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ENHANCEMENT OF SOLUBILITY, DISSOLUTION AND STABILITY PROPERTIES OF GRISEOFULVIN

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BY

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DECLARATION

I, GIFTY BONNEY, do hereby declare that all the experimental work in this research was carried out by me and that references have been duly acknowledged. This thesis either in whole or in part has not been presented for any other degree elsewhere.

……. …….

GIFTY BONNEY Date

(Student)

I declare that I have supervised the student in undertaking the study reported herein and I confirm the student has my permission to present it for assessment.

…… ……

DR. RICHARD K. AMEWU Date

(Principal Supervisor)

……. ……...

DR. ABDUL K. BRIMAH Date

(Supervisor)

DEDICATION

I dedicate this work to my parents Mr. and Mrs. Bonney and all other persons who supported me in diverse ways to come this far.

ACKNOWLEDGEMENT

The Lord, God Almighty has brought me this far, He has always been my guide and am sincerely grateful to Him.

I thank my supervisors especially Dr. Richard K. Amewu, who acted not only as academic mentor but adviser too.

My special gratitude to the management of Ernest Chemists Limited (Manufacturing division) especially, Mr. Mark S. Owiredu, Mr. Dzigbordi Y. Agbitor, Mr. William Adjei, Ms. Orlia F. Lumor, for all the tutorials, exposure, and letting me do the research in the facility.

In addition, I also thank the following staff for their support: warehouse, dispensary, granulation, tableting, coating and quality control. Well done, God bless you all and raise the company to greater level.

I acknowledge and thank my husband, Mr. Edem K. Dussi for his supports, prayers and encouragement, and all the entire Bonney and Dussi families.

ABSTRACT

Good aqueous solubility and permeability of a drug is essential for the desired concentration (bioavailability) to be achieved in systemic circulation. Poor solubility of drugs remains a major challenge in pharmaceutical firms. Such drugs lead to poor bioavailability, dissolution rates, stability, permeation through membrane and extensive presystemic metabolism. Oral route remains the preferred choice of drug administration but the poor solubility of Griseofulvin, a class II drug of Biopharmaceutics Classification System (BCS) threatens its use as an oral therapy.

This study addressed the challenge by developing various formulations to enhance the solubility and dissolution properties of Griseofulvin tablets. Four different formulations of Griseofulvin were investigated using the wet granulation method. *Formulation A* contained gelatin as a binder and *formulations B, C* and *D* contained PVP with varying amount of SLS. At both the granules and tablets stages, the relevant test parameters were carried out. The formulations with PVP (***B, C,*** and ***D***) gave better dissolutions than that of the gelatin (***A***). Dissolution values for *formulation A* tablets were lower while *formulations B, C, D* tablets gave values above the standard 80 %. Of the three PVP-*formulations D* emerged the best formulation which was adopted to produce a confirmatory batch.

Tablets of this confirmatory batch were subjected to stability testing as done previously and further to both ICH accelerated and real time conditions. After both studies (6 months for accelerated and 12 months for real time), there was no significant change in accordance to ICH definition. The control samples also confirmed the dissolution of *formulation D* to the original *formulation* A.

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LIST OF ABBREVIATIONS

1. ^{13}C NMR – Carbon-13 NMR
2. ^{1}H NMR – Proton NMR
3. A^{1}_{1} – Molar Absorptivity
4. API – Active Pharmaceutical Ingredients
5. BCS – Biopharmaceutics Classification System
6. $CDCl_3$ – Deuterated Chloroform
7. CMC – Critical Micelle Concentration
8. DEPT – Distortionless Enhancement by Polarisation Transfer
9. DMF – Dimethylformamide
10. ECL – Ernest Chemists Limited
11. FBD – Fluid Bed Dryer
12. FTIR – Fourier Transform Infra-Red
13. GI – Gastrointestinal tract
14. GMP – Good Manufacturing Practices
15. HPC – Hydroxypropyl Cellulose
16. HPMC – Hydroxypropylmethyl Cellulose
17. HSQC – ^{1}H-^{13}C Heteronuclear Single Quantum Correlation
18. ICH – International Conference on Hamonisation
19. IR – Infra Red
20. KBr – Potassium bromide
21. KMT –Mean Kinetic Temperature
22. Kp – Kilo pond
23. LOD – Loss on drying
24. MC – Methylcellulose

25. NLT – Not Less Than

26. NMR – Nuclear Magnetic Resonance

27. NMT – Not More Than

28. PEG – Polyethylene Glycol

29. PVA – Polyvinylalcohol

30. PVP – Polyvinylpyrrolidone

31. RMG – Rapid Mixer Granulator

32. SLS – Sodium Lauryl Sulphate / Sodium dodecyl sulphate

33. SOP – Standard Operating Procedure

34. USP – United States Pharmacopoeial

35. UV/VIS – Ultra Violet / Visible

CHAPTER ONE

1.0 INTRODUCTION

Oral administration remain the common and convenient route employed in drug delivery system (patient acceptance, cost effective, least sterility constraints and laxity in the design of dosage form) (Yellela, 2010). Poor bioavailability of drugs however has been the major challenge with these oral dosage forms. The poor oral bioavailability usually results from poor solubility and low permeability of these drugs (Edward & Li, 2008).

Solubility of drugs still remains the main challenge in all pharmaceutical set-ups. The absorption of an oral drug depends on the physiochemical properties, nature, anatomy and physiology of the drug absorption site (Lennernäs, Abrahamsson, Persson, & Knutson, 2007). Orally administered poor water-soluble drugs regularly show poor bioavailability. Due to the absorption of such drugs in the gastrointestinal tract, this can be a rate-limiting step. Hence, it is important to enhance the dissolution rate for such drugs (Sugimoto, Okagaki, Narisawa, Koida, & Nakajima, 1998).

Biopharmaceutics Classification System (BCS), is a scientific classification of a drug and drug substance based on its aqueous solubility and intestinal permeability (Lennernäs et al., 2007). This BCS correlates *in vitro* dissolution and *in vivo* bioavailability of drug products (Amidon, Lennernas, Shah, & Crison, 1995; Khadka et al., 2014). Amidon et al (1995) classified oral drug products into four classes (**I**, **II**, **III** and **IV**). Drugs in class I are those of high solubility and high permeability (Cheng et al., 2004), (examples include **Paracetamol, Propranolol, Metoprolol, Diltiazem, Verapamil**) (Reddy & Karunakar, 2011). These drug substances are well absorbed with their absorption rate mostly higher than the excretion rate. However, the rate-determining step is due to the rapid dissolution and gastric-emptying rate of the drug (Wagh & Patel, 2010).

Class II on the other hand are drugs of high permeability with low solubility (examples are **Griseofulvin, Ketoconazole, Glibenclamide, Danazol, Nifedinpine, Naproxen, etc.**) (Amidon et al., 1995). The *in vivo* dissolution becomes the rate-limiting step for absorption in this class and have been the recent focus for solubility enhancement by researches with several formulation approaches emerging (Khadka et al., 2014; Sumit Kumar, Bhargava, Thakkar, & Saahil Arora, 2013). Permeability of class III drug has been the rate-limiting step for absorption (Reddy & Karunakar, 2011) due to rapid dissolution. High solubility and low permeability are characteristic properties of this class (examples include **Atenolol, Metformin, Neomycin B**). Low permeability and low solubility are characteristic of drugs belonging to class IV (examples include **Hydrochlorothiazide, Furosemide, Taxol**). Their poor bioavailability make them problematic for effective oral administration (Reddy & Karunakar, 2011).

Reports indicate that 40 % of marketed drugs and about 65 % of candidate compounds in pipeline belong to BCS Class II (Fong, Ibisogly, & Bauer-Brandl, 2015). Most active pharmaceutical ingredients (API) show inadequate physical and chemical properties (aqueous solubility, stability) and sometimes biopharmaceutical (dissolution rate, permeability) properties which significantly limit their bioavailability and hence oral delivery (Ambrogi et al., 2012; D. Kumar, Chirravuri, & Shastri, 2014).

Currently, an important area of pharmaceutical research is the transporting of drug substance to the target site in the biological systems. The role of drug delivery system is not only limited to the drug package but also cause a required change in therapeutic efficacy and safety during the transporting of drug molecules to the desired site in the most appropriate approach (Jadon, Gajbhiye, Jadon, Gajbhiye, & Ganesh, 2009). Drugs with low dissolution rates require special formulations containing carrier excipients to improve their efficacy.

The poor aqueous solubility of these drugs e.g. Griseofulvin remains a major challenge (Phanchaxari, Sumit, & Shaktish, 2011). Efforts to overcome this are of great importance in drug discovery and formulations (Hecq, Fanara, Vranckx, & Amighi, 2005). Nonetheless, several types of 'enabling formulations' and 'solubilization technologies' have been developed and reported to enhance the rate of dissolution, and dissolved-drug levels in an effort to attain the desired extent and rate of oral absorption (Fong et al., 2015).

Improving the dissolution rate and consequently the bioavailability can be achieved by increasing the drug's surface area (Babu & Nangia, 2011; Fong et al., 2015; Oka et al., 2015). Other approaches include the use of mesoporous silica particles to enhance dissolution and oral bioavailability through either salt formation, micronization, co-solvency, cyclodextrin complexation, hydrotropy, micellar solubilization, pH modification, solid dispersions, nanosuspensions, or spherical crystallization etc., (Alladi & Shastri, 2015; Chavda, Patel, & Anand, 2010; ECL, 2016; D. Kumar et al., 2014).

Solid dispersion, another efficient method for increasing the drug dissolution rates is carried out by melting method or solvent method. Two components, a poorly water-soluble drug and a water-soluble polymer are involved in this method. Examples are polyvinylpyrrolidone (PVP), polyethylene glycol (PEG), hydroxypropyl cellulose (HPC), hydroxypropylmethyl cellulose (HPMC), methylcellulose (MC), polyvinylalcohol (PVA), etc., (Sugimoto et al., 1998).

The use of excipients to increase dissolution and membrane permeation of drug is an old approach in optimizing its oral bioavailability (Jane, Jian-Hwa, & Mahesh, 2006). This work is focussed on the concept of particle size and excipient technology as well as wet granulation method. Granulation forms an intrinsic stage in manufacturing process of a large quantity of pharmaceutical solid dosage forms (Oka et al., 2015). A percentage amount of polyvinylpyrrolidone (PVP) was used with varying percentage amount of sodium lauryl

sulphate (SLS) to enhance the solubility and dissolution of the griseofulvin tablets. Stability studies were performed to ascertain the tablets dissolution with time.

1.1 STATEMENT OF PROBLEM

Griseofulvin is the oral antifungal agent of choice for the treatment of dermatophytoses (Araujo, Flowers, & King, 1990). Mycovin 500 tablets (Griseofulvin B.P.500 mg) produced by Ernest Chemists Limited (ECL) have low dissolution rate at the initial stage of manufacturing (ECL, 2016). Griseofulvin, an example of BCS Class II shows drug low solubility and high permeability drugs (Chavda et al., 2010) with elimination half-life of Griseofulvin is 9-24 hr (Sweetman SC, 1999). Within this period, it is expected that Griseofulvin is absorbed, distributed into target tissues, metabolised and eliminated from the body.

Out of 12 batches produced during 2013 - 2015, 8 batches had dissolution rates between 50 – 69 %, 3 batches 70 – 88 % and a batch 80 – 89 % (ECL, 2016). Tablets with dissolution rates < 80 % are reported to suffer low solubility, poor absorption, increased half-life and slow elimination. High solubility of Griseofulvin will result in higher absorption into target tissues. One major approach to enhance the pharmacological response of Griseofulvin is by reformulation in order to improve solubility, dissolution and absorption rates.

1.2 AIM

To prepare various formulations using combinations of PVP and SLS in order to improve the solubility (dissolution) and stability conditions of Mycovin 500 tablets manufactured by the Ernest Chemist Limited (ECL).

1.3 SPECIFIC OBJECTIVES

i. To reformulate the Mycovin 500 (Griseofulvin B.P. 500 mg) by replacing the binder and varying the concentration of SLS.

ii. To monitor the granulation and control kneading during the reformulation processes.

iii. To determine the various dissolution rates of the different formulations of the Griseofulvin tablets.

iv. To standardise the formulation of Griseofulvin 500 mg for ECL changing or varying product components and altering their percentage levels for various batches of the product.

v. To subject the Mycovin 500 tablets to ICH accelerated and real time stability conditions.

vi. To assess the friability, hardness, moisture content, disintegration time, assay and dissolution with time for the adopted formulation.

1.4 JUSTIFICATION

The elimination half-life of Griseofulvin is 9 to 24 hours (Sweetman SC, 1999). Within this period, it is expected that Griseofulvin is absorbed, distributed into target tissues, metabolised and eliminated from the body. High solubility of Griseofulvin will result in higher absorption into target tissues with enhanced metabolism and elimination.

When one of the batches, produced the Ernest Chemist Limited that passed the dissolution test (80 – 89 %) was subjected to accelerated stability test under elevated environmental factors - temperature & humidity, it failed. It is therefore necessary to investigate the stability of Griseofulvin under prevailing environmental conditions (**real time**) and elevated conditions (**accelerated**) to provide justification for the stated expiry date. Accelerated studies give an idea about the impacts of extreme environmental conditions outside the real conditions on the product (Bajaj, Sakhuja, Singla, & Bajaj Principal, 2012; CPMP/ICH/380/95, 1998; ECL, 2016). This will provide vital information for the storage, shelf life, packaging and transport conditions (CPMP/ICH/380/95, 1998).

