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“DEVELOPMENT OF TOPICAL OIL/PEG CREAMS OF VORICONAZOLE”

A Thesis

Presented for the

Master of Science

Pharmaceutical sciences emphasis Pharmaceutics and Drug delivery

Degree

The University of Mississippi

ABHISHEK SHETTAR

December 2019

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ABSTRACT

Creams are heterogenous biphasic semi-solid preparations consisting of oil and aqueous phases, wherein one phase is dispersed in the other and stabilized using a suitable emulsifier. The active pharmaceutical ingredient is either dissolved or dispersed in continuous or dispersed phase of the formulation. Water is the most common and the major constituent in the preparation of creams. However, certain moisture sensitive drugs like mechlorethamine, acyclovir, itraconazole and ketoconazole are liable to degradation in presence of water thereby decreasing the stability of the formulation. Hence, there is a need for formulation suitable for incorporating water sensitive drugs. Creams prepared with polyethylene glycol instead of water could be an alternative to creams prepared using water to improve the stability of the drug. Moreover, PEGs are generally used as drug penetration enhancers and solubilizers in cream preparations. The objective of present study was to develop and optimize Oil in PEG creams suitable for incorporating moisture sensitive drugs and to investigate the feasibility of twin-screw processor in the continuous manufacturing of topical semi-solid cream formulation.

Voriconazole (VRC) was selected as a model drug for preparation of Oil/PEG cream consisting of PEG 2000, PEG 400 and propylene glycol after performing drug-exciipient compatibility studies. Twin screw processor with a 10 mm co-rotating twin screw configuration was used for preparation of oil/PEG creams. Creams prepared using twin-screw processor were characterized for the following: Differential Scanning Calorimetry (DSC), pH, Solvent activity (a_s), viscosity, drug content uniformity, *in vitro* drug release testing (IVRT) and *in vitro* permeation testing (IVPT) using human cadaver skin.

DEDICATION

I would like to dedicate this thesis to my parents
Shivashankar Shettar, Sarita Shettar and my brother
Akash Shettar for all their support and sacrifices.

ACKNOWLEDGMENTS

I would like to express my gratitude to my advisor Dr. S. Narasimha Murthy, for all his constant support, time and valuable advices throughout my master's degree. I am grateful to Dr. Walter Chambliss and Dr. Michael Repka for agreeing to be a part of my committee, in spite of their busy schedule and helping me with the valuable inputs.

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I am grateful to all the members of Dr Murthy's group, Purnendu, Dr Vijay, Apoorva, Srinivas and Maha, for their unconditional help and support. A special thanks to Srinivas, Apoorva and Dr Vijay for helping me throughout my project and for their constant motivation which helped me to complete my project on time.

Huge thanks to all the senior graduate students, friends and family.

TABLE OF CONTENTS

Contents

ABSTRACT	ii
DEDICATION	iii
ACKNOWLEDGMENTS	iv
TABLE OF CONTENTS	v
LIST OF ABBREVIATIONS.....	vii
LIST OF TABLES.....	viii
LIST OF FIGURES	ix
CHAPTER I: INTRODUCTION	1
Advantages of preparation of creams using twin-screw processor over homogenization	1
Active Pharmaceutical Ingredient	2
CHAPTER II: MATERIALS AND METHODS.....	4
Materials	4
Methods	4
Screening of the Excipients	4
Fourier Transform Infrared Spectroscopy (FT-IR)	4
Preparation and optimization of Oil/PEG cream	5
Preparation of selected formulation using Twin screw processor	6
Hot melt extrusion method	6
Characterization of Oil/PEG creams:	8
Differential Scanning Calorimetry (DSC) Analysis	8
pH measurement	8
Solvent Activity (a_w) measurements	9
Viscosity measurement	10
Method of analysis	10
High Performance Liquid Chromatography (HPLC)	10
Spectrophotometric analysis	11
Drug-content uniformity	12
In-vitro release testing (IVRT)	12
Drug-stability in receiver medium	13
In-vitro drug release test	15

<i>In-vitro</i> permeation testing (IVPT).....	16
CHAPTER III: RESULTS AND DISCUSSION.....	18
Screening of excipients	18
Fourier Transform Infrared Spectroscopy (FT-IR).....	18
Preparation and optimization of Oil/PEG cream	20
Preparation of selected formulation using Twin screw processor.....	23
Differential scanning calorimetry (DSC)	24
pH measurement.....	26
Solvent Activity (aw) measurements	27
Viscosity measurements	28
Drug-content uniformity.....	28
<i>In-vitro</i> release testing (IVRT)	29
Drug-stability in receiver medium	30
<i>In-vitro</i> drug release test.....	31
<i>In-vitro</i> permeation testing (IVPT).....	32
CHAPTER IV: CONCLUSION	35
LIST OF REFERENCES.....	36
VITA	39

LIST OF ABBREVIATIONS

VRC	Voriconazole
PEG	Polyethylene Glycol
PG	Propylene glycol
ACN	Acetonitrile
PBS	Phosphate buffer solution/saline
HME	Hot Melt Extrusion
HPLC	High pressure/performance liquid chromatography
UV/VIS	Ultraviolet/Visible spectroscopic detection
FT-IR	Fourier Transform Infrared Spectroscopy
DSC	Differential Scanning Microscopy
RPM	Rotations/revolutions/rate per minute
FDC	Franz-Diffusion cells
IV-RT	<i>in-vitro</i> release testing
IV-PT	<i>in-vitro</i> permeation testing
SC	Subcutaneous
API	Active pharmaceutical ingredient
O/W or W/O	Oil in Water or Water in Oil
O/PEG	Oil in Polyethylene glycol
DMSO	Dimethyl Sulfoxide
aw	Solvent activity
AUC	Area under the curve
RSD	Relative standard deviation

LIST OF TABLES

Table 1. Composition of formulation used to screen Oil/PEG cream.....	6
Table 2. Processing parameters for the manufacturing of creams by Hot melt extrusion.	8
Table 3. Processing parameters for the HPLC method.	11
Table 4. Various aqueous media with different concentrations for solubility studies.	13
Table 5. Membrane for IVRT.	14
Table 6. IVRT parameters.....	16
Table 7. Compositions containing PEG 1450 and PEG 400, Subjected for Freeze thaw cycle. .	21
Table 8. Compositions containing PEG 2000 and PEG 400, Subjected for Freeze thaw cycle. .	21
Table 9. Compositions containing PEG 3350 and PEG 400, Subjected for Freeze thaw cycle. .	21
Table 10. Compositions containing PEG 4500 and PEG 400, Subjected for Freeze thaw cycle.	22
Table 11. pH of the manufactured formulations.	27
Table 12. Solvent activity (a_w) of the manufactured formulations.....	28
Table 13. Viscosity measurement of the manufactured formulations.....	28
Table 14. Drug content uniformity of the manufactured formulations.	29
Table 15. Solubility studies results of various aqueous media with different concentrations.	29
Table 16. Drug stability in receiver medium.....	30
Table 17. Membrane inertness study results.	31
Table 18. Comparison of AUC ($\mu\text{g}/\text{cm}^2$) and J_{max} ($\mu\text{g}/\text{cm}^2/\text{h}$).	34

LIST OF FIGURES

Figure 1. Structure of voriconazole.....	3
Figure 2. Omicron 10 [Mini] with Do/Di ratio 1.71 for manufacturing of the Cream formulation.	7
Figure 3. Water activity meter (Series 3E, USA)	9
Figure 4. Brookfield viscometer (DV-II+).....	10
Figure 5. Franz diffusion cell.....	15
Figure 6. FT-IR of voriconazole, excipients and F9A.....	19
Figure 7. FT-IR of voriconazole, excipients and F9B.....	19
Figure 8. FT-IR of voriconazole, excipients and F10A.....	20
Figure 9. FT-IR of voriconazole, excipients and F10B.....	20
Figure 10. Freeze thaw stability cycle.....	21
Figure 11. Microscopic images of formulation F11 and F12.....	23
Figure 12. Microscopic images of formulation F9 and F10.....	23
Figure 13. Representation of the Hot melt extrusion setup.....	24
Figure 14. DSC of pure components and F9A.....	25
Figure 15. DSC of pure components and F9B.....	25
Figure 16. DSC of pure components and F10A.....	26
Figure 17. DSC of pure components and F10B.....	26
Figure 18. <i>In-vitro</i> drug release profile of the formulations.....	32
Figure 19. <i>In-vitro</i> drug permeation profile of the formulations.....	34

CHAPTER I: INTRODUCTION

Creams are heterogenous semi-solid preparations, where oil in water (O/W) or water in oil (W/O) is dispersed and stabilized using a suitable emulsifier. O/W creams are most commonly used topical formulation for various dermatological disorders. Moisture sensitive drugs like mechlorethamine, acyclovir, itraconazole and ketoconazole are liable to degradation in water phase. Hence, there is a need for developing cream formulation using non-aqueous hydrophilic component. Polyethylene glycol (PEG) is a polyether compound which is produced by the interaction of ethylene oxide with ethylene glycols and water. PEG has many applications, from industrial manufacturing to medicine. PEG is also used as an excipient in many pharmaceutical products. PEG is a product of condensed ethylene oxide and hydrophilic in nature. Moreover, PEGs are generally used as drug penetration enhancers and solubilizers in cream preparation. In the current study, PEG was used in preparation of creams instead of water, in order to overcome the degradation of moisture sensitive drugs^{[1][2]}.

Advantages of preparation of creams using twin-screw processor over homogenization

Creams are prepared by homogenization of the ingredients which involves applying sufficient energy to disperse one phase into another^[1]. However, homogenization method for preparation of creams has several disadvantages; 1. Homogenization is a multistep process, hence time and resources invested in each batch is more resulting in lesser productivity and efficiency, 2. Homogenization is a batch process; hence variations are observed between batch to batch process. In this project, we investigated the application of hot melt extrusion (HME) technology in

preparation of topical semi-solid creams for continuous manufacturing of a topical semi-solid cream formulation. HME provides many advantages over conventional method like homogenization technique for preparation of creams, such as reduced processing time as it is continuous process or one-step process. Moreover, the variations involved in preparation of creams using twin screw extruder are less compared to homogenization technique. Moreover, no additional agitators and scrapers are required since mixing action is performed by the screw elements in the barrel. The screw elements also aid in particle size reduction. Additionally, the processing parameters could be customized to obtain products with desired characteristics^[3] with the high yield and minimum material loss.

