
CN	Cellulose nitrate membrane filters
Da	Bulk density
Dc	Tapped density
DM β CD	2,6-dimethyl- β -cyclodextrin
DSC	Differential scanning calorimetry
FS	Fluorescence spectrophotometric
FTIR	Fourier-Transform Infrared spectroscopy
G2 β CD	Diglucosyl- β -cyclodextrin
GCI	Good compression index
HPLC	High Performance Liquid Chromatography
HPMC	Hydroxypropyl methylcellulose
HPMC	Hydroxypropyl methylcellulose (Pharmacoat®)
HP β CD	Hydroxypropyl- β -cyclodextrin
IC	Carr index
Icd	Cohesion index
Ie	Inter-particle porosity
IH	Hausner index

IP	Parameter index
IPP	Parameter profile index
KBr	Potassium bromide
KRB	Krebs-Ringer bicarbonate buffer
LC-MS	Liquid chromatography mass spectrometry analysis
log P	Partition coefficient value
LY	Lucifer Yellow
MCC	Microcrystalline cellulose (Avicel® pH 200)
Mg Stearate	Magnesium stearate
P _{app}	Apparent permeability coefficient
PVP VA64	Vinylpyrrolidone-vinyl acetate copolymer (Kollidon® VA64)
R ²	Correlation coefficient
RMβCD	Randomly methylated β-cyclodextrins
SBE4βCD	Sulfobutyl ether β-cyclodextrin with 4 degrees of substitution
SBE7βCD	Sulfobutyl ether β-cyclodextrin with 7 degrees of substitution
SBEβCD	Sulfobutyl ether β-cyclodextrin
SeDeM EDS	SeDeM expert diagram system
SGF	Simulated gastric fluid
styrene DVB	Styrene divinylbenzene copolymer
t''	Powder flow
XRPD	X-ray Powder Diffraction spectroscopy

CHAPTER 1: INTRODUCTION

1.1 Dissertation layout

This dissertation consists of a total of five chapters, of which the first chapter serves as an introduction and provides a brief overview of the study, together with the research problem, aim and objectives.

Chapter 2 contains a literature overview on the origin and physicochemical properties of curcumin, followed by the principles of absorption of solid oral dosage forms after administration, the pharmaceutical strategies that will be incorporated to improve the solubility and delivery of curcumin during this study, an introduction to the SeDeM expert diagram system (EDS) and lastly a short discussion on *ex-vivo* permeation studies.

The materials and methods of this study are described in Chapter 3 where after the results and elucidations thereof are discussed in Chapter 4.

The study is summarized and concluded in Chapter 5 and provides future recommendations for further investigation.

All ancillary documents, which include the certificate for ethics approval (NWU-00501-20-A5) and certificates of analysis for the curcumin reference standard, curcumin raw material and hydroxypropyl- β -cyclodextrin are provided in addenda A, B, C and D, respectively. Additional ancillary data is given in addendum E.

1.2 Background

1.2.1 Curcumin: Origin, uses and physicochemical properties

Curcuma longa, universally known as turmeric, is a plant that is cultivated in the tropical parts of Asia mainly for the food industry as a spice (Van Wyk & Wink, 2004). Turmeric is renowned for its use as a culinary spice in curry and mustard powders, but is also often used as a vegetable dye in food and textiles (Van Wyk, 2005). Furthermore, turmeric has been used for medicinal purposes as it exhibits a wide variety of pharmacological activities such as anti-inflammatory, anti-oxidant and anti-tumour effects (Goel *et al.*, 2008, Hewlings *et al.*, 2017, Salehi *et al.*, 2019 and Fadus *et al.*, 2017).

These pharmacological effects are attributed to phytochemicals known as curcuminoids, which can be extracted from the rhizomes of *C. longa* (Van Wyk & Wink, 2004). Curcumin is one of the three main curcuminoids found in *C. longa* that has anti-cancer and anti-inflammatory actions (Basnet *et al.*, 2011 and Sharma *et al.*, 2005). However, the therapeutic application of curcumin is severely limited due to its unfavourable physicochemical properties (Tønnesen^b *et al.*, 2002). Curcumin is a hydrophobic molecule (Aggarwal *et al.*, 2008) that exhibits poor aqueous solubility (0.011 $\mu\text{g/ml}$) in

both acidic and neutral environments (Liu *et al.*, 2016 and Kawabata *et al.*, 2011). In addition to its poor solubility, curcumin has been found to undergo rapid chemical degradation upon exposure to both neutral and alkaline environments and is also susceptible to photo-degradation (Liu *et al.*, 2016 and Basnipar *et al.*, 2018). Conversely, curcumin is relatively stable in acidic environments (Tønnesen^a *et al.* 1985 and Price & Buescher, 1997).

Consequently, there is a need to apply pharmaceutical strategies in an effort to overcome these shortcomings of curcumin. One promising strategy is to use functional excipients in the formulation of a drug delivery system for curcumin, which is able to increase its solubility (Kawabata *et al.*, 2011), enhancing its membrane permeability (Baki *et al.*, 2015) and/or reducing its metabolism (Panakanti *et al.*, 2015). Tønnesen^b *et al.* (2002) found that the solubility of curcumin can be markedly increased upon complexation with cyclodextrins, while the degradation of curcumin can be delayed by confining the curcumin dosage form to an environment with a relatively low pH (Wang *et al.*, 1997). In terms of curcumin's chemical stability, the environment of the stomach with a relative pH of 1.5 – 2.0 (Martini *et al.*, 2013) is much more favourable with regards to chemical stability than that of the intestines with a pH of 6.0 – 7.5 (Martini *et al.*, 2013).

1.2.2 Complexation with cyclodextrin

Cyclodextrins have hollow, truncated cone-like molecules with a hydrophilic outer surface and hydrophobic core (Arun *et al.*, 2008). Cyclodextrin can accommodate a lipophilic drug molecule within its cone cavity and thereby increases the amount of drug available at the site of absorption (Jansook *et al.*, 2011).

β -cyclodextrin consists of 7 glucose units and has a cavity diameter of 0.7 nm, which is suitable to include most drug molecules (Ashford, 2013). However, β -cyclodextrin has some restrictions regarding solubility and is potentially nephrotoxic. The solubility of β -cyclodextrin can be improved by modifying the hydroxy groups with various other functional groups (Arun *et al.*, 2008). By modifying β -cyclodextrin to hydroxypropyl- β -cyclodextrin (HP β CD), the molecule's solubility is fundamentally increased (Gidwani *et al.*, 2015), which in turn reduces the risk for nephrotoxicity (Hedges, 2009).

1.2.3 Multiple unit dosage forms: The mini-tablet-in-capsule system

When numerous small units (e.g. mini-tablets) are combined to form a single dosage form (e.g. capsule), it is referred to as a multiple unit dosage form (Alderborn, 2013 and McConnel *et al.*, 2013). Furthermore, a biphasic multiple-unit drug delivery system can be produced by combining mini-tablets with different release rates (Mounika *et al.*, 2015). Usually, a biphasic drug delivery system consists of both immediate-release tablets combined with modified-release tablets in one dosage form.

An immediate release dosage form works on the principle of rapid drug release by disintegrating promptly after oral administration (Ding, 2016 and Alderborn, 2013), whereas modified-release tablets gradually release a drug over a prolonged period of time (Fassihi, 2017). In addition, the site of drug release can also be controlled by modifying a dosage form. An example of dosage forms that release the drug exclusively in the stomach are gastro-retentive formulations.

1.2.4 Gastro-retentive dosage forms

Gastro-retentive dosage forms are retained in the stomach by different mechanisms such as floating on the stomach's contents, adhering to the mucous layer or expanding to such a large size that it cannot move through the pyloric valve. These dosage forms are formulated by integrating functional excipients such as hydroxypropyl methylcellulose (HPMC). When HPMC comes into contact with gastric fluids, it reacts to form a highly swellable gel layer around the tablet (Rao *et al.*, 2012 and Sahu *et al.*, 2011). The low-density gel layer lends buoyancy to the dosage form and enables the dosage form to float on top of the gastric fluids (Garg *et al.*, 2008). Gastro-retention provides a prolonged residence time within the stomach and thereby allowing extended time for drug release from the dosage form in the stomach environment (Streubel *et al.*, 2006).

1.2.5 SeDeM expert diagram

The SeDeM expert diagram system (EDS) is a pre-formulation method that is usually applied during the formulation of direct-compressible tablets. The system is used to determine the characteristics of powders to evaluate its suitability for formulation by measuring twelve parameters, which are classed into five factors (refer to Table 1-1). The parameters are measured and compared to pre-specified limit values where after calculations and conversions are made and transferred to a SeDeM polygon diagram (Pérez *et al.*, 2006 and Suñé-Negre^a *et al.*, 2008).

Table 1-1: Classification of parameters according to factors as evaluated by the SeDeM expert diagram system (Adapted from Suñé-Negre^b *et al.*, 2011).

Factor	Parameter
Dimension	• Bulk density • Tapped density
Compressibility	• Inter-particle porosity • Carr index • Cohesion index
Flowability	• Hausner index • Angle of repose • Powder flow
Lubricity/stability	• Loss on drying • Hygroscopicity
Lubricity/dosage	• Particles < 50 µm • Homogeneity index

1.3 Research problem

Curcumin is the main active compound found in *C. longa* that is therapeutically used as an anti-oxidant, for the treatment of inflammatory diseases and for the treatment of certain cancers. However, the systemic delivery of curcumin by means of oral administration is severely limited due to its poor water solubility and its susceptibility to chemical degradation. The chemical stability of curcumin is pH dependent and keeping it in an acidic environment is favourable in terms of stability. The unfavourable physico-chemical properties of curcumin can be overcome by formulation approaches. Curcumin is a hydrophobic molecule that can be incorporated into the hydrophobic core of cyclodextrin molecules by means of the formation of inclusion complexes. These inclusion complexes are capable of improving the aqueous solubility of curcumin through solubilisation. By formulating curcumin into gastro-retentive dosage forms, it can be kept in the acidic environment of the stomach for prolonged time periods where it is more stable as compared to the neutral and alkaline environments of the small intestine. Multiple-unit dosage forms such as the mini-tablet-in-capsule drug delivery system provide additional advantages such as less variability in drug delivery rates, less irritation and a lower chance for dose dumping.

1.4 Aim and objectives

The aim of this study was to utilise formulation approaches to overcome some of curcumin's unfavourable physico-chemical properties in order to improve its delivery via the oral route of drug administration. These approaches include the formation of inclusion complexes between curcumin and hydroxypropyl- β -cyclodextrin (HP β CD), the design of a floating type gastro-retentive dosage form, the production of a multiple-unit drug delivery system (i.e. a mini-tablet-in-capsule drug delivery system) and the optimisation of direct mini-tablet compression by utilising the SeDeM EDS.

The aim of the study was met by completing the following specific objectives:

- Formation of inclusion complexes between curcumin and hydroxypropyl- β -cyclodextrin (HP β CD) and chemical characterisation of these complexes by means of Fourier-Transform Infrared spectroscopy (FTIR), differential scanning calorimetry (DSC), microscopic analysis, crystallographic analysis by means of X-ray Powder Diffraction (XRPD) spectroscopy and solubility studies.
- Determining the most suitable filler material and quantity thereof for the production of direct-compressible curcumin containing mini-tablets by using the SeDeM EDS.
- Preparing four different direct-compressible mini-tablet formulations. Two immediate release formulations of which one contains curcumin alone and the other contains the curcumin-HP β CD complex and two gastro-retentive formulations of which one contains curcumin alone and the other contains the curcumin-HP β CD complex.
- Evaluating the four mini-tablet formulations in terms of their physical properties (mass variation, hardness, friability, disintegration) and dissolution properties with an additional buoyancy evaluation of the two gastro-retentive formulations.
- Evaluating the efficacy of the functional excipients in terms of curcumin delivery across synthetic cellulose nitrate (CN) membrane filters mounted on a modified Sweetana-Grass diffusion apparatus.

1.5 Ethical aspects

Initially, it was planned to use excised pig stomach tissue for permeation studies for which ethics approval was obtained (NWU-00501-20-A5). However, it was decided to use synthetic membranes instead, which are considered acceptable surrogates for drug delivery studies across excised pig intestinal tissues (Corti *et al.*, 2006 and Fensham *et al.*, 2019). The study did not involve any humans or animals. Furthermore, this study did not involve the harvesting of any plant material, all curcumin extracts were purchased from suppliers. All research was performed according to Good Laboratory Practices and general health and safety guidelines.

CHAPTER 2: LITERATURE OVERVIEW OF *CURCUMA LONGA* AND BIOPHARMACEUTICAL ASPECTS OF CURCUMIN

2.1 Botany and common medicinal uses of *Curcuma longa*

Curcuma longa, commonly known as turmeric, is a perennial herb of the ginger family (Zingiberaceae). The turmeric plant (Figure 2-1) is stemless and leafy with white and yellow flowers (Van Wyk & Wink, 2004). *C. longa* is cultivated in the tropical parts of Asia and it is thought to be indigenous to India (Van Wyk, 2005).

The Swedish botanist, Linnaeus, was first to describe domestically cultivated turmeric with the botanic name of *C. longa* (Chattopadhyay *et al.*, 2004). The taxonomic position of the turmeric plant in the plant kingdom classification is outlined in Table 2-1.

Table 2-1: The taxonomic position of the turmeric plant in the scientific classification system (Chattopadhyay *et al.*, 2004).

Kingdom	Plantae
Subkingdom	Tracheobionta
Superdivision	Spermatophyta
Division	Magnoliophyta
Class	Liliopsida
Subclass	Commelinids
Order	Zingiberales
Family	Zingiberaceae
Genus	<i>Curcuma</i>
Species	<i>C. longa</i>

C. longa (turmeric) has an annual growth cycle, developing from a planted rhizome to being harvested, spread roughly over eight to nine months. With each new crop, the planted rhizome enlarges and produces several new secondary rhizomes from its auxiliary buds. The rhizomes are deemed mature and ready for harvest upon senescence of the plant's foliage - when the leaves and stems become yellow and deteriorate. The rhizome clusters are raised from the soil where after the roots and foliage are removed. The new season's growth (as seen in Figure 2-1-B-i) is removed from the rhizome cluster and stored for planting the next crop while the matured rhizome from the previous season's growth (as seen in Figure 2-1-B-ii) is dried and ground to extract turmeric powder (Buescher & Yang, 2000).

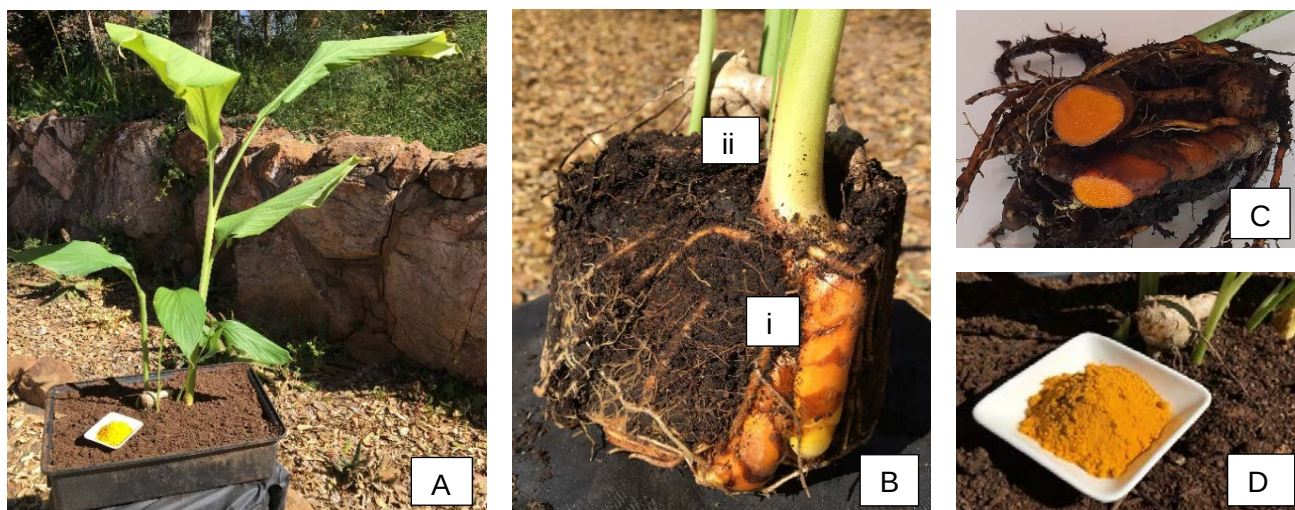


Figure 2-1: Photographic images of *Curcuma longa* at various stages of its growth cycle. (A) the *C. longa* plant on the verge of senescence, (B) shows the rhizome cluster with (i), the new season's rhizome and (ii), a mature rhizome from the previous season's growth, (C) the mature rhizome upon harvest and (D) commercially available turmeric powder extract from a dried *C. longa* rhizome.

Ground *C. longa* rhizome (turmeric) is used as a culinary spice and this is probably the most commonly known use of this plant, since it is a key ingredient in curry and mustard powders. Furthermore, due to the bright colour of the flesh of the turmeric plant's root or rhizome, it is often used as a vegetable dye in food stuff and textiles (Van Wyk, 2005).

The *C. longa* plant (turmeric) has a variety of medicinal uses due to its pharmacological activities (Goel *et al.*, 2008, Hewlings & Kalman, 2017 and Salehi *et al.*, 2019), which include anti-inflammatory (Fadus *et al.*, 2017), anti-oxidant, anti-microbial, anti-protozoal, anti-malarial, anti-proliferative, anti-venom, anti-angiogenic, anti-tumour, anti-ageing effects. Due to its general health benefits, it can also be administered as a supplement (Hewlings & Kalman, 2017). The pharmacological activities of the turmeric plant can mainly be attributed to specific phytochemicals found in the plant's rhizomes, which are known as curcuminoids (Van Wyk & Wink, 2004).

Numerous studies have been conducted on the safety of curcumin. In most of these studies it was suggested that dosages as high as 8 g per day can generally be considered as safe in humans (Cheng *et al.*, 2001 & Mirzaei *et al.*, 2016). In a dose escalation study performed by Lao *et al.*, (2006), curcumin was administered in doses up to 12 g to adult humans without any adverse effects.

2.2 Phytochemicals found in *Curcuma longa*

The curcuminoid compounds found in *C. longa* include curcumin, desmethoxycurcumin and bisdemethoxycurcumin. All three these curcuminoids are usually present in different concentrations in commercially available *C. longa* (turmeric) extract. The composition of *C. longa* (turmeric) commercial grade extract in terms of curcuminoid content is usually in the following order: curcumin (77%), desmethoxycurcumin (17%) and bismethoxycurcumin (3%) (Basnet *et al.*, 2011 and Sharma *et al.*, 2005). The chemical structures of the three curcuminoid compounds that are found in the *C. longa* plant (turmeric) are shown in Figure 2-2.

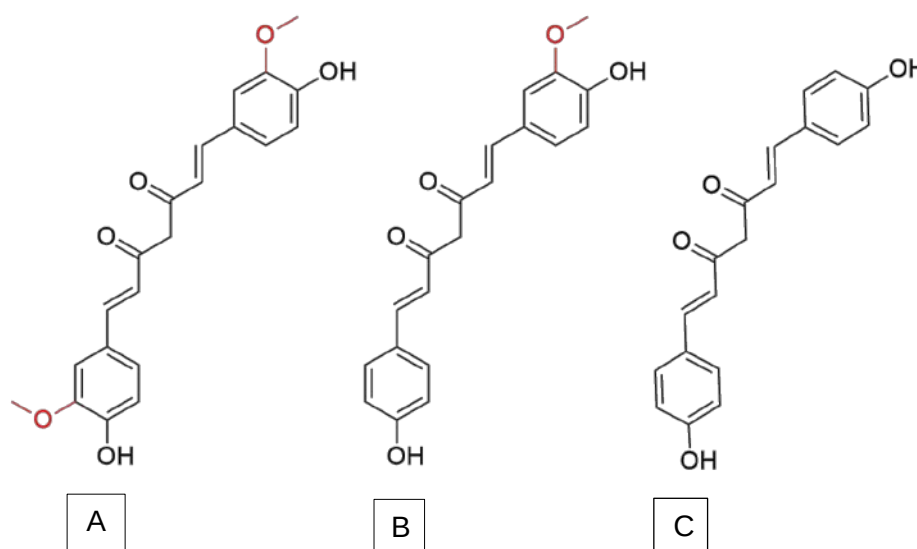


Figure 2-2: Chemical structures of the main curcuminoids found in *Curcuma longa* rhizome: (A) curcumin (diferuloylmethane or diferulylmethane), (B) desmethoxycurcumin (demethoxycurcumin, monodemethoxycurcumin) and (C) bisdemethoxycurcumin (didemethoxycurcumin).

The curcuminoid compounds extracted from *C. longa* (turmeric) have been approved as Generally Recognised as Safe (GRAS) by the Food and Drug Administration in accordance with Section 201(s) of the Federal Food, Drug and Cosmetics Act (GRAS Notice (GRN) No. 686). Due to the relative safety and large range of medicinal applications of turmeric as well as its culinary use as spice in food, *C. longa* has become a plant species of economic importance (Leong-Škorníčková *et al.*, 2008).

2.3 Curcumin (the main curcuminoid in *Curcuma longa*)

2.3.1 Physicochemical properties of curcumin

Curcumin is a lipophilic polyphenolic compound with the chemical name of 1,7-bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione that has a molecular weight of 368.38 g/mol and has a melting point of approximately 183°C. Curcumin is a bis- α , β -unsaturated β -diketone that exhibits keto-enol tautomerism and cis-trans isomerism. It is available as a bright yellow crystalline powder (Basnet *et al.*, 2011, Sharma *et al.*, 2005 and Slika & Patra, 2020).

Under physiological conditions, curcumin can exist in two tautomeric forms as illustrated in Figure 2-3. Transition from one tautomeric form to another is pH dependant. Curcumin acts as a potent H-atom donor in acidic to neutral environments ($\text{pH} \leq 7.4$), where the keto tautomeric form predominates. However, in an alkaline environment ($\text{pH} \geq 8.0$), the phenolic part of the molecule acts as an electron donor and the enol tautomeric form predominates (Basnet *et al.*, 2011 and Jovanovic *et al.*, 1999).

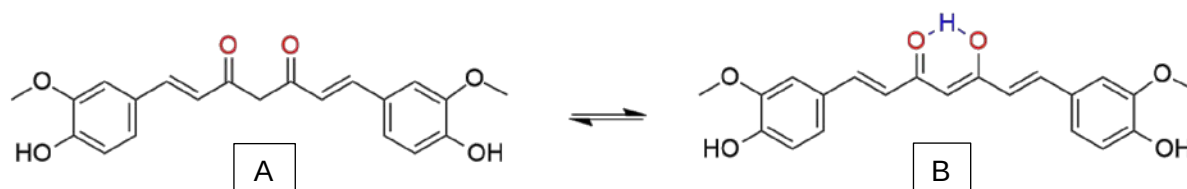


Figure 2-3: The (A) bis-keto and (B) enolate tautomeric forms of curcumin (Basnet *et al.*, 2011).

2.3.1.1 Solubility of curcumin

The poor solubility ($0.011 \mu\text{g/ml}$) of curcumin in water (Liu *et al.*, 2016 and Kawabata *et al.*, 2011) is due to the hydrophobic properties of this compound caused by the hydrocarbon bridge and the two phenolic or aromatic groups (Aggarwal & Sung, 2008). Although highly insoluble in water, curcumin can be dissolved in other solvents such as ethanol, methanol, acetone or dimethylsulfoxide (DMSO) (Amalraj *et al.*, 2017 and Prasad *et al.*, 2014). Curcumin has a relatively high partition coefficient (log P) value, which has been reported as being in the range of 2.3 to 3.2 (Heger *et al.*, 2014, Tomren *et al.*, 2007 and Priyadarsini, 2014). This log P value indicates that the compound is more lipophilic of nature and would dissolve in organic solvents rather than aqueous solvents (Bhal, 2011).

Curcumin is characterised as a weak Brønsted acid (Priyadarsini, 2014). The molecule possesses three protons, which are ionisable in water. There are two phenolic protons and one enolic proton of which the pKa values are 10.5 and 8.5, respectively (Ma *et al.*, 2019). At acidic pH values ($\text{pH} < 1.0$), curcumin is in its protonated form (H_4A^+) and the solution is red of colour. When pH values range from 1.0 – 7.0, the compound is largely found in its neutral form (H_3A) and the solution is yellow of colour (curcumin is highly insoluble in water at this pH range). When the pH is increased to values > 7.5 the compound's colour shifts to orange (Kumavat *et al.*, 2013, Stankovic, 2004 and Slika & Patra, 2020).

2.3.1.2 Stability of curcumin

It has been found that curcumin degrades rapidly upon exposure to neutral or alkaline environments in solution and is also susceptible to photochemical degradation (Liu *et al.*, 2016 and Baspinar *et al.*, 2018). Tønnesen^a *et al.* (1985) conducted alkaline degradation studies on curcumin solutions over a pH range of 7 – 10. In these alkaline environments, initial degradation products (ferulic acid and feruolylmethane) started to develop after merely 5 min. Feruolylmethane forms yellow-brown condensation products, which undergo hydrolysis and then develops vanillin and acetone products. In another study, curcumin was incubated in a 0.1 M phosphate buffer at pH 7.2 and after 30 min approximately 90% of the compound had been decomposed (Wang *et al.*, 1997).

In contrast, curcumin is relatively stable in acidic pH environments. Kumavat *et al.*, (2013) conducted a curcumin degradation study and found that 96.47% of curcumin was present in the solution after 60 min at a pH value of 1.2 (See Table 2-2 below). Delayed degradation due to more acidic environments as opposed to the more rapid degradation in alkaline environments is also supported by other scientific publications (Liu *et al.*, 2016, Tønnesen^a *et al.*, 1985 and Wang *et al.*, 1997).

Table 2-2: Amount of residual curcumin that remained after 60 min in solutions with different pH values (Kumavat *et al.*, 2013).

pH value	% curcumin after 60 min
7.4	3.83
7.0	47.87
1.2	96.47

It has been proposed by Kumavat *et al.* (2013) that the increased stability of curcumin in acidic environments can be due to presence of the conjugated diene structure. When the pH of the solution is increased to neutral or basic conditions, the proton is removed from the phenolic group and results in the destruction of the structure. Wang *et al.* (1997) suggested that by lowering the pH of the environment or adding an anti-oxidant such as ascorbic acid, N-acetylcysteine or glutathione, the degradation of curcumin can be markedly delayed.

The therapeutic use of curcumin is limited due to its low water solubility in both acidic or neutral aqueous environments, rapid hydrolytic degradation in alkaline media and susceptibility to photo-degradation (Tønnesen^b *et al.*, 2002).

2.3.2 Curcumin bioavailability

In a pharmacokinetic study where curcumin was administered orally (500 mg/kg) and intravenously (10 mg/kg) to rats, the absolute oral bioavailability of curcumin was determined to be only 1%. The absolute bioavailability was calculated from the area under the plasma concentration time curves (AUC) obtained after the oral and intravenous administrations, while compensating for dose differences. The AUC's were 7.2 ± 1.2 min $\mu\text{g/ml}$ and 3.6 ± 0.6 min $\mu\text{g/ml}$ for intravenous (10 mg/kg) and oral (500 mg/kg) doses, respectively (Yang *et al.*, 2007). Several pharmacokinetic studies in humans have indicated that curcumin reached relatively low blood plasma levels, exhibited limited distribution to tissues, was susceptible to excessive first pass metabolism and high elimination rates with short half-life values (Slika & Patra, 2020).

The extremely low bioavailability of curcumin can be attributed to its poor water solubility, poor absorption and membrane permeability, rapid first pass metabolism, fast clearance and elimination rates (Ma *et al.*, 2019 and Wang *et al.*, 2019). The low oral bioavailability of curcumin is possibly one of the main reasons why this pharmacologically active compound could not produce acceptable therapeutic outcomes. Several strategies have been implemented to improve the bioavailability of curcumin such as the preparation of submicron suspensions, phosphatidine complexes, numerous nano-formulations and technologies to increase its solubility such as solid dispersions, micelles, cyclodextrin complexes and conjugates (Imaizumi, 2016 and Ma *et al.*, 2019).