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 OVERVIEW OF ORAL DRUG ADMINISTRATION

Orally administered drug first disintegrates, dissolves in gastric and/or intestinal fluids (to form a solution for easy absorption), permeates the membranes of the Gastrointestinal (GI) tract to reach the target site (Sandeep Kumar & Singh, 2016; Von Orelli & Leuenberger, 2004). Absorption of drug from the GI tract can be limited by various factors including poor solubility and poor permeability of the drug molecule (Sandeep Kumar & Singh, 2016; Mohini, Godse, & Saudagar, 2013). Hence a drug must be very soluble as well as possess an acceptable bioavailability. Solubility of a drug therefore forms a critical parameter to attain the required concentration to reach the target site for its pharmacological response (Parve, Shinde, Rawat, Rathod, & Waghmode, 2014). Hence, a drug's solubility and dissolution remain the principal ideas of any physicochemical science such as pharmacokinetic and biopharmaceutical considerations in therapy of any medicine (Thorat, Gonjari, & Hosmani, 2011).

The BCS groupings of drug substances based on their aqueous solubility and intestinal permeability had been useful in research and drug development (Parve et al., 2014; Reddy & Karunakar, 2011).

Examples of some structures of drug substances shown below (**Figure 2.1**)

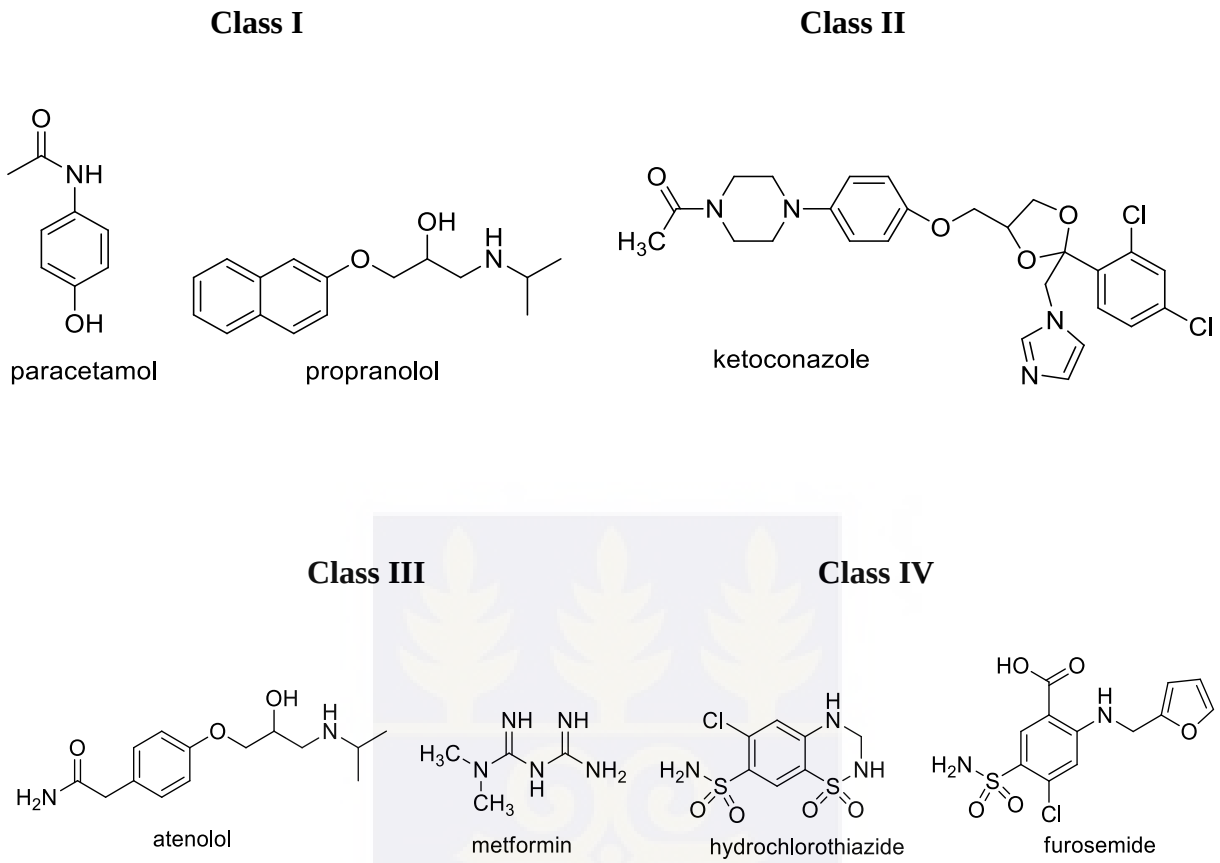

Figure 2.1: Selected BCS classified drugs.

Poorly soluble drugs continue to be a challenge in drug research and development as solubility and permeability remain key factors used in deciding the *in-vivo* absorption of such drugs (Kadam, D. M. Shinkar, & B. Saudagar, 2013; Vemula, Lagishetty, & Lingala, 2010). The *in vitro* dissolution study result of a drug can be closely related to the result of its *in vivo* dissolution (Sung-Hyun & Hoo-Kyun, 2006). Therefore, *in vitro* dissolution has become a significant element in the development of drug.

For both Classes II and IV (poorly soluble drugs), poor solubility and poor dissolution in the aqueous GI fluids is a limiting factor to the *in vivo* bioavailability (FDA, 1997; Khadka et al., 2014). This creates an erratic and inadequate absorption resulting to low and insufficient bioavailability when orally administered (Sandeep Kumar & Singh, 2016; Thorat et al., 2011).

These can however be altered and/or modified by a number of enhanced methodologies to improve solubilisation, dissolution and consequently improve their bioavailability (Parve et al., 2014; Vemula et al., 2010). The improvement of drug solubility, dissolution and hence its oral bioavailability pose major challenging phases of drug development process most importantly in cases of oral drug delivery system (Chokshi, Zia, Sandhu, Shah, & Malick, 2007; Kadam et al., 2013).

2.2 GRISEOFULVIN

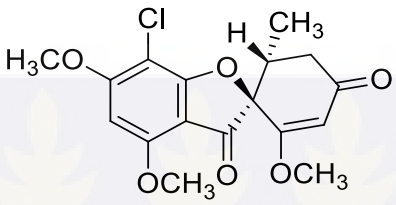

Figure 2.2: Structure of Griseofulvin

According to the BCS, Griseofulvin (**Figure 2.2**) belongs to class II drug – poorly soluble but able to permeate gastrointestinal mucosa (Elbary, Salem, & Maher, 2011; Issam et al., 2007). Griseofulvin is an antifungal drug substance produced by the growth of certain strains of *Penicillium griseofulvum* (Jadhav, Kate, & Payghan, 2014). An antifungal agent is a drug substance that selectively eliminates fungal pathogens with minimal toxicity to the host. They are either fungicidal or fungistatic, and mainly used in the treatment and prophylaxis of fungal infections (mycoses) (Dixon & Walsh, 1996; Sweetman SC, 1999). Griseofulvin been used for more than 30 years in the treatment of dermatophyte infections with other uses including treatment of malignant and inflammatory diseases (Finkelstein, Amichai, & Grunwald, 1996).

Griseofulvin is absorbed primarily in the duodenum. The microsize griseofulvin has variable bioavailability (25 to 70 %) when administered orally but taking it with a high-fat meal increases the bioavailability (Issam et al., 2007; Sweetman SC, 1999).

Griseofulvin concentrates in nails, skin, hair, skeletal muscle, fat and liver. It binds to tubulin, interfering with microtubule function inhibiting mitosis and oxidatively demethylated and conjugated with glucuronic acid, principally in the liver (Dash & Mishra, 2012) . The major metabolite, 6-desmethylgriseofulvin (**Figure 2.3**), is microbiologically inactive (Finkelstein et al., 1996).

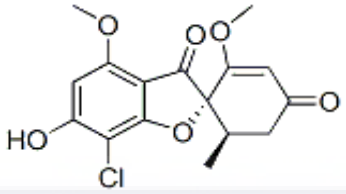

Figure 2.3: Structure of 6-desmethylgriseofulvin

The peak serum level for griseofulvin requires about 4 h after administration of a single dose of 250 mg of ultramicrosize griseofulvin, or 500 mg of microsize griseofulvin, with biological half-life ($t_{1/2}$) 9-24 h (Issam et al., 2007).

2.3 SOLUBILITY IMPROVEMENT TECHNIQUES

Several techniques have been used to enhance the solubility of poorly-water soluble drugs (Elbary et al., 2011; Issam et al., 2007). Thus increasing the dissolution rate resulting in improved oral absorption and bioavailability of these drugs (Mohini et al., 2013). Drug solubility improvement techniques employed by the pharmaceutical institutions include salt formation, co-solvency, hydrotropy, cyclodextrin complexation, micronization, micellar solubilization, solid dispersions, nanosuspensions, spherical crystallization, liquisolid methods, self-emulsifying or self-micro emulsifying Systems, pH modification (Alladi & Shastri, 2015; D. Kumar et al., 2014; Sandeep Kumar & Singh, 2016). In addition, methods including the use of surfactants, water-soluble carriers, polymeric conjugates, suitable polymorph and anhydrous/organic solvate forms have also been reported to enhance the solubility and dissolution properties of drugs (Issam et al., 2007; Yoo et al., 2000).

The solubilization techniques, are categorized into physical modification, chemical modification and other techniques as shown in **Table 2.1** (Parve et al., 2014).

Table 2.1: Methods to enhance solubility of poorly soluble drugs (Anupama Kalia & Mayur Poddar, 2011)

Physical Modification				
Reduction of Particle size	**Crystal Habit Modification**	**Drug Dispersion in carriers**	**Complexation**	**Solubilisation by surfactants**
a. Micronization b. Nanosuspension • Homogenization • Wet milling c. Sono crystallization d. Supercritical fluid process f. Spray drying	a. Polymorphs b. Pseudo Polymorphs	a. Eutectic mixtures • Hot plate method • Solvent evaporation method • Hot-melt extrusion • Melting-solvent method	a.Usage of complexing agents • Inorganic Coordination • Chelates • Metal-olefin • Inclusion •Molecular complexes	a. Microemulsions b. Self-micro emulsifying drug delivery systems
Chemical Modification				
Soluble prodrugs			Salt formation	
Other Techniques				
Cocrystallisation	Cosolvency	Hydrotrophy	Solubilizing agents	Nanotechnology approaches

In these techniques, excipients play important roles in the enhancement of drug's solubility and dissolution rate. Extensive research on polymers, superdisintegrants and surfactants in recent years are being explored for the dissolution enhancement in drugs (Sandeep Kumar & Singh, 2016).

Particle technology technique alter physicochemical, micrometrics and biopharmaceutical properties of poorly soluble drugs, hence improving their aqueous solubility. Nonetheless, among the several approaches to enhancing solubility, particle size reduction and modifying

crystal habit are the commonest processes of physical modifications to increase drug solubility (Savjani, Gajjar, & Savjani, 2012).

2.3.1 Particle size reduction

The surface area to volume ratio increases as particle size becomes smaller (Sandeep Kumar & Singh, 2016). Conventional methods of particle size reduction (e.g comminution and spray drying) rely upon mechanical stress to disaggregate the active substance (Savjani et al., 2012). Reduction of particle size creates an effective, reproducible, and economic means of enhancing the solubility (Khadka et al., 2014). Adversely, mechanical forces inherent to comminution frequently impart substantial extents of physical stress upon the drug product facilitating degradation (Savjani et al., 2012).

2.3.1.1 Micronization / Nanonization

This is another conventional technique for the particle size reduction where coarse drug powder is transformed into an ultrafine powder possibly ranging from 2 - 5 µm of mean particle size with a little fraction below 1 µm size range (Khadka et al., 2014; Rawat, Kumar, & Mahadevan, 2011). Micronization increases the drug dissolution rate since an increase in surface area and saturation solubility results from reduction of the particle size to sub-micron level. This improves the rate of dissolution and hence bioavailability of the drugs. The micronization is done by milling techniques using jet mill, rotor stator colloid mills etc., (Blagden, Matas, Gavan, & York, 2007; Parve et al., 2014; Thorat et al., 2011). This approach is mostly used to increase solubility of BCS class II drugs such as Spironolactone, Griseofulvin, Progesterone and fenofibrate (Leleux & Williams, 2013). Micronization of these drug substances improved their digestive absorption attributes, oral bioavailability and clinical efficacy. For instance, a report indicated micronized fenofibrate exhibited more than 10-fold (1.3 to 20 %) increase in dissolution at 30 min biorelevant media (Chaumeil, 1998; Sandeep Kumar & Singh, 2016; Vogt, Kunath, & J.B. Dressman, 2008).

2.3.2 Solubilization by surfactants

The addition of surfactants in pharmaceutical drug formulations often improves the dissolution performance of poorly soluble drugs, which has been the basic and ancient approach. The usage of most surfactants such as sodium dodecyl sulphate, polysorbates, polyoxyethylated glycerides, tweens, spans, etc. as excipients and carriers for dissolution enhancement are very successful (Parve et al., 2014). Reduction of surface tension by surfactants increase the dissolution rate of lipophilic drugs in aqueous medium (Vemula et al., 2010; Ventosa-andrés & Fernández, 2012). When the concentration of surfactants exceed their critical micelle concentration, CMC (0.05 – 0.10 %) micelles are observed. These micelles entrap the drug within, a process known as **micellization**, which enhance solubility of these poorly soluble drugs. This has been used extensively as an alternative for the dissolution of most poorly soluble drugs (Desai & Park, 2004; Mohini et al., 2013; Vemula et al., 2010).

