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Functionality And Role Of Different Fatty Alcohols In Topical O/W Cream Formulation

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FUNCTIONALITY AND ROLE OF DIFFERENT FATTY ALCOHOLS IN TOPICAL O/W CREAM FORMULATION

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by

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ABSTRACT

Fatty alcohols are excipients that are abundantly used in various pharmaceutical formulations. The objective of the present study was to evaluate the impact of incorporating different fatty alcohols on microstructural and formulation characteristics of topical o/w clotrimazole cream formulation. The formulations containing different fatty alcohols as Kolliwax® CSA 50 (CSA 50), Kolliwax® CSA 70 (CSA 70), Kolliwax® MA (MA), Kolliwax® CA (CA), Kolliwax® SA (SA) were prepared by selecting suitable RPM and homogenization time. The M.P. of API and fatty alcohols were determined using DSC technique. The prepared cream formulations were evaluated for their phase separation. The various tests like pH, water activity measurement, work of adhesion measurement, globule size distribution, *In vitro* permeation study and rheological study were performed for the characterization of the formulation. All formulations were made in two batches and were subjected to stability conditions for 3 month at two conditions, 25°C/60% RH and 40°C/75% RH respectively. The initial and 3 month samples were characterized using tests like, pH, water activity measurement and globule size distribution.

The results for the tests like pH, water activity were found to be comparable in all cream formulations with different fatty alcohols. In phase separation study, the drug was found to have partitioned predominantly in the lipid phase with negligible amount present in the aqueous phase of the cream formulations. The work of adhesion values for all fatty alcohols except SA were found to be in the same range of values. From the results of globule size distribution, it can be

concluded that CSA50, CSA70, MA and CA were found to form stable o/w cream formulation with a globule size range 2 to 6µm. SA when used alone in cream formulation, failed to produce a cream formulation with stable globules. All fatty alcohols containing creams showed yield stress values ~ 10 Pa, except for the SA sample, which displayed a very low yield stress. The results of the rheological studies revealed that CSA 50 and CSA 70 form relatively stable microstructure. As, all products are shear thinning and have comparable yield stress, the rheological characteristics clearly reflect the microstructural similarities in the formulations. The results of finite and infinite IVPT studies revealed that the permeation of clotrimazole from all the o/w topical cream formulations could be enhanced when MA and CA are used in the formulation. The cream products prepared with different fatty alcohols maintained same characteristics at two storage conditions. SA was found to be one of the less efficient fatty alcohol in terms of forming a good o/w cream in the present case.

DEDICATION

I dedicate my thesis to my loving and caring family. I am thankful to my parents, Sudhir and Smita Bhide who gave me constant motivation and unconditional support throughout this Master's program. I am also grateful to my lovely elder sister Swapna for acting as an emotional anchor during this process.

ACKNOWLEDGMENTS

I would like to thank the committee members, who gave their valuable opinions and time to make this thesis more precise and accurate. I am grateful to Dr. S. Narasimha Murthy, my advisor, for his patience and constant motivation to finish this work on time. I also thank Dr. Dr. Michael A. Repka and Dr. Seongbong Jo for being the part of the committee.

I wish to thank my department and other staff for helping me out for doing my research work. I would like to thank all of my colleagues, Dr. Murthy Group, Abhijeet Maurya, Purnendu Sharma, Vijay Kumar Shankar, Anitha Reddy, Srinivas Ajjarapu, Apoorva Panda, Marey A. Almaghrabi, Alia Alshammari and Dr. Srinath Rangappa for their unconditional help and support. I would like to thank Tasnim Fatima for always supporting me and guiding me throughtout the project. I would like thank Gauri Shadambikar, Pranav Ponkshe, Sushrut Marathe and Akshaya Tatke for always staying by my side throughout the project.

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Finally yet importantly, I want to thank my friends Apurva Purohit, Renuka Sarnaik and my caring brother Soham Erande for staying with me in all situations of life.

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CHAPTER I

INTRODUCTION

Pharmaceutical cream formulations are heterogeneous semisolid systems in which drug is either dissolved or dispersed in water in oil (w/o) or oil in water (o/w) emulsion. They are widely used in topical application of the drugs[1]. An o/w cream besides being non-occlusive in nature also has emollient properties as it can lead to deposition of lipids and moisturizers on and into the stratum corneum[2].

The basic ingredients used in cream formulations are emollients, viscogens, emulsifying agents, vehicles, penetration enhancers. It is extremely important to select appropriate combination of these ingredients to formulate a stable topical cream formulation. Fatty alcohols are excipients that are abundantly used in various pharmaceutical formulations. Chemically, fatty alcohols are aliphatic hydrocarbons with a hydroxyl group at the terminal position and are derived from either plants or animal oils and fats.

Inactive Ingredient database of FDA has 44 listings of approved NDA topical formulations with different fatty alcohols like myristyl alcohol, cetyl alcohol, stearyl alcohol or cetostearyl alcohol[3]. There are also approved generic topical semi-solid products that contain different fatty alcohols which can be identified at US National institute of Health Daily Med database[4]

Fatty alcohols play multiple roles leading to development of topical products of desired efficacy, safety and stability. Fatty alcohols are used in creams to impart the desired sensorial and functional characteristics to the formulation. They can influence texture characteristics and improve the rheological properties of topical products. They act as solubility enhancers for some of the poorly soluble APIs. They can also play the role of permeation enhancer by improving the drug penetration into as well as across the skin.

Fatty alcohols were found to influence the rate of metamorphosis of the cream product after application on the skin. The rate of drying and metamorphosis of the product on the skin surface determines the extent of dermal absorption of therapeutic agents. The fatty alcohols also found to influence the spreadability, spreading area, skin cooling and work of adhesion properties of the topical products. Incorporation of fatty alcohols as a part of any pharmaceutical or cosmetic cream formulation helps the formulator to impart the desired characteristics to the product by the way of changing the fatty alcohol type and ratio. The type of vehicle used in the topical formulation can also play an important role in permeation of drug. The solubility of drug in different components of formulation and in stratum corneum can influence partitioning of drug from the dosage form[5,6].

Clotrimazole is an azole derivative having a broad-spectrum antifungal activity. The drug is known to act by binding to cytochrome P 450 enzymes, which are required for demethylation of lanosterol to ergosterol. Ergosterol concentrations get reduced leading to damaged and leakage in the cell membranes leading to the prevention of fungal growth[7]. The chemical structure of clotrimazole is as shown in Figure 1[8].

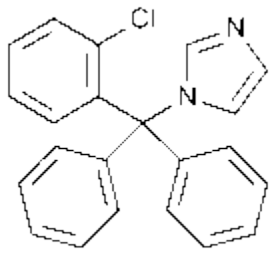


Figure 1: Chemical structure of clotrimazole

It is used to treat skin infections like athlete's foot, jock itch, ringworm. It also can be used to treat a skin condition called as pityriasis and for fungal infection that causes a lightening or darkening of the skin in the area of the neck, chest, arms or legs[9].

Clotrimazole is commonly formulated in the form of topical cream and most of these formulations are available over the counter. Few examples of FDA approved products containing clotrimazole as an active ingredient are described in Table 1.

Table 1: FDA approved products containing clotrimazole

Product Name	Strength	Dosage form/route	Company
MYCELEX[(10)]	1%	Solution; topical	Bayer Healthcare
MYCELEX-7[(11)]	1%	Cream; vaginal	Bayer Healthcare LLC
CLOTRIMAZOLE[(12)]	1%	Cream; vaginal	Taro

There is a need of studying the influence of commercially available pharmacopoeial grade fatty alcohols on a drug-containing cream modeled after commercially available clotrimazole formulations as a majority of the studies reported have been based on simple model systems with water, emulsifier and fatty alcohols.

Hence, the present study focused on evaluating influence of fatty alcohols on the characteristics and performance of the topical o/w cream formulation containing clotrimazole.

CHAPTER II

OBJECTIVE OF THE STUDY

The objective of the present study was to evaluate the influence of fatty alcohols on formulation characteristics and dermal delivery of clotrimazole. Clotrimazole containing topical O/W cream formulations with different fatty alcohols were prepared and characterized.

Research Strategy: The formulations containing different fatty alcohols such as Kolliwax®CSA 50(CSA 50), Kolliwax® CSA 70 (CSA 70), Kolliwax®MA (MA), Kolliwax® CA(CA) , Kolliwax® SA (SA) were prepared at set homogenization protocol. The API and fatty alcohols were characterized by determination of M.P. by DSC. The various tests like pH, water activity measurement, work of adhesion measurement, globule size distribution and rheological study were performed for the characterization of the formulation.

In vitro permeation studies at finite and infinite dose conditions were performed across porcine epidermis model using Franz diffusion cells. All formulations were made in two batches and were subjected to stability conditions for 3 months. Formulations were kept at two conditions, 25°C/60% RH and 40°C/75% RH respectively. The initial and 3 month samples were characterized for pH, water activity measurement and globule size distribution.

CHAPTER III

MATERIALS AND METHODS

Materials

Clotrimazole (≥ 98%) was gifted by YRROW CHEM PRODUCTS, Mumbai. Kolliwax®CSA 50 (cetostearyl alcohol 50), Kolliwax® CSA 70 (cetostearyl alcohol 70), Kolliwax®MA (myristyl alcohol), Kolliwax® CA (cetyl alcohol), Kolliwax® SA (stearyl alcohol), Kollisolv™ PG (propylene glycol), Kollicream™ OD (octyldodecanol) and Kolliphor™ CS 20 (macrogol cetostearyl ether 20) were gifted by BASF. Euxyl 320 and Nile Red were purchased from Sigma Aldrich. High Performance Liquid Chromatography (HPLC)-grade solvents like methanol, ethanol were purchased from Fisher Chemicals, USA. Porcine skin was obtained from Pontotoc Slaughterhouse, Pontotoc, MS, USA. The wide mouth containers (10 oz.) were purchased from U Line suppliers.

Methods

Table 2: Preparation of clotrimazole containing o/w formulations

Ingredient	Description	Purpose	w/w%
Kollisolv™ PG	Propylene glycol	Solvent	8
Fatty alcohol#	Fatty alcohol	Viscogen	9
Kollicream™ OD	Octyldodecanol	Emollient	6
Kolliphor™ CS 20	Macrogol cetostearyl ether 20	Emulsifier	1
Active Ingredient	Clotrimazole (clz)	API	1
Water	-	Solvent	75
Euxyl 320	Phenoxyethanol	Preservative	q.s.
Nile Red		Dye	0.0005

Table 3: The list of fatty alcohols with their brand names

Fatty alcohol[#]	
Brand Name	**Chemical Name**
Kolliwax® CSA 50	Cetostearyl alcohol 50
Kolliwax® CSA 70	Cetostearyl alcohol 70
Kolliwax® MA	Myristyl alcohol
Kolliwax® CA	Cetyl alcohol
Kolliwax® SA	Stearyl alcohol

The formula for the cream preparation was used as mentioned in Table 2 and fatty alcohols used in the study were as shown in Table 3. Aqueous and lipid phase were heated separately at 60°C. Lipid phase was added slowly with the constant speed to the aqueous phase. Both the phases were homogenized for 30 minutes at 3000 RPM in Silverson Homogenizer L5M-A (Silverson Machines INC, USA). The constant temperature was maintained during the transfer and homogenization of both the phases. Propylene glycol along with the Clotrimazole API was added at the end of the 30 minutes followed by the cooling program. Cooling program- 60°C to 25°C in 20 minutes was given using VWR cooling/heating probe and throughout the cooling, speed of 3000 RPM in Silverson Homogenizer L5M-A was maintained. These batches were made in the quantity of 300 gm. The brief steps involved in the preparation of topical O/W cream were as shown in Figure 2.