2.4 Drug absorption process and factors influencing it

Drugs administered via an extravascular route of administration such as solid oral dosage forms, must dissolve before it can be absorbed into the systemic circulation (Ashford, 2013 and Gerck *et al.*, 2016). Drug absorption from solid oral dosages is dependent on a series of potentially rate limiting processes. For conventional, immediate release oral dosage forms such as tablets these processes include disintegration of the tablet and subsequent release of the drug particles, dissolution of the drug particles in the aqueous gastric/intestinal fluids and permeation of the drug across the epithelial cell membranes into the blood circulation surrounding the gastro-intestinal tract as illustrated schematically in Figure 2-4 (Suarez *et al.*, 2016).

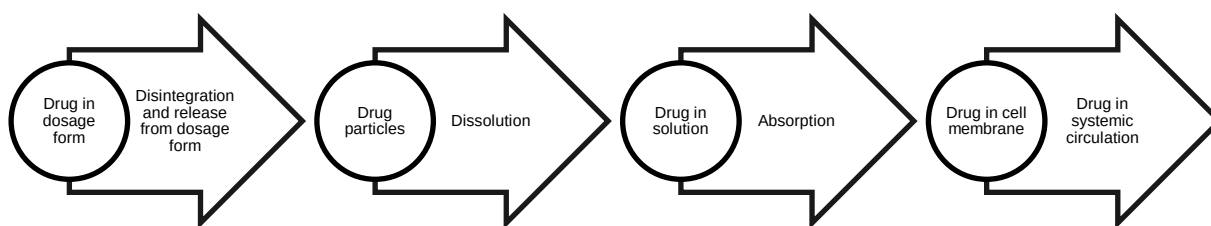


Figure 2-4: Schematic presentation of the rate processes of drug bioavailability as adapted from Suarez *et al.*, (2016).

Drugs that are considered poorly soluble in aqueous media (< 100 µg/ml) are limited by the rate at which they dissolve (Hörter & Dressman, 2001). Drug dissolution can be affected by several physicochemical and physiological factors in the gastrointestinal tract. These factors can be identified from the following Noyes-Whitney equation (Ashford, 2013 and Dokoumetzidis & Macheras, 2006);

$$\frac{dm}{dt} = \frac{DA(C_s - C)}{h} \quad \text{Equation 2-1}$$

Where, dm/dt is the rate of dissolution of the drug particles, D is the diffusion coefficient of the drug in solution in the gastrointestinal fluids, A is the effective surface area of the drug particles that are in contact with the gastrointestinal fluids, h is the thickness of the diffusion layer surrounding each drug particle, C_s is the saturation solubility of the drug in solution in the diffusion layer and C is the concentration of the drug present in the gastrointestinal fluid.

The rationale behind drug molecules moving from a region with high concentration to a low concentration region can be described by Fick's law of diffusion in the following equation (Gerk *et al.*, 2016).

$$\frac{dQ}{dt} = \frac{DAK}{h} (C_{GI} - C_p) \quad \text{Equation 2-2}$$

Where; dQ/dt is the diffusion rate, D is the diffusion co-efficient, A is the surface area of the biological membrane, K is the lipid-water partition coefficient of the drug present in the biologic membrane which controls drug permeation, h is the thickness of the membrane and $C_{GI}-C_p$ is the concentration gradient between the gastrointestinal tract and blood plasma in systemic circulation.

Thus, an increase in drug solubility will increase the saturation solubility of the drug in the diffusion layer surrounding drug particles and subsequently increase the concentration gradient and also the dissolution rate. The solubility of weak electrolytes is subject to their ionisation constants, pK_a and the pH value of the dissolution medium (Hörter *et al.*, 2001).

A solubility-pH profile can be produced for a drug molecule, which indicates its potential solubility at various pH values. A weak basic drug is more soluble in an acidic environment as it will be in an ionised state while the opposite will be true for a weak acidic drug which will be more soluble in a more alkaline environment (Suarez *et al.*, 2016)

For a drug that is administered orally, it has to cross the epithelium of the gastrointestinal tract in order to reach the blood circulation. According to the pH-partition theory, ionisable drugs only diffuse across biological membranes in their unionised form. Thus, the molecule should be in its unionised, lipophilic form rather than its ionised, hydrophilic form to permeate the membrane, but it should be in the ionised form for optimum solubility. Fortunately, there is an equilibrium between the ionised and unionised species of drug molecules. The absorption of the unionised species disturbs the equilibrium that exists between the ratio of ionised to unionised species. To replace the unionised molecules in order to regain equilibrium, some of the ionised species are converted to unionised species, which then become available to be absorbed through the membrane. Because this process occurs continuously, it permits the absorption of an ionisable drug to some extent even in unfavourable pH conditions where the drug is mainly in the ionised state (York, 2013 and Jambhekar, 1995).

2.5 The Biopharmaceutics Classification System (BCS)

The Biopharmaceutics Classification System (BCS) categorises active pharmaceutical ingredients (or drugs) based on their solubility and intestinal membrane permeability properties (refer to Table 2-3 below).

Table 2-3: The Biopharmaceutics Classification System for conventional, immediate release tablets as proposed by Amidon (1995).

Class	Solubility	Permeability
I	High	High
II	Low	High
III	High	Low
IV	Low	Low

According to the BCS, the terms “high solubility” and “high membrane permeability” can be defined as follows (Mehta *et al.*, 2017):

- A drug is considered highly soluble, when the highest dose strength is soluble in 250 ml or less of aqueous media over a pH range of 1.0 to 7.5 at $37^{\circ}\text{C} \pm 1^{\circ}\text{C}$.
- A drug is considered highly permeable when 90% or more of an orally administered dose is absorbed based on a mass balance determination or when compared to an intravenous reference dose.

Drugs are classified into the following classes according to the BCS:

Class I drugs with high solubility and permeability are generally well absorbed from the gastrointestinal tract. For these immediate release drugs, the dissolution rate is usually quite rapid meaning that gastric emptying will be the rate limiting factor. Class II drugs are poorly soluble but highly permeable. The poor solubility of the drug can lead to irregular absorption, with dissolution as the rate limiting step. Class III drugs are highly soluble but have very poor permeation capabilities. Dissolution will theoretically be rapid while absorption can be subject to factors such as gastrointestinal transit time or luminal contents. The final class, class IV drugs, present substantial complications for oral delivery as these drugs are neither soluble nor permeable (Amidon, 1995).

Upon a literature review, it was found that there is inconsistency regarding the BCS classification of curcumin, because it is classified to fall either in class II or class IV. Although numerous publications such as John *et al.*, (2013), Wahlang *et al.*, (2011), Her *et al.*, (2018) and Kawabata (2011) consider curcumin a class IV compound, there are also publications that classify curcumin as a class II drug including de Souza Ferreira & Bruschi (2019), Wan *et al.*, (2011), Hu *et al.*, (2015) and Jannin *et al.*, (2015). It is well known that curcumin has poor bioavailability and certain pharmaceutical strategies can be implemented to overcome this problem.

2.6 Pharmaceutical strategies to overcome the poor bioavailability of compounds

Different formulation approaches have been utilised to improve the delivery of compounds that suffer from limited bioavailability after oral administration (Liu *et al.*, 2016). One approach is to add functional excipients to the dosage form that can increase the active ingredient's solubility (Kawabata *et al.*, 2011), enhance its membrane permeability (Baki *et al.*, 2015) and/or reduce its metabolism (Panakanti *et al.*, 2015).

Numerous strategies have successfully been implemented to increase the solubility of a compound such as pH modification, particle size reduction, self-emulsification, solid dispersions, amorphization, inclusion complex formation amongst others (Kalepu & Nekkanti, 2015 and Kawabata *et al.*, 2011). Since curcumin has extremely low solubility (which will be addressed in this study), selected strategies that can be used to improve the solubility of compounds will be discussed in more detail below.

2.6.1 Complex formation with cyclodextrins

Cyclodextrins are cyclic oligosaccharides, which are molecularly structured as hollow truncated cones. The outer surface of the cyclodextrin cone is hydrophilic, while the inner cavity of the cone is hydrophobic. The hydrophilic nature of the outer surface of the cyclodextrin molecule consequently causes it to be water-soluble, while the hydrophobic core gives it the ability to accommodate a lipophilic molecule. Thus, cyclodextrins are used to increase the solubility of hydrophobic compounds (e.g. curcumin) by forming a reversible inclusion complex as illustrated in Figure 2-5, 'hiding' the drug within the cavity of the cone (Arun *et al.*, 2008). By implementing strategies such as complexation with cyclodextrins, an increased drug bioavailability and more consistent blood plasma levels can be achieved with lower and fewer administered doses (Jansook *et al.*, 2011).

A reversible complex infers that the drug will be released from the cyclodextrin molecule and become available to interact with the target receptor in order to cause a pharmacological action (Murdan, 2013). In the case of hydrophobic compounds, cyclodextrin complexes increase the amount of dissolved compound molecules available at the surface of the epithelium (Jansook *et al.*, 2011), which can be transported across the membrane as the complex or the molecule can dissociate from the complex just before permeation through the lipid membrane (Haimhoffer *et al.*, 2019).

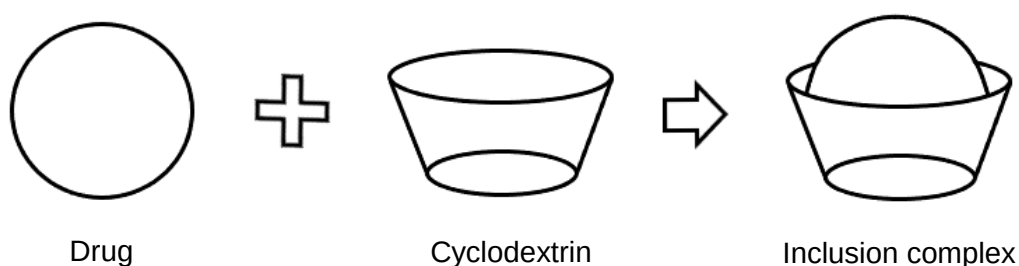


Figure 2-5: A graphical representation of a drug molecule and a cyclodextrin cone which form an inclusion complex, subsequently 'hiding' the drug molecule within the cyclodextrin cone cavity (Adapted from Arun *et al.*, 2008).

Cyclodextrins are produced from starch and comprise of glucopyranose units arranged in ring form, that exist as α -cyclodextrin (6 glucose units), β -cyclodextrin (7 glucose units) and γ -cyclodextrin (8 glucose units). The diameter of the cavity depends on the number of glucose units present in the ring, consequently, α -cyclodextrin has a cavity diameter of approximately 0.55 nm, while β -cyclodextrin and γ -cyclodextrin have cavity diameters of about 0.70 nm and 0.90 nm, respectively (Murdan, 2013). The size of the cavity of β -cyclodextrin is suitable for most drug molecules and will usually form reversible inclusion complexes with lipophilic drug molecules (Ashford, 2013).

Due to restrictions in terms of solubility and safety concerns of β -cyclodextrins such as potential nephrotoxicity, the need for modified cyclodextrins became clear. The solubility of β -cyclodextrins can be improved by substituting the H-bond forming hydroxyl group with various functional groups (Arun *et al.*, 2008). There are five general types of modified β -cyclodextrins with pharmaceutical significance as shown in Table 2-4.

Table 2-4: Types of modified β -cyclodextrins (Rajewski *et al.*, 1996)

Modified cyclodextrin type	Example
Methylated and alkylated cyclodextrins	2,6-dimethyl- β -cyclodextrin (DM β CD) and randomly methylated β -cyclodextrins (RM β CD)
Hydroxyalkylated cyclodextrins	2-hydroxypropyl- β -cyclodextrins (HP β CD)
Branched cyclodextrins	Glucosyl, diglucosyl (G2 β CD), maltosyl, and dimaltosyl
Carboxymethyl cyclodextrins	CM- β -CD
Sulfoalkylether cyclodextrins	Sulfobutyl ethers (SBE β CD) of β -CD with degrees of substitution of 4 and 7, SBE4 β CD and SBE7 β CD, respectively

2.6.1.1 2-hydroxypropyl- β -cyclodextrins (HP β CD)

Hydroxyalkyl derivatives of cyclodextrins, such as hydroxypropyl- β -cyclodextrin (HP β CD), are formed by reactions between cyclodextrins and either propylene or ethylene oxide. The reaction leads to substitution of the C-2, C-3 and C-6 hydroxyl groups with hydroxypropyl (Gao *et al.*, 2019). The amount of substitution present, as well as the position at which the substitution occurs, varies in commercially available products. Generally, there are between 3 and 10 hydroxyalkyl groups present per cyclodextrin molecule. Thus, the R-groups illustrated on the HP β CD structure below, can represent either a hydroxypropyl substitution or the initial, unchanged hydroxyl group (Hedges, 2009). The chemical structures of β -cyclodextrin and modified HP β CD are shown in Figure 2-6.

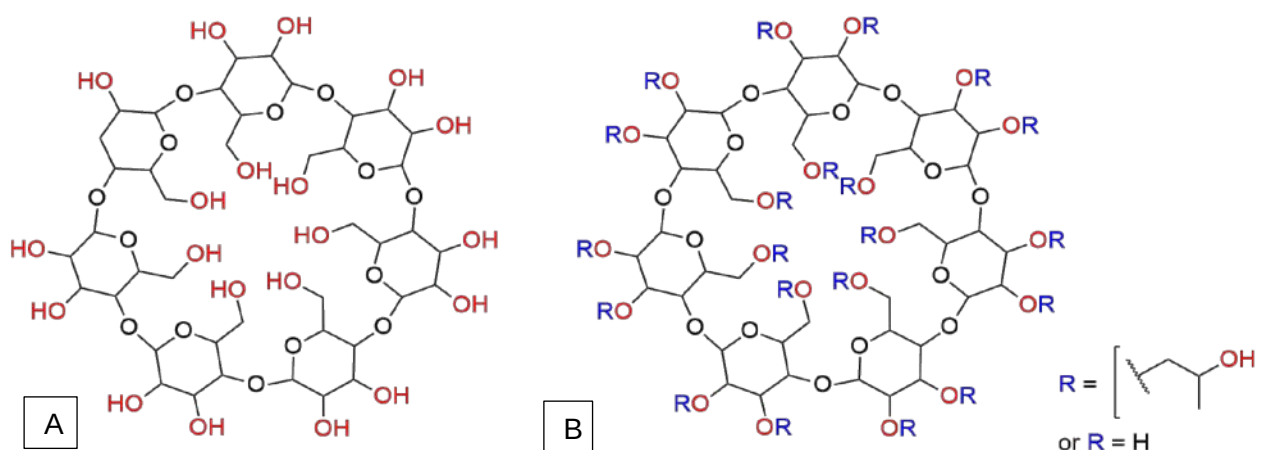


Figure 2-6: (A), chemical structure of β -cyclodextrin (Arun *et al.*, 2008) and (B), its derivative, hydroxypropyl- β -cyclodextrin (USP). The potential substitution of the hydroxyl group for hydroxypropyl group is represented by the R-group seen in the hydroxypropyl- β -cyclodextrin structure.

Upon modification of natural β -cyclodextrin to HP β CD, solubility is increased from around 18.5 mg/ml to ≥ 600 mg/ml (Gidwani *et al.*, 2015). This considerable increase in solubility of the cyclodextrin, significantly lowers the risk for renal damage as no crystallization occurs in the kidneys (Hedges, 2009).

Tønnesen^b *et al.* (2002) conducted a study on cyclodextrin complexation with curcumin and found that curcumin-cyclodextrin inclusion complexes led to an increase in curcumin solubility by a factor of 10^4 . The cavity of HP β CD is suitable for including the bulky side groups of the curcumin molecule and shows a higher increase in solubility than with natural β -cyclodextrin or its methylated derivate (Yadav *et al.*, 2009 and Jantarat *et al.*, 2014).

Complexation of curcumin with cyclodextrins corresponds with a 1:1 (guest:host) inclusion ratio (Patro *et al.*, 2014), but as explained by both Hegge *et al.* (2009) and Baglole *et al.* (2005), a 1:2 stoichiometry will be more likely given that a curcumin molecule is symmetrical with two identical phenyl groups, available for binding at both ends (See Figure 2-7). This 1:2 stoichiometry is also supported by other authors such as Tang *et al.* (2002) and Singh *et al.* (2009).

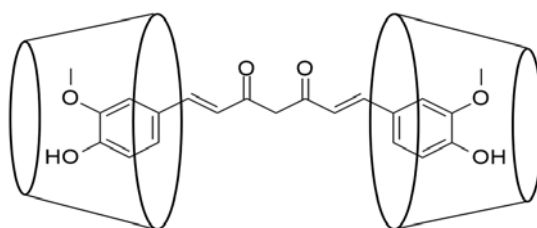


Figure 2-7: Graphical representation of the proposed 1:2 complex formed by curcumin and cyclodextrin (Adapted from Baglole *et al.* (2005))

2.6.2 Multiple-unit dosage forms

Multiple-unit dosage forms are drug delivery systems, which consist of several smaller units that each contains a portion of the drug dose (Alderborn, 2013 and McConnel & Basit, 2013). Multiple-unit dosage forms are characterised by more predictable and more specific drug delivery patterns as compared to that of conventional single-unit dosage forms. Generally, these systems lead to an increase in drug bioavailability, predictable gastrointestinal transit time, less gastrointestinal disturbances, more consistent plasma concentration levels, less inter- and intra-subject variability and greater product safety (Kumar and Teotia, 2018 and Fassihi, 2017).

2.6.2.1 Mini-tablet-in-capsule system

The mini-tablet-in-capsule system is a multiple-unit drug delivery system where conventional tableting methods are used to produce mini-tablets, which are loaded into hard gelatine capsules for oral administration (Alderborn, 2013).

Mini-tablets are an attractive alternative to conventional tablets due to the following advantages (de Brabander *et al.*, 2000, Lingam *et al.*, 2008, Mounika *et al.* 2015 and Lopes *et al.*, 2017):

- A larger surface area per volume, which leads to more efficient drug release.
- Decreased risk for dose dumping, local irritation and toxicity associated with high drug concentrations due to a higher dispersion of the smaller units throughout the gastrointestinal tract.
- Less subject to gastric emptying effects, which causes lower inter- and intra- subject variability.
- The multiple units offer high drug loading with higher modifiability in terms of release rate.
- Multiple units ensure that the failure of an individual unit won't be detrimental to the therapeutic effect of the entire dosage form.
- Fairly simple to manufacture, especially when compared to other multiple-unit dosage forms such as pellets.
- More reproducible than pellets or granules due to a more uniform shape and size, smoother surface, low porosity and high attainable strength which leads to improved compressibility.

By incorporating different mini-tablets into a capsule, a biphasic (fast/slow) delivery system can be created where different drug release rates can be achieved from one dosage form. The therapeutic advantage of implementing a biphasic system is less fluctuation in plasma levels as the primary rapid drug release provides a fast onset rise in drug plasma levels followed by a secondary steady release, maintaining drug plasma levels over an extended period of time (Mounika *et al.*, 2015).

The release of a drug substance from a dosage form can be modified in order to achieve a desired therapeutic effect. These effects are achieved by physically, chemically or biologically altering the site of drug release of a dosage form, delivery pattern and/or apparent absorption of a drug substance. Standard approaches of oral modified-release delivery include, delayed-release, site-specific or timed release, extended release and programmed release (Qiu *et al.*, 2009).

Extended release dosage forms are formulated to deliver a drug substance to a specific part of the gastrointestinal tract (e.g. the stomach) for absorption. These dosage forms are designed to gradually release the drug substance over a period of several hours (Fassihi, 2017).

2.6.2.2 Gastro-retentive dosage forms

Due to the instability and low solubility of curcumin in alkaline environments such as those found in the small intestine, a gastro-retentive dosage form will have been proven to prolong the residence time of the drug in the stomach, thereby ensuring better stability as well as solubility of curcumin in the acidic environment (Sahu *et al.*, 2011 and Streubel *et al.*, 2006).

Floating matrix type gastro-retentive formulations are retained in the stomach by incorporating a highly swellable, gel-forming cellulosic hydrocolloid such as hydroxypropyl methylcellulose into an oral dosage form. Upon contact with gastric fluid, the swellable excipient forms a colloidal gel layer around the tablet's surface. As the tablet floats on the gastric contents the drug will gradually and continually diffuse through the gel layer. The drug release rate is reduced by the relatively short diffusion distance through the gel layer, which is kept thin by continuous abrasion of the outer part as water penetrates the matrix core. The gel barrier maintains a relative density lower than that of the surrounding gastric fluids and the swollen polymer can entrap air, which in turn contributes to the buoyancy of the dosage form (Garg *et al.*, 2008, Nokhodchi *et al.*, 2012, Rao *et al.*, 2012 and Sahu *et al.*, 2011).

Controlled release, floating drug delivery systems usually exhibit a delayed response in drug release, which means there will be an initial lag period. Furthermore, this system is highly dependent on the amount of gastric fluid and other gastric contents such as food (Pawar *et al.*, 2012), it is therefore imperative that the patient takes this dosage form with an sufficient amount of water.

2.7 SeDeM expert diagram system

The SeDeM expert diagram system (EDS) is a pre-formulation tool that can be used to determine the suitability of an active pharmaceutical ingredient for formulation into direct-compressible tablets by analysing specific characteristics of the powder. Consequently, it is possible to detect the characteristics of the active pharmaceutical ingredient that need to be adjusted by addition of other powders (excipients) to facilitate direct compression into a tablet (Pérez *et al.*, 2006 and Suñé-Negre^a *et al.*, 2008).

The system quantitatively and experimentally determines the suitability of a powder for direct compression by investigating 12 parameters (powder properties that are further processed into 5 incidence factors are specified in Table 2-5). Each parameter has a pre-specified limit value.

Table 2-5: Parameters and equations of the SeDeM expert diagram (adapted from Suñé-Negre^b et al., 2011).

Incidence	Parameter	Symbol	Unit	Equation	Limit
Dimension	Bulk density	Da	g/ml	$Da = P/Va$	0 - 1
	Tapped density	Dc	g/ml	$Dc = P/Vc$	0 - 1
Compressibility	Inter-particle porosity	Ie	–	$Ie = Dc - Da/Dc \times Da$	0 - 1.2
	Carr index	IC	%	$IC = (Dc - Da/Dc) \times 100$	0 - 50
	Cohesion index	Icd	N	Experimental	0 - 200
Flowability / powder flow	Hausner index	IH	–	$IH = Dc/Da$	3 - 1
	Angle of repose	(α)	°	$\alpha = \tan^{-1} h/r$	50 - 0
	Powder flow	"	s	Experimental	20 - 0
Lubricity / stability	Loss on drying	%HR	%	Experimental	10 - 0
	Hygroscopicity	%H	%	Experimental	20 - 0%
Lubricity / dosage	Particles < 50 μ m	%Pf	%	Experimental	50 - 0%
	Homogeneity index	(I θ)	–	$I\theta = Fm/100 + \Delta Fmn$	0 - 2×10^{-2}

After the parameter values are obtained and processed, conversions are made to provide values that will be used to draw SeDeM diagrams (as seen in Figure 2-8). The diagram is represented by a 12-sided polygon where the radius values are connected with linear segments.

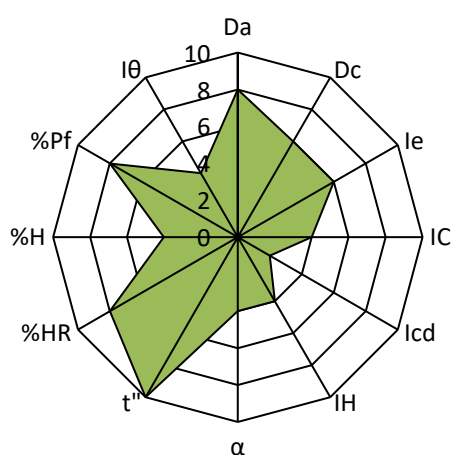


Figure 2-8: Example of a SeDeM diagram or polygon based on calculated radius values.

2.8 Techniques to evaluate drug delivery: permeation studies

The *in silico* or computational determination of drug permeation following drug release from a solid oral dosage form based only on the physicochemical properties of the drug molecule such as pKa, molecular weight, solubility and dissolution rate oversimplifies the rationale behind drug permeation across biological membranes (Joubert *et al.*, 2017). The most accurate method to determine the true permeation capabilities of a substance is by means of *in vivo* studies as these models take physiological properties such as gastric fluid, bile, chemical or bacterial degradation and metabolism into account. Whole animal studies are, however, severely limited by the availability of subjects, costs and ethics related issues, therefore alternative absorption models have been developed, which include; tissue-based, cell-based and cell-free models (Berben^a *et al.*, 2018, Tukker, 2000 and Westerhout *et al.*, 2014).

Ex vivo studies, using excised tissues, have been implemented as an easier and more cost-effective alternative to whole animal studies. The use of human tissue (typically obtained from bariatric surgery) is favoured for *ex vivo* studies, however, availability of these tissues is limited. Thus, animal tissues (either laboratory animals or from an abattoir) are often utilised during these studies (Joubert *et al.*, 2017 and Nunes *et al.*, 2015). A brief tissue viability window and inter-individual differences such as age, diet, pathology etc. potentially affect tissue morphology and often results in both intra- and inter-laboratory variability (Joubert, *et al.*, 2017 and Westerhout *et al.*, 2014) subsequently reducing this model to a low- to moderate-throughput system (Berben^a *et al.*, 2018).

In vitro, epithelial cell monolayers such as colonic adenocarcinoma (Caco-2) cell line models exhibit transport pathways that correlate to pathways present within the gastrointestinal tract. Although cell monolayer models present with functional properties similar to human intestinal epithelium, use of this model is restricted by the lengthy duration of cell growth cycles, high monetary cost, risk of microbial contamination and both intra- and inter-laboratory variability (Corti *et al.*, 2006 and Volpe, 2010).

Therefore, the most attractive method for permeation testing is a cell-free system such as a synthetic membrane model. This model is beneficial as it is simple, time- and cost-effective and provides reproducible results (Berben^b *et al.*, 2018). As most drugs are primarily absorbed by means of passive diffusion, synthetic membranes offer a high-throughput option for the preliminary determination of drug permeation (Corti *et al.*, 2006).

Side-by-side diffusion chambers are routinely used to perform permeation studies (Westerhout *et al.*, 2014 and Nunes *et al.*, 2016). One of the first uses of side-by-side diffusion cells was described by Ussing (Persson, 2016) and later modified by Grass & Sweetana (1988). For this method, small sections of tissue are clamped between two acrylic chambers, each filled with a buffer solution preheated to 37°C. Carbogen gas (a mixture of 95% oxygen and 5% carbon dioxide) is continuously

bubbled through the buffer solution to maintain tissue viability and ensure good hydrodynamics. The drug substance or dosage form is then added to one of the chamber compartments where after the amount of drug accumulated in the other chamber is measured as a function of time (Tukker, 2000, Grass & Sweetana, 1988).

Permeation studies of curcumin across Caco-2 cell lines found passive diffusion to be the main mechanism of drug transport (McLellan, 2018 and Yu *et al.*, 2011), therefore a synthetic membrane model will be appropriate for the current study. A previously published study conducted on a lipophilic drug compound over a selection of synthetic membranes concluded that membranes composed of cellulose nitrate presented with an apparent permeability coefficient (P_{app}) comparable to excised porcine intestinal tissue (Fensham *et al.*, 2020). For that reason, curcumin permeation studies will be conducted by mounting synthetic cellulose nitrate membrane filters in a modified Sweetana-Grass diffusion apparatus.

2.9 Summary of literature review on curcumin

Curcuma longa (turmeric) has many pharmacological activities of which include anti-inflammatory and anti-cancer effects (Van Wyk & Wink, 2004). These medicinal effects are due to the phytochemicals found in the plant's rhizome which are known as curcuminoids. There are three curcuminoids present in commercially available turmeric powder of which curcumin is the most abundant (77%) (Basnet *et al.*, 2011 and Sharma *et al.*, 2005).

Effective therapeutic plasma levels after oral administration of curcumin is deterred by the compound's poor aqueous solubility and rapid degradation in alkaline environments (Tønnesen^b *et al.*, 2002), therefore, demanding formulation approaches to be utilised in an effort to overcome these limitations and potentially improve bioavailability.