2.3.3 Solid dispersion

Solid dispersions was first introduced in 1961 by Sekiguchi et al who investigated the generation and dissolution performance of eutectic melts of a sulphonamide drug and a water-soluble carrier (Sekiguchi & N. Obi, 1961). In this process, a poorly soluble drug is dispersed in a highly soluble solid hydrophilic matrix to enhance the dissolution of the drug and yield eutectic (non-molecular level mixing) or solid solution (molecular level mixing) products (Sekiguchi & N. Obi, 1961; Vemula et al., 2010). These groups of solid products consist of at least two different components, a hydrophilic matrix and a hydrophobic drug. Polyvinylpyrrolidone (Povidone, PVP), polyethylene glycols (PEGs), Plasdone- S630 are the most commonly used hydrophilic carriers for solid dispersions. Surfactants (eg. Tween-80, docusate sodium, Myrj-52, Pluronic-F68, and SLS) are used mostly in the formulation of solid dispersion (Sandeep Kumar & Singh, 2016; Mohini et al., 2013).

Since solubility remains the key physical attribute for a drug's oral bioavailability, the choice of a suitable solubility enhancement technique is critical in ensuring good formulation (Sandeep Kumar & Singh, 2016).

2.4 EXCIPIENTS AND THEIR USES

Any natural or synthetic substance formulated alongside the API in drug production is known as an excipient or additive. The purposes of using excipients are vast and including long-term stabilization, bulking up solid formulations (bulking agents / fillers / diluents) and therapeutic enhancement on the API in the final dosage form (such as facilitating drug absorption, reducing viscosity, or enhancing solubility) (Bhattacharyya, Schuber, Sheehan, & Roger William, 2006).

In tablet preparations, the excipients play significant roles in the drug formulation process. For instance the while the use of diluents increase the bulk of the formulation, binders cause the adhesion of the powdered drug and pharmaceutical substances, anti-adherents / lubricants aid in the smooth tableting process, disintegrating agents promote tablet break - up after administration, and coating agents improve stability, control disintegration, and/or enhance appearance (Gad, 2008). These defined functional roles by excipients in pharmaceutical dosage forms has been extensively reported (Katdare & Chaubal, 2006): enhancing stability of the API(s) in finished dosage forms, modulating solubility and bioavailability of the API (s), maintaining pH and osmolarity of liquid formulations, acting as antioxidants, emulsifying agents and aiding API(s) maintain a preferred polymorphic form or conformation.

2.4.1 Gelatin

Gelatin is used in various pharmaceutical formulations, soft or hard gelatin capsules, food products and photographic emulsions. It is mainly used in formulation as coating agent, film-former, gelling agent, suspending agent, tablet binder, viscosity-increasing agent (*Handbook of Pharmaceutical Excipients*, 2000).

2.4.2 Povidone (PVP)

PVP, a synthetic polymer consisting of 1 - vinyl - 2 – pyrrolidinone monomer units (**Figure 2.4**). It is white to creamy - white fine powder with varying molecular weight grades which differ in their degree of polymerization (*Handbook of Pharmaceutical Excipients*, 2000).

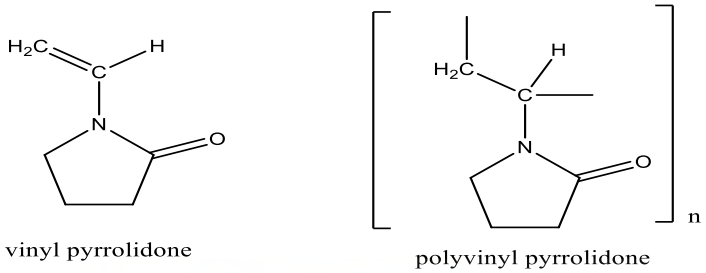

Figure 2.4: Monomer and Polymer of Vinylpyrrolidone

PVP is soluble in water, GI fluids, alcohol, and isopropyl alcohol (IPA) and forms viscous solutions and gels (Gad, 2008). PVP is often used as a binder in most tablet formulation to give harder granulates with good flowability, higher binding and low friability as compared to other binders in wet granulation process. PVP property increases the dissolution of poorly soluble active ingredients by forming water-soluble complexes with these active substances and subsequently increase the bioavailability (Chowhan, 1980; Kadajji & Betageri, 2011; Sugimoto et al., 1998; Wang & Chowhan, 1990).

2.4.3 Sodium Lauryl Sulphates (SLS)

SLS also known as sodium dodecyl sulphate, $CH_3(CH_2)_{11}SO_4Na$ (*Handbook of Pharmaceutical Excipients*, 2000), has number of functional uses in pharmaceutical preparations. SLS can be used as an emulsifying agent, modified-release agent, penetration enhancer, solubilizing agent, tablet and capsule lubricant.

2.4.4 The effect of excipients and manufacturing process on dissolution

Formulation and manufacturing process variables influence drug quality (Yekpe et al., 2015). Various studies showed that attributes of certain materials and manufacturing process

parameters affect the results of dissolution tests performed on finished products (D'Souza, Lozano, Mayock, & Gray, 2010; Yekpe et al., 2015). The excipient choice and production method strongly have an impact on the quality of the final drug product (Yekpe et al., 2015). Therefore, the quantity and choice of pharmaceutical excipients to use are highly dependent on the final dosage forms (T. Kumar et al., 2012).

Hence, the implementation of these strategic approaches requires an in-depth understanding of the intrinsic characteristics of individual APIs, the desired formulation properties, the physicochemical properties of suitable excipients, and the stability of drug molecules in the presence of pharmaceutical excipients on oral dosage forms (Bhattacharyya et al., 2006; Jane et al., 2006).

2.5 GRANULATION PROCESS

Granulation is a process where the primary powder particles are made to adhere to form larger, multiparticle entities called granules, creating bonds between them. Granulation forms the central part of the manufacturing process and thus, an integral step in the manufacturing of a large volume of pharmaceutical solid dosage forms (Oka et al., 2015). Dry granulation and wet granulation methods are the two main types of granulation employed in manufacturing of drugs. The process of wet granulation involves the formation of granules by the addition of a granulating liquid onto a powder bed under the influence of an impeller (in a high-shear granulator), screws (in a twin screw granulator), etc., (Lee et al., 2015) but not in dry granulation. Often, dry granulation are employed in areas where drug substance involve are moisture and heat sensitive. Wet granulation is preferred (about 70 %) over the dry granulation (Rahmanian & Ghadiri, 2013). This is because in wet granulation there is a better control of drug content uniformity at low drug concentrations, control of product bulk density and ultimately compatibility(brittle fracture), even for high drug contents are achievable (Faure, York, & Rowe, 2001).

2.6 DISSOLUTION

Dissolution testing is an *in vitro* method that characterizes how an API is extracted out of the dosage form solid-state matrix into the dissolution medium (mimicking the GIT). It indicates the efficiency of *in vivo* dissolution but does not provide any information on drug substance absorption. Dissolution testing is essential for all solid orally administered drugs, useful in all phases of drug development for product release and stability testing. Dissolution testing plays a vital role in analytical test, and not limited to detecting physical changes in an API and in the formulated product.

A vital property of solid dosage form is the *in vitro* dissolution rate of the drug substance. This is an initial stage to assess the quality of a certain compound and a guide to new formulation (von Orelli & Leuenberger, 2004). Recent attention has been focused on the significance of this test of oral dosage forms and in the development of new formulations (Sung-Hyun & Hoo-Kyun, 2006). Dissolution testing is requirement for all solid oral Pharmacopoeial dosage forms in which absorption of the drug is necessary for the product to exert the desired therapeutic effect ("USP 38-NF 33," 2015).

2.7 STABILITY TESTING AND STUDIES

Unstable product degradation into toxic decomposed products can results in loss of activity up to a level of 85 %. This may result in failure of the therapy. It has therefore become a legal requirement to provide data for certain types of stability tests for the regulatory agencies before approval of a new product (Bajaj et al., 2012).

The main goal of stability studies is to provide evidence that a drug substance will remain potent within recommended acceptable limits in the course of the retest period if stored under recommended storage conditions as per ICH Q1A (**Table 2.2)** (CPMP/ICH/380/95, 1998; FDA, 1998).

Finished pharmaceutical products stability depends on several factors. These factors include environmental factors (temperature, humidity, light) and product-related factors (both physical and chemical properties of the excipients and API, the composition and dosage form, the manufacturing process, container-closure system and the properties of the packaging materials) (Koop, 2006).

Table 2.2: Summary of the main objectives of stability testing

Objective	Type of study	Use
Identify and adopt the suitable formulation and container closure system	Accelerated	Developing of the drug product
Establish the shelf-life and storage condition	Real-time and accelerated	Developing of the product and registration dossier
Authenticate the claimed shelf-life	Real-time	Registration dossier
Substantiate that no adverse changes had occur in the formulation and manufacturing process that can have an impact on the stability of the product	Accelerated and real-time	Quality Assurance / Quality Control

(WHO, 1996)

Drug formulations, stability and storage conditions depend on the climatic zone. The world has been divided into four (**4**) climatic zones based on the Mean Kinetic Temperature (KMT) (Kunzle, Schreiber, & Gomez, 2009).

The intended market and the climatic conditions in the area where the drug product will be used is considered for the design of the stability testing programme. The four (**4**) climatic zones are shown in **Table 2.3** in order to categorise worldwide stability (Malik, Kumar, Renu, Suni, & Kumar Tarun, 2011).

Table 2.3: Classification of climatic zones with standard storage conditions

Climatic zones	Designation	Countries	ICH stability conditions
I	Temperate (moderate)	Great Britain, Canada, Northern Europe, U.S., Japan, Russia,	Long term studies (real time): 25 °C ± 2 60 % ± 5 Accelerated studies: 40 °C ± 2 75 % ± 5
II	Subtropical	USA, Southern Europe	
III	Hot/Dry	Iran, Iraq Sudan	Long term studies (real time): 30 °C ± 2 65 % ± 5
IV	Tropical (hot/humid)	Brazil, Ghana, Indonesia, Nicaragua, Philippines	*Zone IVa – 30 °C ± 2 / 65 % ± 5; Zone IVb – 30 °C ± 2 / 75 % ± 5* Accelerated studies: 40 °C ± 2 75 % ± 5

(Source: ICH Q1A (R2) and ICH QIF)

CHAPTER THREE

3.0 METHODOLOGY

3.1 RESEARCH ACTIVITIES

The research study was in four phases: *Phase I, Phase II, Phase III and Phase IV.* Each phase involved specific activities carried out in the course of the study.

3.1.1 Phase I

In Phase I, four different formulations of the Mycovin (Griseofulvin B.P. 500 mg) tablets were prepared and coded as *formulation A, formulation B, formulation C* and *formulation D.* At each stage of the formulation, critical test attributes or parameters were performed.

3.1.2 Phase II

In phase II, a confirmatory batch from the best formulations in the Phase I *(formulation D)* was prepared, compressed into tablets and various parameters were tested and processed into the primary commercial package.

3.1.3 Phase III

The freshly prepared batch of Mycovin 500 tablets were divided into three parts.

The first part was subjected to stability study under ICH Accelerated storage conditions of temperature 40 °C ± 2 and relative humidity 75 % ± 5 (FDA Ghana, 2015) in the accelerated stability chamber (Serial No. E010026, manufactured by Sanyo Gallenkamp PLC). The second part was placed in the real time stability room with ICH Real-Time storage condition of temperature 30 °C ± 2 and relative humidity 75 % ± 5 (FDA Ghana, 2015). The final part was set to serve as a control.

3.1.4 Phase IV

In Phase IV, all the results were grouped according to the tests and the various deductions and findings were made.

3.2 MATERIALS AND METHODS

3.2.1 Materials

Griseofulvin micronized (Attar Global), PVP (Clonoose), Sodium Lauryl Sulphate (SLS) (H. Carroux), Gelatin, Isopropyl Alcohol (IPA) and other excipients were of pharmaceutical grade. Methanol (Merck Specialities), Absolute Ethanol (Merck Specialities) and any analytical chemicals used were of analytical reagent grade.

3.2.2 Process summary

The formulation consisted of Griseofulvin micronised (API) and all other excipients (such as binder, lubricant, diluents, disintegrant, etc,). The tablet production requires wet granulation with the various activities: shredding, sifting, drying, milling, blending with extra-granulator excipients, compression and packaging occurring.

The full details of some ingredient quantities in the various formulations and processes are not disclosed due to confidentiality agreement but these information are not considered key to demonstrate the impact of formulation on dissolution profile.

Four main key areas were assumed and considered in order to solve or improve the dissolution rates of the Mycovin (Griseofulvin) 500 tablets:

i. *Formulation A (* 0.79 % Gelatin (binder) and 0.24 % SLS)

This was the original formulation of ECL. The assumption made was that the binder (Gelatin) may not necessarily be the cause of the problem of poor dissolution but uncontrolled (prolong) kneading during granulation process. Kneading (**process where the granulating liquid is added during the granulation process**) time may have effect on the process. Hence, the original formulation was maintained with kneading process and time controlled and monitored. 0.75 kg of gelatin was present in the total weight (95.00 kg) of the formulation representing 0.79% as well as 0.225 kg representing 0.2 % of SLS.

ii. *Formulation B (*2.05 % PVP (binder) and 0.24 % SLS (0.225kg)

In this formulation, all quantities of ingredient in *formulation A* were maintained expect Gelatin. It was assumed that the Gelatin (binder in *formulation A*) may be the contributing factor of the low dissolution in the tablets. (ECL, 2016). Gelatin was replaced with PVP known to improve dissolution of class II drugs (*Handbook of Pharmaceutical Excipients*, 2000).

iii. *Formulation C (*2.05 % PVP and 0.47 % SLS)

In this formulation, the quantity of SLS (0.225 kg) in *formulation B* was doubled to 0.450 kg representing an increased from 0.24 % to 0.47 %. The quantities of all other ingredients were the same as in *formulation B*. SLS, a type of surfactant is known to enhance the solubility and hence dissolution of drugs substance especially class II drugs (Ventosa-andrés & Fernández, 2012).

iv. *Formulation D (*2.05 % PVP and 0.95 % SLS.)