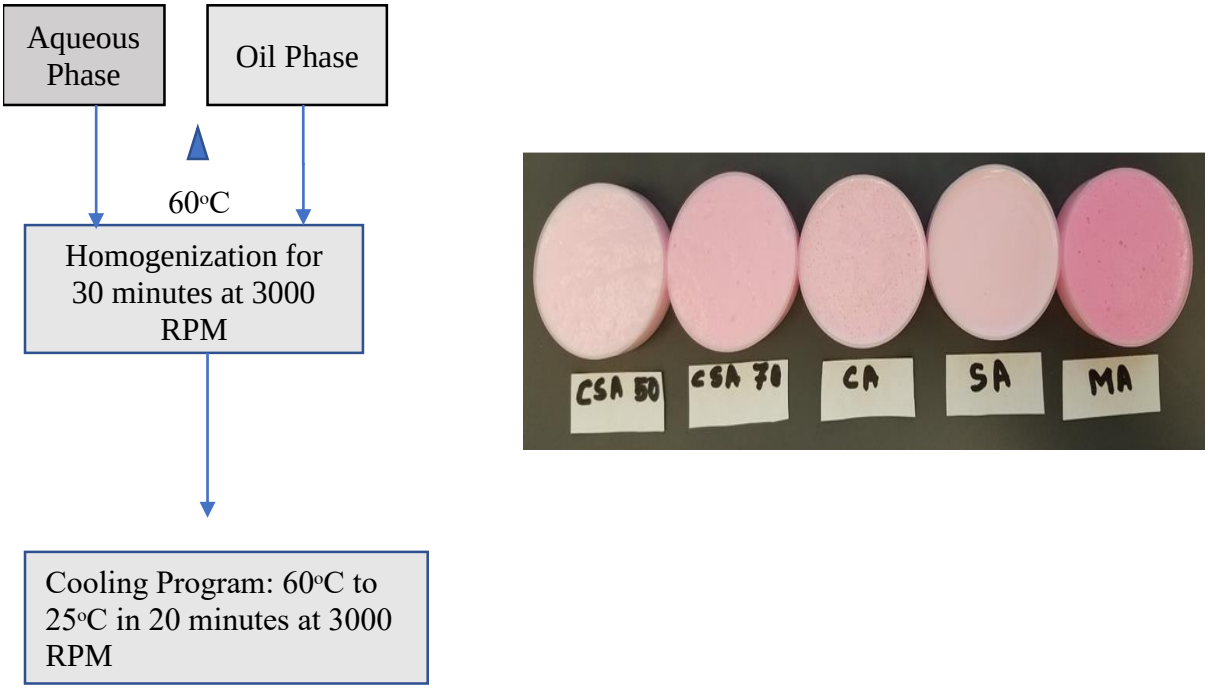


Figure 2: Steps in preparation of topical o/w cream

HPLC Analysis

An isocratic HPLC method was developed for the quantification of clotrimazole[3]. The experiment was performed 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 µl, and a 2487 dual absorbance UV detector. The 10mmM solution of dibasic potassium phosphate was made and was used with acetonitrile. The mobile phase had acetonitrile and buffer (3:1 v/v) and was delivered at a flow rate of 1 ml/min. Then, 20 µl of the injection was eluted in column. The calibration curve was prepared using different concentrations of API in the range of 1µg to 10ng and methanol: water (90:10 v/v) was used as a solvent. LOD, LOQ were determined.

Measurement of M.P.using DSC

The M.P. of all five fatty alcohols were determined using DSC 25 (TA Instruments Delaver, USA) technique. The M.P. of the API was also determined. Around 7 mg of powdered sample was placed in Tzero Hermetic Pan and was sealed with Tzero lid. The pan was placed in the sample chamber along with the reference pan and analysis was done by using Ramp method with the rate of 10^{o}C/min.

pH measurement

The pH of the creams was measured by using Mettler Toledo Inc. USA pH meter. The probes used in the study are METTLER TOLEDO InLab®Science Pro, 0….12.0, 0….100^{o} C and METTLER TOLEDO InLab®Micro, 0….12.0, 0….100^{o}C. 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 thrice. In between every individual measurement, the pH using buffer standard of pH 4.0 and using buffer standard of pH 10.0 after the next measurement was noted.

Phase separation study

The study was performed by weighing 1 gm of each sample in eppendorf. The samples were subjected to centrifugation at 50,000 RPM in Beckman Coulter centrifugation instrument (Beckman Coulter Inc USA). All formulations were centrifuged for 8 hrs. The separated oil phase and aqueous phase were weighed separately. The 2mg of oil phase was diluted to 50 ml with methanol and aqueous phase was diluted by adding 2 ml of methanol. The samples were subjected to HPLC analysis. The distribution of drug in both the phases was evaluated.

Water Activity measurement

The water activity of all cream formulations was measured by using AQUALAB water activity meter series 3 and 3TE (Series 3E, USA) as shown in Figure 3. All measurements were performed at 32° C. The instrument was calibrated using DI water and the water activity of each formulation was recorded. The DI water was taken in sampling cup and kept in the sampling chamber of the instrument. The values were noted thrice and the instrument was found to be suitable if the water activity for DI water comes equal to 1.00. The samples were analyzed in triplicate and the average of the values was reported.

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

Work of Adhesion

The test was performed using TA-XT2i Texture Analyser (Texture Technologies Corp) as shown in Figure 4. TA3 1" dia cylinder,w.radius,acrylic probe as shown in Figure 5. was used for the measurement of work of adhesion of the samples[10,11].

Figure 4: Texture Analyzer (TAXT2i, Texture Tech. Corp., Scarsdale, NY)

Globule size measurement

The globule size of the cream formulation was measured by using Axio Cam HR3 (Carl Zeiss Microscopy, Germany) bright field microscopy at the magnification scale of 50 x 2.5. The thin smear of the cream was prepared with the help of coverslip on the glass slide and observed immediately under the microscope. For each cream, the mean diameter of randomly distributed 100 globules was measured using the Zen Lite software. The globule size measurement was done by calculating d10, d50 and d90 values for each cream.

Rheological Study

The rheology of all samples were studied using TA Instruments HR2 Rheometer (TA Instruments, New Castle, DE) at 32 °C with 20 mm parallel plate with 600 grit sandpaper and solvent trap.

In vitro Drug Permeation Study

A fresh porcine skin was brought from a local slaughterhouse. The abdominal skin regions were taken and shaved using an electric shaver. The hairless skin was cut into small rectangular pieces. Then, the pieces were covered with aluminium foil and immersed in a water bath maintained at 60 ºC for 2 min. After 2 min, the aluminium foil was removed and the epidermis was carefully mounted by hand on clean glass slides. Then, the epidermis was kept for 12 h until it was completely dried and then was stored in a refrigerator at 5 ºC for future use. The *in vitro* skin permeation study was performed using Franz diffusion cells with an effective diffusion area of 0.64 cm2. A hairless porcine epidermis was sandwiched between the donor and receiver medium with the SC side facing the donor compartment. The receiver compartment (5ml)/were filled with an ethanol/PBS solution (30:70) as a reservoir solution[12] and was maintained at 37 °C.

The dose for infinite dose study was 300 mg/cm^2, and that for finite dose study was 10 mg /cm^2 respectively. The infinite dose study was carried out as the dose is several fold higher than the clinically relevant dose and at such high dose, the influence of drying and metamorphosis of the product would be negligible. The steady state flux is a good parameter to understand the formulation and API related properties in relevance to drug delivery. The finite dose studies would be close to clinically relevant dose and it would reveal the way the formulation would behave and perform in real life situation.

During the IVPT studies, the entire receiver compartment was fluid was withdrawn at the time points of 0,2,4,8 and 12h and replaced by equal quantities of fresh medium. The amount of clotrimazole in these samples was quantified and determined using HPLC. The cumulative amount of drug permeated at different time points was plotted and steady state flux was calculated from

the slope of the line in infinite dose study. Whereas in finite dose study, the midpoint average flux was calculated and plotted to determine the maximum flux (J_{max}) and AUC_{0-t}

The porcine epidermis was selected as the stratum corneum has been reported to be closely resemble in terms of structure and composition with human stratum corneum[13]. The porcine skin is found to have a comparable drug permeability with human skin[14]. Porcine skin has shown histological as well as biochemical similarities with human skin[14,15,16,17].

Stability Studies

The quantity of 20 gm from each drug-containing batch was kept for stability studies. The samples were kept at conditions of 25°C/60% RH and 40°C/75% RH for 3 months in stability chambers. The samples were withdrawn and analysed for initial and 3 months.

Following tests were performed at each time interval,

1. Microscopy i.e. globule size measurement
2. pH determination
3. Water activity measurement

CHAPTER IV

RESULTS AND DISCUSSION

HPLC analysis

Table 4: HPLC results for different clotrimazole standard preparations

Concentration (µg/ml)	Area
1	472432
0.75	278827
0.5	255131
0.25	133342
0.1	56464
0.05	25702
0.025	18783
0.01	11072

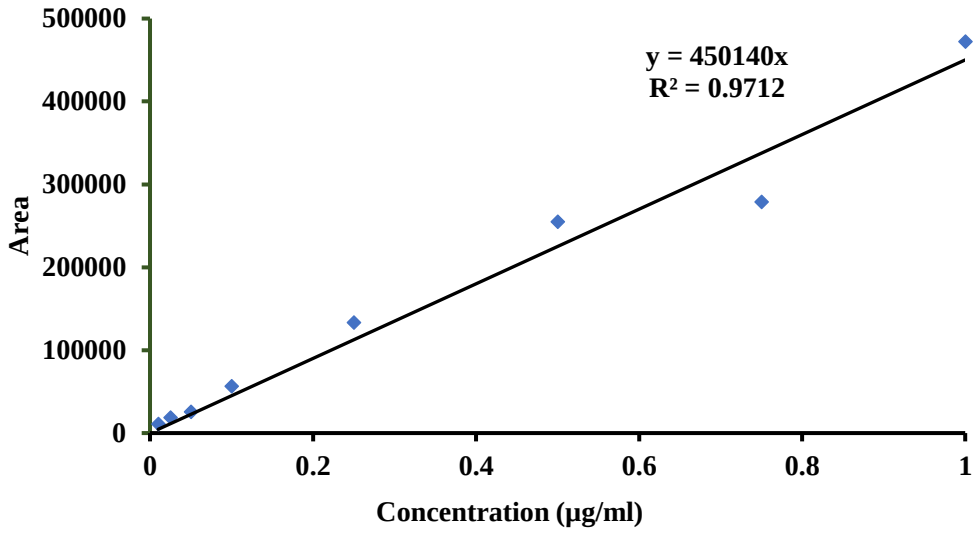


Figure 5: Clotrimazole standard calibration curve

From Table 4**,** the LOQ was found to be at 10 ng. The concentration lower than 10 ng was detectable but was not being able to get quantified by the HPLC method. As shown in Figure 5, the calibration curve was plotted and the value of R^2 was found to be 0.97 which indicated that regression line correctly fits with the data.

