FORMULATION AND EVALUATION OF GASTRO-RETENTIVE FLOATING TABLETS OF GRISEOFULVIN

by

JONATHAN TINOTENDA CHANYANDURA

[redacted]

DISSERTATION

Submitted in fulfilment of the requirements for the degree

MASTERS OF PHARMACY

in

PHARMACEUTICS

in the

FACULTY OF HEALTH SCIENCES

(School of Health Care Sciences)

at the

UNIVERSITY OF LIMPOPO

SUPERVISOR: Mr. MS Poka

CO-SUPERVISOR: Prof PH Demana

2018

DECLARATION BY CANDIDATE

“I hereby declare that the dissertation submitted for the degree MPHARM: Pharmaceutics, at the University of Limpopo, is my own original work and has not previously been submitted to any other institution of higher education. I further declare that all sources cited or quoted are indicated and acknowledged by means of a comprehensive list of references”.

Jonathan Tinotenda Chanyandura

[redacted]

DEDICATION

This work is dedicated to Mr MS Poka;

A truly great mentor, hard to find.

ACKNOWLEDGEMENTS

More than words can say, I would like to express my gratitude to:

- My supervisor, Mr MS Poka for his guidance, support and building me to be a better researcher. I am grateful for the skills he imparted, for guidance, wisdom and knowledge shared. His kindness is amazing.

- My co-supervisor, Prof PH Demana for his valuable support and advice all the way throughout the project.

- Dr S Khamanga from Rhodes University for taking me through designing of experiments using Design Expert®.

- Prof W Liebenberg from North-West University for helping with differential scanning calorimetry (DSC) experiments.

- Prof NZ Nyazema, Mr GM Mohlala, Mr TL Manyama, Mrs EM Masupye, Dr SH Ginindza and Mr M Ginindza for their support and guidance.

- University of Limpopo, the Department of Pharmacy and colleagues for support.

- Miss DM Malepe for believing in me and standing by me.

- Family and friends for cheering me on.

- The Lord Almighty, for He enables.

ABSTRACT

Griseofulvin is an antibiotic fungistatic drug used in the treatment of dermatophyte and ringworm infections. About 50% of a dose of griseofulvin passes the gastro-intestinal tract unabsorbed and is excreted in faeces. Since griseofulvin is highly soluble in acidic pH, a gastro-retentive floating matrix system was developed to control dissolution rate and thereby enhance solubility in an effort to develop an improved and convenient dosage form.

Preformulation studies included selection of excipients and evaluation of their compatibility with griseofulvin. Using the chosen excipients, floating tablets of griseofulvin were formulated. Floating tablets containing 100 mg of griseofulvin were prepared by wet granulation technique with varying ratios of Methocel™, Accurel MP and Polyvinylpyrrolidone as determined by Design Expert software. Pre and post-compression studies, buoyancy capability and dissolution studies were carried out to assess the influence of the tablet components.

Results obtained revealed that a density of less than 0.00091 g/cm^3 was necessary for tablet floatation. Tablets that float immediately upon contact with dissolution medium and continue floating for over 12 hours were achieved with at least 28% Accurel MP by mass of the tablet. Dissolution studies revealed that an increase in tablet hardness reduced the rate of griseofulvin release only up to 120 minutes. From 120 minutes onwards, tablet hardness had no significant influence on griseofulvin release from tablets. Methocel™ had the most significant influence on griseofulvin release. The amount of Methocel™ included in the formulation was indirectly proportional to the rate of griseofulvin release.

Using Design Expert software, optimized formulation was achieved with 1% Polyvinylpyrrolidone, 30% Methocel™, 60% Accurel MP and hardness ranging between 8 – 9 N. Pre and post-compression parameters of the optimized tablets were found to be within pharmacopoeial limits and thus compressed tablets were of acceptable quality. Tablets produced floated immediately upon contact with the medium and remained floating for at least 12 hours. Griseofulvin was released from the optimized tablets in a near zero order fashion, with a total of 80.8% griseofulvin released at the end of the 12 hour dissolution test period. Results of accelerated stability studies indicated potential stability of the manufactured tablets months.

CONTENTS

		PAGE
DECLARATION BY CANDIDATE		i
DEDICATION		ii
ACKNOWLEDGEMENTS		iii
ABSTRACT		iv
LIST OF ABBREVIATIONS		xii
LIST OF ANNEXURES		xiii
LIST OF TABLES		xiv
LIST OF FIGURES		xvi
CONFERENCE PRESENTATIONS		xix
CHAPTER 1: INTRODUCTION		
1.1	BACKGROUND AND JUSTIFICATION	1
1.2	RESEARCH PROBLEM	4
1.3	AIM	4
1.4	OBJECTIVES	4
CHAPTER 2: DERMATOPHYTE INFECTIONS		
2.1	INTRODUCTION	6
2.2	DERMATOPHYTES	6
2.2.1	Infection mechanism of dermatophytes	6
2.2.2	Dermatophytes' adhesion to and invading superficial skin tissues	7
2.2.3	Dermatophytes' growth on keratinized tissue	7
2.2.4	Immune response to dermatophyte infection	8
2.2.5	Signs and symptoms of dermatophytes infection	9
2.2.6	Diagnosis of dermatophytes infection	10

2.2.7	Treatment of dermatophytes infection	10
2.3	GRISEOFULVIN	11
2.3.1	Drug identity	11
2.3.2	Pharmacological action	12
2.3.3	Mycology	13
2.3.4	Mechanism of action	13
2.3.5	Griseofulvin potential as therapeutics for cancer	13
2.3.6	Pharmacokinetic properties	14
2.3.6.1	Absorption	14
2.3.6.2	Distribution	15
2.3.6.3	Metabolism	15
2.3.6.4	Excretion	16
2.3.7	Drug-drug interactions	16
2.3.8	Drug-food interaction	16
2.3.9	Contraindications	18
2.3.10	Adverse effects	18
2.4	BIOPHARMACEUTICS CLASSIFICATION OF GRISEOFULVIN	19
2.5	SUMMARY	20

CHAPTER 3: GASTRO-RETENTIVE DRUG DELIVERY SYSTEMS

3.1	INTRODUCTION	21
3.2	ORAL DRUG DELIVERY	21
3.3	THE GASTRO-INTESTINAL TRACT AND DRUG DELIVERY	21
3.4	GASTRO-RETENTIVE DRUG DELIVERY SYSTEMS	24

3.4.1	Anatomical and Physiological aspects of Gastro-retentive drug delivery	24
3.4.2	Factors controlling gastric retention of dosage forms	27
3.4.2.1	Density of dosage form	27
3.4.2.2	Size of dosage form	28
3.4.2.3	Food intake	28
3.4.2.4	Nature of food present in the stomach	28
3.4.2.5	Effect of gender, posture and age	29
3.4.3	Advantages of Gastro-retentive Drug Delivery System	29
3.4.3.1	Improved patient compliance	29
3.4.3.2	Enhanced biolavailability	29
3.4.3.3	Site specific drug delivery	30
3.4.3.4	Controlled drug delivery	30
3.4.4	Disadvantages of Gastro-retentive Drug Delivery System	30
3.4.4.1	Gastric mucosa irritation by drugs	30
3.4.4.2	Gastro-retentive drug delivery is not suitable for all drugs	31
3.4.4.3	Floating drug delivery systems require high fluid level in stomach	31
3.4.5	Potential drug candidates for Gastro-retentive Drug Delivery	31
3.4.6	Unsuitable drugs for Gastro-retentive Drug Delivery	31
3.5	TYPES OF GASTRO-RETENTIVE DRUG DELIVERY SYSTEMS	32
3.5.1	Bio/Muco-Adhesive systems	32
3.5.1.1	Electronic theory	32
3.5.1.2	Wetting theory	33
3.5.1.3	Adsorption theory	33

3.5.1.4	Diffusion theory	33
3.5.1.5	Fracture theory	34
3.5.1.6	Mechanical theory	34
3.5.2	Expandable systems	36
3.5.3	High density systems	38
3.5.4	Magnetic systems	38
3.5.5	Super porous hydrogels	39
3.5.5.1	Conventional Super Porous Hydrogels (CSPH)	40
3.5.5.2	Super Porous Hydrogels Composites (SPHC)	40
3.5.5.3	Super Porous Hydrogels Hybrids	41
3.5.6	Floating systems	41
3.5.6.1	Non effervescent systems	42
3.5.6.2	Effervescent systems	47
3.6	GASTRO-RETENTIVE PREPARATIONS AVAILABLE IN INTERNATIONAL MARKET	50
3.7	METHODS TO IMPROVE ORAL ABSORPTION OF GRISEOFULVIN	51
3.8	SUMMARY	53
CHAPTER 4: MATERIALS AND METHODS		
4.1	INTRODUCTION	54
4.2	MATERIALS	54
4.2.1	Accurel MP	54
4.2.2	Methocel™	54
4.2.3	Polyvinylpyrrolidone (PVP k-30)	55
4.2.4	Magnesium stearate	55
4.3	DRUG – EXCIPIENT COMPATIBILITY STUDIES	55

4.4	MANUFACTURING METHOD OF GRISEOFULVIN FLOATING MATRIX TABLETS	56
4.5	EVALUATION OF FLOATING MATRIX TABLETS OF GRISEOFULVIN	58
4.5.1	Pre-compression studies	58
4.5.1.1	Loss on drying	58
4.5.1.2	Bulk density	58
4.5.1.3	Tapped density	59
4.5.1.4	Carr's index and Hausner ratio	59
4.5.1.5	Angle of repose	60
4.5.2	Post-compression studies	61
4.5.2.1	Tablet dimensions	61
4.5.2.2	Hardness	62
4.5.2.3	Weight variation test	62
4.5.2.4	Friability	62
4.5.3	Buoyancy/floating test	63
4.5.4	Tablet density	63
4.5.5	Assay	64
4.5.6	*In-vitro* dissolution testing	64
4.6	SUMMARY	65

CHAPTER 5: RESULTS AND DISCUSSION

5.1	INTRODUCTION	66
5.2	COMPATIBILITY STUDIES	66

5.2.1	Differential scanning calorimetry thermogram of pure griseofulvin	67
5.2.2	Compatibility of griseofulvin with Accurel MP	67
5.2.3	Compatibility of griseofulvin with Methocel™	68
5.2.4	Compatibility of griseofulvin with magnesium stearate	69
5.2.5	Compatibility of griseofulvin with PVP k-30	70
5.3	FORMULATION DESIGN	71
5.4	PRE-COMPRESSION STUDIES	73
5.5	POST COMPRESSION STUDIES	75
5.6	ASSAY	77
5.7	BUOYANCY STUDIES	78
5.7.1	Analysis of variance on lag time	82
5.7.2	Analysis of variance on floating time	83
5.7.3	Influence of Accurel MP on tablet buoyancy	84
5.8	DISSOLUTION STUDIES	85
5.8.1	ANOVA analysis of factors influencing drug release at 120 minutes	87
5.8.2	ANOVA analysis of factors influencing drug release at 720 minutes	91
5.9	OPTIMIZATION OF FORMULATION	94
5.9.1	Pre-compression parameters of the optimized batch	97
5.9.2	Post compression parameters of the optimized batch	97
5.9.3	Buoyancy studies	98
5.9.4	Dissolution studies	99
5.10	CONCLUSION	100
CHAPTER 6: CONCLUSSIONS AND RECOMMENDATIONS		
6.1	FORMULATION DEVELOPMENT	101

6.2 RECOMMENDATIONS 103

REFERENCES 104

LIST OF ABBREVIATIONS

LOD	-	Loss on drying
PVP	-	Polyvinylpyrrolidone
HPMC	-	Hydroxypropylmethyl cellulose
DSC	-	Differential Scanning Calorimetry
ANOVA	-	Analysis of variance
Methocel™	-	Methylcellulose
USP	-	United States Pharmacopeia
BP	-	British Pharmacopeia
MCC	-	Medicine Control Council

LIST OF ANNEXURES

		PAGE
ANNEXURE A	STANDARD CALIBRATION CURVE	125

LIST OFTABLES

		PAGE
Table 2.1	Oral antifungal agents for the treatment of dermatophytosis	11
Table 2.2	The Biopharmaceutics Classification System	19
Table 3.1	Phases of migrating myoelectric complex (MMC)	27
Table 3.2	Marketed preparations of Gastro retentive technologies	50
Table 4.1	Effect of Carr's index and Hausner ratio on flow properties	60
Table 4.2	Comparison between angle of repose and flow properties of solids	61
Table 4.3	Specification for weight variation of solid dosage forms	62
Table 5.1	Factors and levels for factorial design	72
Table 5.2	Composition of griseofulvin floating tablet formulations generated by Design Expert	73
Table 5.3	Pre-compression parameters of griseofulvin tablets formulation runs	74
Table 5.4	Post compression parameters of griseofulvin tablets formulation runs	76
Table 5.5	Assay of griseofulvin tablets	78
Table 5.6	Buoyancy capabilities of griseoufulvin tablets formulation runs	79
Table 5.7	Composition of optimized formulation	97
Table 5.8	Pre-compression parameters of optimized griseofulvin floating tablets	97
Table 5.9	Post compression parameters of optimized griseofulvin floating tablets	98
Table A1	Calibration data of griseofulvin at 296 nm	125

LIST OF FIGURES

Figure 2.1	The schematic route of dermatophytes entry into the host system and onset of immune response	8
Figure 2.2	Classical ringed lesions caused by dermatophytes	9
Figure 2.3	Chemical structure of Griseofulvin	12
Figure 2.4	Proposed molecular mechanism(s) of griseofulvin in Cx43-dependent apoptosis of tumor cells	14
Figure 2.5	Mechanism of lipid digestion and drug solubilisation in postprandial intestine	17
Figure 3.1	Diagrammatic presentation of the alimentary canal	22
Figure 3.2	Anatomy of the stomach	25
Figure 3.3	Motility pattern in gastro-intestinal tract (GIT)	26
Figure 3.4	Bio-adhesion system	35
Figure 3.5	Swellable tablet in the stomach	36
Figure 3.6	Modified swelling system	37
Figure 3.7	High density systems	38
Figure 3.8	Generations of super porous hydrogels	41
Figure 3.9	Floating system	42
Figure 3.10	Hydrodynamically balanced system	43
Figure 3.11	Floating drug delivery system with microporous membrane and floatation chamber	44
Figure 3.12	Cross-section of single and twin screw extruder barrel	46
Figure 3.13	Hollow microspheres	47
Figure 3.14	Volatile liquid containing system	48
Figure 3.15	Principle mechanism of floating by carbon dioxide gas releasing method	49
Figure 4.1	Processing flow for manufacturing of griseofulvin floating matrix tablets	57
Figure 5.1	Differential scanning calorimetry thermogram of griseofulvin	67
Figure 5.2	Thermograms of griseofulvin, Accurel MP and 1:1 (w/w) griseofulvin / Accurel MP mixture	68

Figure 5.3	Thermograms of griseofulvin, Methocel™ and 1:1 (w/w) griseofulvin / Methocel™ mixture	69
Figure 5.4	Thermograms of griseofulvin, magnesium stearate and 1:1 (w/w) griseofulvin / magnesium mixture	70
Figure 5.5	Thermograms of griseofulvin, PVP-k30 and 1:1 (w/w) griseofulvin / PVP-k30 mixture	71
Figure 5.6a	Statistical analysis of model of fit for lag time using design expert	81
Figure 5.6b	Statistical analysis of model of fit for floating time using design expert	81
Figure 5.7	Analysis of variance for the influence of factors on lag time	82
Figure 5.8	Analysis of variance for the influence of factors on floating time	83
Figure 5.9	Influence of Accurel MP on lag time and total floating time	85
Figure 5.10a	*In-vitro* drug release profile of the formulation runs with 25% Methocel™	85
Figure 5.10b	*In-vitro* drug release profile of the formulation runs with 50 to 75% Methocel™	86
Figure 5.10c	*In-vitro* drug release profile of the formulation runs with100% Methocel™	86
Figure 5.11	Statistical analysis of model fit using design expert for drug release up to 120 minutes	87
Figure 5.12	Analysis of variance for the influence of factors on drug release up to 120 minutes	88
Figure 5.13	Relationship between each factor and griseofulvin release up to 120 minutes	89
Figure 5.14	Combined effect of Methocel™ and Accurel MP on initial release of griseofulvin	91

Figure 5.15	Statistical analysis of model fit using design expert for drug release up to 720 minutes	91
Figure 5.16	Analysis of variance for the influence of factors on drug release up to 720 minutes	92
Figure 5.17	Effects of the selected factors on final drug release	93
Figure 5.18	Combined effect of Methocel™ and Accurel MP on the final release of griseofulvin	94
Figure 5.19	Optimization using Design Expert Software	95
Figure 5.20	Optimized formula chosen by Design expert Software	96
Figure 5.21	Tablets assessed for buoyancy capabilities	99
Figure 5.22	In-vitro drug release profile of the optimized batch	99
Figure A1	Calibration curve of griseofulvin at 296 nm	125

CONFERENCE PRESENTATIONS

J.T. Chanyandura, M. Poka, P.H. Demana Formulation and evaluation of gastro-retentive floating tablets of griseofulvin. *All Africa Congress on Pharmacology and Pharmacy*. (2016). Misty Hills Hotel and Conference Centre, Muldesdrift, Johannesburg, South Africa (Podium presentation)

J.T. Chanyandura, M.S. Poka, P.H. Demana. Formulation and evaluation of gastro-retentive floating tablets of griseofulvin. *1st Faculty of Health Sciences Research Day, (2016),* University of Limpopo, Turfloop, South Africa. (Podium presentation)

J.T. Chanyandura, M.S. Poka, Formulation and evaluation of gastro-retentive floating tablets of griseofulvin. *2nd School of Health Sciences Research Day, (2015),* University of Limpopo, Turfloop, South Africa. (Poster presentation)

CHAPTER 1

INTRODUCTION

1.1 BACKGROUND AND JUSTIFICATION

Administration of drugs by oral route is one of the important methods to impart systemic effects (Sravani *et al*., 2012: 23). This route is cheaper to use than other routes such as the parenteral and rectal routes, the patient can easily administer medication by themselves, it is pain free and absorption takes place along the whole length of the gastro-intestinal tract (Verma *et al*., 2010: 54). However, this route has disadvantages which include the possibilities of irregular absorption of drugs which can be caused by interaction of drugs with other contents of the gastro-intestinal tract, large particle size of the drug, decreased lipid-water solubility of the drug and the short residence time of the drug in the gastro-intestinal tract. There has been efforts to develop controlled-release drug delivery systems which release drugs at predetermined, predictable and controlled rate, thereby allowing for enhanced drug absorption (Narang, 2011: 1).

Controlled-release systems control or limit the entry of drugs into the blood stream. Most controlled-release dosage forms contain drugs that are easily absorbed throughout the entire gastrointestinal tract. However, some drugs tend to be absorbed in specific areas, principally due to their low permeability or solubility in the intestinal tract, their chemical instability, the binding of the drug to the gut contents, as well as to the degradation of the drug by microorganisms present in the colon (Upadhyay *et al*., 2014: 432). The past three decades have seen the pursuit and exploration of gastro-retentive dosage forms. These are the systems that retain in the stomach for a sufficient time interval against all the physiological barriers and release active moiety in a controlled manner (Foda, 2011: 94). Gastric retention provides advantages such as the delivery of drugs with narrow absorption windows in the small intestinal region. Also, longer residence time in the stomach is advantageous for local action in the upper part of the small intestine (Kumar *et al*., 2013: 150).

Griseofulvin is an antibiotic fungistatic drug used in the treatment of dermatophyte and ringworm infections (Medical Economics Co., 1999a). It has anti-mitotic properties, owing to its interference with the normal polymerization of microtubule protein (Rebacz *et al*., 2007: 6345). Griseofulvin is a metabolic product of *Penicilium grisofulvum* (Jiang *et al*., 2012: 973). It is a white to pale cream odourless or almost odourless tasteless powder (BP, 2014: I-1085). Griseofulvin is very slightly soluble in water (0.2 g/L at 25 ºC), sparingly soluble in ethanol and methanol, soluble in acetone, chloroform and dimethylformamide (BP, 2014: I-1085). Despite its use for dermatophytes infections, griseofulvin is administered only orally due to its poor penetration of the skin. The absorption of griseofulvin from the gastro-intestinal tract is highly variable and continues over a prolonged period of time (Merisko-Liversidge *et al*., 2003: 117). About 10-50% of a dose of griseofulvin is excreted almost exclusively as metabolites in the urine and the remainder in the faeces for about four to five days after administration. Plasma levels of griseofulvin peak in about four hours after administration and it has an elimination half-life of about twenty-four hours (Merck manual, 2010).

To enhance the solubility and absorption of griseofulvin in pharmaceutical preparations, it is normally mixed with a non-toxic, water soluble polymer such as polyvinylpyrrolidone or hydroxypropyl cellulose and spray-dried before treatment with a wetting agent such as sodium lauryl sulphate or benzalkonium chloride. The resulting material is characterized as ‘microsized’ crystals of griseofulvin (Desai & Soon-Shiong, 2003: 1). This has influenced griseofulvin to be commercially available as tablets containing 250 mg or 500 mg microsize or 125 mg or 165 mg ultramicrosize crystals of griseofulvin. It is also available as capsules containing 250 mg microsize griseofulvin and as an oral suspension containing 125 mg/5 ml microsize griseofulvin (Medical Economics Co, 1999a). The various dosage forms described above are ways of maximizing the oral delivery, release and absorption of griseofulvin. At present there are no gastro-retentive floating tablets of griseofulvin available both on the national and international market.

A number of gastro-retentive drug delivery approaches are being designed. These include high density sinking systems that are retained in the bottom of the stomach, floating systems, muco-adhesive systems that cause bioadhesion to stomach mucosa, swellable, expandable or unfoldable systems which limit emptying of the dosage forms through the pyloric sphincter of stomach, superporous hydrogel systems and magnetic systems. In particular, floating drug delivery systems (FDDS) are aimed to retain drugs in the stomach and are useful for drugs that are poorly soluble or unstable in intestinal fluids (Geetha *et al*., 2012: 1). These systems have a bulk density less than that of gastric fluids and so remain buoyant in the stomach without affecting the gastric emptying rate for a prolonged period of time. While the system floats on the gastric contents, the drug is released slowly at a desired rate (Shaha *et al*., 2009: 66).

Effervescent systems are prepared with swellable polymers and an effervescent component containing sodium carbonate, citric acid and/or tartaric acid is usually included. Upon contact of the system with gastric fluid, carbon dioxide is liberated by the acidity of gastric contents and is entrapped in the gelyfied hydrocolloid. This produces an upward motion of the dosage form and maintains buoyancy (Geetha *et al*., 2012: 3).

The concept of floating tablets is mainly based on the matrix type drug delivery system. Matrix based tablet formulations are popular and easy to formulate on a commercial scale in industry (Kar *et al*., 2009: 54). Preparation of matrix tablets involves either direct compression of blends of drug, retardant material, effervescent component or retardant drug blends which are granulated prior to compression. Matrix devices consist of a drug dispersed homogenously throughout a polymer matrix. The use of simple formulation techniques to develop a cost effective floating drug delivery system for griseofulvin is significant in developing countries like South Africa. It leads to improved drug utilization, improved health and production of cost-effective affordable medicine. In the study, floating matrix tablets were formulated and evaluated for the retention and sustained release of griseofulvin.

1.2 RESEARCH PROBLEM

When drugs intended for systemic use are administered orally, it is desirable that they are adequately absorbed and reach their site of action at a particular concentration to effect a pharmacological action. It is of paramount importance to know the pharmacokinetic properties of drugs. The Biopharmaceutics Classification System (BCS) is a system that was developed to a group of drugs based on the three major factors governing bioavailability i.e dissolution, solubility and permeability (Gothoskar & Khangaonkar, 2005).

Griseofulvin falls into BCS class two, a class that contains drugs that are problematic for effective oral administration. These drugs have low solubility and low permeability, and are usually not well absorbed through the intestinal mucosa and a high variability is expected (Reddy & Karunakar, 2011: 31). About 50% of a dose of griseofulvin passes the gastro-intestinal tract unabsorbed and is excreted in faeces (Merck Manual, 2010). This is quite predictable with immediate release tablets where the short residence time of the low soluble griseofulvin, in stomach and small intestine, limits its dissolution. A floating matrix system could be necessary to target the slow release of griseofulvin in the stomach in order to extend its dissolution to ultimately improve its absorption into the body system.

The research question in this study therefore was: could a floating matrix system sufficiently sustain the release of griseofulvin in the stomach?

1.3 AIM

The aim of this study was to formulate, evaluate and optimize gastro-retentive floating griseofulvin tablets.

1.4 OBJECTIVES

The aim of this study was achieved by setting the following objectives:

- To investigate the compatibility of griseofulvin and excipients to be used

- To develop floating griseofulvin matrix tablets
- To characterize the physical properties and quality of the tablets
- To determine the buoyancy capabilities of the floating drug delivery system
- To evaluate and optimize the *in-vitro* release properties of griseofulvin from the floating tablets
- To determine the stability of the optimized formulation under accelerated stability conditions.

CHAPTER 2

DERMATOPHYTE INFECTIONS

2.1 INTRODUCTION

Chapter two dwells on literature review. It gives an overview of dermatophyte infections. Further, it expounds on the properties and characteristic of griseofulvin (such as pharmacokinetics and pharmacodynamics), the standard treatment for dermatophytosis in South Africa.

2.2 DERMATOPHYTES

Dermatophytes are a group of morphologically and physiologically related fungi that have the capacity to invade keratinized tissue such as skin, hair and nails of humans and other animals (Shrivastav *et al.,* 2013: 2136). These fungi belong to the genera Microsporum, Trichophyton and Epidermophyton. Members of Microsporum and Trichophyton cause illness in both humans and animals (Merck Manual, 2006). Dermatophytosis, the disease caused by these fungi, is common worldwide (Outerbridge, 2006: 128) and has veterinary and public health relevance (Cafarchia *et al.,* 2012: 396). Dermatophytes affect approximately 20-25% of the world's population and are responsible for 30% of all skin fungal infections. In addition, it is considered to be the third most common skin disorder in adults (Venturini *et al.,* 2012: 1144). The distribution of these fungi varies considerably, depending on geographical area of provenience and other epidemiological factors such as age, sex, seasons, etc (Iwen *et al.,* 2002: 96).

2.2.1 Infection mechanism of dermatophytes

Dermatophyte infection in humans occurs through contact with contaminated products or specimens, such as soil, hair, or animal epidermal scales (pets, work animals, or fur farming), as well as frequent contact with affected individuals (Mendez-Tovar, 2010: 185). This contact can be with family members, in work situations (people who live in dormitories or barracks), or by sharing personal belongings such as combs, shoes, or clothing. Predisposing factors include young

age, immunosuppression, nutritional deficits, high temperature and humidity (DeBoer *et al.,* 2002: 1532). Any skin trauma resulting from increased moisture, injury by ectoparasites or scratches due to pruritus, playing or aggressive behaviour and clipping is important for facilitating infection. Owing to anatomical or environmental factors, some areas of the body are more susceptible to the development of dermatophyte infections. The fungi under the distal nail area remains in contact with the skin and nails for several hours or even days. This allows for the growth and eventual invasion of the keratin layers of the nail. The spaces between the fingers, where factors such as sweat, maceration and alkaline pH are present, provide the proper environment for the development of fungal diseases (Mendez-Tovar, 2010: 185).

2.2.2 Dermatophytes' adhesion to and invasion of human superficial skin tissues

Dermatophytes require keratin for growth and therefore infect hair, nails and superficial skin, all of which are rich in keratin. These organisms do not usually invade resting hairs since the essential nutrients they need for growth are absent or limited. Hyphae spread in hair and keratinized skin, and ultimately develop into infectious arthrospore (Microsporum, 2005: 1). In *in-vitro* models, *Trichophyton mentagrophytes* has been shown to require approximately 6 hours to adhere firmly, even though germination begins after 4 hours. In other models using layers of fingernail keratin, adherence occurred after 6 hours and germination of the conidia and branching after 16 hours. Other experiments using skin cross sections have shown that 12 hours is necessary for adherence, 16 hours for germination, and 72 hours for invasion of the stratum corneum (Vermout et al., 2008: 268).

2.2.3 Dermatophytes' growth on keratinized tissue

Dermatophytes are provided with an arsenal of enzymes (keratinases, metallproteases, and serine proteases) aimed at the digestion of the keratin network into assimilable oligopeptides or amino acids (Scazzocchio, 2000: 126). The production of these enzymes is induced by the substrate on which they develop. Dermatophytes normally form only asexual spores that develop within the

hyphae. However, dermatophytes can also produce asexual spores that develop outside the hyphae.

2.2.4 Human immune response to dermatophyte infection

Infections by dermatophytes generally induce both immediate hypersensitivity as well as cell mediated or delayed type hypersensitivity. The immune response is characterized by the action of macrophages as effector cells and by some key cytokines like interferon-y (IFN-y). The immune response that is raised, and especially the degree of inflammation, varies according to the dermatophyte species, to the host species, and to the pathophysiological status of the host. Two factors account for the differences in the degree of immunologic response. The first factor is the type of metabolites and enzymes released by the agent: the more foreign they are, the greater the molecular weight and complexity of the antigen they have, hence the immune response is more vigorous. The second factor is immunosuppression, caused by the metabolites in anthropophilic dermatophytes (Serrano-Gomez *et al.,* 2004: 5635). The schematic route of entry of dermatophytes into the host system and onset of immune response in the response to the pathogen entry is shown in Figure 2.1.

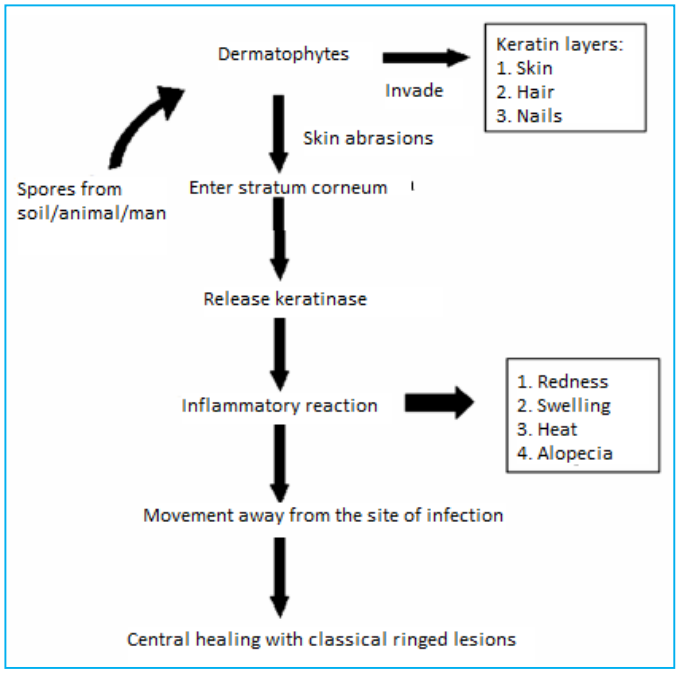


Figure 2.1: The schematic route of dermatophytes entry into the host system and onset of immune response (Lakshmipathy & Kannabiran, 2010: 727)

2.2.5 Signs and symptoms of dermatophytes infection

The customary signs of inflammatory reactions such as redness (rubor), swelling (induration), heat and alopecia (loss of hair) are seen at the infection site. Inflammation causes the pathogen to move away from the site of infection and take residence at a new site. This movement of the organism away from the infection site produces the classical ringed lesion as shown in Figure 2.2 (Degreef 2008: 258). Most often, there is little or no inflammation; asymptomatic or mildly itching lesions with a scaling, slightly raised border remit and recur intermittently. Occasionally, inflammation is more severe and manifests as sudden vesicular or bullous disease (usually of the foot) or as an inflamed boggy lesion of the scalp (kerion) (Merck Manual, 2006).

The infections caused by dermatophytes are commonly referred to as “tinea” or “ring-worm” infections due to the characteristic ringed lesions (White *et al.,* 2008: 1238). Based on the site of infection the tinea infections are referred to as: tinea capitis (scalp); tinea corporis (body); tinea cruris (groin); tinea pedis (feet); tinea manuum (hands); tinea barbae (affecting the beard areain males); tinea faciei (face); and tinea unguium (nails) (Gupta *et al.,* 2008: 355).

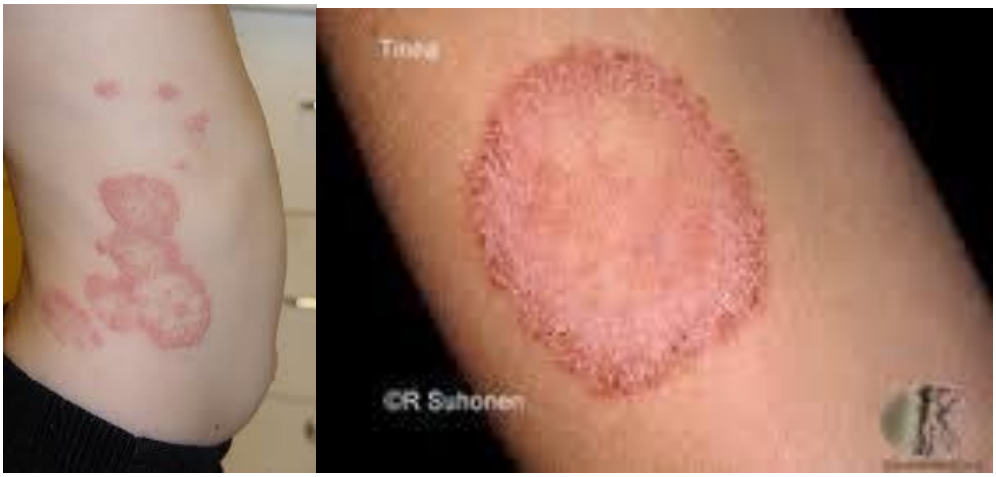


Figure 2.2: Classical ringed lesions caused by dermatophytes (Degreef, 2008: 258)

2.2.6 Diagnosis of dermatophytes infection

Diagnosis of dermatophyte infection is based on clinical appearance and site of infection and confirmed by skin scrapings and demonstration of hyphae on potassium hydroxide wet mount. Identification of specific organisms by culture is unnecessary except in cases of scalp infection (where an animal source may be identified and treated) and nail infection (which may be caused by a nondermatophyte). Culture may also be useful when overlying inflammation and bacterial infections are severe and/or accompanied by alopecia. Differential diagnosis includes folliculitis decalvans, bacterial pyodermas, and entities that cause scarring alopecia, such as discoid lupus, lichen planopilaris, and pseudopelade (Merck Manual, 2006).