Formulation D had all quantities of ingredients in *formulation C* maintained except SLS increased from 0.450 kg (0.47 %) to 0.900 kg (0.95 %).

3.2.3 Design of experiment

The experiment was carried out at half-batch size of approximately 95 kg. The raw materials were grouped into two parts. **Part 1** consisted of the Griseofulvin micronised, Lactose, Pregelatinised starch, PVP and Gelatin (converted into granulating liquid prior to use) and methyl- and propyl-hydroxybenzoate. The **Part 2** constituted all other excipients included in the granulation process.

The summary of the four different batches prepared were coded as follows:

i. *Formulation A* contained 0.79 % Gelatin and 0.24 % SLS.

ii. *Formulation B* contained 2.05 % PVP and 0.24 % SLS.

iii. *Formulation C* contained 2.05 % PVP and 0.47 % SLS.

iv. *Formulation D* consisted of 2.05 % PVP and 0.95 % SLS.

3.3 PROCEDURE

Good Manufacturing Practices (GMP) were incorporated in this experiment. Clean equipment, machinery and all other internationally accepted SOP's were followed. **Figure 3.1** below illustrates the steps carried out to attain consistency in all the four formulations:

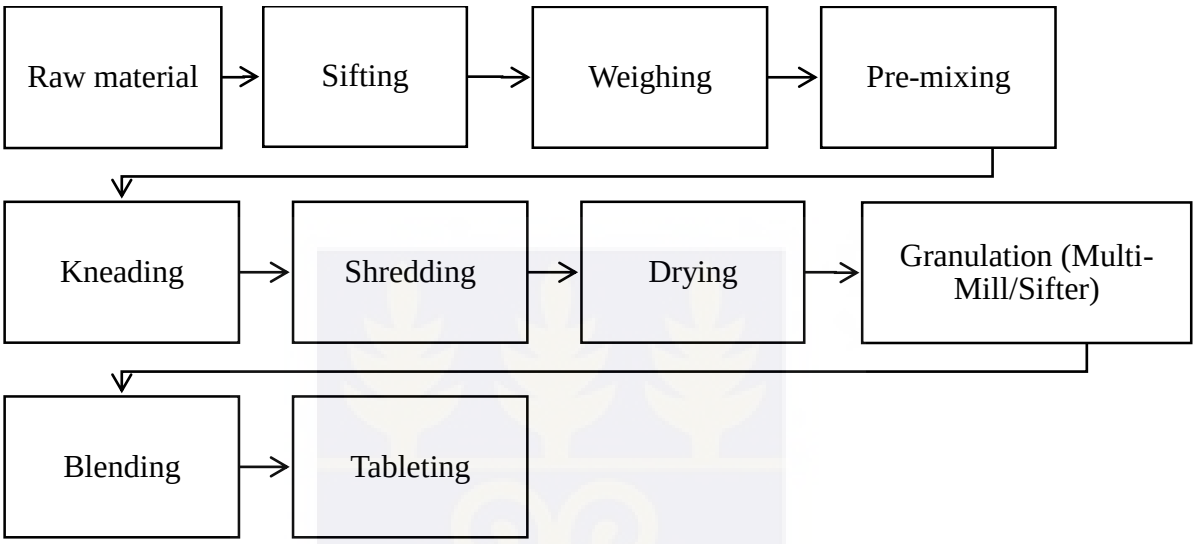

Figure 3.1: Flow Chart of Processes involved in the experiment

3.3.1 Sifting

Aerosil 200, Sodium Lauryl Sulphate, Sodium Starch Glycolate, Purified Talc, Magnesium Stearate were passed through mechanical sifter using 20 mesh before weighing / dispensing. Sifting before dispensing is key in order to loosen the ingredients (raw material) and thereby aerating them. Lactose was also milled in the multi-mill using 1.00 mm sieve to obtain uniform size distribution.

3.3.2 Granulating Liquid

The mixing of powders with water forms stronger bonds, but these powders fall apart once dried. Due to this limitation, a binder is frequently dissolved in solvent/water mixture to obtain a granulating liquid/solution. The gelatin was then dissolved in IPA/water mixture to form the granulating liquid for *formulation A.* On the other hand, PVP was dissolved in water to form

granulating liquid for *formulations B, C* and *D*. These granulating solutions were prepared several hours before the start of the formulation in order to achieve a homogeneous solution. Once the granulating liquid is mixed with the powder, a granular form of mass is obtained after drying.

3.3.3 Pre-mixing

This process involved the mixing of **Part 1** ingredient (except the granulating liquid) in the Rapid Mixer Granulator (RMG, serial number 019/57, Kevin Engineering PVT Ltd.), for 5 min effective time with adequate compressed air.

3.3.4 Kneading

The granulating liquid was added to the pre-mixed component using the main mixer at low speed for 5 min, and high speed for 5 mins. This process generated wet mass of larger sizes.

3.3.5 Shredding

The wet mass (obtained from the kneading process) were of unequal larger size particles. By shredding these wet mass through the multi mill using 10 mm sieve.

3.3.6 Drying

The wet mass shredded was dried in the Fluid Bed Dryer (FBD, serial number 111/Y/00-01, Cadmach Machinery Co. PVT Ltd.) at 75 – 90 °C for an hour. The attained dried mass was passed through 18 mesh using the mechanical sifter. The residue was milled through the multi-mill using 3 mm sieve.

3.3.7 Blending

The **Part 2** (except magnesium stearate) was added to dried mass, blended in the mixer for 20 min and extra 10 min when magnesium stearate was added. All the four formulations were subjected to same granulation conditions and processes.

3.3.8 Tableting

The granules obtained were then compressed as tablets between the range 633 mg ± 3 % (614 – 652mg) with a compression force of 3 tons.

3.4 ANALYTICAL TESTING

The analytical tests (identification, spectroscopic measurement, moisture content, assay, moisture content, disintegration time, dissolution test, hardness, friability test) were carried out in order to ascertain the physical and chemical properties of the API-Griseofulvin and Griseofulvin tablets.

3.4.1 Physicochemical parameters of API-Griseofulvin

The physicochemical parameters of API-Griseofulvin included various identification tests (solubility, identification, acidity, loss on drying and assay) and these were performed as per the British Pharmacopoeia guidelines (BP, 2014a).

Fourier Transform Infra-Red (FTIR) spectroscopy was carried out on the sample. About 5 mg Griseofulvin was thoroughly mixed with about 100 mg IR powder of potassium bromide (KBr), compacted to form a disk and mounted in a suitable holder in Perkin Elmer FT-IR Spectrometer Spectrum Two (Model: L1600401 Spectrum Two DTGS, Serial number: 104799). The spectrum was recorded and compared with a Griseofulvin standard.

The API and standard Griseofulvin were analysed using the Nuclear Magnetic Resonance (NMR), (Brucker 500 MHz) with other NMR measurements such as Proton NMR (^{1}H NMR), Carbon-13 NMR (^{13}C NMR), 135 Distortionless Enhancement by Polarisation Transfer (DEPT) and ^{1}H-^{13}C Heteronuclear Single Quantum Correlation (HSQC).

3.4.2 Moisture Content

The moisture content for granules was determined using the HG53 Halogen Moisture Analyzers (Mettler Toledo) at 100 °C for 1.00 g of granules. The grounded tablets was also determined for moisture following the same procedure as granules.

3.4.3 Tablet Weights

The tablet weights (and any other weighing done) were determined using the Mettler Toledo Analytical scale, model ME204. The average weight of tablets was deduced from weight of randomly sampled twenty tablets.

3.4.4 Hardness Test

The tablets hardness was determined using Hardness Tester Apparatus (Pharma Test, Germany), Model PTB111E-500. Five tablets each were subjected for the hardness tester and the crushing strength of the tablet was measured.

3.4.5 Friability

Twenty randomly sampled tablets were weighed and transferred into the Friability Tester (Copley Scientific, model FRV 2000). These tablets were exposed to turnings of repeated shocks as they fell in each turn within the tester for 100 turns of 4 min duration (at a speed of 25 revolutions per min). The resultant tablets were dusted from any fragments and reweighed. The difference in weight was taken and percent loss in weight was calculated. The loss due to the shocks is a degree of tablet friability expressed in percentage.

3.4.6 Disintegration Time

Disintegration tests were performed according to the procedure described in the USP (USP23–NF18, 1994) using a disintegration apparatus (Pharma Test-Germany, model - PTZ AUTO 2) and 700 mL purified water was the test medium at 37 ± 0.5 °C for a set of six tablets.

3.4.7 Assay

One averaged weight of Mycovin 500 tablet is equivalent to Griseofulvin B.P. 500 mg. Hence equivalent weight of grounded tablets containing 0.10 g of Griseofulvin content was weighed, 50 mL of absolute ethanol was added and shaken for 15 min (using Stuart Orbital Shaker model SSL1). This was made up to the graduated mark of the 100 mL volumetric flask using same solvent, mixed and filtered (through Whatman filter paper # 41). 1 mL of the filtrate was pipetted and diluted to 100 mL with same solvent. The absorbance was measured using Perkin Elmer UV/VIS Spectrometer (Lambda 25) at a wavelength absorbance of 291 nm taking the 686 as the value of Molar Absorptivity, A^1_1 (1 %, 1 cm) at 291 nm.

3.4.8 Granules and tablet dissolution

Dissolution testing was carried out using Model DIS 6000 (of Copley Dissolution Bath) as directed in the British Pharmacopeia (BP, 2014b) employing paddle at 100 rpm for 45 min using 1000 mL purified water containing 1.5 % ($^w/_v$) of Sodium Lauryl Sulphate, at a temperature of 37 $^oC \pm 0.5$. Six averaged weight for tablet (of granules were weighed) and six tablets were placed in the vessels. After 45 min, 25 mL of the samples were drawn, 1 mL of the filtrate was pipetted and diluted with (4:1) methanol / water solution for all the six. The absorbance of the resulting solutions were measured using Perkin Elmer UV/VIS Spectrometer (Lambda 25) at a maximum wavelength absorbance at 291 nm taking the 725 as the value of A^1_1 at 291 nm.

3.4.9 Stability Time Points

Accelerated stability studies covered maximum period of six (6) months (CPMP/ICH/380/95, 1998; WHO, 1996) from the start of the month from which the product was placed into the accelerated chamber. The time points were three months intervals but the first month (1 month) was included to capture any declined results. Hence, the time points are 0 (start date or initial), 1 month, 3 months and 6 months are the time points.

The real time studies covered a longer period of one year with time points being three months intervals thus 0, 3, 6, 9, 12 months (WHO, 1996, 2009). In each of the due month, all the parameters ascribed in the earlier analytical tests were performed on the Mycovin 500 tablets.

CHAPTER FOUR

4.0 RESULTS AND DISCUSSION

Griseofulvin is an important antifungal agent that has recently received attention due to its antiproliferative activity in mammalian cancer cells (Petersen et al., 2017).

4.1 PHYSICOCHEMICAL PARAMETERS OF API-GRISEOFULVIN

The testing of the physicochemical parameters of API and/or excipients are critical in the pharmaceutical set up. This process authenticates the purity and acceptance of the drug substances and excipients for use. The various pharmacopoeias detail the required tests and specifications of any of such drug substances and excipients. In order to determine the physicochemical parameters for Griseofulvin, a selection of physicochemical assessment was performed and the results are summarised (**Table 4.1)**. The results were compared to the British Pharmacopoeia (2014) specification.

Table 4.1: Identification and Analysis of API-Griseofulvin

TEST	RESULTS	SPECIFICATION*
Description / Appearance	Almost white amorphous powder	Almost white amorphous powder
Solubility - Water	Insoluble	Insoluble
Alcohol	Slightly soluble	Slightly soluble
Dimethylformamide	Freely soluble	Freely soluble
Identification B: 5 mg of Griseofulvin dissolved in 1 mL of 1 M $H_2SO_{4\,(aq)}$ and 5 mg of powdered $K_2Cr_2O_7$	A dark red colour observed	A dark red colour must develop
Acidity 0.25 g of Griseofulvin in 20 mL 96 % $C_2H_5OH_{(aq)}$ and 0.1mL phenolphthalein solution.	0.36 mL of 0.02 M $NaOH_{(aq)}$ changed the colour to pink	NMT 1.0 mL of 0.02 M $NaOH_{(aq)}$ must change the colour to pink
Loss on drying 1.00 g of Griseofulvin at 105 °C	0.21 %	NMT 1.00 %
Assay (Griseofulvin content)	99.42 %	97.00 % - 102.00 %

*Testing and specification in accordance to the British Pharmacopeia (BP, 2014a)

NMT- Not More Than

NLT – Not Less Than

Griseofulvin was insoluble in water however showed solubility in organic solvents (e.g dimethylformamide (DMF) or absolute ethanol) (**Table 4.1**). Loss on drying (LOD) is the most ascribed test attribute for APIs. LOD measures both the volatile matter and water/moisture content. Several steps in the synthesis and manufacturing of APIs retain some by-products of traced impurities, solvents and water in the final product-APIs. LOD above the threshold-limit of 1.00 % could indicate the high possibility of an impure substance and the potential of facilitating microbial growth and activities. LOD analysis of the batch of Griseofulvin was

found to be 0.21 % which fell within the recommended limit (NMT 1.00 %). The Griseofulvin content 99.42 % was within the acceptable limit.