Physicochemical properties of fatty alcohols

M.P. for different fatty alcohols were found to be as shown in Figure 6. Different physicochemical properties of fatty alcohols were as shown in Table 4.

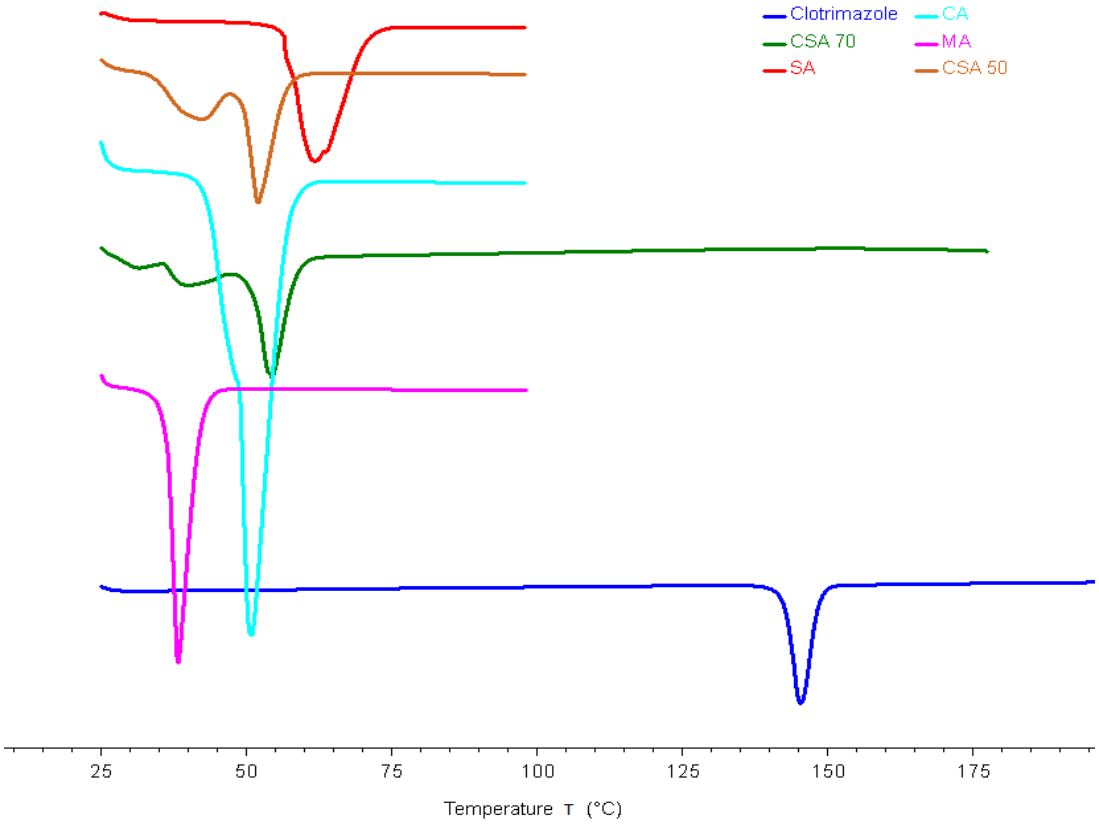


Figure 6: M.P. determination of fatty alcohols using DSC technique

Table 5: Physicochemical properties of fatty alcohols

	CSA 50	CSA 70	CA	SA	MA
M.P	51.97°C	54.3°C	49.3°C	59.5°C	38°C
C-chain length [21-24]	16-18	16-18	C16	C18	C14
Avg. Mol. Wt.[21-24]	512.94 g/mol	512.94 g/mol	242.43 g/mol	270.48 g/mol	214.39 g/mol
log P [22-24]	7.53	7.25	6.83	8.22	6.03

Measurement of pH of topical o/w cream formulations

Table 6: pH values for topical o/w cream formulation

Name	pH
CSA 50	5.75 ± 0.05
CSA 70	5.89 ± 0.02
CA	5.79 ± 0.02
SA	5.79 ± 0.01
MA	5.79 ± 0.01

Clotrimazole is weakly basic in nature[18]. As per the pH/partition theory, the unionized drug has more ability to permeate compared to the ionized form of the drug. We have earlier found that the pH of the topical products could change post application on the skin to homeostasis with the pH of the skin, pH 5. As in this case, the initial pH of the formulation is close to 5, there is less concern about any change in the ionized/unionized drug ratio due to meager changes in the pH of the formulation.

Phase distribution study

Table 7: Phase separation study for topical o/w cream formulation

		Clotrimazole in each phase(µg)	Clotrimazole in each phase/gm of sample(mg)	Total amount of drug (mg)
CSA 50	Oil Layer	8577.60	8.58	9.19
	Intermediate waxy Layer	599.93	0.60	
	Aqueous Layer	8.71	0.01	
CSA 70	waxy Oil Layer	9040.232	9.04	9.27
	Intermediate waxy Layer	22.09	0.22	
	Aqueous Layer	3.14	0.00	
CA	Oil Layer	10852.86	10.85	11.21
	Intermediate waxy Layer	348.04	0.35	
	Aqueous Layer	4.50	0.00	
SA	Oil Layer	10775.13	10.78	10.98
	Intermediate waxy Layer	204.60	0.20	
	Aqueous Layer	1.29	0.00	
MA	Oil Layer	11246.40	11.25	11.35
	Intermediate waxy Layer	102.33	0.10	
	Aqueous Layer	0.70	0.00	

In case of all the creams, the drug was found to have partitioned predominantly in the lipid phase with negligible amount present in the aqueous phase as seen in Table 7.

Water Activity measurement

Table 8: Water activity measurement of clotrimazole containing o/w creams with different fatty alcohol

Name	Water Activity (a_w)
CSA 50	0.97 ± 0.00
CSA 70	0.97 ± 0.00
CA	0.96 ± 0.00
SA	0.97 ± 0.00
MA	0.99 ± 0.01

Water activity values give idea about the microbial stability of the formulation[19]. Water activity is simply defined as the ratio of the vapor pressure of pure water (100% equilibrium relative humidity) over the vapor pressure of the sample. Water activity can have influence on the drug permeation from the formulation as it was found that water activity gradient between outer layers of skin and formulation vehicle can change the amount of water loss from skin[19]. Being o/w cream in nature, the values were found to be higher as shown in Table 8. The water activity values were found comparable for all fatty alcohols.

Work of adhesion

Table 9: Work of adhesion measurement of Clotrimazole containing o/w creams with different fatty alcohols

Name	Work of Adhesion (g.sec)
CSA 50	27.48 ± 0.68
CSA 70	20.50 ± 0.04
CA	26.70 ± 0.69
SA	17.04 ± 0.37
MA	25.75 ± 0.62

As shown in Table 9, work of adhesion values for all fatty alcohols except SA were found to be in the same range of values. Work of adhesion could be the sensitive tool for determination of parameters like spreadability of the cream formulation.

The Young- Dupre equation, gives an idea about the surface tensions of liquid, soild and surface tension at the interface of solid-liquid along with the contact angle between them[20],

$$W_{ab} = \gamma 1(1+ \cos\theta)$$

W_{ab} = work of adhesion between two phases, γ = surface tension and $\cos\theta$ = contact angle

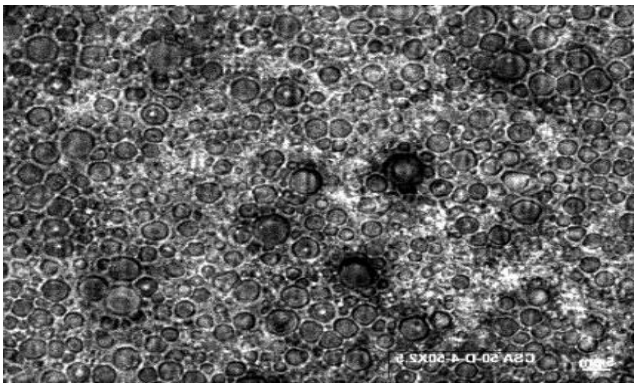
The work of adhesion is an interfacial phenomenon as it helps in determining force required to separate solid surface from the liquid surface.

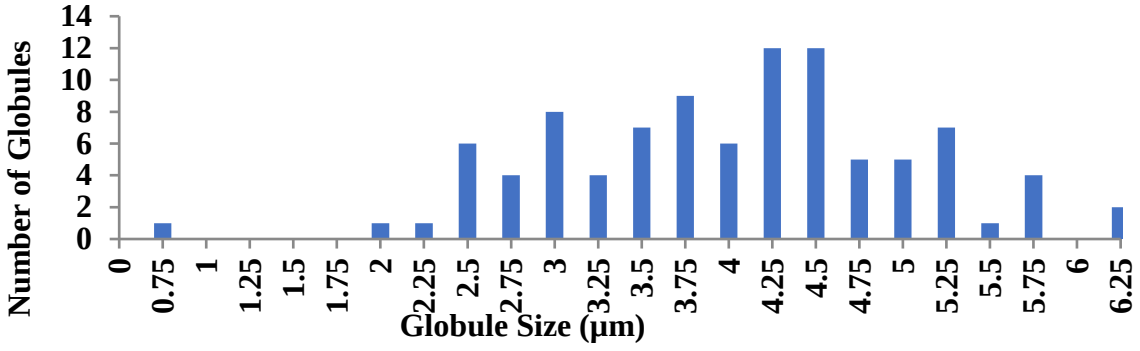
From the results mentioned in Table 9, the SA has low work adhesion indicating the more spreadability of the formulation.