2.2.7 Treatment of dermatophytes infection

Treatment of dermatophyte infection involves primarily oral and/or topical formulations (Gupta *et al.,* 2008: 361). In the last 50 years numerous drugs have been introduced for the treatment of superficial infections. The choice of treatment is determined by the site and extent of the infection, the species involved as well as by the efficacy and safety profile, and kinetics of the drugs available. For localised non-extensive lesions caused by dermatophytes topical therapies with an imidazole, allylamines, tolnaftate, morphine derivatives and others are generally used (Del Palacio *et al.,* 2000: 150).

The use of oral antifungals is effective where the tinea involvement is extensive or chronic, or where application of a topical is not feasible. For tinea unguium (onchomycosis) and tinea capitis, oral therapies are the primary treatments provided. The oral compounds with the potential for treating dermatophytosis are shown in table 2.1.

Table 2.1: Oral antifungal agents for the treatment of dermatophytosis (Del Palacio *et al.,* 2000: 150)

Class	**Drug**
Antibiotics	Griseofulvin, Amphoterecin B Natamycin
Azoles	Ketoconazole, Econazole,iconazole, Oxiconazole
Triazoles	Itraconazole, Fluconazole, Voriconazole
Allylamines	Terbinafine,
Antimetabolites	5-Fluorocytosine (5-FC)
Other topical agents	Tolnaftate, Undecylenic acid, Benzoic acid, Quiniodochlor, Ciclopirox olamine, Sod. thiosulfate.

Griseofulvin is still currently the gold standard for treatment of dermatophytosis (excluding tinea unguium) since being introduced clinically in the sixties following the observations of Gentles (Del Palacio *et al.,* 2000: 150).

2.3 GRISEOFULVIN

Griseofulvin is a metabolic product of *Penicilium griseofulvum* (Wen-xue *et al.,* 2012: 973). It is a white to pale cream odourless or almost odourless tasteless powder (BP, 2014: I-1085).

2.3.1 Drug identity

Primary Name: Griseofulvin

IUPAC Systemic Name: 7-Chloro-2',4,6-trimethoxy-6'β-methylspiro[benzo-furan-2(3H),1'-[cyclohexene]-3,4'-dione

CAS Reg. no: 126-07-8

Chemical structure:

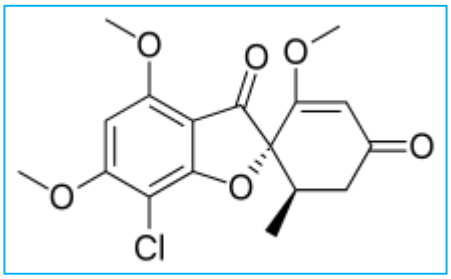


Figure 2.3: Chemical structure of Griseofulvin (Pradhan *et al.,* 2011: 68)

Molecular Formula	$C_{17}H_{17}ClO_6$
Molecular Weight	352.77
Solubility	Griseofulvin is very slightly soluble in water (0.2 g/L at 25 ºC); sparingly soluble in ethanol and methanol; soluble in acetone, chloroform and dimethylformamide (BP, 2014: I-1085).
Log P	2.18
Melting range	217 ºC – 224 ºC (USP 2014: 3196)
Identification	A 5 mg sample is dissolved in 1 ml of sulphuric acid and 5 mg of powdered potassium dichromate is added. A wine red colour is produced.
Sources	Griseofulvin is typically produced by the growth of certain strains of *Penicillium griseofulvum* (BP, 2014). A method for the synthesis of griseofulvin from dimethoxyphenol has been reported (Brä se *et al.,* 2009: 3974).

2.3.2 Pharmacological action

Griseofulvin is a systemic antifungal agent that is effective against the common dermatophytes. Griseofulvin is fungistatic rather than fungicidal, except in young active cells (Merck manual).

2.3.3 Mycology

Griseofulvin has antifungal activity against the following dermatophytes, although there is species and strain variability in susceptibility.

A: *Trichophyton rubrum, T. tonsurans, T. mentagrophytes, T. interdigitalis, T. verrucosum, T. megnini, T. gallinae, T. crateriform, T. sulphureum and T. schoenleinii*

B: *Microsporum audouinii, M. canis, M. gypseum*

C: *Epidermophyton floccosum*

Griseofulvin has no activity against dermatophyte fungi of other genera, non-dermatophyte fungi, yeasts, gram positive bacteria, or gram negative bacteria. If any of these are cofactors in the pathology of infection, suitable additional therapy will be required for their eradication (Brä se *et al.,* 2009: 3974).

2.3.4 Mechanism of action

Dermatophytes concentrate griseofulvin by an energy-dependent process. Griseofulvin then disrupts the mitotic spindle by interacting with the polymerized microtubules in susceptible dermatophytes. This leads to the production of multinucleate fungal cells. The inhibition of nucleic acid synthesis and the formation of hyphal cell wall material may also be involved. The result is distortion, irregular swelling, and spiral curling of the hyphae (Merck manual, 2010).

2.3.5 Griseofulvin potential as therapeutics for cancer

Interestingly, griseofulvin was long ago found to reduce the number of tumor cells induced by croton oil in mice (Bladt *et al.*, 2013: 11342) and to inhibit, alone or associated with other anticancer drugs, *in-vitro* proliferation of cancer cell lines (Panda et al., 2005: 9883). It has been shown that in addition to its classical effect on microtubule stability, griseofulvin is able to induce the expression of connexin 43 (Cx43), a tumor-suppressor gene known to participate in apoptosis regulation (Mauro *et al.,* 2013: 481). Griseofulvin also stimulates the caspase-dependent cell

apoptosis in human germ cell tumor cells. Griseofulvin that enters into the cell could: (i) reduce the association between Cx43 and microtubules by altering microtubule function; (ii) enhance Cx43 translocation or a part of protein within the nucleus; (iii) control gene expression conducing to activation of the mitochondrial caspase pathway leading to apoptosis, as shown in Figure 2.4.

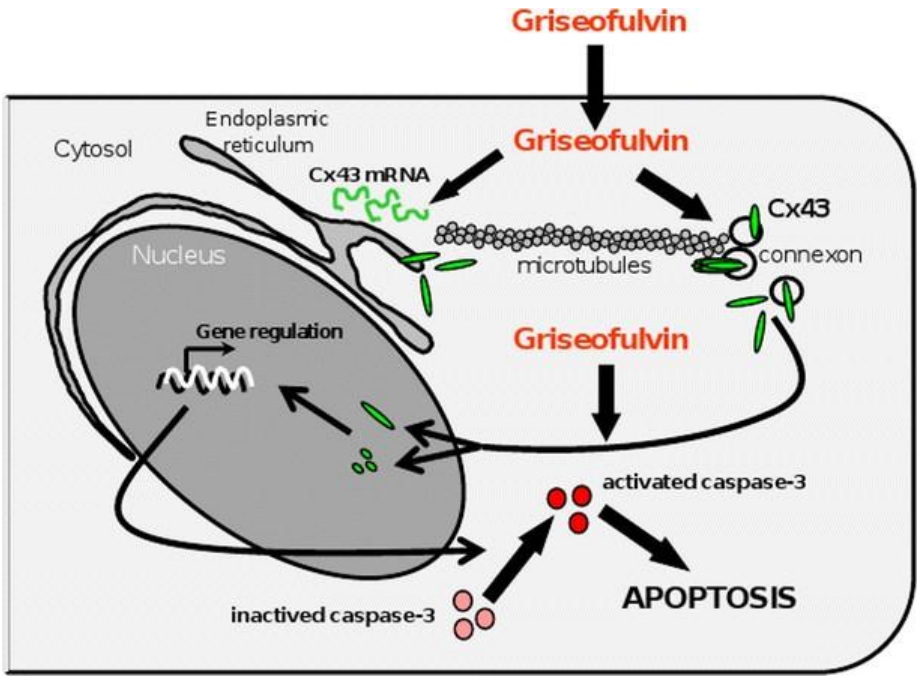


Figure 2.4: Proposed molecular mechanism(s) of griseofulvin in Cx43-dependent apoptosis of tumor cells (Mauro et al., 2013: 489).

2.3.6 Pharmacokinetic properties

The pharmacokinetic properties of griseofulvin are divided into absorption, distribution, metabolism and excretion.

2.3.6.1 Absorption

Griseofulvin is absorbed in the first part of the small intestine. Its absorption continues over a prolonged period and is variable and incomplete (Merck manual, 2010). Griseofulvin has good absorbability in rat intestine if it is dissolved, supporting that the permeability of griseofulvin is also good in humans (Persson *et al.,* 2005: 2141). The rates of disaggregation and dissolution in the gastro-intestinal tract limit the bioavailability of griseofulvin. On average, less than 50% of the oral

dose is absorbed. High-fat meals, margarine, or propylene glycol significantly enhance gastro-intestinal absorption of griseofulvin. A reduction in particle size will increase the rate and extent of the absorption. Following oral administration there is a phase of rapid absorption, and thereafter a phase of slower prolonged absorption (Persson *et al.,* 2005: 2141).

The dose of griseofulvin depends on preparation used. In general, microcrystalline preparations are given in a dose of 500 to 1000 mg/day depending on body weight. In children, a dose of 10 mg/kg per day is generally used. With ultramicronized preparation, dosage may range between one half and two thirds of the microsized variety (Lee *et al.,* 2007: 551). Peak plasma levels, 0.5 µg/ ml – 1.5 µg/ ml after a 500 mg dose, and 1.5 µg/ ml – 3.0 µg/ ml after a 1000 mg dose, are reached in 2-4 hours, and are maintained for some 10-20 hours (Lee *et al.,* 2007: 551).

2.3.6.2 Distribution

The volume of distribution is about 0.7 L/Kg, and griseofulvin is 80% bound to plasma proteins, predominantly serum albumin. Griseofulvin is deposited in keratin precursor cells within 4-8 hours of administration per oral. Sweat and transdermal fluid loss appear to play an important role in griseofulvin transfer in the stratum corneum. When these cells differentiate, griseofulvin remains bound and persists in keratin, making it resistant to fungal invasion. For this reason, new growth of hair, nails, or horn is the first to become free of fungal infection. As fungus-containing keratin is shed, it is replaced by normal skin and hair. Only a small fraction of a dose of griseofulvin remains in the body fluids or tissues (Merck manual, 2010).

Griseofulvin crosses the placenta, and may be excreted in breast milk. There is selective deposition of griseofulvin in newly formed keratin of hair, skin, and nails, which gradually moves to the surface of these appendages (Merck manual, 2010).

2.3.6.3 Metabolism

Griseofulvin is oxidatively demethylated to its inactive metabolites, 6-desmethylgriseofulvin and conjugated with glucuronic acid, principally in the liver. The major metabolite, 6-desmethylgriseofulvin, is microbiologically inactive (Chooi *et al.,* 2010: 190).

2.3.6.4 Excretion

The terminal plasma half-life of griseofulvin ranges from 9.5 - 21 hours, with considerable intersubject variability. The majority of the dose, as 6-desmethylgriseofulvin or the glucuronide conjugate, and other metabolites is excreted in the urine, with less than 1% administered dose being excreted as unchanged griseofulvin. The remainder of the dose, principally as metabolites, is excreted in bile and faeces. Renal insufficiency does not lead to accumulation (Lee *et al.,* 2007: 552).

2.3.7 Drug-drug interactions

Lipids increase the gastrointestinal absorption of griseofulvin. Because of this, it is normally recommended that griseofulvin be co-administered with a fatty meal. Barbiturates decrease the absorption and antifungal activity of griseofulvin (Merck manual, 2005). Griseofulvin is a microsomal enzyme inducer and may depress plasma levels, and therefore the efficacy, of concomitantly administered medicinal products that are metabolised by cytochrome P450 3A4 (Persson *et al.,* 2005: 2141). The combined use of ketoconazole and griseofulvin may lead to hepatotoxicity (Merck manual, 2005).

2.3.8 Drug-food interactions

The constituents of the mixed micellar phase in lipid digestion and absorption impact on the intestinal permeability of poorly water-soluble drugs via three major processes. First, lipid digestion products and bile salts characteristic of the fed state may alter the intrinsic permeability of the intestinal membrane leading to increased penetration via paracellular or transcellular routes. Second,

solubilisation of poorly water-soluble drugs within bile salt micelles may facilitate diffusion through the unstirred water layer leading to increased absorption. Third, and conversely, solubilisation may decrease the intermicellar "free" fraction of drug that could lead to a decrease in absorption (Porter *et al.,* 2008: 678). Poorly water-soluble drugs that are co-administered with either food or lipid-based formulations can benefit from the processed involved with lipid digestion through enhanced wetting and solubilisation facilitated by the conditions present in the postprandial intestine, as shown in Figure 2.5.

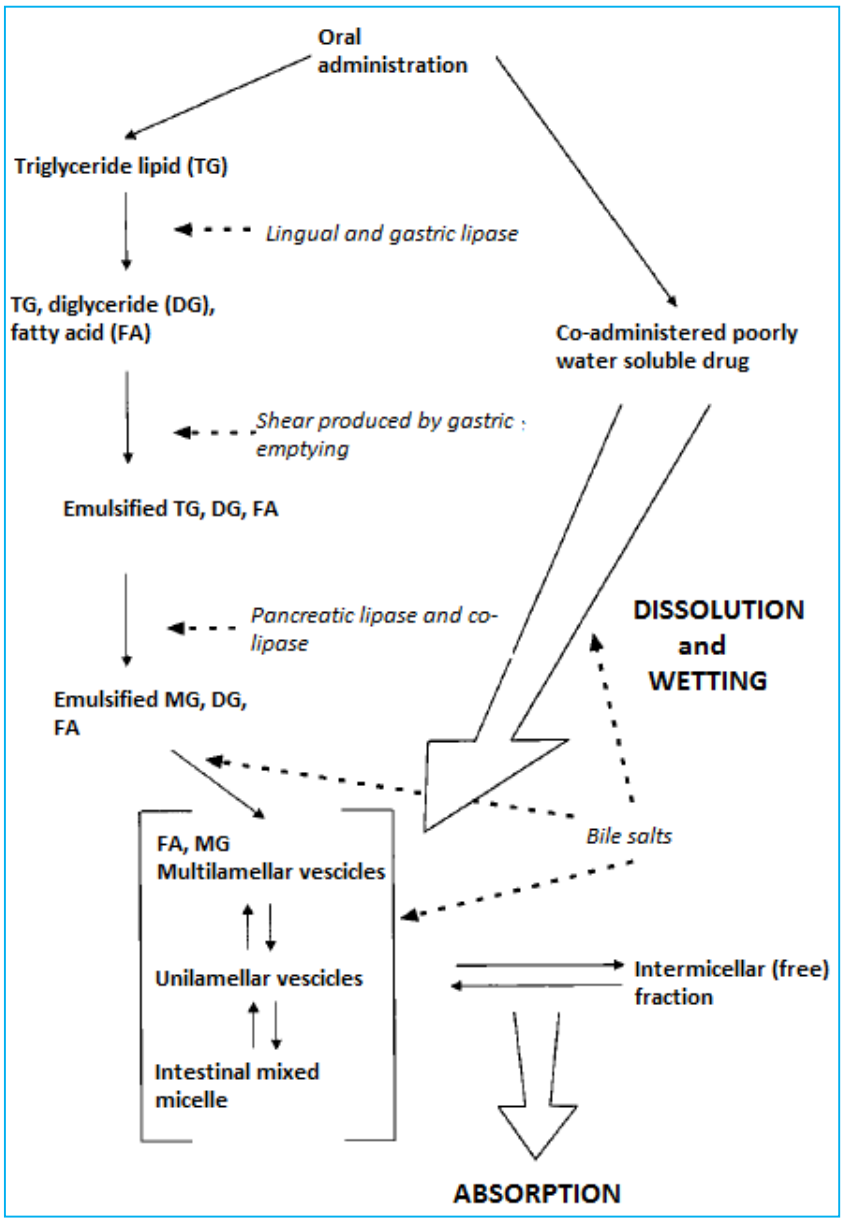


Figure 2.5: Mechanism of lipid digestion and drug solubilisation in postprandial intestine (Porter *et al.,* 2008: 676)

The enhanced solubility and dissolution of griseofulvin in supramicellar concentrations of bile salts is the primary basis for its enhanced absorption (Persson *et al.,* 2005: 2141).

2.3.9 Contraindications

Griseofulvin is contraindicated in patients who have:

- Hypersensitivity to griseofulvin or to any of the excipients
- Porphyria
- Severe hepatic impairment
- Systemic lupus erythematosus
- Pregnancy
- Breastfeeding

2.3.10 Adverse effects

Common:

- Mild and transient headaches
- Skin rashes
- Gastrointestinal effects
- Altered taste sensation
- Dry mouth

Uncommon:

- Severe allergic reactions (angio-oedema)
- Severe dermatoses (Stevens-Johnson syndrome, systemic lupus erythematosus, exfoliative dermatitis, photosensitivity)
- Blood dyscrasias (leucopenia)
- CNS symptoms (dizziness, paraesthesia, severe headache, confusion, impaired judgement, depression, irritability)
- Fatigue
- Proteinuria
- Hepatotoxicity
- Candidiasis (Rossiter, 2016: 83)

2.4 BIOPHARMACEUTICS CLASSIFICATION OF GRISEOFULVIN

The Biopharmaceutics Classification System (BCS) is a scientific framework that was developed to group of drugs based on the three major factors governing bioavailability i.e dissolution, solubility and permeability (Gothoskar & Khangaonkar, 2005). It allows for the prediction of *in-vivo* pharmacokinetics of oral immediate-release drug products by classifying drug compounds into four classes (Table 2.2 below) based on their solubility related to dose and intestinal permeability in combination with the dissolution properties of the dosage form (Gothoskar & Khangaonkar, 2005). The objectives of the BSC are:

- To improve the efficiency of the drug development and review process by recommending a strategy for identifying expendable clinical bioequivalence test.
- To recommend a class of immediate-release solid oral dosage forms for which bioequivalence may be assessed based on *in-vitro* dissolution tests.
- To recommend methods for classification according to dosage form dissolution along with the solubility-permeability characteristics of the drug product.

Table 2.2: The Biopharmaceutics Classification System (Reddy & Karunakar, 2011: 33)

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

Griseofulvin falls into BCS class II (Fujioka *et al.,* 2008: 38). It is poorly water-soluble, and displays a dissolution rate-limited absorption pattern in humans and animals (Wei *et al.,* 2008: 103). The results of the experiments done by Yamamoto revealed that higher dissolution rate of griseofulvin from a ground mixture with microcrystalline cellulose is associated with higher bioavailability (Fujioka *et al.,*

2008: 37). This observation gives additional evidence for the proposition that the dissolution process is the rate-limiting step in the absorption of griseofulvin. The limited solubility of griseofulvin in water causes its incomplete absorption after oral administration (Rasenack & Müller, 2004: 5).

Many drugs, including griseofulvin, exhibit poor solubility in water, and absorption in the gastrointestinal tract is very low, because of the particles' big size (Hu *et al.,* 2003: 1725). The dissolution rate is directly proportional to the surface area of the drug, which in turn increases with decreasing particle size (Horter & Dressman, 2001: 76). The main emphasis for solubility enhancement till to date in the pharmaceutical fraternity relies on particle size reduction, as reflected by Ostwald-Frenendlich equation (Jambhrunkar, 2014: 710).

Particle size reduction techniques such as comminution (crushing, grinding and milling), micronization and spray drying are conceived to be efficient, reproducible and economically viable. However, particle size reduction leads to induction of physical stress on drug particles, tendency to agglomerate on standing and its inapt for thermolabile drugs. Several delivery systems such as surfactant complexes, liposomes, hydrogels, and polymeric nanoparticles have been developed but suffer from synthesis complexity and poor biological stability. Several studies addressed the solubility and/or dissolution enhancement of griseofulvin using processes such as solid dispersions and, complexation with cyclodextrin, microemulsions and deformable membrane vesicles (Jambhrunkar, 2014: 709).

2.5 SUMMARY

Chapter two outlined dermatophyte infection in human beings. Furthermore, it highlighted dermatophyte infection treatment by griseofulvin as the drug of choice in South Africa. Information on the properties of griseofulvin was presented including its drawbacks.

CHAPTER 3

GASTRO-RETENTIVE DRUG DELIVERY SYSTEMS

3.1 INTRODUCTION

Chapter three gives literature review on gastro-retentive drug delivery systems. First, it gives an overview of the gastro-intestinal tract and drug delivery. It then expounds on the different types of gastro-retentive drug delivery systems and techniques available, their advantages and limitations. Furthermore, chapter three highlights the various technologies that have been exploited for the delivery of griseofulvin.

3.2 ORAL DRUG DELIVERY

Development in pharmaceutical technology has provided viable dosage alternatives that can be administered via different routes of administration. Various routes that are used include oral, parenteral, topical, nasal, rectal, vaginal and ocular (Bhardwaj *et al.,* 2011: 300). To date, oral administration is the most convenient and preferred route of any drug delivery to the systemic circulation (Kumar *et al.,* 2013: 150). This is because the oral route provides ease of administration, more flexibility in designing, ease of production and low cost (Bhardwaj *et al.,* 2011: 300). Approximately 50% of the drug delivery systems available in the market are oral drug delivery systems (Sarojini & Manavalan, 2012: 2).

3.3 THE GASTRO-INTESTINAL TRACT AND DRUG DELIVERY

Drugs administered orally pass through and are absorbed along the gastrointestinal tract (GIT). The GIT is essentially a tube of about nine metres that runs through the middle of the body from the mouth to the anus and includes the throat (pharynx), oesophagus, stomach, small intestine (consisting of the duodenum, jejunum and ileum) and large intestine (consisting of the cecum, appendix, colon and rectum) (Kumar *et al.,* 2013: 151), Figure 3.1. The GIT is a continuous muscular tube, which functions to take in nutrients and eliminate waste

by such physiological processes as secretion, motility, digestion, absorption and excretion. The walls of the GIT, from stomach to large intestine, have the same basic arrangement of tissues, the different layers, from outside to inside, comprising of serosa, longitudinal muscle, intermuscular plane, circular muscle, submucosa, muscularis mucosae, lamina propria and epithelium. The GIT presents a large surface area which is a perfect environment for the delivery and absorption of drugs (Zate *et al.,* 2010: 1229).

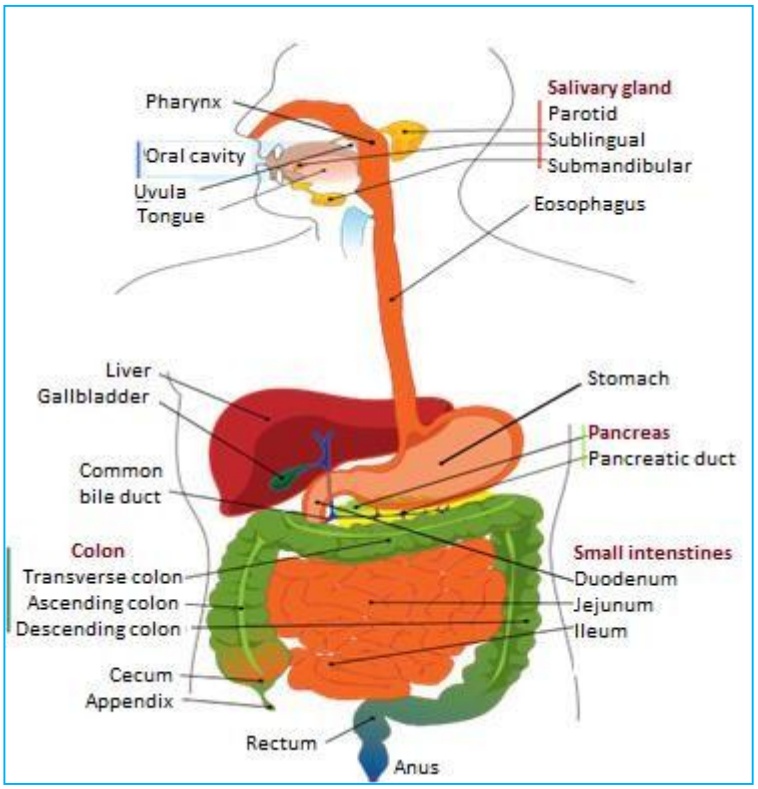


Figure 3.1: Diagrammatic presentation of the alimentary canal (Kumar *et al*., 2013: 152)

As drugs are administered orally or through any other route, some may provide effective treatment following the administration of a single dose. However, the duration of most illnesses, for example dermatophytosis, is longer than the therapeutic effect produced by the administration of a single dose of a drug in a conventional dosage form, i.e a dosage form which is formulated to give rapid and complete drug release (Collett & Moreton, 2007: 330). Such illnesses require multiple dosage regimens where doses of the drug are administered on a repetitive basis over a period of time determined by the nature of the illness. The achievement and maintenance of a concentration of a drug at the appropriate

site(s) of action which is both clinically efficacious and safe for the desired duration of treatment is then the aim of drug therapy. Proper selection of the dose size and the dosage time interval is crucial in ensuring that a multiple-dosage regimen provides steady-state concentrations of drug in the body which are both clinically efficacious and safe. The effects of dose size and frequency of administration are summarized as follows:

- The magnitude of the fluctuations between the maximum and minimum steady-state amounts of drug in the body is determined by the size of dose administered or, more accurately, by the amount of drug absorbed following each dose administered.
- The magnitude of the fluctuations between the maximum and minimum steady-state plasma concentrations is an important consideration for any drug that has a narrow therapeutic range. The more frequent administration of smaller doses is a means of reducing the steady-state fluctuations without altering the average steady-state plasma concentration.
- The average maximum and minimum amounts of drug achieved in the body at steady state are influenced by either the dose size, the dosage time interval in relation to the biological half-life of the drug, or both. The greater the dose size and the smaller the dosage time interval relative to the biological half-life of the drug, the greater are the average, maximum and minimum steady-state amounts of drug in the body. (Collett, 2007: 330).

Drugs that are easily absorbed from the gastrointestinal tract and have short half-lives are eliminated quickly from the systemic circulation. Frequent dosing of these drugs is required to achieve suitable therapeutic activity. However, frequent dosing of these conventional dosage forms has drawbacks which include:

- Poor patient compliance, increased chances of missing the dose of a drug with short half-life for which frequent administration is necessary.
- The unavoidable fluctuations of drug concentration may lead to under medication or over medication.
- A typical peak-valley plasma concentration time profile is obtained which makes attainment of steady-state condition difficult.

- The fluctuations in drug levels may lead to precipitation of adverse effects especially of a drug with small therapeutic index whenever over medication occurs. (Ratilal *et al.,* 2011: 1701).

To avoid this limitation, the development of oral sustained or controlled release delivery systems is an attempt to release the drug slowly into the gastrointestinal tract and maintain an effective drug concentration in the systemic circulation for a long time (Badoni *et al.,* 2012: 33). Past decades have further seen the development of targeted drug delivery systems which deliver drugs at specific sites of the GIT. Examples of such advancement include colon specific drug delivery system and gastro-retentive drug delivery systems. However, gastro-retentive delivery systems are more attractive than colon specific systems especially for drugs that are absorbed in the small intestines.

3.4 GASTRO-RETENTIVE DRUG DELIVERY SYSTEMS

Optimum gastro-retentive drug formulations can be defined as systems which are retained in the stomach for a sufficient time interval against all the physiological barriers, release active moiety in a controlled manner, ultimately being metabolized in the body (Foda & Ali, 2011: 94). Gastro-retentive systems can remain in the gastric region for several hours and hence significantly prolong the gastric residence time of drugs. Many drugs categorised as once-a-day delivery have been demonstrated to have suboptimal absorption due to dependence on the transit time of the dosage form, making traditional extended release development challenging (Kumar *et al.,* 2013: 150). Prolonged gastric retention therefore helps to improve bioavailability, reduce drug waste, and improve solubility of drugs that are less soluble in a high pH environment (Upadhyay *et al.,* 2014: 431).

3.4.1 Anatomical and Physiological aspects of Gastro-retentive drug delivery

The stomach is suitable for use as a ‘depot’ for sustained release dosage forms and an understanding of its anatomy and physiology helps in appreciating the mechanisms of gastro-retentive drug formulations. The stomach is a J-shaped

enlargement of the GIT which can be divided into four anatomical regions: cardia, fundus, body and antrum (pylorus), Figure 3.2. The main function of the stomach is to store and mix food with gastric secretions before emptying its load (chyme) through the pyloric sphincter and into the small intestine at controlled rate suitable for digestion and absorption (Zate *et al.,* 2010: 1229).

The proximal part of the stomach made of fundus and body acts as a reservoir for undigested material, whereas the antrum is the main part for mixing motions and acts as a pump for gastric emptying by propelling actions. In addition to longitudinal and circular muscle, the stomach has a third muscle layer known as the “oblique muscle layer”, which is situated in the proximal stomach, branching over the fundus and higher regions of the gastric body. The different smooth muscle layers are responsible for performing the motor functions of the GIT, i.e gastric emptying and intestinal transit. The stomach has a volume of about 1.5 litres after a meal and a range of 250-500 millilitres in inter-digestive phases. It produces 2 litres among the 8 litres of all liquid present in gastrointestinal tract (Upadhyay *et al.,* 2014: 435).

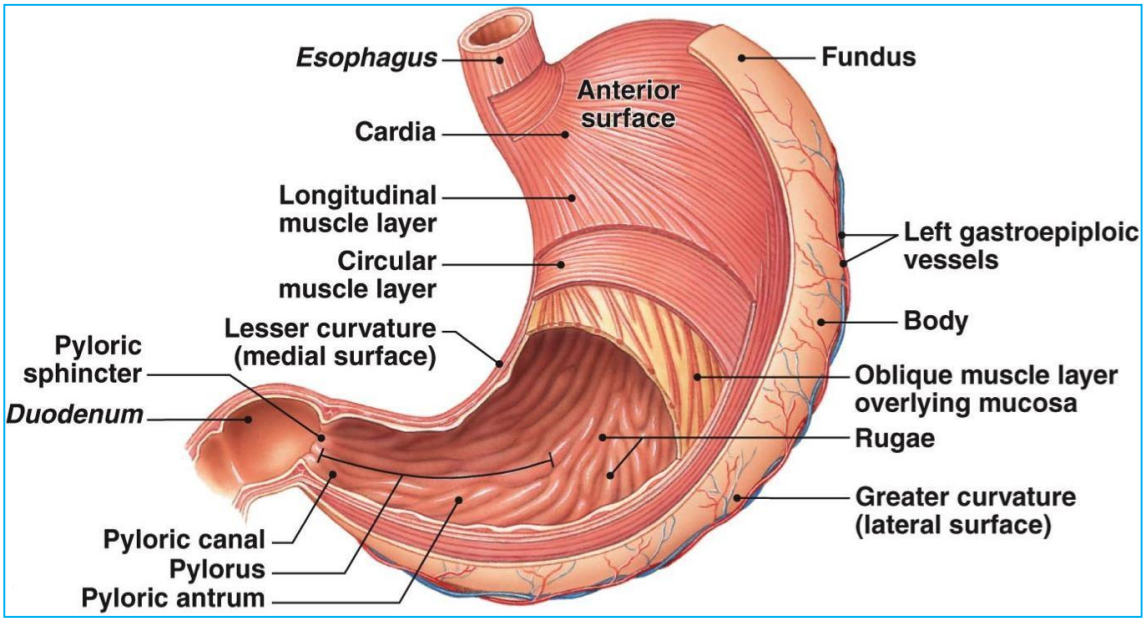


Figure 3.2: Anatomy of the stomach (Upadhyay *et al.,* 2014: 435)

Gastric emptying occurs during fasting as well as fed states. The pattern of motility is however, distinct in the two states. During the fasting state an inter-digestive series of electrical events take place, which cycle both through stomach and intestine every two to three hours. This is called the inter-digestive myoelectric cycle or migrating myoelectric cycle (MMC), which is further divided into following four phases as described by (Nayak *et al.,* 2010: 2). Figure 3.3 and Table 3.1 show the four phases of MMC.

- Phase I (basal phase)
- Phase II (preburst phase)
- Phase III (burst phase)
- Phase IV

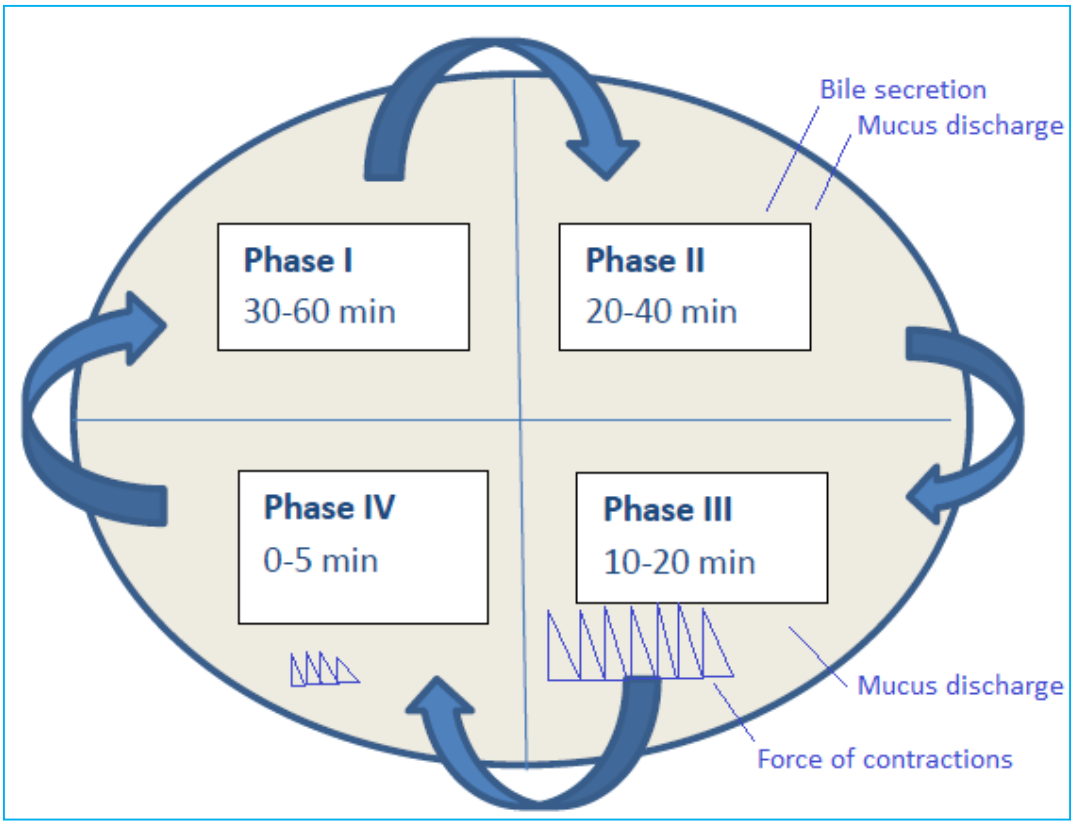


Figure 3.3: Motility pattern in GIT (Kumar, 2012: 126)

Table 3.1: Phases of migrating myoelectric complex (MMC) (Talukder & Fassihi, 2004: 1023)

Phase	Description
Phase I	It is a quiescent period lasting from 30 to 60 minutes with no contractions.
Phase II	It consists of intermittent contractions that gradually increase in intensity as the phase progresses, and it lasts about 20 to 40 minutes. Gastric discharge of fluid and very small particles begin later in this phase.
Phase III	This is a short period of intense distal and proximal gastric contractions (4-5 contractions per minute) lasting about 10 to 20 minutes; these contractions, also known as “house-keeper wave,” sweep gastric contents down the small intestine.
Phase IV	This is a short transitory period of about 0 to 5 minutes, and the contractions dissipate between the last part of phase III and quiescence of phase I

3.4.2 Factors controlling gastric retention of dosage forms

Various factors influence the retention of dosage forms in the stomach. These include the anatomy and physiology of the body and some aspects of the dosage form itself. Below listed are various factors which play a major role in determining the retention of dosage forms in the stomach.