By forming an inclusion complex with curcumin and a cyclodextrin such as hydroxypropyl- β -cyclodextrin (HP β CD), the poor aqueous solubility of curcumin can be markedly improved (Tønnesen^b *et al.* 2002). The instability of curcumin in alkaline environments can be overcome by formulating floating matrix type gastro-retentive mini-tablets that will be incorporated into a capsule system. Formulating gastro-retentive tablets will be beneficial as this will retain the dosage form in the acidic environment of the stomach where the curcumin compound is known to be more stable and additionally providing a prolonged residence time could potentially increase the drug's bioavailability (Sahu *et al.*, 2011 and Streubel *et al.*, 2006).

CHAPTER 3: MATERIALS AND METHODS

3.1 Introduction to materials and methods

Oral administration of curcumin results in relatively low plasma levels, which can be ascribed to the poor aqueous solubility and rapid degradation of curcumin within alkaline environments (Tønnesen^b *et al.*, 2002). This necessitates the application of formulation approaches to overcome these undesirable biopharmaceutical characteristics of curcumin.

Therefore, the purpose of this study was to prepare an inclusion complex of curcumin in hydroxypropyl- β -cyclodextrin (HP β CD) in order to improve the poor aqueous solubility of curcumin. Additionally, the instability of curcumin in alkaline environments was addressed by formulating floating matrix type gastro-retentive mini-tablets, which retain the dosage form in the acidic environment of the stomach where curcumin is more stable (Sahu *et al.*, 2011 and Streubel *et al.*, 2006). The curcumin-HP β CD inclusion complex has been chemically characterised by means of different analytical techniques to confirm complex formation. The solubility of the curcumin-HP β CD inclusion complex was measured and compared to that of curcumin raw material as well as a physical mixture of curcumin and HP β CD. Direct-compressible mini-tablets have been formulated by making use of the SeDeM EDS. The SeDeM EDS was used to predict the quantity of corrective excipients to add to the active ingredient in order to prepare direct-compressible mini-tablets. After different types of curcumin and curcumin-HP β CD inclusion complex containing mini-tablets were prepared, they were evaluated in terms of their physical properties, curcumin release behaviour (dissolution profiles) and curcumin delivery across synthetic membranes in a simulated gastric fluid environment.

The materials and methods used to complete these experiments are described in this chapter. A quantitative experimental design was used to execute this research project in order to obtain results that could be used to reach a conclusion regarding improvement of curcumin delivery by means of solubility enhancement and gastro-retentive formulation design.

3.2 Materials

The materials used for this study and the manufacturers/suppliers from which each material was purchased are given in Table 3-1, below. The purity of the chemicals is indicated where applicable and the purity of curcumin was confirmed by analysis in comparison to a reference standard.

Table 3-1: Summary of the materials used for this study and the manufacturers/suppliers from which they were purchased.

Material	Manufacturer/supplier
Curcumin (98%)	Industrial Analytical Pty Ltd (Johannesburg, RSA)
Curcumin reference standard (99.9%)	ChromaDex (Johannesburg, RSA)
Hydroxypropyl- β -cyclodextrin (HP β CD)	DB Fine Chemicals Pty Ltd. (Johannesburg, RSA)
Lucifer Yellow (LY)	Sigma Aldrich (Johannesburg, RSA)
Magnesium stearate	Sigma Aldrich (Johannesburg, RSA)
Hydroxypropyl methylcellulose (HPMC - Pharmacoat®)	Shin-Etsu Chemical Co. Ltd. (Tokyo, Japan)
Styrene divinylbenzene copolymer (styrene DVB)	Sigma Aldrich (Johannesburg, RSA)
Vinylpyrrolidone-vinyl acetate copolymer (PVP VA64 - Kollidon® VA64)	BASF (Ludwigshaven, Germany)
Microcrystalline cellulose (MCC - Avicel® pH 200)	DB Fine Chemicals Pty Ltd. (Johannesburg, RSA)
Talc	Associated Chemical Enterprises (ACE) (Johannesburg, RSA)
Aerosil®	DB Fine Chemicals Pty Ltd. (Johannesburg, RSA)
Ac-di-sol®	DB Fine Chemicals Pty Ltd. (Johannesburg, RSA)
Krebs-Ringer bicarbonate buffer (KRB)	Sigma Aldrich (Johannesburg, RSA)
Sodium bicarbonate	Sigma Aldrich (Johannesburg, RSA)
Hydrochloric acid (37%)	Merck (Sandton, RSA)
Sodium chloride	Associated Chemical Enterprises (ACE) (Johannesburg, RSA)
Acetonitrile (gradient grade)	Merck (Sandton, RSA)
Methanol (gradient grade)	Merck (Sandton, RSA)
Methanol	Burdick & Jackson (Johannesburg, RSA)
Water	Burdick & Jackson (Johannesburg, RSA)
Formic acid (99%)	Merck (Sandton, RSA)
Cellulose nitrate (CN) membrane filters	Merck (Sandton, RSA)
Costar® 96-well plates	The Scientific Group (Randburg, RSA)
HPLC vials and caps	Seperations (Randburg, RSA)
Membrane filters (cellulose nitrate)	Anatech Analytical Technology (Johannesburg, RSA)

3.3 Methods

3.3.1 Analytical methods for sample analysis

3.3.1.1 Liquid chromatography mass spectrometry analysis (LC-MS)

Curcumin samples were analysed on an LC-MS apparatus. The LC system consisted of an Agilent 1290 Infinity binary pump and a 1260 Infinity autosampler fitted with a Phenomenex Kinetix (2.6 μ m, C18, 100 x 2.1 mm) column. Samples were separated with an (50:50 v/v) isocratic mobile phase consisting of acetonitrile/formic acid (0.1% v/v) and water/formic acid (0.1% v/v) at a flow rate of 0.200 ml/min and a column temperature of 40°C. ESI-MS data acquisition was performed in positive mode using an Agilent Jetstream ESI probe and a 6470 triple quadrupole detector with capillary voltages set to 4000 V. Drying gas (N₂) and sheath gas temperatures were set at 300°C with a flow rate of 5 l/min and 6 l/min, respectively. Curcumin was quantified by multiple-reaction monitoring of the precursor-product ion combination of 369.1 $\rightarrow$ 145 m/z.

3.3.1.2 Fluorescence spectrophotometric analysis

Lucifer yellow (LY) samples, obtained from the membrane integrity test performed on the synthetic cellulose nitrate membrane filters used during the permeation studies, were analysed with a Spectramax® Paradigm plate reader with excitation and emission wavelengths pre-set to 485 nm and 525 nm, respectively (adapted from Rozehnal *et al.*, 2012 and van Niekerk *et al.*, 2018).

3.3.2 Validation of liquid chromatography – mass spectrometry (LC-MS) and fluorescence spectrophotometric (FS) analyses methods

As per requirements set out by the International Conference on Harmonisation of Characteristics (ICH) document (ICH Q2(R1), 2005) on the validation of analytical procedures; both the LC-MS and fluorometric spectrophotometer analytical methods used during curcumin quantification, have been validated by means of linearity, range, repeatability (intra-day) and intermediate (inter-day) precision and accuracy. The LC-MS analytical method was also validated in terms of specificity.

3.3.2.1 Linearity

Linearity refers to a direct correlation between the concentration of the analyte and the response of the instrument. Linearity is verified by statistical methods such as performing a regression on a line obtained from plotting instrument measurement as a function of concentration. A correlation coefficient (R^2) $\geq$ 0.998 is considered acceptable for linearity (BP, 2019; FDA (ORA-LAB. 5.4.5), 2020; Shabir, 2003).

Serial dilutions of both curcumin and LY were prepared and analysed by the respective instruments. The dilutions ranged from 750 ng/ml to 10 ng/ml for curcumin and 50 µg/ml to 1.56 µg/ml for LY. After analysis, a calibration curve was set up on Microsoft Excel® to determine the correlation coefficient (R^2) for each molecule and corresponding instrument.

3.3.2.2 Precision

Precision confirms the proximity of agreement between a series of separate measurements of homogenous samples under the same operating conditions (BP, 2019; ICH Q2(R1), 2005).

Both repeatability (intra-day) and intermediate precision (inter-day) validations were conducted. After results were obtained, the percentage relative standard deviation (% RSD) value was determined as described below (Shabir, 2003). Precision was determined by calculating the standard deviation and % RSD of the measurements acquired during analysis. The % RSD was determined using Equation 3-1 (Shabir, 2003).

$$\% \text{ RSD} = \text{SD} / \text{Ave} \times 100 \quad \text{Equation 3-1}$$

Where % RSD is the relative standard deviation expressed as a percentage, SD is the standard deviation between sample detection levels and Ave is the average sample detection value at each time point.

3.3.2.2.1 Intra-day

Three samples of different concentrations were analysed in triplicate under the same operating conditions within 24 h. In addition, nine samples of the same concentration were analysed to confirm the repeatability of the analytical methods.

3.3.2.2.2 Inter-day

Inter day precision was performed to determine variations within the same laboratory (Rambla-Alegre *et al.*, 2012). The evaluation was performed in triplicate, over three separate days. The same analytical instrument was used under the same experimental conditions for both analytical methods.

An acceptable % RSD limit was established for this study by reviewing previously validated curcumin analysis methods similar to the analytical method used in this study (USP, 2015). From the literature review, % RSD values for intra-day precision ranged from 1.8% to 14.4%, while the % RSD values for inter-day precision ranged from 0.6% to 10.9% (Liu *et al.*, 2006, Yang *et al.*, 2007, Ramalingam *et al.*, 2014, Ma *et al.*, 2015, Li *et al.*, 2018 and Song *et al.*, 2019). Based on these published values, it was decided that a % RSD value equal to or less than 15% for intra-day and 10% for inter-day precision will be deemed acceptable for this study.

3.3.2.2.3 Accuracy

Accuracy is also known as the trueness of a value (Shabir, 2003). This conveys the closeness of agreement of the measured value to the accepted reference or true value (Rambla-Alegre *et al.*, 2012).

Accuracy was confirmed by comparing the average response values of a large series of samples of three different concentrations to the true response value of a corresponding standard sample. The difference between the sample response values was determined by calculating the percentage recovery (Equation 3-2) for each concentration (ICH Q2(R1), 2005).

$$\text{Percentage recovery} = \text{observed/expected} \times 100 \quad \text{Equation 3-2}$$

The validated method is considered accurate if the mean recovery value falls within 90% to 110% of the standard sample (Shabir, 2003).

3.3.2.2.4 Specificity

Specificity refers to the ability of an analytical technique to analyse a particular compound without substantial interference from other entities such as excipients, impurities or degradation products (BP, 2019; ICH Q2(R1), 2005).

Specificity was determined for the LC-MS analytical method by measuring the response of a physical mixture sample which contain the analytes curcumin and HP β CD in the stoichiometric ratio used during formulation and then comparing these measurements with those of a standard sample containing only curcumin (Shabir, 2003).

3.4 Purity tests of curcumin vs reference standard

The purity of curcumin raw material which was used as the active ingredient in this study, was determined by comparing the concentration of the same quantity of a curcumin reference standard as determined by means of high-performance liquid chromatography (HPLC) analysis where after the percentage recovery of curcumin raw material was determined. The isocratic chromatographic elution of curcumin samples was performed on an Agilent 1100 series quaternary core pump and autosampler system, set-up similar to the method described by Jayaprakasha *et al.* (2002). Curcumin samples were quantified at 425 nm with a diode array ultraviolet detector. The mobile phase consisted of methanol, a mixture of water and acetic acid and acetonitrile (5%, 35% and 60% v/v, respectively) with a flow rate of 1 ml/min for 8 min.

3.5 Preparation of inclusion complexes of curcumin with hydroxypropyl- β -cyclodextrin (HP β CD)

An inclusion complex of curcumin with HP β CD was prepared in a 1:2 stoichiometric ratio similar to the common solvent evaporation technique as described by Yadav *et al.* (2009) and Jantarat *et al.* (2014). Briefly, sufficient curcumin and HP β CD as needed for a 1:2 stoichiometric ratio namely 4.00 g and 29.87 g, respectively, were both dissolved in methanol (750 ml) by gently agitating the solution until clear. A substantial volume of methanol was added to the solution to ensure that the total amount of curcumin and HP β CD was completely dissolved in order for the respective compounds to freely interact with one another. The solution was placed on a magnetic stirrer in a fume hood and left overnight to evaporate at room temperature ($23 \pm 2^\circ\text{C}$). The dry powder obtained (Figure 3-1-A) after evaporation was gently ground with a mortar and pestle and sieved to attain a powder with relatively uniform particle consistency. Thereafter, the curcumin-HP β CD inclusion complex powder obtained (Figure 3-1-B) was placed in an amber container to protect it from light and it was stored in a fridge until used in the experiments.

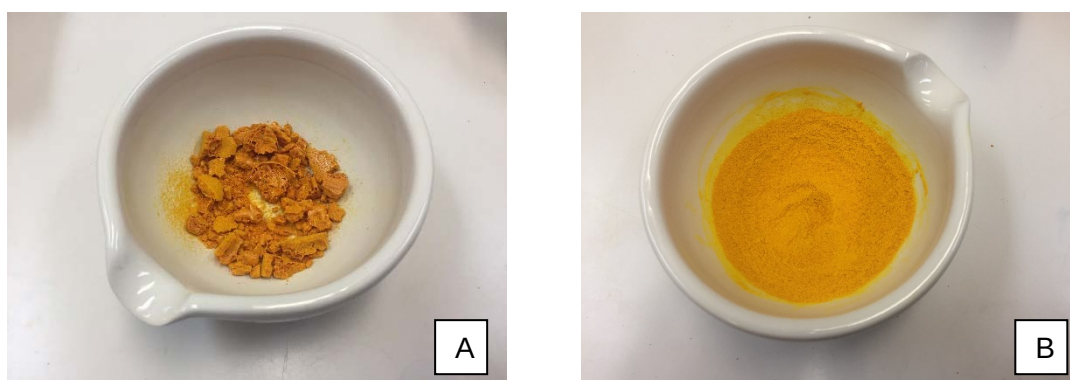


Figure 3-1: Photographs of the solid curcumin-HP β CD inclusion complex material obtained after overnight evaporation (A); and the final, ground and sieved curcumin-HP β CD inclusion complex powder obtained (B).

A physical mixture of curcumin and HP β CD was prepared by thoroughly mixing curcumin and HP β CD in a 1:2 molar ratio (1.00 g and 7.47 g, respectively) with a pestle in a mortar.

3.6 Characterisation of inclusion complexes with hydroxypropyl- β -cyclodextrin (HP β CD)

Complex formation between curcumin and HP β CD was confirmed and characterised by means of Fourier Transform Infrared Spectroscopy (FTIR), Differential Scanning Calorimetry (DSC), X-ray Diffraction (XRD) spectroscopy, visual inspection under a microscope and solubility studies. The characterisation results obtained for the curcumin-HP β CD inclusion complex was compared to both curcumin and HP β CD individually, as well as to a physical mixture of curcumin and HP β CD.

3.6.1 Fourier-Transform Infrared spectroscopy (FTIR)

Fourier-Transform Infrared (FTIR) spectroscopic spectra were recorded on a Shimadzu IR Prestige-21 spectrophotometer (Shimadzu, Kyoto, Japan) over a range of 400 - 4000 cm^{-1} . Potassium bromide (KBr) was used as a background analyte. A diffuse reflectance method was applied by combining each sample with KBr using a mortar and pestle and measuring its IR spectrum in a reflectance cell.

3.6.2 Differential Scanning Calorimetry (DSC)

A Mettler DSC 3⁺ (Mettler Toledo, Greifensee, Switzerland) apparatus was used to record the DSC thermograms. Powder samples, weighing roughly 3 – 5 mg were placed in closed aluminium 40 μl crimp cells (with pin-holes) and heated to an end temperature conditional to the melting point of the relevant API and/or mixture, at a heating rate of 10°C/min, with a nitrogen gas flow rate of 35 ml/min.

3.6.3 X-ray Powder Diffraction (XRPD) spectroscopy

X-ray powder diffraction patterns were obtained using a PANalytical Empyrean diffractometer (PANalytical, Almelo, Netherlands). The measurement conditions were: target, Cu; voltage, 40 kV; current, 30 mA; divergence slit, 2 mm; anti-scatter slit, 0.6 mm; detector slit, 0.2 mm; monochromator; scanning speed, 2°/min (step size, 0.025°; step time, 1.0 sec).

3.6.4 Visual inspection of hydroxypropyl- β -cyclodextrin (HP β CD) and a physical mixture of curcumin and HP β CD under a microscope

The microscopic analysis was performed with a Nikon Eclipse E4000 microscope, fitted with a Nikon DS-Fi1 camera (Nikon, Japan). A small amount of sample was placed on the centre of the slide and viewed under the microscope. A sample of the prepared curcumin-HP β CD complex was visually compared to a physical mixture sample containing curcumin and HP β CD in a 1:2 molar ratio.

3.6.5 Solubility studies

Solubility studies were performed on curcumin and on the inclusion complex of curcumin in HP β CD to confirm whether it has produced a noticeable increase in the solubility of curcumin. Oversaturated samples were prepared by weighing an amount in excess of the expected solubility for each powder in amber glass tubes. The tubes were then loaded on to a rotating aluminium axis in a water bath with a temperature of 37°C ($\pm$ 2°C). Samples were removed and analysed after 2, 4, 8, 24 and 48 hours. Solubility data was reported in duplicate as previously published (Prakash, 2013).

3.7 Characterisation of powder flow (active ingredient and excipients) according to the SeDeM expert diagram system (EDS)

The following powder flow parameters (Table 3-4) were evaluated for the active ingredient (curcumin), the prepared curcumin-HP β CD inclusion complex and each selected excipient (to be included in a gastro-retentive drug delivery system) using the methods described by the British Pharmacopoeia (BP, 2019), Pérez *et al.* (2006) and Suñé-Negre^a *et al.* (2008).

Table 3-2: List of active ingredients and selected excipients for the evaluation of powder flow parameters

Active ingredients	Curcumin
	Complex of curcumin in HP β CD
Selected excipients	Microcrystalline cellulose
	Hydroxypropyl methyl cellulose
	Vinylpyrrolidone-vinyl acetate copolymer
	Styrene divinylbenzene copolymer

3.7.1 Bulk density (Da) and Tapped density (Dc)

The volumes of the relevant active ingredient and each excipient were measured by placing a powder sample of approximately 25 g into a dry 250 ml glass graduated measuring cylinder (markings readable in 2 ml increments). The powder was levelled within the cylinder without compaction, where after the initial or unsettled apparent volume of powder was recorded by reading it off the applicable marking (BP, 2019). The bulk density (Da) of the powder was then calculated using the following equation:

$$Da = P/V_a \quad \text{Equation 3-3}$$

Where bulk density (Da) is expressed as the ratio of net weight (P) to the initial volume of the powder (V_a).

The measuring cylinder was attached to a mounted plate auto tap apparatus (Erweka SVM 223 tapped volumeter, Erweka, Germany). The apparatus was operated for 1250 taps, after which, the volume of the powder was recorded by reading it off the applicable marking. The tapped density (Dc) of the powder was calculated using the following equation:

$$Dc = P/V_c \quad \text{Equation 3-4}$$

Where; tapped density (Dc) is expressed as the ratio of net weight (P) to the final volume of powder after tapping (V_c).

3.7.2 Inter-particle porosity (Ie)

The inter-particle porosity was calculated from the bulk density and tapped density values using the following equation (Suñé-Negre^a *et al.* 2008):

$$I_e = D_c - D_a / D_c \times D_a \quad \text{Equation 3-5}$$

Where; I_e is the inter-particle porosity, D_c is the tapped density and D_a is the bulk density.

3.7.3 Compressibility index (IC%)

The compressibility index or Carr's index was calculated from the bulk density and tapped density values using the following equation (BP, 2020):

$$IC = (D_c - D_a / D_c) \times 100 \quad \text{Equation 3-6}$$

Where; IC is the compressibility index (Carr's index), D_c is the tapped density and D_a is the bulk density.

3.7.4 Hausner ratio (IH)

The Hausner ratio was calculated from the bulk density and tapped density using the following equation (BP, 2020):

$$IH = D_c / D_a \quad \text{Equation 3-7}$$

Where; IH is the Hausner ratio, D_c is the tapped density and D_a is the bulk density.

3.7.5 Cohesion index (Icd)

Powder samples were compressed at each powder's respective maximum compression force by a Korsch XP1 (Korsch AG, Germany) single station tablet press, where after the mean hardness (N) was determined with an Erweka TBH 425 TD hardness tester (Erweka, Germany) unit. A suitable lubricant mixture was added to abrasive powder samples to improve compressibility as previously suggested. The lubricant mixture composition is described in Table 3-3 (Pérez *et al.*, 2006).

Table 3-3: Standard lubricant composition for abrasive powders, which were added to the powders where needed (Pérez *et al.*, 2006)

Lubricant	Concentration
Talc	2.36% w/w
Aerosil 200	0.14% w/w
Magnesium stearate	1.00% w/w

3.7.6 Angle of repose (α)

The angle of repose was determined by passing 25 g of each powder sample through a funnel, allowing the powder to flow onto a flat base surface to form a cone shaped heap. The specific funnel used was 9.5 cm in length, starting with an upper diameter of 7.2 cm which tapers down and ends at the bottom of the spout at a diameter of 1.8 cm. The funnel was positioned at a height of 6 cm above the base surface and the powder was allowed to flow through at its own pace without additional agitation. The height and diameter of the powder heap was then measured using a graduated ruler (BP, 2020; Pérez *et al.*, 2006). The angle of repose was calculated using the following equation (BP, 2020):

$$\alpha = \tan^{-1} h/r \quad \text{Equation 3-8}$$

Where; α is the angle of repose calculated by the tangent of the height (h) and radius (r) of the powder heap.

3.7.7 Flowability (t'')

Powder samples (25 g) were placed in the funnel of a granulate flow tester (Erweka GTL, Erweka, Germany) apparatus with the shutter closed. The shutter was opened allowing the sample to flow through a 10 mm orifice, during which the flow rate of the powder sample was calculated in g/sec. This test was performed in triplicate on each powder and the mean values were calculated (s/25 g).

3.7.8 Loss on drying (%HR)

Powder samples (1 g) were dried in an oven at $105 \pm 2^\circ\text{C}$ for 24 h, until a constant weight was obtained. Measured weight values of each powder were compared before and after drying to determine the percentage mass lost (BP, 2020).

3.7.9 Hygroscopicity (%H)

Powder samples (1 g) were placed in open containers and stored in a climatic chamber (Binder KBF240, Binder, Germany) with an ambient relative humidity of approximately $76 \pm 2\%$ at a temperature of $22 \pm 2^\circ\text{C}$ for 24 h (Pérez *et al.*, 2006). The hygroscopicity of each powder was established by comparing the initial weight of each powder sample to the weight of the relevant sample following the 24-hour storage period.

3.7.10 Particle size (%Pf)

In this study, particle size of the powder particles was determined by the laser diffraction method as previously described (Scholtz *et al.*, 2017). Laser diffraction is used as an alternative to the sieve method of particle size determination due to its improved reproducibility supported by easier, quicker, more accurate and cost-effective application (Malvern Instruments Worldwide, 2017).

A Malvern Mastersizer 2000 instrument was used to measure the particle size of each powder. A Hydro 2000SM dispersion unit was fitted on to the Mastersizer 2000 instrument and a suitable dispersant (a liquid in which the particles don't dissolve) was used for each sample. The measurements were categorised by size and the percentage of particles classed as 0 µm to 50 µm, 50 µm to 100 µm, 100 µm to 212 µm, 212 µm to 355 µm and particles larger than 355 µm (Scholtz *et al.*, 2017).

3.7.11 Homogeneity index (Iθ)

The following equation (Pérez *et al.*, 2006) was applied to the data acquired from the particle size analysis to calculate the relative homogeneity index values.

$$I\theta = \frac{F_m}{100 + (d_m - d_{m-1})F_{m-1} + (d_{m+1} - d_m)F_{m+1} + (d_m - d_{m-2})F_{m-2} + (d_{m+2} - d_m)F_{m-2} + \dots + (d_m - d_{m-n})F_{m-n} + (d_{m+n} - d_m)F_{m+n}} \quad \text{Equation 3-9}$$

Where; $I\theta$ represents the relative homogeneity index that indicates the particle-size homogeneity in the range of the fractions under investigation;

F_m represents the percentage of particles in the majority range;

F_{m-1} is the percentage of particles in the range immediately below the majority range;

F_{m+1} is the percentage of particles in the range immediately above the majority range;

n is the order number of the fraction under investigation, within a series, with respect to the majority fraction;

d_m is the mean diameter of the particles in the majority fraction;

d_{m-1} is the mean diameter of the particles in the fraction of the range immediately below the majority range;

d_{m+1} is the mean diameter of the particles in the fraction of the range immediately above the majority range.

3.7.12 Conversion of numerical values obtained from the parameters to radius (r) values for diagram construction

The numerical values obtained from the powder flow parameters described above were converted to radius values (r) on a scale ranging from 1 to 10. The conversion limits are shown in Table 3-4.

Table 3-4: Conversion of powder flow parameter limit values into radius values (r). Adapted from Pérez *et al.*, (2006).

Incidence	Parameter	Symbol	Limit value (v)	Radius (r)	Factor applied to (v)
Dimension	Bulk density	Da	0-1 g/ml	0-10	10v
	Tapped density	Dc	0-1 g/ml	0-10	10v
Compressibility	Inter-particle porosity	le	0-1.2	0-10	10v/1.2
	Carr index	IC	0-50%	0-10	v/5
	Cohesion index	lcd	0-200 N	0-10	v/20
Flowability / powder flow	Hausner index	IH	3-1	0-10	[10 – (10v/3)]
	Angle of repose	(α)	50-0°	0-10	10 – (v/5)
	Powder flow	t''	20-0 s	0-10	10 – (v/2)
Lubricity / stability	Loss on drying	%HR	10-0	0-10	10 – v
	Hygroscopicity	%H	20-0%	0-10	10 – (v/2)
Lubricity / dosage	Particles < 50 μ m	%Pf	50-0%	0-10	10 – (v/5)
	Homogeneity index	(I θ)	0-2 x 10 ⁻²	0-10	500v

After the radius values were determined, a diagram (or polygon) was drawn by connecting the radius values with linear segments. This polygon produced by these values represents the characteristics of the powder's parameters and whether these characteristics are acceptable for formulation into a direct-compression dosage form. To compute a numerical value in order to establish whether the powder is acceptable for direct compression, indices were calculated based on the SeDeM EDS as described hereafter.

3.7.13 Parameter index (IP)

The parameter index was calculated with the following equation (Pérez *et al.*, 2006):

$$\text{Parameter index (IP)} = \frac{\text{Number of parameters with } r \geq 5}{\text{Total number of parameters}} \quad \text{Equation 3-10}$$

An IP value ≥ 5 indicates that the selected powder should be acceptable for direct compression.

3.7.14 Parameter profile index (IPP)

The parameter profile index (IPP) value was calculated by means of the following equation (Pérez *et al.*, 2006):

$$\text{Parameter profile index (IPP)} = \text{Mean value for all the parameters} \quad \text{Equation 3-11}$$

An IPP value ≥ 5 indicates that the selected powder should be acceptable for direct compression.

3.7.14.1 Good compression index

The good compression index (GCI) value was calculated by means of the following equation (Pérez *et al.*, 2006):

$$\text{Good compression index (GCI)} = \text{IPP} \times f \quad \text{Equation 3-12}$$

Where f is;

$$f = \frac{\text{Polygon area}}{\text{Circle area}} \quad \text{Equation 3-13}$$

A GCI value ≥ 5 indicates that the selected powder should be acceptable for direct compression.

3.8 Preparation of mini-tablet immediate release and gastro-retentive delivery systems

Four different curcumin containing mini-tablet formulations were prepared for this study, two immediate release formulations and two gastro-retentive formulations. Each mini-tablet in capsule system (consisting of 10 mini-tablets in a hard gelatine capsule) contained 50 mg curcumin or the equivalent thereof for the curcumin-HP β CD inclusion complex, divided throughout all the mini-tablets in the delivery system. Each delivery system comprised of 2 immediate release mini-tablets and 8 sustained release matrix type mini-tablets. The mini-tablets were prepared based on the data obtained from the SeDeM EDS and a previously published study (Fensham *et al.*, 2020) and then formulated by direct compression using a Korsch XP1 (Korsch AG, Germany) single station tablet press.