Globule size measurement

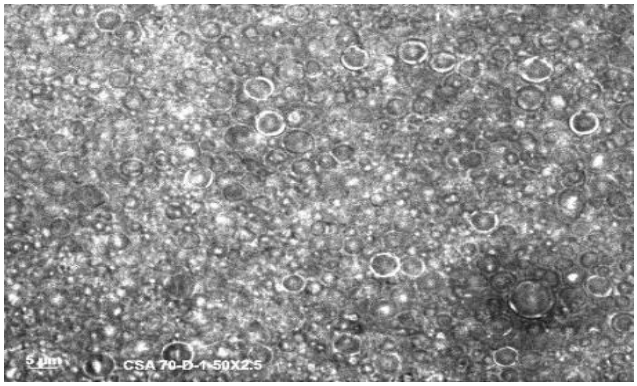
Table 10: Globule size measurement of clotrimazole containing o/w creams with different fatty alcoholsITIAL

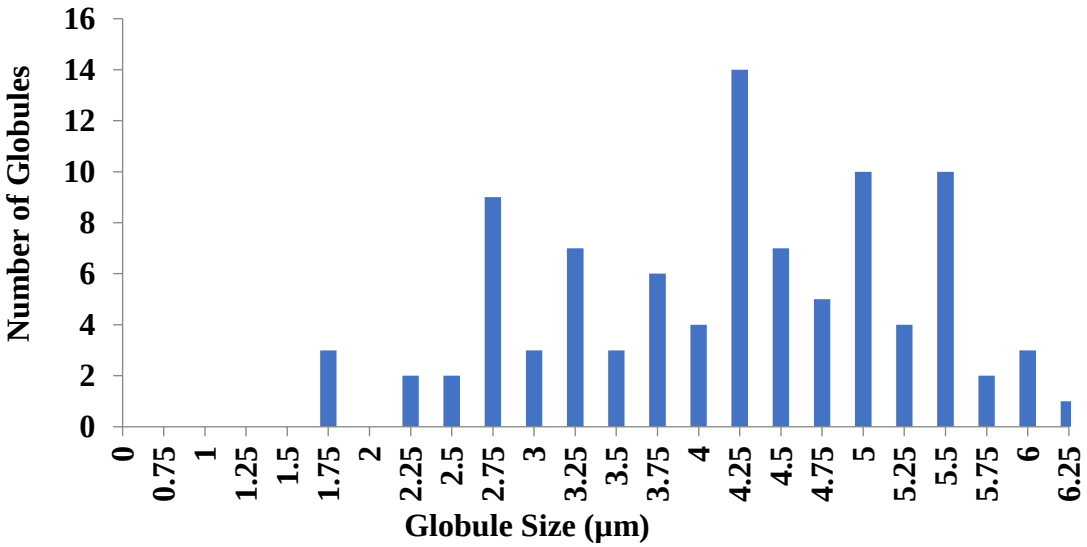
Name	d_{10} (µm)	d_{50} (µm)	d_{90} (µm)
CSA 50	2.61 ± 0.00	4.05 ± 0.07	5.45 ± 0.21
CSA 70	2.50 ± 0.14	3.90 ± 0.42	5.20 ± 0.57
CA	2.55 ± 0.07	3.75 ± 0.35	4.90 ± 0.71
SA	N.A.	N.A.	N.A.
MA	2.05 ± 0.07	3.05 ± 0.07	3.80 ± 0.10



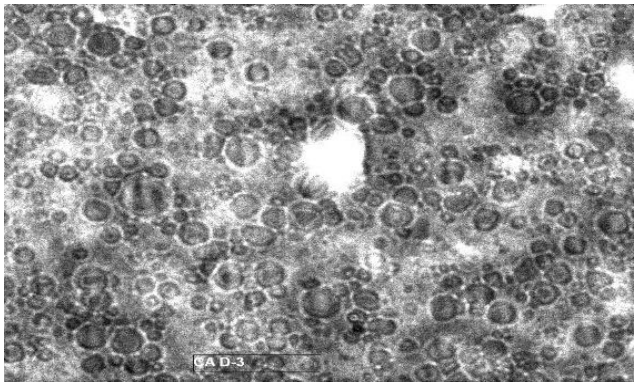


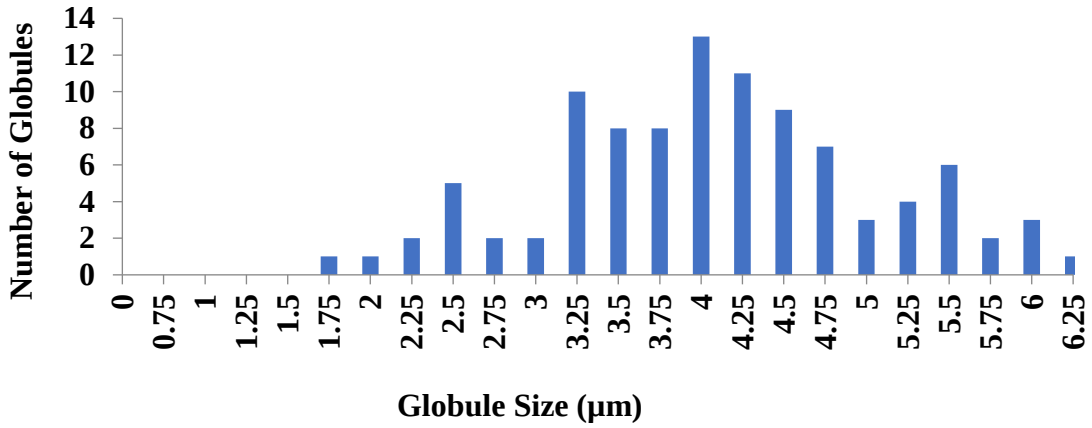
7 (a). CSA 50



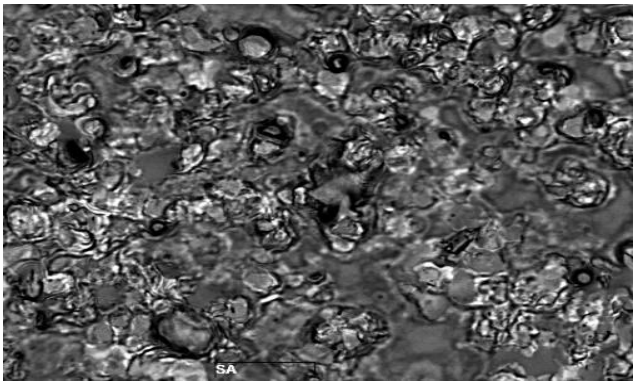


7 (b). CSA 70

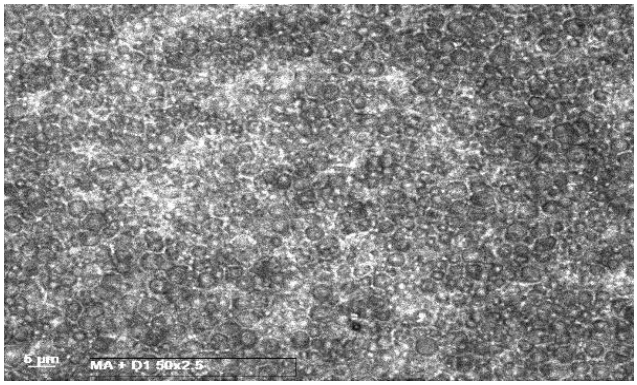


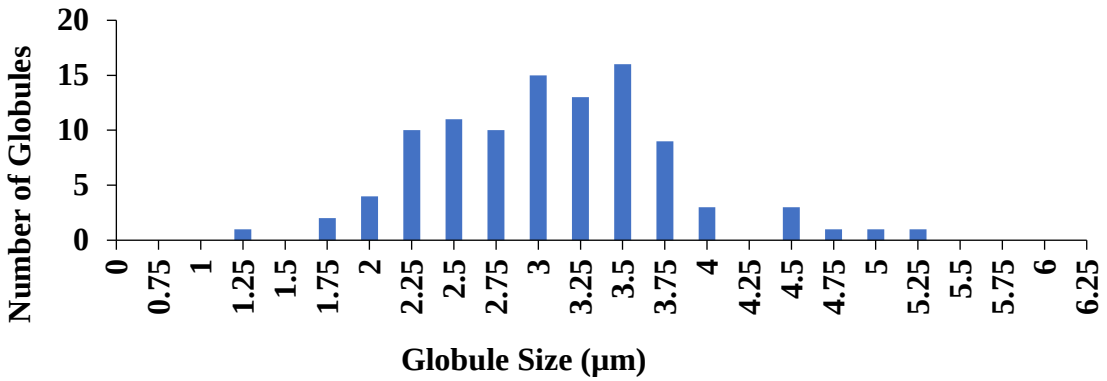


7 (c).CA



7 (d). SA





7(e). MA

Figure 7: Photomicrographs and globule size distribution for clotrimazole creams containing different fatty alcohols

As shown in Table 10 and Figure 7, the globule size distribution of creams containing CSA 50 was found to have d10, d50 and d90 as 2.6 ± 0.01 , 4.0 ± 0.06 and 5.4 ± 0.23µm respectively. In case of CSA 70, CA and MA the globule size values did not differ significantly from that of

CSA 50. However in cream containing SA, emulsification was not complete and globules were found to be irregular and larger in size.

From the results of globule size distribution, it can be concluded that CSA50, CSA70, MA and CA were found to form stable o/w cream formulation with a globule size range 2 to 6 µm. SA when used alone in cream formulation, failed to produce a cream formulation with stable globules.

Rheological study

In semisolid emulsions, surfactants and fatty alcohol contribute towards formation of viscoelastic gel network which helps in stabilizing the oil droplets into the water phase. Type of surfactant whether ionic or nonionic along with its homologous composition affect the structure of the gel network. This phenomenon results in giving particular appearance and rheological stability to the formulation[21,22].The surfactant emulsifiers reduce the surface tension which helps in easy breakdown of droplets during the emulsification process. This process facilitates formation of viscoelastic gel network. Higher mixed emulsifier concentrations lead to linkage of crystalline and gel phases forming a semisolid cream[23]. The mixtures which form lamellar phases in the bulk can form strong viscoelastic films at the interface[23,24]. Fatty alcohols have tendency of swelling in the presence of nonionic surfactant. Surfactant gets interpositioned between the alcohol molecules and alpha crystals of alcohol resulting in swelling because of the hydration POE chains of the surfactant[20,25,21].