3.4.2.1 Density of dosage form

Dosage forms having a density lower than that of gastric fluid float on gastric juice and are retained in the stomach. A density of less than 1.0 gm/cm^3 is required to exhibit floating property. However, the floating tendency of the dosage form usually decreases as a function of time, as the dosage form gets immersed into the fluid, as a result of the development of hydrodynamic equilibrium (Baumgartner et al., 2000: 126).

3.4.2.2 Size of dosage form

The size of the dosage form is another factor that influences gastric retention. The mean gastric residence times of non-floating dosage forms are highly variable and greatly dependent on their size, which may be small, medium, and large units (Chandra *et al.,* 2013: 23). In fed conditions, the smaller units get emptied from the stomach during the digestive phase and the larger units during the housekeeping waves. In most cases, the larger the size of the dosage form, the greater will be the gastric retention time because the larger size would not allow the dosage form to quickly pass through the pyloric antrum into the intestine. Thus the size of the dosage form appears to be an important factor affecting gastric retention (El-Kamel *et al.,* 2002: 14).

3.4.2.3 Food intake

Food intake and frequency of feeding have a profound effect on the gastric retention of dosage forms. The presence or absence of food in the stomach influences the gastro-retention time of the dosage form. Usually, the presence of food increases the gastro-retention time of the dosage form and increases drug absorption by allowing it to stay at the absorption site for a longer time. Food habits affect the gastro-retention time in the following ways: Fed or unfed state – under fasting conditions, the gastro-intestinal motility is characterized by periods of strong motor activity or the migrating myoelectric complex (MMC) that occurs every 1.5 to 2 hours. The MMC sweeps undigested material from the stomach and, if the timing of administration of the formulation coincides with that of the MMC, the gastro-retention time of the unit can be expected to be very short. However, in the fed state, MMC is delayed and gastro-retention time is considerably longer. Gastro-retention time can increase by over 400 minutes when successive meals are given compared with a single meal due to the low frequency of MMC (Baumgartner et al., 2000: 126).

3.4.2.4 Nature of food present in the stomach

Feeding of indigestible polymers or fatty acid salts can change the motility pattern of the stomach to a fed state, thus decreasing the gastric emptying rate and

prolonging drug release. Gastro-retention time can be increased by four to ten hours with a meal that is high in proteins and fats (El-Kamel *et al.,* 2002: 14).

3.4.2.5 Effect of gender, posture and age

A study by Mojaverian *et al* found that females showed comparatively shorter mean ambulatory gastro-retention time than males, and the gastric emptying in women was slower than in men. The authors also found no significant difference in the mean gastro-retention time for individuals in upright, ambulatory and supine state. On the other hand, in a comparative study in humans by Gansbeke *et al.,* the floating and non-floating systems behaved differently. In the upright position, the floating systems floated to the top of the gastric contents and remained for a longer time, showing prolonged gastro-retention time. But the non-floating units settled to the lower part of the stomach and underwent faster emptying as a result of peristaltic contractions, and the floating units remained away from the pylorus. However, in the supine position, the floating units are emptied faster than non- floating units of similar size (Chauhan *et al.,* 2013: 219).

3.4.3 Advantages of Gastro-retentive Drug Delivery System

Gastro-retentive drug delivery systems were introduced to overcome limitations of drug delivery by conventional methods. A number of merits are identified for using gastro-retentive drug delivery systems, and they include the following.

3.4.3.1 Improved patient compliance

Combining multiple doses in a single gastro-retentive drug delivery system greatly reduces the frequency of dosing. This results in better patient compliance (Bhardwaj *et al.,* 2011: 302).

3.4.3.2 Enhanced biolavailability

Gastro-retention enhances bioavailability despite first pass effect. Fluctuations in plasma drug concentration are avoided and a desirable plasma drug concentration is maintained by continuous drug release. Gastro-retentive drug

delivery systems improve the solubility of drugs that dissolve better in acidic environment (Gaba *et al.,* 2008: 122).

3.4.3.3 Site specific drug delivery

Gastro-retentive drug delivery helps in achieving local delivery of drugs to the stomach and proximal part of small intestine. Gastric retention time is increased for topical application of drugs to the stomach. This is quite advantageous for drugs such as antacids which are intended to act locally in the stomach (Bhardwaj *et al.,* 2011: 302).

3.4.3.4 Controlled drug delivery

Gastro-retentive drug delivery systems can be designed to release drugs in a controlled manner for a prolonged period. Another advantage is that floating drug delivery systems such as microspheres release drug uniformly and there is no risk of dose dumping (Gaba *et al.,* 2008: 122).

3.4.4 Disadvantages of Gastro-retentive Drug Delivery System

Although gastro-retentive drug delivery systems provide a number of merits as highlighted above, these systems still have some drawbacks which include the following.

3.4.4.1 Gastric mucosa irritation by drugs

Drugs that irritate or cause gastric lesions on slow release are not favoured for gastro-retentive drug delivery. This is because gastro-retentive systems prolong the residence time of such drugs and thereby increasing irritation of the gastric mucosa (Bhardwaj *et al.,* 2011: 302).

3.4.4.2 Gastro-retentive drug delivery is not suitable for all drugs

A number of drugs are not suitable for delivery by gastro-retentive drug delivery systems. Drugs with limited acid solubility will not dissolve in the stomach and hence cannot be delivered by gastro-retentive drug delivery systems (Badoni *et al.,* 2012: 39).

3.4.4.3 Floating drug delivery systems require high fluid level in stomach

A sufficiently high level of fluid is required to suspend floating drug delivery systems. Almost all floating drug delivery systems need to take in fluid so they can swell and sustain drug release. Without high level of fluid in the stomach, floating drug delivery systems will not float and work effectively (Badoni *et al.,* 2012: 39).

3.4.5 Potential drug candidates for Gastro-retentive Drug Delivery

- Drugs acting locally in the stomach such as misoprostol and antacids.
- Drugs that are poorly soluble at alkaline pH such as diazepam, verapamil hydrochloride and griseofulvin.
- Drugs with a narrow window of absorption such as furosemide and riboflavin.
- Drugs which are absorbed rapidly from the GIT such as metronidazole.
- Drugs that are unstable in the intestinal or colonic environment such as captopril and ranitidine.
- Drugs that disturb normal colonic microbes such as antibiotics against Helicobacter *pylori* (Badoni *et al.,* 2012: 37).

3.4.6 Unsuitable drugs for Gastro-retentive Drug Delivery

- Drugs that have very limited acid solubility such as phenytoin.
- Drugs that suffer instability in the gastric environment such as erythromycin.
- Drugs intended for selective release in the colon such as 5-amino salicylic acid (Nayak *et al.,* 2010: 4).

3.5 TYPES OF GASTRORETENTIVE DRUG DELIVERY SYSTEMS

Various techniques are used to retain drug delivery systems in the stomach. These systems can be used to sustain or control the release of drugs for delivery in the stomach.

3.5.1 Bio/Muco-Adhesive systems

Bioadhesion can be defined as the state in which two materials, at least one of which is biological in nature, are maintained together for a prolonged time period by means of interfacial forces (Smart, 2005: 1556). Bioadhesive or mucoadhesive systems bind with gastric epithelial cell surface or mucin and produces an increase in gastro-retention time of a dosage form, Figure 3.4. The mechanism of mucoadhesion is made up of two stages: the contact stage and the consolidation stage. The contact stage is characterized by the contact between the mucoadhesive and the mucous membrane, with spreading and swelling of the formulation, initiating its deep contact with the mucous layer (Carvalho *et al.,* 2010: 3). As the particle approaches the mucous surface, it will come into contact with repulse forces (osmotic pressure, electrostatic repulsion) and attractive forces (van der Waals forces and electrostatic attraction). Therefore, the particle must overcome this repulsive barrier (Smart, 2005: 1556). In the second stage; the consolidation stage, the mucoadhesive materials are activated by the presence of moisture. Moisture plasticizes the system, allowing the mucoadhesive molecules to break free and link up by weak van der Waals and hydrogen bonds. There are theories adapted from studies on the performance of several materials and polymer-polymer adhesion which explain the mucoadhesion phenomenon (Smart, 2005: 1557).

3.5.1.1 Electronic theory

The electronic theory depends on the assumption that the bioadhesive material and the target biological material have different electronic surface characteristics. Based on this, when two surfaces come in contact with each other, electron transfer occurs in an attempt to balance the Fermi levels, resulting in the formation of adouble layer of electrical charge at the interface of the bioadhesive and the

biological surface. The bioadhesive force is believed to be present due to the attractive forces across this double layer (Tangri & Madhav, 2011: 37).

3.5.1.2 Wetting theory

The wetting theory applies to liquid systems which present to the surface in order to spread over it. This affinity can be found by using measuring techniques such as the contact angle. The general rule states that the lower the contact angle then the greater the affinity. The contact should be equal or close to zero to provide adequate spreadability (Vinod *et al.,* 2012: 11).

3.5.1.3 Adsorption theory

According to the adsorption theory, the mucoadhesive device adheres to the mucus by secondary chemical interactions, such as in Van der Waals and hydrogen bonds, electrostatic attraction or hydrophobic interactions. Hydrogen bonds, which are prevalent interfacial forces in polymers containing carboxyl groups are considered the most important in the adhesive interaction phenomenon (Vinod *et al.,* 2012: 11).

3.5.1.4 Diffusion theory

Diffussion theory describes the interpenetration of both polymer and mucin chains to a sufficient depth to create a semi-permanent adhesive bond. It is believed that the adhesion force increases with the degree of penetration of the polymer chains. This penetration rate depends on the diffusion coefficient, flexibility and nature of the mucoadhesive chains, mobility and contact time. The adhesion strength of a polymer is reached when the depth of penetration is approximately equivalent to the polymer chain size. In order for diffusion to occur, it is important that the components involved have good mutual solubility, that is, both the bioadhesive and the mucus have similar chemical structures. The greater the structural similarity, the better the mucoadhesive bond (Vinod *et al.,* 2012: 11).

3.5.1.5 Fracture theory

Fracture theory is by far the most accepted theory on bioadhesion. It explains the forces required to separate two surfaces after adhesion has taken place. It measures the maximum tensile stress produced during detachment (Tangri & Madhav, 2011: 37).

3.5.1.6 Mechanical theory

Mechanical theory considers adhesion to be due to filling of the irregularities on arough surface by a mucoadhesive liquid. Moreover, such roughness increases the interfacial area available to interactions thereby aiding dissipating energy and can be considered the most important phenomenon of the process (Vinod *et al.,* 2012: 12). It is unlikely that the mucoadhesion process is the same for all cases and therefore it cannot be described by a single theory. All theories are relevant to identify the important process variables (Lee *et al.,* 2000: 855).

A mucoadhesion promoting agent or polymer is added to the formulation which helps to promote the adhering of the active pharmaceutical ingredient to the oral mucosa. The agent can have additional properties like swelling so as to promote the disintegration when in contact with the saliva. Mucoadhesive polymers can be categorized into materials which undergo matrix formation or hydrogel formation by either a water swellable material or a water soluble material. The adherence of the drug/carrier in a mucoadhesive drug delivery system to the mucous membrane is promoted by suitable carrier polymers. These carrier polymers are classified as:

- Hydrophilic polymers: Contain carboxylic group and possess excellent mucoadhessive properties. These are poly vinyl pyrrolidine (PVP), methyl cellulose, sodium carboxy methyl cellulose and hydroxyl propyl cellulose.
- Hydrogels: These swell when in contact with water and adhere to the mucus membrane. They include: carbopol, polyacrylates, chitosan, tragacanth, pecyin, acacia and eudragit analogues (Tangri & Madhav, 2011: 42).

The binding of polymers to the mucin/epithelial surface can be divided into three categories:

- Hydration – mediated adhesion: Certain hydrophilic polymers have the tendency to imbibe large amount of water and become sticky, thereby acquiring bioadhesive properties. The prolonged gastro-retention of the bio/muco-adhesive delivery system is further controlled by the dissolution rate of the polymer.
- Bonding – mediated adhesion: Adhesion of polymers to mucus/epithelial cell surface involves varying bonding mechanisms. Physical or mechanical bonds can result from deposition and inclusion of the adhesive material in the crevices of the mucosa. Secondary chemical bonds, contributing to bioadhesive properties, consist of dispersive interactions (i.e van der waals interactions) and stronger specific interactions, which include hydrogen bonds (Jassal et al., 2015: 82).
- Receptor – mediated adhesion: Certain polymers have the ability to bind to specific receptor sites on the cell surface. The receptor mediated events serves as a potential approach in bio/muco-adhesion, hence enhancing the gastric retention of dosage forms (Jassal *et al.,* 2015: 82).

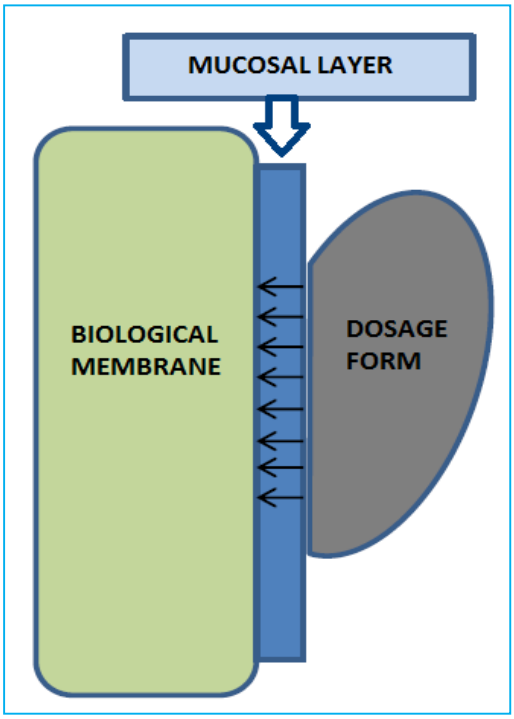


Figure 3.4: Bio-adhesion system (Kumar et al., 2012: 130)

The phenomenon of mucoadhesion is a novel controlled drug delivery approach. It has excellent accessibility and rapid onset of action. However, it has its limitations including the occurrence of local ulceration effects due to prolonged contact of drugs possessing ulcerogenic properties. Adhesion of preparations onto the mucous membrane can be impaired by the mucociliary clearance system. This clearance system, a natural defence mechanism of the body against the deposition of impurities onto the mucous membrane, can also remove preparations (Carvalho *et al.,* 2010: 6). Lack of a good model for in vitro screening to identify drugs suitable for administration by mucoadhesion is one of the major limitations in the development of this phenomenon. Patient acceptability in terms to taste, irritancy and mouth feel has to be accessed as well (Tangri *et al.,* 2011: 461). The systems may bind to other mucosal lining like the oesophagus.

3.5.2 Expandable systems

These are the dosage forms formulated in small 'collapsed' configuration to enable convenient oral intake; expand in the stomach to prevent passage through the pyloric sphincter; and finally get back to a small form when retention is no longer required, after the gastro-retentive drug formulation has released its active ingredient thereby enabling evacuation (Klausner *et al.,* 2003: 146). Expansion results from either swelling or from unfolding due to mechanical shape memory.

Swelling usually results from osmotic absorption of water and the dosage form becomes too big to pass through the pyloric sphincter. As a result, the dosage form is retained in the stomach for a long period of time, as shown in Figure 3.5.

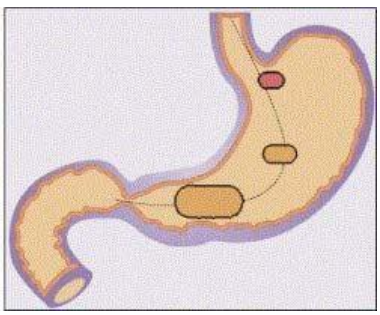


Figure 3.5: Swellable tablet in the stomach (Kumar *et al.,* 2012: 130)

Urquhart and Theeuwes developed a swelling system exhibiting a 2-50-fold volume increase. Further modification has been done to formulate a dosage form that comprises a body (A) shaped for oral administration and a hydrogel (B) which expands in the presence of gastric fluids thereby releasing tiny tablets (C) for controlled drug delivery (Klausner et al., 2003: 149), as shown in Figure 3.6.

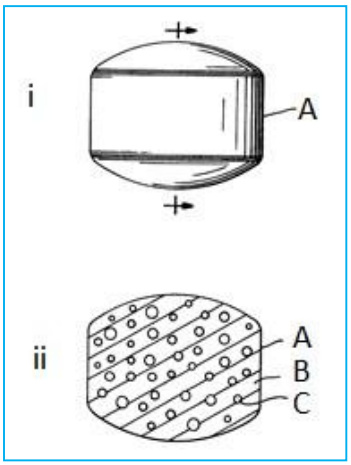


Figure 3.6: Modified swelling system. i. As seen from side view and ii. As seen in the direction of the arrows (Klausner *et al.,* 2003: 149)

A study of unfolding devices characterized by different erodibility, mechanical properties, sizes and geometries was conducted by Caldwell (Klausner *et al.,* 2003: 149). The developed geometric configurations were continuous stick, ring, tetrahedron, planar disc, planar multilobe and string. The devices developed sufficiently resisted forces applied by the stomach, thus preventing rapid passage through the pylorus; allowed for free passage of food while in residence in the stomach; and had the *in-vivo* circumference larger than 5 cm to ensure gastro-retentivity.

Swelling systems have an appreciable advantage of suppressing the burst phase of the inter-digestive myoeletric cycle, due to their increased size, thereby increasing the residence time of the formulation in the stomach (Bardonnet *et al.,* 2006: 8). However, these swelling systems have some drawbacks which include the permanent retention of rigid, large single-unit expandable drug delivery dosage

forms which may cause brief obstruction, intestinal adhesion and gastropathy (Garg & Sharma, 2003: 163).

3.5.3 High density systems

Sedimentation is the concept involved in high density systems. These systems with a density around 3 g/cm^3 have the property to sink to and remain in the pyloric region and can withstand the peristaltic movements of the stomach, as shown in Figure
3.7. High density systems incorporate high dense excipients to increase the density of the whole system. Excipients commonly used to increase the density of the system are: barium sulphate, zinc oxide, titanium dioxide and iron powder (Nayak *et al.,* 2010: 4).

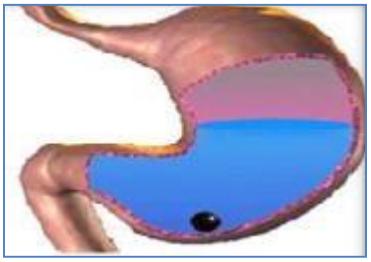


Figure 3.7: High density systems (Kumar *et al*., 2012: 130)

The effectiveness of high density systems in human beings was not observed (Nayak *et al.,* 2010: 4). These systems are technically difficult to manufacture with a large amount of drug because the dry material used to make the system interacts with the gastric fluid to release its drug contents. As such, no high density system has been marketed (Sharma & Sharma, 2014).

3.5.4 Magnetic systems

These systems appear as small gastroretentive capsules containing a magnetic material, whose elimination from the stomach is prevented by the interaction with a sufficiently strong magnet applied to the body surface in the region of the stomach. Although magnetic systems seem to provide satisfactory results, there is a problem of placing the magnet externally at the right position with great accuracy

and precision. This provides a risk of compromising patient compliance. Also, gastric residence time has been observed to differ depending on whether the patient is in a fed or fasting state. Gastric motility and peristaltic waves influence the extent to which these systems retain in the stomach (Bardonnet *et al.,* 2006: 9).

3.5.5 Superporous hydrogels

Superporous hydrogels are systems which swell instantly in the stomach and maintain their integrity in the harsh stomach environment, while releasing the pharmaceutical active ingredient (Omidian *et al.,* 2007: 317). Hydrogels have been used widely in various pharmaceutical and biomedical applications, controlled drug delivery being one of the main areas of research utilizing hydrogels. A hydrogel is a three-dimensional cross linked polymer network which is bonded physically or chemically and is insoluble in water but swells in the presence of water. Hydrogels with effective pore sizes in the range of 10 to 100 nm are termed as microporous hydrogels and pore sizes in the range of 100 nm to 10µm are termed as macroporous hydrogels (Gemeinhart *et al.,* 2000: 617).

For dried hydrogels to swell, water has to be absorbed into the glassy matrix of the dried hydrogels. The swelling kinetics of the dried hydrogels thus depends on the absorption of water occurring by a diffusion process and the relaxation of the polymer chains in the rubbery region. The rate limiting factor with these hydrogels has been to some extent the slow swelling of the dried hydrogels; it takes at least several hours to attain equilibrium swelling. This slow swelling property is advantageous in controlled drug delivery systems, but many of the pharmaceutical applications need fast swelling property (Harika *et al.,* 2011: 334).

Superporous hydrogels are porous hydrophilic crosslinked structures with the ability of absorbing aqueous fluids up to a few hundred times their own weight. Maximum swelling is generally reached in a fraction of a minute with superporous hydrogels having average pores of 200 µm in size. The unique property of size independent fast swelling kinetics of superporous hydrogels is accounted for by

their inter-connected open cellular structure. The open porous structure allows extremely fast absorption of water into the centre of the dried matrix by capillary force (Turakhiya *et al.,* 2013: 47). Superporous hydrogels possess three unique properties that conventional hydrogels do not have:

- First, the swelling rate is extremely fast. Regardless of the size of the dried superporous hydrogels, the full swelling is complete in a matter of a minute.
- Second, superporous hydrogels swell to very large sizes, and the weights of the fully swollen superporous hydrogels are orders of magnitude higher than the weights of dried superporous hydrogels.
- Third, the swelling superporous hydrogels can exert significant expansion force during swelling, despite the fact that the solid content is only a percentage of the total weight (Harika *et al.,* 2011: 330).

Because these hydrogels absorb a large volume of environmental fluids, which expand their volume considerably over a very short period of time, their sheer bulk hinders their transport to the next organ via the narrow pylorus. This unique property allows them to be used as gastric retentive carriers, providing sustained release through long residence in the stomach (Gupta & Shivakumar, 2010: 258). The development of super porous hydrogels has continued and three generations have evolved as follows and as shown in Figure 3.8.

3.5.5.1 Conventional Super Porous Hydrogels (CSPH)

This first generation was prepared by Chen *et al.,* in the year 1999. These super porous hydrogels are polymerized and cross linked with different vinyl monomers and they rewire a foaming agent, foam stabilizer and a foaming aid, along with these different wetting agents are also added to increase the water absorption rate to less than a minute.

3.5.5.2 Super Porous Hydrogels Composites (SPHC)

These second generation super porous hydrogels were developed by Park *et al* in 2001 to overcome the lack of desirable mechanical properties in conventional

CSPHs, by the addition of superdisintegrants into the formulation. The composite is a matrix which contains both dispersed phase and continuous phase.

3.5.5.3 Super Porous Hydrogels Hybrids

The third generation of super porous hydrogels are improved versions of the second generation, and developed based on super porous hybrids for synthesizing super porous hydrogels which have high mechanical and elastic properties, as shown in Figure 3.8. Super porous hydrogels hybrids are prepared by adding a hybrid agent that can be cross-linked after super porous hybrid is formed. The hybrid agent is a water-soluble or water-dispersible polymer that can form a cross-linked structure through chemical or physical cross-linking. Examples of hybrid agents are polysaccharides including sodium alginate, pectin, chitosan or synthetic water-soluble hydrophilic polymers such as polyvinyl alcohol (Harika *et al.,* 2011: 331).

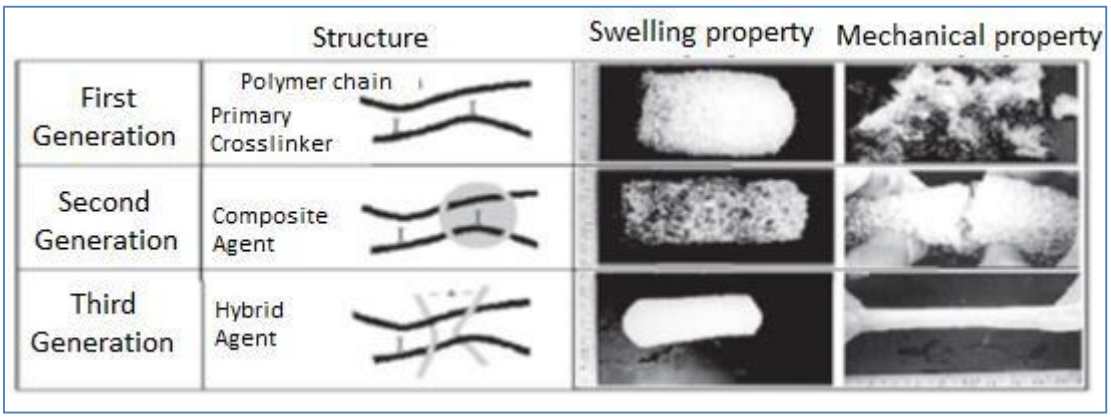


Figure 3.8: Generations of super porous hydrogels (Turakhiya *et al.,* 2013: 51)

Through the incorporation of biodegradable crosslinkers, the super porous hydrogel degrades in the body, preventing obstruction within the gastrointestinal tract.

3.5.6 Floating systems

Floating drug delivery systems (FDDS) have a bulk density less than gastric fluids and so remain buoyant in the stomach without affecting gastric emptying rate for a prolonged period of time, as shown in Figure 3.9. While the system is floating on

the gastric contents, the drug is released slowly at the desired rate from the system (Chandra *et al.,* 2013: 21). After release of drug, the residual system is emptied from the stomach. This results in an increased gastro-retention time and a better control of the fluctuations in plasma drug concentration. FDDS can be divided into non-effervescent and effervescent systems.

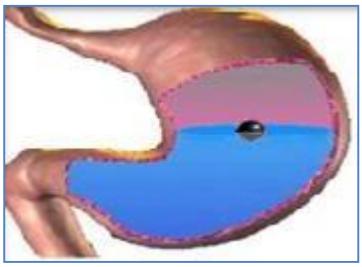


Figure 3.9: Floating system (Kumar *et al.,* 2012: 128)

3.5.6.1 Non effervescent systems

This type of system, after swallowing, swells unrestrained via imbibition of gastric fluid to an extent that it prevents their exit from the stomach. This system can be further divided into four sub-types:

3.5.6.1.1 Colloidal gel barrier system

This system incorporates a high level of one or more gel-forming highly soluble cellulose types of hydrocolloid, for example, hydroxpropyl cellulose, hydroxyethyl cellulose, hydroxypropyl methyl cellulose, polysaccharides and matrix-forming polymer such as polycarbophil, polyacrylate and polystyrene. The polymer is mixed with drug to form a single-unit dosage form and the system is usually administered in a gelatine capsule (Tariq *et al.,* 2014: 1557). On coming in contact with gastric fluid, the hydrocolloid in the system hydrates and forms a colloid gel barrier around its surface (Seth *et al.,* 1984: 313). This gel barrier controls the rate of fluid penetration into the device and consequently release of the drug. As the exterior surface of the dosage form goes into the solution, the gel layer is maintained by the adjacent hydrocolloid layer becoming hydrated. The air trapped in by the swollen polymer maintains a density less than unity and confers buoyancy to the dosage form (Sahil *et al.,* 2011: 20). As the system remains buoyant on the stomach content, gastro-retention time prolongs and maximizes the amount of drug

that reaches it absorption sites in the solution form ready for absorption, as shown in Figure 3.10.

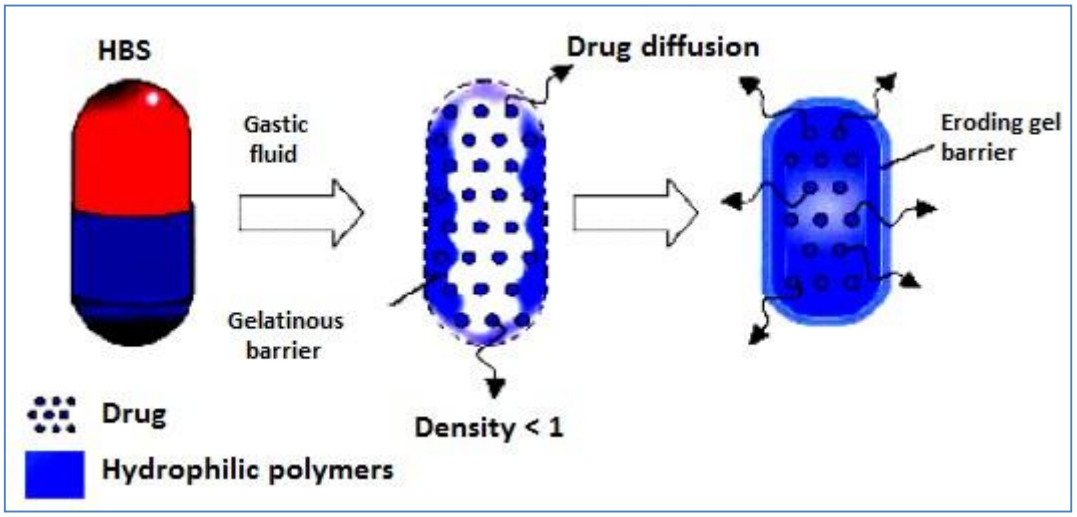


Figure 3.10: Hydrodynamically balanced system (Kumar *et al.,* 2013: 154)

Madopar, an anti-Parkinson's disease medicine based on the colloidal gel barrier system was marketed during the 1980's. Effective drug deliveries depend on the balance of drug loading and the effect of polymer on its release profile. Several strategies have been tried and investigated to improve efficiencies of the floating hydrodynamically balanced systems (Bardonnet *et al.,* 2006: 3).

3.5.6.1.2 Microporous compartment system

This technology is based on the encapsulation of a drug reservoir inside a microporous compartment with pores along its top and bottom walls. The peripheral walls of the drug reservoir compartment are completely sealed to prevent any direct contact of gastric surface with the undissolved drug. In the stomach, the floatation chamber containing entrapped air causes the delivery system to float over the gastric content, as shown in Figure 3.11. Gastric fluid enters through the aperture, dissolves the drug and carries the dissolved drug for continuous transport across the intestine for absorption (Kumar *et al.,* 2013: 154).

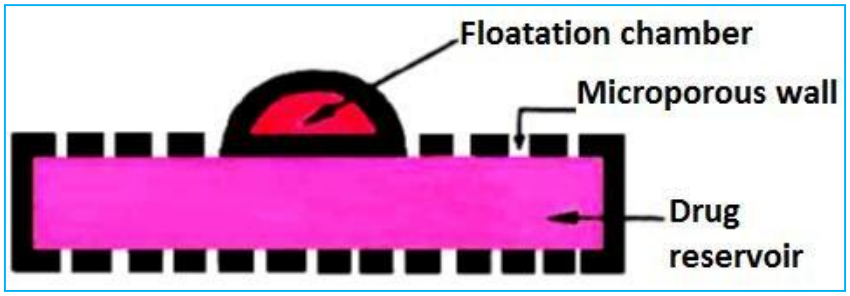


Figure 3.11: Floating drug delivery system with microporous membrane and floatation chamber (Kumar *et al.,* 2013: 154)

3.5.6.1.3 Alginate beads

Multi-unit floating dosage forms have been developed from freeze-dried calcium alginate. Spherical beads of approximately 2.5 mm in diameter can be prepared by dropping sodium alginate solution into aqueous solution of calcium chloride, causing the precipitation of calcium alginate. The beads are then separated, snap-frozen in liquid nitrogen, and freeze-dried at -40 ºC for 24 hours, leading to the formation of a porous system, which can maintain a floating force for over 12 hours (Sahil *et al.,* 2011: 21).

3.5.6.1.4 Highly porous systems

A relatively newer group of porous carriers that are low-density solids with open or closed pore structures and provide large exposed surface area have been used for drug loading. Their hydrophobicity varies from completely hydrophilic carriers, which immediately disperse or dissolve in water, to completely hydrophobic ones, which float on water for hours. Owing to a wide range of useful properties, porous carriers have been used in pharmaceuticals for many purposes including development of novel drug delivery systems such as floating drug delivery systems and sustained drug delivery systems, improvement of solubility of poorly water soluble drug and enzyme immobilization (Ahuja & Pathak, 2009: 599). The useful properties of these porous carriers include a high surface area, tunable pore sizes with narrow distributions, stable uniform porous structures and well-defined surface properties thus allowing for the absorption of drugs and drug release in a reproducible and predictable manner (Sher et al., 2007: 73). In floating drug

delivery systems, the addition of a low density polymeric carrier results in a matrix with a density less than 1 g/cm^3. This ensures retention of the formulation in the stomach. Examples of exploited porous carriers include porous silicon dioxide (Sylysia® 550, 320), polypropylene foam powder (Accurel®), porous calcium silicate (Florite®), magnesium aluminometa silicate (Neusilin® S2, NS2 N, US2), porous ceramic and calcium carbonate (Sher *et al.,* 2007: 73).

Streubel *et al.,* (2003: 39) developed single unit, floating controlled drug delivery systems consisting of polypropylene foam powder, matrix-forming polymers, drug and filler. The highly porous foam powder provided low density and excellent *in-vitro* floating behaviour of the tablets. All foam powder-containing tablets remained floating for at least 8 hours in 0.1 N HCL at 37 ºC. The tablets eroded upon contact with the release medium, and the relative importance of drug diffusion, polymer swelling and tablet erosion for the resulting release patterns varied significantly with the type of matrix former. The release rate could effectively be modified by varying the “matrix-forming polymer/foam powder” ratio, the initial drug loading, the tablet geometry (radius and height), the type of matrix-foaming polymer, the use of polymer blends and the addition of water-soluble or water-insoluble fillers. The floating behaviour of the low density drug delivery systems could successfully be combined with accurate control of the drug release patterns.

