FORMULATION AND IN VITRO EVALUATION OF GRISEOFULVIN CREAM FOR TREATMENT OF TINEA PEDIS.

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FORMULATION AND IN VITRO EVALUATION OF GRISEOFULVIN CREAM FOR TREATMENT OF TINEA PEDIS.

By

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A Dissertation submitted in partial fulfillment of the requirements for the Degree award of Master of Pharmacy in Industrial Pharmacy of the Muhimbili University of Health and Allied Sciences.

October, 2024

CERTIFICATION

The undersigned certify that they have read and hereby recommend the acceptance of the dissertation entitled "Formulation and in vitro evaluation of griseofulvin cream for the treatment of tinea pedis" in partial fulfillment of the requirements for the degree of Master of Pharmacy in Industrial Pharmacy from Muhimbili University of Health and Allied Sciences.

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DEDICATION

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ABSTRACT

Background: Tinea pedis have been treated using Griseofulvin tablet as oral dosage form in part of Africa including Tanzania. In Ghana Griseofulvin was incorporated into shea-butter to make the drug spread easy to infected region of skin. The approach to seek formulation of griseofulvin cream in Tanzania is due to the rising need to reducing side effects induced by griseofulvin tablet, among the primary and secondary school children infected by tinea pedis when administered the drug orally.

Objective: To formulate and in vitro evaluate griseofulvin cream for treating Tinea pedis.

Material and method: White vaseline from Caesar and Loretz GmbH; Polysorbate 80 (Tween 80) from Sigma Aldrich (Switzerland); Sorbitan oleate (Span 80) from Sigma (USA); distilled water q.s. and Griseofulvin powder was purchased and bought from North Korea, were used in formulation of cream. Toluene, diethyl ether for mobile phase, ethyl acetate for diluent were bought in Dar es salaam. Tests and analysis for rheological properties were determined using Kinexus rheometer (Malvern) at temperature of 25ºC, with a geometry spindle CP4/40 SR1454SS (KJ3100277), Potato agar, *Trichophyton rubrum*, and incubator from ITM-Muhas. Albino rats from school of Pharmacy-Muhas. Physical properties were observed and verified against specifications. pH was measured using electronic pH conductometer in which 1gm was diluted to 100mls of distilled water. Accelerated stability test for cream was done using stability chamber from day 0 to day90, then test performed using HPTLC. Antifungal activity tested against *Trichophyton rubrum* which causes tinea capitis in which Agar plate diffusion method was done using potato agar and activity studied for 5days. Well diameters were 15mm, cream weight was 100.2mg, control was pure cream.

Results: Formulations 1,2 and 3 had desirable trends of rheological properties in term of viscometry, frequency sweep, three step shear rate, creep and recovery. Meanwhile formulation F4 had less desirable viscometry, thixotropic, creep and recovery properties. pH trends were stable, ranging from 5.9 to 7. Formulations were stable from day 0 to 90 under accelerated temperature conditions of 40ºC± 5ºC and humidity conditions of 75%±5%.

The assay trend showed a range from 100.62% on day 0 to 99.68% on day 90. Antifungal activity of Griseofulvin cream had shown a very strong activity against *Trichophyton rubrum* with zone of inhibition of 37.5mm. Irritation effect of the cream was very slight with an average edema and erythema score of 1.17.

Conclusion and recommendations: This was the first formulation of a semisolid griseofulvin product in Tanzania, focusing on essential performance parameters such as rheological and physical properties, pH, and stability behavior. The first three formulations demonstrated desirable rheological properties, with formulation F3 showing the most favorable trend. The pH values adhered to specifications ranging from 5 to 8, and all formulations exhibited a stable shelf-life trend. Formulation showed strong antifungal activity against Tinea pedis.

Keywords: *Griseofulvin cream, Rheological properties, Hydrophilic lipophilic balance value, physical appearance, pH, Antifungal activity*

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LIST OF ABBREVIATIONS

ICH: International Conference on Harmonization

EMA: European Medicine Agency

HLB: Hydrophilic lipophilic balance value

LOQ: limit of Quantification

LOD: Limit of detection

G`: G prime modulus of elastic properties

G``: G double prime of viscous properties

pH: Measure of acidity and alkalinity, term as potential of Hydrogen, scale range from 1-14, with 7 being neutral

MUHAS: Muhimbili University of Health and Allied science.

UiB: University of Bergen

W/O: Water to Oil ratio

P.Cx: Pharmaceutical Codex

HPTLC: High performance thin layer chromatograph

F1-F4: Formulation 1 to 4

J_E: Compliance

HLB: Hydrophilic-Lipophilic balance

SOP: School of Pharmacy

DEFINITION OF KEY TERMS

Creep compliance: a ratio of the measured strain to the applied stress or inverse modulus

Yield value: a rheological property that measures the resistance of a material to flow. higher yield value tends to be thicker and more viscous. They spread easily and are quickly absorbed into the skin. That is viscosity or thickness of the cream

Yield stress: a stress applied to the cream before it starts to spread, in which below yield stress the sample will deform elastically and above yield stress the cream will spread like a liquid.

Hydrophilic-Lipophilic Balance value: a measure of balance between the hydrophilic (water loving) and lipophilic (oil-loving) components in a substance.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

Dermatological diseases, particularly fungal skin infections, are widespread globally. Utilizing topical therapy proves advantageous in treating these infections, enabling targeted drug delivery to the infection site and minimizing systemic side effects(1). Formulation of griseofulvin cream had diversity following the complex nature of the skin due to variation in environmental conditions in parts of the Tanzanian region(2).

The formulation of quality cream involves selecting excipients, such as emulsifiers, which play a crucial role in modifying the thermodynamic activity of griseofulvin to facilitate enhanced diffusion. This alteration aids in overcoming the physical barrier of the skin and promoting deeper penetration.

Several rheological tests were conducted on a 10% w/w griseofulvin cream, including viscometry (flow curve), yield point measurements (ramp test), frequency sweeps (oscillatory tests), three-step shear rate analysis, as well as creep and recovery tests. These tests were employed to assess the distinct properties of four pharmaceutical-grade formulations, facilitating easy identification of their characteristics (3).

The selection of emulsifiers/surfactants based on HLB values had an important role in viscosity of the griseofulvin cream, as any inaccuracy in HLB calculation may lead to liquid crystalline phases for the formulated cream. Thus consistency of the cream was contributed due to proper selection of the emulsifier and other variables that were considered during design of study(4).

The griseofulvin cream has a complex rheological properties in which it characterized by reversible shear breakdown, viscoelasticity and thixotropy(5). Rheological properties of the griseofulvin cream 10% w/w from this study can also be demonstrated as a formulation tool in determining the performance of semisolid dosage form during storage, stability and

spreadability during application to the patient`s skin. These parameters can also be employed in preliminary preparation and formulation of specifications as per regulatory guidelines.

The formulation strategies were to explored the effectiveness of delivering griseofulvin to infected skin areas(1, 5, 6). The objective of formulating griseofulvin cream enabled drug to be delivery efficiently and conveniently to localized skin regions of foot and nails(7).

The griseofulvin cream is currently produced and marketed in Asian countries, mostly in India. Its availability depends on demand and is only produced upon request from pharmaceutical industries. In the African region, like in Ghana, the griseofulvin cream is produced using a local excipient like shea-butter to enable the medicine to flow and spread on the skin based on their geographic conditions and availability of excipients, in order to treat tinea pedis for school children and rural people (5, 8).

In Tanzania, the medicine is not registered in a semisolid form (9). The young children in primary and secondary schools are mostly affected by tinea capitis and pedis. The disease is currently treated using griseofulvin tablets, which can cause toxic side effects, and the dosing regimen tends to compromise compliance in most children.

Griseofulvin has poor water bioavailability as well as low water solubility. It is effective when used in topical application due to its lipophilic properties (1, 10). Griseofulvin has high permeability and belongs to Class II in the Biopharmaceutical Classification System (11).

The topical cream delivery system is an additional advantage for griseofulvin, as it would reduce the side effects and toxicity associated with overdosing through oral administration, and would increase compliance in children most affected by tinea pedis(6, 10-12).

1.2 Problem statement

Common fungal infections of tinea pedis affect a wide population of children and young teenagers in both rural and urban areas, particularly among students in primary and secondary schools in Tanzania. Various factors, such as differences in races, ages/skin types, environmental conditions, and challenges associated with inaccurate fungal infection diagnoses and the toxicity from oral dosage forms across different geographic regions of African countries, including Tanzania, have facilitated the formulation and evaluation of griseofulvin cream(8).

The existing gap in Tanzania is evident in the reliance on griseofulvin tablets (oral solid dosage form) for the treatment of tinea pedis, improper fungal diagnosis through the use of multitherapy, and an increase in toxicity and non-compliance among children following treatment using oral dosage forms(9).

In order to address this knowledge gap, it is essential to develop a new formulation based on Tanzania's geographic regions to address the shortcomings and future prevention of multi-fungal resistance by formulating griseofulvin cream with desirable texture, spreadability, and stability.

The formulation of griseofulvin cream would enable the treatment and prevention of the spread of tinea by formulating a cream with desirable properties based on Tanzania's geographic conditions (tropical regions) through the analysis of its rheological properties, physical properties, pH, and it`s stability(13).

1.3 Rationale of the study

The research study aimed to formulate and evaluate the optimal combination of griseofulvin, oil, water, and emulsifiers for creating a cream to treat fungal infections, which will reduce systemic side effects while addressing early-stage fungal resistance.

The choice of topical griseofulvin is motivated by the desire to minimize systemic adverse effects in children. Skin texture varies by race and environmental conditions necessitating a

specialized topical formulation with desirable physical characteristics, rheological properties, pH, stability and antifungal activities (2, 14, 15).

1.4 Research questions

i. What are the optimal rheological properties required for griseofulvin cream formulations to ensure easy spreadability and desirable texture, taking into account the environmental conditions of Tanzania?

ii. How do varying amounts of water and oil content in antifungal cream formulations affect their physical and rheological properties?

iii. What is the impact of pH on the stability of the cream?

iv. What effect to the cream following accelerated stability study over period of time?

1.5 Objective

1.5.1 Broad objective

To formulate and in vitro evaluate cream containing a griseofulvin for antifungal activity.

1.5.2 Specific objective

i. To determine the optimal rheological properties of griseofulvin cream formulations for achieving the desired spreadability and texture.

ii. To evaluate the effects of varying water-to-oil contents on the physical and rheological properties of antifungal cream formulations.

iii. To determine the pH of cream formulation.

iv. To determine accelerated stability of the cream formulation throughout a specified storage period.

1.6 Literature review

1.6.1 Rheological and physical properties of antifungal cream formulations

The rheological characteristics of the cream are impacted by the formulation type, either water-in-oil or oil-in-water, and by precise HLB (Hydrophilic-Lipophilic Balance) values as per emulsion theory. This aids in determining the cream's quality and cost-effectiveness concerning oil usage. Regions like India and parts of Asia often prefer water-in-oil formulations, leveraging readily available oils like Vaseline to attain optimal rheological attributes at a lower expense. In contrast, in Tanzania, most pharmaceutical oil lines, including Vaseline, are imported(12).

The formulation of griseofulvin cream for topical application has emerged from the basic concepts of pharmaceutical emulsions, in which emulsions consist of two phases that resist mixing due to non-zero surface tension. These emulsions may incorporate additional hydrophilic or lipophilic fluids facilitated by surface-active substances. Each phase can also encompass multiple components(12, 16-18). Emulsions are used in drug delivery, contributing to the improved solubility of drugs with low water solubility.