3.9 Evaluation of mini-tablets

Each mini-tablet formulation was evaluated by performing physical tests on a specific number of tablets randomly selected from each batch.

3.9.1 Mass variation

A total of 20 mini-tablets were weighed individually and the mass of each tablet was documented. The average mass of the 20 mini-tablets was calculated where after the individual mass of each tablet was compared to the average mass calculated. No more than 2 tablets may deviate by more than 5% of the average mass and not one may deviate by 10% (BP, 2019).

3.9.2 Disintegration

Six mini-tablets were randomly selected and placed in a tablet disintegration apparatus (Erweka ZT 123, Erweka, Germany), prefilled with thermostat regulated water (36.5 – 37.5°C). The apparatus was operated for a total of 15 min. All six mini-tablets must disintegrate within the 15 min period for the formulation to pass the test for immediate release tablets (BP, 2019). Slower disintegration (> 15 min) was acceptable for the matrix type mini-tablets since sustained release was specifically targeted for these mini-tablets.

3.9.3 Friability

A total number of 10 mini-tablets were randomly selected from each formulation after which the total weight was determined. The tablets were then placed in the drum of a friabilitor (Erweka TAR 220, Erweka, Germany) and rotated 100 times at a rotation speed of 25 rpm for a total of 4 min. The tablets were removed from the drum and the total weight determined. The friability was then calculated by using Equation 3-14. A maximum loss of weight of less than 1% was considered acceptable (BP, 2019).

$$\text{Friability (\%)} = \frac{\text{Initial tablet weight} - \text{Weight after friability}}{\text{Initial tablet weight}} \times 100 \quad \text{Equation 3-14}$$

3.9.4 Hardness and diameter

The diameter and mechanical strength of 10 randomly selected mini-tablets was measured on a hardness tester (Erweka TBH 425 TD, Erweka, Germany).

3.9.5 Mini-tablet assay

The assay for curcumin content was determined for each of the four different mini-tablet formulations, respectively. Briefly, a mini-tablet was randomly selected from each formulation, crushed and powdered with a mortar-pestle and transferred to a volumetric flask. The crushed powder was made up to volume with acetonitrile in a volumetric flask and dissolved by sonicating the solution for 10 minutes. Thereafter, the solution was diluted to an appropriate concentration and analysed according to the LC-MS analytical method as previously described in section 3.3.1.1.

3.9.6 Buoyancy

The *in vitro* buoyancy experiments were performed on the gastro-retentive mini-tablet formulations in accordance with a previously published method (Basak *et al.*, 2007). A tablet was placed in 80 ml of simulated gastric fluid (SGF) adjusted to pH 1.2. The buoyancy (or floating) ability of the tablets was determined by means of visual observation of floatation over a 5 h period.

3.9.7 Dissolution studies

Dissolution tests on the mini-tablets were done in triplicate using a Distek Model 2500 dissolution bath (Distek, NewJersey) with a USP type II apparatus (i.e. paddle). The final dosage forms (i.e. mini-tablet in capsule drug delivery systems) consisting of a combination of both immediate release and gastro-retentive mini-tablets was inserted into each dissolution flask filled with 600 ml, preheated, dissolution medium (SGF solution, pH 1.2) while the paddles rotated at a speed of 50 rpm. A sample (2 ml) was taken from each flask at time intervals of 15, 30, 60, 120, 180, 360 and 480 min. The samples were analysed on a LC-MS, where after the percentage curcumin released from the dosage form was plotted as a function of time. Dissolution data was reported in duplicate as previously published (Keller *et al.*, 2020).

3.10 Permeation studies

Synthetic membrane models are often employed for permeation testing as these models are an attractive alternative to *ex vivo* and *in vitro* models (Fensham *et al.*, 2020). Synthetic membranes overcome challenges such as high monetary cost, complicated and lengthy preparation periods, intra- and inter-laboratory variability, limited cell or tissue viability (Joubert, *et al.*, 2017 and Westerhout *et al.*, 2014) and provide reproducible results (Berben^b *et al.*, 2018).

A permeation study of a lipophilic compound conducted with cellulose nitrate (CN) synthetic membrane filters (Whatman, pore size 0.1 μm , diameter 47 mm) reported an apparent permeability coefficient (P_{app}) value closely related to the P_{app} value obtained from a permeation study of the same compound on excised porcine intestinal tissue (Fensham *et al.*, 2020). Therefore, curcumin permeation studies were conducted by mounting synthetic CN membrane filters (Whatman, pore size 0.1 μm , diameter 47 mm) in a modified Sweetana-Grass diffusion apparatus. Curcumin powder, the prepared inclusion complex of curcumin in HP β CD and both the mini-tablet dosage forms were evaluated and subsequently reported in duplicate as previously published (Hayeshi *et al.*, 2006).

3.10.1 Preparation of media

To simulate the acidic environment of the stomach, the dissolution and permeation studies were performed with a SGF (pH 1.2) solution prepared according to the recommended composition and method of the British Pharmacopoeia (BP, 2019). The SGF was used in the apical (donor) compartment of the diffusion chamber, while Krebs Ringer Bicarbonate (KRB) Buffer was used in the basolateral (acceptor) compartment to imitate the neutral pH of the blood system during permeation studies.

3.10.2 Preparation of synthetic membranes for permeation studies

Cellulose nitrate (CN) synthetic membrane filters (Whatman, pore size 0.1 μm , diameter 47 mm) are relatively brittle and often damaged during handling, therefore each membrane was cut and shaped to fit properly onto the half-cells of the modified Sweetana-Grass diffusion apparatus as shown in Figure 3-2-A. The pre-shaped membranes were carefully fitted between the half-cell spikes and sealed in place with vacuum grease. The half-cells were assembled (Figure 3-2-B) and placed between the heating blocks (maintaining a temperature of 37°C), where after each half-cell was filled with the appropriate pre-heated SGF. The apical chambers were filled with 40 ml pre-heated SGF while the basolateral chambers were filled with 40 ml pre-heated KRB buffer. Carbogen gas (95% O₂ and 5% CO₂) was connected to the chambers and bubbled through the buffer solutions throughout the study.

3.11 Evaluation of membrane integrity with Lucifer Yellow (LY)

The integrity of the synthetic membranes used during unidirectional transport studies was verified by performing a membrane integrity test with marker molecule, Lucifer Yellow (LY).

Lucifer Yellow (LY) was used as marker molecule to verify the membrane integrity of the membranes for the permeation study over the entire period of the permeation studies (i.e. 300 min). A 50 $\mu\text{g/ml}$ LY solution was added to each apical chamber (Figure 3-2-C and 3-2-D), where after, a 200 μl sample was withdrawn from the basolateral chamber every 30 min and replenished with pre-heated KRBB without delay. Samples were transferred to a Costar 96-well plate and quantified by fluorescence spectrophotometry (SpectraMax® Paradigm® plate reader) at an excitation wavelength of 485 nm and an emission wavelength of 525 nm (Rozeňnal *et al.*, 2012; van Niekerk *et al.*, 2018). Permeation studies were reported in duplicate as previously published (Hayeshi *et al.*, 2006).

Unilateral permeation studies were performed by placing the powder samples or mini-tablet dosage forms in the apical chamber filled with 40 ml pre-heated SGF. A 2 ml sample was withdrawn from the basolateral (acceptor) chamber filled with KRBB following a 30, 60, 120, 180, 240 and 300 min time period. Following each withdrawal, without delay, the basolateral chamber was replenished with 2 ml preheated KRBB. The samples were then analysed by means of the LC-MS analytical method as previously described.

3.11.1.1 Processing of permeation data

The following calculations as described below were performed on the data obtained from the permeation studies.

3.11.1.1.1 Percentage transport

After applying corrections for dilution, the transport of curcumin across the synthetic cellulose nitrate membrane was expressed as a percentage and then plotted as a function of time to produce a percentage transport curve (Fensham *et al.*, 2020).

$$\% \text{ Transport} = \frac{\text{Concentration at specific time point}}{\text{Concentration in initial solution}} \times 100 \quad \text{Equation 3-15}$$

3.11.1.1.2 Apparent permeability coefficient (P_{app})

The apparent permeability coefficient (P_{app}) values for curcumin were calculated using the following equation (Tukker, 2000 and Zhao *et al.*, 2016);

$$P_{app} = \frac{dc}{dt} \left(\frac{1}{A \cdot 60 \cdot C_0} \right) \quad \text{Equation 3-16}$$

Where; P_{app} is the apparent permeability coefficient ($\text{cm} \cdot \text{s}^{-1}$), $\frac{dC}{dt}$ is the permeability rate (the amount of curcumin that permeated across the membrane, per min), A is the membrane surface area (cm^2) and C_0 is the initial apical chamber concentration (mg/ml).

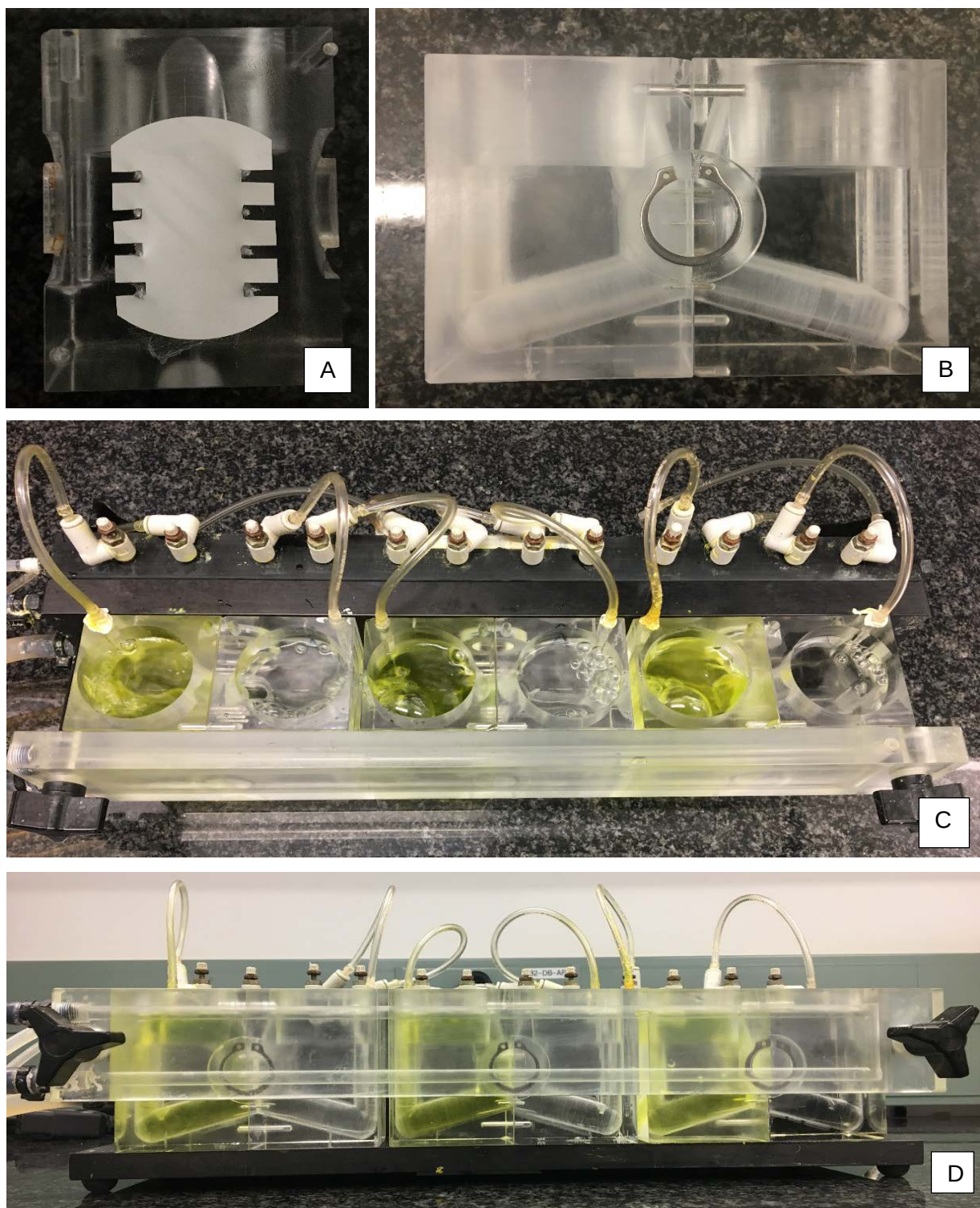


Figure 3-2: Photographs from top-left; A) cellulose nitrate membranes were cut and shaped to fit within the spikes of the half-cells. B) An assembled diffusion cell. C) Top-view of diffusion cells placed between heating blocks and connected to the carbogen gas. D) Front-view of diffusion cells containing marker molecule, LY.

3.12 Summary of materials and methods

The methods that have been described in this chapter include the validation of the LC-MS analytical method that was used to determine the curcumin concentrations in samples obtained from the different experiments during the research project. The solvent evaporation method of how an inclusion complex was formed between curcumin and HP β CD in a 1:2 stoichiometric ratio is described as well as how the resultant powder was chemically characterised by means of DSC, FTIR and XRPD. The method on how the mini-tablet formulations were designed according to the results obtained from powder flow experiments according to the SeDeM EDS is also given in detail. Furthermore, the methods on how the immediate release as well as matrix type sustained release gastro-retentive mini-tablets were prepared and evaluated are described in detail.

CHAPTER 4: RESULTS AND DISCUSSIONS

4.1 Introduction to results and discussions

Both the liquid chromatography mass spectrometry (LC-MS) and fluorescence spectrophotometric (FS) analyses method were validated before commencing the study to ensure that the analytical methods produce reliable and accurate sample analysis throughout the study. An inclusion complex was formed between curcumin and HP β CD in a 1:2 stoichiometric ratio through a common solvent evaporation method. The prepared inclusion complex was characterised by means of FTIR, DSC, visual microscopic analysis, XRPD and solubility studies in an acidic environment (pH 1.2). After the formation of an inclusion complex was confirmed, mini-tablet formulations were designed and subsequently manufactured according to results obtained from powder flow experiments as per the SeDeM EDS. The mini-tablet dosage forms were then evaluated through dissolution and permeation studies, in order to determine whether curcumin delivery has been improved by means of the multiple unit dosage form system.

4.2 Validation of liquid chromatography – mass spectrometry (LC-MS) and fluorescence spectrophotometric (FS) analyses methods

Both the LC-MS and FS analytical methods were validated in accordance with parameters set out by the International Conference on Harmonisation (ICH Q2(R1), 2005).

4.2.1 Validation results for liquid chromatography – mass spectrometry (LC-MS) analytical method

The standard curve obtained for the evaluation of linearity presented with good linear regression with a slope of 42.327 and a correlation coefficient (R^2) value of 0.999 over a range of 1.95 ng/ml to 500 ng/ml (Figure 4-1).

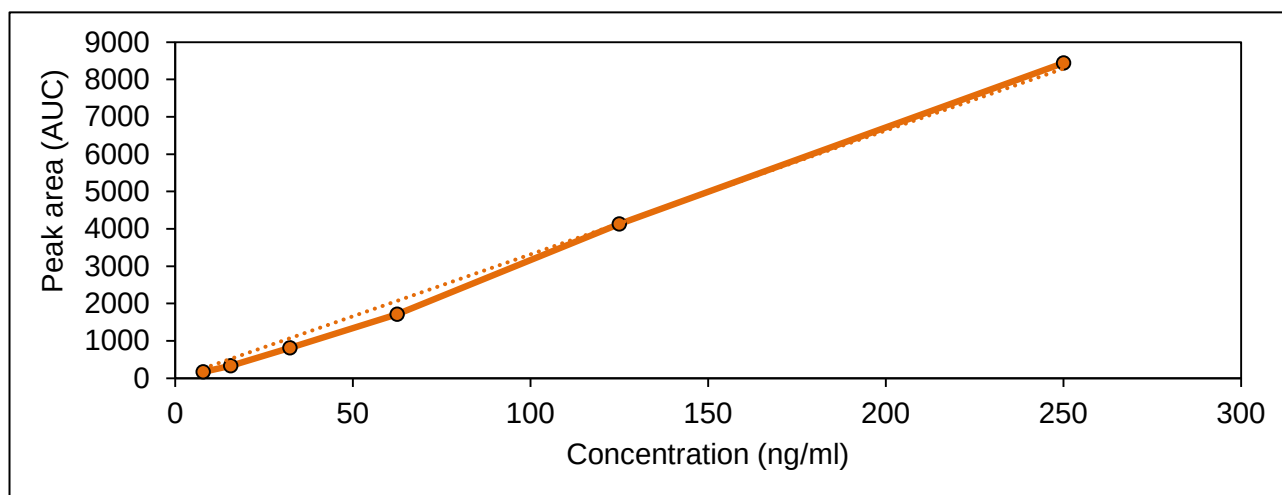


Figure 4-1: Standard curve for curcumin obtained by a liquid chromatographic mass spectrometric (LC-MS) analytical method where peak area is plotted as a function of concentration

The results obtained for the validation of the LC-MS analytical method in terms of range, linearity, precision, accuracy and specificity are summarised in Table 4-1. Both intra-day and inter-day precision delivered an acceptable percentage relative standard deviation (%RSD), altogether with values equal to or lower than 3.441%. The curcumin concentrations obtained during determination of accuracy of the analytical method exhibited values between 104% and 107% for three selected concentrations, which were within the limits of $100 \pm 10\%$. The percentage curcumin recovery value obtained during determination of specificity was within the 80 to 120% range.

The LC-MS analytical method for curcumin therefore complied with specifications for all the validation parameters tested.

Table 4-1: Validation data obtained for liquid chromatographic mass spectrometric (LC-MS) analytical method for curcumin

Characteristic	Parameter	Limit	(ng/ml)	Measurement
Range	Correlation coefficient (R^2)	0.995	1.95 - 500	0.999
Linearity	Correlation coefficient (R^2)	0.998	1.95 - 500	0.999
Intra-day Precision (Repeatability)	Standard Deviation	≤ 5	7.8125	3.441
	Standard Deviation	≤ 5	15.625	1.270
	Standard Deviation	≤ 5	32.25	1.971
Inter-day Precision	Standard Deviation	≤ 5	7.8125	3.388
	Standard Deviation	≤ 5	15.625	2.041
	Standard Deviation	≤ 5	32.25	1.769
Accuracy	Percentage Recovery	90 - 110%	7.8125	105.025
	Percentage Recovery	90 - 110%	15.625	104.449
	Percentage Recovery	90 - 110%	32.25	107.015
Specificity	Percentage Recovery	80 - 120%	100	88.277

4.2.2 Validation results for fluorescence spectrophotometric method

After regression analysis of the standard curve obtained for curcumin on the fluorescence spectrophotometer, a slope of 3401057.7273 and an acceptable correlation coefficient (R^2) value of 0.999 was obtained (Figure 4-2).

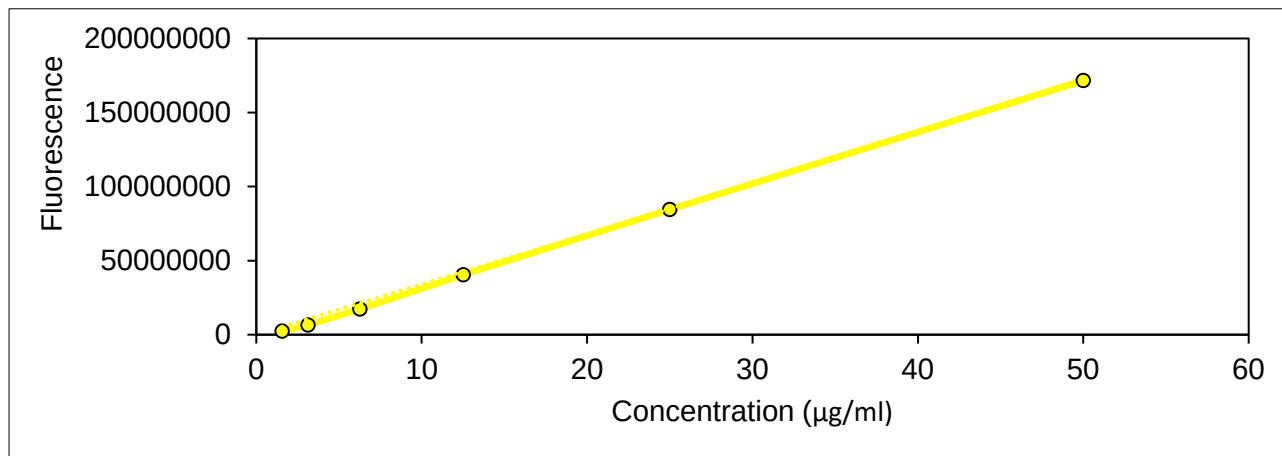


Figure 4-2: Standard curve for the determination of the linear range for marker molecule, Lucifer Yellow (LY), on a Spectramax® paradigm plate reader where fluorescence value is plotted as a function of concentration

The results obtained for the validation of the fluorescence analytical method in terms of range, linearity, precision and accuracy are summarised in Table 4-2.

The fluorescence spectrophotometric analytical method complied with both intra- and inter-day precision specifications of replicate samples with percentage relative standard deviations (% RSD) values of $\leq 0.800\%$ and $\leq 1.643\%$, respectively. The accuracy of the method in terms of curcumin recovery was determined to be 95.127% to 97.769% (thus, within the limits of $100 \pm 10\%$). The fluorescence spectrophotometric analytical method was therefore deemed suitable for the determination of marker molecule, LY.

Table 4-2: Validation data for fluorescence spectrophotometric analytical method for the determination of Lucifer Yellow (LY) samples

Characteristic	Parameter	Limit	($\mu\text{g/ml}$)	Measurement
Range	Correlation coefficient (R^2)	0.995	1.56 - 50	0.999
Linearity	Correlation coefficient (R^2)	0.998	1.56 - 50	0.999
Intra-day Precision (Repeatability)	Standard Deviation	≤ 5	12.5	0.438
	Standard Deviation	≤ 5	25	0.479
	Standard Deviation	≤ 5	50	0.800
Inter-day Precision	Standard Deviation	≤ 5	12.5	1.643
	Standard Deviation	≤ 5	25	1.547
	Standard Deviation	≤ 5	50	1.574
Accuracy	Percentage Recovery	90 - 110%	12.5	97.769
	Percentage Recovery	90 - 110%	25	95.127
	Percentage Recovery	90 - 110%	50	96.485

4.3 Purity tests of curcumin vs reference standard

The purity of curcumin raw material (Industrial Analytical Pty Ltd, RSA), which was used as the active ingredient in this study, was determined by comparing the concentration of the same quantity of a curcumin reference standard as determined by means of HPLC analysis. The percentage recovery of curcumin raw material was determined to be 102.390% compared to the curcumin reference standard. The curcumin raw material was therefore established to be of acceptable purity to be used for preparation of the inclusion complexes and formulation of the mini-tablets for this study.

4.4 Characterisation of inclusion complexes with hydroxypropyl- β -cyclodextrin (HP β CD)

The results obtained from the characterisation of the curcumin-HP β CD complex with FTIR, DSC, XRD, visual inspection under a microscope and solubility studies in comparison to that of curcumin alone, HP β CD and a physical mixture prepared in a similar stoichiometric ratio as used in the complex are shown and discussed in the sub-sections below.

4.4.1 Fourier-Transform Infrared spectroscopy (FTIR)

The position and prevalence of distinct peaks of functional groups present on the FTIR spectra obtained for the curcumin-HP β CD complex was compared to that of the curcumin raw material and HP β CD alone as well as that of a physical mixture of these two compounds. An overlay of the respectively obtained FTIR spectra can be seen in Figure 4-3 followed by a discussion on the position and prevalence of distinct peaks.

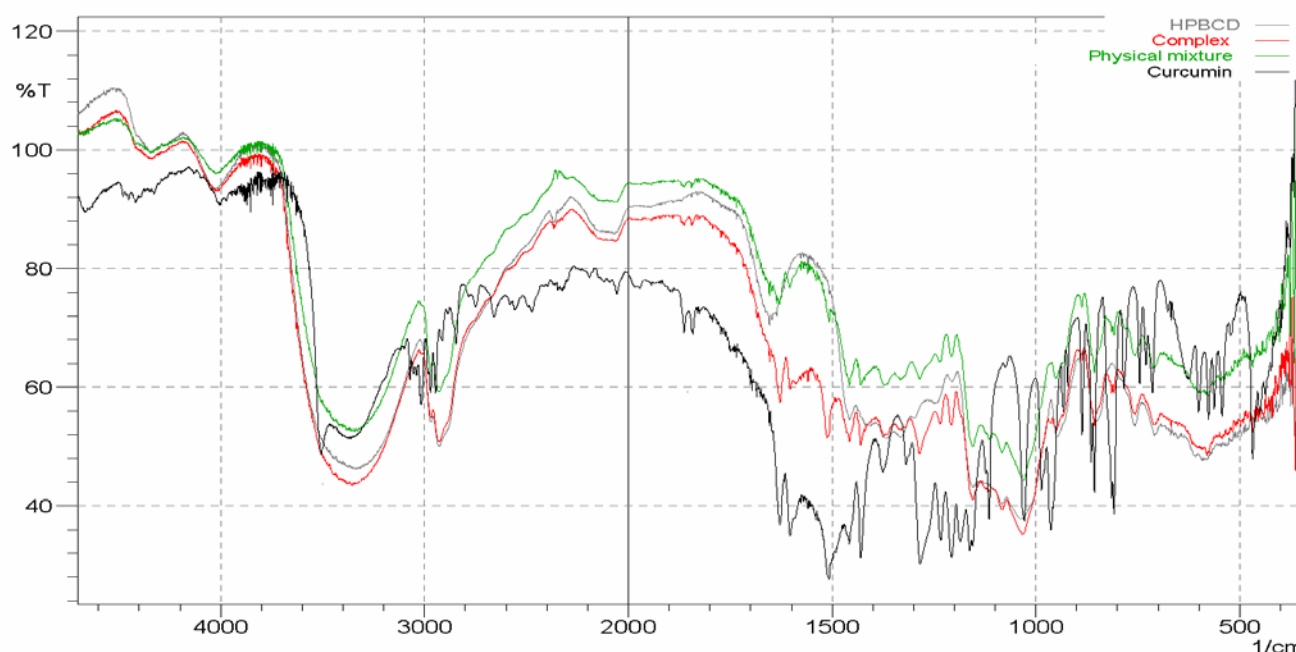


Figure 4-3: Superimposed FTIR spectra for the prepared curcumin-hydroxypropyl- β -cyclodextrin (HP β CD) complex (red curve), curcumin raw material (black curve), HP β CD (grey curve) and a physical mixture of curcumin and HP β CD (green curve)

The FTIR spectra (Table 4-3) revealed the absence of the curcumin ether peak (1114.9 cm^{-1}) for the complex, whilst it was clearly present for the curcumin raw material and physical mixture. The HP β CD cyclic ether peak (1082.11 cm^{-1}) was visible in the FTIR spectra of both the curcumin-HP β CD complex and physical mixture with minor movement in peak position (1083.08 and 1084.04 cm^{-1} , respectively) suggesting a masking effect that could possibly be the result of inclusion of curcumin within the HP β CD cavity. Furthermore, a deviation was observed in the position of the curcumin aromatic ring stretch peak (1507.43 cm^{-1}), which was unchanged within the physical mixture's FTIR spectrum but shifted to 1512.26 cm^{-1} within the complex's FTIR spectrum.

Table 4-3: Comparison of distinct peaks (cm^{-1}) visible in FTIR spectrum for the curcumin in HP β CD complex, curcumin, HP β CD and a physical mixture

Class of compound	Region	Complex	Curcumin	HPBCD	Physical mixture
Ether (R-OR')	C-O-C stretch	n.a.	1114.90	n.a.	1114.90
		1083.08		1082.11	1084.04
Aromatic ring	Aryl-C-H & ring C=C stretch	1512.26	1507.43		1507.43

FTIR analysis alone cannot be used conclusively to confirm inclusion complex formation between curcumin and HP β CD as there are many coinciding functional groups within curcumin and HP β CD structures, which makes it difficult to differentiate between assigned peaks for the curcumin-HP β CD complex and physical mixture spectra. The results from the other physical chemical characteristic tests such as DSC should therefore also be taken into consideration.