3.5.6.1.5 Hot melt extrusion (HME)

Hot melt extrusion is the process of applying heat and pressure to melt a polymer and force it through an orifice in a continuous process. HME is a well-known process, developed to produce polymer products of uniform shape and density, and its industrial application dates back to the 1930’s (Patil *et al.,* 2016: 22). HME has more recently been applied to the health-care industry where it is used to manufacture medical devices and to mix active pharmaceutical ingredients (APIs) with polymers to enhance the API’s bioavailability or prepare precursors for thermoplastic drug-eluting devices such as subcutaneous and intraocular implants and intravaginal rings. HME is carried out using an extruder - a barrel containing one or two rotating screws that transport material down to the barrel. There are two

types of extruders: single and twin screw extruders, as shown in Figure 3.12. Single screw extruders are primarily used for melting and conveying polymers to extrude them into continuous shapes, whereas twin screw extruders are used for melt-mixing polymers with additional materials and for devolatization.

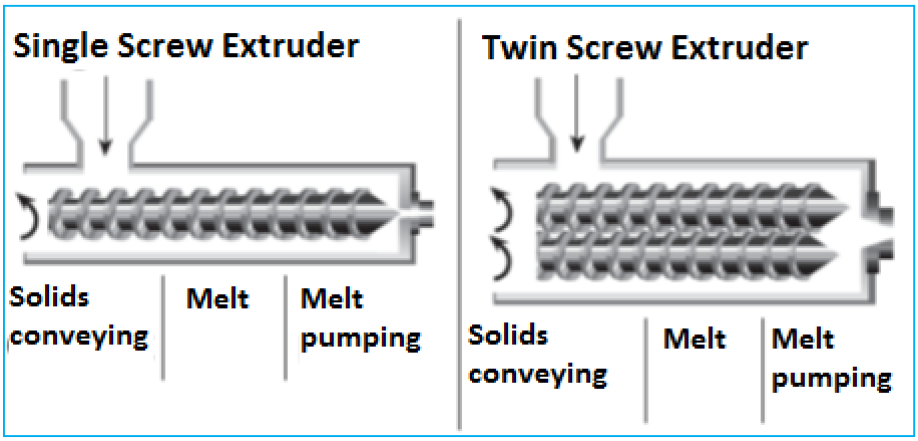


Figure 3.12: Cross-section of single and twin screw extruder barrel (Patil *et al.,* 2016: 34).

In the production of pharmaceutical formulations, which require homogeneous and consistent mixing of multiple formulation ingredients, a twin screw extruder is preferred because the rotation of the intermeshing screws provides better mixing to produce a homogeneous solid containing finely dispersed API particles, or a solid-solution of API in polymer. Poorly soluble actives can often be enhanced in their solubility and thereby in their bioavailability, e.g. by molecularly dispersing them in a polymeric carrier. A hot-melt extrusion process always yields a solid dispersion. Since melt extrudates can have a high density and a low porosity, they can be used for controlled-release dosage forms as well, if suitable polymer is used. Polymethacrylate polymers are the most commonly used polymers for this process, because of their thermoplastic properties. (Fukuda *et al.,* 2006: 122) produced tablets by HME. Sodium bicarbonate was added in the formulation that produced carbon dioxide gas through thermal degradation. Carbon dioxide was entrapped within the tablet matrix, resulting in a highly porous, buoyant tablet.

HME is currently generating more and more interest as the percentage of poorly soluble new chemical entities in drug development is constantly increasing. As an

emerging technology, HME's potential has not been fully explored yet. One of the challenges of HME is that the polymer to be used in the formulation has to have appropriate thermoplastic behaviour, and yet the number of such polymers approved for pharmaceutical use is limited (Kolter *et al.,* 2012).

3.5.6.1.6 Hollow microspheres (microballoons)

Hollow microspheres are in a strict sense, spherical empty particles without a core, as shown in Figure 3.13. These microspheres are characteristically free flowing powders consisting of proteins or synthetic polymers, ideally having a size less than 200 micrometers. These systems contain an outer polymer shell loaded with drug. The outer polymer shell is made up of polymers like polycarbonate, cellulose acetate, calcium alginate and agar. The microspheres are less dense and have sufficient buoyancy to float over gastric contents and remain in the stomach for prolonged periods (Bhowmik *et al.,* 2009).

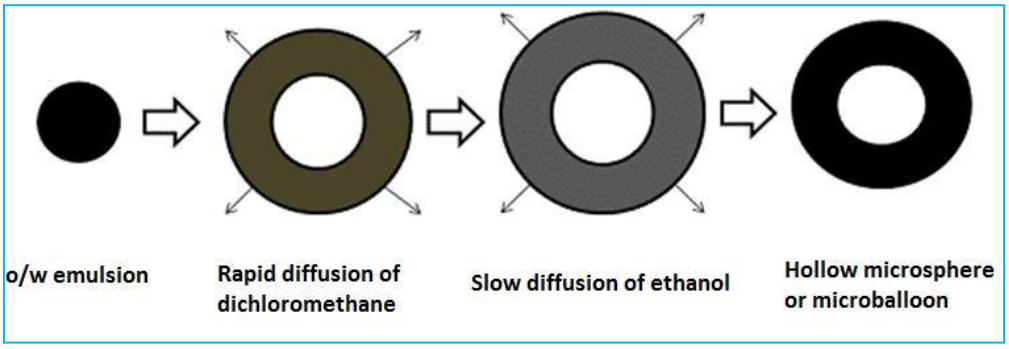


Figure 3.13: Hollow microspheres (Kumar *et al.,* 2012: 129)

At present hollow microspheres are considered to be one of the most promising buoyant systems because they combine the advantages of multiple-unit system and good floating (Nayak *et al.,* 2010: 5).

3.5.6.2 Effervescent systems

These buoyant systems utilize matrices prepared with swellable polymers such as Methocel™, polysaccharides (e.g. chitosan), effervescent components (e.g. sodium carbonate, citric acid or tartaric acid). Floatation of the drug delivery system

in the stomach can be achieved by incorporating a floating chamber filled with vacuum, air or an inert gas. Gas can be introduced into the floating chamber by the volatilization of an organic solvent (e.g. ether or cyclopentane) or by the carbon dioxide produced as a result of an effervescent reaction between organic acids and carbonate-bicarbonate salts. When the system comes in contact with gastric fluids it releases carbon dioxide, causing the formulation to remain and float in the stomach (Khan, 2013: 632).

3.5.6.2.1 Volatile liquid containing systems

These systems consist of two chambers separated by an impermeable, pressure-responsive, movable bladder, as shown in Figure 3.14. The first chamber contains the drug and the second chamber, contains volatile liquid e.g. ether or cyclopentane that gasifies at body temperature to cause inflation. The device inflates, floats in the stomach, allowing the drug to be continuously released from the reservoir into the gastric fluid with time (Kumar *et al.,* 2013: 155). The device may also consist of a bioerodible plug made up of polymers like PVA and polyethylene that gradually dissolve causing the inflatable chamber to release gas and collapse after a predetermined time to permit the spontaneous ejection of the inflatable system from the stomach.

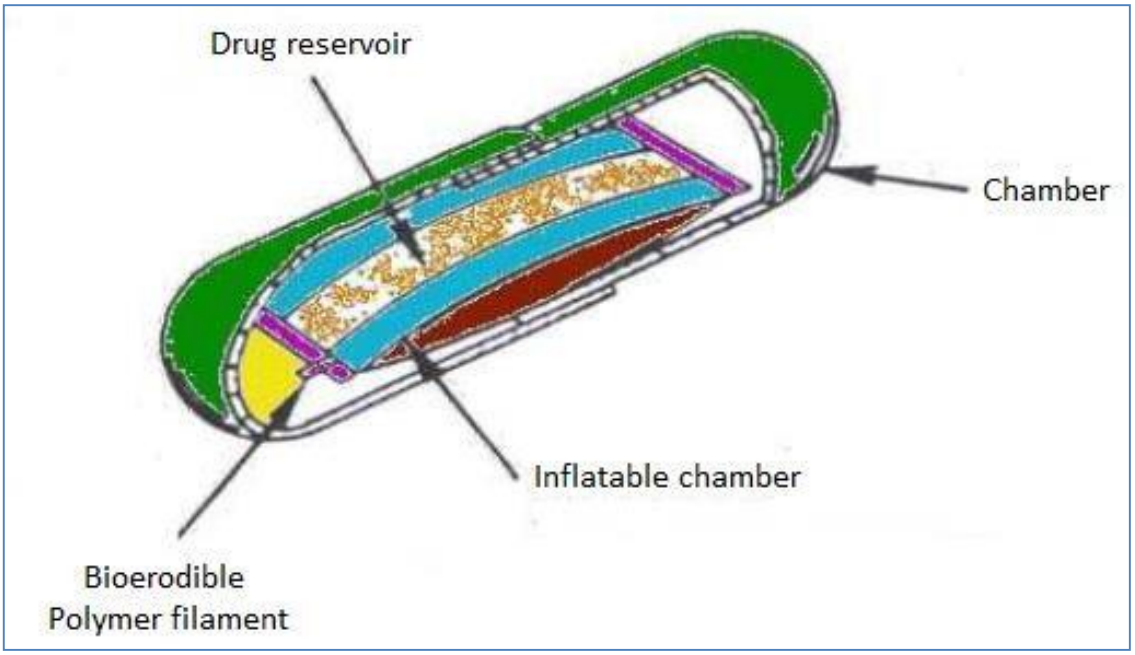


Figure 3.14: Volatile liquid containing system (Kumar *et al.,* 2012: 129)

3.5.6.2.2 Gas generating systems

The underlying principle of the gas generating systems is the effervescent reaction between carbonate/bicarbonate salts and citric/tartaric acid to liberate carbon dioxide, which gets entrapped in the gelled hydrocolloid layer of the systems thus decreasing its specific gravity and making it to float over chime (Khan, 2013: 633), as shown in Figure 3.15.

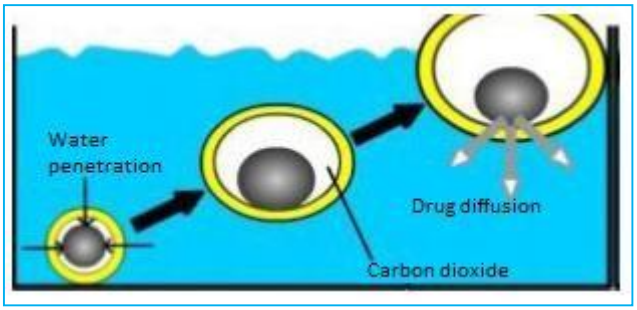


Figure 3.15: Principle mechanism of floating by carbon dioxide gas releasing method (Kumar *et al.,* 2012: 129)

A multiple-unit type of floating pill, which generates carbon dioxide gas, has been developed by Ichikawa *et al*. The system consisted of sustained release pills as seeds surrounded by double layers. The inner layer was an effervescent layer containing both sodium bicarbonate and tartaric acid. The outer layer was swellable membrane layer containing mainly polyvinyl acetate and purified shellac. The effervescent layer was divided into two sub layers to avoid direct contact between sodium bicarbonate and tartaric acid. Sodium bicarbonate was contained in the inner sublayer and tartaric acid was in the outer layer. When the system was immersed in a buffer solution at 37 ºC, it sank at once in the solution and formed swollen pills, like balloons, with a density much lower than 1 g/ml. the system was found to float completely within 10 minutes and approximately 80% remained floating over a period of 5 hours irrespective of pH and viscosity of the test medium. While the system was floating, a drug (p-amino benzoic acid) was released (Choi et al., 2002: 82).

3.6 GASTRO-RETENTIVE PREPARATIONS AVAILABLE IN INTERNATIONAL MARKET

Currently there are no gastro-retentive drug delivery systems on South African market. However, there are a number of marketed preparations of gastro-retentive technologies available in the international market, as shown in Table 3.2.

Table 3.2: Marketed preparations of Gastro retentive (Maity *et al*., 2014: 14)

Brand name	Drug	Type of GRDD
Glumetza	Metformin	Polymer Based
proQuin XR	Ciprofloxacin	Polymer Based
Cifran OD	Ciprofloxacin (1g)	Gas generating Floating Form
GabapentinGR	Gabapentine (In Phase-III clinical trials) Accordion Pill TM	Polymer Based
Baclofen GRS	Baclofen	Coated multi-layer floating & swelling system
Coreg CR (Carvedilol)	Carvedilol	Gastro retention with osmotic system
Madopar	Levodopa and benserzide	Floating, CR Capsule
Topalkan	Aluminium magnesium antacid	Floating Liquid Alginate
Valrelease	Diazepam	Floating Capsule
Almagate flatcoat	Antacid	Floating Liquid Form
Liquid gavison	Alginic acid and sodium bicarbonate	Effervescent floating liquid alginate preparation
Cytotec	Misoprostol (100mcg/200mcg)	Bilayer floating Capsule
Conviron	Ferrous Sulphate	Colloidal gel forming FDDS

3.7 METHODS TO IMPROVE ORAL ABSORPTION OF GRISEOFULVIN

Over the years research has been directed towards enhancing the solubility and dissolution rate of griseofulvin in an effort to develop an improved and convenient dosage form. As indicated earlier in this chapter, one of the known methods of increasing the rate of dissolution and absorption of griseofulvin is by reducing particle size through micronization. Micronization is a term used to describe size reduction where the resulting particle size is less than 10 microns. Micronization involves acceleration of particles so that grinding occurs by particle- to-particle impact or impact against a solid surface. However, particle size reduction is considered expensive and often leads to aggregation and agglomeration of particles resulting in poor wetabilities. This problem has been minimized by solid dispersions of the drug with water-soluble carriers (Chiou & Reigelman, 1970: 1378), but the high surface energy of the particles produced tend to limit their physical stability. On the other hand, the enhancement of griseofulvin absorption in the presence of fat or triglycerides led Carrigan and Bates (1973: 1477) to develop the oil-in-water emulsion. This has been considered as the best delivery system for griseofulvin.

The solubility of griseofulvin in distilled water at 37 ºC was investigated and found to be 12.9 mg/ml (Tur *et al.,* 1997: 66) which is in good agreement with the published value by Vojnovic *et al* 1993. Similar results 11.3 µg/ml was obtained in simulated intestinal fluid (Tur *et al.,* 1997: 66). On the other hand, the solubility of griseofulvin was significantly increased at the same temperature in simulated gastric fluid 23.4 µg/ml. Tur et al's results demonstrated that griseofulvin dissolves better in the acidic medium of the stomach than in the water or alkaline environment of the intestine. The passage of griseofulvin into the neutral or alkaline region of the GIT could result in a potential decrease in the dissolution and absorption rates. It follows that there is a clear advantage to be gained if griseofulvin drug particles were to be retained in the stomach for a prolonged period of time. This gives more time for the drug to dissolve in the stomach where its dissolution is optimal (Tur *et al.,* 1997: 66).

In another experiment, Tur *et al.,* 1997 investigated the use of biodhesive polymer to improve the bioavailability of griseofulvin. From the results obtained from their experiment, it was concluded that the presence of a bioadhesive polymer increases the bioavailability of griseofulvin in rabbits, producing a satisfactory plasma concentration profile over 24 hours. The significance was accounted for by the delaying effect on the gastric emptying process caused by binding of the bioadhesive polymer to the gastric mucin/epithelial cell surface. The delay could have allowed only small amounts of undissolved drug to be emptied into the small intestine, as compared to the rapid emptying of the drug particles in the absence of a bioadhesive polymer. The prolonged detainment in the acidic medium of stomach further promotes the dissolution and absorption of griseofulvin (Tur *et al.,* 1997: 66). However, the mucoadhesive drug delivery system has disadvantages which include patient acceptability in terms to taste, irritancy and mouth feel which needs to be checked (Tangri *et al.,* 2011: 38).

Another technique that has been used to improve the solubility of griseofulvin is the preparation of liquisolid compacts. A liquisolid system refers to formulations formed by converting liquid drugs (oil), drug suspensions or drug solution in non-volatile liquid vehicle into dry, non-adherent, free-flowing and compactible powder mixtures. This is usually obtained by mixing the liquid medication (drug and liquid vehicle) with a carrier excipient that forms a thin layer around the drug particles. The obtained liquid medication-carrier system is blended with an adsorbing agent (commonly known as a coating agent) so as to get an apparently dry looking, free flowing powder mix that can be easily compacted into tablets. Various grades of cellulose, starch, lactose are used as the carriers, whereas very fine silica powder is used as the coating material (Elkordy *et al.,* 2012: 123). In a study by (Elkordy *et al.,* 2012: 122) the liquisolid technique was utilized to modify drug release rate using griseofulvin as a model. The study provided evidence of possible control of drug release from liquisolid tablets by proper choice of the liquid vehicle. In addition, it has been suggested that liquisolid formulations may not only help in reduction of the dose size but also may help in production of sustained release griseofulvin formulations by choosing a suitable vehicle to control the drug release (Elkordy *et al.,* 2012: 131).

3.8 SUMMARY

From the literature survey, it appears that the improved bioavailability of griseofulvin is mainly due to either promoting the drug dissolution or delaying the gastrointestinal emptying process which could increase the total amount of drug absorbed. Therefore, the use of a floating effervescent matrix system for the purpose of keeping the drug for a prolonged period of time in the stomach poses great interest. Floating drug delivery systems are simple and require conventional equipment for manufacture. The use of simple formulation techniques to develop a cost effective floating drug delivery system for griseofulvin would be significant in developing countries like South Africa.

CHAPTER 4

MATERIALS AND METHODS

4.1 INTRODUCTION

This chapter deals with the materials, apparatus, reagents and methodology used for the characterization of floating tablets of griseofulvin. The methodology includes a description of the pre, post compression, compatibility, buoyancy and stability studies of the developed formulations

4.2 MATERIALS

Griseofulvin was supplied by Aspen Pharmacare (South Africa). Accurel MP, a low density polymer was purchased from Membrana (Germany), Methocel™ (HPMC K100) which is a rate controlling polymer was donated by Colorcon (England), polyvinylpyrrolidone (PVP K-30), a binder was obtained from Fluka (United States), Magnesium stearate a lubricant was purchased from BDH Chemicals Ltd (England), and Ethanol (South Africa) was used as a granulating agent.

4.2.1 Accurel MP

Accurel MP, polypropylene foam powder, is a low density polymer used in floating drug delivery systems. When included in a matrix, Accurel MP adsorbs the drug, excipients and entraps air. The entrapped air reduces the density of the formulation and allows the unit to float when exposed in an aqueous environment (Al-Achi *et al.,* 2013: 77).

4.2.2 Methocel™

Methocel™, also known as hydroxypropyl methylcellulose (HPMC), is a water-soluble, non-ionic cellulose ether. It retains chemical stability over a pH range of 3.0-11.0 and resists enzymatic degradation (Dow, 2000: 3). HPMC has a cellulose backbone with ether linked methoxyl and hydroxypropyl side group substituents attached through ether linkages to the cellulose chain hydroxyl groups.

HPMC is used as a rate controlling polymer. When HPMC in a matrix is exposed to an aqueous medium, HPMC hydrates rapidly, relaxing the chain to form a viscous gelatinous layer at the surface of the tablet. The formed gelatinous layer controls the penetration of additional water into the tablet. As the outer gel layer hydrates fully, it erodes and dissolves. As HPMC molecules beneath the gel layer hydrate, a new inner layer replaces it and becomes cohesive enough to retard the influx of water and in turn controls drug diffusion (Dow, 2000).

4.2.3 Polyvinylpyrrolidone (PVP k-30)

Polyvinylpyrrolidone, also known as povidone is a binder used in both wet and dry granulation. Polyvinylpyrrolidone facilitates agglomeration of powder material to form granules of desired hardness and size (Cantor *et al.*, 2008: 285).

4.2.4 Magnesium stearate

Magnesium stearate is the most common lubricant used in tableting. It is a white powder, which is insoluble in nature. Magnesium stearate reduces both wall friction and internal friction of powder and granules. This makes materials (powder/granules) glide better and be non-adherent, thereby enhancing flowability (Li & Wu, 2014: 27).

4.3 DRUG – EXCIPIENT COMPATIBILITY STUDIES

The potential physical and chemical interactions between drug and excipients can affect the chemical, physical, therapeutical properties and stability of a dosage form (Patel *et al.,* 2015: 14). Compatibility studies are carried out to investigate the potential interactions between drug and excipients before formulation. Compatibility studies between griseofulvin and excipients were done using differential scanning calorimetry (DSC).

DSC is a thermo-analytical technique used to analyse the thermal behaviour of active pharmaceutical ingredients. DSC measures the difference in the amount of

heat required to increase the temperature of a sample and reference over time. When a sample experiences a physical or chemical transformation, more or less heat flows to it than to the reference to maintain both at the same temperature (Kodre *et al.,* 2014: 11). Heat flow measurements essentially give quantitative and qualitative information about physical and chemical changes that involve endothermic or exothermic processes or changes in heat capacity (Bhusnure, 2016: 443). The method in this study is similar to that reported in literature (Kodre *et al.,* 2014: 12).

A DSC-60 Shimadzu (Kyoto, Japan) instrument was used to record the DSC thermograms. Samples, weighing approximately 3 - 5 mg were placed in aluminium crimp cells and heated to 300 °C with a heating rate of 10°C/min, with a nitrogen gas flow of 35 ml/min. Thermograms obtained in DSC were analysed based on the changes in appearance, disappearance or shift of endothermic or exothermic peaks of griseofulvin-excipient mixtures as compared to the pure griseofulvin and excipients.

4.4 MANUFACTURING METHOD OF GRISEOFULVIN FLOATING MATRIX TABLETS

The formulae of griseofulvin floating tablets were determined using Design Expert 9.0 (USA, 2013), a design of experiment software. The use of Design Expert 9.0 in this study was carried out as described by Khamanga (Khamanga & Walker, 2011: 1039). A total of 25 runs were generated. Floating tablets containing 100 mg of griseofulvin were prepared by wet granulation technique with varying ratios of Methocel™, Accurel MP and Polyvinylpyrrolidone (PVP k-30) as determined by Design Expert software. Magnesium stearate was kept at 1% for all the formulations.

Griseofulvin and all the other excipients were weighed accurately for a batch size of 200 tablets. To avoid segregation of materials, Accurel MP and other excipients were separately passed through an 850 µm sieve in order to obtain finer particles of similar size. Materials were transferred into a 1000 ml plastic beaker and mixed

thoroughly with a bowl and stand mixer (KENWOOD, United Kingdom) mixer for 10 minutes to form a homogeneous mixture. To the above powder blend, 60% ethanol (granulating agent) was added followed by mixing until the end point of granulation was observed. The wet granules were then passed through an 850 µm sieve, transferred onto a stainless steel plate covered with aluminium foil and placed in an oven. The granules were dried at 40 ºC (Labotec, South Aftrica) until the moisture content was less than 1.5%. The dried granules were transferred into a 1000 ml plastic beaker and blended with previously weighed and screened magnesium stearate for three minutes using a spatula. The final granules were compressed using a 12 mm diameter compression tooling on a Cadmach compression machine (India). Figure 4.1 below illustrates the processing flow for manufacturing of griseofulvin matrix tablets.

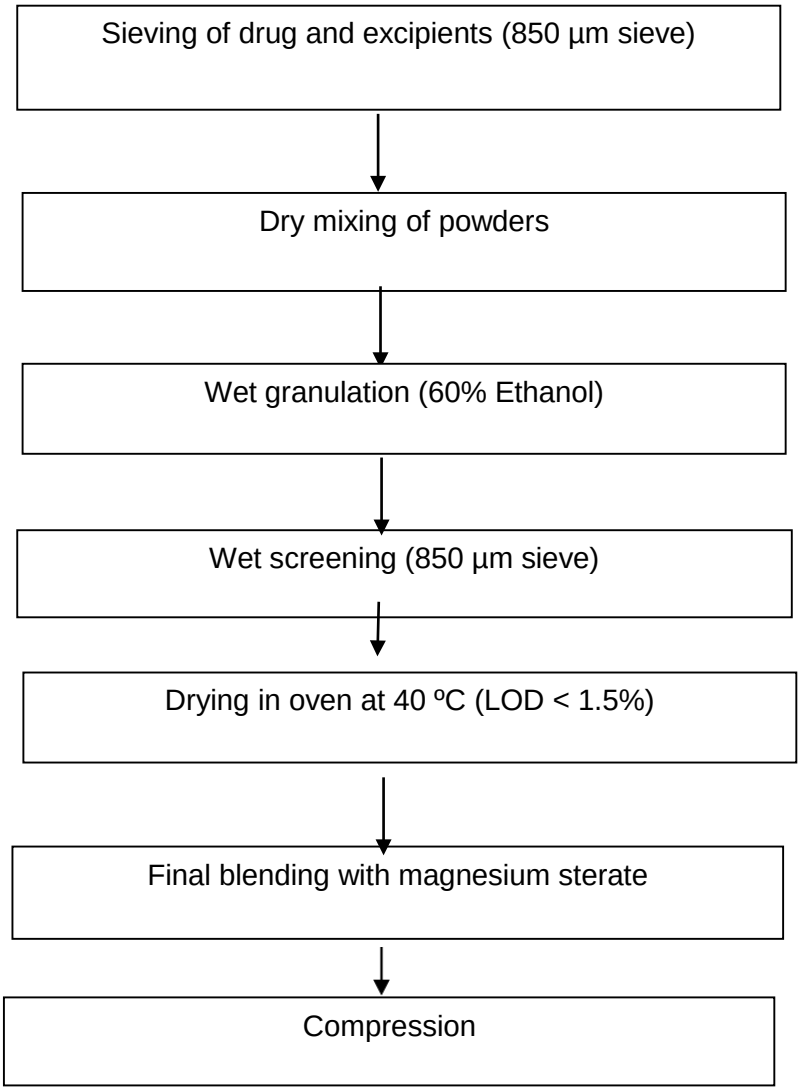


Figure 4.1: Processing flow for manufacturing of griseofulvin floating matrix tablets

4.5 EVALUATION OF FLOATING MATRIX TABLETS OF GRISEOFULVIN

All formulations were tested for both pre- and post-compression parameters following methods in the United States Pharmacopoeia (USP). Readings were done in triplicate, mean and standard deviations were calculated.

4.5.1 Pre-compression studies

Pre-compression studies were done to assess the flow properties of granules before compression. The flow property of powder mixture or granules is important for the uniformity of the mass of tablets (Morin & Briens, 2013: 1159). Therefore bulk density, tapped density, angle of repose, loss on drying and compressibility of the granules were analysed before compression of the tablets, as per methods described by (Wells & Aulton, 2007: 168).

4.5.1.1 Loss on drying

Loss on drying test measures the quantity of water in a sample when the sample is dried under specific conditions. Loss on drying is the loss of mass expressed as percentage of the initial weight. Loss on drying was carried out as per USP method (USP, 2014: 369). 1.0 g powdered tablet samples of each formulation runs were weighed into previously dried empty containers. The samples were spread evenly and dried at 105 ^{o}C in a Labcon drying oven (Laboratory Consumables and Chemical Supplies CC, Johannesburg, South Africa) for one hour. After drying, the containers were cooled to room temperature in a desiccator and reweighed. Loss on drying was calculated by determining the percentage difference in the sample mass.

4.5.1.2 Bulk density

Bulk density is the ratio of total mass of powder to the bulk volume of powder. It was measured by pouring the weighed powder into a measuring cylinder. It is expressed in g/ml and was calculated by equation 1 (Varun Kumar & Ajaykumar., 2013: 241).

$$D = M / V_0 \text{ --------- (Equation 1)}$$

Where;

D is bulk density

M is the mass of powder,

V_0 is the bulk volume of the powder.

4.5.1.3 Tapped density

The tapped density is an increased bulk density attained after mechanically tapping a 250ml graduated cylinder containing the powder sample. Measurement of tapped density was done using the method descried by (Wells & Aulton, 2007: 168). The cylinder was tapped 500 times to obtain the initial volume (V_0). It was further tapped 750 times and the tapped volume was measured (V_f). If the difference between the two volumes is less than 2% V_f, is the final tapped volume. If the % difference is more than 2%, an addition 1250 taps may be required. It is expressed in g/ml and is given by

$$DT = M / V_f \text{ --------- (Equation 2)}$$

Where;

TD is tapped density

M is the mass of powder,

V_f is the tapped volume of the powder

4.5.1.4 Carr's index and Hausner ratio

Hausner ratio and Carr's index are the two most commonly used methods to measure the compressibility of powders. Both Carr's index and Hausner ratio were determined by using the bulk density and tapped density of the powder. The Hausner ratio and Carr's index (also known as percentage compressibility index) were calculated according to the equation 3 and equation 4 respectively (Wells & Aulton, 2007: 355).

$$\text{Hausner ratio} = V_o / V_f \text{ ---------- (Equation 3)}$$

$$\text{Carr's index (\%)} = [(V_o - V_f) \times 100] / V_o \text{ --------- (Equation 4)}$$

For the compressibility index percentage and Hausner ratio, the generally accepted scale of flowabillity is given in Table 4.1.

Table 4.1: Effect of Carr's index and Hausner ratio on flow properties (USP, 2014: 298)

Compressibility Index (%)	**Compressibility**	**Hausner Ratio**
≤10	Excellent	1.00–1.11
11–15	Good	1.12–1.18
16–20	Fair	1.19–1.25
21–25	Passable	1.26–1.34
26–31	Poor	1.35–1.45
32–37	Very poor	1.46–1.59
>38	Very, very poor	>1.60

4.5.1.5 Angle of repose

The friction forces in a loose powder can be measured by the angle of repose, the maximum angle possible between the surface of a pile of powder and horizontal plane is equal to coefficient of friction between the particles (Ghosh & Jasti, 2004: 149). Measurement of angle of repose was done as per method described by (Wells & Aulton, 2007: 168). The sample was poured onto a horizontal surface passing through a funnel orifice, from a fixed height of 5 cm and the angle of the resulting pyramid was measured. The funnel orifice was selected through which the sample flowed slowly and reasonably constantly. For the angle of repose, the generally accepted scale of flowability is given in Table 4.2 below. Equation 5 was used to calculate the angle of repose of the granules.

$$\tan \theta = h / r \text{ --------- (Equation 5)}$$

Where;

θ = angle of repose,

h = height of the powder cone and

r = radius of the heap of the cone.

Table 4.2: Comparison between angle of repose and flow properties of solids (USP, 2014: 298)

Flow Property	Angle of Repose (degrees)
Excellent	25–30
Good	31–35
Fair - aid not needed	36–40
Passable - may hang up	41–45
Poor - must agitate, vibrate	46–55
Very poor	56–65
Very, very poor	>66

4.5.2 Post-compression studies

Tablets were evaluated for the post-compression parameters which include physico-chemical parameters like weight variation, thickness, diameter, hardness, friability, *in-vitro* drug release (dissolution) and assay. Manufactured tablets were evaluated in terms of the following physical properties:

4.5.2.1 Tablet dimensions

The thickness and diameter of tablets are important for uniformity of tablet size. These were determined using a Vernier calliper (Fragram, South Africa), as per USP method (USP, 2014: 1144).

4.5.2.2 Hardness

The hardness of tablets predicts the resistance of tablets to breakage under conditions of storage, transportation and handling before use (USP, 2014: 1146). Tablet hardness was measured as per USP method (USP, 2014: 1146). Ten tablets were randomly selected and hardness was measured by a hardness tester (Schleuniger, Switzerland).

4.5.2.3 Weight variation test

Uniformity of weight is an in process test parameter which ensures consistency of dosage units during compression. Weight variation test was carried out as per USP method (USP, 2014: 492). Twenty tablets were selected randomly and weighed individually to check for weight variation. The average weight and standard deviation (SD) was calculated. The individual weights of tablets were compared to the average weight. Tablets pass the test if not more than two tablets fall outside the percentage limit and if no tablet differs by more than two times the percentage limit (USP, 2014: 492). The percentage deviation mentioned in Table 4.3 for weight variation was used. A 7.5% maximum difference was allowed as all formulations developed in this study weighed between 130 to 324 mg.

Table 4.3: Specification for weight variation of solid dosage forms (USP, 2014: 492)

Average Weight Of Tablet	**Percentage Weight Variation**
130mg or less	10%
More than 130mg and less than 324mg	7.5%
324mg or more	5%

4.5.2.4 Friability

Friability determines the resistance of tablets to shipping or breakage under conditions of storage, transportation and handling before usage. Friability test was performed as per USP method (USP, 2014: 1145). Twenty tablets were weighed and their weight was recorded. The tablets were placed in a Roche friabilator (Roche, USA) and rotated at the speed of 25 rpm for 100 revolutions. The tablets were dusted after the 100 revolutions and then weighed as per USP method. The

percentage loss was calculated according to the following equation.

$$\% \text{ Friability} = \frac{\text{Initial weight of tablets} - \text{Final weight of tablets}}{\text{Initial weight of tablets}} X\ 100 \text{ ----- (Equation 6)}$$

Acceptance criteria: not more than 1 %

4.5.3 Buoyancy/floating test

Buoyancy is both the floating lag time and total floating time. Floating lag time (FLT) is the time taken for a tablet to rise on medium surface, and total floating time is the floating duration a tablet takes on medium surface. Buoyancy test was performed as per method published by Taghizadeh (Taghizadeh *et al.*, 2013: 3). To determine the floating lag time, 6 tablets were put in 100 mL of 0.1 N HCL in a beaker, and the time required for each tablet to rise to the surface was measured. Then, the duration of each tablet that remained on the surface were determined as total floating time. Mean and standard deviation were calculated for the measurements obtained from 6 tablets.

4.5.4 Tablet density

Tablet density is a vital parameter for floating tablets. Tablets float in the stomach when they are less dense than gastric fluid. Tablet density was calculated as done by (Seth *et al*., 2013: 607) using the following equation.