Active Pharmaceutical Ingredient

In this project, voriconazole (Fig. 1) an antifungal drug was selected for the study. Voriconazole has a tendency to undergo neutral hydrolytic conditions, hence this project was designed to incorporate voriconazole in oil/PEG creams^{[31][38][39]}. Voriconazole is a triazole antifungal medication used to treat serious fungal infections. It is used to treat invasive fungal infections that are generally seen in patients who are immunocompromised. These include invasive candidiasis, invasive aspergillosis, and emerging fungal infections caused by *Scedosporium apiospermum* (asexual form of *Pseudallescheria boydii*) and *Fusarium* spp. including *Fusarium solani*. Fungal plasma membranes are similar to mammalian plasma membranes, differing in having the nonpolar sterol ergosterol, rather than cholesterol, as the principal sterol. Membrane sterols such as ergosterol provide structure, modulation of membrane fluidity, and possibly control of some physiologic events. Voriconazole effects the formation of the fungal plasma membrane by indirectly inhibiting the biosynthesis of ergosterol. This results in plasma membrane permeability changes and inhibition of growth.

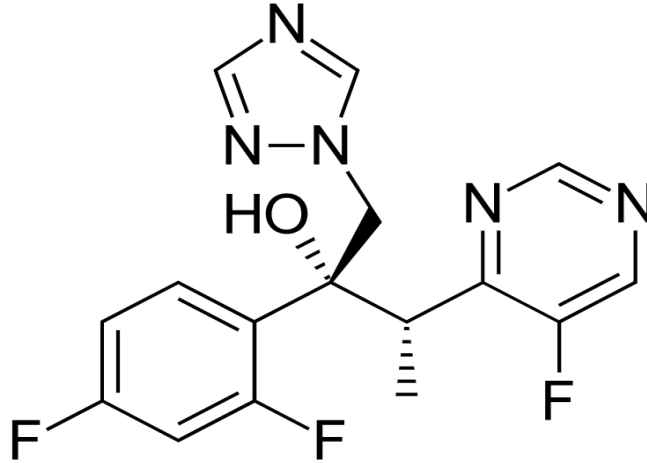


Figure 1. Structure of voriconazole

Mechanism of action: Voriconazole binds and inhibits ergosterol synthesis by inhibiting CYP450-dependent 14-alpha sterol demethylase. The inhibition of 14-alpha sterol demethylase results in a depletion of ergosterol in fungal cell membrane.

Physicochemical properties of voriconazole:

The melting point of voriconazole was found to be 127-130 °C, log P value was 1, and voriconazole do not show any degradation at dry heat 70 °C ^[37]. Voriconazole is soluble in solvents like propylene glycol.

CHAPTER II: MATERIALS AND METHODS

Materials

PEG 4500 and light mineral oil were purchased from PCCA (Houston, TX), PEG 2000 was purchased from Sigma-Aldrich, now Millipore Sigma (Darmstadt, Germany), PEG 400 was purchased from BASF (New Jersey, USA), Labrafil M1944 CS, Tefose 63 and Transcutol P were kindly gifted by Gattefosse (Saint Priest, France), Menthol and Camphor were purchased from Ward's Science (Rochester, New York, USA) and Voriconazole (Batch no. RVK6H-QO) was purchased from VWR (New Jersey, USA). All other chemicals and solvents (Methanol and Acetonitrile) used were of HPLC grade. DI water was used in all studies and obtained from a MilliQ water filtration unit.

Methods

Screening of the Excipients

Before formulation development, it is important to screen the excipients in order to develop a stable formulation. Initial strategy included screening of the excipients for the base formulation. Screening of the excipients was done by FTIR studies to identify any chemical interactions.

Fourier Transform Infrared Spectroscopy (FT-IR)

Understanding of physicochemical interactions of an active pharmaceutical ingredient (API) and pharmaceutically inactive ingredients (excipients) in the dosage forms is an integral part of pre-formulation studies, to develop a new dosage form. Although excipients are pharmacologically

inert, they can interact with drugs in the dosage form to affect drug product stability, hence drug excipient compatibility studies were performed to study the interactions [5]. Voriconazole and excipients were analyzed using Cary 600 series Fourier transform infrared spectrometer, Agilent Technologies (FT-IR).

Preparation and optimization of Oil/PEG cream

Oil/PEG creams were prepared by varying different grades of PEGs in combination with propylene glycol as aqueous phase. The Oil/PEG creams F1 through F32 (table 1) was prepared by keeping a constant amount of Voriconazole, propylene glycol, Labrafil M 1944 CS, light mineral oil, and Tefose 63; and by varying the amount of high molecular weight/solid PEGs (PEG 4500, PEG 3350, PEG 2000, and PEG 1450 and low molecular weight/liquid PEG (PEG 400). The Oil/PEG creams were prepared by adding the oil phase containing Tefose 63 (emulsifier) to the hydrophilic phase at 70 °C by employing high shear homogenization technique. Labrafil M 1944 CS was used in the oil phase because it is an excellent water dispersible surfactant, solubilizer and bioavailability enhancer. Labrafil M 1944 CS is often used as co-emulsifier in topical formulations along with Tefose 63 in antifungal creams [32]. The prepared creams were subjected to freeze thaw stability studies, where the creams were exposed to 3 cycles of freeze and thaw and evaluated visually for the phase separation of the formulation. Creams which did not undergo phase separation were selected for the further studies.

Table 1. Composition of formulations used to screen Oil/PEG creams

Formulation	Proportion of PEGs	Oil Phase
F1	PEG 1450: PEG 400 (15:85)	Labrafil M1944 CS
F2	PEG 1450: PEG 400 (15:85)	Light mineral oil
F3	PEG 1450: PEG 400 (20:80)	Labrafil M1944 CS
F4	PEG 1450: PEG 400 (20:80)	Light mineral oil
F5	PEG 1450: PEG 400 (30:70)	Labrafil M1944 CS
F6	PEG 1450: PEG 400 (30:70)	Light mineral oil
F7	PEG 1450: PEG 400 (50:50)	Labrafil M1944 CS
F8	PEG 1450: PEG 400 (50:50)	Light mineral oil
F9	PEG 2000: PEG 400 (15:85)	Labrafil M1944 CS
F10	PEG 2000: PEG 400 (15:85)	Light mineral oil
F11	PEG 2000: PEG 400 (20:80)	Labrafil M1944 CS
F12	PEG 2000: PEG 400 (20:80)	Light mineral oil
F13	PEG 2000: PEG 400 (30:70)	Labrafil M1944 CS
F14	PEG 2000: PEG 400 (30:70)	Light mineral oil
F15	PEG 2000: PEG 400 (50:50)	Labrafil M1944 CS
F16	PEG 2000: PEG 400 (50:50)	Light mineral oil
F17	PEG 3350: PEG 400 (15:85)	Labrafil M1944 CS
F18	PEG 3350: PEG 400 (15:85)	Light mineral oil
F19	PEG 3350: PEG 400 (20:80)	Labrafil M1944 CS
F20	PEG 3350: PEG 400 (20:80)	Light mineral oil
F21	PEG 3350: PEG 400 (30:70)	Labrafil M1944 CS
F22	PEG 3350: PEG 400 (30:70)	Light mineral oil
F23	PEG 3350: PEG 400 (50:50)	Labrafil M1944 CS
F24	PEG 3350: PEG 400 (50:50)	Light mineral oil
F25	PEG 4500: PEG 400 (15:85)	Labrafil M1944 CS
F26	PEG 4500: PEG 400 (15:85)	Light mineral oil
F27	PEG 4500: PEG 400 (20:80)	Labrafil M1944 CS
F28	PEG 4500: PEG 400 (20:80)	Light mineral oil
F29	PEG 4500: PEG 400 (30:70)	Labrafil M1944 CS
F30	PEG 4500: PEG 400 (30:70)	Light mineral oil
F31	PEG 4500: PEG 400 (50:50)	Labrafil M1944 CS
F32	PEG 4500: PEG 400 (50:50)	Light mineral oil

Preparation of selected formulation using Twin screw processor

Hot melt extrusion method

Creams were prepared by co-rotating twin screw extruder (OMICRON 10, STEER Life) (Fig. 2).

For this method all the ingredients of aqueous phase and oil phase were weighed accurately and the mixture was heated on a hot plate maintained at a temperature of $70\pm 5^{\circ}\text{C}$.



Figure 2. Omicron 10 [Mini] with Do/Di ratio 1.71 for manufacturing of the Cream formulation. Based on the optimization of the screw with diameter ratio (Do/Di) 1.71 was selected for the extrusion process. Processing parameters for twin screw processor are described in Table 2. Screw speed was set to 200 RPM. Feeder speed was set to 30 rpm to feed the material at 1.8 g/min, and 0.595 - 1.19 mm particle size of PEG 2000 were used. Optimization of the peristaltic pump was done to give proper feed at feeding zone and zone 2. For the optimization of the peristaltic pump weight of material dispensed per minute vs RPM of the peristaltic pump was noted. A peristaltic pump set to a flow rate of 45 RPM was used to dispense the aqueous phase at the feeding zone and peristaltic pump set to a flow rate of pump 20 RPM was used to dispense the oil phase at the zone 2. After the optimization of both peristaltic pumps, the aqueous phase containing a 15:85 mixture of PEG 2000 and PEG 400 was passed through the feeding zone. The peristaltic pump dispensing the aqueous phase was set to an RPM of 45. The oil phase was introduced at zone 2 for proper

mixing with the aqueous phase. A peristaltic pump dispensing the oil phase was set to an RPM of 20. The RPM was adjusted according to the percentage of composition in aqueous phase (75%) and oil phase (25%) as needed. At the end zone (collecting zone) creams which were prepared were collected in a container and allowed to cool down to room temperature. The creams were then refrigerated for further study.

Table 2. Processing parameters for the manufacturing of creams by Hot melt extrusion.

PARAMETERS	DESCRIPTION
Equipment	OMICRON 10 with Do/Di ratio 1.71, STEER Life
Screw design	Co-rotating twin screw extruder
Barrel Diameter	10 mm
Barrel temperature	Zone 1 - 70 °C
	Zone 2 - 40 °C
RPM	200
Torque	0-3 Nm/Shaft

Characterization of Oil/PEG creams:

Differential Scanning Calorimetry (DSC) Analysis

DSC was performed to analyze the glass transition temperature and melting point of pure ingredients and selected cream formulations. Thermograms were obtained using TA Instruments Discovery Series DSC 25, New Castle, DE, USA. Analysis was performed over the temperature range of 20°C to 200°C at 10°C increments. The nitrogen purge was set at 20°C/min. Samples were weighed accurately (around 5 mg) in an aluminum pan, sealed with a hermetic lid. TRIOS software was used as a means to analyze the solid-state characteristics and thermal stability of the voriconazole, excipients and the formulations.

pH measurement

The pH of the creams was measured by using Mettler Toledo InLab®Micro pH probe (Electrolyte 3 mol/L KCl). The pH meter was calibrated using standard buffers with the known pH of 4.0, 7.0

and 10.0 respectively. The pH of each cream formulation was measured in triplicate (n=3). In between every individual measurement, pH reading of the standard buffer pH 4.0 was taken followed by next reading and a standard buffer of pH 10.0 respectively.

Solvent Activity (a_w) measurements

The solvent activity of all cream formulations was measured by using AQUALAB water activity meter series 3 and 3TE (Series 3E, USA) (Fig. 3). All measurements were performed at 32°C.