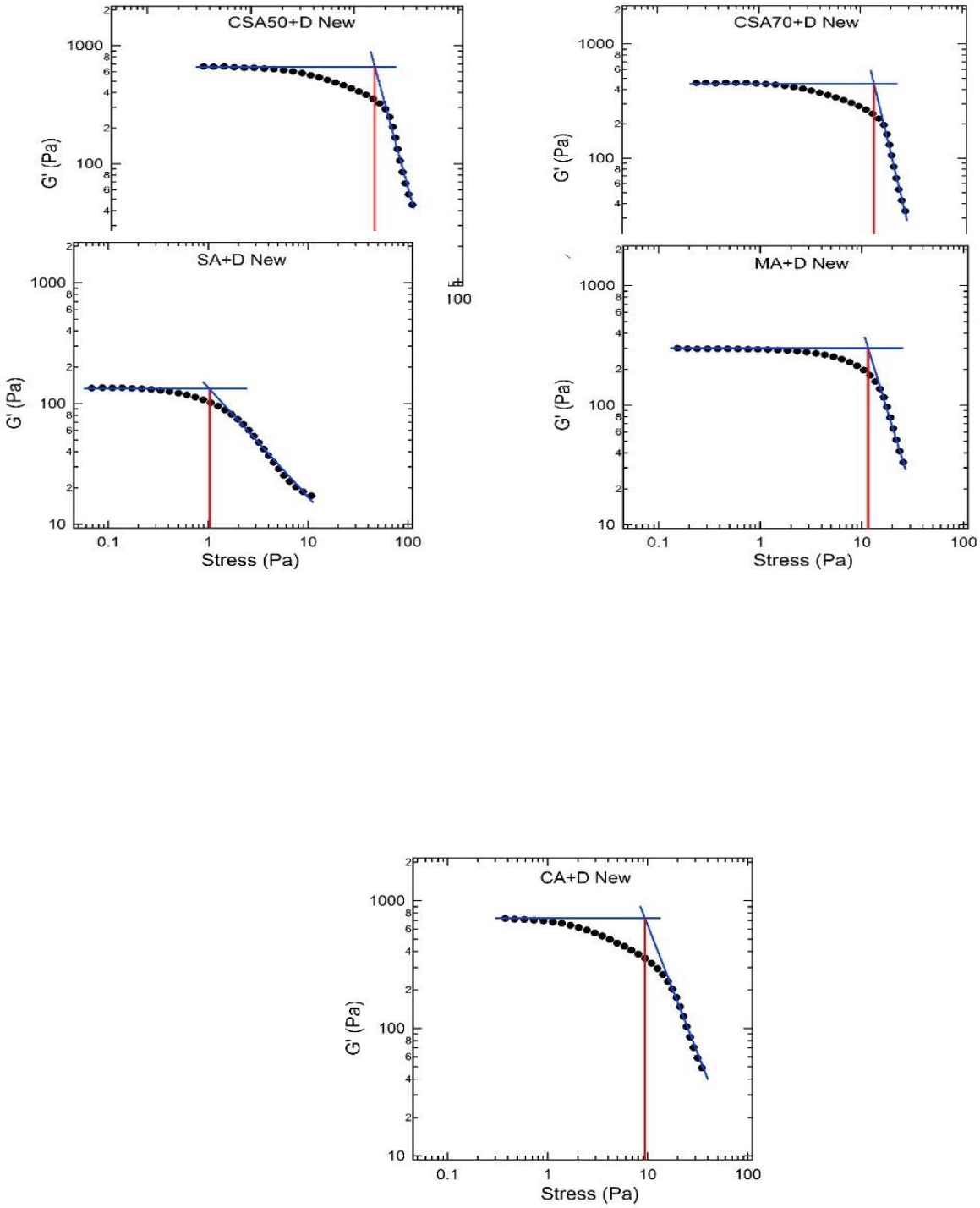


Figure 8: Yield Stress study for clotrimazole creams containing different fatty alcohol

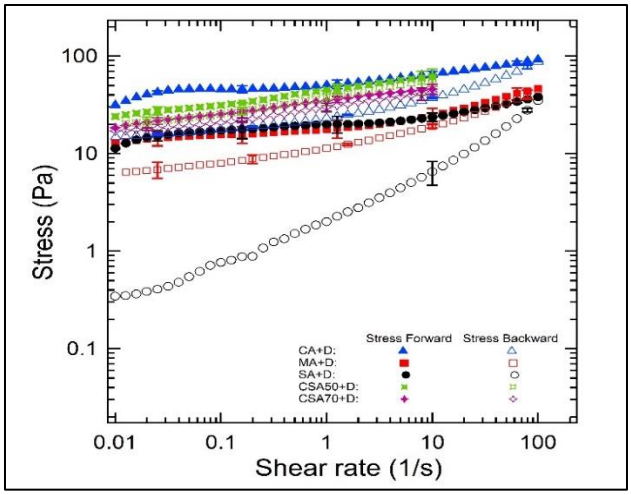


Figure 9: Stress vs shear rate study for clotrimazole containing o/w creams with different fatty alcohols

Table 11: Viscosities at different shear rates of clotrimazole containing o/w creams with different fatty alcohols

	0.01 (1/s)	**At the shear rate: 20 s^{-1}**	**Yield Stress (Pa)**
CSA50	2397	N/A	16
CSA70	1838	N/A	13
CA	3137	3.6	9
SA	1132	1.3	1
MA	1276	1.5	12

Previous studies have reported reduction in consistency of formulations containing cetyl alcohol and stearyl alcohol during the storage period[21]. The yield stress values as shown in Figure 8 and Table 11. All fatty alcohols containing creams showed yield stress values ~10 Pa, except for the SA sample, which displayed a very low yield stress. When stress vs shear rate was plotted, there was increasing shear rate (forward) with decrease in viscocity for all samples, indicating that the samples were shear thinning as seen in Figure 9. The hysteresis has been found to be highest in SA sample. CSA 50 and CSA 70 samples did not display any significant hysteresis indicating that their ability to impart robustness to the products. Shear thinning behaviour of cream formulation gives an idea about the spreadability of the formulation and it is an indicative of stability of the formulation[26].

The results of the rheological studies revealed that CSA 50 and CSA 70 form relatively stable microstructure. This could be attributed to the fact that cetostearyl alcohol can stabilize the emulsion due to lamellar gel network. Also, cetostearyl alcohol provides stability to the formulation due to chain-length mismatch which is not found in the pure cetyl alcohol nor stearyl alcohol. Chain-length mismatch also reduces the melting enthalpy in comparison to pure fatty alcohols[27]. As, all products were found to be shear thinning and have comparable yield stress, the rheological characteristics clearly reflect the microstructural similarities in the formulations.

***In vitro* Drug permeation study using infinite dose study for o/w cream formulations**

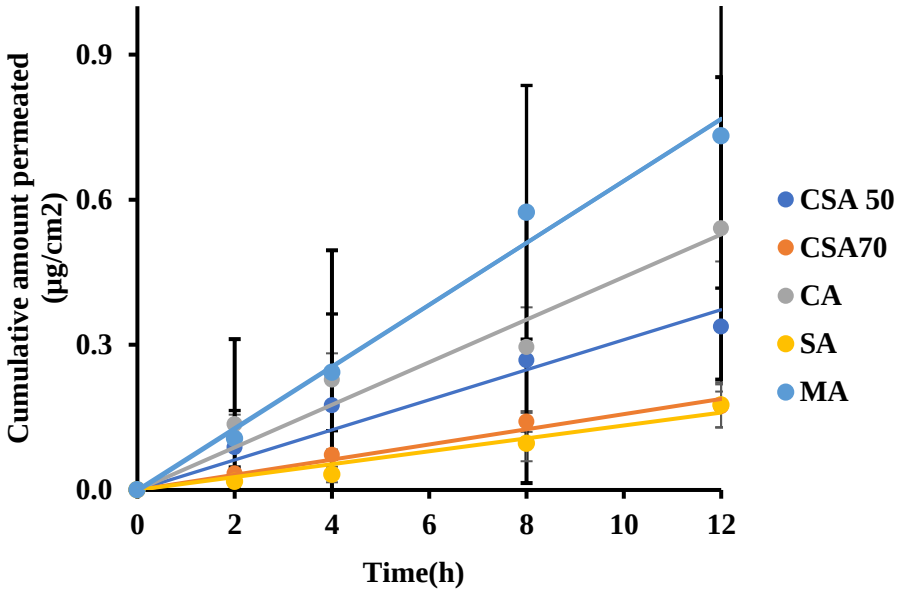


Figure 10: IVPT profile of clotrimazole at Infinite Dose from clotrimazole containing O/W cream

[n=2 donors (3-6 replicate each donor ±SEM)]

Table 12: Clotrimazole O/W cream infinite dose IVPT study Steady state flux values and physicochemical properties of fatty alcohols

Fatty alcohol	Steady state flux ($\mu g/cm^2/h$)	M.P.(^oC)
CSA 50	0.028 ± 0.011	51.97
CSA 70	0.015 ± 0.004	54.3
CA	0.041 ± 0.021	49.3
SA	0.015 ± 0.004	59.5
MA	0.064 ± 0.028	38

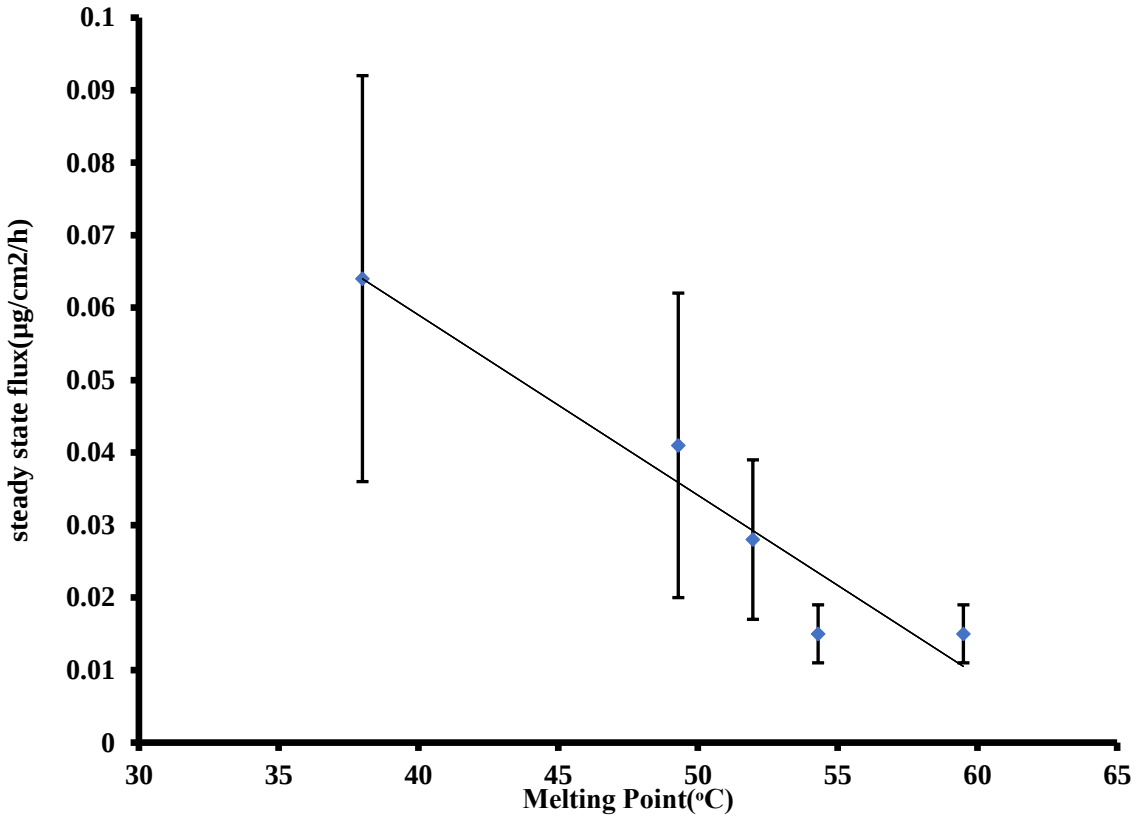


Figure 11: The relationship between steady state flux and M.P. of each fatty alcohol

Fatty alcohols have shown an ability to act as effective permeation enhancers of drugs in many formulations[14]. The steady state flux of drug from o/w creams containing different fatty alcohols was determined from the cumulative release versus flux data as shown in Table 12 and Figure 10. The results clearly show that the type of fatty alcohol incorporated has a significant impact on the drug permeation from o/w creams. Creams incorporated with MA and CA resulted in higher permeation of clotrimazole than those prepared with CSA50.