4.4.2 Differential scanning calorimetry (DSC)

The melting point for each respective powder was measured and compared in order to determine whether there are conclusive differences between the prepared complex and the other powders which could confirm the formation of an inclusion complex. From the DSC thermograms (Figure 4-4), HP β CD had a melting point of 353.05°C, while curcumin and the physical mixture had corresponding melting points of 184.40°C and 184.36°C, respectively. The curcumin-HP β CD complex, on the other hand, exhibited a lower melting point of 182.87°C. This shift in melting point suggests the formation of an inclusion complex between curcumin and HP β CD.

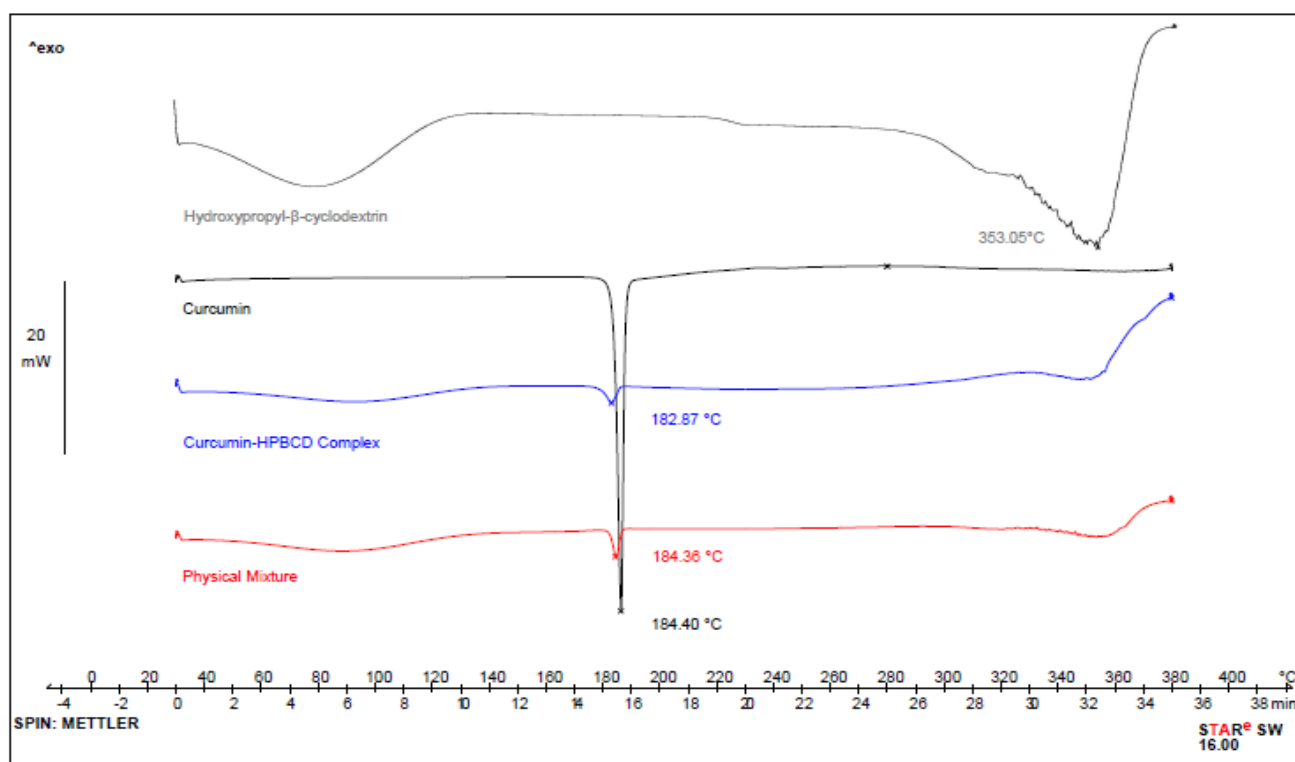


Figure 4-4: DSC thermogram overlay of melting points (°C) for hydroxypropyl-β-cyclodextrin (grey curve), curcumin (black curve), curcumin-HPBCD complex (blue curve) and a physical mixture (red curve).

In support of the shift in melting points, the enthalpy required to transfer from physical states was calculated for the curcumin-HP β CD complex and the physical mixture. The DSC thermograms showing the melting points and enthalpy of and curcumin-HP β CD complex and the physical mixture are shown in Figures 4-5 and 4-6.

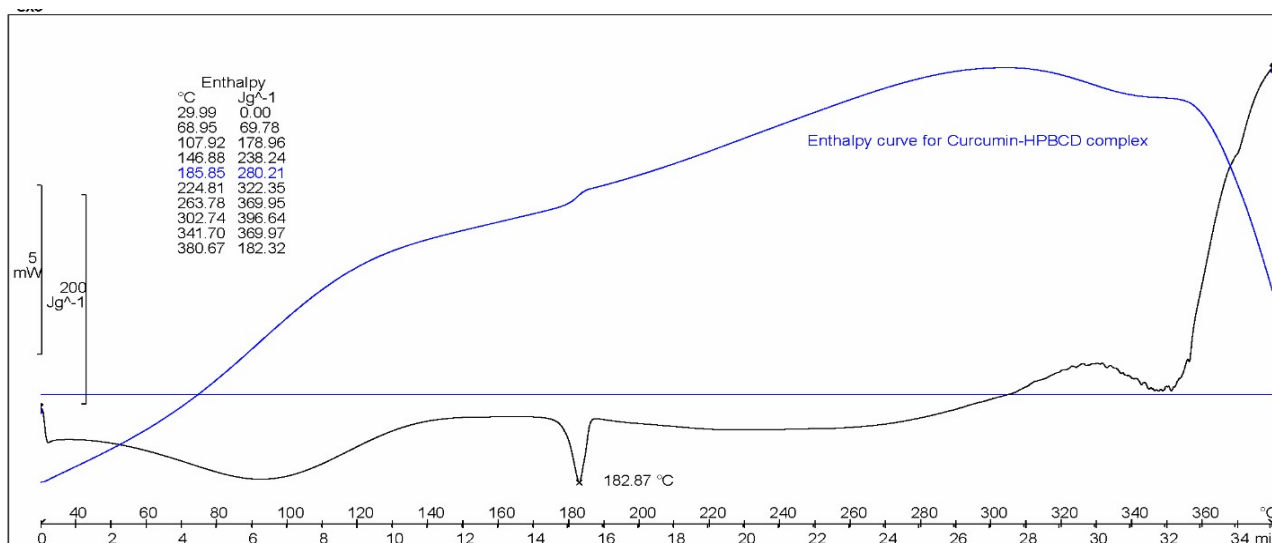


Figure 4-5: DSC thermograms of the melting point and enthalpy required for transition of the curcumin-HP β CD complex from solid state to liquid state

In addition to liquefaction occurring at a lower temperature for the curcumin-HP β CD complex, a much higher enthalpy (280.21 Jg⁻¹) was required for the transition of the curcumin-HP β CD complex (Figure 4-5) from the solid state to liquid state as compared to that of the physical mixture (199.90 Jg⁻¹) (Figure 4-6). The difference in enthalpy values is ascribed to Van der Waals bonds formed within the curcumin-HP β CD complex, which require more energy to surpass the solid state.

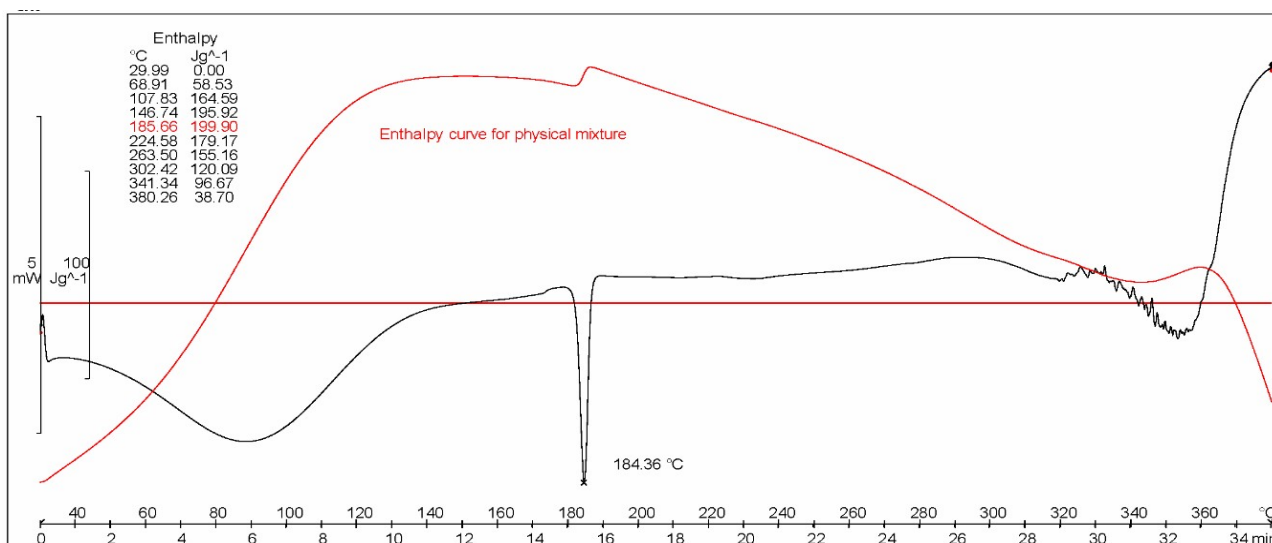


Figure 4-6: DSC analysis of the melting point and enthalpy required to transition a physical mixture of curcumin and HP β CD from solid state to liquid state

4.4.3 X-ray powder diffraction (XRPD) pattern

XRPD studies were performed to determine the structural composition (crystalline or amorphous) of the respective powders. The XRPD analysis of the respective powders (Figure 4-7) indicated that the curcumin raw material is highly crystalline, while HP β CD presented with an amorphous appearance. Both the physical mixture and the curcumin in HP β CD complex present with a similar combination of jagged crystalline peaks as observed for curcumin, which are compressed along the amorphous curvatures of the HP β CD pattern. Upon inspection of peak positions, it can be noted that there are more crystalline peaks present within the physical mixture, correlating closer to the peaks present in curcumin, while the curcumin in HP β CD complex has slightly fewer peaks along the curvature possibly owed to a slight masking effect caused by HP β CD. A definite differentiation could not be made between the physical mixture and the formulated curcumin-HP β CD complex by means of XRPD analysis only.

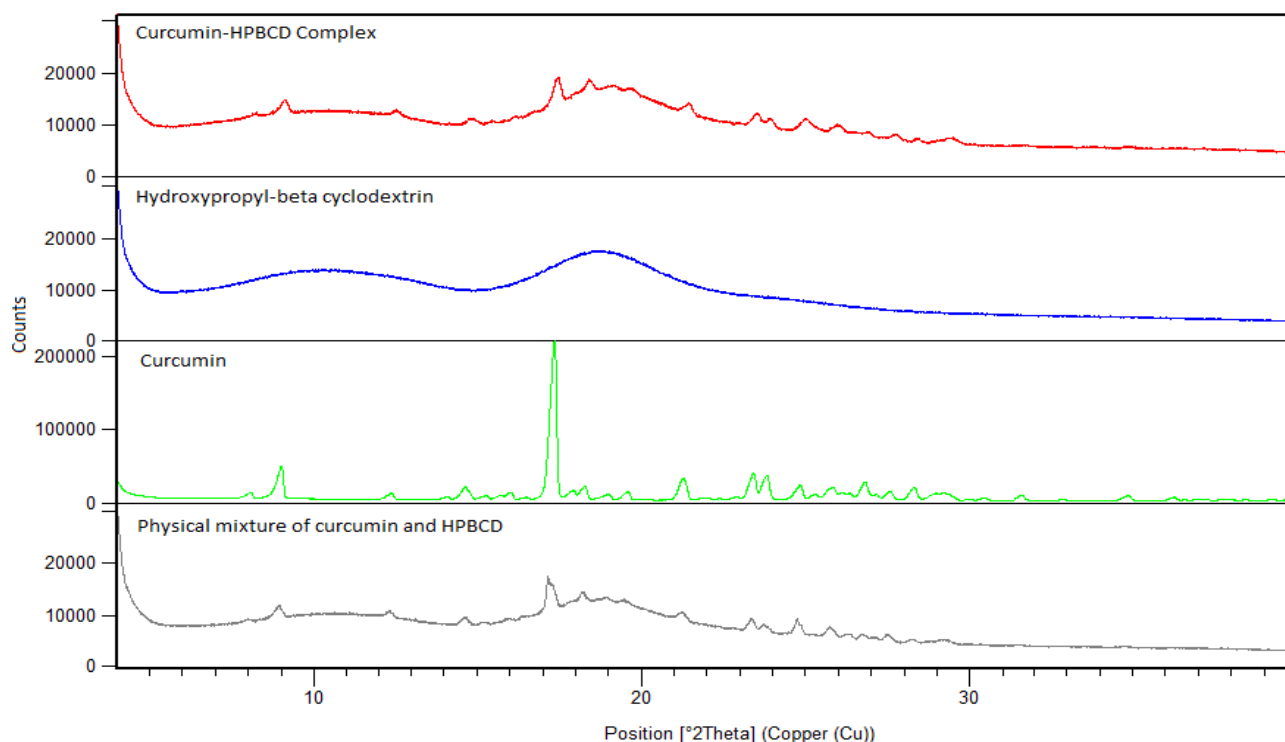


Figure 4-7: X-ray powder diffraction (XRPD) pattern of curcumin-HP β CD complex, curcumin hydroxypropyl- β -cyclodextrin (HP β CD) and a physical mixture of curcumin and HP β CD

4.4.4 Visual inspection of hydroxypropyl- β -cyclodextrin (HP β CD) and a physical mixture of curcumin and HP β CD under a microscope

Upon visual inspection under a Nikon Eclipse E4000 microscope, fitted with a Nikon DS-Fi1 camera (Nikon, Japan), there is a perceptible difference between a simple physical mixture and an inclusion complex between curcumin and HP β CD. Note Figure 4-8-A and 4-8-B, on the left the well-defined, yellow elongated crystals of curcumin can be seen intermixed with the translucent-white rounded components of HP β CD. Whereas, the complex on the right presents as agglomerates of yellow granulates.

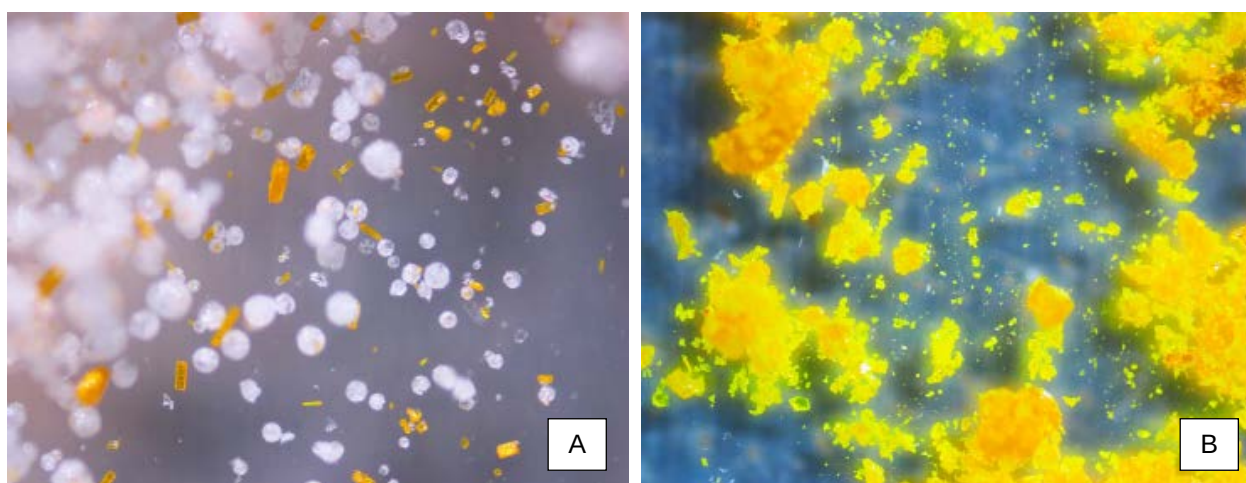


Figure 4-8: Microscopic image of a simple physical mixture of curcumin and HP β CD (A) and the formulated curcumin-HP β CD complex (B)

4.4.5 Solubility studies

Solubility studies were performed on the prepared curcumin-HP β CD complex and curcumin raw material alone, which could be used to determine whether the formed inclusion complex successfully produced a noticeable increase in the solubility of curcumin. The first differentiation could be made directly after mixing of the respective powders with the solvent (SGF, pH 1.2). It was observed that the curcumin-HP β CD complex stained the inner glass wall of the solubility tubes to a yellow shade while there was no change in colour to the inner surface of the solubility tubes containing curcumin (Figure 4-9-A). Figure 4-9-B is a photograph of the samples loaded onto the rotating aluminium axis within the water bath after a few hours' rotations. Note the bright yellow, foamy consistency of the complex samples (first three from left and last three to the right) in comparison to the faded orange curcumin samples (three middle samples) which contain agglomerates of powder. These powder agglomerates visibly display the hydrophobicity of curcumin.

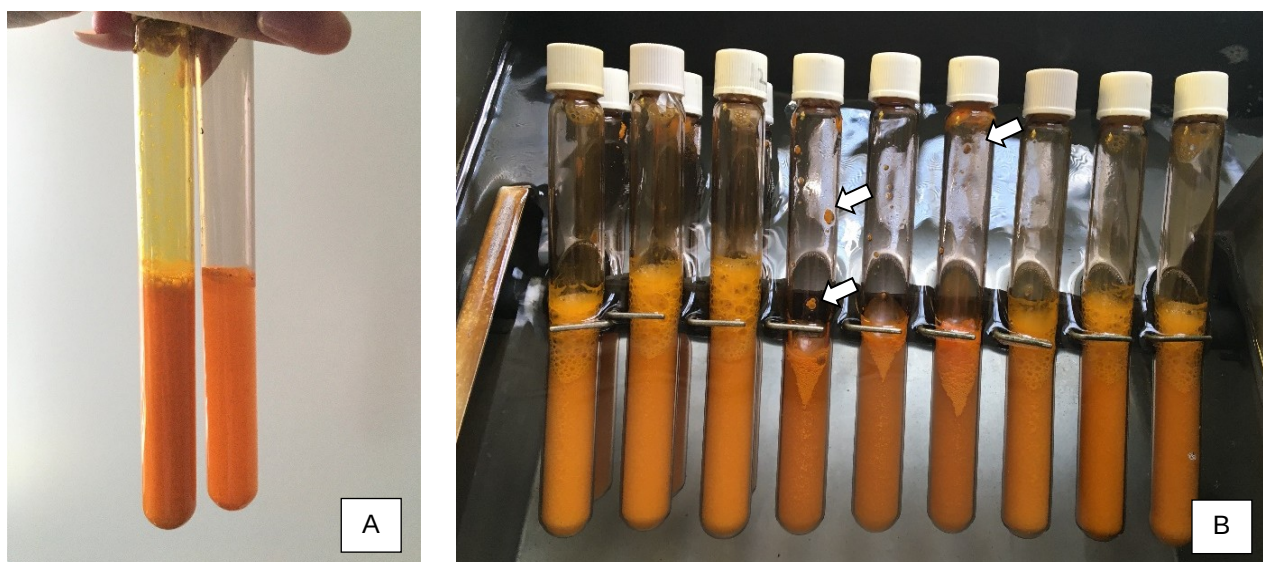


Figure 4-9: Photographic images of (A) the visible yellow staining caused by the curcumin-HP β CD complex compared to the absence of staining of the solubility tube containing curcumin. (B) Solubility tubes loaded onto a rotating aluminium axis within a water bath ($37^{\circ}\text{C} \pm 2^{\circ}\text{C}$). From the left, three tubes containing curcumin-HP β CD complex and three tubes containing curcumin followed by three tubes containing curcumin-HP β CD complex. The white arrows indicate the powder agglomerates present within the curcumin-simulated gastric fluid (SGF) solution.

There is also a clear macroscopic difference in the colour of the samples withdrawn over the 48-h time period (Figure 4-10). The samples containing curcumin appeared completely transparent, whereas the colour of the samples of the curcumin-HP β CD complex was a bright yellow.

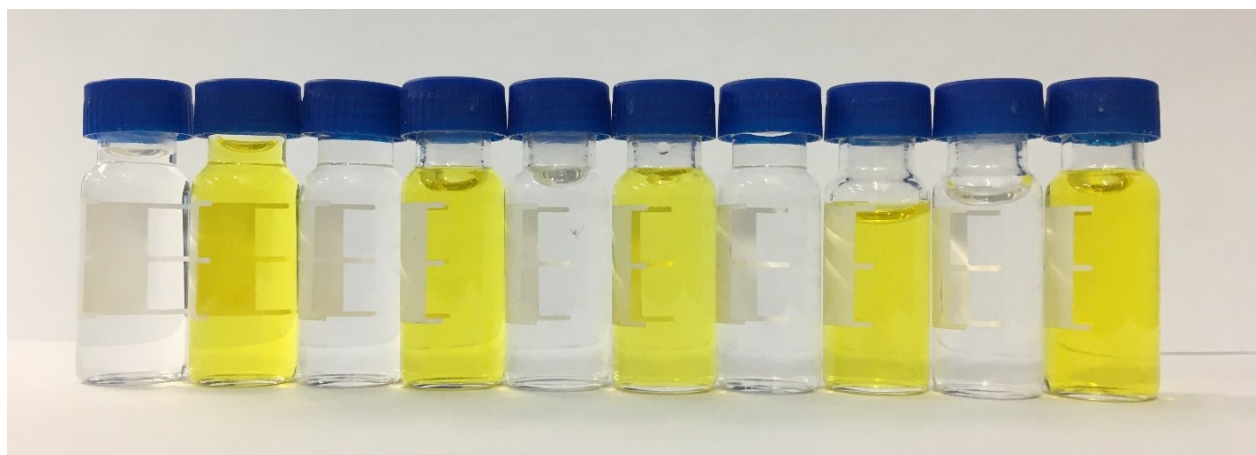


Figure 4-10: Photograph of curcumin (transparent samples) and inclusion complex (yellow toned samples) solubility samples for each time point over 48 hours. From left, samples withdrawn after 2, 4, 8, 24 and 48-h time periods.

Analysis of these samples ascertained a total solubility of 0.000069 mg/ml for curcumin and a total solubility of just over 2 mg/ml for the curcumin-HP β CD complex (Table 4-4). The solubility of curcumin was therefore increased 30 000-fold by the curcumin-HP β CD complex. From this visual observation in conjunction with other measurements (e.g. DSC), it can be deduced that an inclusion complex of curcumin in HP β CD has successfully formed and that the formed complex has led to an enormous increase in solubility of curcumin. This is in line with previous findings where cyclodextrin inclusion complexes increased the solubility of curcumin by a factor of 10^4 (Tønnesen^b *et al.*, 2002).

Table 4-4: Summary of data acquired from solubility studies performed on curcumin and the prepared complex

Solubility parameter	Curcumin	Complex
Time (hours)	(ng/ml)	(ug/ml)
2	0.295	7.508
4	0.329	11.447
8	0.365	11.437
24	3.110	210.234
48	6.995	233.344
Total amount (weight per 10 ml)	69.948	2333.442

After analysis of the samples, solubility graphs were plotted as a function of time (Figure 4-11). Both curcumin and the curcumin-HP β CD complex displayed a delayed solubility for the first eight hours of the study where after, the solubility of curcumin increased 10-fold and the solubility of the curcumin-HP β CD complex 18-fold up to the 48 h time point. The curcumin solubility graph presented with a steep increase in solubility after the 8 h time point. However, merely 70 ng of the total oversaturated amount of curcumin (5 mg/ml) went into solution after 48 hours. Whereas, the curcumin-HP β CD complex solubility graph presented with a more gradual increase in solubility with a total of 2 mg of the oversaturated amount of curcumin-HP β CD complex (50mg/ml) in solution after 48 h.

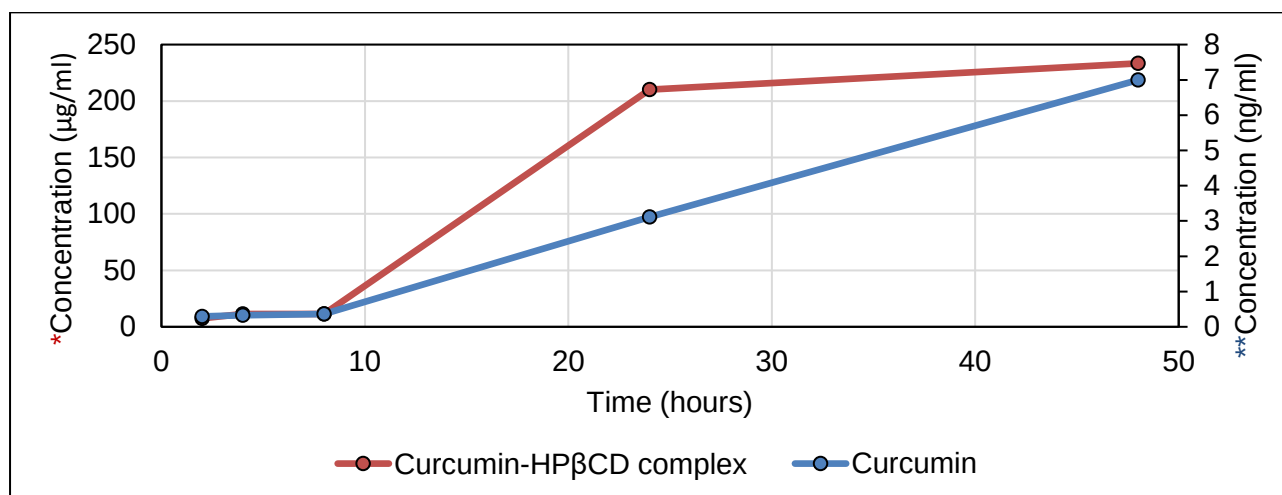


Figure 4-11: Solubility curves for the curcumin-HPβCD complex (red curve) and curcumin (blue curve) in simulated gastric fluid (pH 1.2) over a 48 hour period. (*The curcumin-HPβCD complex graph is expressed as μg/ml while the **curcumin graph is expressed as ng/ml). (Error bars omitted for reasons of clarity, data available in appendices).

4.5 Characterisation of powder flow (active ingredient and excipients) according to the SeDeM expert diagram system (EDS)

The successful production of tablets is highly dependent on the flowability of each powder substance within the formulation (Aulton, 2013). The angle of repose, compressibility index (Carr's index) and Hausner ratio are some of the most commonly reported powder flow parameters and indices (BP, 2020). The limits for these parameters are summarised in Table 4-5.

Table 4-5: Summary of powder flow properties and the corresponding values for angle of repose, compressibility index (Carr's index) and Hausner ratio (BP, 2020)

Flow property	Compressibility index (%)	Hausner ratio	Angle of repose (°)
Excellent	1 - 10	1.00 - 1.11	25 - 30
Good	11 - 15	1.12 - 1.18	31 - 35
Fair (aid not needed)	16 - 20	1.19 - 1.25	36 - 40
Passable (may hang up)	21 - 25	1.26 - 1.34	41 - 45
Poor (must agitate, vibrate)	26 - 31	1.35 - 1.45	46 - 55
Very poor	32 - 37	1.46 - 1.59	56 - 65
Very, very poor	> 38	> 1.60	> 66

The experimentally obtained powder flow data of curcumin and the curcumin-HPβCD complex for the three abovementioned parameters and indices are given in Table 4-6. Evaluation of these parameters suggested that curcumin has very poor to very, very poor powder flow properties while the curcumin-HPβCD complex has fair to passable powder flow properties. This evaluation determined that curcumin would most likely have difficulty producing a uniform dosage form as filling of the tablet dies will be inconsistent, potentially entrapping an excess of air which could lead to

capping, lamination, lubrication issues and variability in tablet weight, while the curcumin-HP β CD complex is primarily considered to be compressible without aid according to the compressibility and Hausner indexes, the angle of repose predicts that the powder could potentially hang up, restricting powder flow.

