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Impact Of Quality Attributes Of Topical Products On Their Performance

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IMPACT OF QUALITY ATTRIBUTES OF TOPICAL PRODUCTS ON THEIR PERFORMANCE

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ABSTRACT

Topical drug delivery such as creams and emulsions are most widely used in the treatment of skin diseases. It offers advantages such as non-invasiveness, direct drug delivery at the site of action, patient compliance and lower cost of treatment. However, topical formulation development faces challenges in the market in terms of critical evaluation parameters for understanding the effect of manufacturing process variables, the role of excipients and the effect of its compositions and skins buffering capacity. The main objective of the first project was to investigate the effect of different manufacturing process variables which can influence the microstructure thereby understanding the influence of process parameters on the performance of these creams. The custom-made formulation with the application of nile red for visualization of globule size in o/w creams was used for this study. The results revealed that the difference in globule size has influenced textural properties and yield stress. This study elucidated the relationship between the globule size and performance of creams. The second study was aimed to evaluate the influence of incremental change in the concentration of a surfactant on the quality attributes and performance of semisolid topical products. The critical quality attributes of these products did not differ significantly but there was a significant difference in their permeation flux-time profile. One of the major reasons was found to be due to the rate of change of thermodynamic activity during the process of evaporative metamorphosis. In the third project, we evaluated the buffer capacity of topical products as a critical quality attribute and impact of buffer capacity on the performance of

topical products. In this project, we investigated the role of the buffering capacity of formulations as a critical quality attribute. Herein we found that skins buffering capacity can alter the pH of formulation on the application site. The results showed that the creams with poor buffer capacity would end up changing their pH and match with the skin's pH. Whereas, the formulations with buffer restricted the physiological interaction of skin with the formulation pH and sustained the original pH of topical product for a longer time.

DEDICATION

This dissertation is dedicated to my mother Smt. Neelu Devi and father Shri Devendra Prasad Sharma, without whose lifelong and tireless encouragement I would have not reached and achieved what I deserved till here. It is their advice, guidance, love and supported that have convinced me that my dreams are worth pursuing who convinced me that my ideas were worth studying.

LIST OF ABBREVIATIONS OR SYMBOLS

Q1	Qualitative sameness
Q2	Quantitative sameness
Q3	Microstructural similarity
FDA	US Food and Drug Administration
O/W	Oil in Water
RLD	Reference listed drug
m	milli
g	grams
µ	micro
L	Liters
h	hour
min	minute
s	second
w/w	weight by weight
w/v	weight by volume
v/v	volume by volume
%	percentage
°C	degree centigrade

rpm	rotation per minute
HPLC	high-performance liquid chromatography
uv	ultra-violet
IVPT	*in vitro* permeation test
IVRT	*in vitro* release test
PBS	Phosphate buffer saline

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CHAPTER I

INTRODUCTION

Skin is the largest organ and an integumentary system of the human body. It accounts for a total surface area of approximately 2 m^2 having thickness ranging from 0.5 to 4 mm or more depending upon the part of body. It performs various functions, such as providing a protective barrier from the external environment (e.g., defending against microbial infection, inhibiting the entry of chemicals and toxins, preventing dehydration), regulating body temperature, and producing vitamin D [1]. The skin is also the most exposed organ and is prone to several physical and environmental stressors. Furthermore, autoimmunity, dysregulation of the stratum corneum regeneration, drug-induced skin hypersensitivity, and many other reasons can result in skin disorders. As such, the skin is susceptible to various disorders and diseases. Topical dermatological products, which can be administered easily and are convenient in terms of portability, are used in treating a variety of skin disorders such as psoriasis, skin cancer, etc. Drug products topically administered via the skin, falls into two broad categories, those applied for local action and those for systemic effects [2, 3]. Local actions include products applied on the surface of the skin, those that exert their actions on the stratum corneum, and those that modulate the function of the epidermis and/or the dermis. Common products in the former category include creams, gels, ointments, pastes, suspensions, lotions, foams, sprays, aerosols, and solutions. Creams, ointments, and gels generally are referred to as semisolid dosage forms [4]. Two categories of tests, product quality tests and product performance tests, are performed with drug products to provide assurance

of batch-to-batch quality, reproducibility, reliability, and performance. Product quality tests are performed to assess critical quality attributes such as assay, identification, content uniformity, pH, microbial limits, and minimum fill and are part of the compendial monograph. Product performance tests are conducted to assess drug release from the finished dosage form. Though the research has advanced at a multi fold rate but there is various parameter that need to explore. Creams are semisolid dosage forms that contain one or more drug substances dissolved or dispersed in a suitable base. This term traditionally has been applied to semisolids that possess a relatively soft, spreadable consistency formulated as either water-in-oil or oil-in-water emulsions. However, more recently the term has been restricted to products consisting of oil-in-water emulsions or aqueous microcrystalline dispersions of long-chain fatty acids or alcohols that are water washable and more cosmetically and aesthetically acceptable. Foams are the emerging topical dosage form which are also emulsified systems packaged in pressurized containers or special dispensing devices that contain dispersed gas bubbles, usually in a liquid continuous phase, that when dispensed has a fluffy, semisolid consistency [5-9].