Figure 3. Water activity meter (Series 3E, USA)

Initially the instrument was calibrated using standards. A whole vial of solution was transferred into a sample cup and was placed in the Aqualab's sample drawer. Two readings were taken to get the a_w within ± 0.003 of the given value. Distilled water was then placed in the drawer and the reading was noted, to ensure that it is within 1.000 ± 0.003 . The instrument was found to be suitable when the water activity for DI water was equal to 1.00. Once the calibration was done, activated charcoal cup was kept in the drawer and the knob was turned on, in order to remove any moisture present before running the samples. The values were noted in triplicate (n=3). In between each reading activated charcoal was placed in the chamber to remove moisture. The samples were analyzed in triplicate and the average of the values was reported.

Viscosity measurement

The viscosity of the prepared formulations was determined using a Brookfield viscometer (DV-II+). The temperature maintained for the study was 21°C. The spindle (3.2 cm) RPM was set to 0.3. The instrument was allowed to stabilize for 15 minutes and readings were noted for each cream formulation.

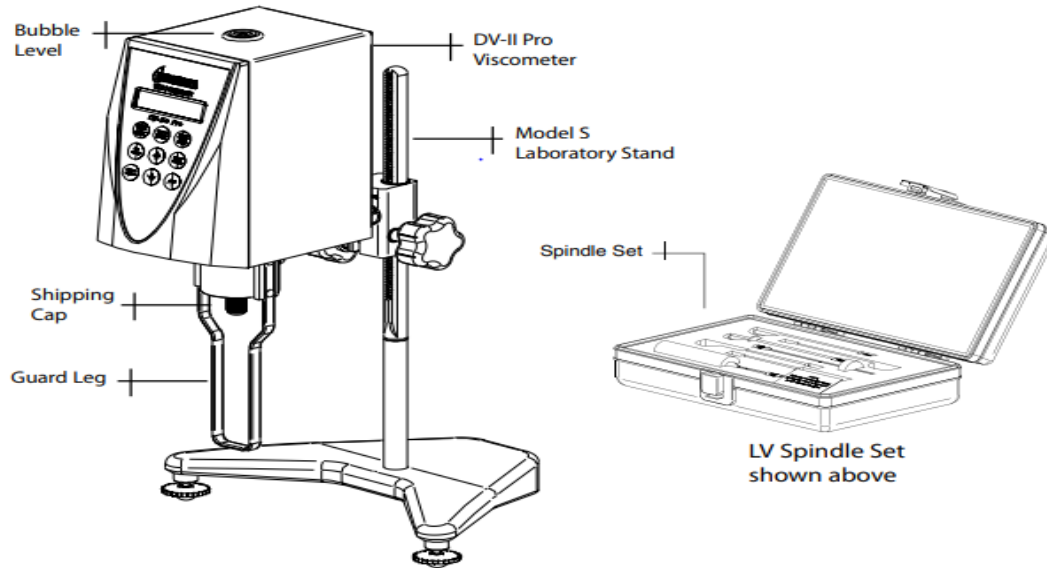


Figure 4. Brookfield viscometer (DV-II+)

LV-4 spindle was fit near the shipping cap of viscometer and the formulations were placed on the guard leg, and the spindle was immersed into a glass vial. All the readings were taken in triplicate (n=3).

Method of Analysis

1. High Performance Liquid Chromatography (HPLC).
2. Spectrophotometric analysis.

High Performance Liquid Chromatography (HPLC)

Analysis of voriconazole was done using a Waters HPLC system (Water 600 Controller, USA)

equipped with a 600-pump unit, a 717 plus auto sampler with an injection valve with a sample loop of 50 μ l, and a 2487 dual absorbance UV detector. Peak area integration was performed using Breeze software. An isocratic HPLC method was developed for the quantification of voriconazole [6,7]. The method was validated for Sensitivity/Specificity, Linearity, Limit of detection (LOD), Limit of quantitation (LOQ), measuring range, accuracy and precision. Processing parameters for the HPLC method is described in table 3.

Table 3. Processing parameters for the HPLC method.

PARAMETERS	DESCRIPTION
Mobile phase	A mixture of Acetonitrile and Water (50:50, v/v)
Column	A reversed-phase C18 column (25 cm \times 4.6 mm i.d., particle size 5 μ m)
Detection wavelength	The UV detector wavelength was set at 256 nm for voriconazole
Flow rate	1.0 mL min ⁻¹
Temperature	23 \pm 1 $^{\circ}$ C
Injection volume	25 μ l
Retention time	3.8 minutes
Run time	9.00 minutes

Spectrophotometric Analysis

The analysis for voriconazole was carried out using a UV/VIS (Ultraviolet/Visible) spectrophotometer (Model-1800, Shimadzu, Japan) at the absorbance maximum (λ_{max}) of voriconazole which was 256 nm using a quartz 96 well plate and against an appropriate blank. The solvents used were the same as the release media. The dilution was necessary in order to get UV absorbance readings below 1 [9].

Voriconazole Content Uniformity

To determine the content uniformity of the creams, 10 mg samples were withdrawn from three different regions of the cream container [e.g. top, middle, bottom] and dissolved in 1 ml of DMSO (Dimethyl Sulfoxide). The mixtures were vortexed using multi-vortexer V-32 Grant-bio for 15 minutes then subjected to sonication using FS60H (Fisher scientific) for 10 minutes. After sonication, the mixtures were centrifuged using AccuSpin Micro 17 Centrifuge (Fisher scientific) for 10 minutes and 100 µl of supernatant samples were collected and diluted up-to 1 ml with DMSO. The concentration of the samples was analyzed using HPLC. All samples were analyzed in triplicate. For analysis, a calibration curve of voriconazole ranging from 0.05 – 100 µg/ml was used. Drug content uniformity was determined by using the following formula:

$$\% \text{ Drug Content} = \frac{\text{Actual Drug}}{\text{Theoretical Drug}} \times 100$$

***In-vitro* Release Testing (IVRT)**

IVRT is well established for characterizing and evaluating the performance of semi-solid dosage forms [33]. IVRT can be a sensitive and discriminating method that is generally responsive to physicochemical changes in semisolid drug products. The IVRT pivotal study comparing the [drug] release rates of the products should be performed in a manner compatible with the general procedures and statistical analysis method specified in the United States Pharmacopeia (USP) General Chapter <1724>, Semisolid Drug Products – Performance Tests [8].

Drug Solubility Studies (Receptor Media Selection):

It is important to determine the solubility and stability of a drug in the receptor media. Drug solubility studies are carried out to determine the ideal composition of the receptor media. To

determine the saturation solubilities of voriconazole, 1 mg of voriconazole was weighed and mixed with 1 ml of various aqueous media with different compositions in 2 ml centrifuge tubes. The aqueous media studied are described in Table 4. The tubes were continuously shaken for 24 hours using a mechanical shaker. Samples were collected and subjected to centrifugation at 13,000 RPM for 10-15 minutes. The resultant supernatants were collected and 10 times diluted with 80% methanol (since voriconazole is poorly soluble in water). 200 μ l sample were transferred into HPLC vials for analysis. All the samples were analyzed in triplicate. A calibration curve of voriconazole ranging from 0.05 – 100 μ g/ml was used for all analysis.

Table 4. Various aqueous media with different concentrations for solubility studies.

Samples number	Media
1	0.2 % Brij S20
2	0.5 % Brij S20
3	1 % Brij S20
4	2 % Brij in 10 % PG
5	0.2 % Brij C20 (AP)
6	2 % PEG 400
7	1 % T80
8	1 % T20
9	10 % PG
10	PBS

Drug-stability in Receiver Medium

A standard stock solution ranging from 100 μ g/ml-1 μ g/ml was prepared. 100 μ l of 100 μ g/ml and 100 μ l of 10 μ g/ml was taken and dissolved in 10 ml of receiver medium (2 % Brij S-20 in 10 % Propylene glycol) respectively. Both samples were vortexed for 5 minutes. From the mixture 500 μ l was collected and another 500 μ l of Acetonitrile (a common solvent used for the analysis of voriconazole) was added and the samples were vortexed. Samples were refrigerated at – 20 °C

(noted as initial concentration/ 0 hr. concentration). Remaining samples were also refrigerated for 48 hours. After 48 hours 500 µl was withdrawn and another 500 µl of Acetonitrile was added and the samples were vortexed. The concentration of the samples was analyzed using HPLC. All the samples were done in triplicate. For analysis calibration curve of voriconazole ranging from 0.1 – 100 µg/ml was used.

Membrane Inertness:

Membrane inertness is done to see if there is any binding of drug to membrane. The membrane selected should offer free resistance to diffusion [8]. For the membrane inertness testing, three different concentration (500 µg/ml (High concentration), 250 µg/ml (Intermediate concentration) and 50 µg/ml (low concentration)) of standard stock solution of drug voriconazole was prepared. Stock solution was centrifuged for 10 minutes. Supernatant was collected from each concentration and added in the scintillation vials (All the samples were taken in triplicate, n=3). The selected membrane (table 5) was cut into small circular shape and 1 ml of sample was withdrawn from the centrifuge tube before soaking the membrane. The membrane was soaked in the scintillation tube and kept aside for 24 hours. After 24 hours 200 µl of sample was withdrawn from each centrifuge tubes and the before and after samples were analyzed using HPLC.

Table 5. Membrane for IVRT.

Membrane for IVRT	
Membrane	Nylon
Pore size	0.22 µm
Diameter	0.47 mm

***In-vitro* drug release test**

Vertical Franz diffusion cells with a volume of 5 ml (contact area: 0.64 cm², Logan Instruments Corp) were used to determine the in vitro drug release of the voriconazole from the formulations (Fig. 5).

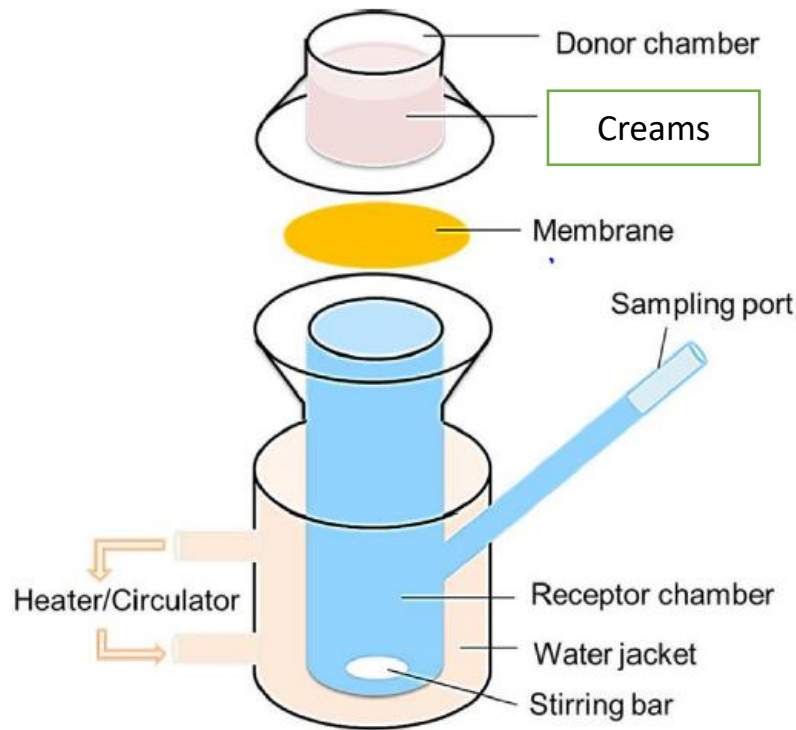


Figure 5. Franz diffusion cell.