4.1.1 Spectroscopic Measurements

To ascertain the authenticity and purity of the raw material acquired for the study a number of spectroscopic measurements were performed. First, Fourier Transform Infra-Red (FTIR) spectrometry measurement on the Griseofulvin sample is summarised in **Figure 4.1** with the standard Griseofulvin as reference.

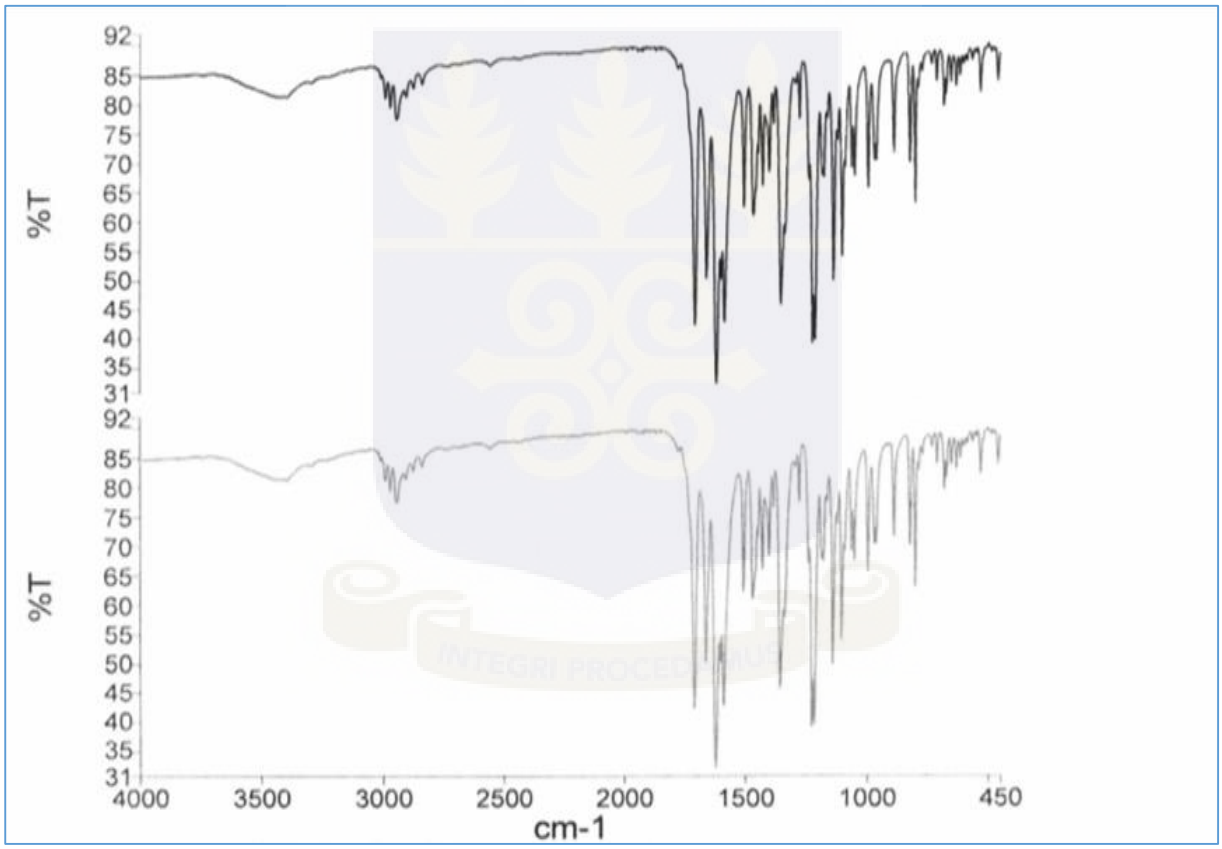

Figure 4.1: IR Spectra of standard and API Griseofulvin-KBr disk

The wavenumbers of the standard Griseofulvin-KBr disk was similar to the sample Griseofulvin-KBr disk with correlation coefficient of 0.9999. Both spectrum showed the principal peaks at wavenumbers 3500 (OH), 2945.1 (CH), 1703.4 (C=C), 1615.7 (C=O), 1583 (C=CH), 1350.3 (OCH), 1211 (C-O), 800.4 (C-Cl) cm^{-1} (KBr) of Griseofulvin (Moffat,

Osselton, & Widdop, 1968). These principle wavenumbers are ascribed to their corresponding functional groups (**Table 4.2).**

Table 4.2: Principal wavenumbers (cm^{-1}) to their corresponding functional groups

Wavenumbers (cm^{-1})	**Functional Group**
3500.2	OH phenolic
2945.1	C-H bond
1703.4	C=C double bond
1615.7	C=O double bond
1583.1	C=CH double bond
1350.3	OCH single bond
1211.2	C-O single bond
800.4	C-Cl bond

Next, both the raw material (API) and standard griseofulvin were analysed using the Nuclear Magnetic Resonance (NMR)**.** A selection of 1D and 2D measurements of the raw material for the study was compared with the standard (**Figure 4.2**).

The singlet at chemical shift 6.15 ppm corresponds to the aromatic proton while that at 5.55 ppm correspond to the cyclohexene proton. The three singlets at 4.04, 4.00 and 3.63 ppm correspond to the methoxy groups and the doublet of doublet at 3.03 ppm correspond to one of the CH_2 proton in the cyclohexene ring, the multiplet between 2.90 - 2.78 ppm correspond to the CH proton, the doublet of doublet at 2.44 ppm correspond to the second CH_2 proton in the cyclohexene ring and the doublet at 0.97 ppm corresponding to the methyl protons.

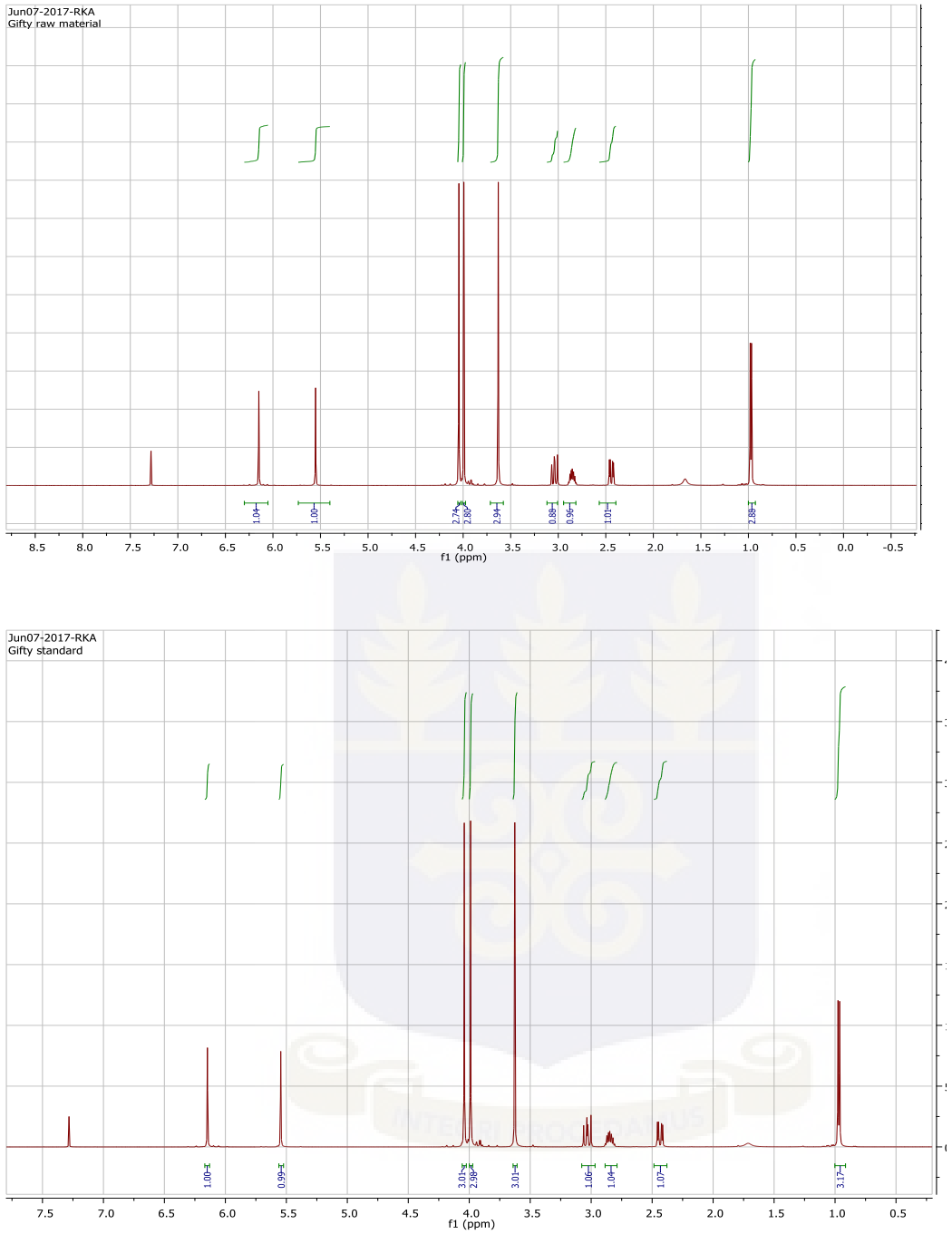

Figure 4.2: ^{1}H NMR of the API and standard Griseofulvin samples

The full characterisation of both ^{1}H NMR, ^{13}C NMR can be seen in **Appendix 1** while the chromatograms of ^{13}C NMR, DEPT and HSQC are in **Appendix 3, 4 and 5** respectively. Comparism of the spectroscopic data collected showed that the raw material is pure and the results compares very well with the standard supplied by the manufacturer.

4.2 FORMULATIONS

Formulation technologies are of great importance in improving the dosage forms of most pharmaceutical products (Frohberg, Nguyen, & Ulrich, 2016). Drug formulations are generally dependent on the physicochemical properties of the drug. Four formulations (*formulations A, B, C* and *D*) were prepared and several parameters were measured and compared. Test parameters were determined at both granules and compressed tablets stages.

4.2.1 Granules

Granules made from *formulations A, B, C* and *D* must have certain limit of moisture content. The level of moisture is critical to overcome over and under wetting of the mass. The moisture content of the granules for the four formulations were determined and the results are summarized in **Table 4.3.** The moisture content values were all found to be within recommended limit of ≤ 3.00 % per formulation requirement by ECL.

Table 4.3: Granules Test Parameters

Test Parameter	***Formulation A***	***Formulation B***	***Formulation C***	***Formulation D***
Moisture content *(NMT 3.00 %)*	1.06	1.01	0.87	0.96
*Assay *(95.00 – 105.00 %)*	97.96 ± 0.02	98.26 ± 0.01	98.11 ± 0.01	98.31 ± 0.01
*Dissolution *(NLT 80.00 %)*	85.64 ± 0.11	94.02 ± 0.05	90.18 ± 0.07	94.31 ± 0.04

*Mean ± sd, **NMT** – Not More Than, **NLT** – Not Less Than

Maintaining the right moisture content is critical in tablet formation. Higher moisture content facilitates microbial growth. Dried under wet mass generates hard granules with moisture

content < 0.5 % creating challenges during compression into tablets. Such compressed tablets cap and chip in the process. Dried over wet mass produce high moisture content granules > 3.00 % with too soft and friable compressed tablets.

Mean dissolution values above 90.00 % was obtained for all the formulations; PVP-*formulations B* (94.02 % ± 0.05), *C* (90.18 % ± 0.07) and *D* (94.31 % ± 0.04), as compared to gelatin- *formulation A* (85.64 % ± 0.11). The assay values were also within the acceptable limit (*95.00 – 105.00 %*) in all the four formulations.