Table 4-6: Summary of experimentally calculated compressibility (Carr's index), Hausner index and angle of repose values for the active ingredients, curcumin and the curcumin-HP β CD complex

Parameter	Symbol	Unit	Curcumin	Flow Property	Complex	Flow Property
Compressibility Index	IC	%	32.87	Very poor	16.23	Fair (aid not needed)
Hausner Index	IH	n.a	1.49	Very poor	1.19	Fair (aid not needed)
Angle of Repose	α	°	42.28	Very, very poor	24.21	Passable (may hang up)

As the aim of this study was to prepare direct-compressible immediate release and controlled released matrix gastro-retentive mini-tablet formulations, the preliminary evaluation of these powder flow parameters indicated the demand for additional excipients. Therefore, curcumin, curcumin-HP β CD complex and selected excipients were evaluated by the SeDeM EDS to determine the possible shortcomings of the active ingredient(s) and the amount of each excipient to be incorporated in order to correct these unfavourable characteristics.

The powder flow parameter values experimentally obtained for curcumin, the curcumin-HP β CD complex and each selected excipient were converted to SeDeM radius/parameter values as shown in Table 4-7 below. Radius/parameter values are consigned to a scale from 1 - 10 and used to construct a SeDeM diagram (section 4.5.1). The SeDeM parameter values were then converted to SeDeM incidence values (Table 4-8) in order to evaluate powder flow deficits for each powder ingredient of the mini-tablet formulations. Incidence values lower than 5 are considered inadequate and require a corrective excipient.

Table 4-7: Summary of SeDeM radius/parameter values calculated from experimentally obtained powder flow parameter values for the construction of SeDeM diagrams. Values were determined for the active ingredients, curcumin and the curcumin-HP β CD complex and selected functional excipients.

SeDeM			Actives		Excipients			
Parameter	Symbol	Unit	Curcumin	Complex	MCC	HPMC	PVP VA64	Styrene DVB
Bulk Density	Da	g/ml	4.387	6.760	3.659	3.659	2.988	6.148
Tapped Density	Dc	g/ml	6.535	8.070	4.314	5.001	3.624	6.413
Inter-particle Porosity	Ie	n.a	6.244	2.001	3.455	6.114	4.891	0.559
Carr Index	IC	%	6.574	3.247	3.035	5.369	3.508	0.825
Cohesion Index	Icd	N	1.250	0.000	10.000	10.000	2.733	0.000
Hausner Index	IH	n.a	7.552	9.031	9.106	8.165	8.937	9.785
Angle of Repose	α	°	1.544	5.158	5.925	5.873	6.254	5.812
Powder Flow	t"	sec	7.183	7.933	7.233	6.183	1.700	9.017
Loss on Drying	%HR	%	7.587	4.705	2.727	4.476	3.499	6.558
Hygroscopicity	%H	%	9.706	7.874	8.906	7.191	2.840	9.960
Particles <50 μ m	%Pf	%	8.563	9.667	9.768	9.109	9.603	10.000
Homogeneity Index	I θ	n.a	7.289	1.273	1.868	3.902	4.092	4.147

*MCC = microcrystalline cellulose, HPMC = hydroxypropyl methylcellulose, PVP VA 64 = vinylpyrrolidone-vinyl acetate copolymer, Styrene DVB = Styrene divinylbenzene copolymer.

** Parameter values lower than 5 are considered inadequate and require a corrective excipient.

Curcumin failed bulk density, cohesion index and the angle of repose parameter in terms of the set limits (Table 4-7). The bulk density value (4.387) indicated that the curcumin powder particles cohered and an external force will have to be applied in order to overcome gravity and initiate powder flow. An inadequate cohesion index (1.250) and angle of repose value (1.544) could lead to brittle tablets and a lack of tablet uniformity as a result of uneven tablet die filling and pliable compression. The markedly low cohesion index value led to failure of the compressibility index (Table 4-8).

However, despite these shortcomings, evaluation of the parameter profile index (Table 4-9) suggested that curcumin may be acceptable for direct compression (6.201).

The curcumin-HP β CD complex failed the inter-particle porosity, cohesion index and loss on drying parameters in terms of the set limits. The high porosity of the complex (2.001) could explain the loss of mass during drying (4.705). In addition, a cohesion value of 0.000 was allocated to the curcumin-HP β CD complex as the minimum value could not be met as defined in the limit set by the SeDeM EDS. Despite addition of the standard lubricant composition (Pérez *et al.*, 2006) the complex powder was too abrasive to be compressed. Failure of these parameters could potentially lead to a lack of moisture within the powder deterring compressibility as predicted by the severely low compressibility incidence value (1.749). The curcumin-HP β CD complex also failed the homogeneity index (1.273). The lack of powder homogeneity can be observed from the microscope photograph (section 4.4.4) of curcumin-HP β CD complex agglomerates of various sizes as well as the particle size distribution data where particle sizes are widespread in relatively even amounts across particles sizes 50 μ m to 335 μ m and above. However, once again, despite these shortcomings, evaluation of the parameter profile index (Table 4-9) suggested that the curcumin-HP β CD complex may be acceptable for direct compression (5.477).

Table 4-8: SeDeM incidence values calculated from parameter values and the determination of incidence deficiencies

SeDeM incidences	API		Excipients			
	Curcumin	Complex	MCC	HPMC	PVP VA64	Styrene DVB
Dimension	5.461	7.415	3.987	4.330	3.306	6.281
Compressibility	4.689	1.749	9.863	8.811	3.711	0.462
Flowability/Powder flow	5.426	7.374	7.421	6.741	5.630	8.205
Lubricity/Stability	8.646	6.289	5.817	5.834	3.169	8.259
Lubricity/Dosage	7.926	5.470	5.818	6.506	6.847	7.073

*MCC = microcrystalline cellulose, HPMC = hydroxypropyl methylcellulose, PVP VA 64 = vinylpyrrolidone-vinyl acetate copolymer, Styrene DVB = Styrene divinylbenzene copolymer.

** Incidence values lower than 5 are considered inadequate and require a corrective excipient.

Despite the low incidence values for both curcumin and the curcumin-HP β CD complex, the final acceptance index; parameter index, parameter profile index and good compression index values were acceptable for the production of direct-compressible tablets (Table 4-9). However, to avoid complications during formulation, suitable excipients were added to improve the low compressibility of both curcumin and curcumin-HP β CD complex (section 4.5.2).

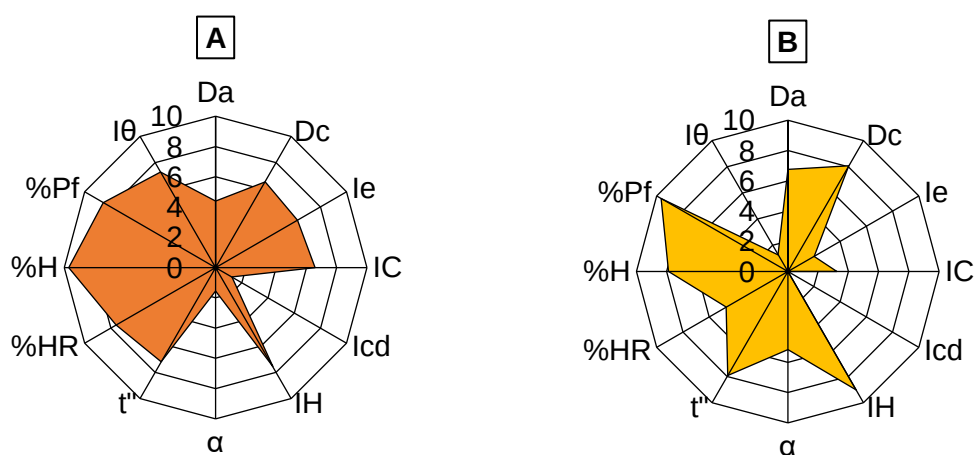
Table 4-9: SeDeM acceptance incidences; parameter index, parameter profile index and good compression index values.

SeDeM acceptance index	Index Limit	API		Excipients			
		Curcumin	Complex	MCC	HPMC	PVP VA64	Styrene DVB
Parameter Index	≥ 0.5	0.750	0.583	0.500	0.750	0.250	0.667
Parameter Profile Index	≥ 5.0	6.201	5.477	5.833	6.254	4.556	5.769
Good Compression Index	≥ 5.0	5.922	5.230	5.570	5.972	4.350	5.509

*MCC = microcrystalline cellulose, HPMC = hydroxypropyl methylcellulose, PVP VA 64 = vinylpyrrolidone-vinyl acetate copolymer, Styrene DVB = Styrene divinylbenzene copolymer.

4.5.1 SeDeM diagrams

Following calculation of the incidence and acceptable index values, SeDeM diagrams were constructed by converting the twelve SeDeM powder flow parameter values to polygons for each active ingredient and the selected excipients (Figure 4-12). Powder suitability is graphically represented within the polygon areas, large shaded areas indicate satisfactory characteristics while small shaded areas indicate inadequacies.



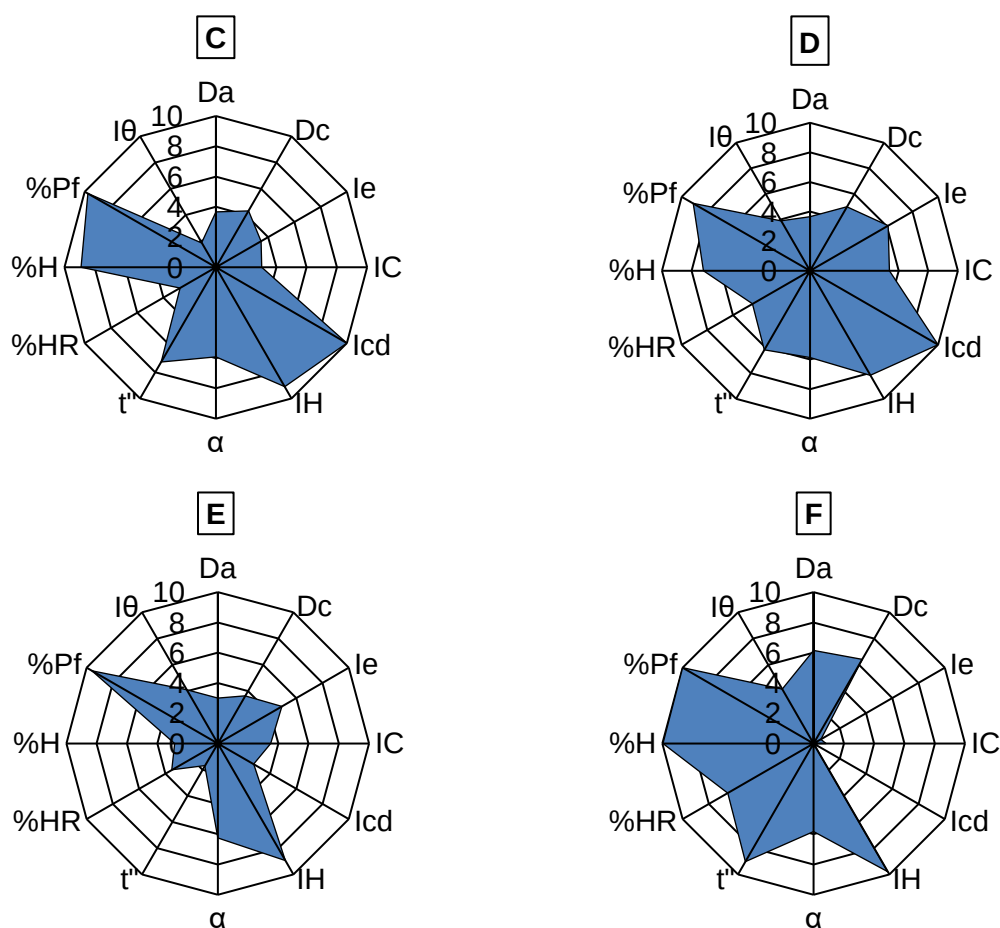


Figure 4-12: The constructed SeDeM diagrams for (A) curcumin and (B) the curcumin-HP β CD complex followed by the diagrams for the excipients; (C) microcrystalline cellulose, (D) hydroxypropyl methyl cellulose, (E) vinylpyrrolidone acetate copolymer and (F) styrene-divinylbenzene copolymer

4.5.2 Calculated percentage excipient to be included in tablet formulation

The parameter and incidence values obtained (shown in Tables 4-7 and 4-8, above), indicated deficiencies regarding the compressibility of both curcumin and curcumin-HP β CD inclusion complex. Both these compounds had incidence values below the required value of five, however, the curcumin-HP β CD inclusion complex's compressibility incidence value was markedly lower than that of curcumin, indicating a greater need for the addition of a corrective excipient. MCC and HPMC were evaluated as potential corrective excipients. MCC was identified as the primary filler for the conventional, immediate release mini-tablet formulations due to its excellent compression properties. HPMC was identified as filler for the controlled released gastro-retentive matrix type mini-tablet formulations. The values in Table 4-10 provide a SeDeM EDS calculated recommendation for the minimum amount (percentage) of each excipient that should be incorporated into each formulation in order to successfully overcome the poor compressibility characteristics of curcumin and curcumin-HP β CD inclusion complex.

Table 4-10: Percentage of each corrective excipient to be included within mini-tablet formulations for curcumin and curcumin HP β CD inclusion complex

Compressibility		
% Excipient to be included in tablet formulation		
Excipient	Curcumin	Curcumin-HP β CD complex
MCC	6.00	40.06277085
HPMC	7.54	46.03171321

The suitability of the selected excipients (MCC and HPMC) is clearly demonstrated in the superimposed polygon areas for the respective powders with curcumin and with the curcumin-HP β CD complex (Figure 4-13). The shaded areas of the excipients overlap the inadequately shaded areas of the mentioned active ingredients. Curcumin compressibility issues could be overcome with both identified excipients, while the curcumin-HP β CD complex needed additional excipients to overcome the lack of homogeneity of the powder particles (section 4.6).

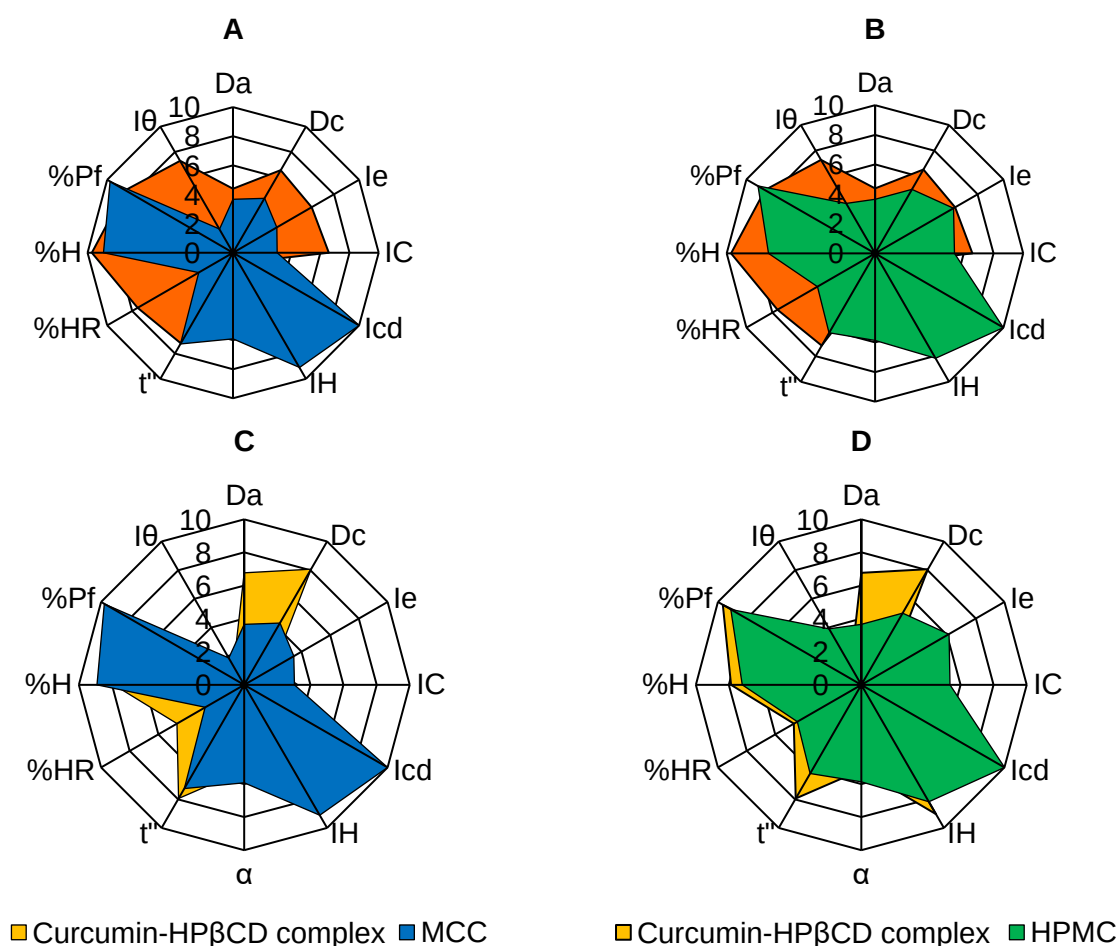


Figure 4-13: Superimposed SeDeM diagram areas to indicate the suitability of selected excipients, microcrystalline cellulose (MCC) and hydroxypropyl methyl cellulose (HPMC) for the correction of poor compressibility characteristics of curcumin and the curcumin-HP β CD complex. (A) curcumin and MCC, (B) curcumin and HPMC, (C) curcumin-HP β CD complex and MCC and (D) curcumin-HP β CD complex and HPMC.

4.6 Preparation of mini-tablet immediate release and gastro-retentive delivery systems

The mini-tablets were formulated using information acquired from previously documented gastro-retentive tablet formulations (Fensham et al., 2020) in combination with the data acquired from the SeDeM EDS in this study. The final mini-tablet formulations were composed of ingredients as shown in Table 4-11.

Table 4-11: Composition of the immediate release and gastro-retentive mini-tablet formulations

Formulation of immediate release mini-tablets		
Excipient	Curcumin (% w/w)	Complex (% w/w)
MCC	91.00	38.00
Mg Stearate	1.00	3.50
Talc	2.36	1.00
Aerosil®	0.14	2.36
Ac-di-sol®	n.a.	3.50
API	5.50	0.14
Formulation of gastro-retentive mini-tablets		
Excipient	Curcumin (% w/w)	Complex (% w/w)
MCC	25.00	7.00
Mg Stearate	0.50	1.00
HPMC	50.50	44.00
PVP VA64	10.00	6.00
Styrene DVB	10.00	6.00
API	4.00	36.00

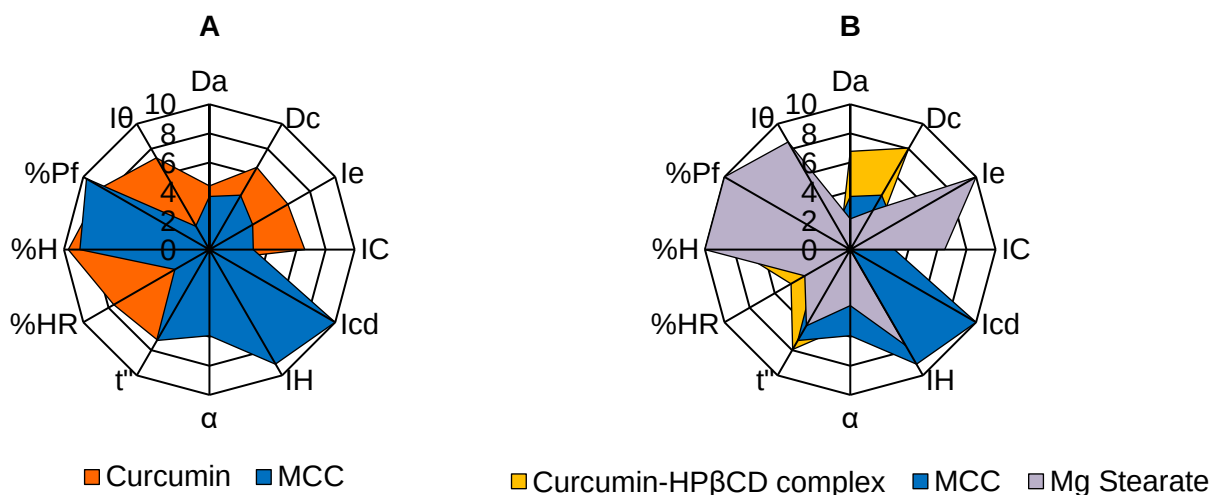
*MCC = microcrystalline cellulose, Mg Stearate = magnesium stearate, HPMC = hydroxypropyl methylcellulose, PVP VA 64 = vinylpyrrolidone-vinyl acetate copolymer, Styrene DVB = Styrene divinylbenzene copolymer, API = active pharmaceutical ingredient (curcumin or curcumin-HP β CD complex).

Photographic images of a final mini-tablet-in-capsule system, the formulated mini-tablets and the floating matrix type gastro-retentive mini-tablets during buoyancy evaluation (section 4.6.1) can be seen in Figure 4-14.



Figure 4-14: (A) A representation of the final mini-tablet in capsule system formulated for this study (B) From left, curcumin-HP β CD complex and curcumin immediate release mini-tablets and curcumin- HP β CD complex and curcumin gastro-retentive mini-tablets (C) Complex (left) and curcumin (right) gastro-retentive mini-tablets floating in acidic buffer (pH 1.2) during the buoyancy test.

As previously stated, the curcumin-HP β CD complex compressibility deficiency could not be corrected by MCC or HPMC alone. Therefore, magnesium stearate was additionally used according to the SeDeM EDS to improve the lack of homogeneity of the complex powder. The superimposed polygon areas for the major excipients in the four final formulations can be seen in Figure 4-15. During the initial production of the immediate release curcumin-HP β CD complex containing mini-tablets, problems were observed with the disintegration of the mini-tablets. Immediate release tablets should completely disintegrate within 15 min; however, the initial formulation did not adhere to this specification. This was addressed with the addition of super-disintegrant, Ac-di-sol®.



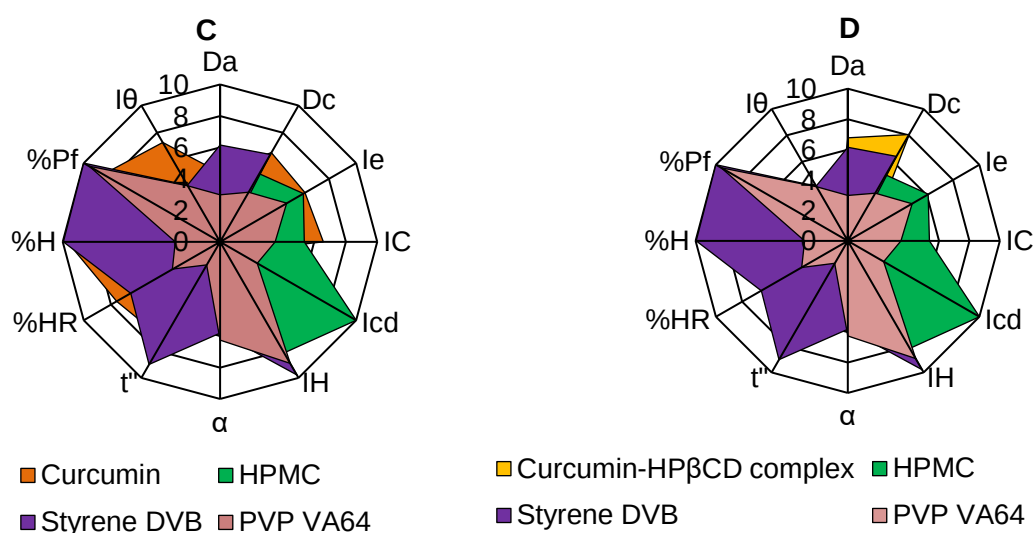


Figure 4-15: Superimposed polygon areas for the major excipients in the four final formulations. Diagrams (A) and (B) represent the gastro-retentive mini-tablet formulations for curcumin and the curcumin-HPBCD complex, respectively. Diagrams (C) and (D) represent the direct-compressible mini-tablet formulations for curcumin and the curcumin-HPBCD complex, respectively.

4.6.1 Evaluation of mini-tablets

The evaluation data for the four mini-tablet formulations are summarised in Table 4-12. All four of the mini-tablet formulations passed the tests for mass variation, tensile strength, thickness and diameter. The immediate release mini-tablets for both curcumin and the curcumin-HPβCD complex displayed no loss in mass following the friability test, while both gastro-retentive mini-tablets had a slight, but acceptable loss in mass following the friability test. This relatively low loss in mass can possibly be owed to the incorporation of the low-density polymer, styrene divinylbenzene copolymer, within the matrix type gastro-retentive mini-tablets formulations. Both immediate-release mini-tablet formulations disintegrated soon after contact with the buffer medium during the disintegration test, whilst the gastro-retentive mini-tablets displayed no disintegration over an extended period of time as expected from these sustained release formulations. Furthermore, the gastro-retentive mini-tablets remained afloat over a period of five hours during the buoyancy test (Figure 4-14-C), where after the mini-tablets disintegrated and settled to the bottom of the beaker. All four mini-tablet formulations returned an acceptable percentage recovery of curcumin during the assay ranging from 99.25 to 111.275%. Following acceptable curcumin content assays for all four mini-tablet formulations, the final dosage form was determined to comprise of two mini-tablet-in-capsule dosages each consisting of two immediate release and eight gastro-retentive mini-tablets for a total dosage of ± 50 mg curcumin.

Table 4-12: Summary of mini-tablet evaluation data for the immediate release and gastro-retentive formulations of curcumin and the curcumin-HP β CD complex, respectively

Physical evaluation parameter			Immediate-release formulations		Gastro-retentive formulations	
Parameter	Value	Limit	Curcumin	Complex	Curcumin	Complex
Mass Variation (BP)	Average mass (g)		0.047	0.045	0.047	0.046
	STDEVP (%)	< 10%	0.001	0.001	0.002	0.001
Disintegration	Time (min)	< 15min	0.420	6.000	>15	>15
Friability	% Friability	< 1%	0.000	0.000	-0.213	-0.219
Tensile strength (USP)	Average strength (N)		22.200	19.000	37.000	17.400
	STDEVP (%)	< 2%	0.632	1.563	1.350	1.713
Thickness	Average thickness (mm)		2.122	1.868	2.136	2.053
	STDEVP (%)	< 2%	0.015	0.027	0.064	0.030
Diameter	Average diameter (mm)		5.951	5.939	5.933	5.919
	STDEVP (%)	< 2%	0.006	0.007	0.009	0.012
Buoyancy	Floatation time (h)		n.a.	n.a.	5.000	5.000
Tablet assay	% Recovery	80 - 120%	99.250	111.275	107.230	105.247

4.7 Dissolution studies

Dissolution studies were performed on the final curcumin and curcumin-HP β CD complex mini-tablet dosage forms (Table 4-13). The respective dosage forms comprised of 2 immediate release and 8 gastro-retentive mini-tablets. The data acquired from the dissolution study was plotted as a function of time to construct a dissolution graph (Figure 4-17). The mini-tablets containing the curcumin-HP β CD complex began releasing its contents as soon as it made contact with the dissolution medium (as seen from the yellowing of the dissolution medium in Figure 4-16). The yellowing of the SGF in the curcumin-HP β CD complex dissolution vessel indicated potentially higher drug release compared to the vessel containing the transparent SGF and curcumin formulation on the left.