Franz-cells were thoroughly washed with the buffer prior to starting release study. Donor cells and rubber rings were washed with 50 % methanol and 50 % water and kept for sonication for 10 minutes. Dried donor cells and rubber rings were used for release study. Franz cells were connected to the consoler and 5 ml of the receiver media was added. Stir bar was placed in each of the Franz cells and turned on the consoler. Consoler was connected to the water jacket and temperature was set to 32 ± 0.5 °C. Nylon membranes were placed on top of the receptor chambers of the cells

following the addition of the release media. Then the donor chambers were mounted on the membranes and clamped tightly. 300 mg samples of the creams were loaded into the donor chambers. The stirring speed of the Franz diffusion cells was set at 600 rpm. At pre-determined time intervals (0, 1, 2, 3, 4, 5 and 6 hours), aliquots of 0.3 ml of the media were withdrawn from the receptor chambers and replenished with fresh media. Processing parameter for IVRT is described in table 6. Voriconazole was estimated spectrophotometrically at 256 nm. UV spectrum of voriconazole was observed for voriconazole transported through the membrane at different time period.

Table 6. IVRT parameters.

Illustrative IVRT parameters	
Parameter	Description
Diffusion cell	5-station Franz diffusion cell
Weight of sample	300 mg of creams
Membrane	Nylon membrane, 0.47 mm diameter and 0.22 μm
Receptor media	2 % Brij S20 in 10 % propylene glycol
Temperature	32 \pm 0.5 $^{\circ}\text{C}$
Sampling aliquot	300 μl
Sampling time	0, 1, 2, 3, 4, 5 and 6 hours

***In-vitro* Permeation Testing (IVPT)**

Drug permeation through skin under in vitro conditions can be used to predict percutaneous absorption in humans ^[10]. For the studies a fresh human cadaver skin (New York fire fighters skin bank) stored in glycerin at a temperature of -20 $^{\circ}\text{C}$ was used. Prior to starting the experiment, the skin was taken out and kept in phosphate buffer solution (PBS) in order to wash the glycerin from the skin.

The *in-vitro* skin permeation study was performed using Franz diffusion cells with an effective diffusion area of 0.64 cm². A human cadaver skin was sandwiched between the donor and receiver media with the subcutaneous (SC) side facing the donor compartment because of uniform diffusion studies. The receiver compartment was filled with 5 ml of receptor media (2 % Brij S-20 in 10 % propylene glycol) and temperature was maintained at 32 ± 0.5 °C. The dose for finite dose study was 10 mg /cm². In the finite dose regime, only a limited amount of the donor formulation is applied to the skin surface. The application of a finite dose supposedly best resembles the *in vivo* situation when applying e.g., a cream. The finite dose studies would be close to clinically relevant dose and it would reveal the way the formulation would behave and perform in the clinical conditions. During the IVPT studies, at pre-determined time points (0, 4, 8, 12, 18, 24, 30 and 36 hours), aliquots of 0.2 ml of the media were withdrawn from the receptor chambers and replenished with fresh media. The amount of voriconazole in these samples was quantified using HPLC. In Finite dose study, the average flux was calculated and plotted to determine the maximum flux (J_{max}) and AUC_{0-t} ^{[11][12]}.

CHAPTER III: RESULTS AND DISCUSSION

Screening of Excipients

Fourier Transform Infrared Spectroscopy (FT-IR)

The FT-IR spectra of the voriconazole, excipients and formulations were analysed to determine the physical (H-bonding) or chemical interactions between API and Excipients. The voriconazole powder exhibited absorption peaks at 3191 cm^{-1} corresponding to the stretching vibrations of OH. The bands at $3000\text{--}2850$, $1600\text{--}1400$, and $1360\text{--}1250\text{ cm}^{-1}$ were assigned to the alkane CH, C = C aromatic and aryl C-N stretches ^{[26][27][28]} as shown in the figure 10, 11, 12 and 13. The formulations showed no major shifting of any functional peaks between the spectra of drug and excipients alone. The characteristics peaks of drug in the formulations were diminished when compared with pure drug peak at same wave number (3191 cm^{-1}). The spectrum shows that there are no drug-excipient interactions in the formulations.

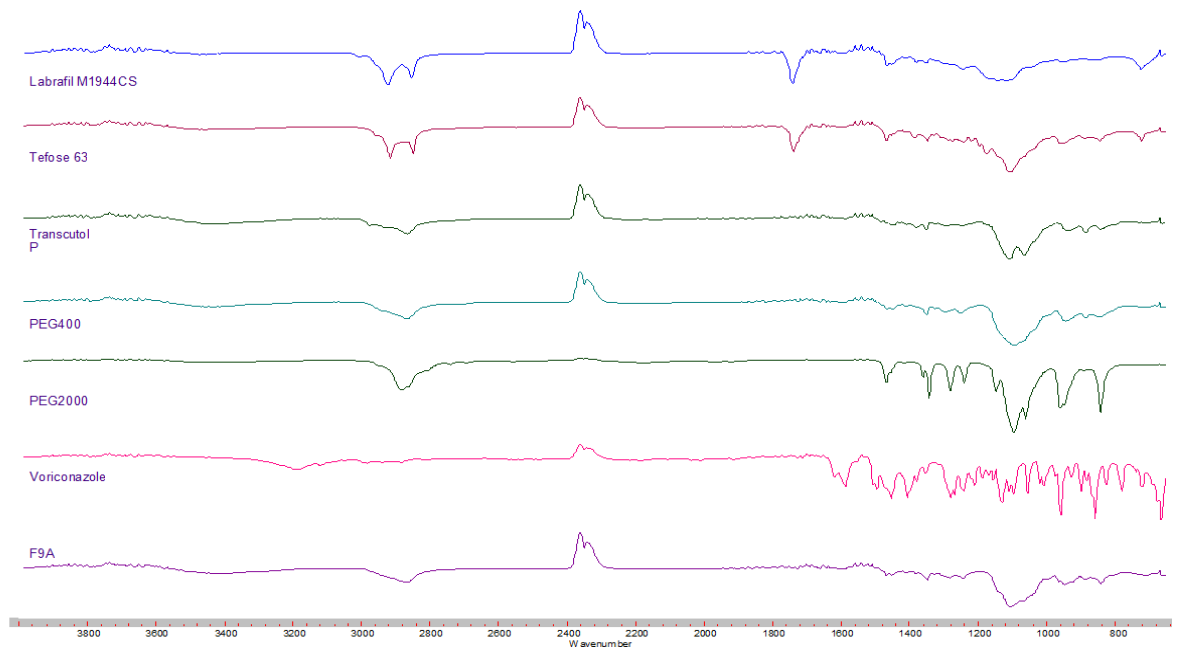


Figure 6. FT-IR of voriconazole, excipients and F9A.

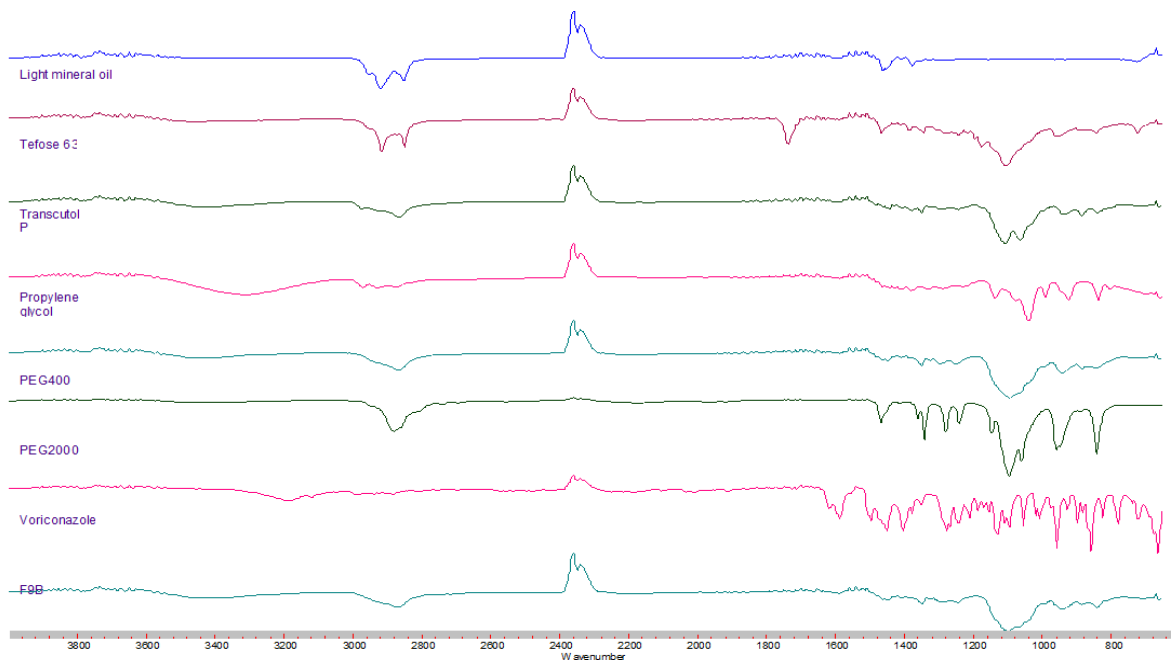


Figure 7. FT-IR of voriconazole, excipients and F9B.