The dissolution profiles for granules formulations **Figure 4.3,** depicts the trend of each formulation granules. The percentage of Griseofulvin drug released from the granules for PVP-*formulations* (*B, C, D)* showed higher trend far above the lower limit than the gelatin-*formulation (A).*

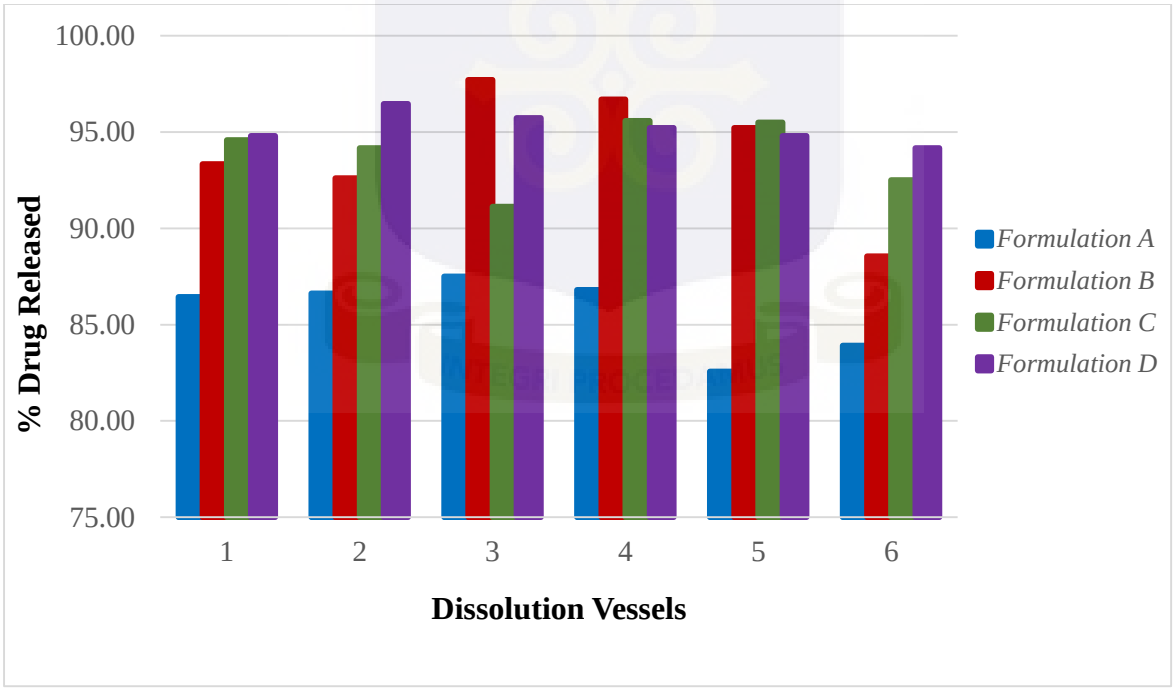

Figure 4.3: Dissolution Profiles for Granules Formulations

4.2.2 Tablet Testing

The quality of compressed tablets depends on several factors such as compression force, the type of binder, disintegrants, etc. The granules from the four formulations were compressed with the same compression force of 3tons into their respective tablets. In order to investigate the quality of the tablets formed, a number of parameters including assay, friability, hardness, moisture content, disintegration time and dissolution were determined, **Table 4.4**.

Table 4.4: Tablet Test Parameters

Parameters *(specification)*	***Formulation A***	***Formulation B***	***Formulation C***	***Formulation D***
Av. tablet weight *(630 mg ± 3 %)*	630.9	633.1	631.0	631.7
Hardness *(NMT 15.0* kP*)*	5.6	4.1	4.9	5.8
Friability *(NMT 1.00 %)*	0.49	>> 1.0, tablets capped and chipped	>> 1.0, tablets capped and chipped	0.40
*Assay *(95.00 – 105.00 %)*	98.80 ± 0.01	99.40 ± 0.01	98.83 ± 0.01	98.40 ± 0.01
Disintegration time *(NMT 15 min)*	4	6	4	7
*Dissolution *(NLT 80.00 %)*	72.01 ± 0.20	87.00 ± 0.10	94.31 ± 0.05	95.19 ± 0.04

*Mean ± sd
NMT – Not More Than
NLT – Not Less Than

Comparing the results in **Table 4.3** with that of **Table 4.4**, the mean dissolution of the granules for *formulations A* and *B* (85.64 % ± 0.11 and 94.02 % ± 0.05 respectively) were relatively greater than that of the mean dissolution of the tablets (72.01 % ± 0.20 and 87.00 % ± 0.10 respectively). However, the mean dissolutions of the tablets for *formulations C* and *D* (94.31 % ± 0.05 and 95.19 % ± 0.04 respectively) were slightly higher than their granules (90.18 % ± 0.07 and 94.31 % ± 0.04 respectively).

Gelatin binds strongly to API and other excipients when used as a binder. It absorbs water and swells up forming a rubber-like pellicle. This may affect the disintegration time of the tablets in which gelatin is a binder. Hence most of the API-Griseofulvin is not readily released/available in the dissolution medium causing less dissolution. PVP on the other hand is a binder but also serves as disintegrant in aqueous solution. This disintegrating property of PVP aids and gives a quicker disintegration.

The disintegrant in compressed tablet absorbs dissolution medium and cause tablets to break up (into disintegrated phase). This process produces particles with larger surface area to facilitate dissolution. However, granules were already in the 'disintegrated phase' and do not require any further time to disintegrate unlike the tablets. Granules from gelatin-*formulation* had higher dissolution values as compared to its compressed tablets. This was due to the high binding nature of gelatin and larger surface area of the granules.

Unlike PVP-*formulations* the granules dissolutions were relatively similar to that of the tablets dissolution with some even being better. There were no significant variation in the weight of the tablets, assay and disintegration time when the various test parameters were investigated (**Table 4.4**). In addition, all the results obtained were within recommended limits.

Table 4.4 further shows the results of hardness measurements performed on the tablets. Tablet hardness provides a guide to product development and as a quality control specification.

Extreme hard/soft tablets are prone to excessive/weak bonding potential between the API and excipient leading to prolong dissolution/friable tablets. The tablet hardness for the formulations was between 4.1 and 5.8 Kp, which were below the 15 Kp acceptable limit. The tablet hardness was relatively linked to their friability. The tablets of *formulations B* (4.1 Kp) and *C* (4.9 Kp) were not adequately hard enough therefore generated chipped, capped and even broken tablets after the friability test. These tablets were found to be too soft (friable) resulting in higher friability above the limit of 1.00 % for *formulations B* and *C* as compared to *formulations A* (5.6 Kp) and *D* (5.8 Kp) whose tablets withstood the friability test.

4.2.3 Formulation Comparison

The dissolutions of the tablets from the various formulations were compared with their respective granules dissolution in order to ascertain the dissolution rates trend. Tablets of *Formulations C* and *D* gave an increased mean dissolution of 93.91 % ± 0.05 and 95.19 % ± 0.04 respectively compared to *formulation A* with mean dissolution of 72.01 % ± 0.20 (**Figure 4.4**).

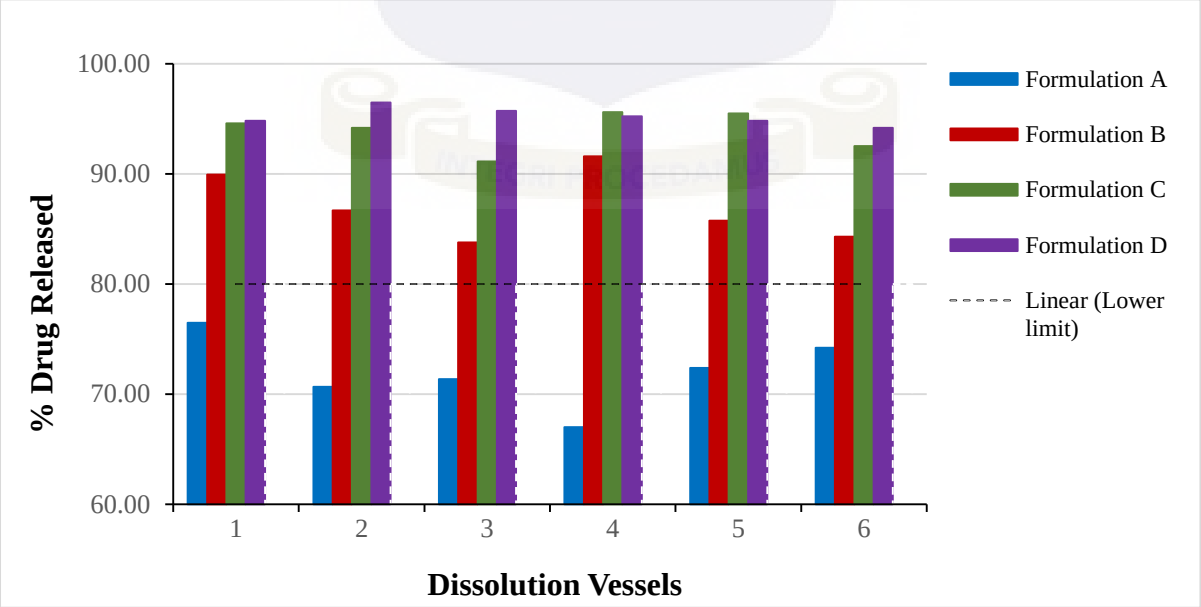

Figure 4.4: Dissolution Profiles for Tablet Formulations

A good dissolution property is the key to an effective and efficient drug. Tested parameters of *formulation A* tablet passed with the exception of the dissolution. The inability of the gelatin to readily release the API-Griseofulvin into the medium within the desired dissolution duration of 45 min was the major challenge in tablet *formulation A*.

All the PVP-*formulations B, C* and *D* gave high percentages of drug released (dissolution) above the recommended 80 %. The PVP-*formulations B*, C and D gave mean dissolutions of 87.00 % ± 0.10, 93.91 % ± 0.05 and 95.19 % ± 0.04 respectively. The gradual increases in the dissolutions of the three *formulations: B, C, D*, (**Figure 4.5**), are attributed to the use of PVP and SLS to enhance their dissolution properties.

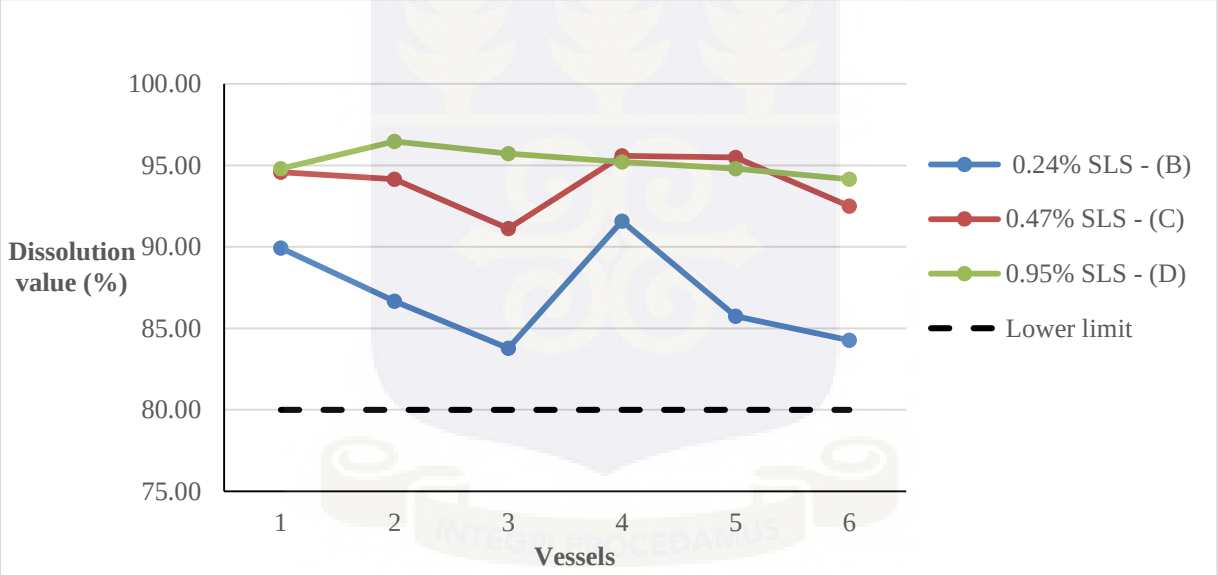

Figure 4.5: Dissolution profile of PVP-Formulations with varied amount of SLS

Within the PVP-*formulations*, an equal amount of 2.05 % PVP was used for all the formulations but with varied amounts of SLS. 0.24, 0.47 and 0.95 % SLS was added to *formulations B, C* and *D* respectively. Examination of the results showed a trend, **Figure 4.5**, where the percentage dissolution increases with increasing amount of SLS in the formulation.

The inclusion of surfactant (SLS) in the formulations are reported to enhance the dissolution performance of class II drugs used in pharmaceutical setting to improve poorly soluble drugs. SLS lowers the surface tension and effectively improve the dissolution of lipophilic drugs such as Griseofulvin in aqueous medium (Ventosa-andrés & Fernández, 2012). The results (**Figure 4.5**) clearly showed a significant improvement in the dissolution as the amount of SLS was increased.

Combining all the data generated for the Mycovin 500 tablet, *formulation D* was adopted as the best formulation to address the poor dissolution challenge encountered by ECL. The chosen formulation was based on all the test parameters: hardness (5.8 Kp), friability (0.40 %), assay (98.40 % ± 0.01), etc. These findings were comparable to the original *formulation A* (hardness 5.6 Kp, friability 0.49 % and assay 98.80 % ± 0.01). The mean dissolution of *formulation D* (95.19 % ± 0.04), was however observed to be an improved formulation compared to that of *formulation A* (72.01 % ± 0.20).

4.2.4 Confirmation of *formulation D*

A new batch of *formulation D* (Phase II) was then prepared and subjected to the same test described above as full scale confirmatory commercial batch of Griseofulvin tablets (**Table 4.5**).

Table 4.5: Tablet result from confirmatory batch of *formulation D*

Test Parameters ***(specification)***	**Test Results**	**Original Data**
Average tablet weight *(630 mg ± 3 %)*	651.5	631.7
Hardness *(NMT 15.0* Kp*)*	5.8	5.8
Friability *(NMT 1.00 %)*	0.26	0.40
Moisture content *(NMT 5.00 %)*	2.11	2.23
*Assay *(95.00 – 105.00 %)*	98.77 ± 0.01	98.40 ± 0.01
Disintegration time *(NMT 15 min)*	9	7
*Dissolution *(NLT 80.00 %)*	93.27 ± 0.05	95.19 ± 0.04

*Mean ± sd **NMT** – Not More Than **NLT** – Not Less Than

Assessments of the results in **Table 4.5** shows all the parameters determined were within the recommended limits and very similar to the original trial *formulation D*. Then the tablets were packed in an Alufoil-PVC blister pack and subjected to ICH accelerated and long term (real time) stability conditions (**Phase III**) and the results discussed below (**Phase IV**).