Critical quality attribute is a parameter or characteristic which ensures the desired quality target product profile. Any deviation in quality attribute can result in inferior product which can deviate the patient's perception about the quality of product and be huge in production point of view for industries.

One of the major quality attributes is globule size of the semisolid O/W type dosage form. Globule size of the active drug substance in semisolid dosage forms is determined and controlled at the formulation development stage. When applicable, semisolid drug products should be tested for any change in the particle size or habit of the active drug substance at the time of batch release and designated stability test time points that could compromise the integrity and/or performance of the

drug product, as appropriate. Another such critical factor is pH of semisolid dosage form. As per US FDA guidelines, semisolid drug products should be tested for pH at the time of batch release and designated stability test time points for batch-to-batch monitoring. Because most semisolid dosage forms contain very limited quantities of water or aqueous phase, pH measurements may be warranted only as a quality control measure, as appropriate [9-13].

A performance test for generic topical drug products must have the ability to measure drug release from the finished dosage form. It must be reproducible and reliable, and although it is not a measure of bioavailability, the performance test must be capable of detecting changes in drug release characteristics from the finished product. The latter have the potential to alter the biological performance of the drug in the dosage form. Those changes may be related to active or inactive/inert ingredients in the formulation, physical or chemical attributes of the finished formulation, manufacturing variables, shipping and storage effects, aging effects, and other formulation factors critical to the quality characteristics of the finished drug product. Product performance tests can serve many useful purposes in product development and in post-approval drug product monitoring.

Development of any quality product involves three critical control over the following:

1. Quality raw material/ API
2. Quantity to be used in optimized formulation
3. Process development

For semisolid topical drug products (creams, emulsions, etc.), the FDA requires Q1 and Q2 similarity for generics. Q1/Q2 similarity may also be recommended when *in vitro* methods for demonstrating bioequivalence are proposed [9, 10, 14]. Their definitions are as follows:

- Q1 (Qualitative sameness): the test product uses the same inactive ingredient(s) as the reference listed drug (RLD)
- Q2 (Quantitative sameness): concentrations of the inactive ingredient(s) used in the test product are within +/-5 % of those used in the RLD

Q1/Q2 sameness may be demonstrated with a formulation table showing the inactive ingredients and their quantities relative to an RLD. However, this approach is not always enough for complex generic drug products. While Q1/Q2 sameness provides a good starting point for matching the performance of an RLD, it is not always enough when it comes to complex generic drug products. For some complex ANDAs, Q3 similarity must also be considered:

- Q3 (Microstructural similarity): the arrangement of matter (physical and structural properties) is like the RLD

Q3 similarity mitigates the risk associated with physicochemical variations in a generic drug product, including, but not limited to:

- Differences in the polymorphic form of a drug
- Differences in rheology
- Differences in crystallinity that can impact drug diffusion

When evaluating products such as emulsions and multi-phase creams, these physicochemical properties can have a significant impact on the performance of the generic drug product. As a result, for topical ANDAs it is recommend supporting with Q3 evaluations as suggested by Shah et al., 2015. With the recent advances in this area, a topical drug classification system has been suggested, which are as follows [2]:

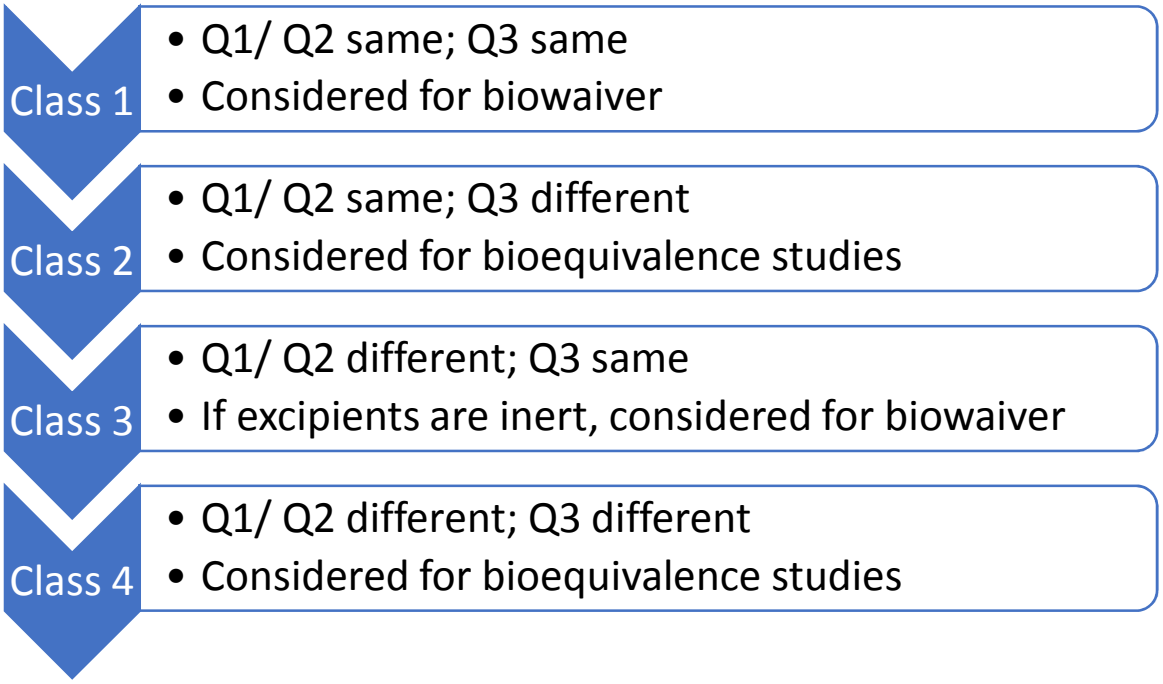


Figure 1: Topical drug classification system

Process Development controls the quality of drug product. In topical manufacturing, mechanical mixers, such as a steel jacketed kettle with agitator are commonly used to prepare semisolid preparations in pharmaceutical industry. The use of mechanical shear or a combination of fusion processes and mixing can be used to facilitate the dispersion or dissolution of the ingredients in the base to form a stable O/W emulsion cream [3]. Emulsion products are prepared by means of a standard two-phase blending process forming either an oil-in-water emulsion or water-in-oil emulsion. Semisolid preparations may require further modifications. Homogenization speed and time can be significant processing factors that may affect physical stability (e.g., coalescence of droplets, phase separation) and homogeneity. Time, temperature, and mechanical energy inputs are the three major variables in the manufacturing of semisolid preparations. The process parameters for these three interrelated factors need to be identified and carefully controlled to produce batches with consistent quality. The initial mixing temperature should be high enough to ensure the intimate mixing of liquid phase and to prevent the premature crystallization and

congealing of its components. The aqueous and oil phases can be mixed by the addition of the discontinuous phase to the continuous phase and addition of the continuous phase to the discontinuous phase. The effect of addition order and the rate of addition on the drug product quality attributes should be evaluated. Furthermore, because cooling rate can influence the final product quality, different cooling rates after melting, mixing, and emulsification steps should be investigated as a process variable.

The stage at which the active ingredient is added to the semisolid mixture may be critical and should be identified. Some active ingredients may be dissolved at high temperature and re-crystallized during the cooling stage, resulting in larger crystals. In this case, the active ingredient can be charged to the cooled down cream base via a powder reduction system or through a slurry addition and simultaneously mixed into the cream base to avoid the re-crystallization problem. A visual check is a useful simple confirmatory step to ensure all solids have dissolved/melted or the phase is uniform before proceeding to next step. Microscopic checks should also be carried out to decide homogenization speed and time for the final product required to ascertain proper incorporation of drug substance to the base and to match the microscopic appearance of the RLD, which includes drug particle size, droplet size, etc.

Thus, apart from excipients used in the formulation design, delivery of the compound into the skin from a topical drug product can be very sensitive to alterations in the manufacturing processes. This is attributable to the fact that the manufacturing process can have a profound impact upon the microstructure of drug product. Thus the goal of process development of generic drug products is to achieve a similar arrangement of matter as the RLD (*i.e.*, Q3, same components in same concentration with the same arrangement of matter (microstructure) as the reference-listed drug), which provides assurance of similar critical quality attributes to those of the RLD [5, 6]**.** Q3

microstructure sameness includes identical rheology, type of emulsion (O/W emulsion, W/O emulsion, and globule size), and physical state of drug in semisolid system (Polymorphic form, solubilized drug *vs.* dispersed solid drug, particle size of drug particles) compared to those of the RLD. Also, it is prudent to provide information to address whether the drug product manufacturing process may alter the polymorphic form of drug particles in the drug product development. It is essential during the process of generic product development to explore the effect of process variables on the drug product quality attributes through an experimental design and to identify a range of process parameters for a robust drug product manufacturing. It is important to be aware of issues and pitfalls of being not Q1/Q2/Q3 linked to the RLD so they can be properly managed to ensure technical and regulatory success. This lead my first project to study identify the range and effect of critical process parameters for a robust topical semisolid dosage form. During formulation development the researcher study determines the optimal concentration of each ingredient, at this stage, generic companies use reverse engineering to identify the concentration of each ingredient. But in many cases qualitative (Q1) and quantitative (Q2) similar products differ in functionality, even though they have similar microstructural(Q3) attributes which has a complex milieu of mesophase and colloidal structure that comprise the final dosage form. Since these semisolid topical formulations are very complex as compared to other dosage forms such as tablets. Excipients used in each topical formulation has its own characteristic function, which may or may not get influenced by the functionality of other excipients in the same formulation. The role of various excipient in the quantitative attribute for topical semisolid dosage forms such as O/W emulsion is undermined and its influence on the performance of the product. It is also very crucial to explore, how systematic variation in quantitative attribute (Q2) of excipient while maintaining qualitative attribute (Q1) and process variables same, can influence the microstructure and

performance attribute of the dosage form. This lead my second project to explore the proactive role of excipients having a quantitative variation which can influence the formulation characteristic. There we want to explore how variation in surfactant profile (which should not have more than (NMT) 5 % deviation to ensure Q2 similarity) is important for the study to develop emulsion. can influence the performance of O/W emulsion based topical dosage form.