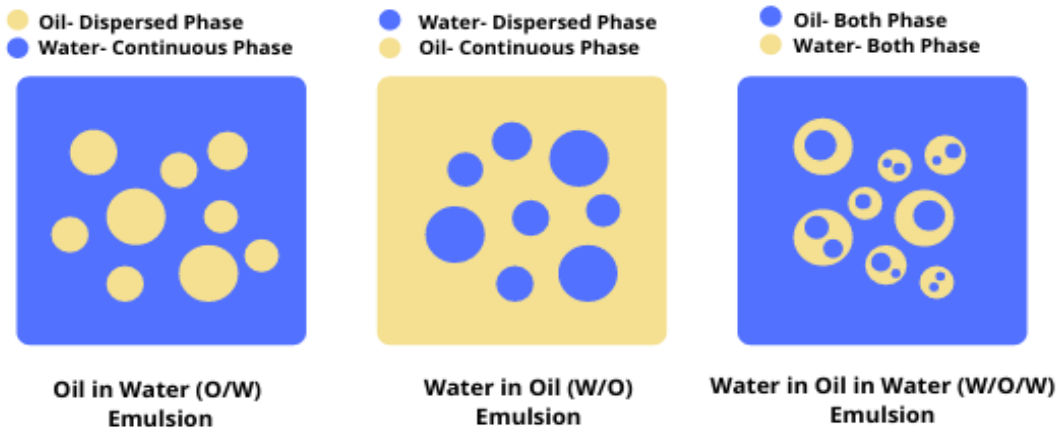


Figure 1: Classification of Emulsion(17)

The HLB (Hydrophilic-lipophilic balance) value of a solvent/substance is a measure of its relative solubility in water and oil. It describes the emulsifying properties of surfactants/emulsifiers which can help to mix water and oil, both together

Based on rheological properties/desirability for pharmaceutical cream, the HLB value was calculated to enable the evaluation of the cream, in which the higher HLB value made it easily absorbed by skin meanwhile the Low HLB values made the cream to be more water resistance(18-20). In order find the proportion in which two emulgents are mixed in order to obtain a certain emulsifying property, the HLB value system was used. Today the HLB scale has been extended up to 50, but most of the pharmaceutically important emulgents range between an HLB value 1 to 20.

Table 1: Ranges of HLB value and their application(21)

HLB range	Application
3-6	W/O emulgents
7-9	Wetting agents
8-18	O/W emulgents

On the HLB scale each emulgent has got a number, the HBL value; high values indicate more hydrophilic properties where as low values indicate that the Lipophilic character of the compound is dominant, as shown in the table 1.1

Table 1.2: HLB value for Oil (Vaseline), w/o cream and prospective emulsifiers(21)

Cream excipient	HLB Value		Emulsifiers/Surfactants	HLB Value
	W/O	O/W		
Petrolatum/Vaseline	5	12	Sorbitan monooleate, NF (Span 80)	4.3
			Polysorbate 80, NF (Tween 80)	15.0

HLB for emulsifiers and Vaseline used in my cream formulations as shown in above table 1.2

Emulsifying agents may be classified into three groups; synthetic surfactants, natural products and finely divided solids; other classifications are also possible, among those of pharmaceutical importance are Synthetic surfactants.

Synthetic surfactants/surface active ingredients may be subdivided according to their ionic character into anionic, cationic and nonionic surfactants(21).

Anionic surfactants ionized in aqueous solution into large anions, which are responsible for their emulsifying ability, and a small cation. They are commonly used for external preparations.

Cationic surfactants ionize in aqueous solution into a large cation, which has emulsifying activity and a small anion.

Nonionic surfactants are the largest group of the synthetic surfactants, they can be used to prepare oil-in-water and water-in-oil emulsions for both external and internal administration. As the member in this group can be greatly varied and combined. It frequently depends on the individual modification of the molecule or the composition of the mixture which emulsion type is formed. The following are the most important compounds used; glycol and glyceryl esters. eg: self-emulsifying monostearin P.Cx, Sorbitan esters which are used in pharmaceutical cream and has a role in its rheological properties.

However, incompatibilities; Anionic surfactants are generally incompatible with cationic surfactants and other cationic compounds and vice versa. Alkali metals and ammonium soaps are incompatible with divalent and trivalent cations. Nonionic surfactants are compatible with anions and cations**.**

1.6.2 Effect of water to oil contents on rheological properties of antifungal cream formulations.

The water-to-oil ratio and emulsifiers in the cream formulation have an impact on the rheological properties and effectiveness of the cream during application to the skin, in order to disrupt the skin's lipid structures or interact with proteins inside the skin cells to help the drug reach effective levels to affect the region. The use of griseofulvin cream on the skin is easy, but it takes longer for the body to absorb the drug compared to taking it through the mouth. It is time now to look into ways to improve drug absorption by using emulsifiers, water-to-oil ratio, and desirable rheological properties that can affect the skin's outer layer From a global perspective, for example in Europe, India, and Korea, there is high quality and availability of oil, and emulsifiers are well assured, while in African countries, such as South Africa and Egypt, are leading, and in Tanzania, there is an existing gap in which most emulsifiers are imported from the global market, which may cause variation and influence the ratio of water

to oil and emulsifiers. However, the choice of a base for antifungal cream is influenced by several factors, such as the desired characteristics of the active ingredient(s) at the target site, the shelf life of the final product, rheological properties, and the environmental conditions during application(22, 23)

1.6.3 Effect of pH on griseofulvin cream formulation

The cream's pH needs to align with specified guidelines, typically falling within the range of 5 to 7. This ensures compatibility with the skin's pH, thereby preserving its natural flora and safeguarding against superficial fungal infections(13). In America and Europe, the pH of human skin tends not to change abruptly due to low temperature and humidity conditions. However, in most African countries, the tropical geographical conditions with high temperature and humidity, including certain regions in Tanzania, can cause the pH of the skin to change and become infected with fungus.

The pH of the cream requires monitoring during storage, as temperature, humidity, and microbial growth may cause it to alter based on environmental and hygiene conditions during preparation, with water content being a significant contributing factor to pH changes (24, 25).

1.6.4 Accelerated stability of the cream formulation

Antifungal cream has to withstand environmental conditions such as temperature and humidity throughout the entire storage period in order to be effective(20). The treatment of tinea capitis and pedis usually takes a long time, about three to four weeks, which requires a stable formulation. For instance, in the past, health professionals used a topical tioconazole along with an oral dosage form of griseofulvin to treat fungal infections of the skin, hair, toes, and nails, which helped people recover faster.

Griseofulvin works well to treat tinea capitis, making it the preferred choice. There are additional advantages when it is formulated as a cream to enhance its effectiveness and stability in the localized region of the infected skin(15). To maintain stability, it is important to understand that rapidly hydrolyzing drugs are more stable in hydrophobic bases compared

to water-based ones, even though the latter may offer better efficacy(1). The formulation of water-in-oil (w/o) cream will depend largely on the delivery method and the choice of the base(26).

In Asia, including India, the stability of griseofulvin cream is higher due to the increased number of pharmaceutical industries that ensure availability and reliability when production demand arises. In Africa, for example, in Ghana, stability is compromised by using shea butter in formulating griseofulvin cream, which is traditionally manufactured(8). Meanwhile, from a Tanzanian perspective, there is currently no pharmaceutical facility producing the drug, creating instability when transporting/importing from other continents like Asia.

Stability studies are crucial for griseofulvin cream, as there are currently challenges associated with using many antifungal creams while there are only a few new antifungal drugs. Given griseofulvin's mechanism, associated side effects, and dosage form, there's a significant demand to develop it into a topical formulation.(27).

The International Conference on Harmonization (ICH) guideline mentioned that for stability testing of new drug substances and products requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance.

1.6.4.1 Antifungal activity, irritation and light effects

David and Stout classified the zone of inhibition in four intensities corresponding to ZOI diameters; >20 mm, very strong; 10-20 mm, strong, 5-10 mm, medium; and <5 mm, no response(28)

Hazard classification for dermal scoring; United Nation globally Harmonized system (GHS) 2003 classification scheme; For irritant- at least 2 animals have an average edema score that is greater than 2.3; for mild irritant- at least 2 animals have average edema score that is between 1.5 and 2.3; for non-irritant-If no more than 1 animal has an average edema score that is greater than 1.5(29).

CHAPTER TWO

2.0 METHODOLOGY

2.1 Study design

This was an experimental study design

2.2 Study area

The experiment was contacted in two sites, at University of Bergen, where full rheology study of griseofulvin cream was contacted using Kinexus rheometry. The second location was the Pharmaceutical Research and Development laboratory within the chemistry department at the School of Pharmacy, Muhimbili University of Health and Allied Sciences (MUHAS). Here, physical, pH, and stability studies were conducted.

2.3 Materials

Griseofulvin of analytical grade was bought by Professor Wei wang and supplied by Korea chemical ltd and Polysorbate 80 (Tween 80) with (HLB=15) from Sigma Aldrich (Switzerland); Sorbitan oleate (Span 80) with (HLB=4.3) from Sigma (USA), White Vaseline with HLB=5 from Caesar and Loretz GmbH; Distilled water obtained from University of Bergen (UiB), Toluene, Diethyl ether for mobile phase, Ethyl acetate for diluent were available at R and D laboratory in the department of chemistry**. Albino rats from school of** Pharmacy-Muhas. Potato agar, *Trichophyton rubrum*, incubator, from Institute of traditional medicine, Muhas.

Table 2: Ingredient and excipients used during preparation

Sn	Description	Purpose
1.	Griseofulvin	Active ingredient
2.	Sorbitan oleate (Span 80) - (HLB=4.3)	Emulsifiers (excipient)
3.	Polysorbate 80 (Tween 80) - (HLB=15)	
4.	Vaseline (HLB=5)	Base w/o (excipients)
5.	Purified/distilled water qs	

Note: Griseofulvin, Emulsifiers and solvents were of analytical grade.

Table 3: Device and equipment

Sn	Device/Equipment description
1.	Rheometer (Brand: *Kinexus-Netzch by Malvern), Spindle type (CP4/40 SR 1454SS)*
2.	Water bath /Hot plate oven *(Heldolph MR3002 from Germany)*
3.	Beakers *(100cc to 500cc VMR Borosilicate glass from Germany)*
4.	Thermometer *(Brand: IKA from Germany)*
5.	pH Conductometer *(Brand: Metrohm. 914 by Swiss made)*
6.	Digital weighing pan *(Mettler Toledo XA204 Delta range- Max wt 81g/220g)*
7.	Spatulas (Stainless steel)
8.	Laboratory mixer/agitator *(IKA -Ultra Turrax T18 Digital ~ 1000rpm to 9000rpm from Germany)*
9.	Dropper (1cc – 2cc)
10.	Storage containers (Plastic/Glass)
11.	Aluminum foil and Wrap soft paper tissues
12.	Stability chamber. Specification (Temperature 40 ºC ±5ºC and humidity of 75±5%)
13.	HPTLC, branded Camag with UV detector and PDA detector, a manual injection facility with the software Vision CAT(Wincat) with serial number 103056
14.	Measuring cylinder 250,500 and 1000mL
15.	Sonicator
16.	Potato agar
17.	*Trichophyton rubrum*
18.	Albino rats
19.	Incubator

2.4 Method

Preparation and formulation

The calculation of HLB value for two emulsifiers was first done using the following equation in order to ensure proper mixing between water and vaseline as follows;

$HLB_O = (W_T HLB_T + W_S HLB_S) / (W_T + W_S)$

Where;

HLB_O is Total Hydrophilic Lipophilic Balance value.