In the present work, it was presumed that the drug permeation could depend on the ability of fatty alcohols to interact with stratum corneum lipids by disrupting the densely packed lipids in the extracellular spaces of the stratum corneum[14].

In 1990, Flynn proposed that physicochemical properties of chemicals can influence their permeation through skin[28]. Potts and Guy established quantitative correlation between physicochemical properties such as molecular weight, log P of molecule and flux across the skin[29].

$$\log k_p = - 2.7 + 0.71 * \log P - 0.0061 * MW$$

k_p= permeability coefficient, log P = partition coefficient, MW = molecular weight

Barratt[30] extended the Potts and Guy equation by using melting point and molecular volume to predict the permeability of the molecules. He performed multiple regression analysis of log PC against log P(partition coefficient),molecular volume and melting point.

$$\log PC = - 0.00484 * MV + 0.71 * \log P - 0.00515 * MP - 2.655$$

PC= permeability coefficient, log P = partition coefficient, MV= molecular volume,

MP =melting point

Hence, the same concept was used to establish the correlation between the steady state flux and melting point of fatty alcohols in the present study. There was an inverse correlation as shown in Figure 11, observed between the steady state flux of drug (which in turn shows the extent of modulation of skin permeability by the fatty alcohol) with melting point of the fatty alcohol incorporated in the cream product. This explains the reason why the permeation of clotrimazole in cream with MA, was found to have more drug permeation than creams having fatty alcohols with higher melting point.

***In vitro* Drug permeation study using finite dose study for o/w cream formulations**

The finite dose permeation flux versus time profile followed a typical up and down curve in all the cases as shown in Figure 12. The J (max) values from CSA 50 and 70 were comparable and was lesser than other fatty alcohols used in neat form as seen in Figure 13 and Table 13. Myristyl alcohol and cetyl alcohol containing formulation resulted in a significantly higher J_{max} values than the other fatty alcohols. The AUC for the formulations containing MA and CA was found to be higher compared to other fatty alcohols as shown in Table 14 and Figure 14. Although T_{max} was different, the J_{max} was comparable between MA and CA.

The results of finite and infinite studies revealed that the permeation of clotrimazole from all the o/w topical cream formulations could be enhanced when MA and CA are used in the formulation.

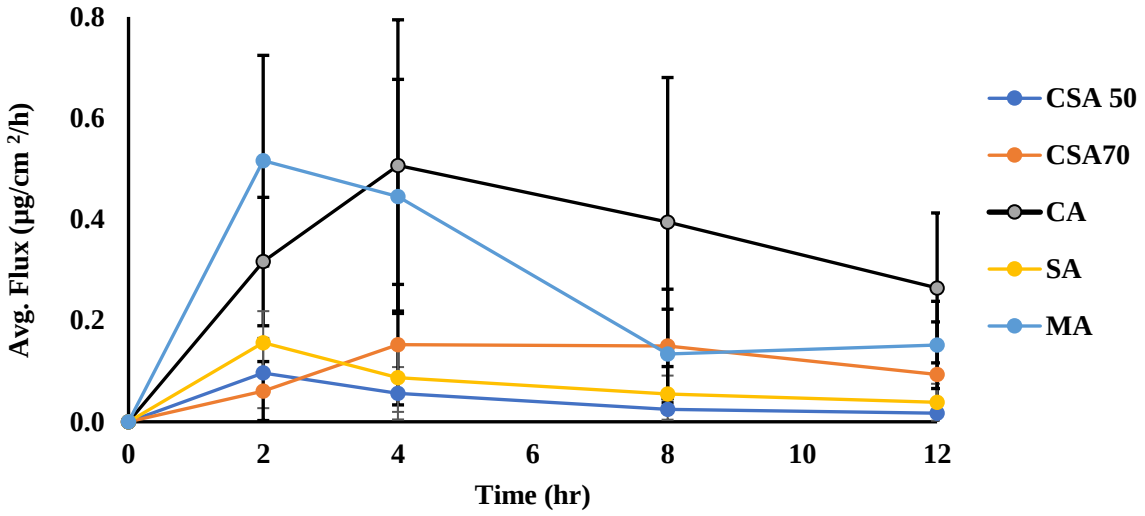


Figure 12: IVPT profile of clotrimazole at finite Dose from clotrimazole containing O/W cream [n=2 donors (3-6 replicate each donor ±SEM)

Table 13: IVPT finite dose study Jmax and standard error of means for clotrimazole creams containing different fatty alcohols

Fatty alcohol	J (max) (µg/cm^2/h) ± SEM
CSA 50	0.102 ± 0.070
CSA 70	0.247 ± 0.110
CA	0.663 ± 0.351
SA	0.166 ± 0.059
MA	0.635 ± 0.279

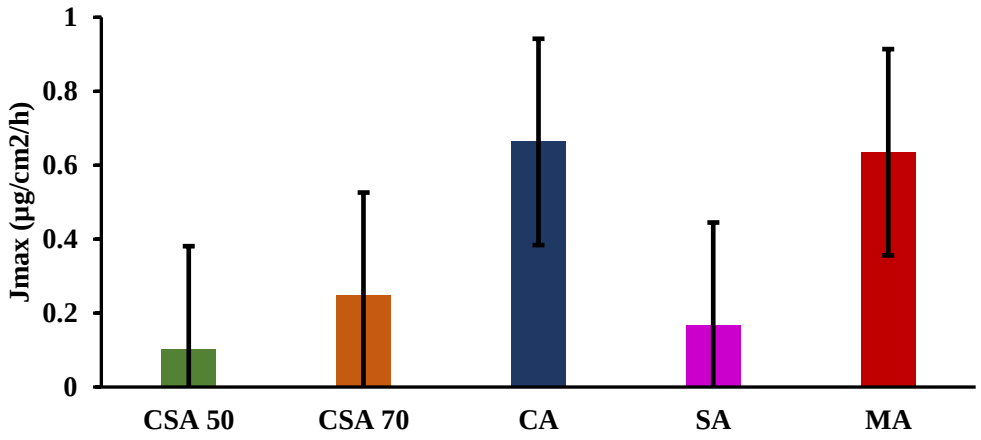


Figure 13: IVPT profile of clotrimazole at finite Dose study J max for clotrimazole containing o/w cream

Table 14: IVPT finite dose study Area under the curve and standard error of means for clotrimazole creams containing different fatty alcohols

Fatty alcohol	AUC (µg/cm^2)
CSA 50	0.184 ± 0.132
CSA 70	0.618 ± 0.443
CA	1.835 ± 1.122
SA	0.321 ± 0.176
MA	1.211 ± 0.602

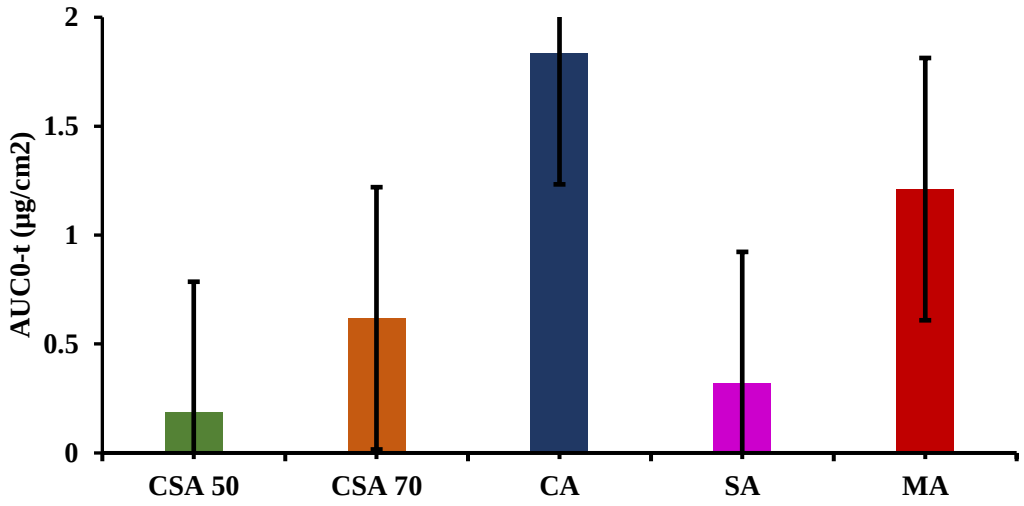


Figure 14: The Area under the curve from IVPT profile of clotrimazole at finite dose for clotrimazole containing o/w cream

Stability Study

Table 15: Results for initial samples of clotrimazole containing o/w creams with different fatty alcohols

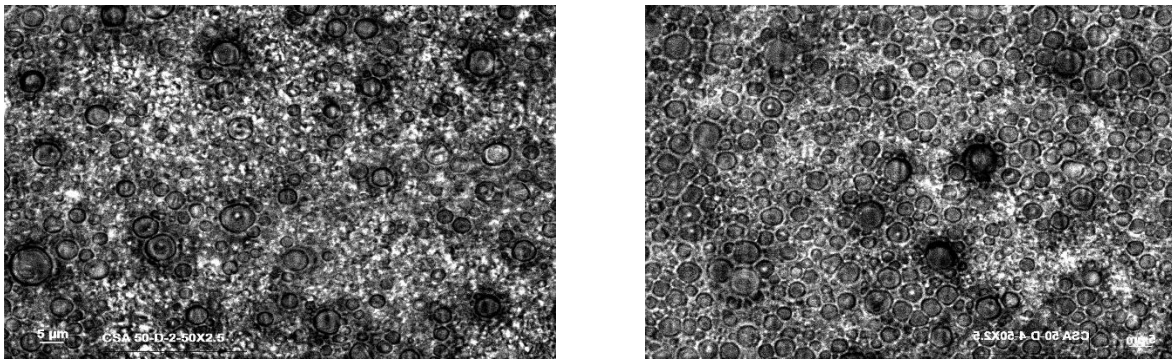
INITIAL					
	CSA 50	**CSA 70**	**CA**	**SA**	**MA**
	Avg.	**Avg.**	**Avg.**	**Avg.**	**Avg.**
pH	5.75 ± 0.05	5.89 ± 0.02	5.79 ± 0.02	5.79 ± 0.01	5.79 ± 0.01
Water Activity (a_w)	0.97 ± 0.00	0.97 ± 0.00	0.96 ± 0.00	0.97 ± 0.00	0.998 ± 0.00
d10 (µm)	2.61 ± 0.00	2.50± 0.14	2.55 ± 0.07	N.A	2 .05 ± 0.07
d50 (µm)	4.05 ± 0.07	3.90 ± 0.42	3.75 ± 0.35	N.A	3.05 ± 0.07
d90 (µm)	5.45 ± 0.21	5.20± 0.57	4.90 ± 0.71	N.A.	3.80 ± 0.10

Table 16: Stability study results for 3-month samples at 25°C/60% RH of clotrimazole containing o/w creams with different fatty alcohols