Apart from development of topical drug product, the intended site of application on skin is also extremely critical. Understanding skin physiology is still a challenge and has been focus of research for dermatologist around the world. When considering the performance of topical drug product, it varies tremendously form person to person, anatomical site and time of application of one person, apart from other conditions like age, disease, etc. The major barrier layer of skin, the stratum corneum, consists of an interstitial lipid pathway and a proteinaceous cellular compartment. Drug molecules penetrate the skin primarily through the tortuous and continuous intercellular path. Transport of topical drugs, especially with the aid of solvents and enhancers used in the formulation, may also occur through a transcellular route, the hair follicles, or sweat ducts. Only the drug in the molecular state can penetrate through the skin. Occluded skin facilitates drug transport through the hydration mechanism. Skin with diseased conditions, such as atopic dermatitis, psoriasis, and warts, may have effects on the barrier property of skin, which must be considered for the drugs geared toward these skin diseases. From a drug delivery perspective, the pH of the formulation and at site of action can play critical role in the driving force for penetration of drug through the skin. The skin pH typically ranges from 5-6 depending on the hydration but is varies from morning to afternoon to evening depending on environmental factors, skin conditions, etc. Skin is known to have buffering ability and considered act as acid mantle to protect our body from harsh environment conditions. The pH of skin is critical in increasing the availability of active

ingredients in therapeutic form and thus providing the thermodynamic state such that a larger driving force for transporting the active through the skin occurs. However, pH of skin changes with hydration and environmental factors. Thus, a drug which is present in a super-saturated solution in topical dosage form is in a metastable state and, hence, may convert back to its stable form. This changes the permeation profile and flux of the drug through skin. Understanding the buffer capacity of skin and topical products, can a critical quality attribute for its efficiency. This lead my last project of thesis, to evaluate and understand the buffering capacity of skin with different formulations prepared at different pH conditions. We also evaluated the impact of different buffering capacity of topical products on the pH of skin in human subjects. Developing and understanding the relationship between the buffering capacity and change in pH of topical products post application on clinical applications [9, 13, 15-17].

CHAPTER II

EFFECT OF CRITICAL PROCESS VARIABLES ON THE CRITICAL QUALITY ATTRIBUTES AND PERFORMANCE OF O/W TOPICAL SEMISOLID CREAMS

Abstract

Topical creams are heterogeneous products in which the dispersed phase is distributed uniformly within a continuous phase. The microstructural characteristics of a cream predominantly depend on the manufacturing process variables. The objective of the present study was to investigate the effect of processing variables on the critical quality attributes of oil in water (O/W) cream products. The second objective was to investigate the influence of microstructural differences in the topical creams on *in vitro* drug permeation. A set of cream products that were compositionally identical were formulated with variations in the manufacturing variables (homogenization speed and duration) to result in microstructural differences across the products. The creams were characterized for their appearance, globule size, rheology, texture properties, stability and *in vitro* drug permeation. It was observed that the systematic variation in manufacturing variables for a cream (Q1/Q2 similar) can progressively influence the Q3 (microstructural) characteristics. We found that homogenization speed during manufacturing of creams had a profound impact on the globule size (1.7 ± 0.05 to 9.9 ± 0.40 μm) and on visual appearance. The difference in globule size has influenced textural properties and yield stress. In addition, the maximum permeation flux decreased with increase in the globule size linearly. This study elucidated the relationship between

globule size and performance of creams.

Keywords: Topical creams, microstructure, critical quality attributes, manufacturing variables, performance parameters.

1. Introduction

Topical semisolid formulations are being widely used for the treatment of local and systemic disorders. Topical products can be formulated in various forms such as ointments, gels, creams, lotions, solutions, and foams[14]. Creams are one of the most commonly used semisolid forms which are generally either oil in water (O/W) or water in oil (W/O) type emulsions. Creams are prepared using different manufacturing processes, each of them is associated with several variables that could impact the quality of the finished product [15-18]. Moreover, the microstructure of products could differ from each other despite the product composition being identical (Q1 and Q2 identical) depending on the variables implemented during manufacturing of creams [15, 19-22].