HLB_T =value for Tween80 (i.e:15)

HLB_S = value for Span80 (i.e:4.3)

W_T = Weight of Tween80 to be used (i.e:0.25g)

W_S =Weight of span to be used (i.e:1.25g)

Then, $HLB_O = ((0.25x15) + (1.25gx4.3)) / (0.25+1.25) = (3.75+5.375)/1.5$

Therefore, Total Hydrophilic lipophilic balance value, for the proposed formulation will be, HLB_O =6.08

The formulation F1(W5O5) began by heating the purified water to about 50°C in a beaker on a heating plate and a watch glass was used to prevent evaporation. 5 grams of griseofulvin were weighed using an analytical balance and were dissolved in purified water and it was further heated up to about 70°C. Meanwhile, an evaluation was done for any water loss after heating. Polysorbate 80 (Tween 80), Sorbitan oleate (Span 80), and Vaseline were weighed as per the summarized table of formulae below and melted in a beaker on a water bath to about 70°C.

Griseofulvin and Purified water Phases were added to Polysorbate 80 (Tween 80), Sorbitan oleate (Span 80), and Vaseline phases with stirring of 4200rpm for 10 minutes, meanwhile a change in the formulation from translucent to a rich white color was observed. Temperature was recorded for griseofulvin and Purified water Phases and Polysorbate 80 (Tween 80), Sorbitan oleate (Span 80), and Vaseline phases.

Continuous homogenization within the mentioned 10 minutes helped to reduce droplet size and likely improved the stability of the formulation, meanwhile the shear rate also depended on the formulation and equipment set up. The critical condition during homogenization was to

ensure air was not introduced into the mixture. After sufficient homogenization, the preparation was allowed for voluntary cooling to about 30°C.

The preparation was transferred to an air-tight container and maintained for at least 48 hours before physical inspection, rheological, and pH tests. The same procedure was repeated for the other remaining formulations; F2 (W3O7), F3 (W2O8) and F4 (W1O9).

Table 4: Formulae and batches adjustment for griseofulvin cream 10% w/w.

Formulation zone Preparation:		**Reference**		
	Ingredients	**Qty (g)**	**Factor**	**Required weight (g)**
I	Griseofulvin	10.0	0.5	5
II	Polysorbate 80 (Tween 80)	0.5		0.25
III	Sorbitan oleate (Span 80)	2.5		1.25
IV	Vaseline	47.5		23.75
V	Purified water	39.5		19.75
	Total (grams)	100.0		50

The formulae above were used to prepare 10% w/w griseofulvin cream.

Calculation for the weight of griseofulvin and it`s excipients based on percentage of weight by weight in order to get the required amount for cream formulation which is 5gram (%w/w) as shown in table 2.3.

Vaseline and purified water will have different ratio for the four formulations, meanwhile emulsifiers will be constant and 5grams of griseofulvin will be used for each of four formulations.

Table 5: Formulations of griseofulvin cream

PS	Ingredients	F1 (W5O5) Weight required(g)	F2 (W3O7)	F3 (W2O8)	F4 (W1O9)
I	Griseofulvin	5.041	5.04	5.06	5.07
II	Polysorbate 80 (Tween80)	0.2548	0.259	0.259	0.256
III	Sorbitan oleate (Span80)	1.252	1.257	1.254	1.256
IV	Vaseline	23.52	28.53	33.52	38.51
V	Purified water	20.3	15.4	10.4	5.09
	Total (grams)	50.3678	50.486	50.493	50.182

Table 6: Summary of formulation method

Steps	Manufacturing procedure:
1	V was heated to 50°C in a beaker on a heating plate. A watch glass or similar was used to prevent evaporation.
2	(I) was dissolved in V and further heated up to about 70°C.
3	II + III + IV was melted in a steel bowl on a water bath to about 70°C
4	(I + V) was added to (II + III + IV) with stirring at 4200rpm for 10minutes. Temp. I + V at mixing was 45^{o}C ±5 ^{o}C
5	Temp. II + III + IV at mixing was 69^{o}C±5 ^{o}C
6	The cream was stirred and allowed to voluntary cooling to about 30°C
7	Cream was confirmed to be homogeneous.
8	Lastly, the formulated cream was filled in the glass tube and covered in order to protect from light.

Analytical method evaluation

An appropriate formulation was chosen based on the evaluation and tests that included physical test, rheological test, pH and homogeneity test(3, 30).

Physical tests were conducted to test the appearance of cream, odor, color and homogeneity. Tests were carried out from day one to day two after formulation, and cream was divided into two portions for further physical test during stability study of 90 days (3months).

Rheological tests were done using Kinexus netzch rheometer with a CP geometry spindle CP4/40 SR1454SS (KJ3100277) and operating temperature 25°C. The appropriate amount of griseofulvin cream was carefully placed on the plate, and overfilled cream was trimmed by a stainless-steel spatula to prevent additional stress from drag along the edges. The Kinexus rheometer was a highly advanced device to regulate the sample rest time within the operating test parameters. Four formulations were compared in flow curve (viscosity), frequency sweep (oscillatory spectrum), shear stress ramp (yield stress), thixotropy (uniformity flow of griseofulvin content in the cream), creep and recovery (deformation point).(3, 24, 31).

- Viscosity Test: the flow curve was run from start shear rate: 0.100 s^{-1} to end shear rate 100.0 s^{-1}, with a sample per decade of 10. Zero shear viscosity was indicated at a plateau when the shear rate approaches zero and an apparent yield stress. The relationship between rate of deformation [shear rate] and force per unit area [shear stress] were used to indicate the flow behavior of a griseofulvin cream. At low shear rate the cream had maintain an irregular order with a high zero shear viscosity resulting from ingredient interactions and the restorative effects of Brownian motion.

  The yield stress of formulated cream was determined in which there was interaction between ingredient and excipients resulting in networking formation which during application to the skin break and allow the cream to flow and spread evening in the skin.

- The frequency sweep test provided detail description of how the formulations behave during storage and application or during rubbing. The starting frequency was 10Hz, the end frequency was 0.1Hz, shear strain is 0.5, and the sample per decade was 10. The storage modulus (G`) indicated the magnitude of energy stored in formulated griseofulvin cream, whereas the loss modulus (G``) indicated the energy loss due to viscous dissipation for the formulations. The resultant modulus (G` and G``) was the function of deformation on a log-log axis in order to establish a spectrum region of the viscoelastic range of each formulation by using angle θ and Tan θ which was calculated for the four formulations as tan θ =G``/G`

- The shear stress ramp test (yield stress test**)** of the four formulations was determined in which the lowest shear stress value above which the cream behaves like a fluid, and below which the materials act as a solid. In this test rheometer will be set with start shear stress 0.00Pa; end shear stress 200Pa; Ramp time will be1:40minute. It will involve gradually increasing the applied shear stress until the material will start to flow, and then will indicate its yield point.

- The three-step shear rate (thixotropic) was determined for the four formulations as shown in the table below;

Table 7: Shear rates used for measuring the thixotropic tests of formulated griseofulvin cream(31).

Formulae	1st Phase (Shear rate in 30sec) (Pa)				2nd Phase (Shear rate in 30sec) (Pa)	3rd Phase (Shear rate in 10 minutes) (Pa)			
F1 (W5O5)	0.1	0.5	0.6	0.7	100	0.1	0.5	0.6	0.7
F2 (W3O7)	0.1	0.5	0.6	0.7	100	0.1	0.5	0.6	0.7
F3 (W2O8)	0.1	0.5	0.6	0.7	100	0.1	0.5	0.6	0.7
F4 (W1O9)	0.1	0.5	0.6	0.7	100	0.1	0.5	0.6	0.7

The first phase demonstrated the formulation at rest before the shear thinning with series of lowest shear stress as indicated in the table above, second phase was applied with highest shear of 100Pa to cause structure deformation within microstructure and third phase with same series of lowest shear rate as of first Phase to allow regaining of microstructure arrangement with time. Thixotropic behavior for the four formulations was then compared for time required to recover 90% of the initial viscosity was employed which was demonstrated as a measure of thixotropy.

- Creep and recovery test was performed in which a standard loading sequence was used to ensure the cream was subjected to a consistent and controllable loading protocol. Series of creep tests were run at different applied stresses between the ramp number that was obtained from the shear stress ramp test for each of the four formulations. Then, each creepy test was stopped after 2 minutes and a recovery test of the same time interval was auto performed by the Kinexus rheometry between the creep tests. The linear viscoelastic region of the prospective curves was used, of which a constant shear stress was applied to a viscoelastic material and the resultant strain response was determined. First, the elastic response was observed, then a delayed elastic response,

and lastly, it was a viscous or steady state (linear) response for a long period, where the slope of the steady state line was equal to the strain rate and a zero shear viscosity of the cream was obtained.

In addition to that, compliance as the function of the elastic component of the cream material was determined, in which the viscous response/steady state (linear) was extrapolated to zero time and the intercept was equal to the equilibrium compliance JE. The true yield stress was expected/obtained when there was no steady state/viscous response.

- **pH measurement and determination;**

pH was measured for each formulation. 1g of cream was carried in volumetric flask and diluted further to 100mls volume with distilled water. The calibrated pH meter was used in measuring and record for each formulation.

- **Accelerated stability study**

Determination of accelerated stability studies for the three months was done using HPTLC in which the method was adopted inhouse from Research and Development Pharm Lab – Muhas and obtained from method validation for quantification purpose.

The mobile phase and developing chamber were prepared by taking 20ml of diethyl Ether and 5mL of Toluene were measured using a measuring cylinder and poured into a 50ml volumetric flask. The flask was closed and shaken well to ensure thorough mixing. A filter paper was cut fitted and put into developing tank to facilitate saturation. 10ml of mobile phase was poured into the developing tank and immediately the lid was closed. Saturation was allowed to take place for 20minutes and scanned wavelength of 299nm was used.

Preparation of stock and working solutions for standard and samples was done by dissolving in a diluent for analysis; the concentration of 0.35mg/mL of the sock standard solution of griseofulvin was prepared it was prepared by weighing 3.5mg of griseofulvin (Reference standard). The weighed drugs were transferred to two separate 10ml volumetric flasks, then a

volume of 5ml of ethyl acetate was added sonicated for about 1minute the volume was made up to mark and flask was named as griseofulvin stock solution.

Preparation of working standard solution was done by taking 5ml of griseofulvin into 10ml volumetric flask, then volume was made to the mark with Ethyl acetate to obtain target concentration of 0.175mg/ml of griseofulvin which was then labeled as griseofulvin working standard solution.

Stock sample solution for griseofulvin cream was obtained by preparing a concentration of 0.35mg/ml, griseofulvin cream contained 17.5mg griseofulvin was weighed accurately and put into a 50ml volumetric flask and 30ml of ethyl acetate was added into the flask and labeled griseofulvin stock sample solution.

The working Griseofulvin(sample) solution was prepared by pipetting 5ml of griseofulvin stock sample solution into 10ml volumetric flask and top up to mark by ethyl acetate, shacked vigorous to make 0.175mg/ml and labelled Griseofulvin working sample solution.

The target concentration was 0.175mg/ml (W/V) from adapted test method based on nominal concentration (LOQ and LOD). That is; if 1g = 100mg of API, then what 'X'g =3.5mg of API, (35mg/0.035g) = 3.5mg of API/20mls which means there is 3.5mg API (Therapeutical effect), thus the total cream weight was 35mg. and obtained from formulae;

Assay = (PA of sample/PA of std) x100%

So from, 35mg of griseofulvin cream was equivalent to 3.5mg of griseofulvin (35mg = 3.5mg W/W) as 5g of API was used to prepare 50g of griseofulvin cream (5g/50g =0.1g/1g). then 3.5mg of API was dissolved in 20mls of Ethyl acetate.