4.3 EVALUATION OF STABILITY OF THE ADOPTED *FORMULATION D*

In the course of a stability studies, a product is expected to be stable for a particular formulation, in a specific container/ closure system, remain within its physical, chemical, microbiological, therapeutic, and toxicological specifications at a defined storage condition (Sachan, Anupam Kumar; Kumar, 2015). The products manufactured are expected to remain potent/active up to their expiry dates stated on their labels.

The expiry dates quoted on the products are arrived at by data from stability studies conducted on them.

Several elements affect drug products stability, these include the intrinsic stability properties of the APIs, the potential interaction between APIs and excipients, the process of manufacturing, the dosage form, the container-closure system and the environmental conditions encountered during transporting (Briscoe & Hage, 2009; Shabir & Arain, 2004).

Other environmental conditions such as temperature, humidity, light, etc. also affect the stability of the product. Hence stability studies are done at the prevailing environmental conditions (**real time/long term**) and elevated conditions (**accelerated**) to provide justification for the stated expiry date and have an idea about excursion of the environmental conditions outside the prevailing condition. The manufactured tablets were subjected to ICH accelerated conditions for zone IVb at 40 °C ± 2 and relative humidity 75 % ± 5.

Table 4.6: Summary results from Accelerated Stability

Storage Time (month)	Friability (%)	Hardness (Kp)	Moisture (%)	*Assay (%)	*Dissolution (%)
0	0.26	5.8	2.11	98.77 ± 0.01	93.28 ± 0.05
1	0.23	5.0	1.74	98.25 ± 0.01	96.76 ± 0.03
3	0.33	5.4	2.04	99.35 ± 0.01	91.56 ± 0.07
6	0.27	6.9	2.11	97.16 ± 0.02	91.63 ± 0.07

*Mean ± sd

Tablets are to have a certain degree of hardness in order to have good friability to withstand external pressure of shock and mechanical abrasions. The lower the tablet friability the better the tablet is able to withstand chipping, abrasion and breakage during manufacturing /handling,

shipping and transportation. The friability of the tablets in **Table 4.6** was found to be 0.23 - 0.33 %, from 0 - 6 months of measurement, below the recommended 1.00 % weight loss suggesting the harsh environment did not have any adverse effect on the tablet friability over the six months period of storage.

In addition, an increase in hardness of 6.9 Kp was determined after 6 month. The increase in hardness, however, had no effect on the friability and dissolution of the tablets after the six months storage duration. Overall the Griseofulvin content (assay) decreased by 1.61 % over the period of six (6) months storage under accelerated condition.

ICH defines 'significant change' in a drug product as a 5 % change in assay from its initial value or failure to meet acceptable criteria for physical attributes (Kunzle et al., 2009). The slight decrease of 1.61 % obtained in the assay under an accelerated condition did not have any adverse effect in the chemical composition of the drug. Griseofulvin content of 97.16 % after six months was within the acceptable limit (95.00 - 105.00 %).

In real time stability studies, the products are generally stored at the ICH recommended storage conditions and monitored until it fails specification (Magari, 2003). Real-time stability studies are carried out at 0, 3, 6, 9, 12 months on the first year, every 6 months on the second year and once every year afterwards (Bhagyashree, Karishma, Sampada, Chandankar, & Kailash, 2015).

The Mycovin (Griseofulvin 500) tablets were also subjected to ICH real time condition (30 °C ± 2 / 75 % ± 5) with control (**retained**) samples at 25 °C ± 2 / 65 % ± 5. In both instances, all the test parameters were measured in 3 months intervals until the 12th month. The results obtained showed no significant change in the real time stability and control assessment (**Table 4.7**).

Table 4.7: Summary results from Real-Time Stability vrs. Control (Retained) Samples.

Real-Time Stability					
Storage Time (month)	**Friability (%)**	**Hardness (Kp)**	**Moisture (%)**	**Assay (%)**	**Dissolution (%)**
0	0.26	5.8	2.11	98.77 ± 0.01	93.28 ± 0.05
3	0.19	5.3	2.19	98.93 ± 0.01	91.75 ± 0.06
6	0.23	5.7	1.84	97.52 ± 0.02	93.70 ± 0.01
9	0.21	5.0	2.07	97.45 ± 0.02	94.74 ± 0.04
12	0.18	5.3	2.32	97.01 ± 0.02	93.96 ± 0.05
Control (**Retained**) Samples					
0	0.26	5.8	2.11	98.77 ± 0.01	93.28 ± 0.05
3	0.26	5.9	2.13	98.79 ± 0.01	93.25 ± 0.05
6	0.27	5.8	2.53	98.91 ± 0.01	94.18 ± 0.04
9	0.25	5.3	2.02	98.47 ± 0.01	93.08 ± 0.05
12	0.23	5.5	1.77	98.25 ± 0.01	93.20 ± 0.05

4.3.1 Stability Comparison

Results obtained for real time stability in **Table 4.7** (**top half**), were very similar to that of the control samples (**bottom half**). There was no significant change, in accordance to ICH definition, in any of the test parameters. This observation satisfies the guideline for storage of pharmaceutical products which directs, for example, that most drugs should be kept below temperature of 30 °C because at this temperature chemical degradation is rare or minimal.

For the assay correlation determinations, a gradual decrease in the assay over the storage duration from 98.77 % to 97.01 % was also observed with a loss of 1.76 % within a period of twelve (12) months for the real time compared to the control of 0.52 %. These differences in assay and dissolution are statistically acceptable (not more than 2 %) in an analytical analysis.

Dissolution stability is regarded a key parameter of quality control. Any significant changes of the *in vitro* release profile of the drug substance during storage may affect its bioavailability (Vidal, Brevedan, Varillas, Simionato, & Pizzorno, 2010). Hence, the absence of such dissolution changes give some assurance that the bioavailability of the drug will remain intact during storage.

The mean dissolution value for both real time and control were also found to be within the recommended limit (80 %) suggesting the tablets withstood environmental factors and still showing excellent *in vitro* dissolution after the 12 months (**Figure 4.6**).

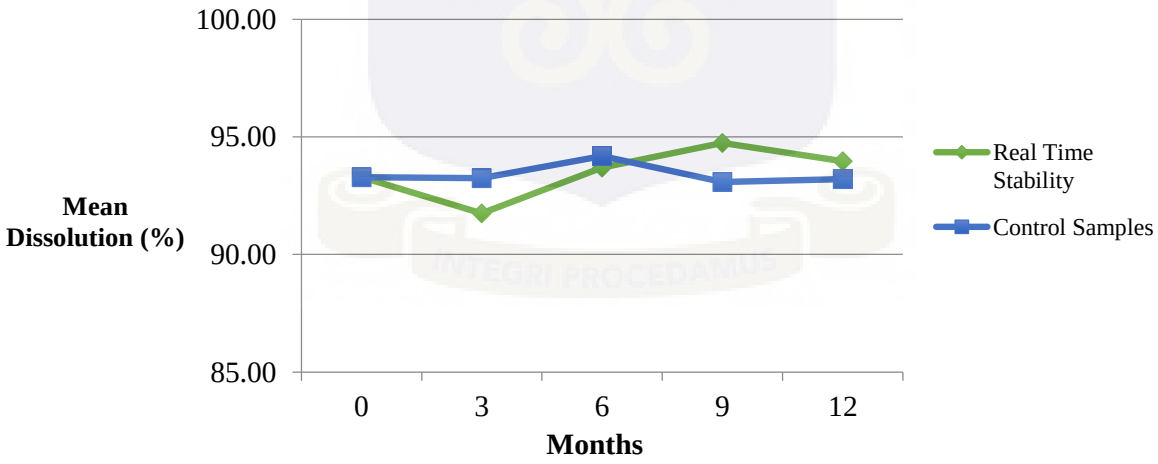

Figure 4.6: Mean Dissolution correlation of Real Time and Control Tablets over 12 months storage duration

With USP definition on dissolution (USP23–NF18, 1994), a rapidly dissolving drug substance is 85% or more of the labelled amount of API dissolve within 30 min using USP Apparatus (basket / paddle) in a 900 mL volume or less of buffer solutions (Gohel, 2005; USP23–NF18, 1994). Hence, in order to ascertain the level of dissolution for the formulated tablets, a dissolution for three time points (15, 30, 45 min) were carried out for the control tablets before the start of the stability studies (0 month) and after the stability studies (12 months) in **Table 4.8**.

Table 4.8: % Mean Dissolution – Time values for control tablets

Months	**% Mean Dissolution**		
	15 min	**30 min**	**45 min**
0	84.23 ± 0.13	91.32 ± 0.07	93.28 ± 0.06
12	82.72 ± 0.13	90.79 ± 0.07	92.38 ± 0.05

At the start of the stability studies (0 month), there was a sharp increase in the mean dissolution from 0 % (0 min) to 84.23 % ± 0.13 (15 min). Resulting in an increase of about 84 % of the dissolved Griseofulvin in the dissolution medium. Between 15 min and 30 min, there was a gradual increase of about 7 % as compared to the time frame between 30 min and 45 min which was only about 2 % of extra dissolved Griseofulvin into the dissolution medium. Similar trend was also seen in the mean dissolution of the tablets after the 12 month storage duration. Nonetheless, the little variation between both mean dissolutions of the tablets could not be considered as a significant change. The data in **Table 4.8** gave an indication that the Griseofulvin tablets dissolution had improved even at 15 min.

CHAPTER FIVE

5.0 CONCLUSION

Four different formulations using combinations of gelatin, PVP and SLS were prepared in order to enhance the solubility (dissolution) and stability conditions of Mycovin 500 tablets manufactured by the Ernest Chemist Limited (ECL). The formulations were investigated using the wet granulation method.

The initial formulation-*Formulation A* contained gelatin as a binder and *formulations B, C* and *D* contained PVP with varying amount of SLS. The formulations with PVP (***B, C,*** and ***D***) gave better dissolutions than that of the gelatin (***A***). Dissolution values for *formulation A* tablets were lower while all the PVP-*formulations B, C* and *D* gave high percentages of dissolution above the recommended 80 %. The PVP-*formulations B,* C and D gave 87.00 % ± 0.10, 93.91 % ± 0.05 and 95.19 % ± 0.04 respectively. The gradual increases in the dissolutions of the three *formulations: B, C, D,* are attributed to the use of PVP and SLS to enhance their dissolution properties.

Of the three PVP-*formulations*, *formulation D* emerged the best with comparable parameters to the original *formulation A*. While the hardness 5.8 Kp, friability 0.40 %, assay 98.40 % ± 0.01 respectively were obtained for the adopted formulation, the original *formulation A* gave hardness 5.6 Kp, friability 0.49 % and assay 98.80 % ± 0.01 respectively. However, the mean dissolution of *formulation D* (95.19 % ± 0.04), was observed to be superior to that of *formulation A* (72.01 % ± 0.20).

Upon subjection of the tablets of the confirmatory batch to both ICH accelerated and real time conditions, no significant changes in the parameters investigated were observed after 6 months for accelerated and 12 months for real time. The friability was found to be between 0.23 - 0.33 % with an increase in hardness of 6.9 Kp observed. In addition, a decrease of 1.61 % (6 months), 1.76 % (12 months) of Griseofulvin content (assay) did not impact any

adverse effect on the chemical composition of the drug over the investigated periods in the stability studies.

In conclusion, this study has addressed the challenge of solubility (dissolution) usually encountered during the manufacture of Griseofulvin by formulation using combinations of gelatin, PVP and SLS.

5.1 RECOMMENDATION

A different approach to this research work should be considered by varying the concentration of PVP against a constant concentration of the SLS.

The use of an HPLC method should also be used for both the assay and dissolution.

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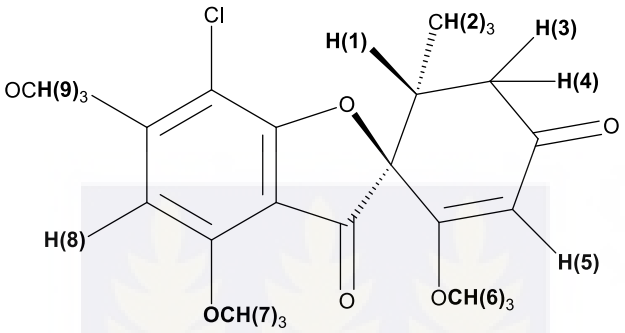
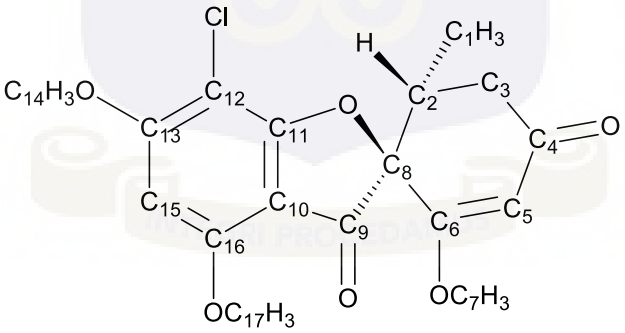
APPENDIX 1

Data for **(1'S,6'R)-7-chloro-2',4,6-trimethoxy-6'-methyl-3H-spiro[benzofuran-2,1'-cyclohex[2]ene]-3,4'-dione**

V_{max} (KBr) cm^3 3500 (OH), 2945.1 (CH), 1703.4 (C=C), 1615.7 (C=O), 1583 (C=CH), 1350.3 (C-O), 1211 (C-O), 800 (C-Cl)



^{1}H NMR (500 MHz, $CDCl_3$) δ_H 6.15 (s, **H8**), 5.55 (s, **H5**), 4.04 (s, **H9**), 4.00 (s, **H7**), 3.63 (s, **H6**), 3.03 (dd, *J* = 16.7, 13.4 Hz, **H4**), 2.89 – 2.78 (m, **H1**), 2.44 (dd, *J* = 16.7, 4.7 Hz, **H3**), 0.97 (d, *J* = 6.7 Hz, **H2**).