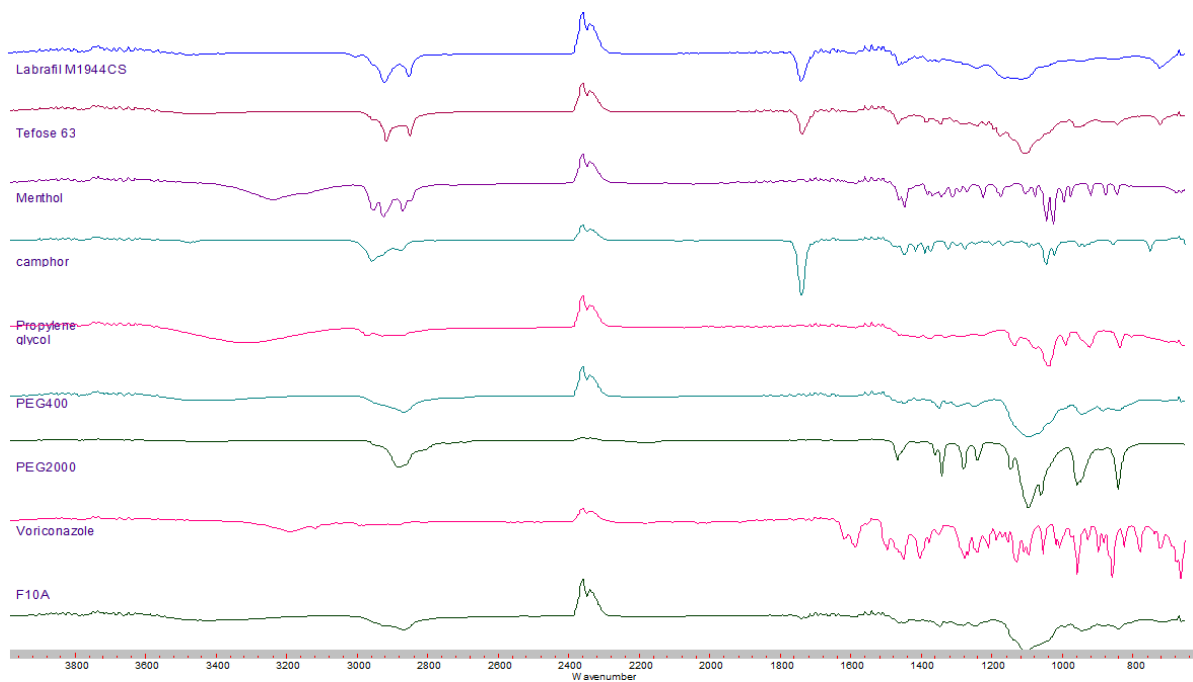


Figure 8. FT-IR of voriconazole, excipients and F10A.

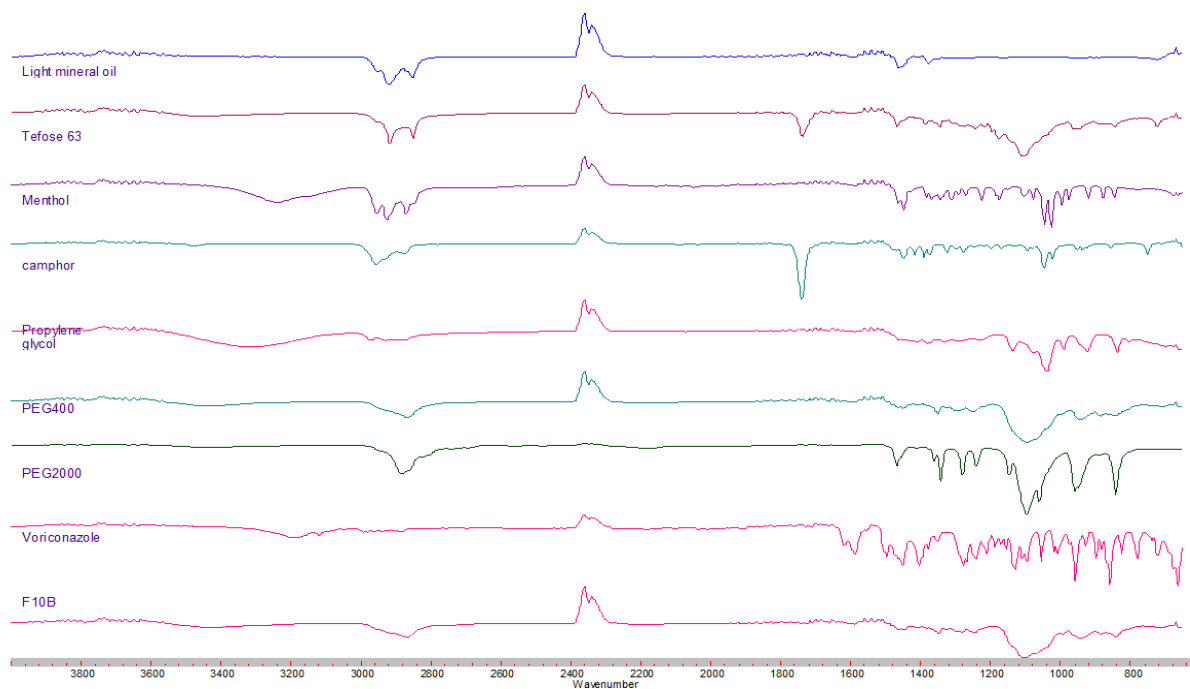


Figure 9. FT-IR of voriconazole, excipients and F10B.

Preparation and Optimization of Oil/PEG Creams

The preliminary screening of the formulations was done based on the stability and the consistency of the formulations. In order to determine the stability, a three cycles of accelerated

stability study was carried out using “freeze thaw stability” and later the formulations were visually inspected for the phase separation and the consistency.



Figure 10. Freeze thaw stability cycle.

Table 7. Compositions containing PEG 1450 and PEG 400, Subjected for Freeze thaw cycle.

Ingredients	Percentage (%) of compositions							
	F1	F2	F3	F4	F5	F6	F7	F8
PEG 1450	15	15	20	20	30	30	50	50
PEG 400	85	85	80	80	70	70	50	50
Propylene Glycol	8	8	8	8	8	8	8	8
API	1	1	1	1	1	1	1	1
Labrafil M 1944 CS*/Light mineral oil**	20*	20**	20*	20**	20*	20**	20*	20**
Tefose 63	5	5	5	5	5	5	5	5

Table 8. Compositions containing PEG 2000 and PEG 400, Subjected for Freeze thaw cycle.

Ingredients	Percentage (%) of compositions							
	F9	F10	F11	F12	F13	F14	F15	F16
PEG 2000	15	15	20	20	30	30	50	50
PEG 400	85	85	80	80	70	70	50	50
Propylene Glycol	8	8	8	8	8	8	8	8
API	1	1	1	1	1	1	1	1
Labrafil M 1944 CS*/Light mineral oil**	20*	20**	20*	20**	20*	20**	20*	20**
Tefose 63	5	5	5	5	5	5	5	5

Table 9. Compositions containing PEG 3350 and PEG 400, Subjected for Freeze thaw cycle.

Ingredients	Percentage (%) of compositions							
	F17	F18	F19	F20	F21	F22	F23	F24
PEG 3350	15	15	20	20	30	30	50	50
PEG 400	85	85	80	80	70	70	50	50
Propylene Glycol	8	8	8	8	8	8	8	8
API	1	1	1	1	1	1	1	1
Labrafil M 1944 CS*/Light mineral oil**	20*	20**	20*	20**	20*	20**	20*	20**

Tefose 63	5	5	5	5	5	5	5	5
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Table 10. Compositions containing PEG 4500 and PEG 400, Subjected for Freeze thaw cycle.

Ingredients	Percentage (%) of compositions							
	F25	F26	F27	F28	F29	F30	F31	F32
PEG 4500	15	15	20	20	30	30	50	50
PEG 400	85	85	80	80	70	70	50	50
Propylene Glycol	8	8	8	8	8	8	8	8
API	1	1	1	1	1	1	1	1
Labrafil M 1944 CS*/Light mineral oil**	20*	20**	20*	20**	20*	20**	20*	20**
Tefose 63	5	5	5	5	5	5	5	5

* Formulation containing Labrafil M 1944 CS in oil phase.

** Formulation containing Light mineral oil in oil phase.

Composition – 75 % aqueous phase (Solid PEG, Liquid PEG, Propylene glycol and API) and 25 % oil phase (Labrafil M 1944 CS/ Light mineral oil and Tefose 63).

All the formulations (F1-F8) or the creams which had combinations of solid PEG (PEG 1450) and Liquid PEG (PEG 400), showed phase separation after accelerated stability studies. Hence formulations having combinations of PEG 1450 and PEG 400 were not selected for the further studies. Formulations (F17-F24) or the creams which had combinations of solid PEG (PEG 3350) and Liquid PEG (PEG 400) and the formulations (F24-F32) or the creams which had combinations of solid PEG (PEG 4500) and Liquid PEG (PEG 400), had a very thick consistency. Hence the formulations having combinations of PEG 3350, 4500 and PEG 400 were not selected for the further studies either. Formulations (F13-F16) had higher percentage of solid PEG (PEG 2000) in the compositions hence the consistency of the formulation was thick and was unable to transfer the formulations into container because of the flakes like consistency. Hence formulations F13-F16 were eliminated. Formulations (F9-F12) had an acceptable consistency and were considered for further characterization. Microscopic images of the formulations (F11 and F12) showed a distorted globule and the formulations (F9 and F10) showed a clear globule (figure.9 and 10). Hence the formulations (F9 and F10) were selected for the further manufacturing and

characterizations.

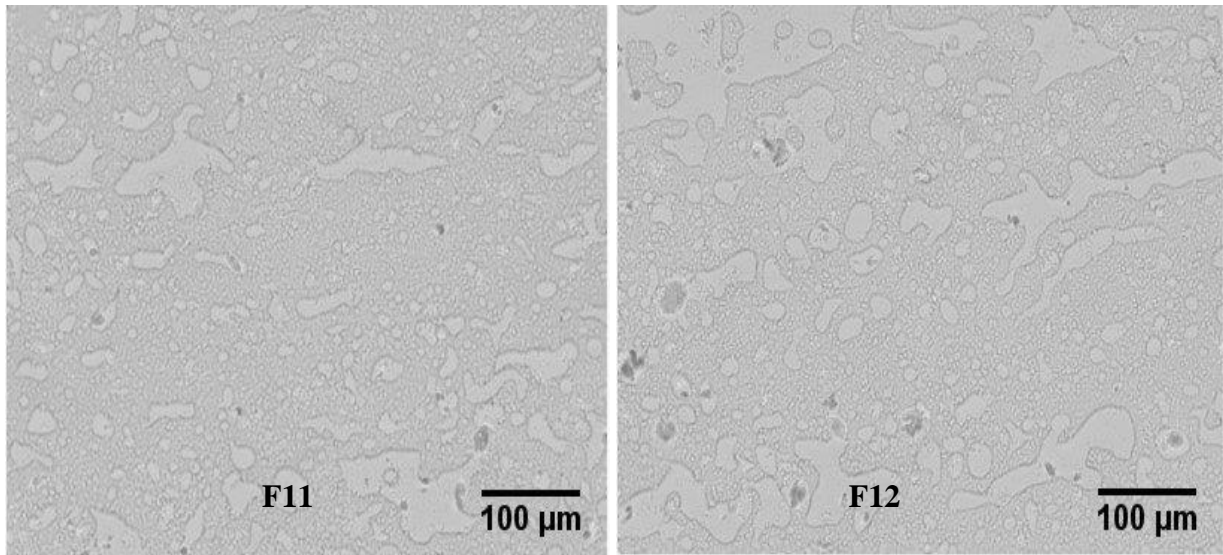


Figure 11. Microscopic images of formulation F11 and F12.