Anti-fungal activities against *Trichophyton rubrum*: Agar plate diffusion method was performed using potato agar and the antifungal activity was studied for 5days. Well diameter was 15mm, and the cream weight was 100.2mg.

Skin irritation test: Done following a stability study using albino rats, and scores were recorded consecutive for 3days.

Light effect: Determined the effect of exposing the cream to sunlight through the window.

CHAPTER THREE

3.0 RESULTS

Evaluation of rheological property of griseofulvin cream for the four formations were compared in viscosity (flow curve), frequency sweep (oscillatory/fingerprint spectrum), shear stress ramp (yield stress), thixotropy (uniformity flow of griseofulvin content in the cream), creep and recovery (deformation point).

3.1 Rheometry

3.1.1 Viscosity (flow curve)

Viscosity curves were generated for the four formulations at 25ºC. The upward and downward flow curves were run from a starting shear rate of 0.100 s^{-1} to an ending shear rate of 100.0s^{-1}, with 10 data points per decade.

The following tables of viscometry shear rate data for the four formulations were obtained after data analysis from the Kinexus rheometer.

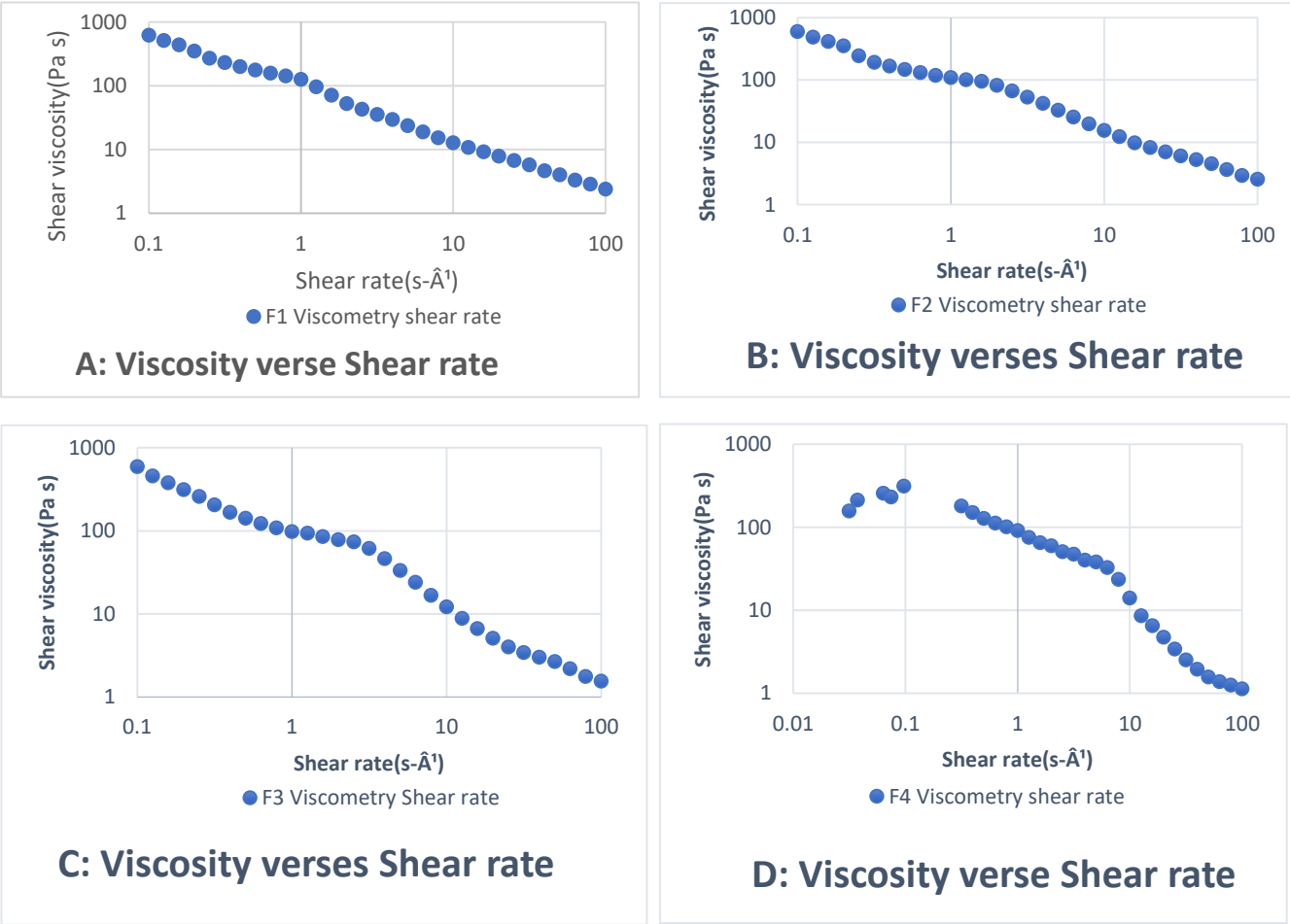


Figure 2: Viscosity verse Shear rate

The formulations exhibit shear thinning behavior, in which their viscosity decreases as the shear rate increases. In zero shear viscosity region, the viscosity shows a plateau as the shear rate approaches zero, while the yield stress indicates an increasing viscosity as the shear rate approaches zero.

Formulation 1,2and 3 have demonstrated a clear curve with distinct zero shear viscosity and yield stress, while Formulation 4 has surge or distortion as shear rate approach zero. This indicates that the formulation 4 cream is harder during application and requires more strength to spread and disappear into the skin. The formulations obviously demonstrated a non-Newtonian fluids behavior. When formulating griseofulvin creams subjected to shear stress,

such as when it is spread or rubbed on the affected and target region of skin, its viscosity decreases, making it easier to apply.

From a formulation standpoint, semisolid dosage forms exhibit varying behaviors at low shear rates while stored in their bottles or containers. However, when subjected to higher shear rates, they tend to display similar viscosities, as they are anticipated to respond similarly under these conditions.

Graph F4 had highly solid content (griseofulvin) to white Vaseline at ratio of W1O9, this made the low shear rate viscosity region disappears between shear rate 0.1 and 1(s-Â¹) and then materials started to yield. This meant the griseofulvin cream materials were in some cases experienced an increase in viscosity after the shear thinning region due to structural rearrangements caused by applied shear, thus leading to flow-induced shear thickening.

3.1.2 Oscillatory test (frequency sweep)

The curve offered a comprehensive overview of the formulations' behavior during storage and application to the affected skin area, as well as detailing the fingerprint spectrum of the griseofulvin cream. The frequency sweep graphs for the four formulations are presented in the accompanying graph.

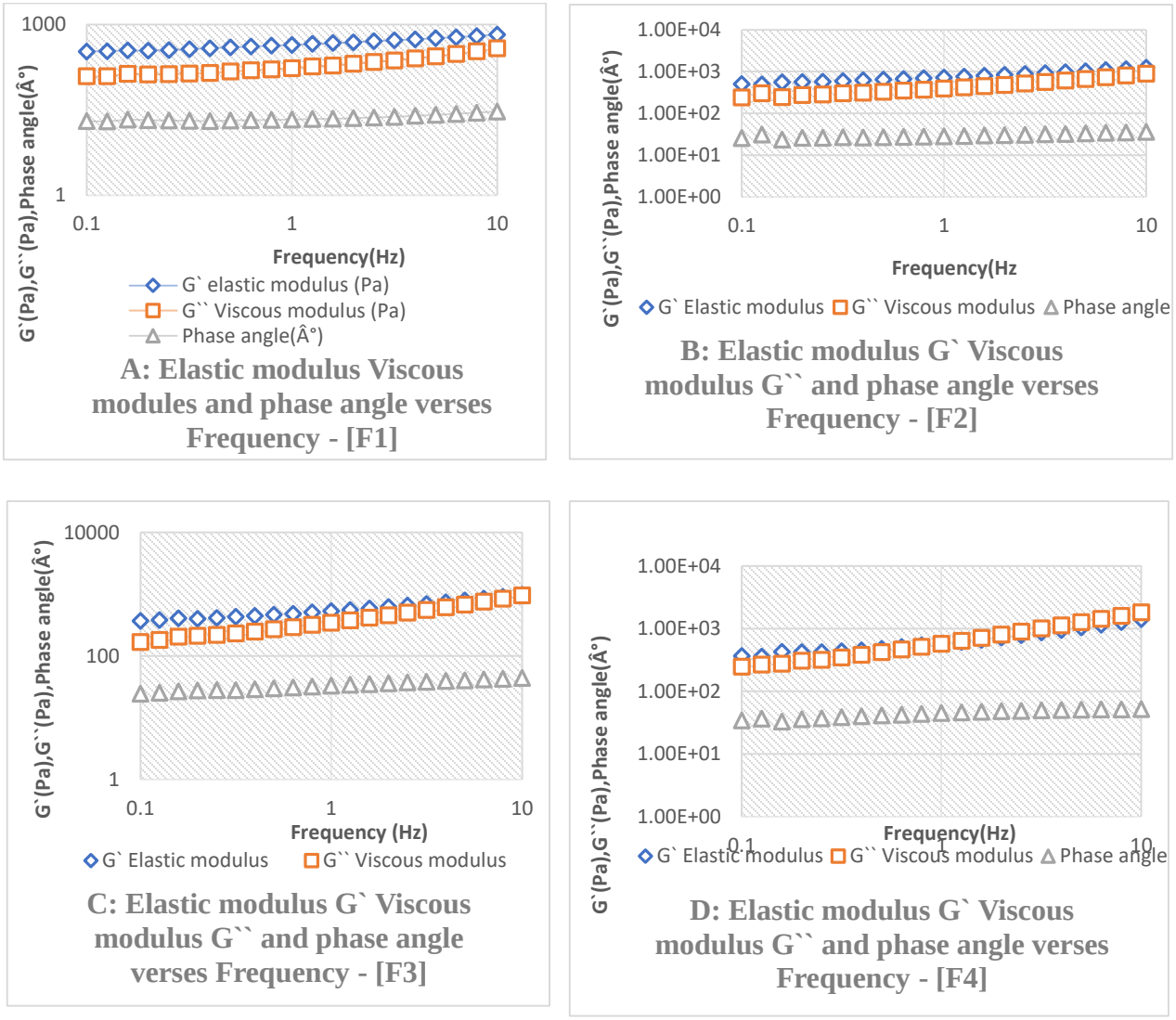


Figure 3: Elastic modulus, viscous modules and phase angle verses Frequency

In F1 the G` modulus (elastic/solid like) was greater than G`` modulus (viscous/liquid like) at low frequencies of less than 1Hz. indicating the cream had viscous properties and it possesses a network structure that needs to be broken down before flow can start, thus indicating it had a yield stress.

For F2: the G` modulus is slightly greater than G`` modulus which also indicated the formulation 2 had a viscous property.

For F3, the G` modulus was equal to the G`` modulus (G`=G``), this meant the moduli crossed over each other at a frequency of 10Hz, which was greater than 1Hz. This indicated that the

shear stress value with have desirable semi-solid properties that can withstand a long storage time. The cream had both elastic and viscous properties, signifying a desirable property when kept in the container. It internal structure had storage of energy that equilibrated with loss of energy. F4 showed that the G' (elastic modulus) was smaller than the G" (viscous modulus) at low frequencies below 1 Hz, indicating that the griseofulvin cream exhibited predominantly elastic properties.