^{13}C NMR (126 MHz, $CDCl_3$) δ_C 197.0 (**C4**), 192.1 (**C9**), 170.8 (**C6**), 169.5 (**C11**), 164.6 (**C16**), 157.8 (**C13**), 105.2 (**C5**), 97.3 (**C10**), 90.8 (**C12**), 89.3 (**C8**), 57.0 (**C15**), 56.7 (**C17**, **C14**), 56.4 (**C7**), 39.9 (**C3**), 36.6 (**C2**), 14.6 (**C1**).

APPENDIX 2

1. In calculating the percentage content of Griseofulvin (Assay) and dissolution, the following processes were observed.

a. Assay: Dilution: $0.10\ g/100\ mL \xrightarrow{1\ mL}$ 100 mL [0.001 % $^{w}/_{v}$]

λmax = 291 nm; A^{1}_{1}=686; Medium (solvent) – Absolute ethanol

Expected Absorbance (E.A): 0.001×686 = 0.686

Observed Absorbance (O.A) = Mean absorbance reading obtained

Hence,

% content: $O.A/E.A$ ×100 %

Example:

Expected Absorbance = 0.001 × 686 = 0.686

Observed Absorbance (i) = 0.662, 0.663, 0.663 mean 0.663

(ii) = 0.674, 0.674, 0.674 mean 0.674

% Content:

(i) $^{0.663}/_{0.686} \times 100 = 96.65\ \%$

(ii) $^{0.674}/_{0.686} \times 100 = 98.25\ \%$

Average % Content = 97.45 %

b. Dissolution: Dilution: $0.50\ g/1000\ mL \xrightarrow{1\ mL} 50\ mL\ [0.001\ \%\ w/v]$

λmax = 291 nm; A^{1}_{1}=725; Medium (solvent) – 4:1methanol/water

Expected Absorbance: 0.001×725 = 0.725

% Dissolution = $O.A/_{E.A}$ ×100 %

Example:

Expected Absorbance = 0.001 × 725 = 0.725

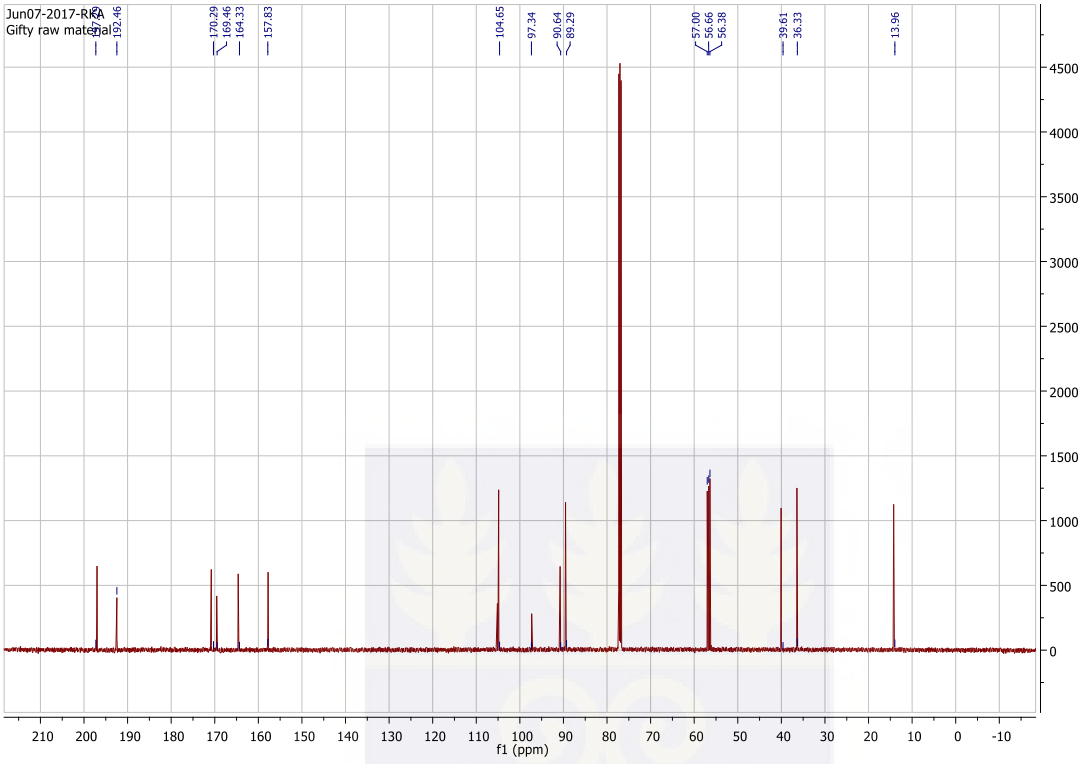
Observed Absorbance (V1) = 0.680, 0.680, 0.680 mean 0.680

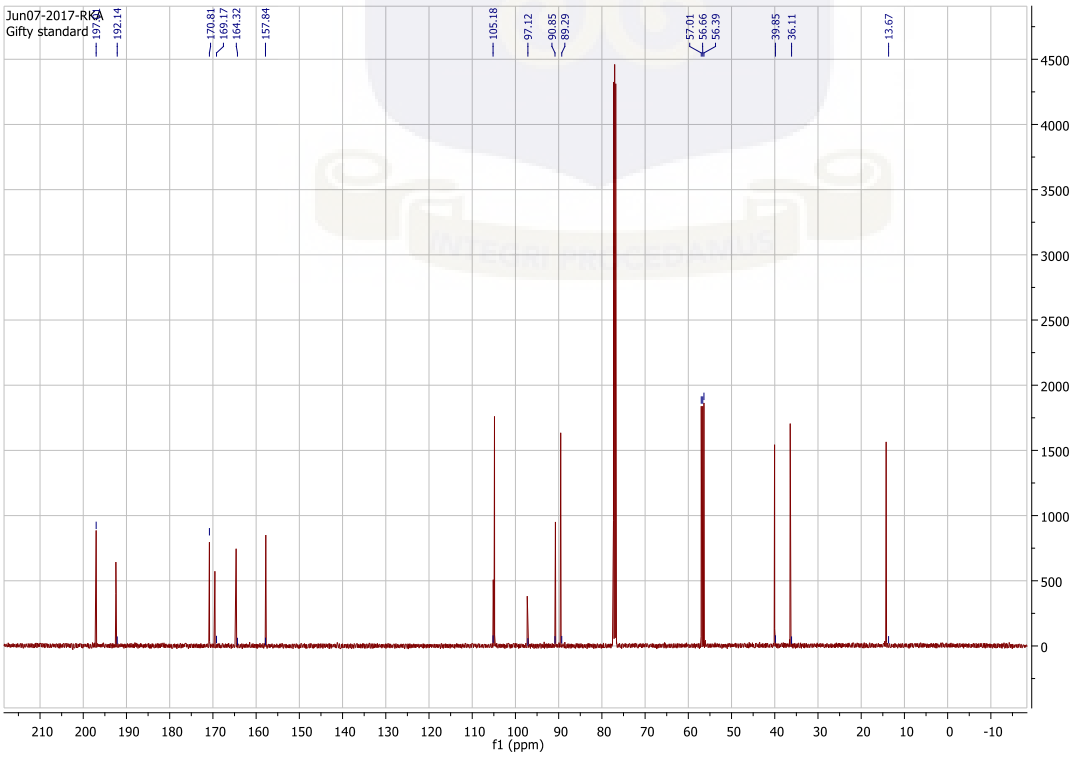
% Dissolution:

$$0.680/_{0.725} \times 100 = 93.79\ \%$$

APPENDIX 3

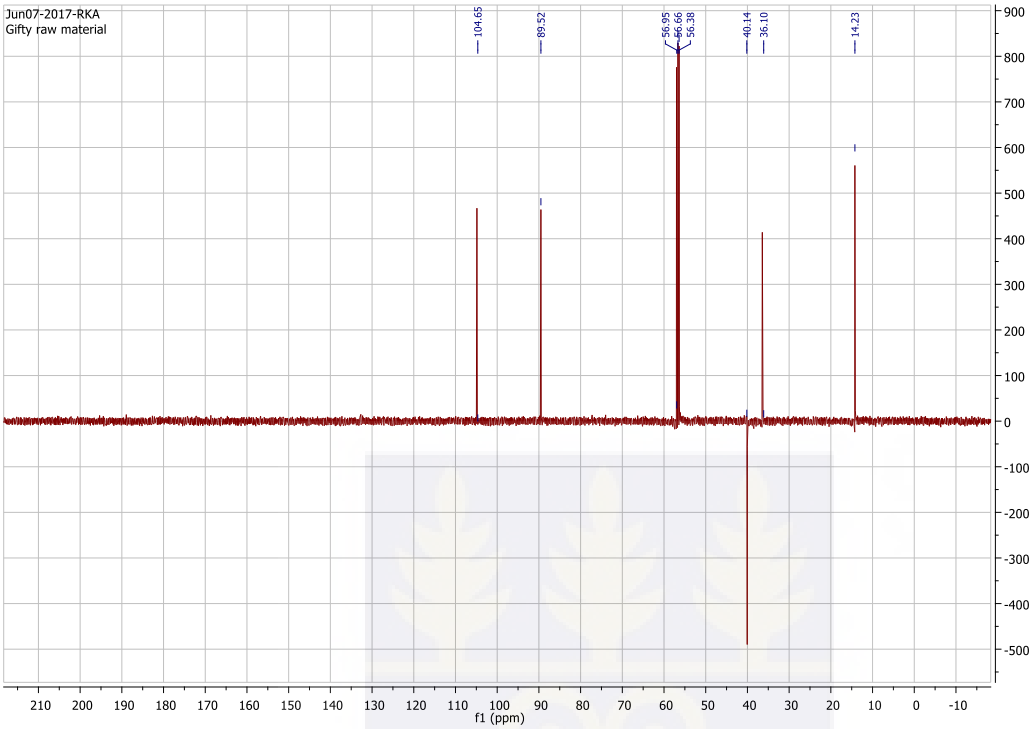
^{13}C NMR OF THE RAW MATERIAL AND STANDARD GRISEOFULVIN

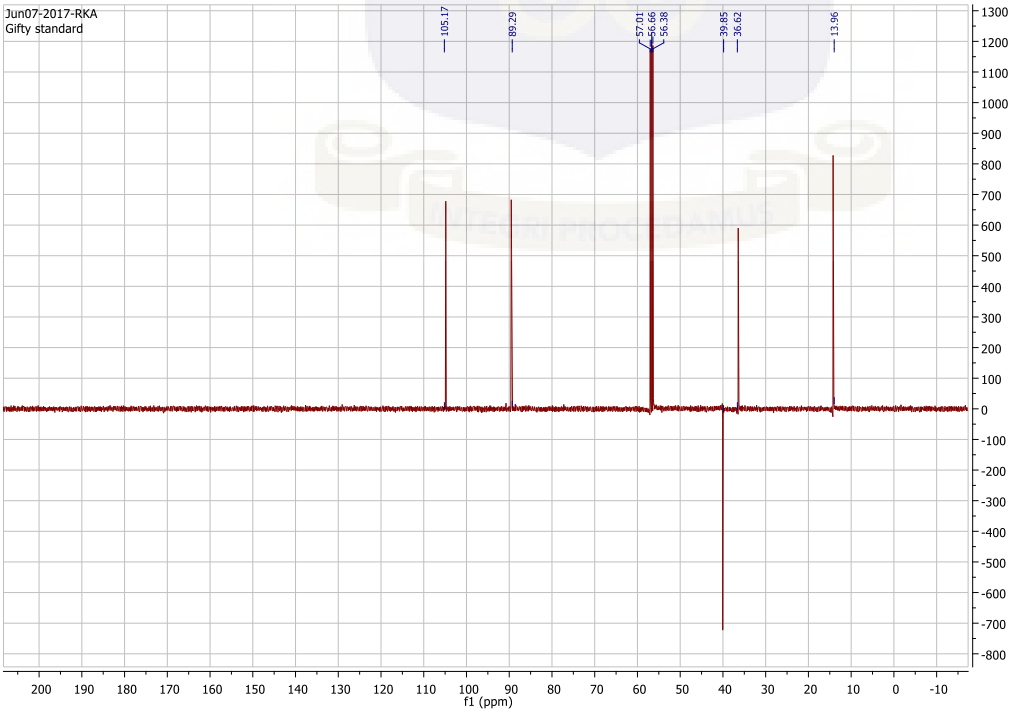




APPENDIX 4

DEPT CHROMATOGRAM OF THE RAW MATERIAL AND STANDARD GRISEOFULVIN





APPENDIX 5

HSQC CHROMATOGRAM OF STANDARD GRISEOFULVIN