The objective of this project was to explore the effect of some of the variables of the homogenization process on the microstructural (Q3) characteristics of topical O/W creams. The objective was to evaluate the impact of microstructural differences on the performance attributes of the product. Therefore, creams with same composition (Q1 and Q2 identical) and with different microstructures (Q3 characteristics) were prepared using different manufacturing conditions and characterized.

2. Material and Methods

2.1. Materials

Nile red was purchased from Sigma-Aldrich, St. Louis, MO, USA. Ethanol 200 proof was purchased from Decon Laboratories, Inc, PA, USA. The formulation excipients Cetostearyl alcohol, Cremophor A25, Cremophor A6 and propylene glycol were from BASF Corporation, NJ, USA. Mineral oil was purchased from Spectrum Chemicals, CA, USA. Purified water was used in all the preparations.

2.2. Preparation of Creams

Nile red, a lipophilic fluorescent dye was incorporated as a marker as it predominantly localizes in the dispersed oil phase in an o/w emulsion [21-24]. The composition of the cream formulation is given in Table 1. 1 mg of nile red was weighed (0.0005% w/w of cream weight) and dissolved in mineral oil prior to preparation of the formulations.

First, the lipid phase components were melted by geometric mixing in a thermostated glass vessel maintained at 80 ºC. Simultaneously, in another glass vessel, aqueous phase components were heated to 80 ºC. Both the phase was mixed together in a thermostated vessel at 80 ºC using a Silverson® homogenizer (Model: L5M-A, Silverson Machines, Inc., MA, USA). The homogenization variables were speed and time. After homogenization for a specified time, a controlled gradual cooling protocol was followed until the temperature of formulation reached 25 ºC in 25 mins.

The homogenization speed selected were 500, 1000, 3000 and 5000 rpm (F1-F4) for a homogenization duration of 20 min. To investigate the effect of homogenization duration on the microstructural characteristics of cream, creams were prepared at 3000 rpm with homogenization duration 10 and 40 min (F5 and F6), as shown in Table 2. One of the cream formulations was prepared at 3000 rpm for 20 min, like other creams, except that the cream was allowed to cool gradually in its own accord without using the programmed cooling process (F7) (Table 2).

Ingredients	Quantity (% w/w)
Cetostearyl alcohol	7
Mineral oil	12
Cremophor A25	1.5
Cremophor A6	1.5
Propylene glycol	8
Water purified	70

Table 1: Composition of cream formulations.

Formulation	Homogenization speed (rpm)	Homogenization time (mins)	Average globule size (μm)
F1	500	20	9.9±4.33
F2	1000	20	5.7±1.14
F3	3000	20	2.9±1.25
F4	5000	20	1.7±0.41
F5	3000	10	4.3±1.34
F6	3000	40	4.4±0.88
F7*	3000	20	4.3±0.99

*Cooling protocol was not followed for F7 cream

Table 2: Variations in process variables incorporated in custom made creams and the average globule size.

2.3. Appearance

2.3.1. Macroscopic appearance

The color difference across the formulations was expressed using a handheld colorimeter (PCE-CSM1, PCE America Inc., USA). It measures different color spaces (L*, a*, b*, C*, h* values) for each of the products. An equal quantity of creams was taken in an individual sample holder for analysis. All color measurements were taken using conditions of standard illuminant D65 and

Specular component included (SCI) while LED blue light source with silicon photoelectric diode sensor was used. The color difference between the different creams was expressed using the parameters which are used to identify color differences using Commission Internationale de L'Eclairage (CIE)[23].

2.3.2. Microscopy and globule size

For microstructural observation under a microscope, 10 mg of cream was applied on a clean glass slide, a film applicator with the gap adjusted to 10 μm between applicator blade (Gardco® Microm II applicator blade, Japan) and glass slide was used to prepare a film. With the application of slight pressure on the slide, the applicator was pulled slowly to form a uniform film on the slide. The slide was observed under a confocal microscope using a 40X objective. The excitation wavelength used for nile red was 515 nm and the emission wavelength were 525-605 nm. 514 nm laser and a BP filter of 530-600 nm were used to view the globules. The images were acquired using Zeiss LSM 510 confocal microscope and processed through Zen Lite software. For each cream, we considered the mean diameter of randomly distributed 50 globules, measured using the Zen Lite software. The d_{10}, d_{50}, and d_{90} are commonly used to represent the midpoint and range of the globule sizes of a given sample [24]. The 10%, 50% , and 90%, 'd' values were determined from the S-curve of cumulative distribution.

2.4. pH measurement

In-Lab Micro® pH probe was calibrated using buffered pH standards of pH 4, 7 and 10. One gram of cream was filled in a container and tapped to remove any entrapped air. A standard buffer solution of pH 4 and of pH 10 was used to check the accuracy of the probe before each measurement. After each measurement, the probe was washed with DI water and 70% ethanol, alternatively in three cycles. Similarly, the pH of the aqueous phase was measured after phase

separation by ultra-centrifugation (TLA55 rotor, Beckman Coulter Inc., USA), at 55000 rpm for 4 h. The pH of each cream and its respective aqueous phase was recorded as an average of three replicate measurements.