The phase angle demonstrated the overall viscoelastic nature of the cream. For the formulations F1, F2, F3 and F4 the phase angle was slightly above 0^o with a slightly reduction in frequencies, indicating the cream was viscoelastic solid at 0Hz. In F3, the G` was largely independent of the frequency, and the phase angle was constant and slightly decreasing with decreasing frequencies, this indicates the griseofulvin cream had had maintained its network structure and will be more stable.

3.1.3 Thixotropy

The results for three-step shear rate were used to study and evaluate the formulated griseofulvin cream for the time required to recover 90% of the defined amount from initial viscosity at a 25^0C. The first stage had a low shear rate of 0.5Pa, which had shown to attain the rest state of the cream that produced a clear curve for the first phase. This was followed by second stage at a high shear rate of 100Pa, at a given time, attained the breakdown of cream structure, comparable to the process of interest. The last, the third stage, had the same low shear of 0.5Pa as the first stage. It was then compared in viscosity recovery followed as a function of time, as shown in the graph below.

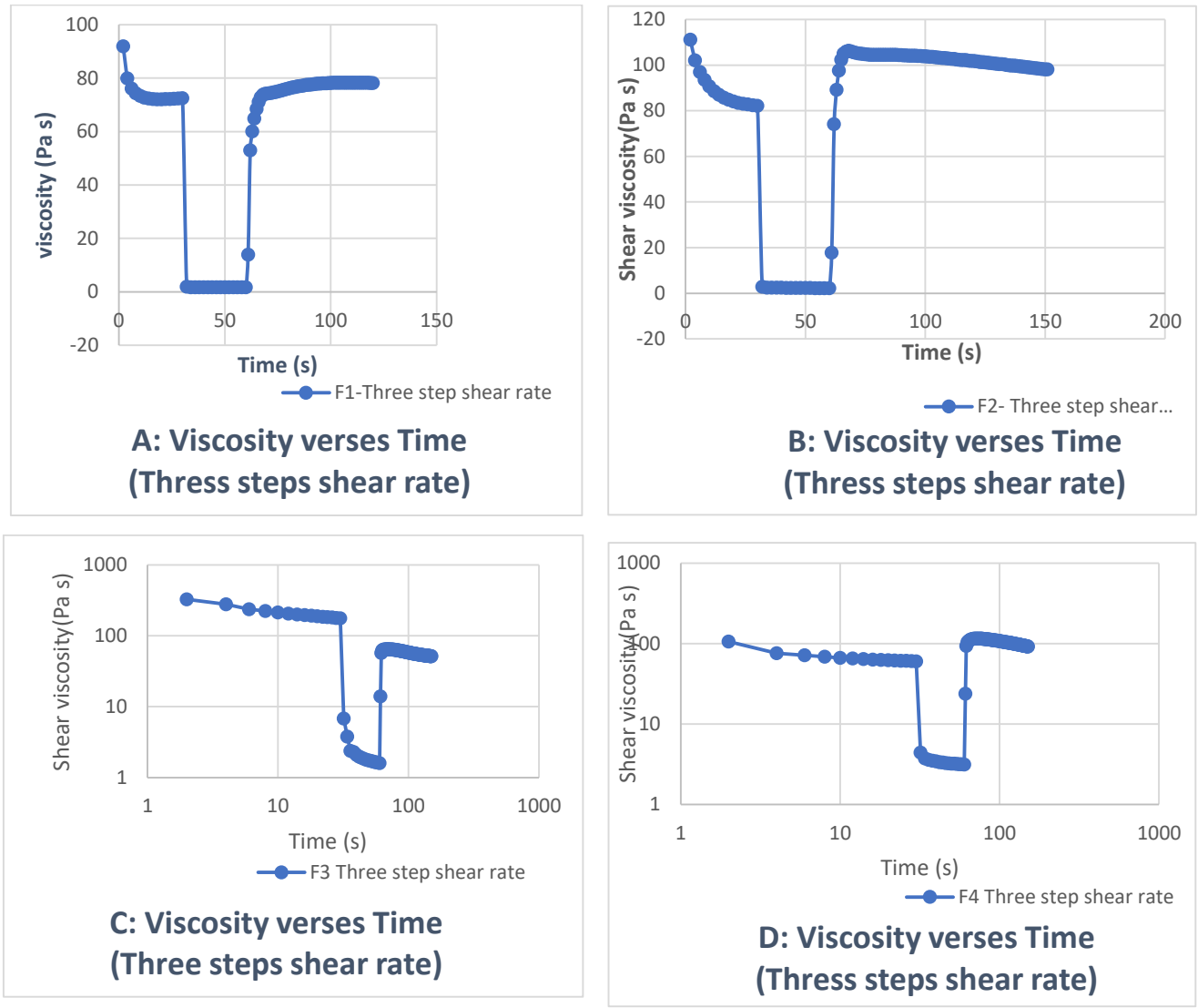


Figure 4: Viscosity verses Time (Three step shear rate)

F1 had a less thixotropic property in which it had loosely lost its internal structure due to the presence of more water content in the cream, making the active ingredient rapidly lose its internal structure arrangement. F2 is also less thixotropic. F3 indicated improved thixotropy properties with an optimal delaying of rebuilding time from the 90% of the initial viscosity when compared to F1, F2 and the least, F4.

The value obtained for the formulation F3 was more positive, indicating a structural breakdown upon shearing that was similar to when the cream would be squeezed from its

packed tube and applied by rubbing it onto the targeted regions. This implied that it could be easily spread, releasing and positioning the cream to the affected area of the infected skin after rubbing off. Meanwhile, F4 had poor thixotropic properties and had less and quicker rebuilding time from the 90% of the initial viscosity, which made it harder to be pumped through its packed tube`s orifice.

3.1.4 Yield stress (shear stress ramp)

This was the minimum shear stress required to initiate the flow and spreading of the cream, So, it is a lowest shear stress value above which cream behaved like a fluid, and below which the materials acts as solid.

The values set to the rheometer were; start shear stress 0.00Pa; end shear stress 200Pa; Rap Time 1:40

Consider the table below for yield stress/value for the four formulations;

Table 8: Yield stress for formulation F1 to F4

Formulae	Yield stress (Pa)
F1 (W5O5)	7.15
F2 (W3O7)	6.143
F3 (W2O8)	4.944
F4 (W1O9)	4.193

3.1.5 Creep and recovery test

The formulation consistency was evaluated and assessed using a series of stresses below and above stress ramp (yield stress) for each formulation. The creep and recovery test also enabled the determination of the viscoelastic properties of the formulations.

The compliance (J) was expressed as reciprocal of the modulus given as J=1/G= strain/t. this was auto calculated from the Kinexus rheometer`s software. G is modulus and γ is strain.
The creep test variables which were used to deliver strain or compliance vs time under very low stresses.

Table 9: Stress ramp (Pa) for compliance verse time

Formulae	Stress Ramp (Pa)	~Lowest stress (Pa)
F1	7.15	6
F2	6.143	5
F3	4.944	4
F4	4.193	3

The high value of creep compliance showed that a formulation had a weaker internal structure. The equilibrium compliance measures the viscoelastic formulation's elastic response to strain. The elastic reformation value represents the elastic component of the viscoelastic behavior. Thus, formulations F2 and F3 were found to be stable and consistent, with the lowest stress of 4 Pa and 5 Pa, respectively, that corresponded to a stress ramp of 4.944 Pa and 6.143 Pa obtained from the Kinexus rheometer.

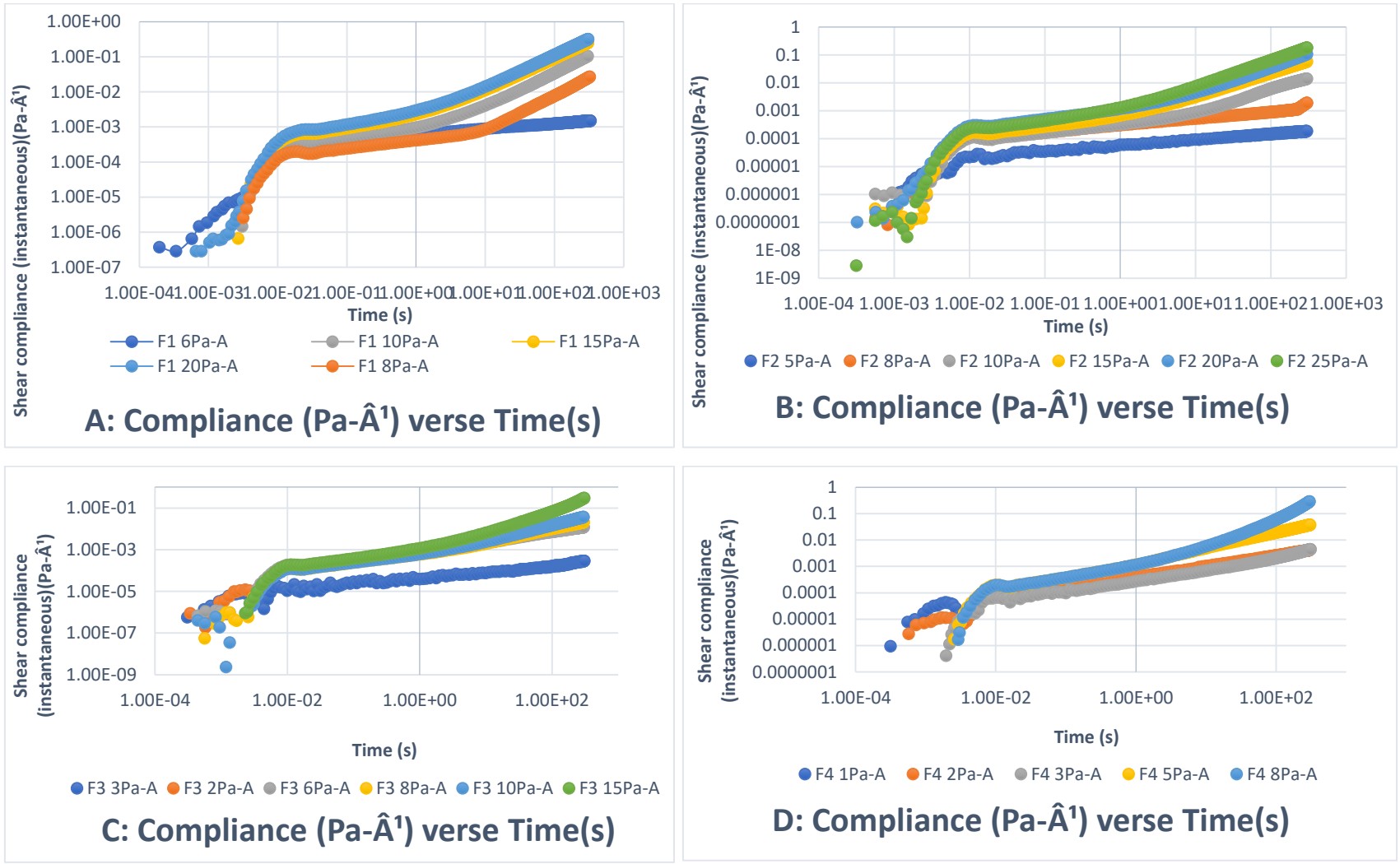


Figure 5: Compliances value verse Time(s)

The primary region of the curve; formulation F1 was found to have compliance of 6Pa-A, which was the enough stress to initiate deformation and weaken the internal structure of the cream, which was then followed by an indicated series of parallel curves. F2 complied at 5Pa-A, F3 complied at 3Pa-A and F4 complied at 2Pa-A, as shown in graph F4, with slow rate that decreases due to strain hardening. The minimum acceptance compliance was between 3 and 5 Pa-A, thus formulation F2 and F3 were compliant.