$V = \pi r^2 h$

$d = m/v$

$d = m/ \pi r^2 h$ ----- (Equation 7)

Where;

V = volume of the tablet (cm^3),

r = radius of the tablet (cm),

h = crown thickness of the tablet (cm) and m = mass of the tablet (mg)

4.5.5 Assay

Assay of the manufactured tablets was performed following BP method (BP, 2014: III-610). Twenty tablets per formulation run were weighed and powdered. To a quantity of powder containing 35 mg of griseofulvin, 60 ml of ethyl acetate was added. The solution was mixed and heated to 60 ^{o}C with shaking for 20 minutes. The solution was allowed to cool and was diluted to 100 ml with ethyl acetate. Afterwards, the solution was centrifuged and two 5 ml aliquots of the clear supernatant liquid were transferred into separate 100 ml graduated flasks. To the first flask, 5 ml of 2M methanolic methanesulfonic acid was added and the solution was allowed to stand at 20 ^{o}C for 30 minutes. The solution was diluted to 100 ml with methanol and labelled solution A. The contents of the second flask were diluted to 100 ml with methanol and labelled solution B. To a third flask, 5 ml of 2M methanolic methanesulfonic acid was added and diluted to 100 ml with methanol. This solution was labelled solution C. The absorbance of each solution was measured at 266 nm. The content of griseofulvin was calculated from the difference between the absorbance obtained with solution A and the sum of the absorbances obtained with solutions B and C and from the difference obtained by repeating the operation using 35 mg of griseofulvin BPCRS (British Pharmacopoeia Catalogue Reference Standard, 2014) in place of the powdered tablets and from the declared content of griseofulvin in griseofulvin BPCRS.

Acceptance criteria: 95.0 % - 105.0 %

4.5.6 *In-vitro* analysis of the griseofulvin release from the floating tablets

In-vitro release of griseofulvin from the tablets was examined as per USP method (USP, 2014: 3198). A dissolution apparatus 2 (paddle apparatus) was used in 900 ml of dissolution medium containing 0.1 N hydrochloric acid and 4 % sodium lauryl sulphate at 37 ^{o}C ± 0.5 ^{o}C and a pH of 1.2. Rotation speed of paddle used was 100 rpm. Samples (5 ml) were taken from the dissolution apparatus at set time intervals: 30, 60, 90, 120, 180, 240, 360, 480 and 720 minutes. The medium was replenished with an equal volume of fresh medium at each time interval. The samples taken were immediately filtered using a 0.45 µm filter. From each 5 ml sample, 1 ml was taken and diluted to 10 ml using dissolution medium. The amount

of griseofulvin dissolved was determined by employing a UV detector at 296 nm. A standard calibration curve of griseofulvin in 0.1 N hydrochloric acid with 4% sodium laurylsulphate was plotted and regression coefficient calculated to validate method (ANNEXURE A). The percentage of the labelled amount of griseofulvin dissolved was calculated using the following equation (Equation 8):

$$\frac{Abs\ sm}{Abs\ st} \times \frac{Std\ dil}{Sm\ dil} \times \frac{Potency}{100} \times \frac{Avg\ wt\ (mg)}{Label\ claim\ (100mg)} \times 100 \quad \text{----------(Equation 8)}$$

Where;

Abs sm = absorbance of sample Abs std = absorbance of standard Std dil = standard dilution

Sm dil = sample dilution

Avg wt = average weight of tablets

4.6 SUMMARY

Methods used to manufacture and analyse floating griseofulvin tablets in this study were done according to the procedures described in the (USP, 2016) and (BP, 2016).

CHAPTER 5

RESULTS AND DISCUSSIONS

5.1 INTRODUCTION

Chapter five gives the results of the compatibility, pre-compression, post-compression, buoyancy, dissolution and stability studies of the floating matrix tablets as described in chapter four. Compatibility studies aimed to study the interaction of griseofulvin with excipients used to identify any physical and chemical incompatibilities. Design Expert Software Version 9.0 was used to generate formulation runs and further assess the influence of formulation and processing parameters on the tablet characteristics. Pre-compression studies determined the flow properties of the granules, post-compression studies assessed the quality of tablets, buoyancy studies determined the floating characteristics of the tablets, dissolution studies revealed the drug release profile, and stability studies evaluated the stability of the tablets under the influence of increased temperature and humidity.

5.2 COMPATIBILITY STUDIES

Differential scanning calorimetry (DSC) thermograms for griseofulvin and excipients chosen were first obtained to analyse the thermal patterns of each pure compound. Exothermic reactions were characterized by upward movement of the thermal line on the thermogram whilst endothermic reactions were characterized by downward movements.

Thermograms of griseofulvin and excipients were interpreted as follows: a change in peak temperature or peak broadening of griseofulvin melting peak was considered as a low degree of incompatibility whilst disappearance of melting peaks for both griseofulvin and excipients or presence of new peaks were regarded as a high degree of incompatibility. A change in the shift of the peaks of substances of at least 10 ^{o}C was considered significant (Heljo, 2007: 6).

5.2.1 Differential scanning calorimetry thermogram of pure griseofulvin

The thermogram obtained for pure griseofulvin showed a peak at 218.9 ºC, onset temperature at 215.69 ºC and endset temperature at 221.01 ºC, as shown in Figure 5.1. Literature reveals that a single sharp melting endotherm occurs for griseofulvin with onset temperature at 216 ºC (Florey, 1983: 234). The peak obtained for pure griseofulvin correlates very well with literature.

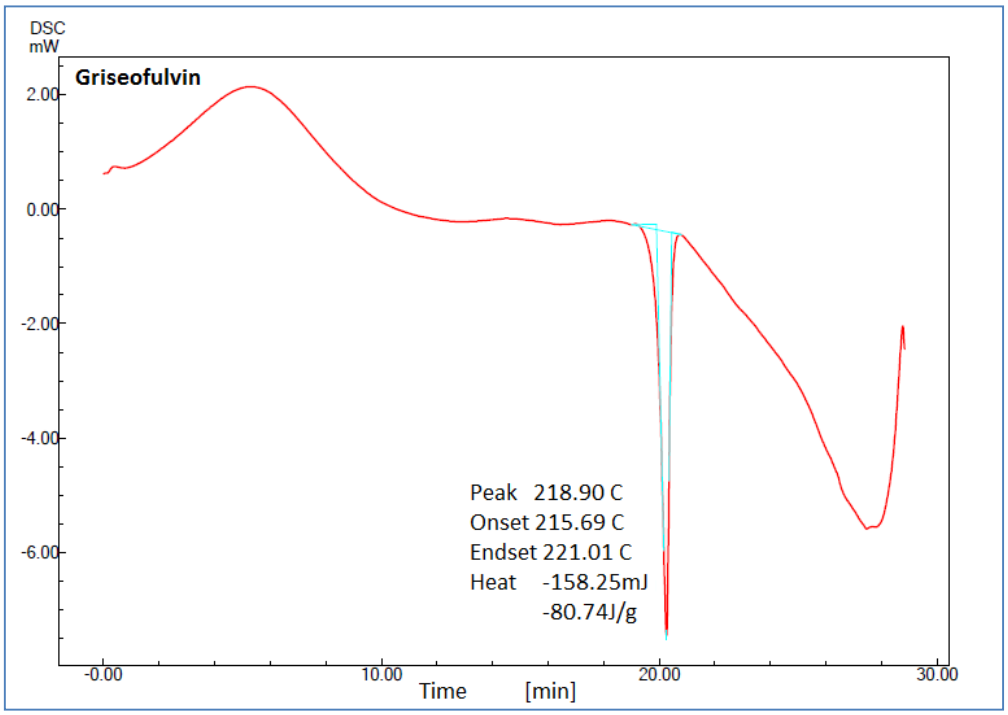


Figure 5.1: Differential scanning calorimetry thermogram of griseofulvin

5.2.2 Compatibility of griseofulvin with Accurel MP

The thermogram of Accurel MP showed an endothermic peak at 160.19 ºC with onset temperature at 152.62 ºC and endset temperature at 168.10 ºC. The thermogram of the 1:1 (w/w) griseofulvin/Accurel MP mixture curve was studied for changes in the characteristic peak of griseofulvin. The thermogram of 1:1 (w/w) griseofulvin /Accurel MP mixture showed a peak at 219.08 ºC with onset temperature at 216.38 ºC and endset temperature at 221.66 ºC, as shown in Figure 5.2. A comparison of the thermogram of griseofulvin to that of 1:1 (w/w) griseofulvin / Accurel MP mixture showed no significant change in enthalpy peak shape or onset, indicating the compatibility of griseofulvin with Accurel MP.

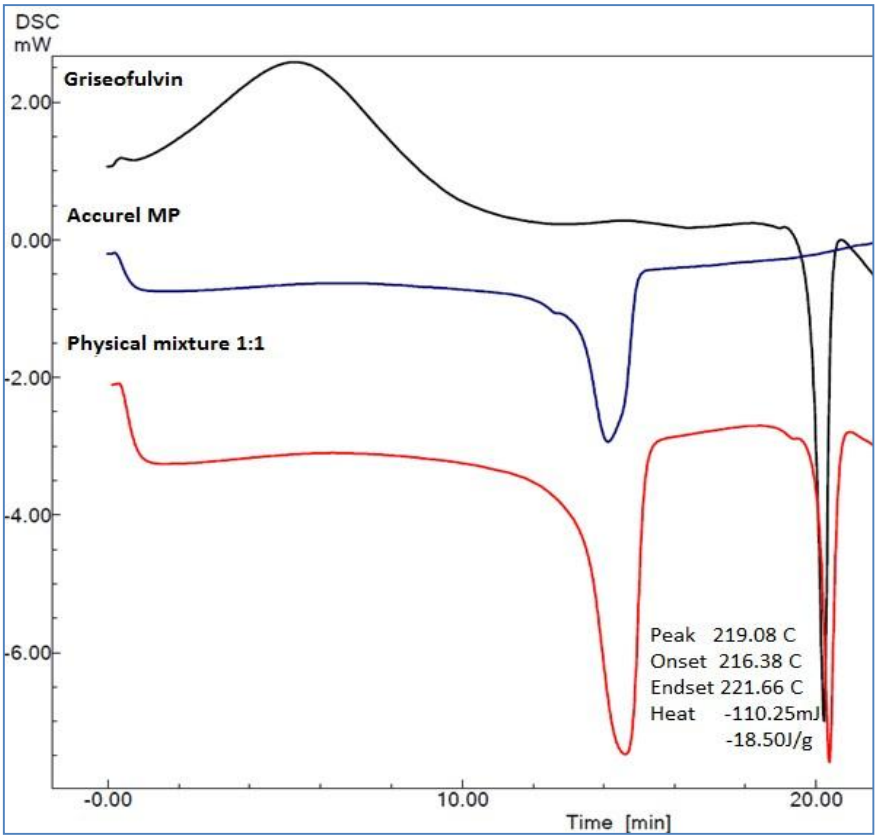


Figure 5.2: Thermograms of griseofulvin, Accurel MP and 1:1 (w/w) griseofulvin / Accurel MP mixture

5.2.3 Compatibility of griseofulvin with Methocel™

The thermogram of Methocel™ showed a broad endothermic peak at 89.37 ºC, with onset at 87.03 ºC and endset at 122.42 ºC. According to (Ahmed *et al.,* 2000: 69), the broad peak may be attributed to vaporization of adsorbed moisture of Methocel™. The thermogram of the 1:1 (w/w) griseofulvin/Methocel™™ mixture curve was studied for changes in the characteristic peak of griseofulvin. As shown in Figure 5.3, the thermogram of the 1:1 (w/w) griseofulvin/ Methocel™ mixture showed a peak at 223.30 ºC, with onset temperature at 219.22 ºC and endset temperature at 225.95 ºC. No significant change was observed in the thermoanalytical profiles of griseofulvin and Methocel™. It was observed that the DSC curve of the griseofulvin / Methocel™ mixture was superposition of griseofulvin and Methocel™, showing that the two are compatible.

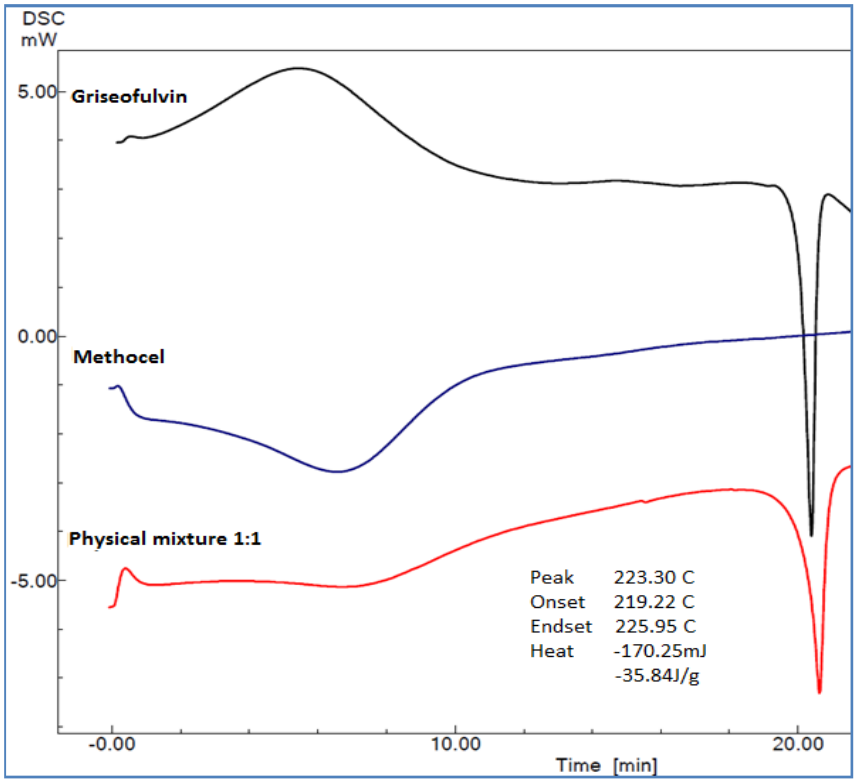


Figure 5.3: Thermograms of griseofulvin, Methocel™ and 1:1 (w/w) griseofulvin / Methocel™ mixture

5.2.4 Compatibility of griseofulvin with magnesium stearate

The thermogram of magnesium stearate showed an endothermic peak at 93.81 ºC, with onset temperature at 91.40 ºC and endset temperature at 96.90 ºC, as shown in Figure 5.4. The endothermic peak of magnesium stearate lies between a temperature range of 70-110 which is characteristic for the dehydration process of magnesium stearate (Ahmed *et al.,* 2000: 69). The thermogram of the 1:1 (w/w) binary mixture curve was studied for changes in the characteristic peak of griseofulvin. The thermogram of the 1:1 (w/w) griseofulvin/magnesium stearate mixture showed a peak at 224.50 ºC, with onset temperature at 219.65 ºC and endset temperature at 228.52 ºC. There was no significant shift in the melting peak of griseofulvin. As well, no significant change was observed in the endothermic peak of magnesium stearate. Therefore, griseofulvin and magnesium stearate were considered compatible.

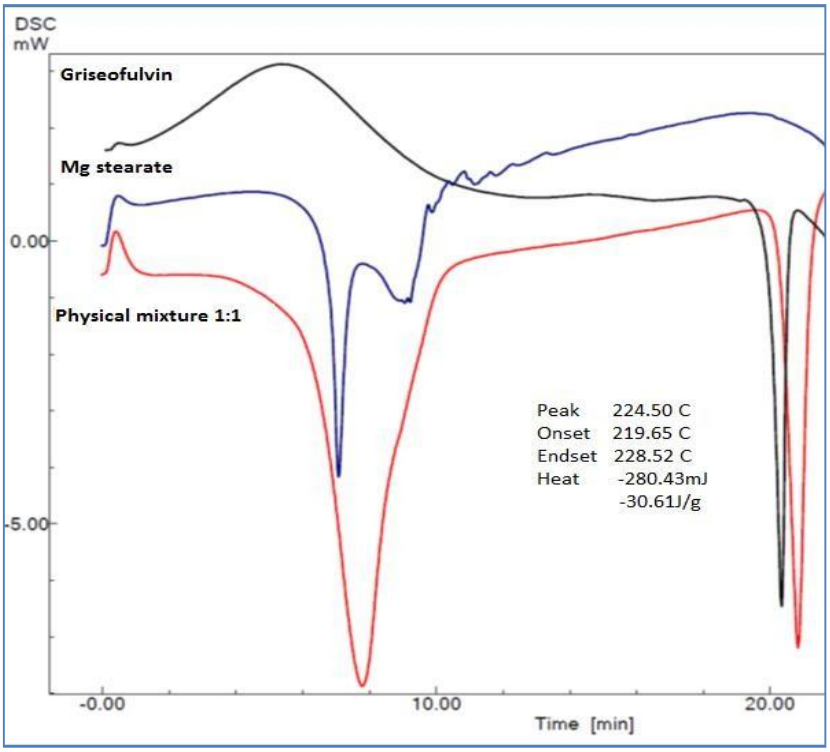


Figure 5.4: Thermograms of griseofulvin, magnesium stearate and 1:1 (w/w) griseofulvin / magnesium stearate

5.2.5 Compatibility of griseofulvin with PVP k-30

The thermogram of PVP k-30 showed an endothermic peak at 108.49 ºC, with onset temperature at 72.28 ºC and endset temperature at 143.63 ºC. Figure 5.5 shows a comparison of the thermograms of pure griseofulvin and PVP k-30 to that of 1:1 (w/w) griseofulvin / PVP k-30 mixture. The thermogram of griseofulvin / PVP k-30 mixture showed a slight broadening of the endothermic peak of griseofulvin (peak temperature 218.21 ºC, with onset temperature at 204.99 ºC and endset temperature at 224.97 ºC) when compared with the thermogram of pure griseofulvin (peak temperature of 218.9 ºC). The slight change in the shape of the endothermic peak of griseofulvin may be an indication of low degree of incompatibility between griseofulvin and PVP k-30.

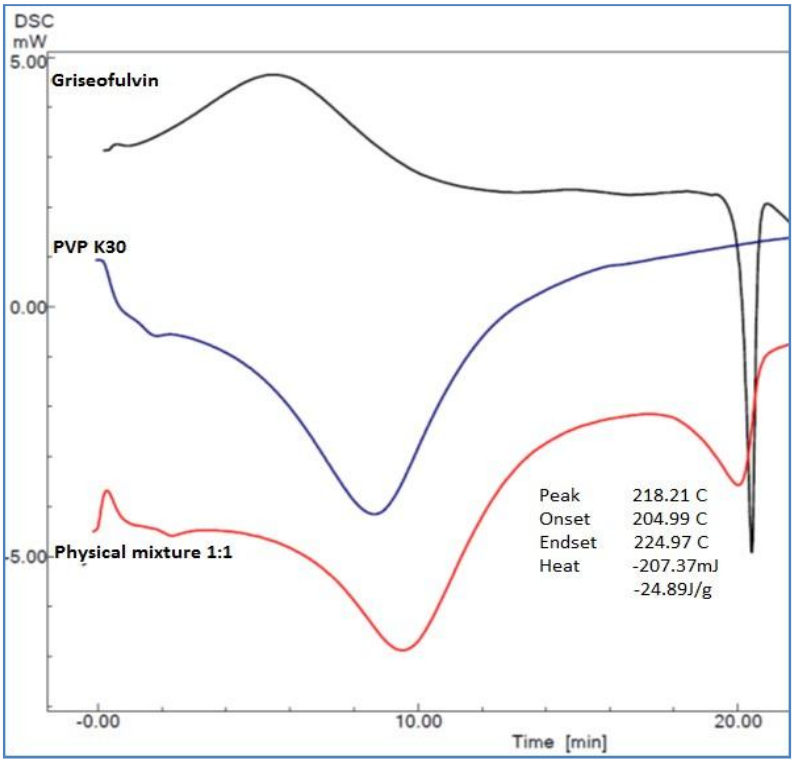


Figure 5.5: Thermograms of griseofulvin, PVP-k30 and 1:1 (w/w) griseofulvin/PVP-k30 mixture

Studies of the differential scanning calorimetry thermograms of griseofulvin and the excipients did not show incompatibility and for the PVP-k30, it appears there was low degree of incompatibility. Neither disappearance of melting peaks for both griseofulvin and excipients nor the presence of new peaks was observed, giving clear indication of compatibility of griseofulvin and the excipients.

5.3 FORMULATION DESIGN

As mentioned in the previous chapter, Design Expert Software Version 9.0 was used to generate formulation runs and further assess the influence of formulation and processing parameters on the tablet characteristics. Factors selected were Methocel™, Accurel MP, Polyvinylpyrrolidone and tablet hardness, as shown in Table 5.1. The responses chosen were lag time, total tablet floating time and percentage drug release at specific time intervals over twelve hours.

Table 5.1: Factors and levels for factorial design

Factor	Levels	
	Lower	Upper
X_1 Methocel (mg)	0	100
X_2 Accurel Mp (mg)	0	100
X_3 PVP k- 30 (%)	1	3
X_4 Tablet hardness (N)	8	12

As shown in Table 5.2, Design Expert Software Version 9.0 generated unique ratios of Methocel™, Accurel MP, Polyvinylpyrrolidone and Tablet hardness, per each run. A total of 25 runs were generated. Methocel™ and Accurel MP had lower limit of 25 mg and upper limit of 100 mg, Polyvinylpyrrolidone ranged between 1 – 3% and Tablet hardness was varied between 8 – 12 newtons. The results of formulation design are presented below in section 5.4 to 5.9.

Table 5.2: Composition of griseofulvin floating tablet formulations generated by Design Expert Software Version 9.0

RUN	Methocel (mg)	Accurel mp (mg)	PVP k – 30 (%)	Hardness (N)
1	65.5	100	2.08	10
2	25	91.375	1	9
3	72.625	25	2.17483	9
4	25	80.5	2.46	12
5	25	100	3	8
6	56.7405	62.5	2	8
7	65.5	100	2.08	10
8	100	25	3	8
9	64	65.125	3	10
10	73	92.5	1	12
11	56.7405	62.5	2	8
12	85	25	1	8
13	25.75	26.875	1	11
14	25	25	2.75	9
15	100	51.3394	1.7	11
16	100	100	3	12
17	100	98.125	3	8
18	100	95.875	1.69317	11
19	72.25	25	2.75	12
20	100	51.3394	1.7	11
21	25	100	1.22	12
22	64	65.125	3	10
23	64	65.125	3	10
24	100	100	1	8
25	54.5133	52.375	1.7238	12

5.4 PRE-COMPRESSION STUDIES

The flow properties of the powder granule mixture of each run were determined by measuring bulk density, tapped density, angle of repose, Hausner ratio and compressibility of the powder before compression, as described in chapter 4.5.1. Table 5.3 shows the results of the pre-compression studies.

Table 5.3: Pre-compression parameters of griseofulvin tablets formulation runs

Batch	BD g/ml	TD g/ml	CI %	H	C	AR (ɵ) ± S.D	FP	LOD %
Run 1	0.18	0.27	17.73	1.18	Fair	37.4 ± 1.3	Fair	0.43
Run 2	0.20	0.21	7.59	1.08	Excellent	32.2 ± 1.4	Good	0.21
Run 3	0.22	0.26	18.00	1.18	Fair	34.5 ± 1.5	Good	0.32
Run 4	0.18	0.21	14.89	1.18	Fair	32.2 ± 0.9	Good	1.65
Run 5	0.17	0.19	10.76	1.12	Good	30.5 ± 0.7	Good	2.71
Run 6	0.19	0.22	13.69	1.16	Good	32.8 ± 1.4	Good	0.54
Run 7	0.18	0.22	19.56	1.24	Fair	33.6 ± 1.3	Good	3.43
Run 8	0.23	0.27	16.67	1.20	Fair	33.3 ± 0.8	Good	2.21
Run 9	0.18	0.21	12.35	1.14	Good	33.9 ± 2.1	Good	0.25
Run 10	0.18	0.21	12.50	1.14	Good	32.4 ± 0.6	Good	1.58
Run 11	0.19	0.22	13.57	1.16	Good	34.4 ± 0.7	Good	2.73
Run 12	0.26	0.30	12.69	1.15	Good	34.9 ± 1.4	Good	4.65
Run 13	0.21	0.25	13.72	1.16	Good	34.4 ± 0.7	Good	2.54
Run 14	0.22	0.27	19.35	1.24	Fair	33.8 ± 1.9	Good	3.44
Run 15	0.19	0.22	14.2	1.17	Good	35.1 ± 0.7	Good	0.87
Run 16	0.19	0.21	11.98	1.14	Good	34.9 ± 1.2	Good	1.74
Run 17	0.18	0.21	13.78	1.16	Good	35.7 ± 1.7	Good	1.32
Run 18	0.18	0.20	12.80	1.14	Good	32.4 ± 1.6	Good	0.13
Run 19	0.20	0.23	14.46	1.17	Good	35.6 ± 1.4	Good	3.72
Run 20	0.19	0.22	12.09	1.14	Good	33.2 ± 2.0	Good	1.93
Run 21	0.16	0.19	14.16	1.16	Good	32.4 ± 1.3	Good	1.21
Run 22	0.19	0.23	14.52	1.17	Good	32.8 ± 1.5	Good	0.34
Run 23	0.19	0.21	12.78	1.15	Good	33.1 ± 1.3	Good	2.38
Run 24	0.19	0.21	13.00	1.15	Good	33.4 ± 1.4	Good	0.67
Run 25	0.19	0.22	13.59	1.16	Good	35.2 ± 1.2	Good	2.43

***BD =** Bulk Density, **TD =** Tapped Density, **CI =** Compressibility Index**, H =** Hausner ratio, **AR =** Angle of Repose, **LOD =** Loss On Drying, **C =** Compressibility, **FP =** Flow Properties & **S.D:** standard deviation**.**

As shown in Table 5.3, all formulations had values of Carr's Index and Hausner ratios of less than 20.0 and 1.25 respectively. Run 2 had excellent compressibility, 18 runs had good compressibility and 6 runs had fair compressibility. Carr's Index and Hausner ratios which reflect the impact of

tapping on particle packing and are influenced by particle size, shape and cohesivity predicted that all the formulations were compressible. Twenty four of the runs had granules with good flowability. The only exception, Run 1, had granules with fair flowability. However, all formulations had an angle of repose less than 40^{O}, an angle acute enough to predict weak intermolecular forces of attraction between granules. It was thus satisfactory to proceed to compression without any further recommendations to aid flowability of the granules. All formulation runs had water content (loss on drying) of less than 5.0 %.

5.5 POST COMPRESSION STUDIES

The quality control aspects of formulated tablets such as weight variation, hardness, diameter, thickness and friability were evaluated as described in chapter 4.5.2 and the results are represented in table 5.4.

Table 5.4: Post-compression parameters of griseofulvin floating tablets formulation runs

B. No:	WV (g) ± S.D	H (N) ± S.D	D (mm) ± S.D	T (mm) ± S.D	F (%)
Run 1	0.2909 ± 0.009	10.1 ± 1.2	12.00 ± 0.1	3.40 ± 0.1	0.14
Run 2	0.2282 ± 0.006	8.8 ± 0.6	12.00 ± 0.1	2.70 ± 0.1	0.06
Run 3	0.2357 ± 0.018	10.8 ± 1.6	12.01 ± 0.1	2.22 ± 0.1	0.08
Run 4	0.2274 ± 0.012	8.6 ± 1.0	12.03 ± 0.1	2.52 ± 0.1	0.10
Run 5	0.2392 ± 0.010	11.3 ± 1.1	12.02 ± 0.1	2.48 ± 0.1	0.05
Run 6	0.2474 ± 0.007	9.0 ± 1.0	12.00 ± 0.1	2.68 ± 0.1	0.21
Run 7	0.2834 ± 0.014	11.4 ± 1.8	12.00 ± 0.1	3.08 ± 0.1	0.12
Run 8	0.2652 ± 0.009	8.6 ± 0.7	12.00 ± 0.1	2.58 ± 0.1	0.06
Run 9	0.2491 ± 0.009	11.7 ± 0.9	12.01 ± 0.1	2.52 ± 0.1	0.08
Run10	0.2981 ± 0.009	12.1 ± 1.3	12.00 ± 0.1	3.20 ± 0.1	0.14
Run11	0.2278 ± 0.017	9.1 ± 0.5	12.00 ± 0.1	2.00 ± 0.1	0.06
Run 12	0.1963 ± 0.014	7.2 ± 1.5	12.00 ± 0.1	1.90 ± 0.1	0.04
Run 13	0.1292 ± 0.005	6.6 ± 1.4	12.00 ± 0.1	1.18 ± 0.1	0.12
Run 14	0.1546 ± 0.008	9.7 ± 0.4	12.00 ± 0.1	1.24 ± 0.1	0.06
Run 15	0.2512 ± 0.010	11.0 ± 1.3	12.04 ± 0.1	2.50 ± 0.1	0.02
Run 16	0.2922 ± 0.013	10.4 ± 0.6	12.00 ± 0.2	3.26 ± 0.1	0.07
Run 17	0.3062 ± 0.013	10.0 ± 0.8	12.00 ± 0.1	3.38 ± 0.1	0.08
Run 18	0.3069 ± 0.014	8.4 ± 0.9	12.02 ± 0.1	3.46 ± 0.1	0.04
Run 19	0.1871 ± 0.009	8.2 ± 0.3	12.00 ± 0.1	1.64 ± 0.1	0.14
Run 20	0.2497 ± 0.016	11.4 ± 1.6	12.00 ± 0.1	2.30 ± 0.1	0.01
Run 21	0.2258 ± 0.009	11.3 ± 1.0	12.00 ± 0.1	2.30 ± 0.1	0.04
Run 22	0.2442 ± 0.010	10.5 ± 1.0	12.03 ± 0.2	2.54 ± 0.1	0.08
Run 23	0.2447 ± 0.006	11.0 ± 0.5	12.00 ± 0.1	2.51 ± 0.1	0.11
Run 24	0.3267 ±0.014	10.0 ± 1.0	12.00 ± 0.1	3.84 ± 0.1	0.05
Run 25	0.2113 ± 0.007	11.6 ± 0.7	12.01 ± 0.1	2.06 ± 0.1	0.08

***WV =** Weight Variation, **H =** Hardness, **D =** Diameter, **T =** Thickness, **T =** Thickness, **F =** Friability & **S.D:** standard deviation**.**

Consistency of tablet weight within each run was assessed by the weight variation test. From a sample of twenty tablets from each run, not more than two tablets were outside a weight range of 7.5% from their mean. This confirmed consistency of weight uniformity and all formulation runs passed weight variation test.

Tablets with high content of Methocel™ were compressed to the required hardness, whereas tablets with high content of Accurel MP compared to Methocel™ were difficult to compress to the required hardness due to its less compressibility associated with reduced friction between particles.

The values of diameter, thickness and hardness for all formulation runs were taken and the deviation of each calculated. None of the deviations of diameter, thickness or hardness exceeded ± 5%. All formulation runs passed the diameter, thickness and hardness test. All formulation runs had friability of less than 1%. Therefore all formulation runs passed friability test and confirmed that the tablets produced were capable enough to resist breakage under stress conditions during handling. The tablets produced were of acceptable quality according to USP standards.

5.6 ASSAY

The assay of griseofulvin content in tablets was conducted as described in chapter 4.5.4. The quantity of griseofulvin in the compressed tablets was calculated and results obtained are shown in table 5.5.

Table 5.5: Assay of griseofulvin tablets

B. No:	Quantity ± SD (mg)	Percentage assay	B.No:	Quantity ± SD mg	Percentage Assay
Run 1	98.7 ± 2.4	98.7%	Run 14	104.6 ± 0.4	104.6%
Run 2	95.7 ± 1.6	95.7%	Run 15	101.6 ± 2.7	101.6%
Run 3	102.9 ± 2.6	102.9%	Run 16	96.4 ± 2.1	96.4%
Run 4	99.4 ± 2.1	99.4%	Run 17	97.8 ± 2.4	97.8%
Run 5	95.7 ± 0.9	95.7%	Run 18	95.3 ± 1.5	95.3%
Run 6	96.6 ± 2.1	96.6%	Run 19	96.3 ± 2.1	96.3%
Run 7	97.9 ± 1.5	97.9%	Run 20	98.1 ± 2.4	98.1%
Run 8	97.0 ± 1.8	97.0%	Run 21	95.3 ± 1.3	95.3%
Run 9	96.2 ± 1.1	96.2%	Run 22	95.5 ± 1.2	95.5%
Run 10	104.6 ± 0.4	104.6%	Run 23	97.6 ± 1.3	97.6%
Run 11	101.6 ± 1.9	101.6%	Run 24	96.2 ± 1.8	96.2%
Run 12	96.4 ± 2.2	96.4%	Run 25	97.8 ± 2.1	97.8%
Run 13	97.8 ± 3.1	97.8%			

The British Pharmacopoeia (BP) specifies that griseofulvin tablets should contain not less than 95.0% and not more than 105.0% of the labelled amount. As shown in table 5.5 above, all formulation runs had percentage griseofulvin content within the acceptable range and hence the formulated tablets complied with the USP specification. The formulated tablets contained the claimed amount of griseofulvin, 100 mg.

5.7 BUOYANCY STUDIES

Buoyancy studies, which consist of determination of both lag time and total floating time were performed on six of the formulated tablets per formulation run to assess the floating characteristics of the tablets. Lag time is the time it takes for the floating dosage forms to float at the surface of a liquid medium. Total floating time is the total time the dosage forms float on the surface of the medium. Table 5.6 shows the results obtained from buoyancy studies.

Table 5.6: Buoyancy capabilities of griseoufulvin tablets formulation runs

Batch number	**Lag time (min)**	**Floating time (hours)**	**Density (g/cm^3)**
Run 1	0	+24	0.00076
Run 2	0	+24	0.00075
Run 3	120	0	0.00094
Run 4	0	+24	0.00079
Run 5	0	+24	0.00085
Run 6	0	+24	0.00082
Run 7	0	+24	0.00081
Run 8	120	0	0.00091
Run 9	2 ± 0.3	+24	0.00087
Run10	0	+24	0.00082
Run 11	120	0	0.00101
Run 12	120	0	0.00091
Run 13	120	0	0.00097
Run 14	120	0	0.00110
Run 15	45 ± 1.5	0	0.00088
Run 16	0	+24	0.00079
Run 17	0	+24	0.00080
Run 18	0	+24	0.00078
Run 19	120	0	0.00101
Run 20	120	0	0.00096
Run 21	0	+24	0.00087
Run 22	0	+24	0.00085
Run 23	0	+24	0.00086
Run 24	0	+24	0.00075
Run 25	50	+24	0.00090

For floating, tablets had to be less dense than the release medium. It was desirable that floating tablets have a lag time of less than 10 minutes. Also, it was desirable that tablets float for at least 24 hours. As shown in Table 5.6, 15 formulation runs produced tablets that floated immediately upon contact with the release medium (zero lag time) and floated for at least 24 hours. It was also observed all the formulation runs that produced tablets that floated had densities less than 0.00091 g/cm^3. Although formulation runs 15 and run 25 had total floating time of 24 hours,

they had lag times of 45 minutes and 50 minutes respectively, and so failed buoyancy test. The rest of the formulation runs (run 3, run 8, run 11, run 12, run 13, run 14, run 19 and run 20) never floated and totally failed the test. The formulation runs that failed buoyancy test had tablets with densities of more than 0.00091 g/cm^3.