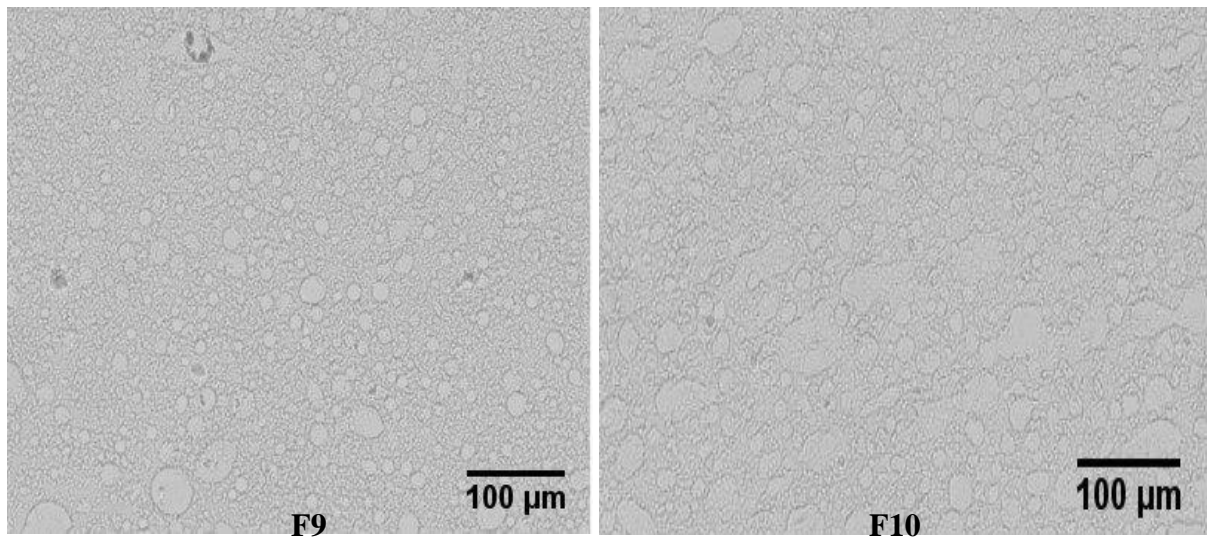


Figure 12. Microscopic images of formulation F9 and F10.

Preparation of Selected Formulations Using a Twin-Screw Processor

The base formulations F9 and F10 were selected for the manufacturing process after screening studies.

Hot melt extrusion (HME): Selected four formulations (F9A, F9B (1 % API) and F10A, F10B (3 % API)) were prepared in bulk using Hot melt extrusion process. Prepared formulations were

subjected to further characterizations.

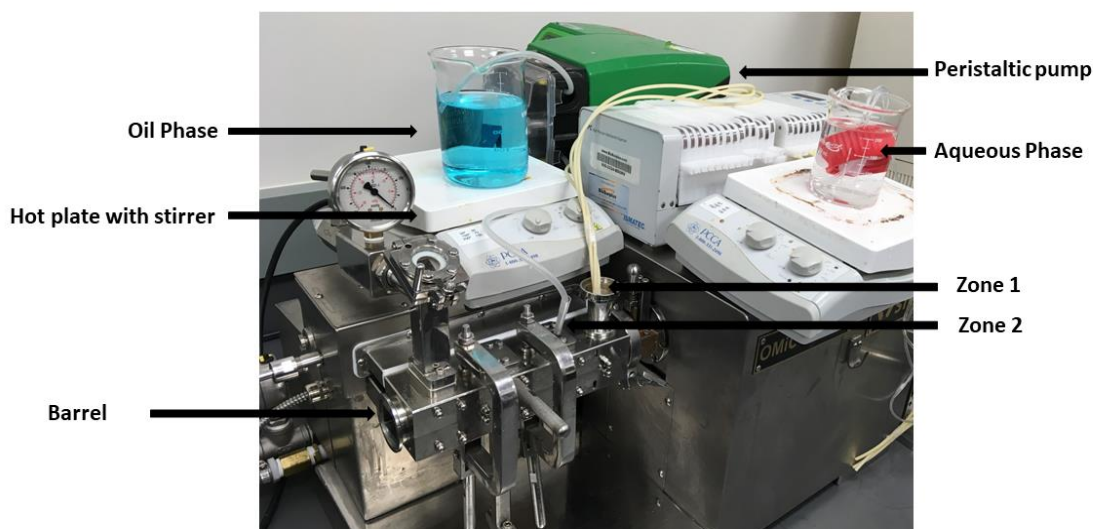


Figure 13. Representation of the Hot melt extrusion setup.

Differential scanning calorimetry (DSC)

DSC was done to determine if voriconazole remains in the amorphous form in the formulation or to know if it is soluble in the formulation (figure.14, 15, 16 and 17). The thermogram obtained showed that the voriconazole had one endothermic peak at 127 °C, which shows that voriconazole exists only in one polymorphic form. Thermogram of the prepared creams, F10B showed complete disappearance of the drug peak which suggests that either all the drug has been dissolved in the base or the increase in temperature during DSC experimentation has solubilized the drug in the base. Thermogram of the creams (F9A, F9B and F10A) showed very low intensity peaks, which indicate that the crystalline peak of voriconazole either disappeared or shifted to lower temperatures. This observation shows that the conversion of voriconazole from crystalline form to amorphous form ^[30].

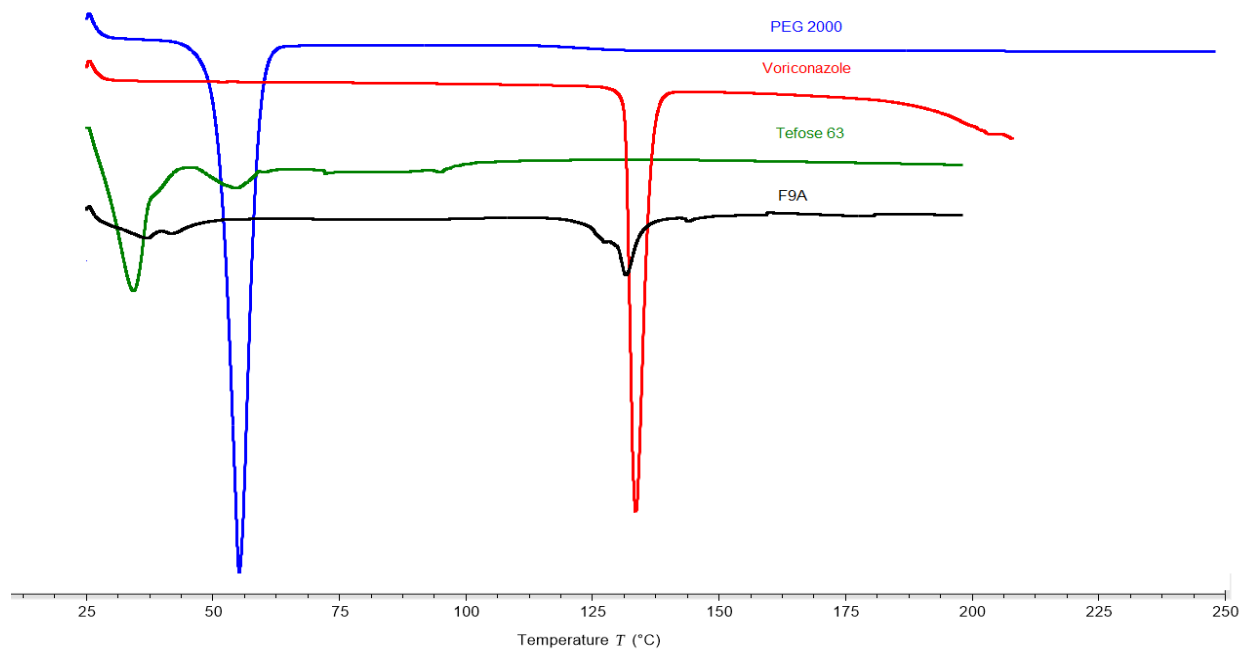


Figure 14. DSC of pure components and F9A.

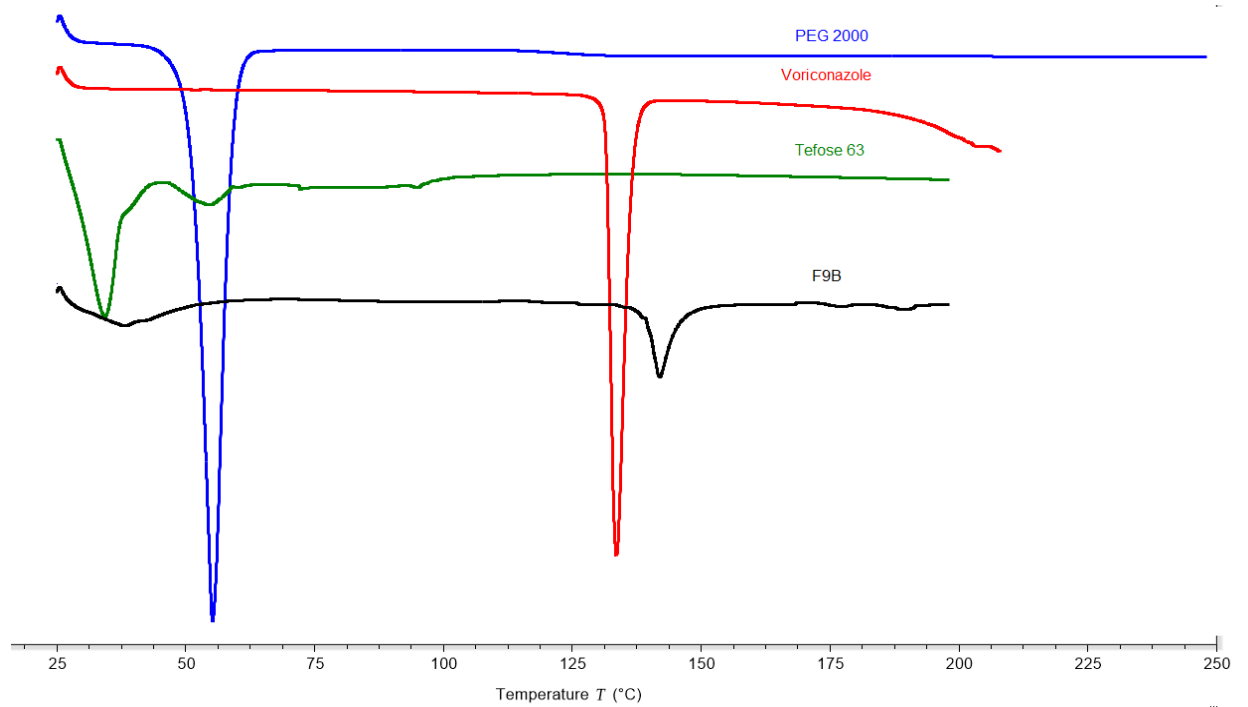


Figure 15. DSC of pure components and F9B.