In the secondary region (steady state deformation); there was the longest and most linear curve, which provided the region with a competitive balance between strain hardening and recovery.

Formulation F4 had a secondary region curve with break points in between and rapidly rise the curve at high shear within a short time, which predicts it will have a shorter period of time of deformation, followed by formulation F3.

Tertiary region; provided detailed information on the microstructural changes, such as necking, accelerated deformation, or internal cracks.(32) In F1 curve, the lines creeped immediately at 8 Pa-Â¹ with no parallel line before creeped curved line. In F2, the curve line creeped at 10 Pa-Â¹, in F3 the curve line also creeped at 10 Pa-Â¹, while in F4 the curve line creeped at 8 Pa-Â¹. These compliances indicate the value in which the microstructure changes occurred, a point where the deformation will be initiated for each griseofulvin cream.

3.2 pH of griseofulvin cream

The pH of creams was recorded in the second day after formulation cream formulation, then followed at the interval of 0,30,60 and 90 days.

Table 10: pH and physical test after formulation

PARAMETER	COLOR				HOMOGENEITY				PH				CRACKING			
Formulae	**F1**	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**
2 days after formulation	C	C	C	C	C	C	C	C	5.52	6.52	6.24	6.53	C	C	C	C
Specification	**White**				**Homogenous**				**5 to 8**				**Non-cracking**			

Table 11: Physical and pH properties during accelerated stability study for the four formulations under 40 ^{o}C ±5^{o}C and humidity of 75±5% for 0,30,60 to 90th days

PARAMETER	COLOR				HOMOGENEITY				PH				CRACKING			
Formulae	**F1**	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**
0 day	C	C	C	C	C	C	C	C	6.4	6.7	6.5	7	C	C	C	C
30th day	C	C	C	C	C	C	C	C	6.6	6.4	6.9	6.9	C	C	C	C
60th day	C	C	C	C	C	C	C	C	6.8	6.7	6.8	7	C	C	C	C
90th day	C	C	C	C	C	C	C	C	6.6	6.6	6.8	7.1	C	C	C	C
Specification	**White**				**Homogenous**				**5-8**				**Non-cracking**			

Note: **C**=Comply **N**=Not comply

3.3 Stability of the cream

Formulations were stable from day 0 to 90 under accelerated temperature condition of 40ºC ± 5ºC and humidity of 75% ± 5% with percentage assay of 100.62% for day0 to 99.68% for day90, which enabled the extension of shelf life to approximately 2 years. The Table and graph below indicate the significant stability of the formulated griseofulvin cream.

Table 12: Stability results formulation F1

Date	Formulation[F1]	Day	Amount% Assay	mg/50g
	F1 [1-1]		100.46	5.023
	[1-2]		100.84	5.042
	[1-3]		100.57	5.0285
26-1-2024	AvF1	0	100.6233333	5.0311667
	F1 [1-1]		100.44	5.022
	[1-2]		100.1	5.005
	[1-3]		100.4	5.02
26-2-2024	AvF1	30	100.3133333	5.0156667
	F1 [1-1]		99.85	4.9925
	[1-2]		99.78	4.989
	[1-3]		99.82	4.991
26-3-2024	AvF1	60	99.81666667	4.9908333
	F1 [1-1]		99.45	4.9725
	[1-2]		99.49	4.9745
	[1-3]		99.33	4.9665
26-4-2024	AvF1	90	99.42333333	4.9711667

Table 13: Stability results formulation F2

Date	Formulation[F2]	Day	Amount% Assay	mg/50g
	F2 [1-1]		100.38	5.019
	[1-2]		100.52	5.026
	[1-3]		100.72	5.036
26-1-2024	AvF2	0	100.54	5.027
	F2 [1-1]		100.19	5.0095
	[1-2]		100.12	5.006
	[1-3]		100.17	5.0085
26-2-2024	AvF2	30	100.16	5.008
	F2 [1-1]		100.04	5.002
	[1-2]		99.78	4.989
	[1-3]		99.72	4.986
26-3-2024	AvF2	60	99.84666667	4.992333
	F2 [1-1]		99.52	4.976
	[1-2]		99.6	4.98
	[1-3]		99.53	4.9765
26-4-2024	AvF2	90	99.55	4.9775

Table 14: Stability results formulation F3

Date	Formulation[F3]	Day	Amount% Assay	mg/50g
	F3 [1-1]		100.55	5.0275
	[1-2]		100.53	5.0265
	[1-3]		100.43	5.0215
26-1-2024	AvF3	0	100.5033333	5.025167
	F3 [1-1]		100.28	5.014
	[1-2]		100.39	5.0195
	[1-3]		100.29	5.0145
26-2-2024	AvF3	30	100.32	5.016
	F3 [1-1]		100.07	5.0035
	[1-2]		100.15	5.0075
	[1-3]		100.14	5.007
26-3-2024	AvF3	60	100.12	5.006
	F3 [1-1]		99.58	4.979
	[1-2]		99.74	4.987
	[1-3]		99.71	4.9855
26-4-2024	AvF3	90	99.67666667	4.983833

Table 15: Stability results formulation F4

Date	Formulation[F4]	Day	Amount% Assay	mg/50g
	F4 [1-1]		100.31	5.0155
	[1-2]		100.25	5.0125
	[1-3]		100.16	5.008
26-1-2024	AvF4	0	100.24	5.012
	F4 [1-1]		100.03	5.0015
	[1-2]		99.88	4.994
	[1-3]		99.79	4.9895
26-2-2024	AvF4	30	99.9	4.995
	F4 [1-1]		99.69	4.9845
	[1-2]		99.67	4.9835
	[1-3]		99.72	4.986
26-3-2024	AvF4	60	99.69333333	4.984667
	F4 [1-1]		99.62	4.981
	[1-2]		99.42	4.971
	[1-3]		99.57	4.9785
26-4-2024	AvF4	90	99.53666667	4.976833

Table 16: Summary results for accelerated stability of griseofulvin cream 10% w/w

	F1		F2		F3		F4	
	Average Assay %		Average Assay %		Average Assay %		Average Assay %	
Day	Amount	mg/50g	Amount	mg/50g	Amount	mg/50g	Amount	mg/50g
0	100.62	5.03	100.54	5.03	100.5	5.03	100.24	5.012
30	100.31	5.02	100.16	5.01	100.32	5.02	99.9	4.995
60	99.82	4.99	99.85	4.99	100.12	5.01	99.69	4.9845
90	99.42	4.97	99.55	4.98	99.68	4.98	99.54	4.977

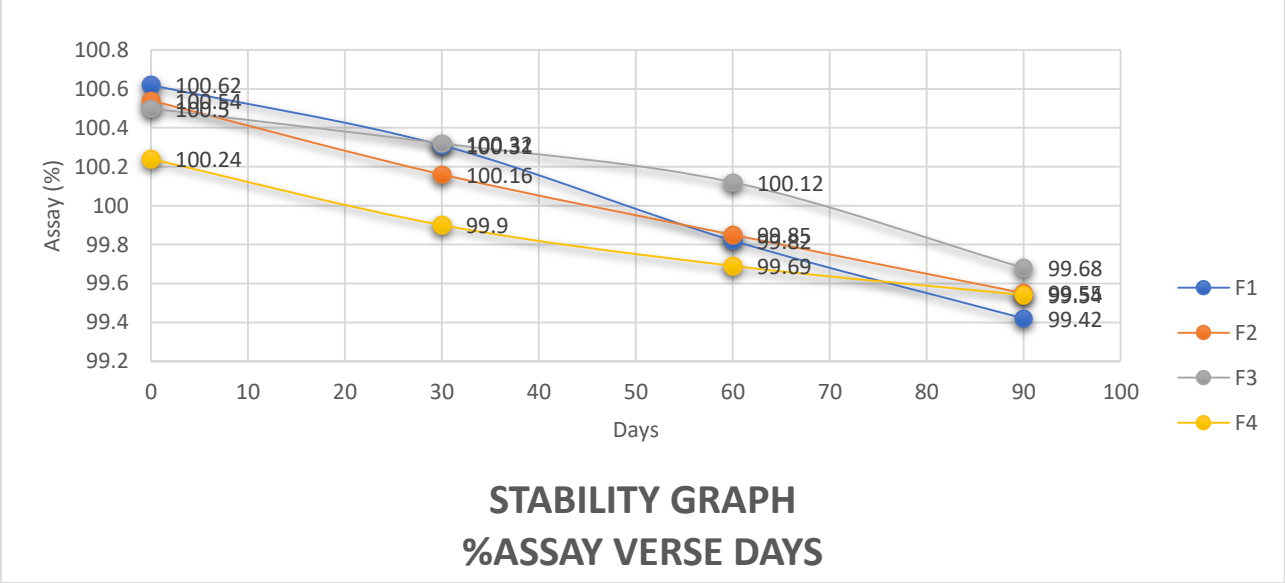


Figure 6: Stability curve indicating percentage assay verse number of days

Griseofulvin cream was significant stable based on the showed trends of 100.62% to 99.68%. This evaluation method was economic, simple and specific.

Anti-fungal activities: It was studied against *Trichophyton rubrum.* Agar plate diffusion was done using potato agar, ZI-Zone of Inhibition were observed after 5days.

Study for *Trichophyton rubrum* was repeated for the same, Agar plate diffusion was repeated using potato agar with two wells. Zone of Inhibition for two wells were also observed after 5days with average diameter of 37.5mm, indicating very strong antifungal activities



Figure 7: Griseofulvin cream activity against *T. rubrum*

Irritation:

Table 17: Irritation effect of griseofulvin cream indication a slight effect

Time [Hours]	**24**				**48**				**72**				**Score**			
Albino Mice Id#	0	I	II	III	0	I	II	III	0	I	II	III	**0**	**I**	**II**	**III**
Edema score	0	1.5	1	1	0	1.5	1.5	1	0	1	1	1	**0**	**1.33**	**1.17**	**1**
Erythema	0	1	1.5	1	0	1.5	1.5	1	0	1	1	1	**0**	**1.17**	**1.33**	**1**

NOTE:

EDEMA;

1=very slight (barely perceptible)

2=well defined

3=moderate to sever

4=severe edema

ERYTHEMA;

1=very slight (barely perceptible)

2=well defined

3=Moderate to severe

4=severe erythema

Light effect:

Analytical package for the griseofulvin cream was opted following expose of package in direct sunlight through the window for more than 90days. The white plastic package was seen to be appropriate as the cream was physical stable compared to amber and glass bottle.





Figure 8: Griseofulvin cream package

CHAPTER FOUR

4.0 DISCUSSION

Evaluation of rheology and physical properties of griseofulvin cream was done at first two days after formulation in which the physical properties were observed to comply with acceptance criteria, having a white color, being homogenous, non-cracking.
Rheological tests for the four formulations showed distinct characteristics. The viscometry result indicated that formulation had a desired flow curve with limited flow/viscosity for formulation F4.

The three-step shear rate, the formulations were characterized by a complex rheological property of a non-Newtonian flow, which showed shear thinning for the four formulations with various thixotropic levels, indicating that the griseofulvin cream had a thixotropic behavior in which there was a complete reduction and degradation in structural strength during a shear load phase. Formulation F3 indicated the suitable thixotropic properties with 90% regaining in initial viscosity, enabling it to held/applied and spread on affected skin, where increasing rubbing(thinning) would allow the drug to penetrate into the skin. Viscosity of the formulations over time indicated the aging and storage time (shelf life), in which the viscosity increased or hardened with increasing time, as in the case of monitoring the change in G`(elastic structure) over time(14).