2.5. Water activity

Water activity was measured using Aqualab® water activity meter (Series 3E, USA). 1 g of cream was dispensed into the sample cup using a positive displacement pipette. The cream was spread uniformly to cover the bottom of sample cup holder evenly using a glass rod. The sample cup holder was placed in the sample chamber until an equilibration value was attained. The sample was later replaced with a cup holder containing activated charcoal in the sample chamber to clear any residual humidity in the chamber.

2.6. Rate of drying

120 mg of cream sample was dispensed and spread uniformly in a plastic cup having surface area 8.0 cm^2 (15 mg/cm^2). These cups were placed an incubator oven (Fisher Scientific Isotemp 550D Incubator Oven) at 32 °C for 150 mins and change in mass was recorded at every 10 mins during the first 60 mins followed by every 30 mins till the weight of the residual content become constant. Thus, the loss on drying at each time point was estimated as percent loss by the weight using the equation:

$$loss\ of\ weight\ \% = [(initial\ weight - dried\ weight)/initial\ weight]\ x100 \quad \ldots\text{equation 1}$$

2.7. Texture analysis

Texture analysis was conducted at room temperature (25 ± 1 °C) using a Texture Analyzer (TA-XT2i, Texture Tech. Corp., Scarsdale, NY) equipped with a TA-3 acrylate cylindrical probe. The test was conducted in compression mode. The conditions for analysis and set of parameters used

are given in Table 3. The texture analyzer is equipped with an analytical probe connected to a motor that enables vertical probe displacements to be measured. The cream was filled in the base plate having aperture of 30 mm diameter fixed to the base plate. The probe penetrates the cream and retracted to measure different texture parameters (work of adhesion, firmness, and stringiness) from the curve. Detectors recorded sensor position and resistance to its displacements. All measurements were recorded in triplicate every time in compression mode[25, 26].

S.No	Parameters	Compression mode
1	Pre-test speed (mm/sec)	2
2	Test speed (mm/sec)	2
3	Post-test speed (mm/sec)	10
4	Trigger type	Auto force
5	Trigger force (N)	0.05
6	Target mode	Distance
7	Distance (mm)	1.5
8	Return distance (mm)	5
9	Points per second	500
10	Probe	TA-3 soft matter acrylic

Table 3: The instrumental setting of Texture Analyzer (TA-XT2i, Texture Tech. Corp., Scarsdale, NY).

2.8. Rheology

Rheological studies were conducted using a TA HR2 rheometer (TA Instruments, New Castle, DE) equipped with 25 mm parallel plate. The experiments were conducted at 32°C and the temperature was controlled using a Peltier stage. To avoid sample slippage at the rheometer plates, the plates were covered with adhesive backed 600 grit sand papers. A gap of 500 μm between the parallel plates was used for the rheological investigations. After loading the samples in between

the plates, the samples were allowed to relax for 5 minutes. The relaxation process was captured using time sweep experiment (low frequency and low strain). Amplitude sweep test and thixotropic loop test were conducted on each sample. The samples were allowed to relax for 5 minutes in between two experiments[21, 22, 24, 25].

2.9. Determination of Spreadable area (S_{max}) study

Spreadable area of each cream was determined on human volunteers as per protocol approved by an institutional review committee of the University of Mississippi. Three operators determined the spreading ability of each cream on five volunteers. The forefinger tip was used to apply the finite amount of creams on the hairless region of the forearm. The cream was spread in a rectangular pattern across a surface area of the skin and the maximum area of spreading was calculated.

2.10. *In vitro* permeation profile

2.10.1. Preparation of porcine epidermis

The freshly excised porcine skin was washed with saline and subcutaneous fat was removed. The epidermis was isolated by heating the skin for 2 min at 60°C. The isolated epidermis was dried and stored at 4 °C, until further use. Before mounting the epidermis for permeation study, it was thawed for 2 h at room temperature. The epidermal barrier integrity was tested by measuring Trans-epidermal electrical resistance (TEER). The epidermis having a TEER value ≥ 20 k Ω cm^2 and without physical damage was used for *in vitro* drug permeation studies.

2.10.2. Experimental setup

Permeation study across the epidermis was performed using vertical Franz diffusion apparatus (Logan Instruments, Somerset, NJ). The epidermis was sandwiched between the two chambers (donor and receiver) of a Franz diffusion cell having an active diffusion area of 0.64 cm^2 with the temperature maintained at 32 ± 1°C using a circulating water bath. The receiver compartment was

filled with 5 mL of freshly prepared hydro-alcoholic solution using phosphate buffered saline (PBS) (pH 7.4) with 20% v/v ethanol. The finite dose of cream was applied (15 mg/cm^2) uniformly across the active diffusion area of the porcine epidermis. The permeation studies were carried out for a period of 32 h and samples were analyzed using fluorescent plate reader (SpectraMax M5, Molecular Devices, USA) at excitation wavelength 515 nm and emission wavelength 525-605 nm.