3M (25°C/65% RH)					
	CSA 50	**CSA 70**	**CA**	**SA**	**MA**
	Avg.	**Avg.**	**Avg.**	**Avg.**	**Avg.**
pH	7.30 ± 0.05	7.23 ± 0.02	7.11 ± 0.01	7.30 ± 0.01	6.87 ± 0.01
Water Activity (a_w)	0.98 ± 0.00	0.97 ± 0.00	0.99 ± 0.00	0.98 ± 0.00	0.98 ± 0.00
d10 (µm)	2.60 ± 0.14	2.45 ± 0.21	1.90 ± 0.28	N.A	2 .30 ± 0.0
d50 (µm)	4.00 ± 0.28	3.80 ± 0.28	3.50 ± 0.00	N.A	3.30 ± 0.14
d90 (µm)	5.45 ± 0.35	5.05± 0.21	5.10 ± 0.14	N.A.	4.25 ± 0.05

Table 17: Stability study results for 3-month samples at 40°C/75% RH of clotrimazole containing O/W creams with different fatty alcohols

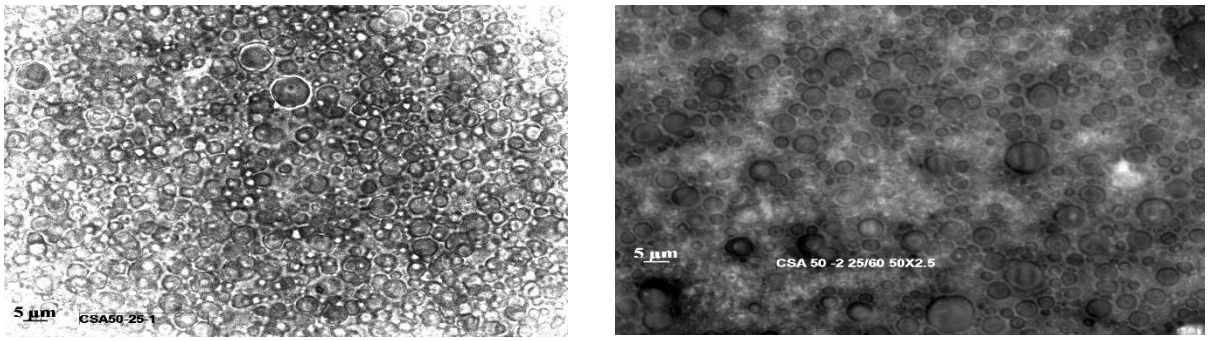
3M (40°C/75% RH)					
	CSA 50	**CSA 70**	**CA**	**SA**	**MA**
	Avg.	**Avg.**	**Avg.**	**Avg.**	**Avg.**
pH	7.15 ± 0.01	6.65 ± 0.01	6.96 ± 0.01	6.89 ± 0.01	6.32 ± 0.01
Water Activity (a_w)	0.97 ± 0.00	0.98 ± 0.00	0.98 ± 0.00	0.98 ± 0.00	0.99 ± 0.00
d10 (µm)	2.25 ± 0.21	2.25 ± 0.21	1.75 ± 0.49	N.A	2 .15 ± 0.07
d50 (µm)	3.85 ± 0.21	3.60 ± 0.00	2.80 ± 0.85	N.A	3.20 ± 0.14
d90 (µm)	5.25 ± 0.21	5.05 ± 0.35	4.25 ± 1.20	N.A.	4.35 ± 0.07



B1 B2

Initial

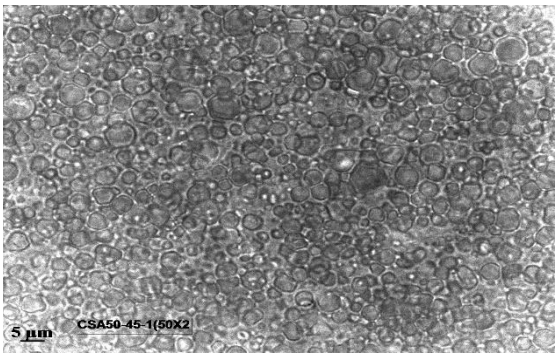
Figure 15: Photomicrograph for initial samples of clotrimazole containing o/w creams with CSA 50

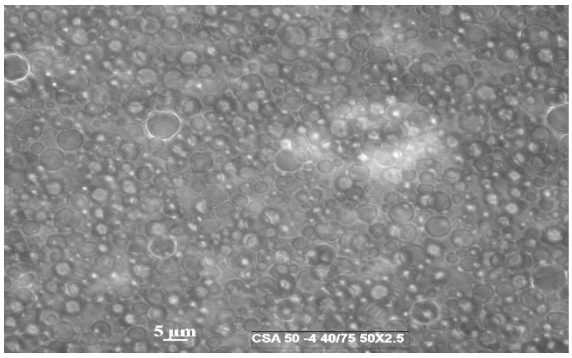


B1 B2

3 Month
25°C/60%RH

Figure 16: Photomicrograph for 3M 25°C/60%RH samples for clotrimazole containing O/W creams with CSA 50

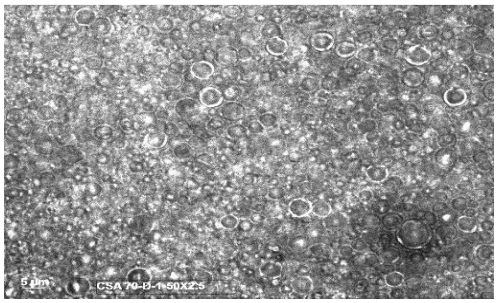


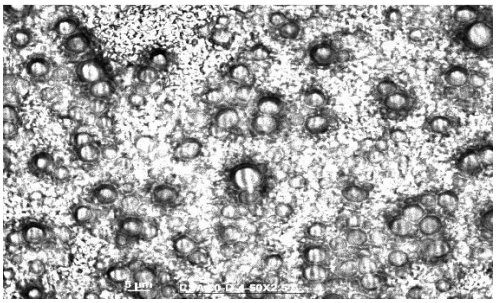


3 Month

B1 **40°C/75%RH** B2

Figure 17: Photomicrograph for 3M 40°C/75%RH samples for clotrimazole containing o/w creams with CSA 50

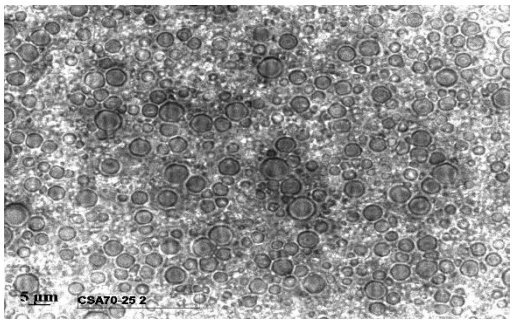


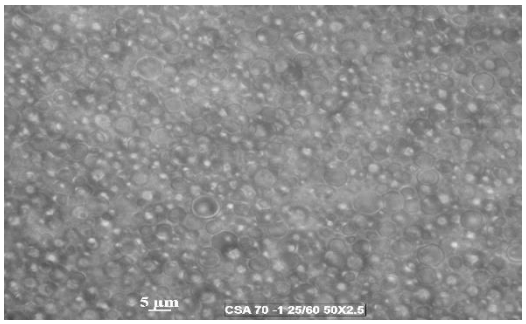


B1 B2

Initial

Figure 18: Photomicrograph for initial samples for clotrimazole containing O/W creams with CSA 70

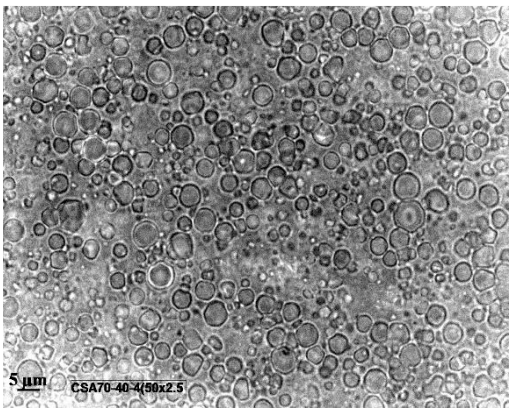


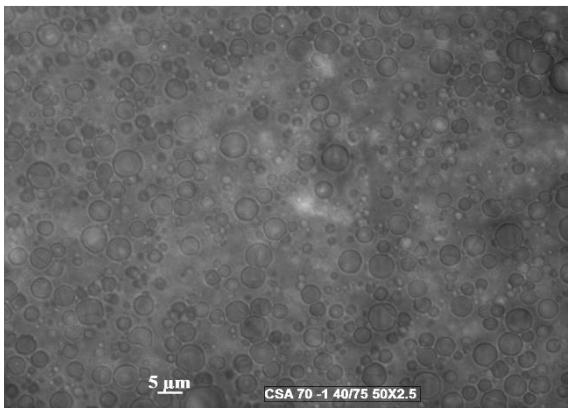


B1 B2

3 Month
25°C/60%RH

Figure 19: Photomicrograph for 3M 25°C/60%RH samples for clotrimazole containing o/w creams with CSA 70

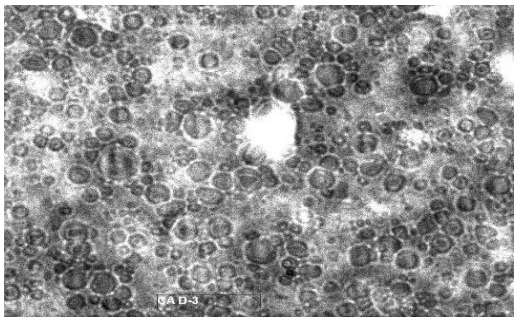


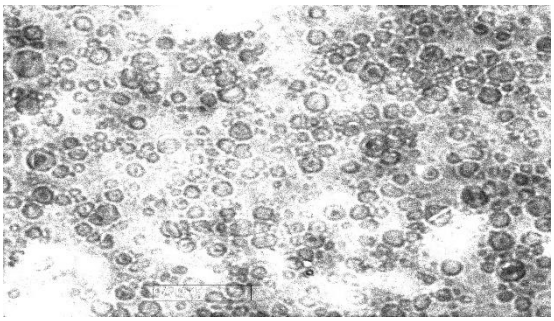


B1 B2

3 Month
40°C/75%RH

Figure 20: Photomicrograph for 3M 40°C/75%RH samples for clotrimazole containing o/w creams with CSA 70

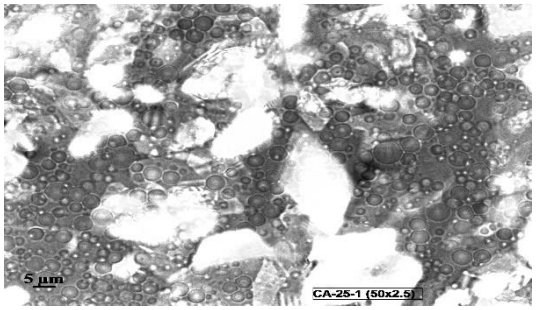


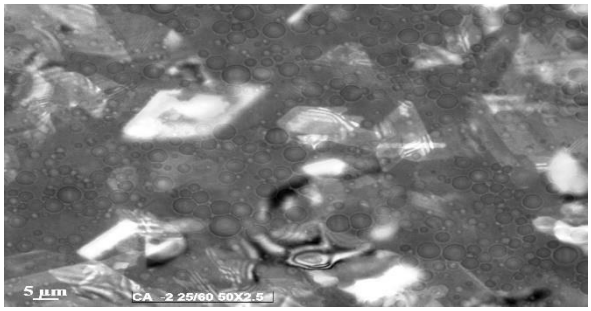


B1 B2

Initial

Figure 21: Photomicrograph for initial samples for clotrimazole containing o/w creams with CA