Frequency sweep/oscillatory test of the griseofulvin cream had shown a stable fingerprint spectrum and a well-micro-structured arrangement that played a role during the storage of the griseofulvin cream in which formulation F3 demonstrated a good and well-formulated cream, which can also be used as a formulation tool to determine the performance of semisolids dosage form during storage, stability and spreadability during rubbing to the infected skin(3).
Determination of linear viscoelastic spectrum in an oscillatory test required the use of a small stress level to achieve consistency in the formulation. Thus, in this test the variation was due to low consistency, as it is usually difficult to use a small stress within the linear viscoelastic region for the oscillatory test. To solve for the variation, the optimal viscoelastic properties of

griseofulvin cream were then determined using creep and recovery test, in which a constant stress obtained from a lowest ramp number of the four formulations, which was below yield stress, was used with a time interval.

Viscoelastic results (frequency sweep-oscillatory) had demonstrated that formulated griseofulvin cream had lamellar phase-like the microstructure. The test optimized the ratio of water and vaseline to selected emulsifiers, physical stability, consistency and homogeneity(33).

The results from the frequency sweep had shown an optimal impact on the physical properties and availability of griseofulvin to the affected and target regions of the patient`s skin. It also demonstrated that the technique used during formulation was optimal and led to an elegant formulated cream(34).

The stress ramp in cream formulation F3 and F2 had shown the shear stress ramp with predominant, suitable solid like-properties that held the network together, thus associated with the yield stress. The viscosity peak at which the cream underwent elastic deformation, thus stretching, indicates that the cream will be easily squeezed, applied and spread on the skin.

In creep and recovery test, formulations F1 and F4 indicated a small value for the creep equilibrium compliance, which means the formulations have a weaker internal structure, as indicated in the tertiary region of the curve. This was then followed by F2 and F3 with large compliance indicating it will take longer for the microstructure to change(33).

The creep compliance test was done at temperature of 25ºC with variable stress ramps, as indicated above. Thus, the studied rheological parameters can also be employed in preliminary preparation and formulation of specifications as per regulatory guidelines.

pH of the griseofulvin cream was found to range from 5.5 to 6 in the first two days after griseofulvin cream formulation. Meanwhile, in the accelerated stability test, it was ranging from 6 to 7 from day 0 to 90, with a marked decrease in pH of 6.1 for formulation F1 and an increase in pH to 7.1 for formulation F4, indicating the dynamic recording in pH of formulations. The pH of creams remained within the accepted criteria(13).

Accelerated stability using high performance thin layer chromatograph method for identification and quantification of griseofulvin cream showed that the formulations were significantly stable, as indicated in the area under the curve and graph trends for the four formulations. The percentage trend ranged 100.68 % on day 0 to 99.68% on day 90.

In addition, the formulations had significant implications in the selection of type formula based on the ratio of water to vaseline for economic purpose during production scale-up for developing countries like Tanzania.

Antifungal activity of griseofulvin cream had shown a very strong activity against *Trichophyton rubrum* with zone of inhibition of 37.5mm. Irritation effect of the cream was very slight with average score of 1.7; the light effect had enabled on appropriate selection of the package container for griseofulvin cream to a white opaque plastic container.

CHAPTER FIVE

5.0 CONCLUSION, RECOMMENDATION AND LIMITATION

5.1 CONCLUSION AND RECOMMENDATION

This was first formulation of semisolid for griseofulvin in Tanzania with promised rheological properties, pH, antifungal activity and stability behavior. Among the four formulations, the third formulation had best and desirable rheological properties, pH, antifungal activity against *T. rubrum* and stable shelf life. The formulated griseofulvin cream had shown strong antifungal activity against *Trichophyton rubrum*, a species responsible for causing Tinea pedis. The irritation effect of the cream was very slight, indicating non-irritant effect.

Formulation exhibits the required cream properties among the novel formulation based on specifications, according to The International Conference on Harmonization (ICH) guideline, it mentioned that for stability testing of new drug substances and products, and it requires that stress testing be carried out to elucidate the inherent stability characteristics of the active substance. This formulation can be used as preliminary preparation in formulation of specifications as per regulatory guidelines.

Additional research is needed to enhance the precision of the yield stress of the griseofulvin cream. Further studies should be conducted for antifungal activity of other species such as *Trichophyton tonsurans, T. violaceum, T. soudanense, and microspores canis, which are* causative agents of Tinea capitis. In vivo evaluating bioavailability levels in animal and recovery tests of griseofulvin cream before recommending its application in humans.

5.2 LIMITATION AND MITIGATION

The stability chamber required to perform the accelerated stability study had a malfunction in the laboratory at school of pharmacy. This demanded me to request a space for a stability chamber at other facilities. Finally, I succeeded in getting permission to use stability chamber at Keko Pharmaceutical Industry. Thus, the problem was mitigated smoothly.

6.0 REFERENCES

1. Pallavi A.Mhatre RNP, Rachana S.Kumar. FORMULATION, DEVELOPMENT AND EVALUATION OF TOPICAL EMULGEL OF GRISEOFULVIN. ISSN: 2231-0541. An International Journal of Advances in Pharmaceutical Sciences. 2014;5(5):2298-308.
2. Aris P, Wei YL, Mohamadzadeh M, Xia XH. Griseofulvin: An Updated Overview of Old and Current Knowledge. DOI: 10.3390/molecules27207034. Molecules. 2022;27(20).
3. Eichner A, Wohlrab J. EMA draft guideline on quality and equivalence of topical products - Ein bereits uberzeugendes Konzept oder noch in den Kinderschuhen?. ISSN: 1610-0379. J Dtsch Dermatol Ges. 2023;21:13-4.
4. Ribeiro HM, Morais JA, Eccleston GM. Structure and rheology of semisolid o/w creams containing cetyl alcohol/non-ionic surfactant mixed emulsifier and different polymers. Int J Cosmet Sci. 2004;26(2):47-59.
5. Cichonska P, Domian E, Ziarno M. Application of Optical and Rheological Techniques in Quality and Storage Assessment of the Newly Developed Colloidal-Suspension Products: Yogurt-Type Bean-Based Beverages. DOI: 10.3390/s22218348. Sensors (Basel). 2022;22(21).
6. Anindya Hana Iradhati MJ. FORMULATION AND PHYSICAL STABILITY TEST OF GRISEOFULVIN MICROEMULSION GEL. Doi.org/10.22159/ijap.2017.v9s1.22_27
7. International Journal of Applied Pharmaceutics. 18 August 2017;9:23-6.
8. Kassem MA, Esmat S, Bendas ER, El-Komy MH. Efficacy of topical griseofulvin in treatment of tinea corporis. DOI: 10.1111/j.1439-0507.2006.01221. Mycoses. 2006;49(3):232-5.
9. B. Ouoba MOD, M. Zuber & J. C. Chaumeil. Feasibility Study of Dermatological Formulations Based on Sheabutter for the Tropical Countries of Africa. DOI: 10.3109/03639049409050224. Drug Development and Industrial Pharmacy. 1994;13:2091 -2 107.

10. TMDA. APPROVED MEDICINES PRODUCT INFORMATION 2024 [updated 1st March, 2024. Available from: https://www.tmda.go.tz/pages/approved-product-information.
11. Martindale SCS. The Complete Drug Reference. ISBN: 978 0 85369 840 1 and 0263-5364. Lambeth High Street, London SEl 7JN, UK 100 South Atkinson Road, Suite 200, Grayslake, IL 60030-7820, USA: Pharmaceutical Press (An imprint of RPS Publishing); 2009. Available from: https://www.academia.edu/8032034/Martindale_The_Complete_Drug_Reference_36th_Edition.
12. Lin Ma1 · Song Guo1 · Jingshu Piao1 · Mingguan Piao1. Preparation and Evaluation of a Microsponge Dermal Stratum Corneum Retention Drug Delivery System for Griseofulvin. DOI: https://doi.org/10.1208/s12249-022-02362-1 2022; 23:[1-16 pp.]. Available from: https://link.springer.com/article/10.1208/s12249-022-02362-1.
13. Jadhav Chaitali KV, Payghan Santosh. Formulation and Evaluation of Antifungal Non-aqueous Microemulsion for Topical Drug Delivery of Griseofulvin. DOI: https://www.researchgate.net/publication/280562932. Published on Web 15/01/2015, wwwinventiin). 2015;2015(1):1-14.
14. Proksch E. pH in nature, humans and skin. DOI:10.1111/1346-8138.14489. J Dermatol. 2018;45(9):1044-52.
15. Franck A. Understanding Rheology of Structured Fluids 2013 [cited 2023 2023].
16. Araujo OE, Flowers FP, King MM. Griseofulvin: a new look at an old drug. Doi10.1177/106002809002400912. DICP. 1990;24(9):851-4.
17. Suitthimeathegorn O, Jaitely V, Florence AT. Novel anhydrous emulsions: formulation as controlled release vehicles. DOI: 10.1016/j.ijpharm.2005.03.028. Int J Pharm. 2005;298(2):367-71.
18. imdip.com. Understanding Emulsion Easily. 2020.
19. Richard B. Stoughton RS, Fe; Richard L. Harris DP, Dea FJ, inventorsTopical Griseoflvin composition and Method. US1977.
20. Quizlet.com. HLB range and Application. 2021.

21. Choudhary A. Stability Problems of Emulsion and Methods to overcome. Pharmaguideline [Internet]. 2008-2021 2023. Available from: https://www.pharmaguideline.com/2021/10/stability-problems-of-emulsions.html.
22. B. Liebsch DSN, S.S.Senya, K.F.Steinhausen. Compounding Manual, School of Pharmaceutical Assistants, Muhimbili Medical center, Dar es salaam, 1980. Muhimbili Medical center, Dar es salaam, : Division of Pharmaceutical Sciences and School of Pharmacy, Muhimbili Medical Center. Dear es salaam, Tanzania; 1980.
23. Aggarwal N, Goindi S, Mehta SD. Preparation and evaluation of dermal delivery system of griseofulvin containing vitamin E-TPGS as penetration enhancer. Doi 10.1208/s12249-011-9722-y. Aaps Pharmscitech. 2012;13(1):67-74.
24. Ahshawat MS, Saraf S, Saraf S. Preparation and characterization of herbal creams for improvement of skin viscoelastic properties. Doi 10.1111/j.1468-2494.2008.00442.x. Int J Cosmet Sci. 2008;30(3):183-93.
25. Mirka Korhonen a LHb, JHc, JY. Rheological properties of creams with four different surfactant combinations-effect of storage time and conditions. International Journal of Pharmaceutics 2001;221 187–96.
26. Council of Europe SC. European Pharmacopoeia-10: Convention on the Elaboration of a European Pharmacopoeia (European Treaty Series No. 50); 2019. Available from: www.webofpharma.com.
27. WHO G. The International Pharmacopoeia, 4th Edition. ISBN: 92 4 156301 X: World Health Assembly resolution, WHA3.10, WHO Handbook of Resolutions and Decisions.; 2006 [cited 2023 20-October-2023]. Available from: https://www.google.no/books/edition/The_International_Pharmacopoeia/s_sBKJNf184C?hl=en&gbpv=1&dq=International+Pharmacopoeia+2023&printsec=frontcover.
28. Ambalal SRM. Griseofulvin and Whitfield's ointment- time for their resurrection as part of multidrug therapy in management of recalcitrant dermatophyte infections. Doi.org/10.18231/j.ijced.2021.018. IP Indian Journal of Clinical and Experimental Dermatology 2021;7(1):86–89. 2021;7(1):86-9.