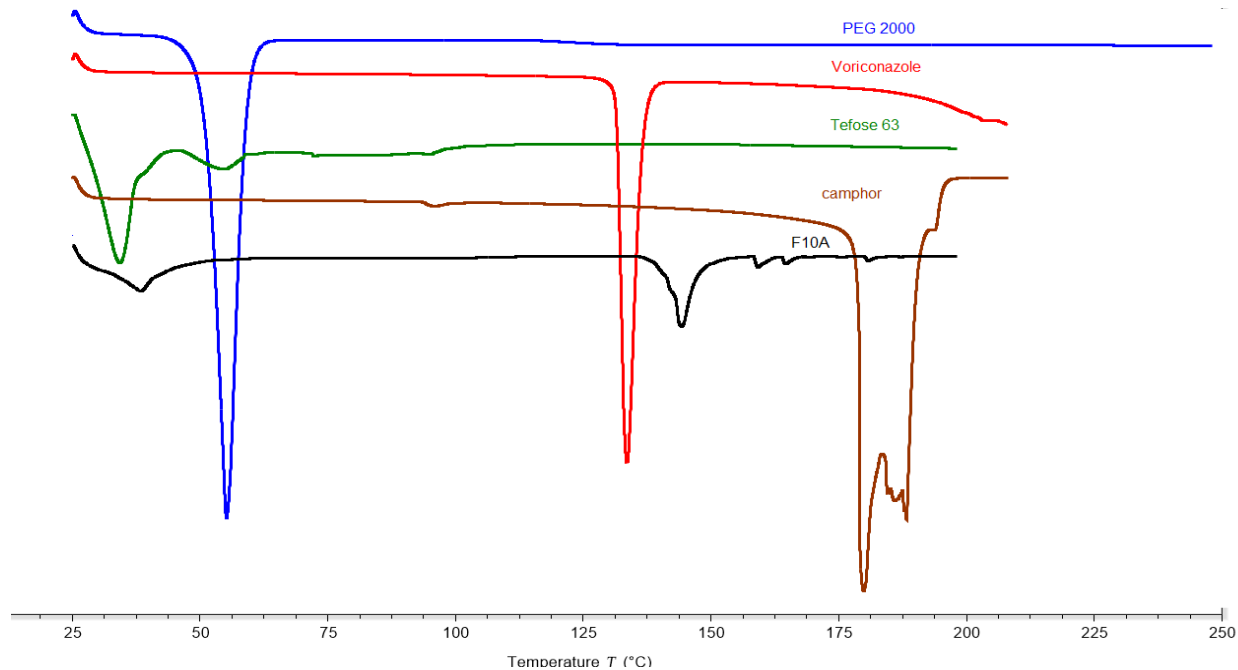


Figure 16. DSC of pure components and F10A.

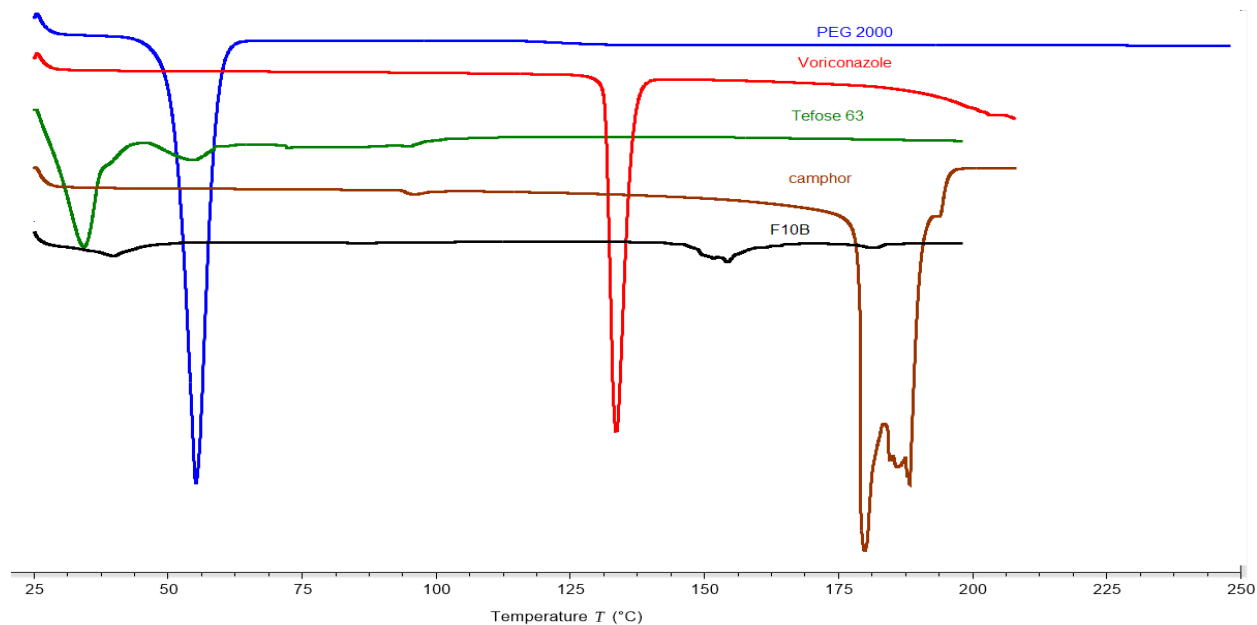


Figure 17. DSC of pure components and F10B.

pH Measurements.

According to the pH/ partition theory, the unionized drug has more ability to permeate compared to the ionized form of the drug. pH of the skin is considered to be around 5.5 and hence acceptable

semisolid formulations should have a pH ranging from 5.5-7.0 to be compatible with the skin. Voriconazole is a weak base (pKa 1.76) [37], the drug remains in the ionized state in the formulation with pH above > 6. It was found that the pH of the topical products can change post application on the skin due to homeostasis [13] [14]. The pH of all the prepared formulations did not differ significantly.

Table 11. pH of the manufactured formulations.

Creams	pH
F9A	6.82±0.016
F9B	6.70±0.009
F10A	6.58±0.025
F10B	6.00±0.001

Solvent Activity (a_w) Measurements

Solvent activity (a_w) is simply defined as the ratio of the vapor pressure of pure water (100% equilibrium relative humidity) over the vapor pressure of the sample. Solvent activity gives a clear understanding about the formulations and its potential for microbial contamination. Solvent activity of the prepared formulations was found to be low as described in table 12, however the formulations did not have water in the compositions. Hence it can be concluded that the solvent activity of the prepared formulations is accurate, since most of the creams are either Oil/Water and Water/Oil creams which will have higher solvent activity. The formulations with low solvent activity have significant influence on hydrodynamics of skin, and this was associated with corresponding impacts on drug permeation and structural changes in stratum corneum layer [15][16][17].

Table 12. Solvent activity (a_w) of the manufactured formulations.

Creams	Solvent Activity (a_w)
F9A	0.288±0.016
F9B	0.251±0.019
F10A	0.193±0.028
F10B	0.173±0.025

Viscosity Measurements

It is essential to determine the viscosity of the formulations as it determines the free flow of the formulation from the container ensuring the ease of administration. A viscosity range of (10 – 60) 10^4 cP is most common for topical formulations i.e., lotions, gels and creams [24][25]. Viscosity of all the formulations were found well within the range.

Table 13. Viscosity measurement of the manufactured formulations.

Samples	Viscosity (cP)
F9A	(24.13±0.5033) X 10^4
F9B	(35.40±1.7088) X 10^4
F10A	(27.53±1.3317) X 10^4
F10B	(34.33±3.0022) X 10^4

Drug Content Uniformity

As mentioned earlier, uniform mixing of the API with cream base is one of the challenging tasks in manufacturing of topical semi-solids. Uniformity of drug content indicates the efficiency of mixing process and the relative standard deviation (RSD) of the drug content is a good indication of the uniformity of the creams. In this study, we found that the drug content of all the prepared creams were within the acceptable range of >90%, the limit set by the USP [34]. In addition, the RSD of the drug concentration calculated based on samples taken from different regions of the

creams was less than 3.5%, indicating adequate uniformity of the drug particles in the creams [21][29].

Table 14. Drug content uniformity of the manufactured formulations.

Creams	Drug Content uniformity
F9A	100.42±0.77
F9B	105.51±2.57
F10A	101.36±0.45
F10B	103.20±1.78

***In-vitro* Release Testing (IVRT)**

Drug Solubility Studies (Receptor media selection):

Prior to starting the IVRT, it is important to select the suitable receptor media which will allow the drug to maintain sufficient sink condition. Screening of different aqueous media with varying concentrations was done before selecting the suitable receptor media for the drug voriconazole [18][19].

Table 15. Solubility studies results of various aqueous media with different concentrations.

Samples number	Media	Solubility (µg/ml)
1	0.2 % Brij S20	648.16
2	0.5 % Brij S20	793.68
3	1 % Brij S20	1033.73
4	2 % Brij S20 in 10 % PG	1369.02
5	0.2 % Brij C20 (AP)	570.16
6	2 % PEG 400	488.90
7	1 % T80	694.19
8	1 % T20	789.44
9	10 % PG	655.75
10	PBS	477.22

As shown in Table 15, voriconazole had a good solubility in 0.5 % BrijTM S-20, 1 % BrijTM S-20,

2 % w/v BrijTM S-20 in 10 % propylene glycol and 1 % T20. But compared to other release medias, voriconazole had the highest saturation solubility in 2 % w/v BrijTM S-20 in 10 % propylene glycol. Hence 2 % w/v BrijTM S-20 in 10 % propylene glycol was selected as the release medium as it completely solubilized voriconazole and had the maximum solubility for voriconazole with respect to other release medias with different surfactants solutions.

Drug-Stability in Receiver Medium

A drug-degradation study is carried out to find if the drug is degraded in the receiver medium. The degradation study was carried out at different concentrations (100 µg/ml and 10 µg/ml) of voriconazole in the receiver medium. As shown in Table 16, voriconazole was not degraded in the receiver medium.

Table 16. Drug stability in receiver medium.

Sample	Stability (%)
100 µg/ml	98.69
10 µg/ml	111.08

Membrane Inertness:

From the literature search it was found that nylon membrane, pore size 0.22 µm and diameter of 0.47 mm had previously been used in a release study of voriconazole [23] formulations. However, it is important to know if there is any resistance to diffusion by the API caused by the membrane and if release is proportional to the concentration of the drug in the formulation. A membrane inertness study was done by using different concentrations of a standard voriconazole stock solution. The samples were refrigerated at -20 °C for 24 hours. Before and after concentration of

drug was analyzed using HPLC. For analysis calibration curve ranging from 0.1 µg/ml to 100 µg/ml was used. Nylon membrane, pore size 0.22 µm and diameter of 0.47 mm membrane was selected because of its inertness to the API. There was negligible absorption of API on the membrane before and after exposure to the drug solution. Hence it is concluded that the membrane is not acting as a rate controlling barrier and the release is absolutely attributable to formulation properties.