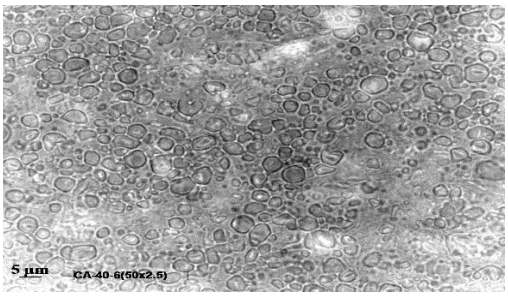


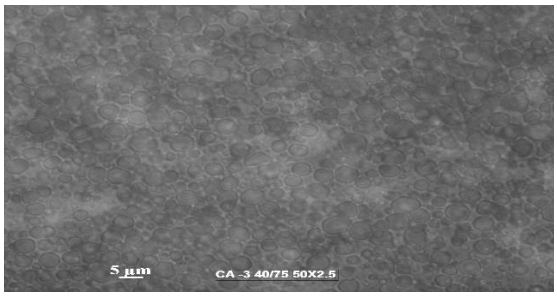


B1 B2

3 Month
25°C/60%RH

Figure 22: Photomicrograph for 3M 25°C/60%RH samples for clotrimazole containing o/w creams with CA

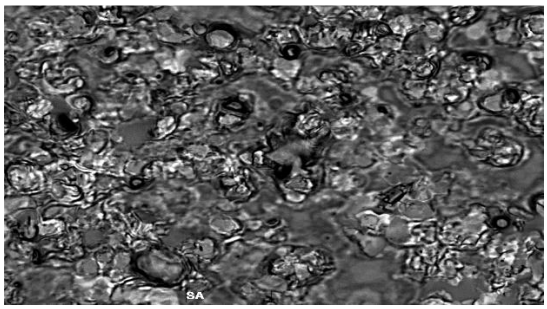


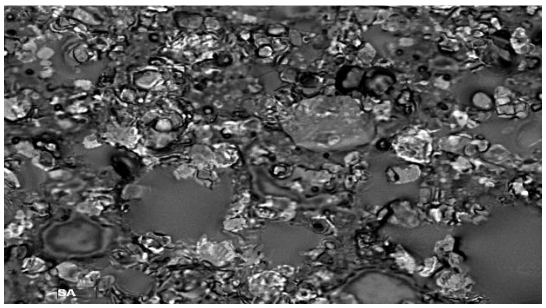


B1 B2

3 Month
40°C/75%RH

Figure 23: Photomicrograph for 3M 40°C/75%RH samples for clotrimazole containing O/W creams with CA

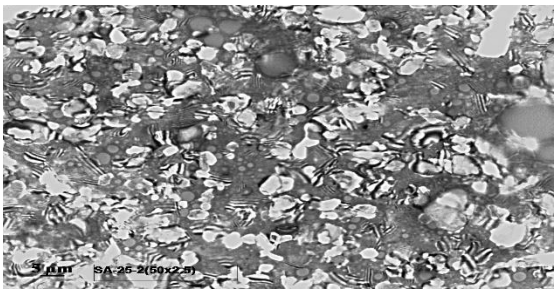


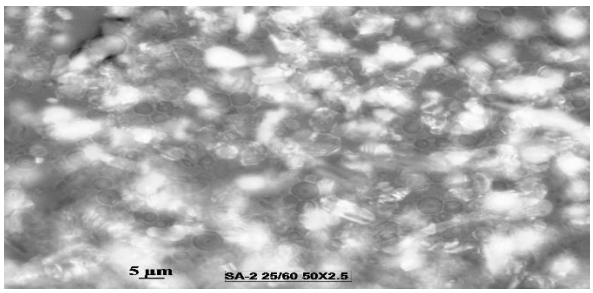


B1 B2

Initial

Figure 24: Photomicrograph for initial samples for clotrimazole containing o/w creams with SA

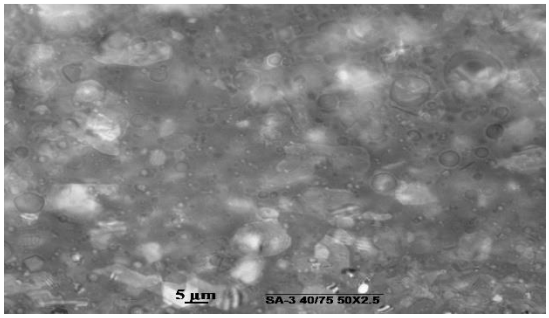




B1 B2

3 Month
25°C/60%RH

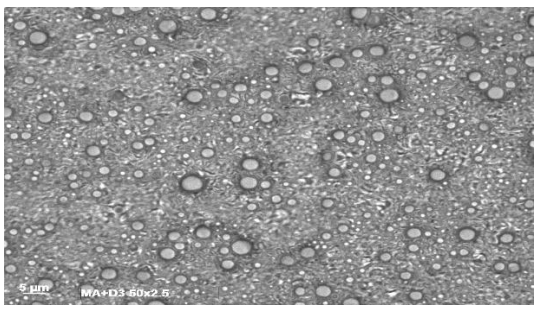
Figure 25: Photomicrograph for 3M 25°C/60%RH samples for clotrimazole containing o/w creams with SA

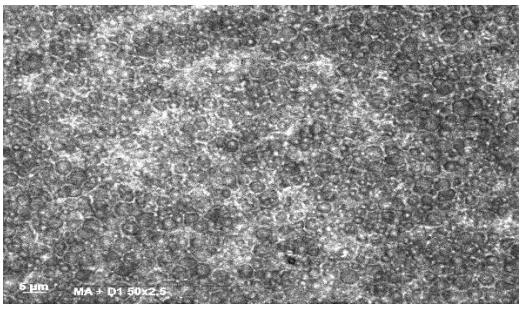


B1 B2

3 Month
40°C/75%RH

Figure 26: Photomicrograph for 3M 40°C/75%RH samples for clotrimazole containing o/w creams with SA

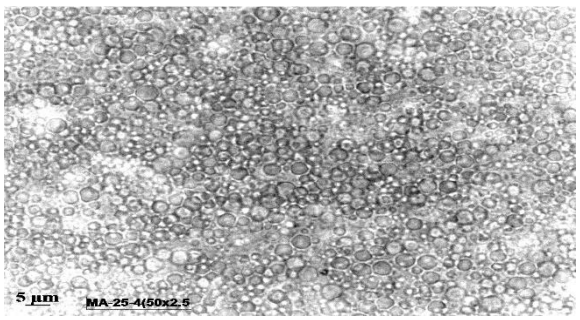


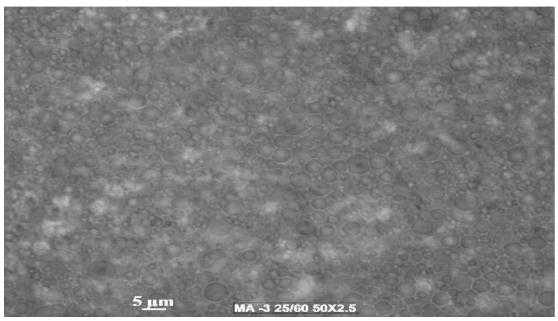


B1 B2

Initial

Figure 27: Photomicrograph for initial samples for clotrimazole containing o/w creams with MA

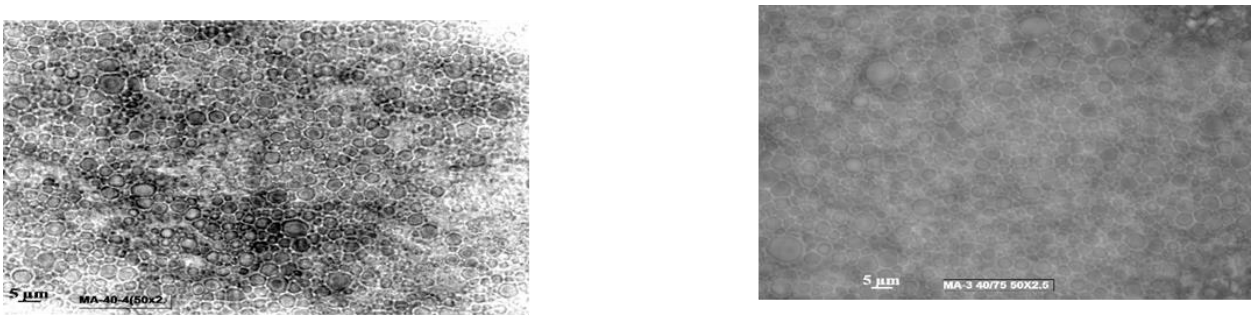




B1 B2

3 Month
25°C/60%RH

Figure 28: Photomicrograph for 3M 25°C/60%RH samples for clotrimazole containing o/w creams with MA



B1 B2

3 Month
40°C/75%RH

Figure 29: Photomicrograph for 3M 40°C/75%RH samples for clotrimazole containing o/w creams with MA

As shown in Table 15-17, the products prior to storage had pH values in the range of 5.75 ± 0.05 to 5.89 ± 0.02 and it did change significantly at both the storage conditions. This change could be because of the change in the partitioning of fatty acids from aqueous phase to lipid phase leading to increase in the pH of the formulation.

The water activity results were not altered and were found to be in the same range of 0.97 ± 0.00 to 0.99 ± 0.00. The d10, d50 and d90 values for initial samples were found to be in the same range as that of 3 month old samples and the globule size distribution was also found to be closer to initial values for all the cream products except for cream product containing SA which showed irregular shaped globules leading to poor globule size distribution. The results are expressed as mean ± SEM in all cases and as mean ± SD in case of globule size distribution. The microscopical images for all samples at both the storage conditions are as shown in Figure 15-30.

The cream products prepared with different fatty alcohols maintain same characteristics at two storage conditions. SA was found to be one of the less efficient fatty alcohol in terms of forming a good o/w cream in the present case.

CHAPTER V

CONCLUSION

The pH, water activity and globule size were found to be in the same range for cream formulations containing different fatty alcohols. CSA 50 and CSA 70 containing creams were found to have stable microstructure during rheological evaluations. Cream containing SA did not form a proper emulsion as evident from microscopic study, texture analysis and rheology. In infinite and finite dose IVPT studies, MA containing cream showed higher transdermal permeation of clotrimazole. A good correlation was found between the steady state flux of clotrimazole and melting point of fatty alcohols. The cream formulations with different fatty alcohols showed stable results of water activity and globule size at both the storage conditions. However, pH values changed significantly during stability study. Hence, it was concluded that fatty alcohols can play different roles like permeation enhancer, stabilizing agent, viscogen in formulation. The formulator can select either the single or combination of fatty alcohols for achieving desired characteristics in the formulation.

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VITA

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