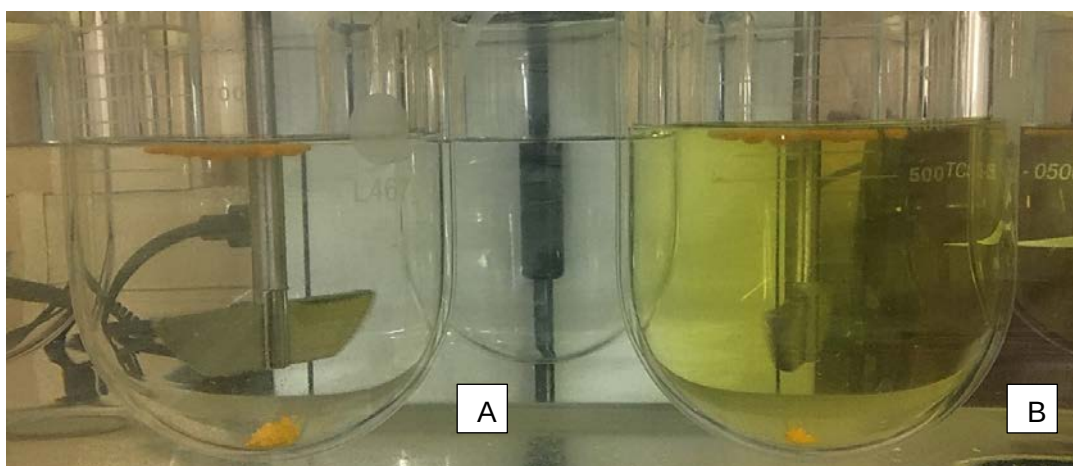


Figure 4-16: Visual comparison of curcumin mini-tablet dosage form (A) and complex mini-tablet dosage form (B), minutes after being placed in the respective dissolution chambers.

A cumulative amount of 9.11% curcumin was released from the curcumin-HP β CD complex dosage form over the entire five-hour dissolution period. The dissolution profile of the curcumin-HP β CD complex dosage form presented with a distinctive two-phase dissolution curve. The initial rapid curcumin release can be ascribed to the relatively quick release from the immediate release mini-tablets, while the second, more gradual increase in concentration was due to the extended drug release from the gastro-retentive mini-tablets.

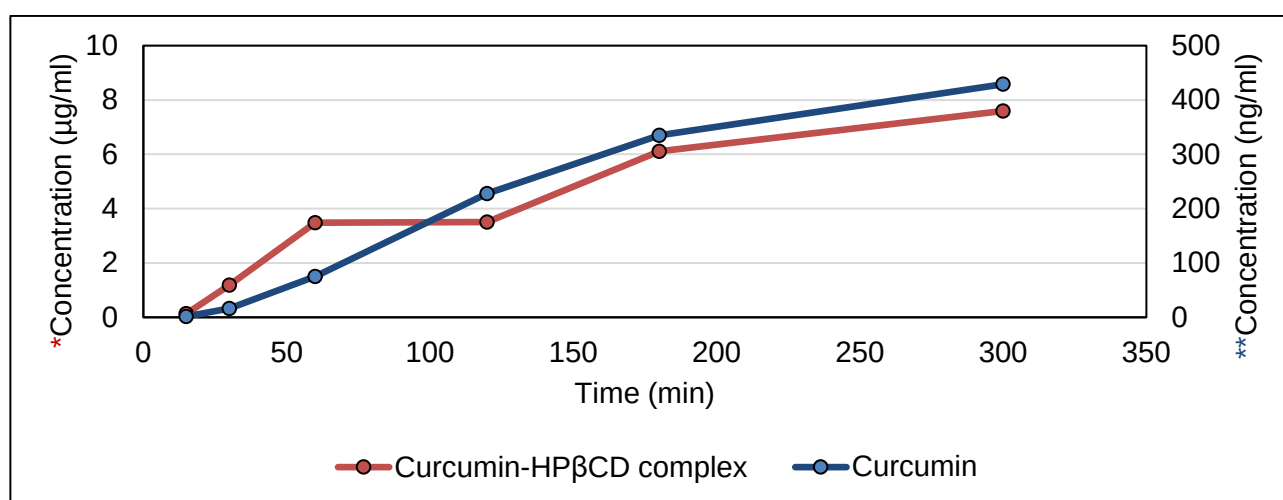


Figure 4-17: Dissolution profile for the curcumin-HP β CD complex (red curve) and curcumin (blue curve) dosage forms plotted as function of time. (*The curcumin-HP β CD complex graph is expressed as $\mu\text{g/ml}$ while the **curcumin graph is expressed as ng/ml). (Error bars omitted for reasons of clarity, data available in appendices).

The curcumin mini-tablet dosage form presented with a markedly lower cumulative drug release compared to the curcumin-HP β CD complex containing mini-tablet formulation as it only exhibited a cumulative percentage curcumin release value of 0.515%. This can be explained by the poor solubility of the curcumin raw material. The curcumin dissolution profile lacked the distinctive immediate and extended drug release phases and instead presented with a gradual increase sigmoid type curve.

The mini-tablet in capsule formulation containing the curcumin-HP β CD inclusion complex produced a nearly 18-fold increase in curcumin drug release as compared to the dosage form containing curcumin alone. The curcumin-HP β CD inclusion complex also presented a biphasic dissolution profile, which was characterised by distinctive immediate and extended drug release phases. However, the overall percentage curcumin release obtained from the curcumin-HP β CD inclusion complex containing dosage form is still considered to be relatively low. More research is therefore required to investigate other approaches to increase the curcumin release further in order to ensure improved bioavailability.

Table 4-13: Summary of dissolution data for curcumin and the prepared curcumin-HP β CD complex dosage forms

Dissolution Parameter	Curcumin	Complex
Time (min)	(ng/ml)	(μg/ml)
15	1.625	0.131
30	16.019	1.186
60	75.232	3.485
120	227.993	3.502
180	334.768	6.107
300	428.986	7.594
Total amount (mg per 600 ml chamber)	0.257	4.557
% Release from dosage form	0.515	9.113

4.8 Evaluation of membrane integrity with Lucifer Yellow (LY)

Prior to conducting the permeation tests on the curcumin, the curcumin-HP β CD inclusion complex and the related mini-tablet formulations, a membrane integrity test was performed on the synthetic cellulose nitrate membranes (Whatman®, Merck, RSA). The integrity test was conducted to ensure that no damage was inflicted upon the brittle membranes during membrane processing namely cutting, shaping and mounting of the membranes on the half-cells of the diffusion chambers. The percentage transport of LY is plotted as a function of time in Figure 4-19.

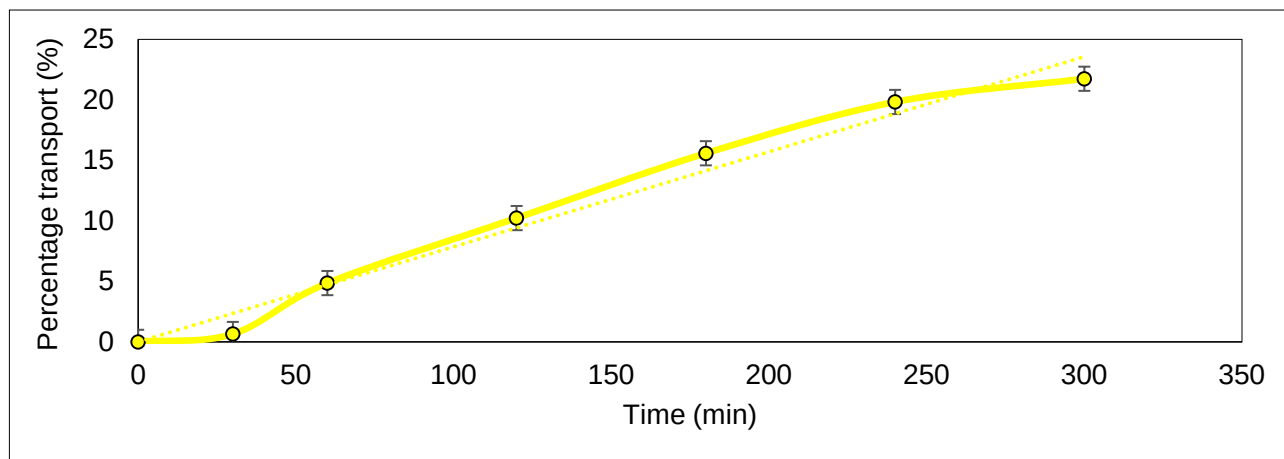


Figure 4-18: Percentage transport of marker molecule, Lucifer Yellow (LY), as a function of time, across synthetic cellulose nitrate membranes for the determination of membrane integrity.

After the 5 hour permeation study period, an average of 72.90% of the LY molecules had permeated across the membrane to the basolateral chamber. From a membrane integrity perspective, an acceptable apparent permeability coefficient (P_{app}) value of 5.70×10^{-6} was obtained, indicating that the membranes were not damaged during handling and preparation. Therefore, all membranes were prepared and affixed as described in Chapter 3 section 3.3.11.

Table 4-14: Summary of the percentage transport, P_{app} value and cumulative transport of marker molecule, Lucifer Yellow, across synthetic cellulose nitrate membranes

Time (min)	Percentage transport (%)
0	0.00
30	0.65
60	4.86
120	10.23
180	15.59
240	19.83
300	21.75
P_{app}	5.70E-06
Cumulative transport	72.90

4.9 Permeation studies across synthetic cellulose nitrate membranes

The average quantity of curcumin that permeated across the synthetic cellulose nitrate membranes was expressed as a percentage and then plotted as a function of time to produce a percentage transport curve. The transport curves of the powder samples and mini-tablet dosage forms for curcumin and the curcumin-HP β CD complex were compared (Figures 4-20 and 4-21).

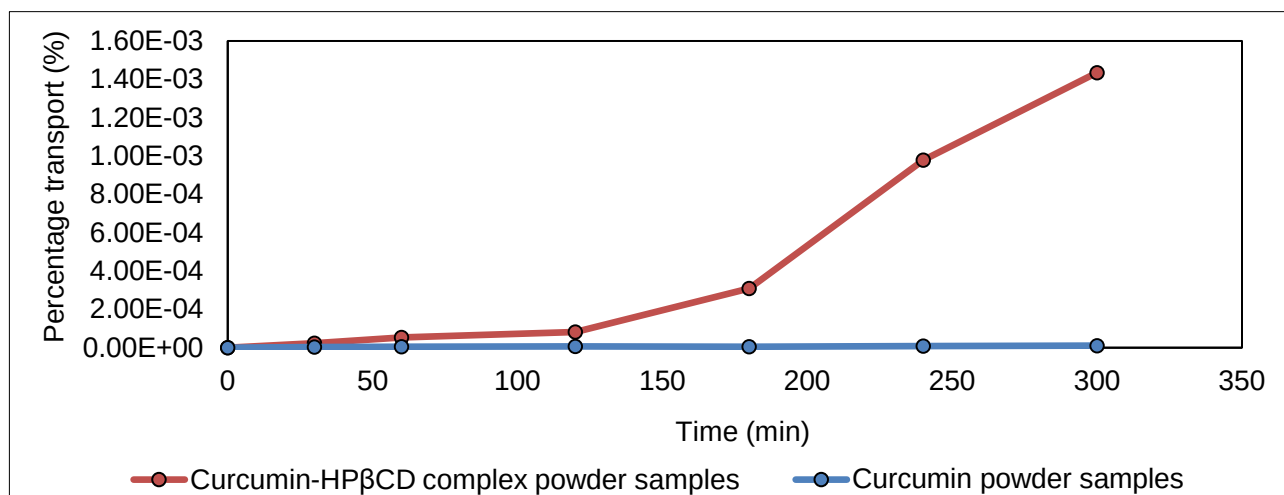


Figure 4-19: Average percentage transport comparison of curcumin powder samples (blue curve) and curcumin-HPβCD complex powder samples (red curve). (Error bars omitted for reasons of clarity, data available in appendices).

Comparison of curcumin with curcumin-HPβCD complex powder samples revealed a noticeable 70-fold increase in the cumulative percentage transport value and 100-fold increase in the P_{app} value obtained by the inclusion complex. This was expected due to the enormous increase in curcumin solubility caused by the inclusion complex.

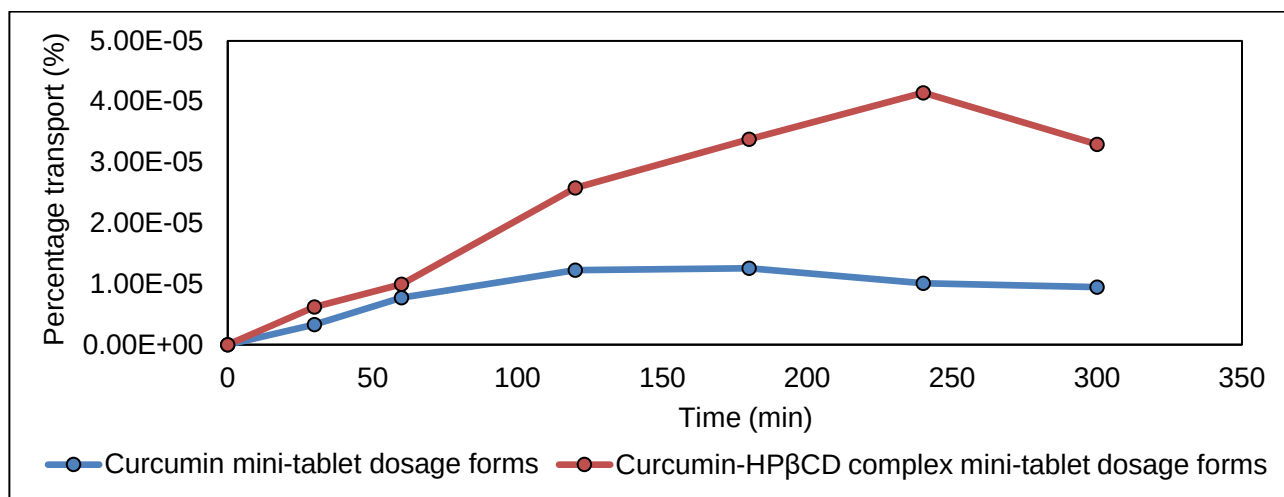


Figure 4-20: Average percentage transport comparison of curcumin mini-tablet dosage forms (blue curve) and curcumin-HPβCD complex mini-tablet dosage forms (red curve). (Error bars omitted for reasons of clarity, data available in appendices).

A nearly 3-fold increase was observed from the cumulative percentage transport and P_{app} values of curcumin mini-tablet formulations compared to the curcumin-HPβCD complex mini-tablet formulations. The percentage transport and P_{app} values are summarised in Table 4-15.

Table 4-15: Summary of the percentage transport (%) values, P_{app} values and cumulative percentage transport values obtained from permeation studies of different forms of curcumin across synthetic cellulose nitrate membranes

Time	Powder		Mini-tablet formulations	
(min)	Complex	Curcumin	Complex	Curcumin
0	0.00E+00	0.00E+00	0.00E+00	0.00E+00
30	2.29E-05	3.54E-06	6.18E-06	3.31E-06
60	5.29E-05	5.41E-06	9.97E-06	7.75E-06
120	8.26E-05	6.33E-06	2.58E-05	1.23E-05
180	3.09E-04	5.36E-06	3.38E-05	1.26E-05
240	9.78E-04	7.88E-06	4.15E-05	1.01E-05
300	1.44E-03	1.09E-05	3.30E-05	9.49E-06
P_{app}	2.68E-10	2.68E-12	1.09E-11	3.50E-12
Cumulative transport (%)	2.88E-03	3.95E-05	1.50E-04	5.55E-05

To elucidate the very low percentage transport obtained from the permeation studies, a visual inspection was performed on photographs taken during the respective transport studies (Figures 4-22 and 4-23). Comparison of a curcumin powder (Figure 4-22-A) with the inclusion complex powder (Figure 4-22-C), clearly illustrated that a hydrophobic layer formed on the surface of the SGF medium by the curcumin powder, while the complex powder readily blended with the SGF medium directly after introduction to the respective apical chambers. From the photographs of the relevant membranes (Figures 4-22-B and 4-22-D), the hydrophobicity of curcumin as opposed to the improved hydrophilicity of the prepared complex powder sample can clearly be observed as there is minimal to barely any adhesion of the curcumin powder sample to the hydrophilic cellulose nitrate membrane, while there is a clear aggregate of the complex powder present on the membrane.

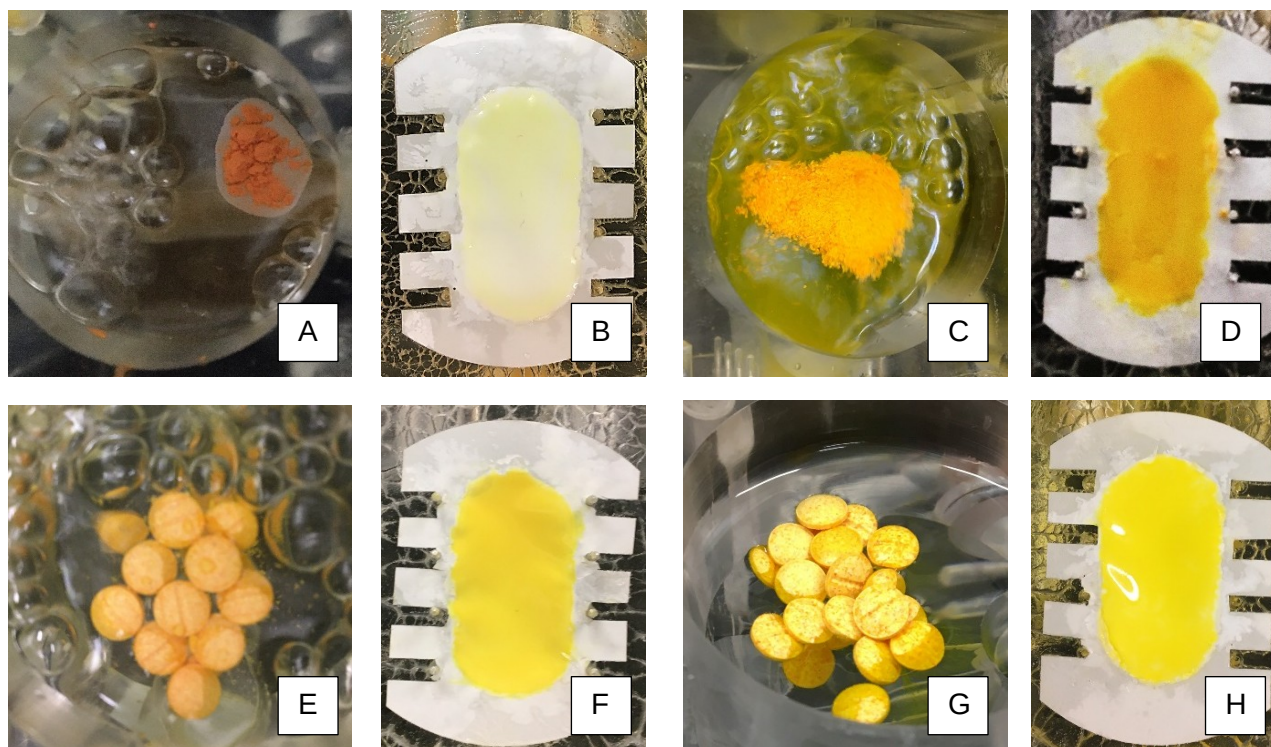


Figure 4-21: Photographic images of each of the four formulations, powder or mini-tablets and their corresponding synthetic membranes following a five-hour permeation study. From top left; (A) curcumin powder, (B) curcumin powder membrane, (C) complex powder, (D) complex powder membrane, (E) curcumin mini-tablets, (F) curcumin mini-tablet membrane, (G) complex mini-tablets, (H) complex mini-tablets membrane.

The curcumin-HP β CD complex containing mini-tablets (Figure 4-22-E) exhibited almost immediate yellowing of the SGF medium (Figure 4-23-B) similar to the dissolution study. However, both mini-tablets formulations revealed clearly visible residues deposited on the synthetic membranes (Figure 4-22-F and 4-22-H) following the permeation study.

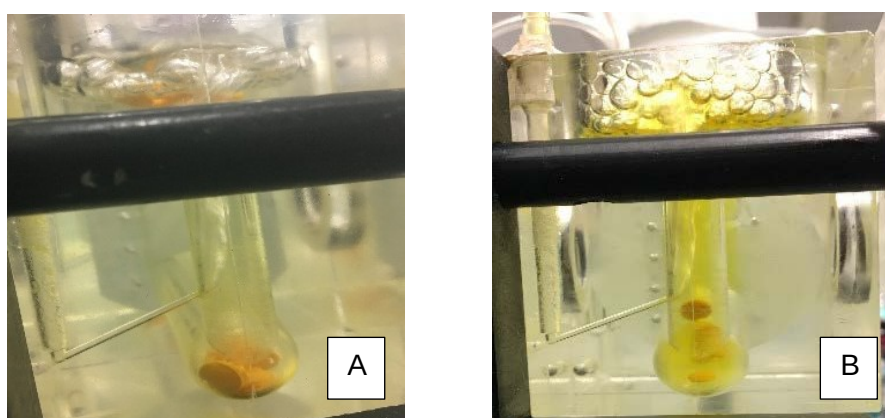


Figure 4-22: Side-view photographic images of (A) curcumin mini-tablets and (B) complex mini-tablets in modified Sweetana-grass diffusion chambers illustrating the yellowing of the simulated gastric fluid (pH1.2) solution caused by the mini-tablets containing the curcumin-HP β CD complex.

Both the curcumin-HP β CD complex powder samples and mini-tablet formulations presented with average percentage transport values of less than 1% (which translates to less than 1 μ g per 40 ml chamber) over the 5 h period, which may be interpreted as similar to the 1% relative bioavailability following oral administration reported in previously published literature (Yang *et al.*, 2007). Although the delivery of curcumin has not been improved in comparison to this previously reported relative plasma concentration, the percentage transport of the curcumin HP β CD complex powder sample was much higher than that of curcumin alone. It is possible that although the inclusion complex markedly increased the solubility of curcumin, the inclusion complex did not cause a similar extent in the increase of the permeation of curcumin across the hydrophilic cellulose nitrate membrane due to adsorbing on the surface of the hydrophilic membrane. The occurrence of the curcumin-HP β CD complex mini-tablet P_{app} value being lower than the P_{app} value of the relevant powder sample can be explained by the more complex release process from the mini-tablets, especially from the matrix type mini-tablets. Figure 4-24 displays photographic images of diffusion chambers containing (A) a curcumin-HP β CD complex powder sample equivalent to a quantity of 50 mg curcumin and (B) the curcumin-HP β CD complex mini-tablets, which have been determined to contain $\pm$ 50 mg curcumin per dosage. Upon inspection of the colour and relative densities of the relevant solutions, the curcumin-HP β CD complex presents with a darker shade of yellow than the mini-tablet formulation. The difference in colour density is a visual indication that the inclusion complex released curcumin faster from the powder than from the mini-tablets.

The smaller difference between the curcumin and complex mini-tablet formulations in terms of P_{app} values (3.50E-12 and 1.09E-11, respectively) when compared to the difference between the two powders' P_{app} values (2.68E-12 and 2.68E-10), could be an indication of unforeseen interactions between the incorporated excipients and the active ingredient, the acidic environment and/or the hydrophilic synthetic membrane.

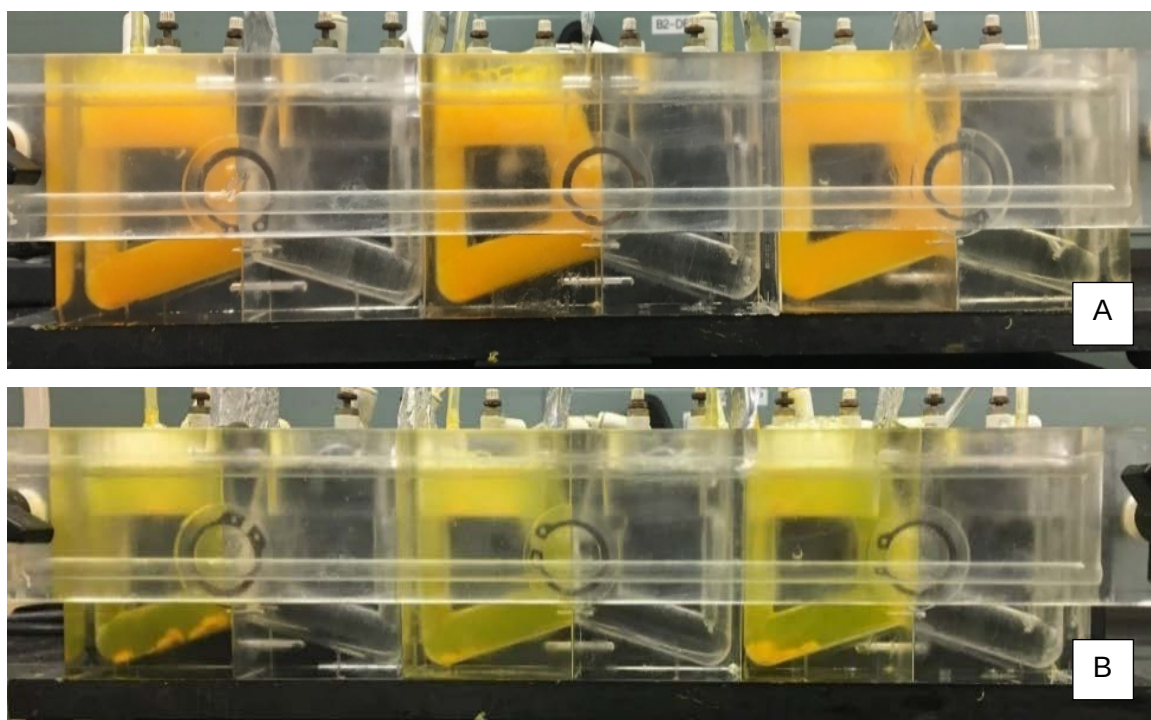


Figure 4-23: Diffusion chambers containing (A) a curcumin-HP β CD complex powder sample equivalent to 50 mg curcumin and (B) the curcumin-HP β CD complex mini-tablet dosage (50 mg)

CHAPTER 5: CONCLUSION AND RECOMMENDATIONS

5.1 Final conclusions

The primary aim of this study was to utilise formulation approaches to overcome some of curcumin's unfavourable physico-chemical properties such as poor aqueous solubility and instability in alkaline environments. These approaches included the formation of inclusion complexes between curcumin and HP β CD, the design of a floating type gastro-retentive dosage form following the optimisation of direct mini-tablet compression by utilising the SeDeM EDS and lastly the production of a multiple-unit drug delivery system (i.e. a mini-tablet-in-capsule drug delivery system).

An inclusion complex of curcumin in HP β CD was successfully formed and characterised. In addition to the FTIR, DSC, visual inspection under a microscope and a crystallographic analysis conducted by means of XRPD, which combined, confirmed the formation of an inclusion complex, the efficacy of the prepared complex was confirmed by conducting solubility studies in simulated gastric fluid (pH 1.2). The solubility studies determined that the solubility of curcumin was successfully increased 30 000-fold after preparing an inclusion complex of curcumin in HP β CD.

The SeDeM EDS effectively aided in the design and optimisation of direct-compressible floating gastro-retentive mini-tablets by determining deficiencies in the powder flow properties of curcumin and the curcumin-HP β CD complex. The SeDeM EDS also identified the amounts of each corrective excipient to be included within the respective formulations in order to overcome these deficits. Four mini-tablet formulations were produced including two immediate-release formulations and two gastro-retentive formulations. An immediate release and gastro-retentive formulation was prepared with curcumin and the curcumin-HP β CD complex respectively. All four formulations passed evaluation tests and resultantly dissolution studies and permeation studies were performed on the dosage forms.

The final mini-tablet-in-capsule dosage forms consisted of 2 immediate release and 8 gastro-retentive mini-tablets per capsule. The therapeutic dosage was selected to comprise of 2 capsules, which contained 20 mini-tablets in total amounting to a drug content of 50 mg curcumin per dosage. These final dosages were used to conduct dissolution and permeation studies.

Dissolution studies of the mini-tablet dosage forms presented with poor drug release profiles followed by low P_{app} values obtained from permeation studies across synthetic cellulose nitrate membranes. Permeation studies were also performed on curcumin and curcumin-HP β CD complex powder samples. The curcumin-HP β CD complex powder samples presented with permeation values 50-times higher than the relevant mini-tablet dosage form. The variance between P_{app} values of the curcumin-HP β CD complex mini-tablet dosages and the powder samples was attributed to the complex sustained release from the mini-tablets.