The effects and influence of formulation variables (Mehtocel, Accurel MP and Polyvinylpyrrolidone) and processing variable (hardness) on the lag time and total floating time of the tablets were statistically evaluated by applying ANOVA using Design Expert® version 9.0 (Stat-Ease, Inc, USA). A model that best fit the collected data was selected based on several statistical parameters including the predicted residual sum of square (PRESS) and p-value. Overally, values of “Prob > F” less than 0.0500 indicate model terms are significant. A model with the least PRESS value indicates a model that best fit the data. As shown in Figure 5.6a below, linear model was the best fit model for lag time. The linear model had a sequential p-value of 0.0003, had a lack of fit value of 0.8541 and the least PRESS value, making it the most significant model. Linear model was as well the best fit model for floating time, as shown in Figure 5.6b. The model had a sequential p-value of 0.0021, lack of fit value of 0.9118 and the least PRESS value.

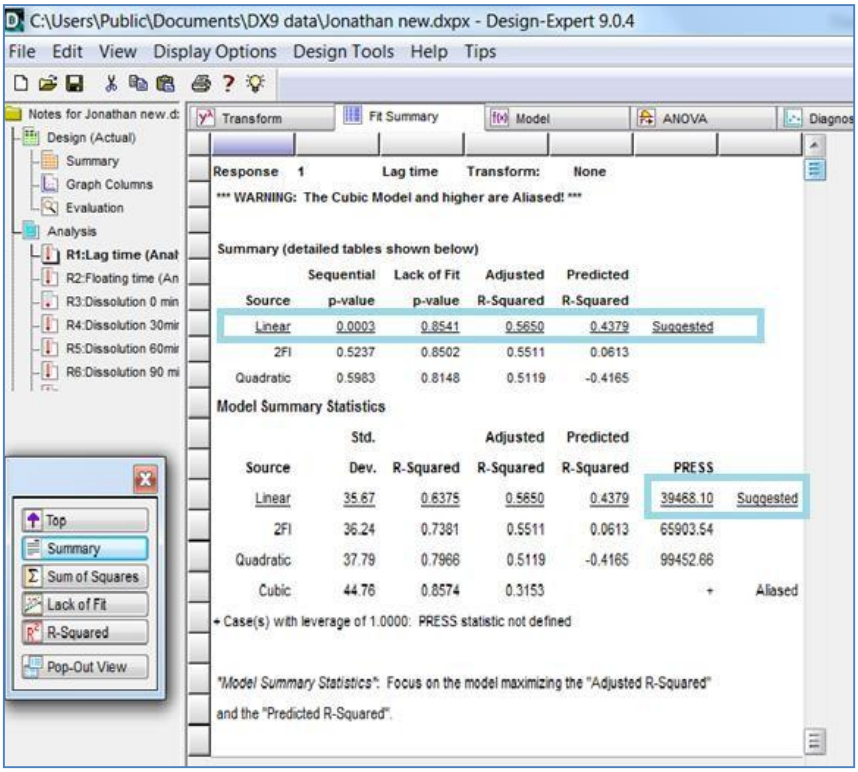


Figure 5.6a: Statistical analysis of model of fit for lag time using design expert.

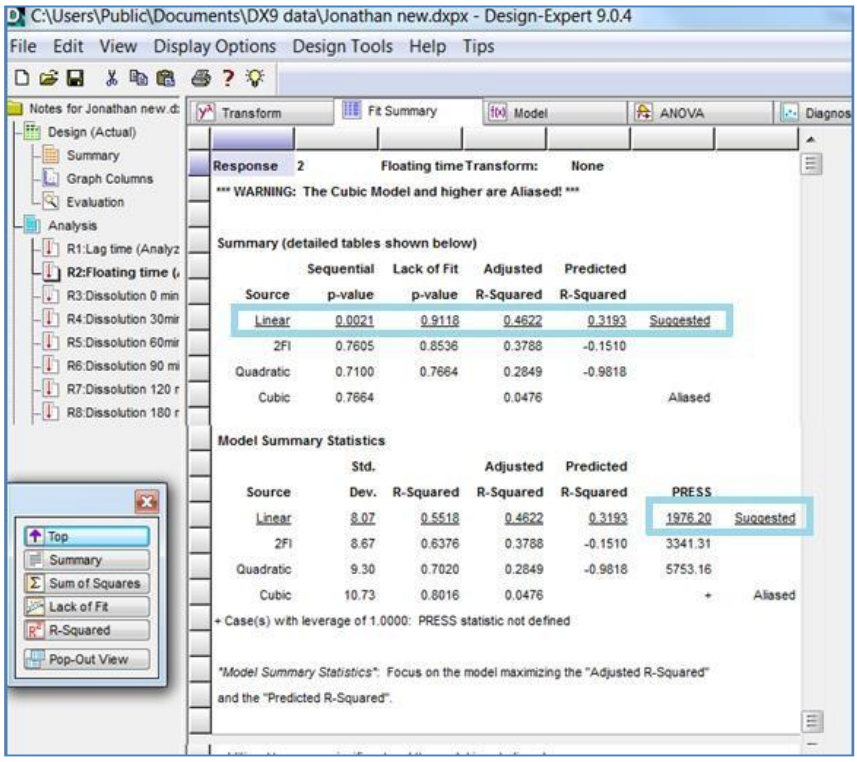


Figure 5.6b: Statistical analysis of model of fit for floating time using design expert.

5.7.1 Analysis of variance on lag time

Using Design Expert 9.0, it was determined that Accurel MP had the most significant influence on lag time. Values of “Probability > F” less than 0.0500 indicate model terms are significant. As shown in Figure 5.7, Accurel MP had a value of “Probability > F” of less than 0.0001, indicating that it had a significant effect on tablet lag time. Whereas for Methocel™ (0.8042), PVP (0.1059), hardness (0.9079) are more than 0.0500 indicating changes in these parameters do not have significant influence on the floating time.

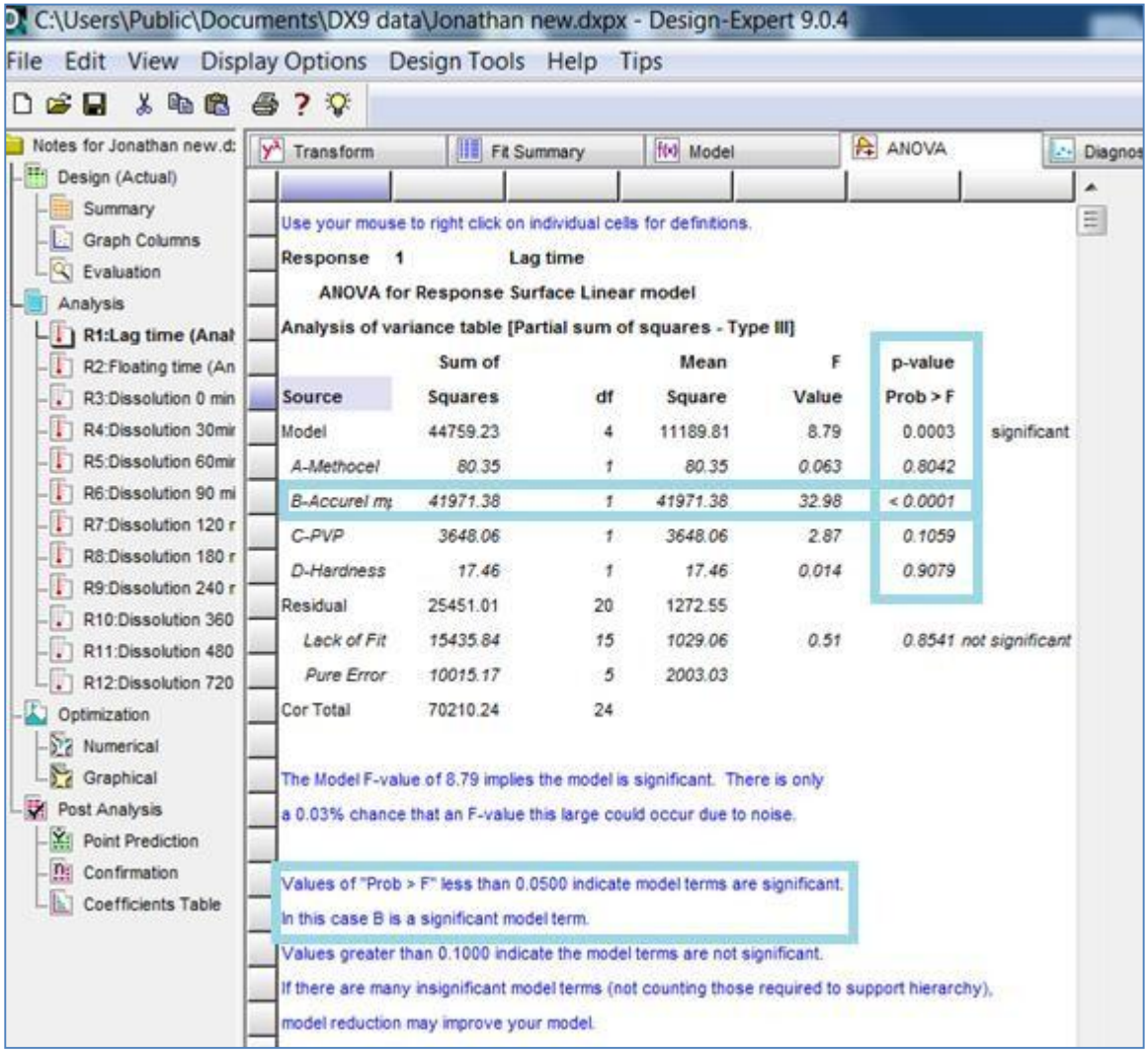


Figure 5.7: Analysis of variance for the influence of factors on lag time

5.7.2 Analysis of variance on floating time

As shown in Figure 5.8, Accurel MP had a value of “Probability > F” of less than 0.0001, indicating that it had a significant effect on tablet floating time. Whereas for Methocel™ (0.6593), PVP (0.2090), hardness (0.6983) are more than 0.0500 indicating changes in these parameters do not have significant influence on the floating time.

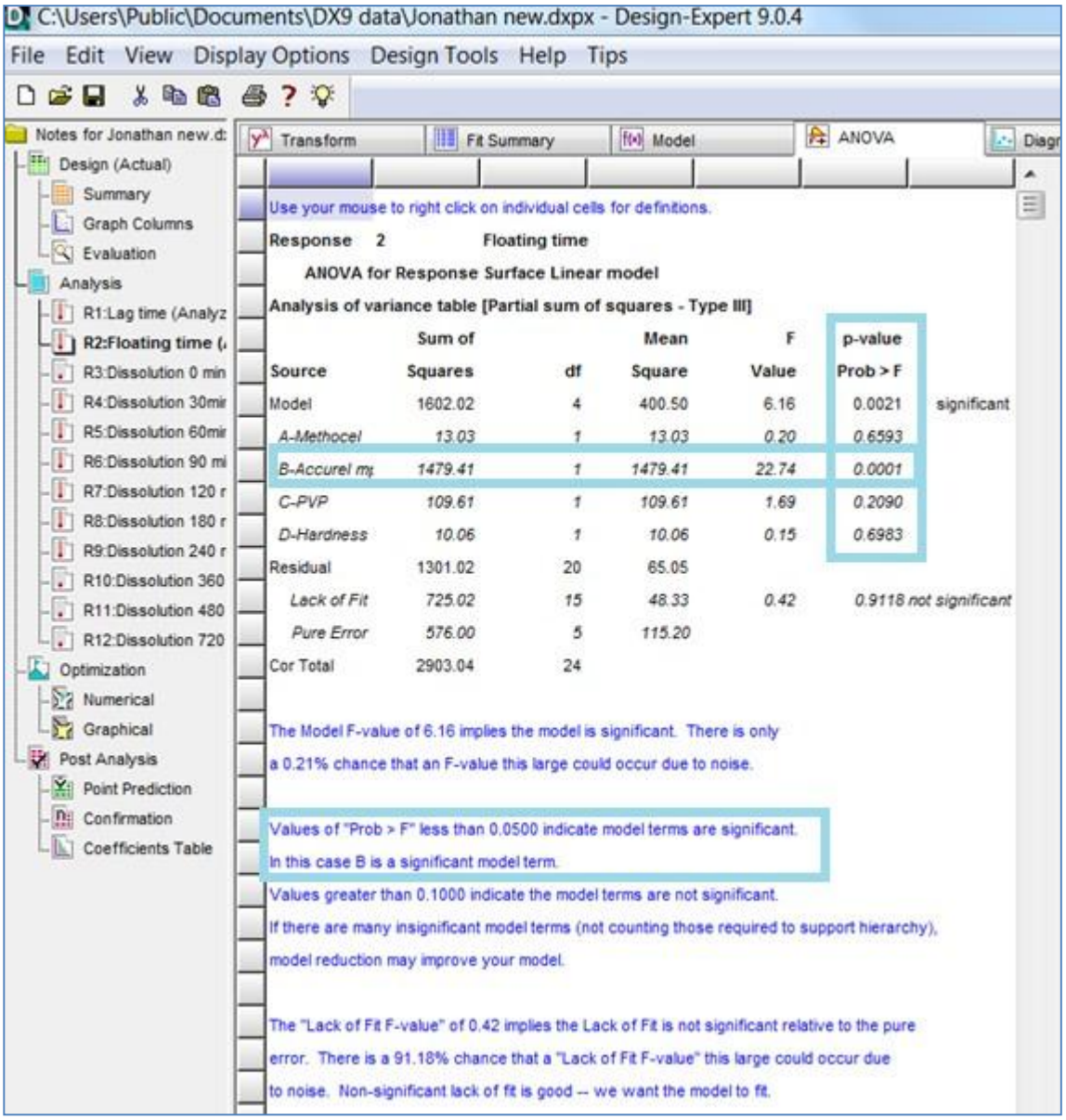


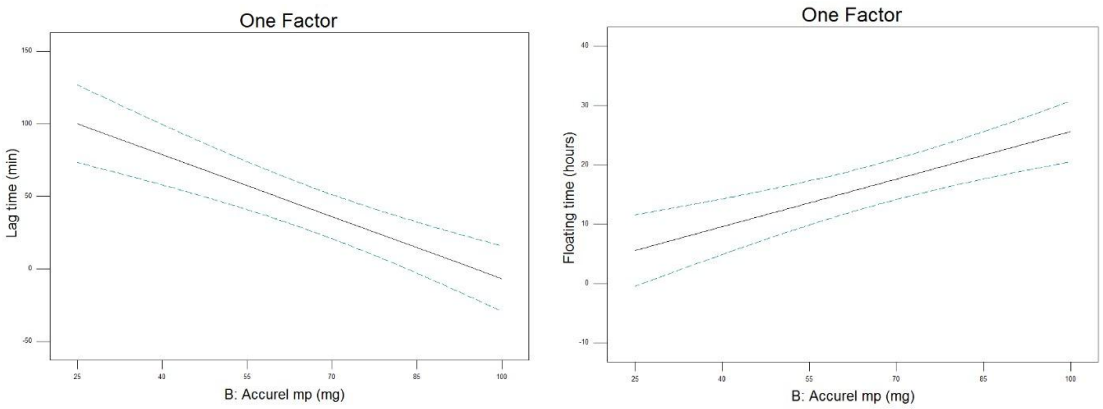
Figure 5.8: Analysis of variance for the influence of factors on floating time

5.7.3 Influence of Accurel MP on tablet buoyancy

Design Expert was used to assess how Accurel MP influenced lag time and floating time and to determine optimized quantity of Accurel MP. As shown in Figure 5.9, an increase in Accurel MP decreased lag time. Accurel MP, a highly porous foam powder provides tablets that have densities that are lower than that of the release medium.

Based on the mass of the tablets, it was observed that approximately 20% w/w of Accurel MP was sufficient to achieve proper floating behaviour for at least 24 hours. All the formulations that had at least 20% w/w Accurel MP floated for at least 720 minutes with the exception of formulation runs 11 and 20 which had percentage by mass of Accurel MP of 28.5 % and 20.4 % respectively. Although these formulation runs had more than 20 % of Accurel MP, they did not float because they had densities more than 0.00091 g/cm^3. For a formulation to produce tablets that float for at least 720 minutes, it had to contain at least 20 % of Accurel MP and the tablets had to have a density of less than 0.00091 g/cm^3. It is also observed that for tablets to float immediately upon contact with release medium, they had to have at least 28 % of Accurel MP.

The exception is seen with formulation runs 15 and 25 which still floated for 720 minutes because they had percentage Accurel MP of more than 20 % (20.4 % for run 15 and 25.3 % for run 25), but because the percentages were less than 28 %, they had lag times of 45 minutes and 50 minutes respectively. It can be concluded that for tablets to float immediately upon contact with release medium and to float for at least 24 hours, they had to contain at least 28 % Accurel MP and had to have densities less than 0.00091 g/cm^3.

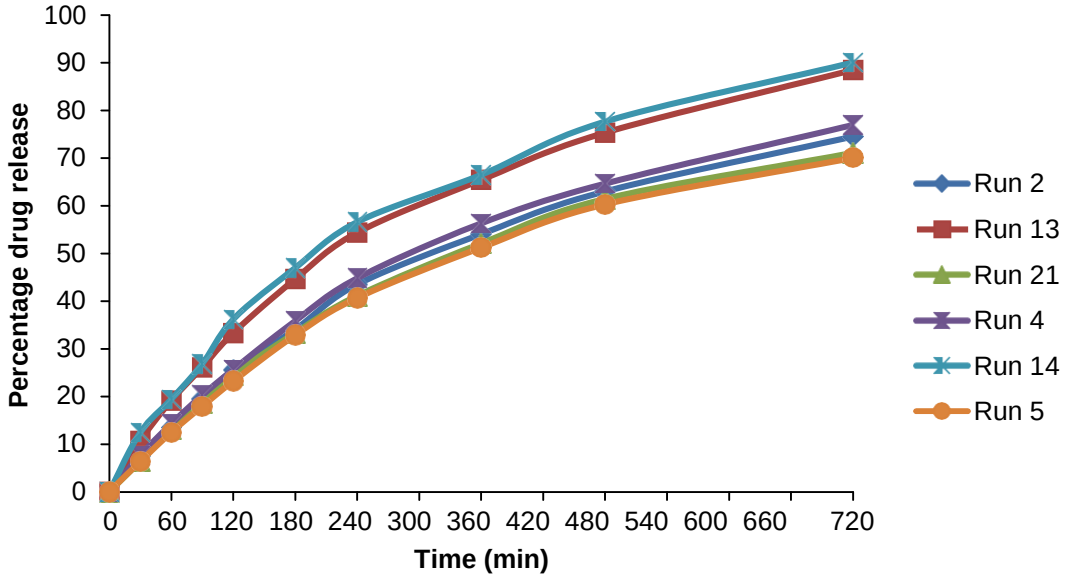


(a) (b)

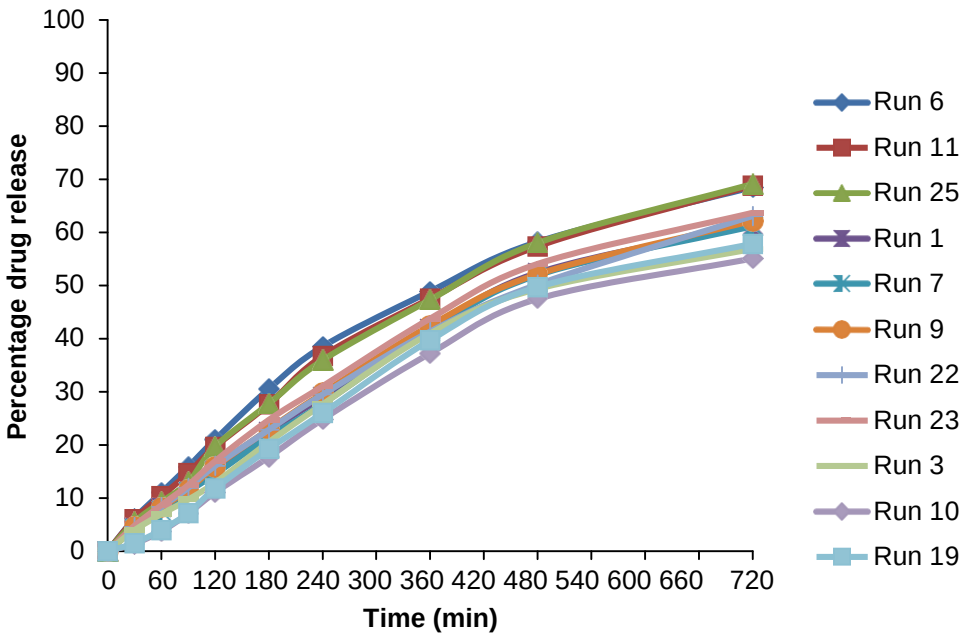
Figure 5.9: Influence of Accurel MP on lag time and total floating time

5.8 DISSOLUTION STUDIES

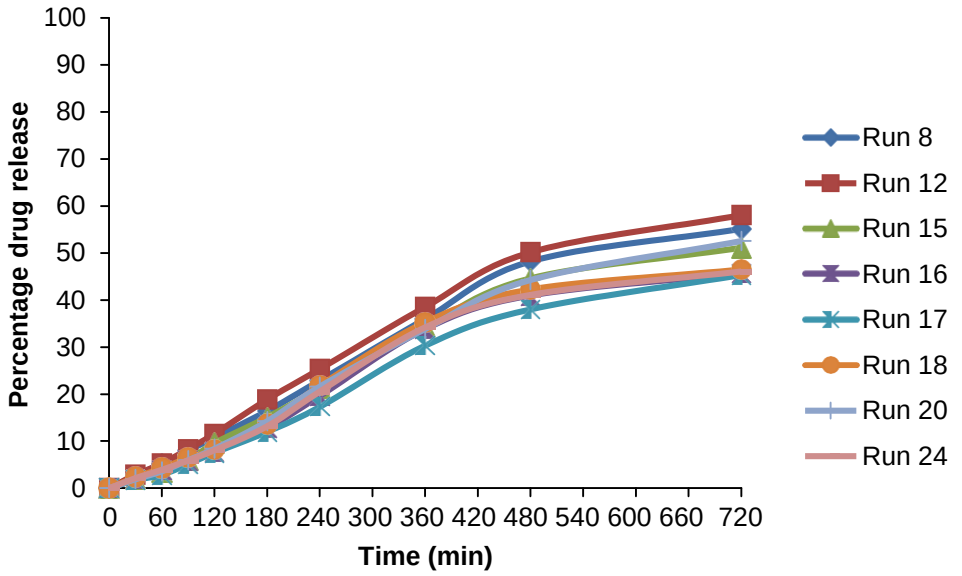
Dissolution studies for all the 25 formulation runs were carried out as described in the previous chapter. The desired drug release pattern for the sustained release of griseofulvin from the floating tablets was zero order, a pattern that is predictable and that releases drug at a constant rate. Also, a zero order release pattern would minimize griseofulvin plasma concentration fluctuations over the time of drug release. Figure 5.10 shows an overview of the *in-vitro* drug release profiles of the formulation runs.



5.10a: *In-vitro* drug release profile of the formulation runs with 25% Methocel^{TM}



5.10b: *In-vitro* drug release profile of the formulation runs with 50 to 75% Methocel™



5.10c: *In-vitro* drug release profile of the formulation runs with100% Methocel™

Highest percentage drug release was achieved by formulation runs that had the least amount of Methocel™ of 25 mg, drug release ranging from 70.1 % to 90.1 % (Figure 5:10a). Formulation run 14 showed the highest final drug release of 90.1%. Formulation 14 had the least amount of Accurel MP, 25 mg, which could have influenced the high drug release rate. Figure 5:10b shows drug release from

formulation runs which had an amount of Methocel™ that ranged from 54 mg to 73 mg. Drug release from these formulations ranged from 55.1 % to 69.1 %. Figure 5:10c shows drug release from formulation runs that had the highest amount of Methocel™, 100 mg. These formulation runs showed the least drug release rate and the least total drug release, ranging from 45.3 % to 58.0 %. Run 17 had the least final drug release of 45.3 %.

5.8.1 ANOVA analysis of factors influencing drug release at 120 minutes

Design Expert was used to describe and further explain the effects the formulation factors (Accurel MP, polyvinnylpyrolidone and Methocel™) and process parameter (tablet hardness) had on drug release profile. A quadratic model was the best fit model to describe the initial drug release profile (time 0 to 120 minutes). As shown in Figure 5.11, quadratic model had a sequential p-value of 0.0290 (less than 0.05), it had the highest lack of fit p-value of 0.0208 and had the least PRESS value of 178.55, qualifying it to be the best fit model.

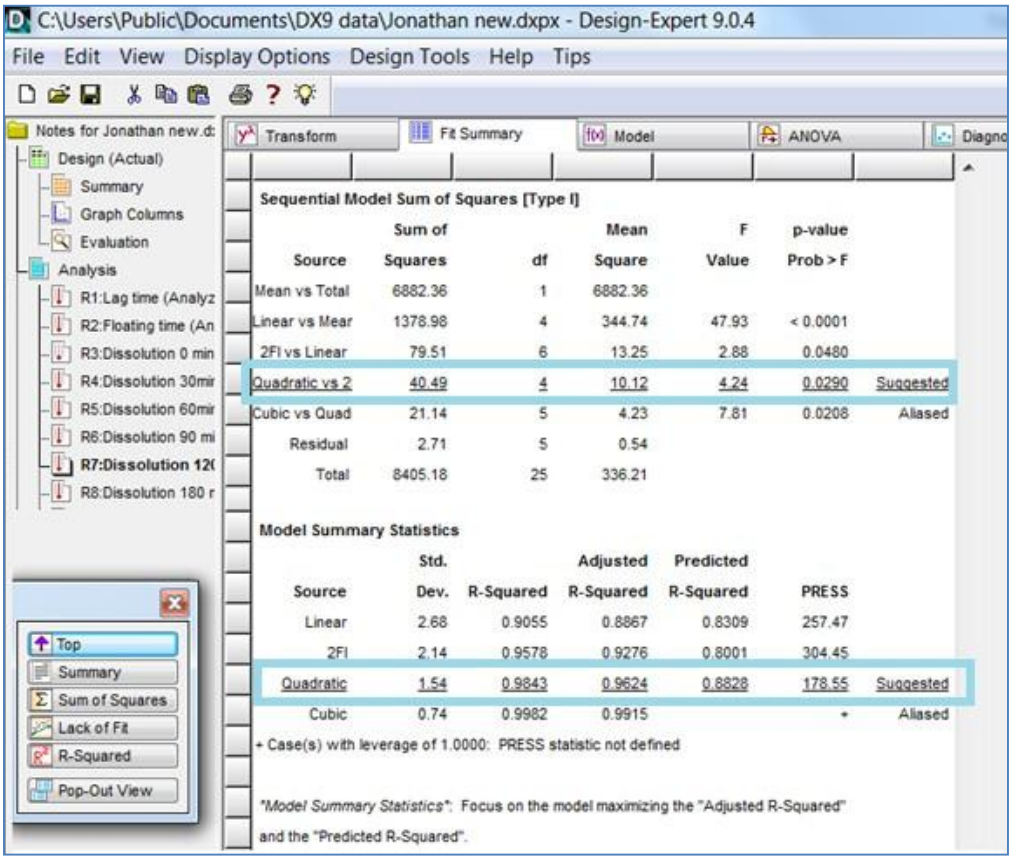


Figure 5.11: Statistical analysis of model fit using design expert for drug release up to 120 minutes

Analysis of Variance (ANOVA) was applied to assess the significance the chosen factors had on drug release. As shown in Figure 5.12, Methocel™, Accurel MP and tablet hardness had significant influence on drug release as indicted by p-values less than 0.05. In contrast, polyvinylpyrrolidone had a p-value of 0.9091, signifying it had no significant effect on drug release.

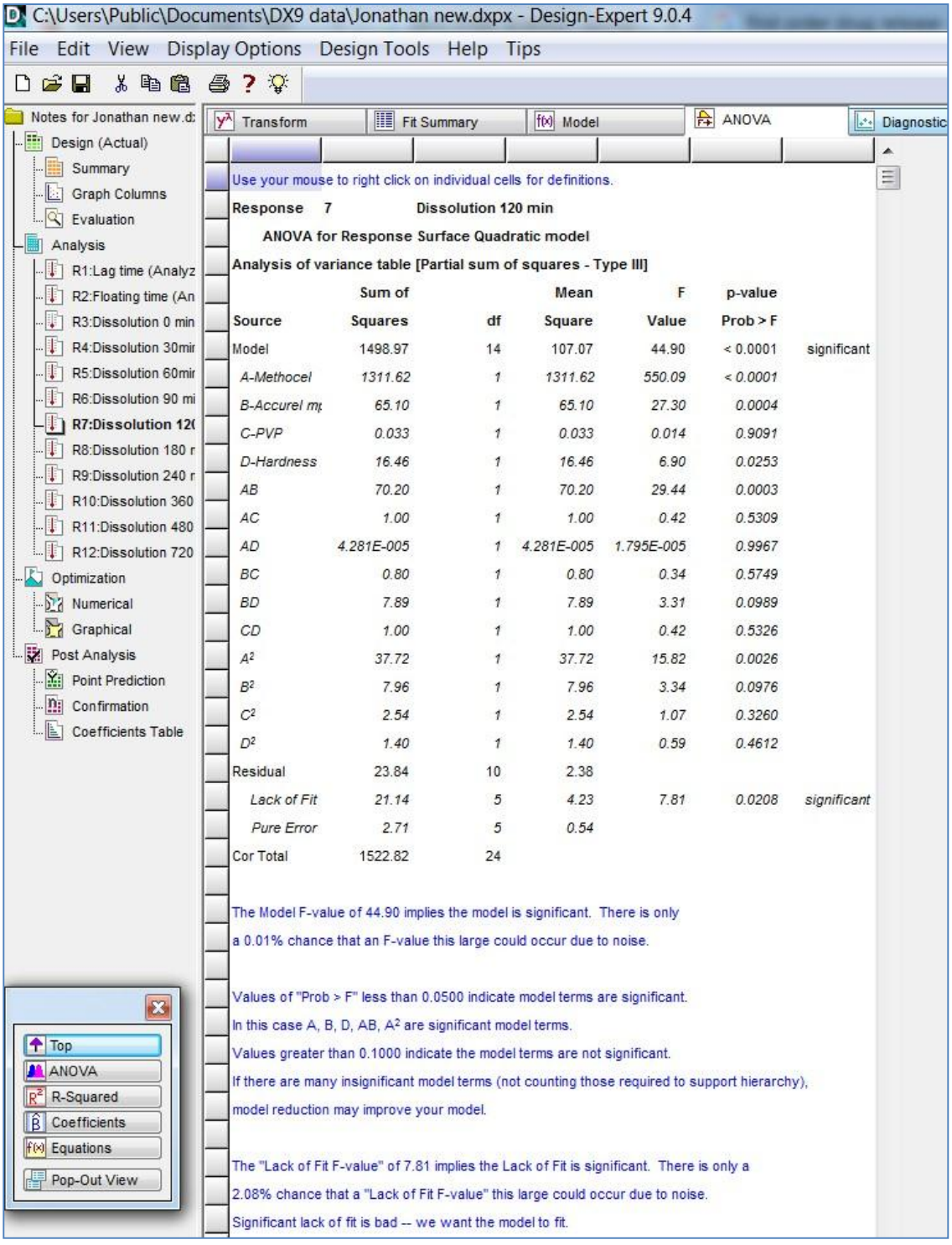
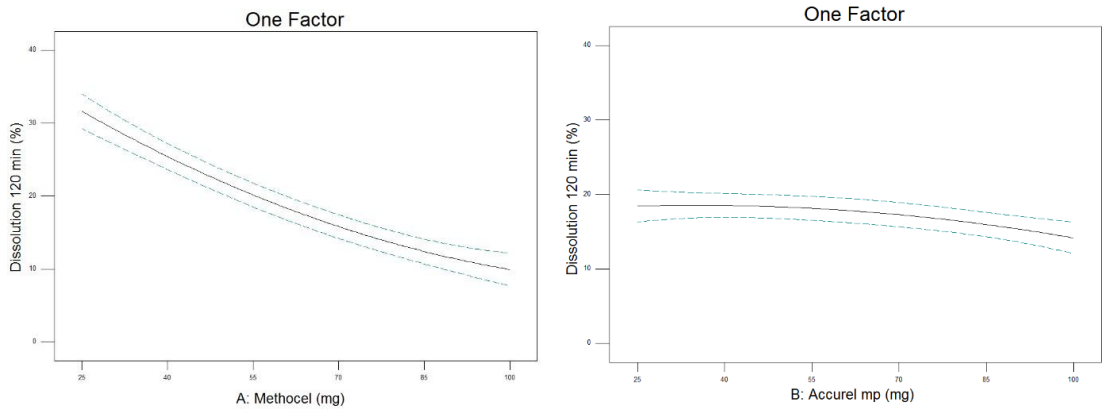
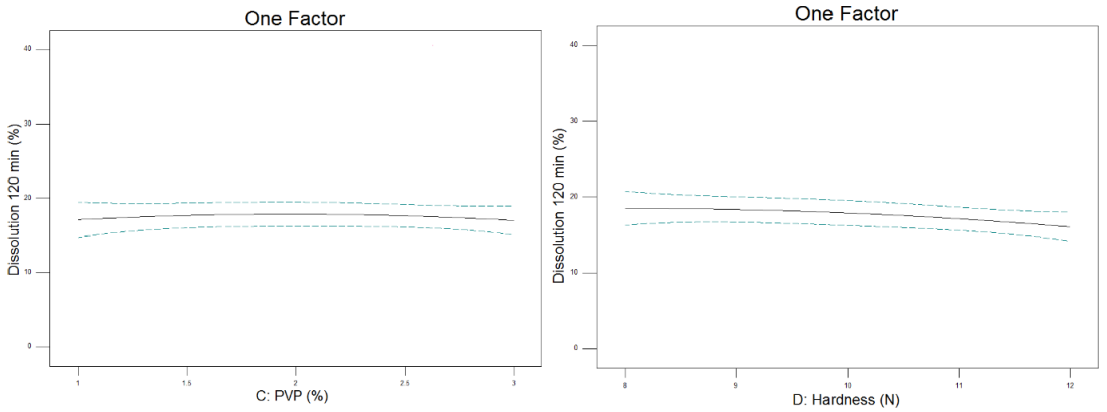


Figure 5.12: ANOVA for the influence of factors on drug release up to 120 minutes



(a) (b)



(b) (d)

Figure 5.13: Relationship between each factor and griseofulvin release up to 120 minutes

As expected, Methocel™, a drug release retardant polymer, had the most significant effect on griseofulvin release. Methocel™ quickly hydrates on the outer tablet skin to form a gelatinous layer, controlling the penetration of water into the tablet. As the outer gel layer fully hydrates and dissolves, a new inner layer replaces it and becomes cohesive and continuous enough to retard the influx of water and controls drug diffusion (Maity *et al.,* 2014: 17).