2.11. Stability study

The stability studies were carried out for all the creams at 25 ± 2 °C, relative humidity 60 ± 5% and 40 ± 2 °C, relative humidity 75 ± 5%, for 90 days. The change in globule size distribution over 90 days was measured to represent the stability of all creams [26].

3. Results and Discussion

3.1. Appearance

Creams prepared were identical in composition. However, their overall appearance (intensity of color) was different from each other indicating differences in the microstructure of the products as shown in Figure 2. The color of the creams was measured using a handheld colorimeter and Table 4 shows the L, a, b, C, h values for each of the products. In L, a, b color space, L indicates the lightness, ‘a’ is red/green coordinate and ‘b’ is yellow/blue coordinate. Whereas, C and h use Cartesian coordinates to represent a color in color space. L, C, and h use polar coordinates where ‘C’ specifies Chroma and ‘h’ denote hue angle. The data in Table 4 distinguishes the color of cream products quantitatively. The differences in the size distribution of dye filled oil globules are likely the reason for the difference in the intensity of the color of the creams. It was evident from the trend lines plotted in Figure 3-a and b, that the Lightness and Hue values decrease with increase in globule size. This study shows that measurement of colorimetric parameters could serve as a primary tool to identify any microstructural differences in formulations.

Figure 2: Appearance of custom-made creams with same composition and different globule size.

Parameter	F1	F2	F3	F4	F5	F6	F7
L	74.5±1.25	78.7±0.24	83.2±0.24	84.0±0.06	83.0±0.35	82.9±0.09	83.0±0.11
a	13.4±0.06	13.4±0.07	10.3±0.26	10.6±0.21	9.5±0.20	11.7±0.09	11.2±0.12
b	3.1±0.07	4.7±0.04	6.4±0.10	8.0±0.06	6.1±0.06	8.1±0.11	7.5±0.08
C	13.8±0.07	14.2±0.07	12.2±0.27	13.3±0.19	11.3±0.20	14.3±0.13	13.5±0.14
h	13.1±0.24	19.4±0.05	31.8±0.29	37.0±0.46	33.0±0.30	34.6±0.17	33.8±0.06

Table 4: Quantitative determination of custom made creams by colorimetric analysis.

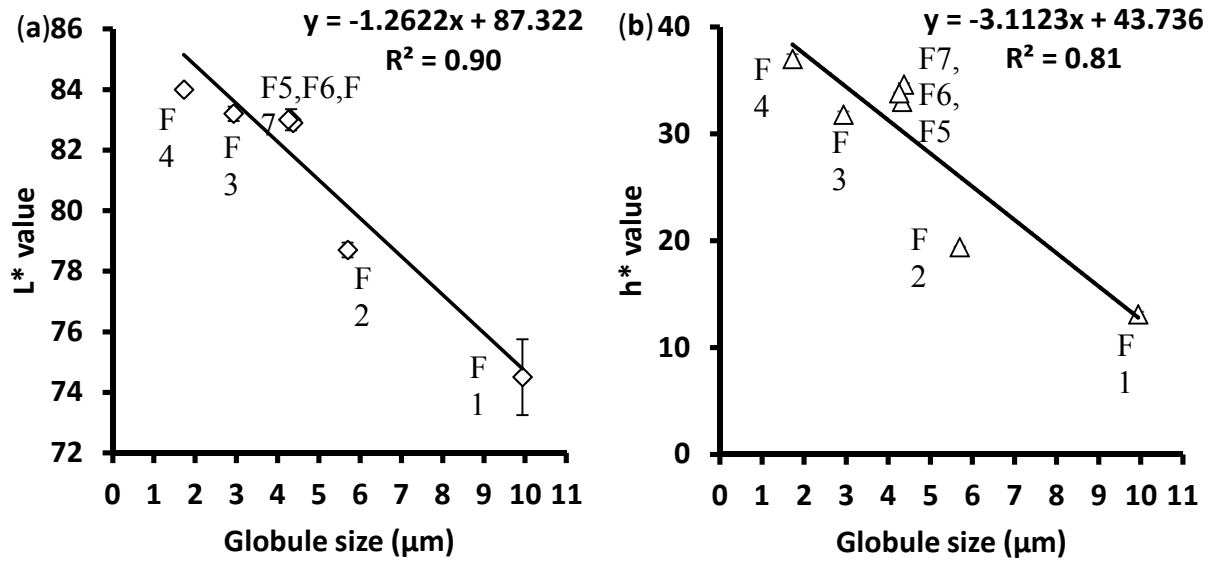


Figure 3:a) The relationship between lightness of creams (L) and globule size; b) The relationship between hue values (h) and globule size of creams.