Table 17. Membrane inertness study results.

Samples	% Adsorbed to membrane
High concentration (500 µg/ml)	0.86
Medium concentration (250 µg/ml)	0.70
Low concentration (50 µg/ml)	0.10

***In-vitro* Drug Release Test**

In-vitro release test was carried out using Franz diffusion cell (FDC), most commonly used in topical semisolid formulations [33]. For the release study, 2 % w/v Brij™ S-20 in 10 % propylene glycol (PG) was used as the receiver media and a nylon membrane (pore size 0.22 µm and diameter of 0.47 mm) was used as the diffusion membrane. As shown in figure. 18. All of the four oil/PEG cream formulations (F9A, F9B, F10A and F10B) prepared by the hot melt extrusion process followed Higuchi's release profile. Hence it can be concluded that hot melt extrusion can be used as continuous process method of preparing an emulsion with the potential of enhancing the solubility of API in the formulation and providing proper distribution of the API throughout the composition. Comparing the four formulations, F10A (slope 3539.62) and F10B (slope 6854.44)

had a higher rate of release profile compared to F9A (slope 1436.55) and F9B (slope 1966.08) (Figure 18).

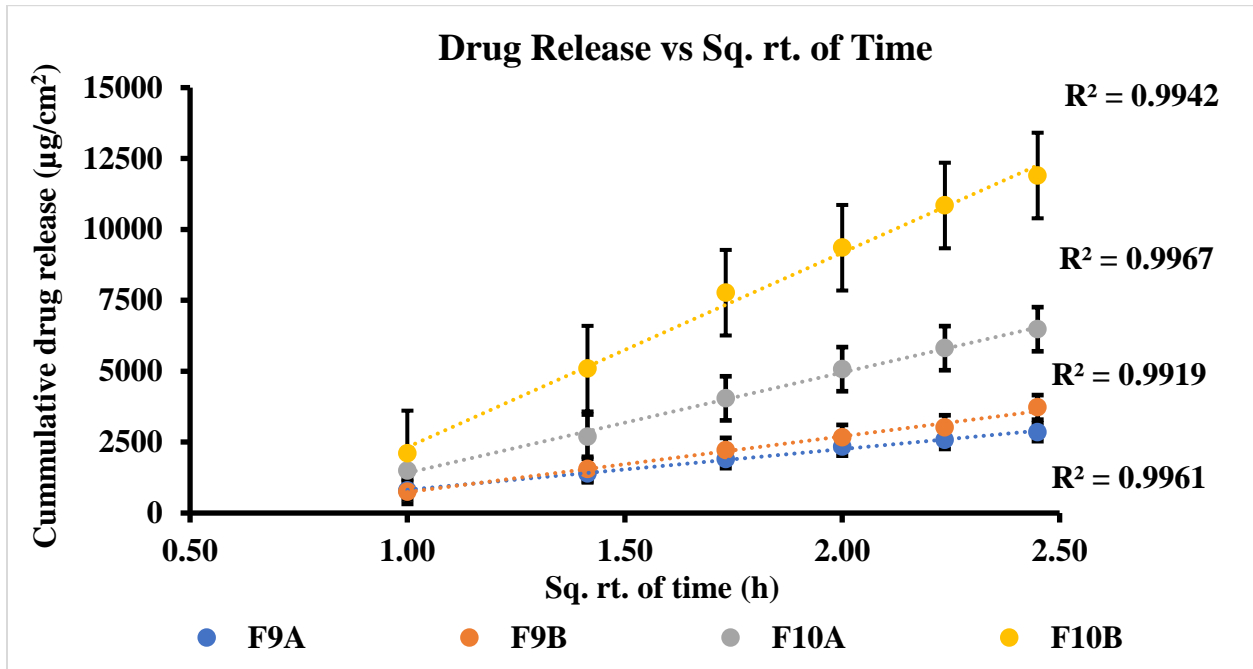


Figure 18. *In-vitro* drug release profile of the formulations.

***In-vitro* Permeation Testing (IVPT)**

After *in-vitro* release test, formulations were subjected to permeation studies. However, for the permeation studies formulations only the formulations which had 3 % voriconazole (F10A and F10B) were selected. Since the water activity of these formulations was low, a low permeation profile was expected. From the literature search it was found that, camphor-menthol (1:1) had previously been used as a permeation enhancer for the formulations having voriconazole [23]. Transcutol P at a low concentration was also explored as a permeation enhancer in the formulations. Transcutol P, is an excellent solvent and powerful solubilizer and penetration enhancer for topical formulations [35]. It is safe to use and has low irritancy inferred by numerous toxicological studies and precedence of use in approved topical medicines [35]. As per the FDA's Inactive Ingredients Database for topical cream formulations, up to 15% w/v of Transcutol P has

been used in FDA approved drug products ^[36]. In the current study only 7.5% w/v of Transcutol P was used as permeation enhancer. For the permeation studies, formulations (F10A2 and F10B2) containing 7.5% w/v camphor-menthol (1:1) were used as positive control, formulations without permeation enhancer was used as negative control, and permeation enhancer activity of formulations (F10A1 and F10B1) containing 7.5% w/v Transcutol P was tested. A finite dose permeation study was carried out ^{[22][23]}.

The finite dose permeation flux versus time profile followed a typical up and down curve in all the formulations. The area under the curve (AUC) of the formulations (F10B1 and F10B2), had five-fold increment compared to the formulations without permeation enhancer; and formulations (F10A1 and F10A2), had three-fold increment compared to the formulations without permeation enhancer. The Jmax values of the formulations shown in Table16. Formulation with permeation enhancer Transcutol P (F10B1) showed the highest flux/ Jmax. Formulation (F10B1) showed nine-fold increment compared to the formulations without permeation enhancer and one-fold increment compared to the formulation with permeation enhancer Camphor-menthol (1:1) (F10A2 and F10B2).

Table 18. Comparison of AUC ($\mu\text{g}/\text{cm}^2$) and Jmax ($\mu\text{g}/\text{cm}^2/\text{h}$).

Samples	AUC ($\mu\text{g}/\text{cm}^2$)	Flux ($\mu\text{g}/\text{cm}^2/\text{h}$)	Tmax (h)
Cream without permeation enhancer (Light mineral oil)	0.699±0.763	0.182±0.098	30±6.0000
Cream without permeation enhancer (Labrafil M 1944 CS)	0.535±0.304	0.115±0.075	22±3.4641
F10A1	1.315±1.038	0.355±0.264	8.67±8.0829
F10B1	2.550±0.789	0.909±0.302	4±0.0000
F10A2	1.562±1.005	0.554±0.393	6.67±4.6188
F10B2	2.552±2.025	0.873±0.751	6.67±4.6188

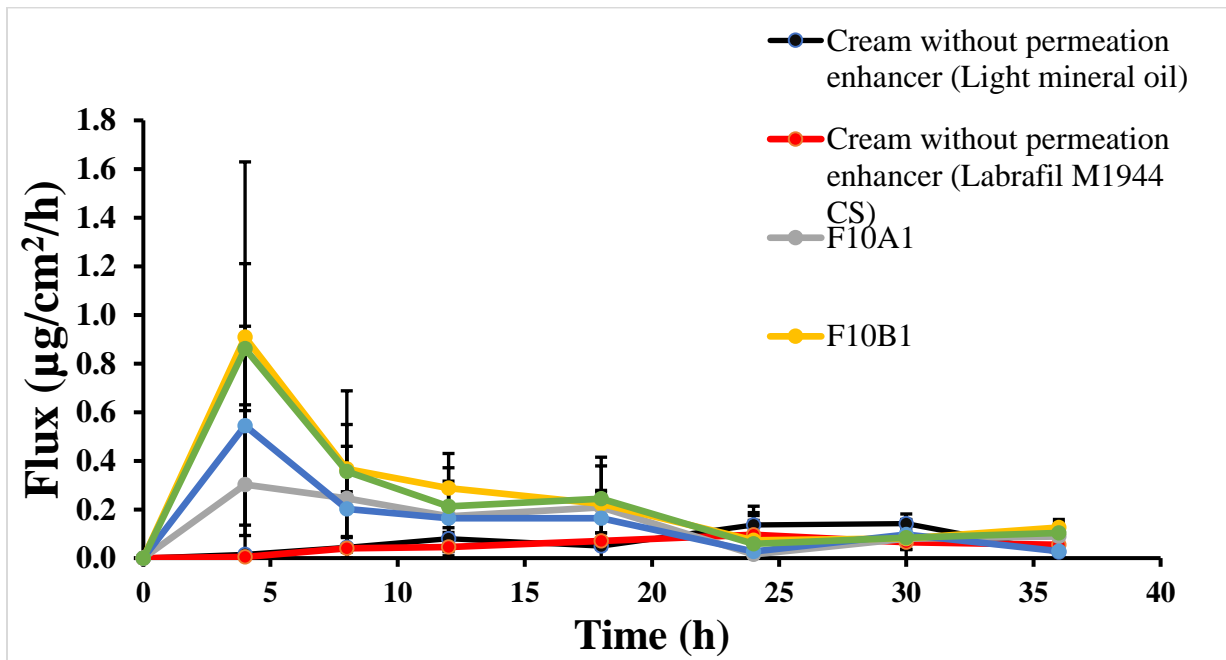


Figure 19. *In-vitro* drug permeation profile of the formulations.

CHAPTER IV: CONCLUSION

Oil/PEG creams of voriconazole were successfully developed in order to overcome the degradations problem of voriconazole in aqueous base formulations (i.e., Oil/Water or Water/Oil). Stable voriconazole topical creams formulations was successfully developed and evaluated. Hot-melt extrusion was used in the manufacturing of voriconazole creams; suggesting the feasibility of using hot melt extrusion as a continuous manufacturing tool for semi-solid formulations. Further, it was found that transcutool P at a lower concentration can be used as permeation enhancers over camphor-menthol (1:1). From the results it was clear that the permeation profile of the formulations with permeation enhancer, transcutool P was much better than the permeation profile of the formulations with permeation enhancer camphor-menthol (1:1).

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VITA

Abhishek Shettar was born in Dharwad, India on 2nd May 1994. He graduated from Government college of Pharmacy with a Bachelor of Pharmacy degree. Upon graduation he worked as a drug safety associate in Quintiles.INC. Bangalore for 2 years. Later he was accepted by the University of Mississippi for the MS in Pharmaceutical sciences program with emphasis on pharmaceutics and drug delivery in Fall 2018-19. Abhishek was elected as graduate school senator for two successive years for the graduate student council (GSC) and is a treasurer for AAPS-UM students' chapter. Abhishek completed the master's program in year and a half, upon graduation he will continue PhD at the University of Mississippi under the guidance of Dr. S. Narasimha Murthy.