An increase in Methocel™ decreased griseofulvin release, Figure 5.13a, with a maximum of 36.2% release corresponding to 25 mg Methocel™ and a minimum of 7.6% release, corresponding to 100 mg Methocel™ after 120 minutes. An increase in Accurel MP slightly decreased the release of griseofulvin, as shown in Figure 5.13b. Accurel MP is highly porous and hydrophobic having open cell structure,

particle size less than 1500 µm, pore size in the range from 5-20 µm, void volume of 70% and has very limited desorption (Ahuja & Patahk, 2013: 241). This could be the reason for the slight decrease in the release of griseofulvin with an increase in the amount of Accurel MP.

Increase in tablet hardness slightly decreased the release of griseofulvin. Although low tablet hardness causes wider pore spaces within the tablet suggesting faster permeation of the dissolution medium, tablets with low hardness swell immediately forming a gel-like layer around the tablet and blocking the surface pores, thereby reducing drug release rate and counteracting the effect of increase pore sizes (Varma *et al.,* 2004: 48). The porosity of matrices is reduced with increasing compression force, leading to slower water uptake and water front movement into the matrix, which in turn, leads to slower drug release. However, the influence of tablet compression force on griseofulvin release from the tablets was less pronounced since tablet hardness was varied over a very small range of 8 N to 12 N.

All above factors were key considerations in optimizing drug release. Polyvinylpyrrolidone had no significant effect on drug release. With this in mind, polyvinyylpyrrolidone could be freely factored in the formulation to enhance binding of granules without modulating drug release. Figure 5.14 shows the combined effect of Methocel™ and Accurel MP on griseofulvin release, with polyvinylpyrrolidone and tablet hardness kept constant. Figure 5.14 overall shows that Methocel™ had the most pronounced effect on drug release whilst Accurel MP had less effect on drug release.

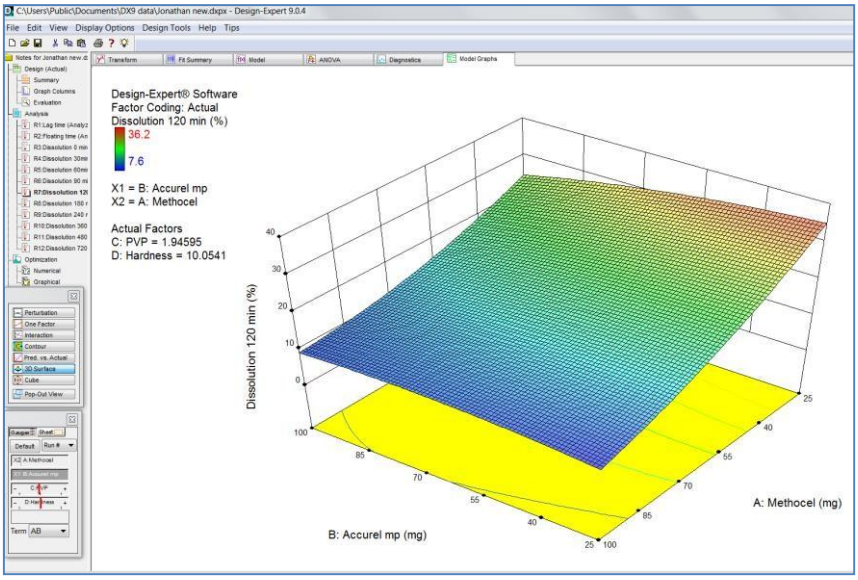


Figure 5.14: Combined effect of Methocel™ and Accurel MP on initial release of griseofulvin

5.8.2 ANOVA analysis of factors influencing drug release at 720 minutes

Griseofulvin release at 720 minutes was assessed using Design Expert. A linear model best fit the data obtained, with a sequential p-value of less than 0.0001, a lack of fit p-value of 0.0004 and had the least PRESS value of 426.91 as shown in Figure 5.15.

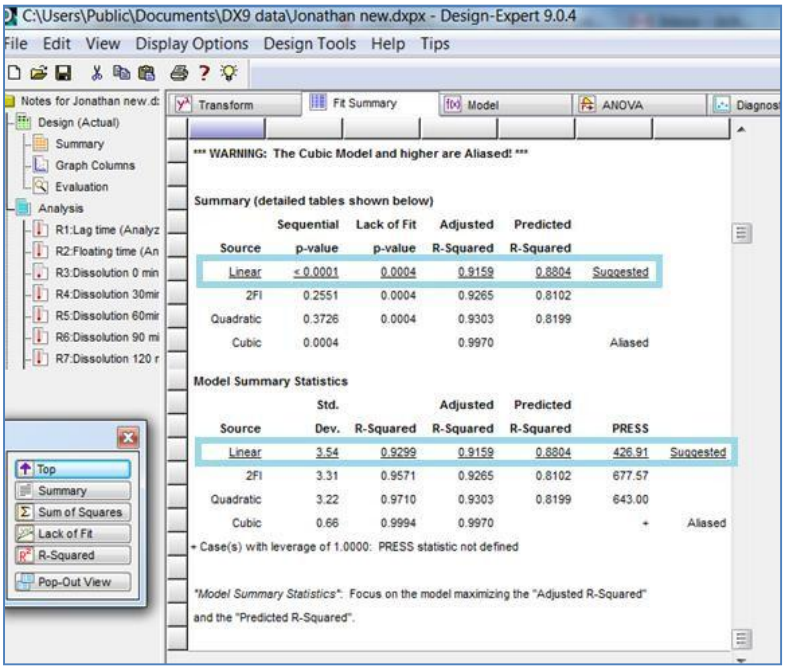


Figure 5.15: Statistical analysis of model fit using design expert for drug release up to 720 minutes

Analysis Of Variance (ANOVA) was applied to assess the effect the chosen factors had on griseofulvin release at 720 minutes, as shown in Figure 5.16. Methocel™ and Accurel MP had p-values of < 0.0001 and 0.0001 respectively and so had significant influence on griseofulvin release. In contrast, polyvinylpyrrolidone and tablet hardness had p-values greater than 0.05 and therefore, had no significant influence on release of griseofulvin.

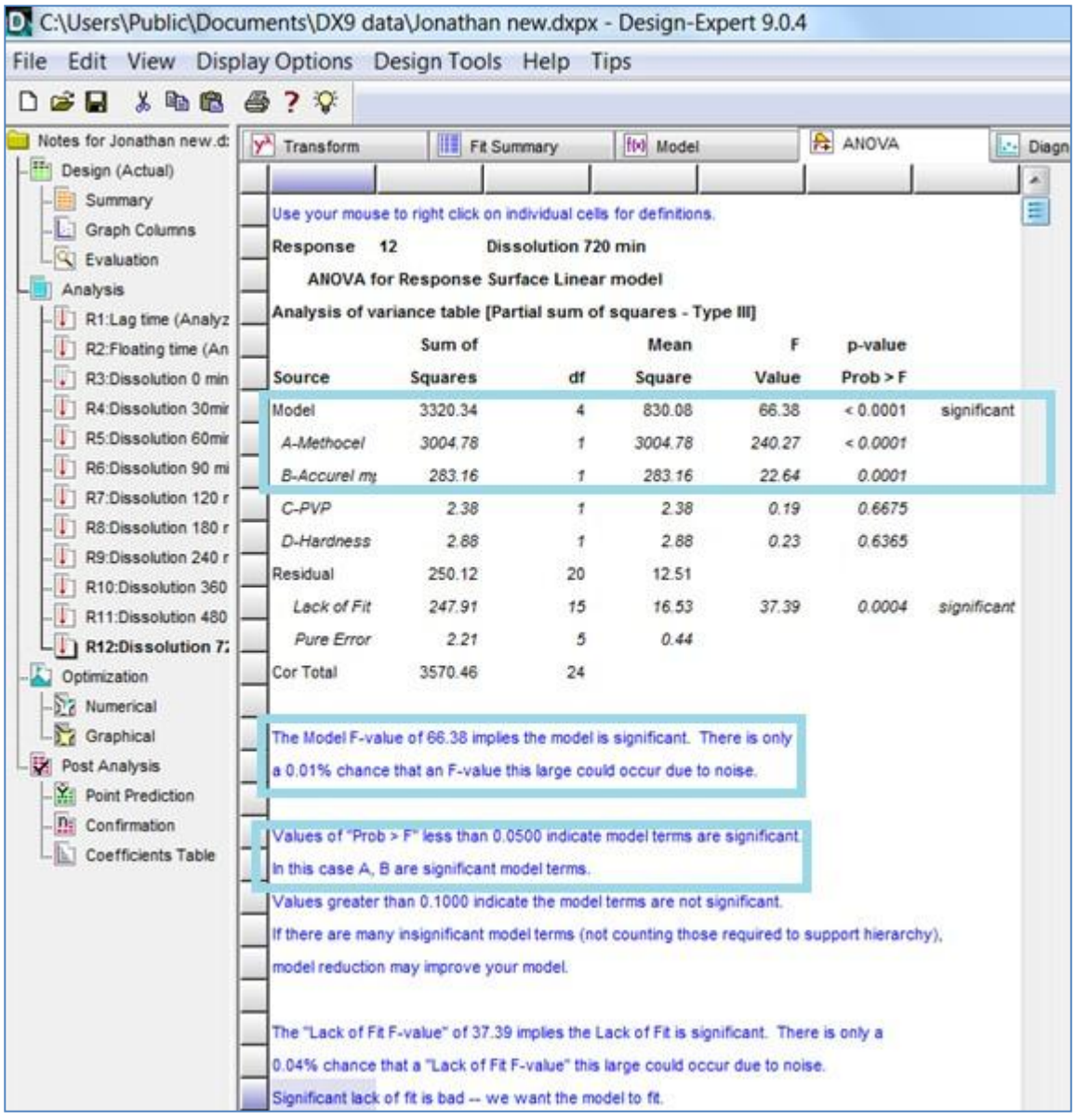
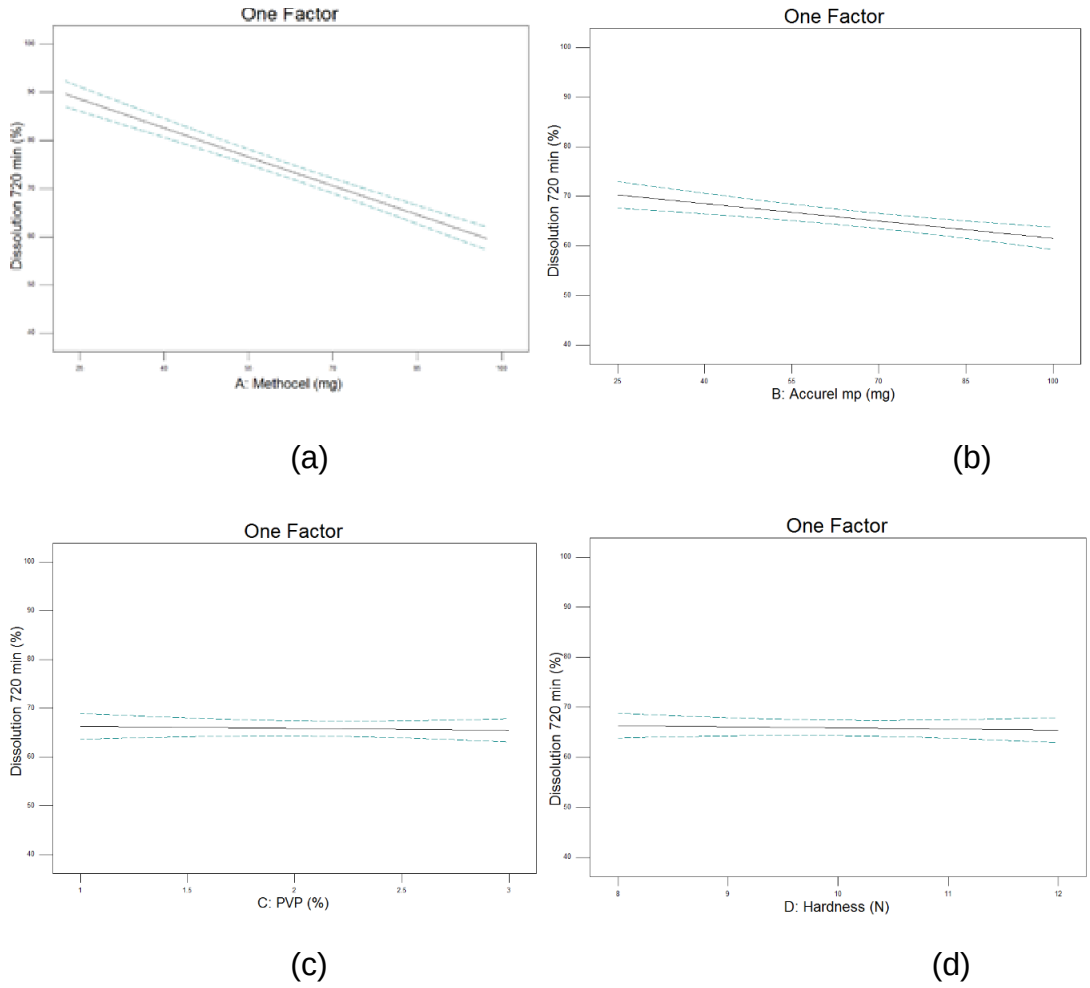


Figure 5.16: ANOVA for the influence of factors on drug release up to 720 minutes

Figure 5.17 shows how each factor affected drug release at 120 minutes. The influence of Methocel™ and Accurel MP on griseofulvin release at 720 minutes

was the same as at the initial drug release (up to 120 minutes). Methocel™ strongly modulated griseofulvin release. An increase in the Methocel™ decreased the release of griseofulvin. The highest percentage griseofulvin release of 90.1 % corresponded to the least amount of Methocel™, 25 mg, as shown in Figure 5.18a. The least percentage griseofulvin release of 50 % corresponded to the highest amount of least amount of Methocel™, 100 mg. An increase in Accurel MP caused a slight decrease in the release of griseofulvin, as shown in Figure 5.18b. Both polyvinylpyrrolidone and tablet hardness had no significant effect on the final release of griseofulvin, Figure 5.18c and d.



(a) (b)

(c) (d)

Figure 5.17: Effects of the selected factors on final drug release

Figure 5.18 shows the combined effect of Methocel™ and Accurel MP on final drug release when both polyvinylpyrrolidone and tablet hardness are kept constant. From the graph, it can be seen that maximum griseofulvin was obtained when 25

mg of Methocel™ and 25 mg of Accurel MP was used. However, a small increase in Methocel™ caused a greater decrease in griseofulvin release rate. In contrast, Accurel MP had less pronounced effect on drug release. Knowledge of the influence of these factors on griseofulvin release was quite essential for optimizing the formulated floating tablets of griseofulvin.

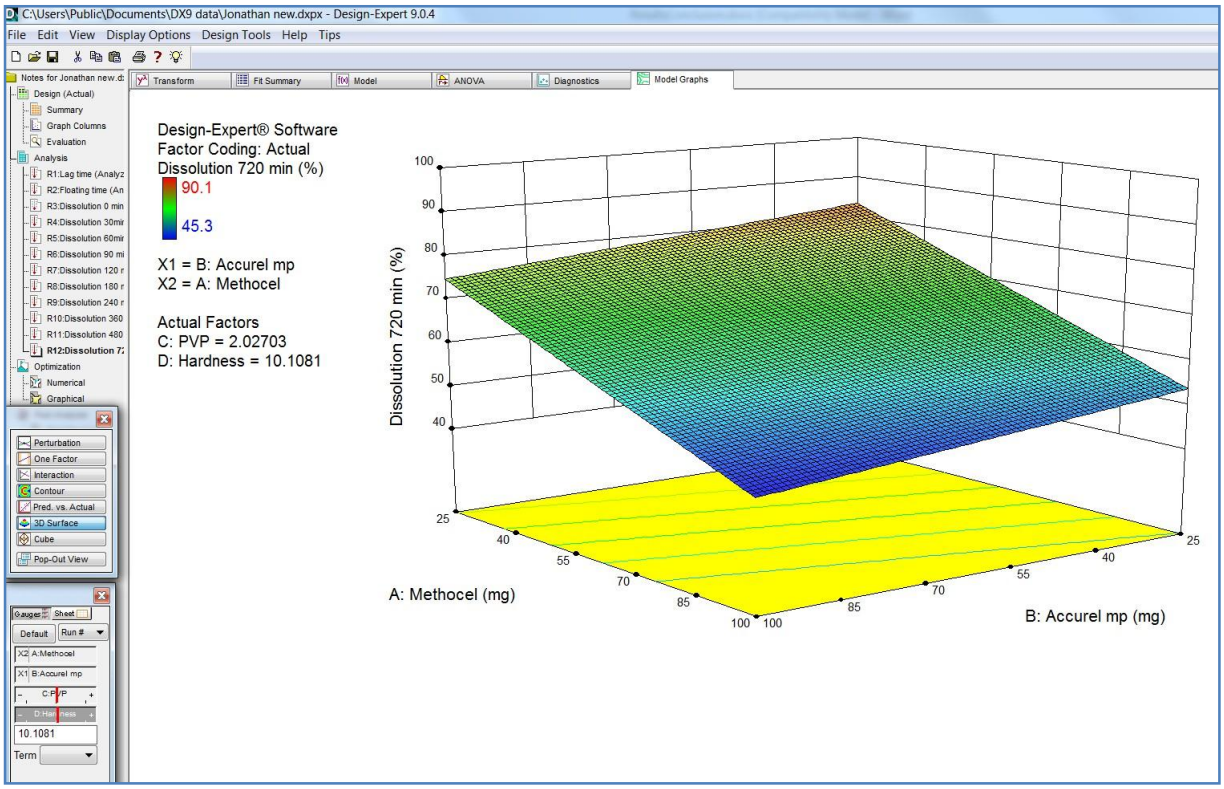


Figure 5.18: Combined effect of Methocel™ and Accurel MP on the final release of griseofulvin

5.9 OPTIMIZATION OF FORMULATION

Knowledge of the influence of the selected factors on both the buoyancy capabilities and drug release profile was used to optimize the characteristics of the formulated floating tablets. In addition to formulating tablets that are of acceptable quality according to USP standards, design and formulation targeted floating tablets with the following characteristics:

- Floating tablets that float immediately upon contact with dissolution medium
- Tablets that remain floating for a minimum of 720 minutes (12 hours)

- Tablets that sustain the release of griseofulvin in a zero order or near zero order pattern
- Tablets that would release at least 75 % of griseofulvin within 720 minutes (12 hours)

With the above ideal characteristics to consider, optimization was done using Design Expert. From the results of buoyancy studies, it was ascertained that for tablets to float immediately upon contact with release medium and to float for at least 12 hours, they needed to contain at least 28 % Accurel MP and had to have densities less than 0.00091 g/cm^3. Design Expert was then used to predict a formulation with the ideal characteristics. Figure 5.19 shows a number of combinations predicted by Design Expert, with various desirabilities close to 1.

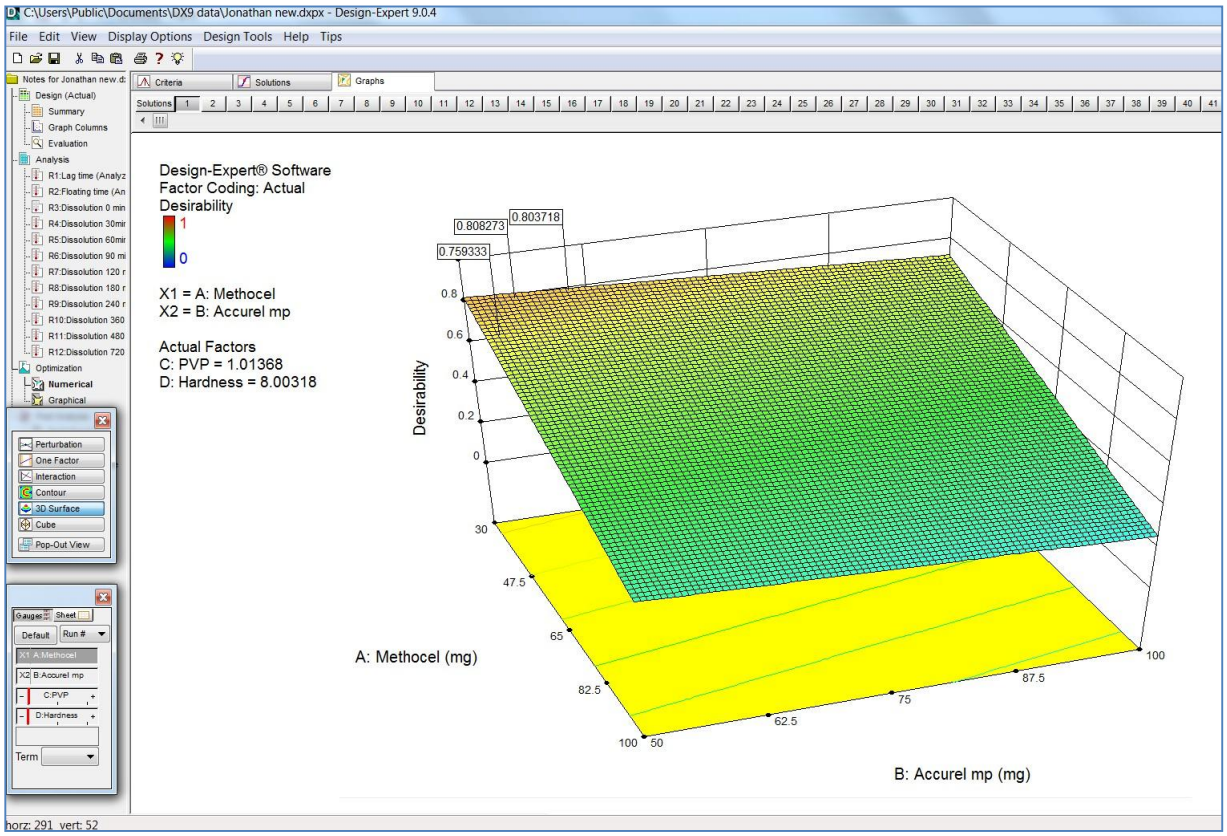


Figure 5.19: Optimization using Design Expert

Optimization was done and one formulation was chosen, as shown in Figure 5.20. This formulation had a desirability of 0.803, proving to be satisfactory.

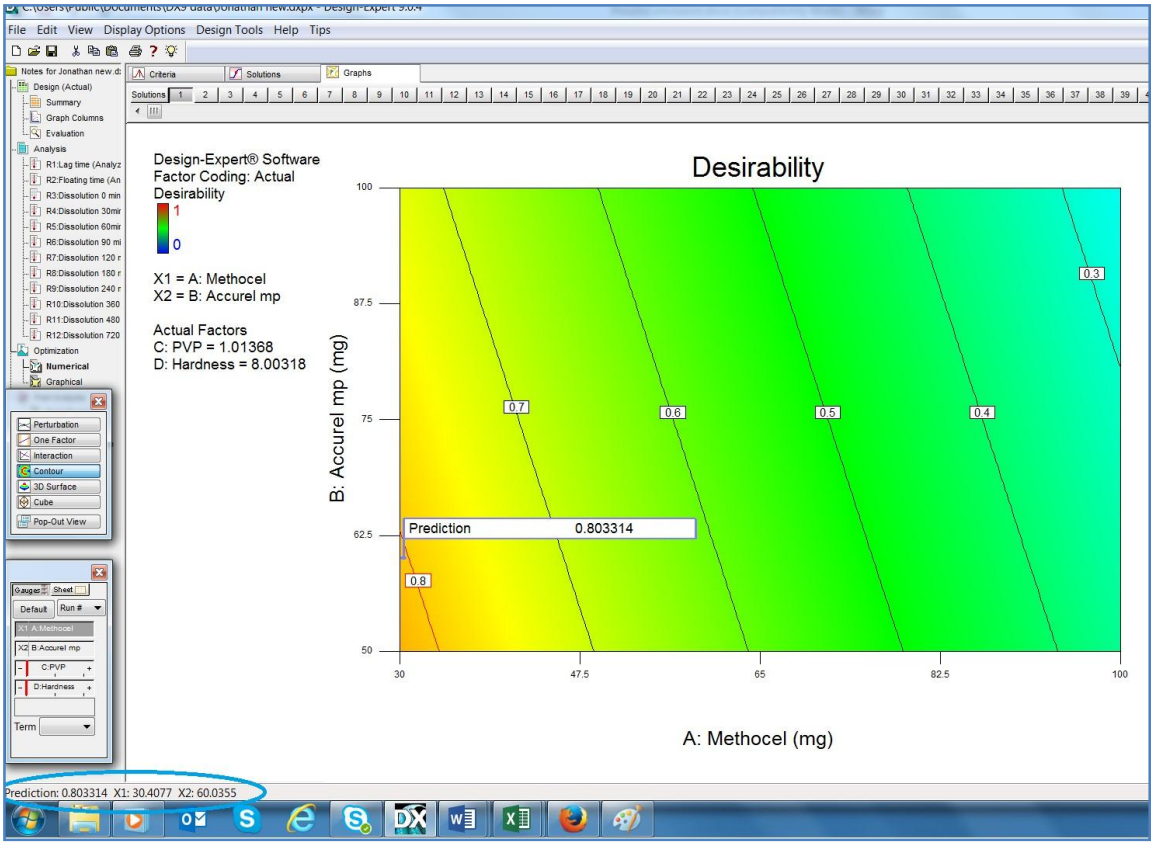


Figure 5.20: Optimized formula chosen by Design expert

Griseofulvin was kept constant at 100 mg. Polyvinylpyrrolidone 1% was chosen, which would provide sufficient binding during granulation. Tablet hardness within a range of 8 – 9 N was chosen and predicted to produce tablets firm enough for handling tablets yet allowing initial griseofulvin release by zero-order. A combination of 30 mg Methocel™ and 60 mg Accurel MP provided the most optimum formulation. The amount of Accurel MP chosen would constitute about 30% by mass of the formulation thereby guaranteeing immediate floatation of tablets and floatation of at least 12 hours. At least 75% final griseofulvin release would be expected after 12 hours with the optimized formulation. Table 5.7 shows the optimized formulation.

Table 5.7: Composition of optimized formulation

Ingredient	Quantity (mg)	Quantity (%)
Griseofulvin	100	52.6
Methocel™	30	15.8
Accurel MP	60	31.5
PVP	1.9	1
Magnesium stearate	1.9	1
Hardness	8-9 N	-
Ethanol	Qs	-

5.9.1 Pre-compression parameters of the optimized batch

The flow properties of the granulated powder mixture of the optimized batch was determined by measuring bulk density, tapped density, angle of repose, Hausner ratio and compressibility of the powder before compression. Table 5.8 shows the results of the pre-compression studies.

Table 5.8: Pre-compression parameters of griseofulvin floating tablets

BD (g/ml)	TD (g/ml)	CI (%)	H	C	AR (ɵ) ± S.D	FP	LOD (%)
0.18	0.196	10.46	1.12	Good	30.1 ± 1.6	Good	0.43

***BD =** Bulk Density, **TD =** Tapped Density, **CI =** Compressibility Index**, H =** Hausner ratio, **T =** Thickness, **F =** Friability, **LOD =** Loss on Drying, **C =** Compressibility, **FP =** Flow Properties & **S.D:** standard deviation**.**

As shown in Table 5.8, granules had a Compressibility Index, Hausner ratio and Angle of Repose of 10.46, 1.12 and 30.1^{O} respectively, values which translate to good flow properties of granules. Therefore, no provisions needed to be made to aid flowability of the granules.

5.9.2 Post-compression parameters of the optimized batch

The quality control aspects of formulated tablets (weight variation, hardness, diameter, thickness, and friability) were evaluated and the results are represented in Table 5.9.

Table 5.9: Post compression parameters of optimized griseofulvin floating tablets

WV ± S.D (mg)	H ± S.D (N)	D ± S.D (mm)	T ± S.D (mm)	F (%)	Assay (%)
0.1941 ± 0.006	8.7 ± 0.7	12.06 ± 0.01	2.03 ± 0.01	0.01	97.4 ± 1.3

***WV =** Weight Variation, **H =** Hardness, **D =** Diameter, **T =** Thickness, **T =** Thickness, **F =** Friability & **S.D:** standard deviation**.**

Consistency of tablet weight was assessed by the weight variation test. From a sample of twenty tablets, none of the tablets had a weight outside a weight range of 0.1976 g to 0.209 g, a range 7.5% about the tablets' mean of 0.1941 g. This confirmed consistency of weight uniformity and the tablets passed weight variation test. Tablets were compressed to a hardness of 8.7 N. The values of diameter, thickness and hardness for the tablets were taken and the deviation of each calculated. None of the deviations of diameter, thickness or hardness exceeded ± 5%. The formulated tablets passed the diameter, thickness and hardness test. Tablets had a friability of 0.01%. Since friability was less than 1%, friability test was passed, confirming that the tablets produced were capable enough to resist breakage under stress during handling. The tablets produced were of acceptable quality according to USP standards.

The assay of the compressed tablets was calculated and found to be 97.4%. The percentage content lies within the acceptable range: 95.0% - 105.0%. Satisfactorily, the compressed tablets contain the claimed amount of griseofulvin, 100 mg, providing evidence of content uniformity.

5.9.3 Buoyancy studies

Tablets produced using optimized formulae float immediately upon contact with the medium, i.e lag time 0 minutes and remain floating for at least 12 hours. Figure 5.21 shows floating tablets being assessed for buoyancy capabilities. Immediate floatation of tablets and floatation for over 12 hours guaranteed that tablets are capable of floating immediately in the stomach and thereby retain in the stomach despite gastric contractions and gastric emptying.

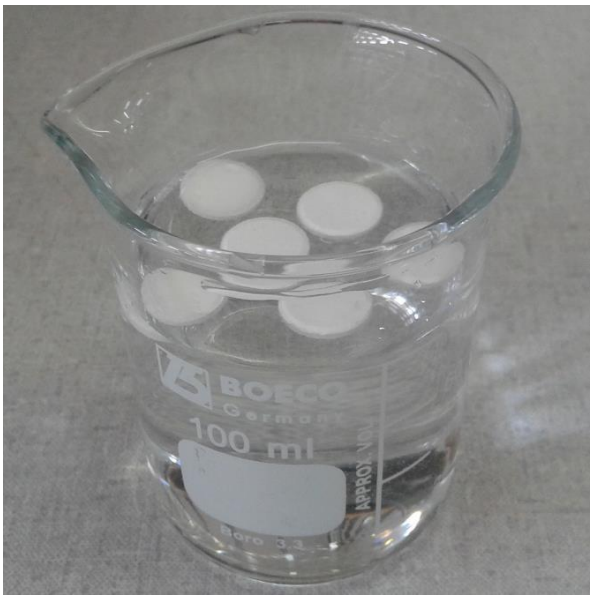


Figure 5.21: Tablets assessed for buoyancy capabilities

5.9.4 Dissolution studies

Dissolution studies for the optimized formulation were carried out as described in the previous chapter. Figure 5.22 shows the in-vitro drug release profile of the optimized formulation.

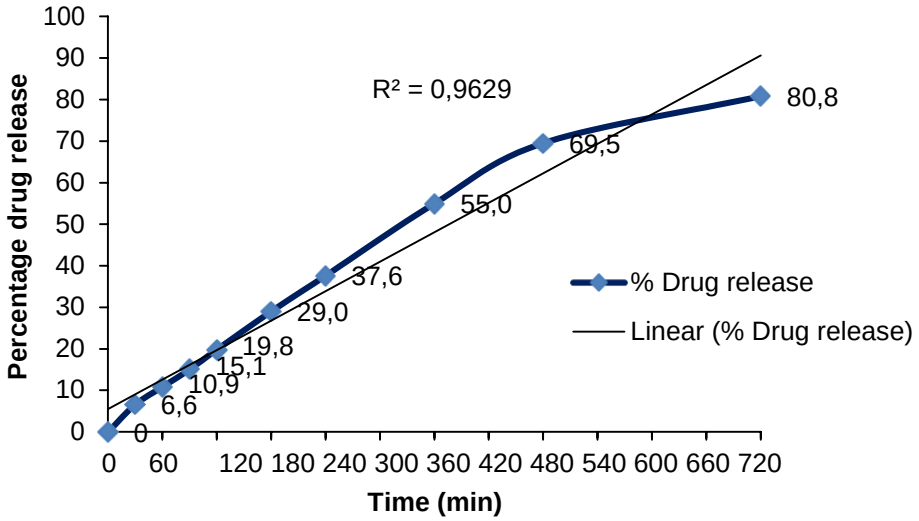


Figure 5.22: *In-vitro* drug release profile of the optimized batch

Griseofulvin is released from the optimized tablets in a near zero order fashion as shown in Figure 5.22, with a total of 80.8% griseofulvin released at the end of the

12 hour dissolution test period. The manner in which griseofulvin is released from the tablets is greatly influenced by the structural characteristics of the gel layer (swelling, uniformity of polymer hydration, diffusion capability, and gel strength), formed by Methocel™ in the formulation. Quick formation of the gel layer to prevent rapid influx of water into the matrix and the high gel strength are key consideration factors in drug release from the Methocel™ matrices (Maity *et al.,* 2014: 20). The optimized formulation, of ratio Methocel™:Accurel MP:hardness 30:60:8 respectively releases griseofulvin at a constant rate. Floating tablets successfully sustained the release of griseofulvin.

5.10 CONCLUSION

Design Expert generated 25 formulation runs which were compressed to assess the influence of Methocel™, Accurel MP, tablet hardness and polyvinylpyrrolidone on buoyancy and drug release. Accurel MP concentration was directly proportional to lag time and total floating time of tablets. Methocel™ concentration was indirectly proportional to release rate of griseofulvin from tablets. The optimized formulation, of ratio Methocel™:Accurel MP:hardness 30:60:8-9 respectively was chosen and floating tablets were compressed. Compressed tablets float immediately upon contact with dissolution medium and float for more than 12 hours. Griseofulvin was released at a constant rate over 12 hours, with 80.8% released within that period. Tablets were of acceptable quality according to USP standards.