29. STOUT Da. Classification zone of inhibition 1971. Available from: https://www.researchgate.net/post/Zone_of_Inhibition_Intensities_by_Davis_and_Stout.
30. (GHS) UNgHs. Hazard classification for dermal scoring and classification scheme 2003. Available from: https://www.ncbi.nlm.nih.gov/books/NBK253967/.
31. Gupta AK, Adam P, Dlova N, Lynde CW, Hofstader S, Morar N, et al. Therapeutic options for the treatment of tinea capitis caused by species:: Griseofulvin versus the new oral antifungal agents, terbinafine, itraconazole, and fluconazole. DOI: 10.1046/j.1525-1470.2001.01978. Pediatr Dermatol. 2001;18(5):433-8.
32. Limited MI. A Basic Introduction to Rheology 2016 [cited 2023. Available from: www.malvern.com/contact.
33. Meck CsCJF. Stress-controlled elastic granular shear flows. DOI: 10.1017/S0022112005005616. 2002;539:273-97.
34. Adeyeye MC, Jain AC, Ghorab MK, Reilly WJ, Jr. Viscoelastic evaluation of topical creams containing microcrystalline cellulose/sodium carboxymethyl cellulose as stabilizer. DOI: 10.1208/pt030208. Aaps Pharmscitech. 2002;3(2):E8.
35. Ramachandran S, Chen S, Etzler F. Rheological characterization of hydroxypropylcellulose gels. Drug Dev Ind Pharm. 1999;25(2):153-61.

APPENDIX I

1.1 Formulae for griseofulvin cream formulation

Formulation zone Preparation:		Reference		
	Ingredients	**Qty (g)**	**Factor**	**Required weight (g)**
I	Griseofulvin			
II	Polysorbate 80 (Tween 80)			
III	Sorbitan oleate (Span 80)			
IV	Vaseline			
V	Purified water			
	Total (grams)			

1.2 Equation for HBL value of emulsifiers

$HLB_O = (W_T HLB_T + W_S HLB_S) / (W_T + W_S)$

1.3 Formulation record

FORMULATION (F1-F4)		%Error			**W/O**	**EMULSIFIER**
		±1%			**Ratio**	**Ratio**
Ingredients	**Required(g)**	0.5	0.5	**Actual (g)**	**W5/O5**	**T2/S8**
Griseofulvin						
Polysorbate 80 (Tween80)						
Sorbitan oleate 80(Span80)						
White vaseline						
Purified water						
SUM						

Appendix II

2.1 Yield stress (Shear stress ramp)

Formulae	Yield stress (Pa)
F1 (W5O5)	
F2 (W3O7)	
F3 (W2O8)	
F4 (W1O9)	

2.2 Creep and recovery

Formulae	Stress Ramp (Pa)	Lowest stress (Pa)
F1		
F2		
F3		
F4		

2.3 Physical and pH results

DATE	PARAMETER	COLOR				HOMOGENEITY				PH				CRACKING			
	Formulae	**F1**	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**	**F1**	**F2**	**F3**	**F4**
26-Jan-24	0 day																
26-Feb-24	30^{th} day																
26-Mar-24	60^{th} day																
26-Apr-24	90^{th} day																
	Specification	**White**				**Homogenous**				**5 to 8**				**Non-cracking**			

2.4 Stability results recording formulation F1 to F4

Date	**Formulation F**	**Day**	**Amount% Assay**	**mg/50g**
	F [1-1]			
	[1-2]			
	[1-3]			
26-1-2024	Average	0		
	F[1-1]			
	[1-2]			
	[1-3]			
26-2-2024	Average	30		
	F [1-1]			
	[1-2]			
	[1-3]			
26-3-2024	Average	60		
	F [1-1]			
	[1-2]			
	[1-3]			
26-4-2024	Average	90		

Appendix III

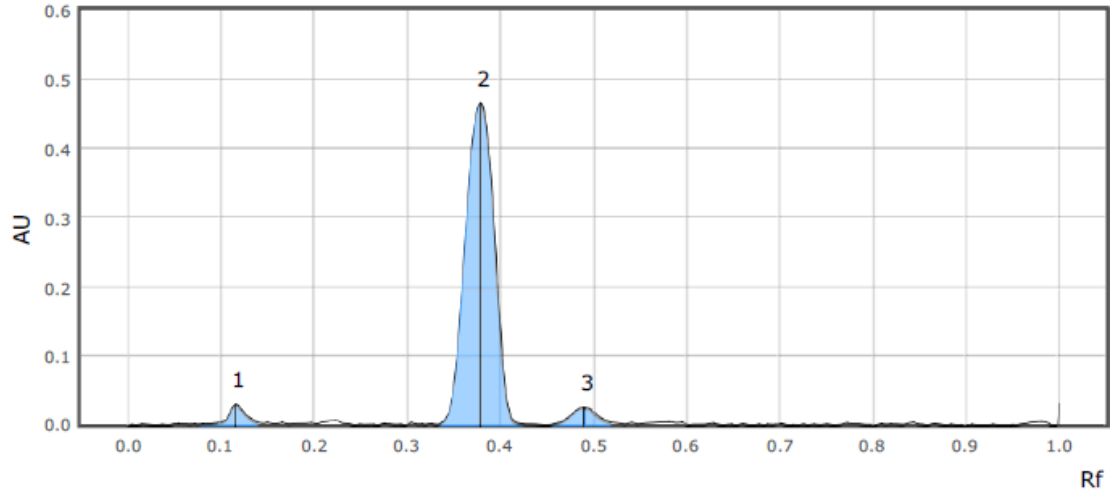


Figure 3.6: Peak area on day 0

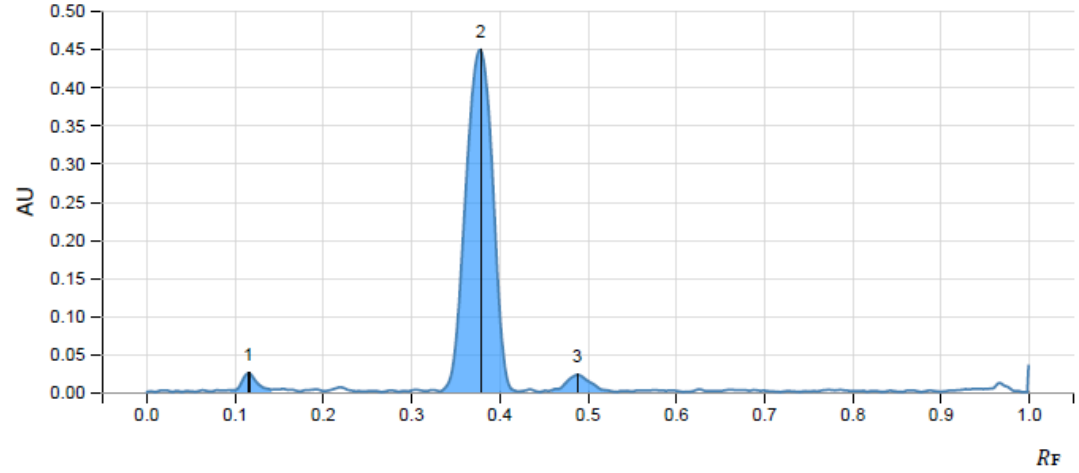


Figure 3.7: Peak area on day 90

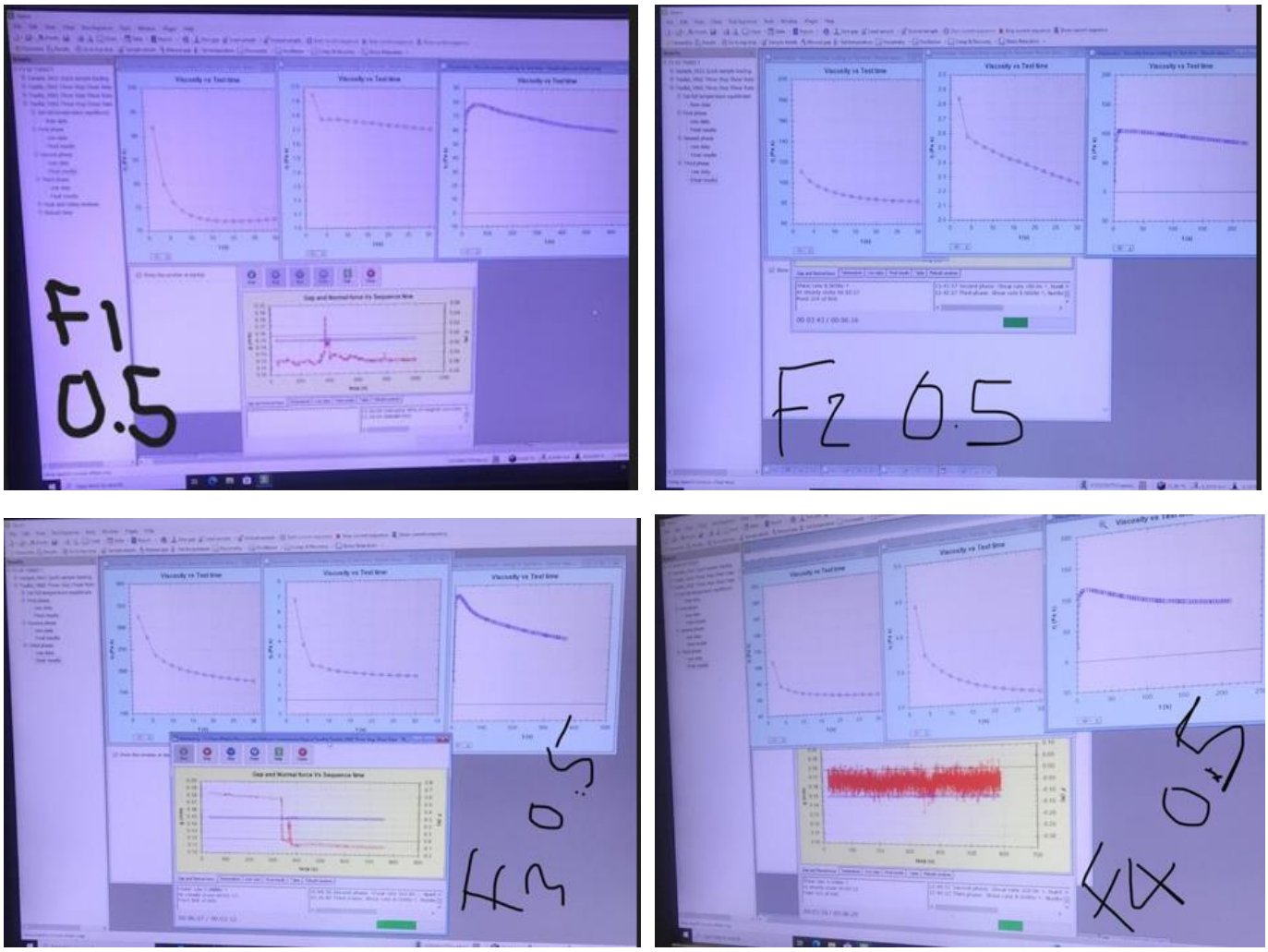


Figure 3.8: The selected 0.5pa for three step shear rate (thixotropic) that gave appropriate curve as shown below from Kinexus-rheometer software visualization.