It was therefore concluded that the primary aim of this study, improving unfavourable physico-chemical properties of curcumin, such as poor aqueous solubility with the utilisation of formulation approaches, was met by preparing an inclusion complex of curcumin in HP β CD. Furthermore, the formulation of floating matrix type gastro-retentive mini-tablets could retain the dosage form within the acidic environment of the stomach and thereby potentially delay and/or prevent instability in the alkaline environment of the small intestine. However, the final dosage forms did not lead to a notable increase in drug delivery across a synthetic cellulose nitrate membrane due to slow drug release and poor permeation. The membrane permeation properties therefore, require further investigation such as inclusion of absorption enhancing agents in the final dosage form.

5.2 Future recommendations

During this study, the formation of an inclusion complex of curcumin in HP β CD showed promise with regard to improving unfavourable physico-chemical properties of curcumin. However, further studies are required such as:

- Determination of the dissolution profiles of curcumin-HP β CD complex from multiple-unit drug delivery systems prepared from powders and beads,
- Investigation into the effect of absorption enhancers such as piperine on membrane permeation across excised animal intestinal tissues;
- The possible improvement of the dissolution profile of the curcumin-HP β CD complex from the matrix type mini-tablets to obtain a higher total cumulative percentage curcumin release,
- Comparison of membrane permeation obtained *in vitro* with bioavailability obtained *in vivo*,
- Investigate the use of bile salts on the solubility and membrane permeation of curcumin,
- Investigate the development of lipid type dosage forms (e.g. solid lipid nanoparticles, nanosomes, liposomes and self-emulsifying drug delivery systems) on the membrane permeability and systemic delivery of curcumin, and

- Investigate the development of soft gelatin capsules filled with oil-based curcumin solutions on the membrane permeation and systemic delivery of curcumin.

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ANNEXURES

ADDENDUM A: ETHICS APPROVAL LETTER OF STUDY



Private Bag X1290, Potchefstroom
South Africa 2520

Tel: 086 016 9698
Web: <http://www.nwu.ac.za/>

North-West University Animal Care, Health and
Safety Research Ethics Committee (NWU-
AnimCareREC)

Tel: 018 299-1208
Email: Ethics-AnimCare@nwu.ac.za (for animal
studies)

09 March 2020

ETHICS APPROVAL LETTER OF STUDY

Based on approval by the North-West University Animal Care, Health and Safety Research Ethics Committee (NWU-AnimCareREC) on 09/03/2020, the NWU-AnimCareREC hereby approves your study as indicated below. This implies that the NWU-AnimCareREC grants its permission that, provided the general conditions specified below are met and pending any other authorisation that may be necessary, the study may be initiated, using the ethics number below.

Study title: Development of curcumin containing direct-compressible gastro-retentive tablets			
Principal Investigator/Study Supervisor/Researcher: Prof JH Hamman			
Student: S van Niekerk - 25971360			
Ethics number:			
N	W	U	-
0	0	5	0
1	-	2	0
-	A	5	
Institution Study Number Year Status			
Status: S = Submission; R = Re-Submission; P = Provisional Authorisation; A = Authorisation			
Application Type: Single study		Risk:	
Commencement date: 09/03/2020		Category 0	
Expiry date: 31/03/2021			
Approval of the study is provided for a year, after which continuation of the study is dependent on receipt and review of a yearly monitoring report and the concomitant issuing of a letter of continuation. A monitoring report is due at the end of March annually until completion.			

General conditions:

While this ethics approval is subject to all declarations, undertakings and agreements incorporated and signed in the application form, the following general terms and conditions will apply:

- The principal investigator/study supervisor/researcher must report in the prescribed format to the NWU-AnimCareREC:
 - Annually on the monitoring of the study, whereby a letter of continuation will be provided annually, and upon completion of the study; and
 - without any delay in case of any adverse event or incident (or any matter that interrupts sound ethical principles) during the course of the study.
- The approval applies strictly to the proposal as stipulated in the application form. Should any amendments to the proposal be deemed necessary during the course of the study, the principal investigator/study supervisor/researcher must apply for approval of these amendments at the NWU-AnimCareREC, prior to implementation. Should there be any deviations from the study proposal without the necessary approval of such amendments, the ethics approval is immediately and automatically forfeited.
- Annually a number of studies may be randomly selected for active monitoring.

- The date of approval indicates the first date that the study may be started.
- In the interest of ethical responsibility, the NWU-AnimCareREC reserves the right to:
 - request access to any information or data at any time during the course or after completion of the study;
 - to ask further questions, seek additional information, require further modification or monitor the conduct of your research or the informed consent process;
 - withdraw or postpone approval if:
 - any unethical principles or practices of the study are revealed or suspected;
 - it becomes apparent that any relevant information was withheld from the NWU-AnimCareREC or that information has been false or misrepresented;
 - submission of the annual monitoring report, the required amendments, or reporting of adverse events or incidents was not done in a timely manner and accurately; and/or
 - new institutional rules, national legislation or international conventions deem it necessary.
- NWU-AnimCareREC can be contacted for further information via Ethics-AnimCare@nwu.ac.za or 018 299 1208

NWU-AnimCareREC would like to remain at your service and wishes you well with your study. Please do not hesitate to contact the NWU-AnimCareREC for any further enquiries or requests for assistance.

Yours sincerely,



Digitally signed by
Christiaan B Brink
Date: 2020.03.18
09:15:09 +02'00'

Chairperson: NWU-AnimCareREC

Current details: (23239522) G:\My Drive\Research and Postgraduate Education\9.1.5.4 Templates\9.1.5.4.2_NWU-AC_EAL.docm
09 March 2020

File Reference: 9.1.5.4.2

ADDENDUM B: CERTIFICATE OF ANALYSIS: CURCUMIN REFERENCE STANDARD



CERTIFICATE OF ANALYSIS


analytics


consulting

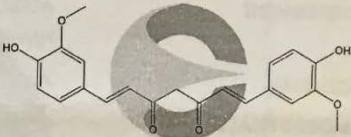

ingredients


standards

For Laboratory Use Only

PRODUCT NAME	Curcumin
PART NUMBER	00003926
STANDARD TYPE	Primary (P) – Compendial Traceable
LOT NUMBER	00003926-515
REPORT NUMBER	CDXA-RSS-3467-03
SAMPLE NUMBER	CDXA-09-1789
DATE OF SAMPLE	04/24/2009
DATE OF RE-EVALUATION	06/09/2017
DATE OF REPORT	07/14/2017

Structure



CHEMICAL NAMES (E,E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; Diferuloylmethane; Haldar; Halud; Indian saffron; Souchet; Terra merita; Yellow root; Yo-kin

CHEMICAL FORMULA C₂₁H₂₀O₆

MOLECULAR WEIGHT (MW) 368.38

CHEMICAL FAMILY Phenolics

CAS NUMBER [458-37-7]

EC#(EINECS) 207-280-5

RTECS MI5230000

ANALYTICAL RESULTS

TEST	METHOD	SPECIFICATION	RESULT
Adjusted Purity	NA	NA	99.9%
HPLC	99.1-03-3.0-000280	NA	99.9%
NMR	99.1-CD-1.0-000122	Conforms to structure	Conforms to structure
Mass Spectrum	Direct Infusion, ESI (-)	Conforms	Conforms
Residual Solvent	99.1-CD-3.0-000115	NA	ND
Water	99.1-34-8.0-000094	NA	ND
Appearance	NA	NA	Orange powder

ADJUSTED PURITY: 99.9% IS BASED ON (100 - 0.0 WATER - 0.0 SOLVENTS) X 99.9% HPLC

TRACEABLE SOURCE	LOT NUMBER	ASSAY RESULT
USP	FOH127	99.9%

STORAGE CONDITIONS

STORAGE Room Temperature in a dry place.

EXPIRATION DATE 06/2022 under the above conditions.

Erik Lightner

Digitally signed by Erik Lightner
DN: cn=Erik Lightner, o=ChromaDex Inc., ou=Quality Assurance,
email=ErikL@chromadex.com, c=US
Date: 2017.08.03 13:33:39 -06'00'

10005 MUIRLANDS BLVD., SUITE G, IRVINE, CA 92618 | T: +1 949-419-0288 | F: +1 949-419-0294 | WWW.CHROMADEX.COM

Page 1 of 4

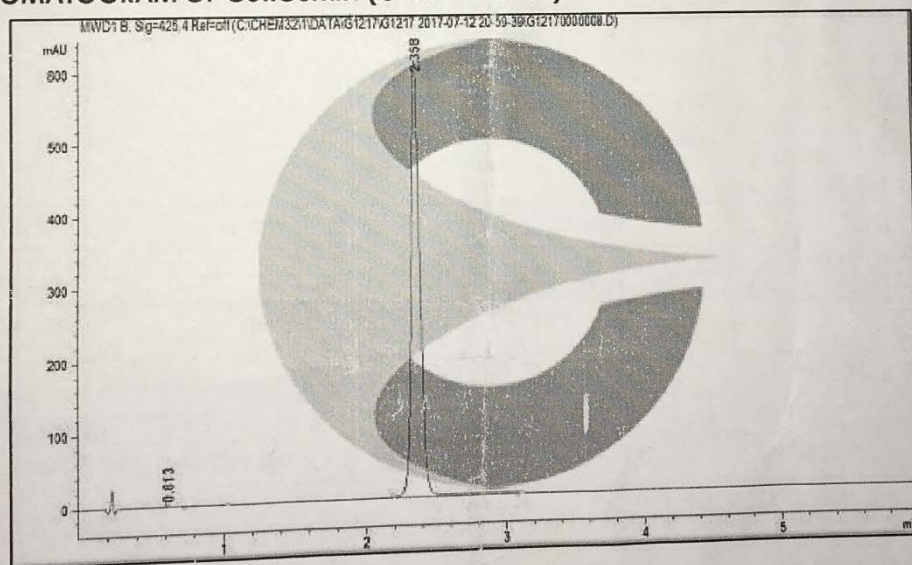
CERTIFICATE OF ANALYSIS

For Laboratory Use Only

ANALYTICAL CONDITIONS

INSTRUMENT	AGILENT 1290 HPLC UV-VIS (MWD) DETECTOR FINNIGAN LCQ-DECA
COLUMN	Agilent Poroshell EC-C18 -- 50 x 3.0 mm, 2.7 μ m particle size.
MOBILE PHASE	A -- 0.01% Phosphoric acid in Milli-Q Water, B -- Acetonitrile; Isocratic 60% B over 6 minutes.
COLUMN TEMP.	35 $^{\circ}$ C
FLOW RATE	1.0 mL/minute
INJECTION VOL.	0.5 μ L
INJECTION CONC.	1.0 mg/mL in Methanol
DETECTION	425 $\pm$ 2 nm

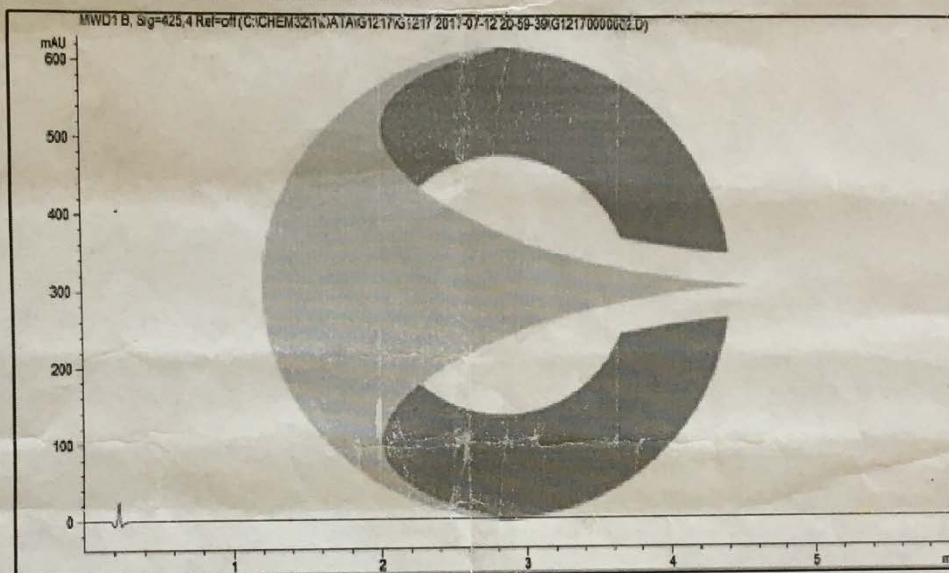
HPLC CHROMATOGRAM OF CURCUMIN (CDXA-09-1789)



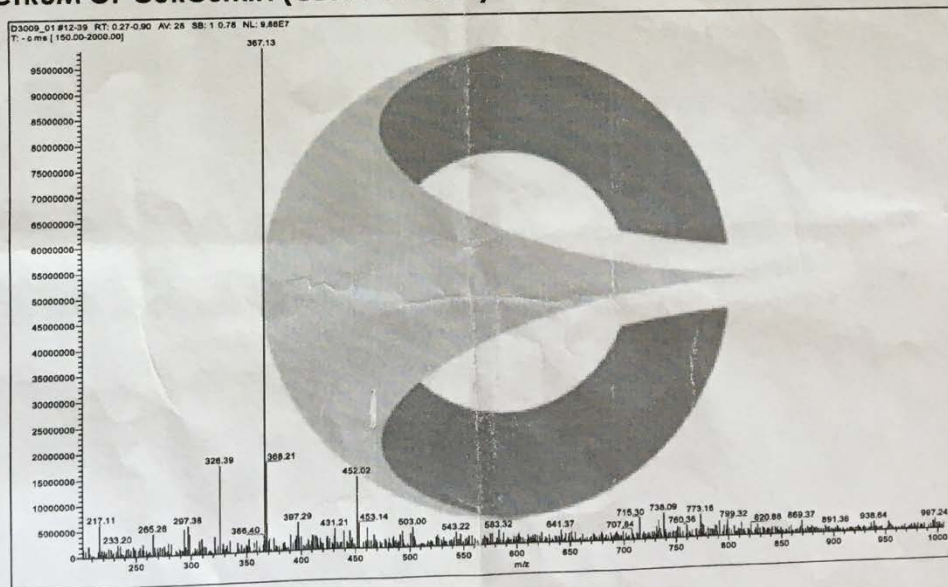
CERTIFICATE OF ANALYSIS

For Laboratory Use Only

HPLC CHROMATOGRAM OF DILUENT BLANK



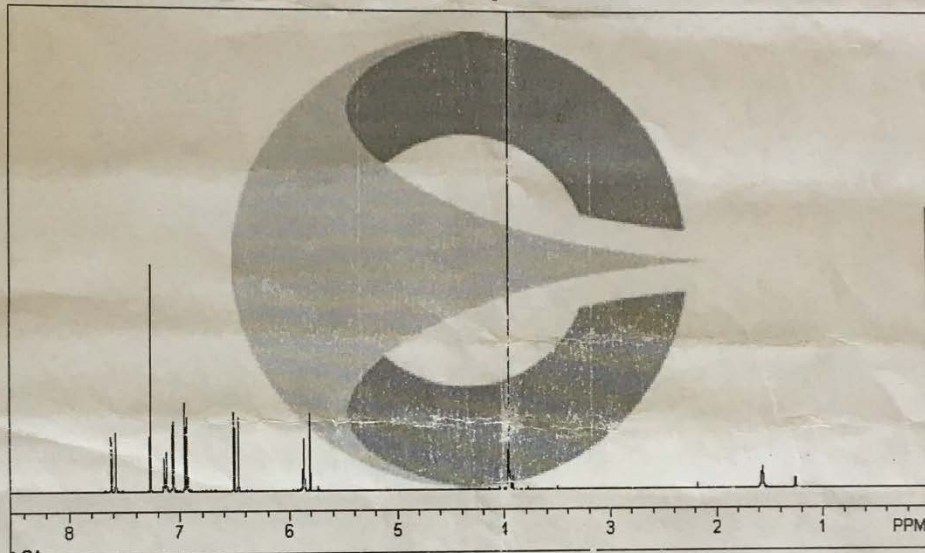
MASS SPECTRUM OF CURCUMIN (CDXA-09-1789)



CERTIFICATE OF ANALYSIS

For Laboratory Use Only

¹H-NMR SPECTRUM OF CURCUMIN (CDXA-09-1789) IN CDCl₃



REVISION HISTORY

<u>Revision History</u>	<u>Date of Revision</u>	<u>Document/Changes</u>
00	05/14/2009	New report
01	05/02/2013	Upgraded PN from P-grade to CT.
02	09/03/2013	Updated expiration date.
03	07/14/2017	Passed re-evaluation by HPLC, KF. Updated expiration date, analytical method, conditions, chromatograms and results. Removed melting point.

ADDENDUM C: CERTIFICATE OF ANALYSIS: CURCUMIN RAW MATERIAL

Toronto Research Chemicals
products for innovative research

20 Martin Ross Ave., Toronto, ON. M3J 2K8 Canada Tel: (416) 665-9696 Fax: (416) 665-4439 E-mail: orders@trc-canada.com Website: www.trc-canada.com

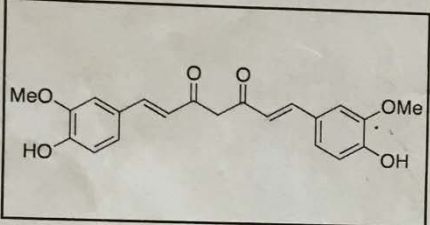
Certificate of Analysis

1. Identification

CAS Number: 458-37-7
Catalogue Number: C838500
Product: Curcumin

Synonyms:
(1E,6E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; (E,E)-1,7-Bis(4-hydroxy-3-methoxyphenyl)-1,6-heptadiene-3,5-dione; C Yellow 15; C.I. 75300; C.I. Natural Yellow 3; Curcuma; Curcumin I; Curcumine; Differuloylmethane; Haidr; Halad;

Structure:



Molecular Formula: C₂₁H₂₀O₆
Molecular Weight: 368.38
Source of Product: N/A

2. Analytical Information

Lot Number: 3-DXX-154-1
Melting Point: 183 °C
Appearance of Product:
Method for Determining Identity:

Purity: 98%

Atmosphere:
Solubility: DMSO (Slightly), Methanol (Slightly)
Stability:
Long Term Storage Condition: Refrigerator
Shipping Condition: This Product Is Stable To Be Shipped At Room Temperature

Additional Information:
TLC Conditions: SiO₂; Dichloromethane : Methanol = 9 : 1; Visualized with UV and Naked Eye; Single Spot, R_f = 0.60.

Purity is based on the analytical results of the tests performed. NMR and TLC may have an accuracy of +/- 2%. Isotopic purity is based on mass distribution observed.

QC Test Date: November 29, 2018
Retest Date: November 27, 2022

Philip Chan, Head of Quality Assurance

APPOINTED DISTRIBUTOR
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ISO9001:2015

ADDENDUM D: CERTIFICATE OF ANALYSIS: HYDROXYPROPYL-B-CYCLODEXTRIN

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Certificate of Analysis

Product Name	(2-HYDROXYPROPYL)-BETA-CYCLODEXTRIN		
Batch No.	20181225	Quantity	1kg
MFG. Date	Dec.25.2018	EXP .Date	Dec.24.2020
CAS No.	128446-35-5	Specs	USP34 Grade

ITEMS	SPECIFICATION	RESULTS
Appearance	White powder	Qualify
Degree of Substitution	2.8-10.5	5.6
Clarity of solution	50%(w/v) water solution is clear	Clear
Identification(IR)	Same absorption bands with USP Hydroxypropyl Betadex RS	Qualify
Loss on Drying	≤10%	4.0%
Conductivity	10%(w/v) water solution ≤200μS/cm	60μS/cm
Propylene oxide	≤1ppm	0.18ppm
Propylene glycol	≤2.5%	0.28%
Sum of Other Impurities	≤1%	0.1%
Betadex	≤1.5%	0.18%
Residue on Ignition	≤0.2%	0.08%
Heavy Metal	≤20ppm	<10ppm
Chloride	≤0.05%	<0.04%
TAMC	≤1000cfu/g	<10cfu/g
TYMC	≤100cfu/g	<10cfu/g
Salmonella/E. Coli	Absent	Absent
Assay	≥98%	99.3%
Conclusion	Conforms to the USP34 Grade	
Remarks	DD-033926	

Quality Control Dept:

REPRESENTED BY:
DB FINE CHEMICALS
Analyst: SPECIALTIES
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ADDENDUM D: ANCILLARY DATA

ANCILLARY DATA FOR THE VALIDATION OF ANALYTICAL METHODS

Table A: Ancillary data for the validation of the liquid chromatography – mass spectrometry (LC-MS) method

Concentration	Peak area (AUC)			
(ng/ml)	A	B	Mean	Standard deviation
3.1	90.29	96.10	93.20	2.90
7.81	176.37	169.58	172.98	3.40
15.63	335.28	333.83	334.56	0.72
32.25	819.42	805.05	812.24	7.19
62.50	1633.90	1782.18	1708.04	74.14
125	4282.25	3969.06	4125.66	156.60
250	8730.20	8144.65	8437.43	292.78

Table B: Ancillary data for the validation of the fluorescence spectrophotometric (FS) analyses method

Concentration	Fluorescence			
(µg/ml)	A	B	Mean	Standard deviation
1.56	2.23E+06	2.31E+06	2.27E+06	4.18E+04
3.13	6.42E+06	6.43E+06	6.42E+06	6.25E+03
6.25	1.74E+07	1.73E+07	1.74E+07	4.90E+04
12.5	4.02E+07	4.13E+07	4.03E+07	1.07E+05
25	8.42E+07	8.49E+07	8.45E+07	3.67E+05
50	1.69E+08	1.74E+08	1.72E+08	2.46E+06

ANCILLARY DATA FOR THE SOLUBILITY STUDIES PERFORMED ON CURCUMIN AND THE PREPARED CURCUMIN-HPBCD COMPLEX

Table C: Ancillary data for the solubility of curcumin and the prepared curcumin-HP β CD complex

Sample	Time	Concentration (ng/ml)			
	(h)	A	B	Mean	Standard deviation
Curcumin-HP β CD complex	2	7135.59	7879.53	7507.56	371.97
	4	13329.60	9565.26	11447.43	1882.17
	8	11383.77	11489.54	11436.65	52.88
	24	207104.50	213362.56	210233.53	3129.03
	48	267705.62	198982.87	233344.24	34361.37
Curcumin	2	0.29	0.30	0.29	0.01
	4	0.30	0.35	0.33	0.03
	8	0.42	0.31	0.37	0.05
	24	4.31	1.91	3.11	1.20
	48	7.00	6.99	6.99	0.01

ANCILLARY DATA FOR THE DISSOLUTION STUDIES PERFORMED ON CURCUMIN AND THE PREPARED CURCUMIN-HPBCD COMPLEX

Table D: Ancillary data for the dissolution of curcumin and the prepared curcumin-HP β CD complex

Sample	Time	Concentration (ng/ml)			
	(min)	A	B	Mean	Standard deviation
Curcumin-HP β CD complex	15	134.06	129.23	131.64	2.42
	30	1159.34	1212.99	1186.16	26.82
	60	3422.39	3548.73	3485.56	63.17
	120	3551.79	3452.29	3502.04	49.75
	180	8802.41	9527.37	9164.89	362.48
	300	9987.78	5200.62	7594.20	2393.58
Curcumin	15	1.30	1.95	1.62	0.33
	30	19.09	12.95	16.02	3.07
	60	72.00	78.46	75.23	3.23
	120	231.72	224.27	227.99	3.73
	180	345.94	323.60	334.77	11.17
	300	463.61	394.36	428.99	34.62

ANCILLARY DATA FOR THE PERMEATION STUDIES PERFORMED ON LUCIFER YELLOW FOR THE DETERMINATION OF MEMBRANE INTEGRITY OF THE SYNTHETIC CELLULOSE NITRATE FILTER MEMBRANES

Table E: Ancillary data for the percentage transport of marker molecule, Lucifer Yellow, across synthetic cellulose nitrate membranes

Time	% Transport			
(min)	Chamber A	Chamber B	Mean	Standard deviation
0	0.00	0.00	0.00	0.00
30	0.41	0.57	0.49	0.08
60	2.06	4.57	3.32	1.26
120	13.06	10.78	11.92	1.14
180	19.82	15.77	17.80	2.02
240	27.00	20.51	23.75	3.24
300	33.02	24.35	28.68	4.33
Slope	1.09E-01	8.40E-02	8.89E-02	
1/A.60.C0	7.25E-05	7.25E-05	7.25E-05	
Papp	7.90E-06	6.09E-06	6.44E-06	

ANCILLARY DATA FOR THE PERMEATION STUDIES PERFORMED ON CURCUMIN AND THE PREPARED CURCUMIN-HPBCD COMPLEX POWDER SAMPLES AND FINAL MINI-TABLET DOSAGE FORMS

Table F: Ancillary data for the percentage transport of curcumin-HP β CD complex powder samples across synthetic cellulose nitrate membranes

Time	% Transport			
(min)	Chamber A	Chamber B	Mean	Standard deviation
0	0.00E+00	0.00E+00	0.00E+00	0.00E+00
30	2.36E-05	2.22E-05	2.29E-05	7.03E-07
60	4.63E-05	5.95E-05	5.29E-05	6.58E-06
120	6.77E-05	9.76E-05	8.26E-05	1.49E-05
180	2.63E-04	3.56E-04	3.09E-04	4.68E-05
240	9.60E-04	9.97E-04	9.78E-04	1.86E-05
300	1.48E-03	1.39E-03	1.44E-03	4.74E-05
Slope	9.25E-02	9.28E-02	3.69E-06	
1/A.60.C0	7.25E-05	7.25E-05	7.25E-05	
Papp	6.71E-06	6.73E-06	2.68E-10	

Table G: Ancillary data for the percentage transport of curcumin powder samples across synthetic cellulose nitrate membranes

Time	% Transport			
(min)	Chamber A	Chamber B	Mean	Standard deviation
0	0.00E+00	0.00E+00	0.00E+00	0.00E+00
30	3.00E-06	4.08E-06	3.54E-06	5.37E-07
60	6.48E-06	4.34E-06	5.41E-06	1.07E-06
120	7.32E-06	5.35E-06	6.33E-06	9.83E-07
180	4.32E-06	6.39E-06	5.36E-06	1.04E-06
240	8.67E-06	7.10E-06	7.88E-06	7.85E-07
300	1.34E-05	8.51E-06	1.09E-05	2.43E-06
Slope	4.10E-08	3.20E-08	3.69E-08	
1/A.60.C0	7.25E-05	7.25E-05	7.25E-05	
Papp	2.97E-12	2.32E-12	2.68E-12	

Table H: Ancillary data for the percentage transport of curcumin-HP β CD complex mini-tablet dosage form samples across synthetic cellulose nitrate membranes

Time	Percentage transport (%)			
(min)	Chamber A	Chamber B	Mean	Standard deviation
0	0.00E+00	0.00E+00	0.00E+00	0.00E+00
30	8.18E-06	4.19E-06	6.18E-06	1.99E-06
60	1.13E-05	8.60E-06	9.97E-06	1.37E-06
120	2.61E-05	2.54E-05	2.58E-05	3.39E-07
180	3.51E-05	3.26E-05	3.38E-05	1.26E-06
240	4.38E-05	3.92E-05	4.15E-05	2.28E-06
300	2.63E-05	3.96E-05	3.30E-05	6.69E-06
Slope	1.45E-07	1.55E-07	1.50E-07	
1/A.60.C0	7.25E-05	7.25E-05	7.25E-05	
Papp	1.05E-11	1.12E-11	1.09E-11	

Table I: Ancillary data for the percentage transport of curcumin mini-tablet dosage form samples across synthetic cellulose nitrate membranes

Time	Percentage transport (%)			
(min)	Chamber A	Chamber B	Mean	Standard deviation
0	0.00E+00	0.00E+00	0.00E+00	00.0E+00
30	0.00E+00	9.92E-06	4.96E-06	4.96E-06
60	7.40E-06	1.10E-05	9.18E-06	1.78E-06
120	1.52E-05	1.16E-05	1.34E-05	1.81E-06
180	1.30E-05	1.33E-05	1.32E-05	1.29E-07
240	1.04E-05	7.40E-06	8.91E-06	1.50E-06
300	8.93E-06	7.51E-06	8.22E-06	7.12E-07
Slope	4.90E-08	4.40E-07	4.82E-08	
1/A.60.C0	7.25E-05	7.25E-05	7.25E-05	
Papp	3.55E-12	3.19E-11	3.50E-12	