CHAPTER 6

CONCLUSIONS AND RECOMMENDATIONS

6.1 FORMULATION DEVELOPMENT

Griseofulvin is an antibiotic fungistatic drug used in the treatment of dermatophyte and ringworm infections (Medical Economics Co., 1999a). Over the years, research has been directed towards enhancing the solubility and dissolution rate of griseofulvin in an effort to develop an improved and convenient dosage form.

This study explored the *in-vitro* dissolution of griseofulvin from floating tablets. Preformulation studies included selection of excipients and evaluation of their compatibility with griseofulvin. Using the chosen excipients, floating tablets of griseofulvin were formulated. The physical characteristics, buoyant capabilities and *in-vitro* drug release profile of the formulated tablets were assessed. Optimization was done and the developed formula was subjected to accelerated stability. This chapter summarizes the results obtained, conclusions made and the recommendations suggested.

A total of 25 runs were generated using Design Expert Software 9.0, specifically varying polymer ratios, binder concentration and compression force. Floating tablets containing 100 mg of griseofulvin were prepared by wet granulation technique with varying ratios of Methocel™, Accurel MP and Polyvinylpyrrolidone as determined by Design Expert software. Magnesium stearate was kept at 1% for all the formulations. Pre- and post-compression studies, buoyancy capability studies and dissolution studies were carried out to assess the influence of the tablet components.

Results obtained revealed that a density of less than 0.00091 g/cm^3 was necessary for tablet floatation. Tablets that float immediately upon contact with dissolution medium and tablets that float for over 24 hours were achieved by increasing the amount of Accurel MP, containing at 28% by mass of Accurel MP. Dissolution

studies revealed that an increase in tablet hardness reduced the rate of griseofulvin release only up to 120 minutes. From 120 minutes onwards, tablet hardness had no significant influence on griseofulvin release from tablets. Methocel™ had the most significant influence on griseofulvin release. The ratio of Methocel™ included in the formulation was indirectly proportional to the rate of griseofulvin release.

Knowledge of the influence of the selected factors on both the buoyancy capabilities and drug release profile was used to optimize the characteristics of the formulated floating tablets.

Using Design Expert, optimization was done and the following formulation was chosen. Griseofulvin was kept constant at 100 mg. Polyvinylpyrrolidone 1% was chosen, which would provide sufficient binding during granulation. Tablet hardness within a range of 8 – 9 N was predicted to produce tablets firm enough for handling tablets yet allowing initial griseofulvin release by zero order. 30 of mg Methocel™ was chosen and predicted to release griseofulvin by zero order release kinetics. An amount of 60 mg Accurel MP was chosen, constituting about 30% by mass of the formulation thereby guaranteeing immediate floatation of tablets for at least 12 hours. Pre- and post-compression parameters of the optimized tablets were found to be within pharmacopeal limits and thus compressed tablets were of acceptable quality. The tablets produced floated immediately upon contact with the medium, and remained floating for at least 12 hours. Griseofulvin was released from the optimized tablets in a near zero-order profile, with a total of 80.8% griseofulvin released at the end of the 12 hour dissolution test period.

6.2 RECOMMENDATIONS

Many aspects were identified to optimize the formulation and evaluation of floating tablets of griseofulvin. The following recommendations are important for future development of this and other similar formulations:

- Griseofulvin is available on the market as 250 mg and 500 mg tablets. However, in this study, 100 mg griseofulvin floating tablets were manufactured due to limited capacity of the compression machine used. It is recommended to formulate both 250 mg and 500 mg floating tablets of griseofulvin and assess influence of excipients on floating behaviour of tablets and release of griseofulvin.
- Use of analytical techniques e.g Fourier transform infrared spectroscopy (FTIR) and microscopy (Transmission electron and Scanning electron microscopy) to analyse interactions between griseofulvin and excipients.
- Model dissolution kinetics (computer modelling) to gain understanding on the release kinetics and mechanism of griseofulvin from the formulated tablets.
- As indicated in literature review, various methods have been exploited to improve oral absorption of griseofulvin. The most successful of these methods is reducing particle size of griseofulvin through micronization and ultramicronization and has seen such marketed micronized griseofulvin tablets. It would be of great value to compare drug absorption from the formulated floating tablets with that from the micronized griseofulvin tablets on the market.
- Although dissolution studies showed drug release in a sustained release fashion, further *in-vivo* studies are recommended to assess the floatation of the tablets over twelve hours and measure bioavailability of griseofulvin from the floating tablets.
- Stability studies need to be carried out in order to determine the fate of the developed formulation when subjected to real time storage conditions.

REFERENCES

AHMED, S., ADEL, A., ALI, A.M. & OMIYA, A. 2000. Compatibility of Itopride HCl with Certain Formulation Excipients. *Unique Journal Of Pharmaceutical And Biological Sciences,* 3: 303-310.

AHUJA, G. & PATHAK, K. 2009. Porous carriers for controlled/modulated drug delivery, *Indian Journal of Pharmaceutical Sciences,* 71 (6): 599-607.

AHUJA, G. & PATHAK, K. 2013. Inclusion of Amiloride Hydrochloride in Microporous Accurel MP 1000: Effect of solvents, drug loading and in vitro release. *Der Pharmacia Lettre,* 5 (5): 241-251.

AHUJA, S. & JIMIDA, M. 2011. Capillary Electrophoresis Methods for Pharmaceutical Analysis. *Elsevier*, 9: 112

AL-ACHI, A., GUPTA, M.R. & STAGNER, W.C. 2013. Integrated Pharmaceutics: Applied Preformulation, Product Design, and Regulatory Science", John Wiley & Sons: 77.

BADONI, A., OJHA, A., GNANARAJAN, G. & KOTHIYAL, P. 2012. Review on gastro-retentive drug delivery system. *The Pharmaceutical Innovation,* 1 (8): 32-40.

BARDONNET, P., FAIVRE, V., PUGH, W., PIFFARETTI, J. & FALSON, F. 2006. Gastroretentive dosage forms: Overview and special case of Helicobacter pylori. *Journal of Controlled Release,* 111 (1): 1-18.

BAUMGARTNER, S., KRISTL, J., VREČER, F., VODOPIVEC, P. & ZORKO, B. 2000. Optimisation of floating matrix tablets and evaluation of their gastric residence time. *International Journal of Pharmaceutics,* 195 (1–2): 125-135.

BHARDWAJ, L., SHARMA, P.K. & MALVIYA, R. 2011. A Short Review on Gastro Retentive Formulations for Stomach Specific Drug Delivery: Special Emphasis on Floating In situ Gel Systems. *African Journal of Basic & Applied Sciences,* 3 (6): 300-312.

BHOWMIK, D., CHIRANJIB, B., CHANDIRA, M., JAYAKAR, B. & SAMPATH KUMAR, K. 2009. Floating drug delivery system-A review. *Der Pharmacia Lettre,* 1 (2): 199-218.

BHUSNURE, O.G. 2016. Solid sample differential scanning calorimetry in biopharmaceutical discovery and development. *World Journal Of Pharmacy And Pharmaceutical Sciences*, 5 (1): 440-454.

BLADT, T.T., FRISVAD, J.C., KNUDSEN, P.B. & LARSEN, T.O. 2013. Anticancer and antifungal compounds from Aspergillus, Penicillium and other filamentous fungi. *Molecule,* 18 (9): 11338-11376.

BP see BRITISH PHARMACOPOEIA.

British Pharmacopoeia 2016. London: British Pharmacopoeia Commission.

BRÄSE, S., ENCINAS, A., KECK, J. & NISING, C.F. 2009. Chemistry and biology of mycotoxins and related fungal metabolites. *Chemical Reviews*. 109 (9): 3903-3990.

CAFARCHIA, C., WEIGL, S., FIGUEREDO, L.A. & OTRANTO, D. 2012. Molecular identification and phylogenesis of dermatophytes isolated from rabbit farms and rabbit farm workers. *Veterinary Microbiology,* 154 (3): 395-402.

CANTOR, S., AUSBURGER, L., HOAG, S. & GERHARDT, A. 2008. Pharmaceutical Granulation Processes, Mechanism, and the Use of Binders in Pharmaceuticals Dossage Form: Tablets. Augsburger and S. W. Hoag, Informa Healthcare USA, Inc. 1: 261-301.

CARRIGAN, P.J. & BATES, T.R. 1973. Biopharmaceutics of drugs administered in lipid-containing dosage forms I: Gi absorption of griseofulvin from an oil-in-water emulsion in the rat. *Journal of Pharmaceutical Sciences,* 62 (9): 1476-1479.

CARVALHO, F.C., BRUSCHI, M.L., EVANGELISTA, R.C. & GREMIÃO, M.P.D. 2010. Mucoadhesive drug delivery systems. *Brazilian Journal of Pharmaceutical Sciences,* 46 (1): 1-17.

CHANDRA, S., KILIMOZHI, D., KUMAR, N.S., KUMAR, S.V., PAVAN-OLETI, CS. 2013. Floating drug delivery system: An overview. *International Journal of Current Pharmaceutical Research*, 5 (3): 19-24.

CHAUHAN, P., PATEL, M. & PATEL, N. 2013. Review on gastroretentive drug delivery systems. *Indian Journal of Drugs and Diseases,* 2 (10): 218-230.

CHIOU, W.L. & RIEGELMAN, S. 1971. Absorption characteristics of solid dispersed and micronized griseofulvin in man. *Journal of Pharmaceutical Sciences,* 60 (9): 1376-1380.

CHOI, B., PARK, H.J., HWANG, S. & PARK, J. 2002. Preparation of alginate beads for floating drug delivery system: effects of CO 2 gas-forming agents. *International*

Journal of Pharmaceutics, 239 (1): 81-91.

CHOOI, Y., CACHO, R. & TANG, Y. 2010. Identification of the viridicatumtoxin and griseofulvin gene clusters from Penicillium aethiopicum. *Chemistry & biology,* 17 (5): 483-494.

COLLETT, J.H. & MORETON, R.C. 2007. Modified-release peroral dosage forms. In: AULTON, M.E. (Editor). Aulton's Pharmaceutics: The design and manufacture of medicines. Elsevier: Philadelphia: 484.

COLLETT, J.H. 2007. Dosage regimens. In: AULTON, M.E. (Editor). Aulton's Pharmaceutics: The design and manufacture of medicines. Elsevier: Philadelphia: 329-330.

DEBOER, D.J., MORIELLO, K.A., BLUM, J.L., VOLK, L.M. & BREDAHL, L.K. 2002. Safety and immunologic effects after inoculation of inactivated and combined live-inactivated dermatophytosis vaccines in cats. *American Journal of Veterinary Research,* 63 (11): 1532-1537.

DEGREEF, H. 2008. Clinical forms of dermatophytosis (ringworm infection). *Mycopathologia.* 166 (5-6): 257-265.

DEL PALACIO, A., GARAU, M., GONZALEZ-ESCALADA, A. & CALVO, M.T. 2000. Trends in the treatment of dermatophytosis. *Biology of dermatophytes and other keratinophilic fungi* Citeseer: 148-158.

DEMIRTÜRK, E. & ONER, L. 2005**.** Evaluation of *in-vitro* dissolution profile comparison methods of immediate release gliclazide tablet formulations. *Journal of Faculty of Pharmacy*, 25: 1-10.

DESAI, N.P. & SOON-SHIONG, P. 2003. Compositions and methods for administration of pharmacologically active compounds. *US Patent*: 1-21.

DOW, T. 2000. Using Methocel™ cellulose ethers for controlled release of drugs in hydrophilic matrix systems. Midland (MI): The Dow Chemical Company: 1-36.

EL-KAMEL, A., SOKAR, M., AL GAMAL, S. & NAGGAR, V. 2001. Preparation and evaluation of ketoprofen floating oral delivery system. *International Journal of Pharmaceutics,* 220 (1): 13-21.

ELKORDY, A.A., ESSA, E.A., DHUPPAD, S. & JAMMIGUMPULA, P. 2012. Liquisolid technique to enhance and to sustain griseofulvin dissolution: effect of choice of non-volatile liquid vehicles. *International Journal of Pharmaceutics,* 434 (1): 122-132.

FLOREY, K. 1983. Profiles of drug substances, excipients and related methodology. *Academic press*: 234.

FODA, N. 2011. Gastroretentive drug delivery systems as a potential tool for enhancing the efficacy of antibiotics: a review. *International Journal of Pharmaceutics and Biological Sciences*, 2 (4): 94-105.

FUJIOKA, Y., METSUGI, Y., OGAWARA, K., HIGAKI, K. & KIMURA, T. 2008. Evaluation of in vivo dissolution behavior and GI transit of griseofulvin, a BCS class II drug. *International Journal of Pharmaceutics,* 352 (1): 36-43.

FUKUDA, M., PEPPAS, N.A. & MCGINITY, J.W. 2006. Floating hot-melt extruded tablets for gastroretentive controlled drug release system. *Journal of Controlled Release,* 115 (2): 121-129.

GABA, P., GABA, M., GARG, R. & GUPTA, G. 2008. Floating microspheres: A review. *Pharmainfo.net,* 6 (5): 121-123.

GARG, S. & SHARMA, S. 2003. Gastroretentive drug delivery systems. *Business Briefing: Pharmatechnology*: 160-166.

GEETHA, A., RAJENDRA KUMAR, J., KRISHNA MOHAN, C. H., SATEESH, V., & RAJU, P.N. 2012. A review on floating drug delivery systems. *International Journal of Pharmaceutical Research and Biomedical Analysis*, 1 (1): 1-13.

GEMEINHART, R.A., PARK, H. & PARK, K. 2000. Pore structure of superporous hydrogels. *Polymers for Advanced Technologies,* 11 (8-12): 617-625.

GHOSH, T.K. & JASTI, B.R. 2004. Theory and practice of contemporary pharmaceutics*,* CRC press: 149.

GOTHOSKAR, A. & KHANGAONKAR, S. 2005. Biopharmaceutical classification of drugs. *Pharmaceutical Review*, 3 (1). [Online] Available at: http://www.pharmainfo.net/reviews/biopharmaceutical-classification-drugs [Accessed: 24/08/2016].

GUPTA, A.K. & COOPER, E.A. 2008. Dermatophytosis (Tinea) and other superficial fungal infections. Diagnosis and treatment of human mycoses: Humana Press Inc. Totowa: 355-381.

GUPTA, N.V. & SHIVAKUMAR, H. 2010. Development of a gastroretentive drug delivery system based on superporous hydrogel. *Tropical Journal of Pharmaceutical Research,* 9 (3): 257-264.

HARIKA, D., SUNITHA, R. & SRIVALLI, K. 2011. Superporous Hydrogels-Versatile Drug Release Retardants. *International Journal of Advances International Pharmaceutical Sciences,* 2 (4): 329-339.

HELJO, P. 2007. Thermo mechanical compatibility study of drug containing siloxone matrices [Online]. Available at: http://www.pharmtech.helsinki.fi/seminaarit/vanhat/esitelma_phelijo.pdf [Accessed: 09/07/2016].

HÖRTER, D. & DRESSMAN, J. 2001. Influence of physicochemical properties on dissolution of drugs in the gastrointestinal tract. *Advanced Drug Delivery Reviews,* 46 (1): 75-87.

HU, G., CHEN, H., CAI, J. & DENG, X. 2003. Solubility and micronization of griseofulvin in supercritical CO2 with cosolvent acetone. *Proceeding 6th International Symp Supercritical Fluid*, 3: 1725-1734.

IWEN, P.C., HINRICHS, S.H. & RUPP, M.E. 2002. Utilization of the internal transcribed spacer regions as molecular targets to detect and identify human fungal pathogens. *Medical Mycology,* 40 (1): 87-109.

JAMBHRUNKAR, S., KARMAKAR, S., POPAT, A., YU, M. & YU, C. 2014. Mesoporous silica nanoparticles enhance the cytotoxicity of curcumin. *RSC Advances,* 4 (2): 709-712.

JASSAL, M., NAUTIYAL, U., KUNDLAS, J. & SINGH, D. 2015. A review: Gastroretentive drug delivery system (grdds). *Indian Journal of Pharmaceutical and Biological Research,* 3 (1): 82.

JIANG, W., GAO, S., YANG, L., ZHANG, X., SUN, H. 2012. Distribution and elimination of griseofulvin residue in rabbit tissues. *Proceedings 10th World Rabbit Congress*, Sharm El-Sheikh, Egypt: 973-977.

KAR, R., MOHAPATRA, S. & BARIK, B. 2009. Design and characterization of controlled release matrix tablets of Zidovudine. *Asian Journal of Pharmaceutical and Clinical Research*, 2 (2): 54-61.

KHAMANGA, S.M. & WALKER, R.B. 2011. The use of experimental design in the development of an HPLC–ECD method for the analysis of captopril. *Talanta,* 83 (3): 1037-1049.

KHAN, R. 2013. GASTRORETENTIVE DRUG DELIVERY SYSTEM-A REVIEW. *International Journal of Pharmaceutical & Biological Sciences,* 4 (2): 630-634.

KLAUSNER, E.A., LAVY, E., FRIEDMAN, M. & HOFFMAN, A. 2003. Expandable gastroretentive dosage forms. *Journal of Controlled Release,* 90 (2): 143-162.

KODRE, K., ATTARDE, S., YENDHE, P., PATIL, R. & BARGE, V. 2014. Differential Scanning Calorimetry: A Review. *Research and Reviews: Journal of Pharmaceutical Analysis*, 3 (3): 11-22.

KUMAR, M.R., SATYANARAYANA, B., PALADUGU, N.D., NEERUKONDAVAMSI, S.M., PASHA, S.I., VEMIREDDY, S. & POLOJU, D. 2013. A comprehensive review on gastro retentive drug delivery system. *Acta Chimica.Pharmaceutics.Indica*, 3 (2): 149-164.

KUMAR, S., JAMIL, F., RAJPUT, M. & SHARMA, S. 2012. Gastro retentive drug delivery system: Features and facts. *International Journal of Research in Pharmaceutical and Biomedical Sciences,* 3 (1): 125-136.

LAKSHMIPATHY, D.T. & KANNABIRAN, K. 2010. Review on dermatomycosis: pathogenesis and treatment. *Natural Science,* 2 (07): 726.

LEE, H.Y., YU, S.A., JEONG, K.H. & KIM, Y.J. 2007. Poly (vinyl pyrrolidone) conjugated lipid system for the hydrophobic drug delivery. *Macromolecular Research*. 15 (6): 547-552.

LEE, J.W., PARK, J.H. & ROBINSON, J.R. 2000. Bioadhesive-based dosage forms: The next generation. *Journal of Pharmaceutical Sciences,* 89 (7): 850-866.

Li, J. & Wu, Y. 2014. Lubricants in pharmaceutical solid dosage forms. *Lubricants,* 2 (1): 21-43.

MAITY, T., NANDY, B., MITTAL, A. & GUPTA, A. 2014. A brief review on recent advances of extended release technology employed to design the oral dosage forms. *International Journal of Medical and Applied Sciences Research*, 1 (1): 12-24.

MAURO, V., CARETTE, D., PONTIER-BRES, R., DOMPIERRE, J., CZERUCKA, D., SEGRETAIN, D., GILLERON, J. & POINTIS, G. 2013. The anti-mitotic drug griseofulvin induces apoptosis of human germ cell tumor cells through a connexin 43-dependent molecular mechanism. *Apoptosis,* 18 (4): 480-491.

MCC see MEDICINES CONTROL COUNCIL.

MEDICINES CONTROL COUNCIL. 2009. Dissolution. [Online] Available at: http://www.mccza.com/genericDocuments/2.07_Dissolution_Jun07_v2.zip [Accessed: 12/08/2016].

MCC see MEDICINES CONTROL COUNCIL.

MEDICINES CONTROL COUNCIL. 2012. Stability testing. [Online] Available at: http://www.mccza.com/documents/b22b99e32.05_Stability_Jul12_v7_1.pdf [Accessed: 25/11/2015].

MEDICAL ECONOMICS CO. 1999a. Fulvicin P/G 165 & 330 tablets (Schering Corp.). In: PDRR: 53rd Ed., Montvale, NJ, Medical Economics Data Production Co. [MicroMedex Online]", *Physicians' Desk Reference*, Montvale, NJ, Medical Economics Data Production Co.

MENDEZ-TOVAR, L.J. 2010. Pathogenesis of dermatophytosis and tinea versicolor. *Clinics in dermatology,* 28 (2): 185-189.

MERCK MANUAL, 2010. Antifungal agents. [Online] Available at: http://www.merckmanuals.com/vet/pharmacolgy/antifungal_agents/griseofulvin.html [Accessed: 02/09/2016].

MERISKO-LIVERSIDGE, E., LIVERSIDGE, G.G. & COOPER, E.R. 2003. Nanosizing: a formulation approach for poorly-water-soluble compounds. *European Journal of Pharmaceutical Science.* 18 (2): 113-120.

Microsporum, T. 2005. Dermatophytosis, Ringworm, Tinea. *The Center for Food Security and Public Health*: 1-13.

MORIN, G. & BRIENS, L. 2013. The effect of lubricants on powder flowability for pharmaceutical application. *AAPS Pharmaceutical Science Technoligy,* 14 (3): 1158-1168.

NARANG, N. 2011. An updated review on: floating drug delivery system (FDDS). *International Journal of Applied Pharmaceutics*, 3 (1): 1-7.

NAYAK, A.K., MALAKAR, J. & SEN, K.K. 2010. Gastroretentive drug delivery technologies: Current approaches and future potential. *Journal of Pharmaceutical Education Research,* 1 (2): 1-12.

OMIDIAN, H., PARK, K. & ROCCA, J.G. 2007. Recent developments in superporous hydrogels. *Journal Of Pharmacy And Pharmacology,* 59 (3): 317-327.

OUTERBRIDGE, C.A. 2006. Mycologic disorders of the skin. *Clinical Techniques In Small Animal Practice,* 21 (3): 128-134.

PANDA, D., RATHINASAMY, K., SANTRA, M.K. & WILSON, L. 2005. Kinetic suppression of microtubule dynamic instability by griseofulvin: implications for its possible use in the treatment of cancer. *Proceedings of the National Academy of Sciences of the United States of America,* 102 (28): 9878-9883.

PATEL, P., AHIR, K., PATEL, V., MANANI, L. & PATEL, C. 2015. Drug-Excipient compatibility studies: First step for dosage form development. *The Pharmaceutical Innovation Journal,* 4 (5): 14-20.

PATIL, H., TIWARI, R.V. & REPKA, M.A. 2016. Hot-melt extrusion: from theory to application in pharmaceutical formulation. *American Association of Pharmaceutical Scientists Technology,* 17 (1): 20-42.

PERSSON, E.M., GUSTAFSSON, A., CARLSSON, A.S., NILSSON, R.G., KNUTSON, L., FORSELL, P., HANISCH, G., LENNERNÄS, H. & ABRAHAMSSON, B. 2005. The effects of food on the dissolution of poorly soluble drugs in human and in model small intestinal fluids. *Pharmaceutical Research,* 22 (12): 2141-2151.

PORTER, C.J., POUTON, C.W., CUINE, J.F. & CHARMAN, W.N. 2008. Enhancing intestinal drug solubilisation using lipid-based delivery systems. *Advanced Drug Delivery Reviews,* 60 (6): 673-691.

PRADHAN, K.K., MISHRA, U.S., MISHRA, D. & SAHU, K.C. 2011. Method development, validation and stability study of griseofulvin in bulk and pharmaceutical dosage form by UV-spectrophotometric method. *Asian Journal of Pharmaceutical Research*, 2 (1): 66-69.

RASENACK, N. & MÜLLER, B.W. 2004. Micron-size drug particles: common and novel micronization techniques. *Pharmaceutical Development and Technology,* 9 (1): 1-13.

RATILAL, D.A., GAIKWARD, P., BANKER, V. & PAWAR, S. 2011. Review on sustained release technology. *International Journal of Research and Pharmaceutical science,* 2 (6): 1701-1708.

RAUWENDAAL, C. 2014. Polymer extrusion*,* Carl Hanser Verlag GmbH Co KG: 1-950.

REBACZ, B., LARSEN, T.O., CLAUSEN, M.H., RONNEST, M.H., LOFFLER, H., HO, A.D. & KRAMER, A. 2007. Identification of griseofulvin as an inhibitor of centrosomal clustering in a phenotype-based screen. *Cancer Research,* 67 (13): 6342-6350.

REDDY, B.B.K. & KARUNAKAR, A. 2011. Biopharmaceutics classification system: a regulatory approach. *Dissolution Technology*, 18 (1): 31-37.

ROSSITER, D. 2016, South African Medicines Formulary, Health and Medical Publishing Group of the South African Medical Association: 83.

SAHIL, K., AKANKSHA, M., SUDEEP, B. & PREMJEET, S. 2011. Floating drug delivery system: A review. *International Research Journal of Pharmacy*, 2 (9): 18–24.

SAROJINI, S. & MANAVALAN, R. 2012. An overview on various approaches to Gastroretentive dosage forms. *International Journal of Drug Development & Research,* 4 (1): 1-13.

SCAZZOCCHIO, C. 2000. The fungal GATA factors. *Current Opinion In Microbiology,* 3 (2): 126-131.

SERRANO-GOMEZ, D., DOMINGUEZ-SOTO, A., ANCOCHEA, J., JIMENEZ-HEFFERNAN, J.A., LEAL, J.A. & CORBI, A.L. 2004. Dendritic cell-specific intercellular adhesion molecule 3-grabbing nonintegrin mediates binding and internalization of Aspergillus fumigatus conidia by dendritic cells and macrophages. *Journal of Immunology*, 173 (9): 5635-5643.

SHAHA, S., PATEL, J., PUNDARIKAKSHUDU, K. & PATEL, N. 2009. An overview of a gastro-retentive floating drug delivery system. *Asian Journal of Pharmaceutical Sciences*, 4 (1): 65-80.

SHARMA, D. & SHARMA, A. 2014. Gastroretentive drug delivery system - a mini review. *Asain Pacific Journal of Health Science*, 1 (2): 80-89.

SHARMA, S. & PAWAR, A. 2006. Low density multiparticulate system for pulsatile release of meloxicam. *International Journal of Pharmaceutics,* 313 (1): 150-158.

SHER, P., INGAVLE, G., PONRATHNAM, S. & PAWAR, A.P. 2007. Low density porous carrier: drug adsorption and release study by response surface methodology using different solvents. *International Journal of Pharmaceutics,* 331 (1): 72-83.

SHRIVASTAV, V.K., SHUKLA, D., PARASHAR, D. & SHRIVASTAV, A. 2013. Dermatophytes and related keratinophilic fungi isolated from the soil in Gwalior region of India and in vitro evaluation of antifungal activity of the selected plant extracts against these fungi. *Journal of Medicinal Plants Research*, 7 (28): 2136-2139.

SMART, J.D. 2005. The basics and underlying mechanisms of mucoadhesion. *Advanced Drug Delivery Reviews,* 57 (11): 1556-1568.

SRAVANI, B., DEVESWARAN, R., BHARATH, S., BASAVARAJ, B. & MADHAVAN, V. 2012. Development of sustained release metformin hydrochloride tablets using a natural polysaccharide. *International Journal Applied of Pharmaceutics*, 4 (2): 23-29.

STREUBEL, A., SIEPMANN, J. & BODMEIER, R. 2003. Floating matrix tablets based on low density foam powder: effects of formulation and processing parameters on drug release. *European Journal of Pharmaceutical Sciences,* 18 (1): 37-45.

TAGHIZADEH D.E., NOORDIN, I.M., KADIVAR, A., KAMALIDEHGHAN, B., FARJAM, A.S. & AKBARI JAVAR, H. 2013. Preparation and characterization of a

gastric floating dosage form of capecitabine. *Biomed Research International,* 2013: 1-8.

TALUKDER, R. & FASSIHI, R. 2004. Gastroretentive delivery systems: a mini review. *Drug Development and Industrial Pharmacy,* 30 (10): 1019-1028.

TANGRI, P. & MADHAV, N.S. 2011. Mucoadhesive drug delivery: Mechanism and methods of evaluation. *International Journal of Pharmaceutical and Biological Sciences,* 2 (1): 458-467.

TARIQ, A., BASHIR, I., KHAN, K.I., MAJEED, I., ZAFAR, N. & SAJID, I. 2014. Structural components of gastro-retentive drug delivery systems. *American Journal of Pharmaceutical Research,* 4 (09): 3863-3871.

TUR, K.M., CH'NG, H. & BAIE, S. 1997. Use of bioadhesive polymer to improve the bioavailability of griseofulvin. *International Journal of Pharmaceutics,* 148 (1): 63-71.

TURAKHIYA JIGNESH, M., SAVANI HITESH, D., PATEL JAINISH, M., AKBARI BHAVESH, V., PRAJAPATI NEHA, G. & SHAH VYOMA, S. 2013. A review superporous hydrogel (SPH)–an approach for controlled drug delivery. *Universal Journal Of Pharmaceutics,*2 (1): 47-58.

UPADHYAY, H.B., PANDYA, S.J. & VYAS, M. 2014. A review on gastroretentive microsphere. *International Journal of Universal Pharmacy and Biological Sciences*, 3 (2): 429-455.

USP see UNITED STATES OF PHARMACOPOIEA.

UNITED STATES OF PHARMACOPOEIA 2016. USP 31-NF 23. Rockville, MD: United States Pharmacopoeial Convention, Inc.

VARMA, M.V., KAUSHAL, A.M., GARG, A. & GARG, S. 2004. Factors affecting mechanism and kinetics of drug release from matrix-based oral controlled drug delivery systems. *American Journal of drug delivery,* 2 (1): 43-57.

VARUN KUMAR, K. & AJAYKUMAR, B. 2013. Formulation and evaluation of rapid disintegration tablets of moxifloxacin HCl. *Der Pharmacia Lettre,* 5 (1): 238-250.

VENTURINI, J., ALVARES, A.M., DE CAMARGO, M.R., MARCHETTI, C.M., DE CAMPOS FRAGA-SILVA, THAIS FERNANDA, LUCHINI, A.C. & DE ARRUDA, MARIA SUELI PARREIRA 2012. Dermatophyte–host relationship of a murine model of experimental invasive dermatophytosis. *Microbes and Infection,* 14 (13): 1144-1151.

VERMA, P., THAKUR, A., DESHMUKH, K., JHA, A. & VERMA, S. 2010. Routes of drug administration. *International Journal of Pharmaceutical Studies Resolutions*, 1 (1): 54-59.

VERMOUT, S., TABART, J., BALDO, A., MATHY, A., LOSSON, B. & MIGNON, B. 2008. Pathogenesis of dermatophytosis. *Mycopathologia,* 166 (5-6): 267-275.

VINOD, K., ROHIT REDDY, T., SANDHYA, S., BANJI, D., VENKATRAM REDDY, B. 2012. Critical review on mucoadhesive drug delivery systems. *Hygeia Journal for Drugs and Medicines,* 4 (1): 1-5.

WEI, B., LIANG, D. & BATES, T.R. 2008. Development and validation of a HPLC method to determine griseofulvin in rat plasma: application to pharmacokinetic studies. *Analytical Chemistry Insights,* 3: 103.

WELLS, J.I. & AULTON, M.E. 2007. Pharmaceutical preformulation. In: AULTON, M.E. (Ed). The design and manufacture of medicines. 3rd Edition. Elsevier: Hungary: 336-360.

WEN-XUE, J., SHU-XIA, G., LI-PING, Y., XIU-LING, Z. & HAI-TAO, S. 2012. DISTRIBUTION AND ELIMINATION OF GRISEOFULVIN RESIDUE IN RABBIT TISSUES. *World Rabbit Congress*, Proceedings 10th: 973-977.

WHITE, T.C., OLIVER, B.G., GRASER, Y. & HENN, M.R. 2008. Generating and testing molecular hypotheses in the dermatophytes. *Eukaryotic Cell,* 7 (8): 1238-1245.

ZATE, S., KOTHAWADE, P., MAHALE, G., KAPSE, K. & ANANTWAR, S. 2010. Gastro retentive bioadhesive drug delivery system: A review. *International Journal of Pharmaceutical Technology Research,* 2 (2): 1227-1235.

ANNEXURE A: STANDARD CALIBRATION CURVE

Table A1: Calibration data of griseofulvin at 296 nm

Concentration (µg/ml)	Absorbance 1	2	3	Average absorbance
0	0	0	0	0
1	0.052	0.052	0.051	0.052
2	0.098	0.098	0.098	0.098
3	0.145	0.146	0.145	0.145
4	0.201	0.201	0.201	0.201
5	0.251	0.252	0.251	0.251
6	0.304	0.305	0.304	0.304
8	0.405	0.405	0.406	0.405
10	0.523	0.522	0.523	0.523

Absorbance = 0.0519x – 0.0048, Regression coefficient $R^2 = 0.9992$

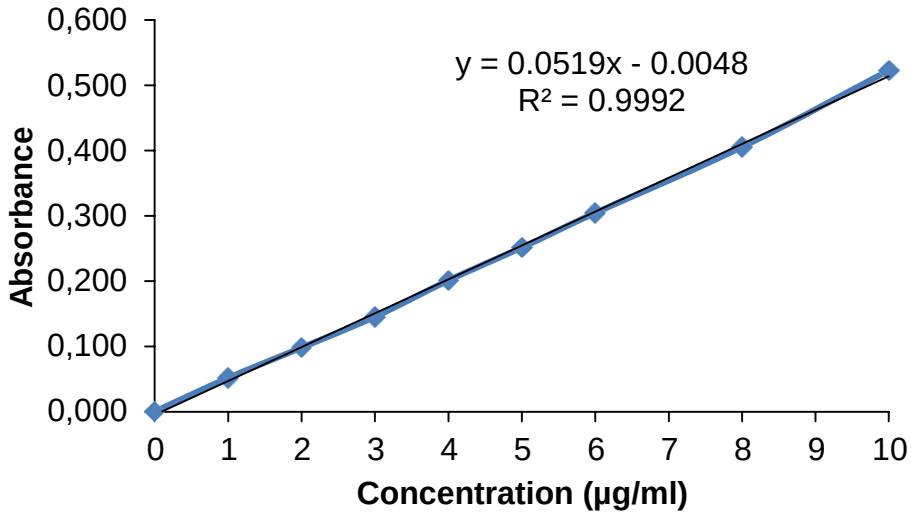


Figure A1: Calibration curve of griseofulvin at 296 nm