3.2.Microscopy and globule size

The homogenization speed was found to have a significant impact on the globule size of the products. The localization of nile red dye in the dispersed oil phase rendered the globules distinctly visible under a confocal fluorescence microscope (Figure 4). At constant homogenization duration, the globule size decreased with an increase in the homogenization speed (Table 2). The average globule size ranged from 9.9 ± 0.40 to 1.7 ± 0.05 µm across the seven cream formulations. At 3000 rpm, the duration of homogenization did not influence the average globule size.

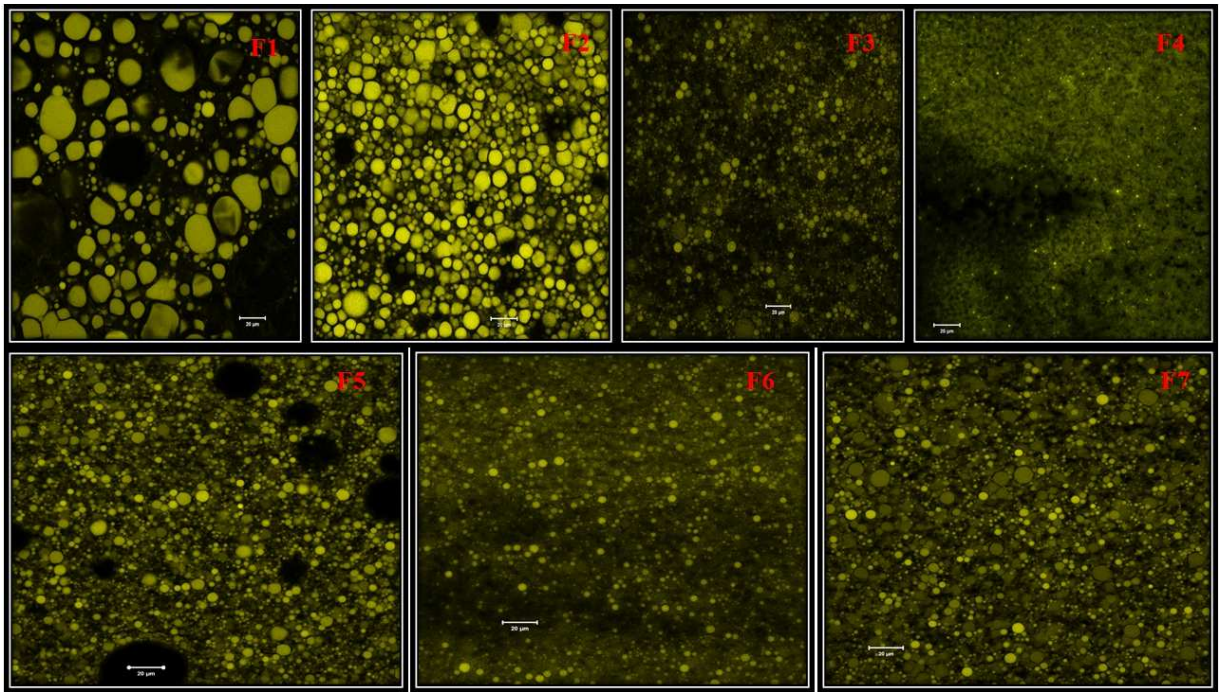


Figure 4: Confocal images of seven custom-made creams at 40X magnification in Zeiss LSM 510 confocal microscope.

3.3.pH of creams

The pH of all creams was in the range of 5.6-5.7. There was no significant difference in the pH between creams and the respective aqueous phases separated by centrifugation. It is evident from

the results that different processing conditions did not result in pH difference in the creams despite the difference in the microstructure.

3.4.Water activity and rate of drying

Water activity represents the thermodynamic energy status of water present in a pharmaceutical system. Water activity would influence the rate of evaporation of water from the applied region leading to rapid metamorphosis changes in the cream. The water activity of all the creams was found to be in the range of 0.937 to 0.944. The changes in critical process parameters did not seem to impact the water activity of creams, likely because the continuous phase comprises of a profound amount of water. There was no significant difference in the drying rate between the creams which could be attributed to the clustering of water activity values of different products within a narrow range. There was about 50% loss in contents in all the creams by the end of 40 min.

Texture analysis

An acrylate texture probe was used to study the textural properties of the creams. Work of adhesion represents the total amount of energy involved in the withdrawal of the probe from the surface and determined by the area under the force versus time curve. The work of adhesion (N.sec) was found to have an inverse relationship with globule size (Figure 5-a and b, respectively). Firmness is determined by penetration of probe where the compression force is determined as the function of deformation. In this parameter, the resistance force of the creams is measured relative to the penetration of a flat circular probe. The firmness of creams showed an increment with a decline in globules size, a similar trend was also observed with the homogenization time. The stringiness is the distance that the cream extended during decompression before separation from the probe. Stringiness did not show any trend with a change in globule size nor with homogenization time. The seven creams differ significantly in firmness and stringiness as given in Figure 6-a-b.

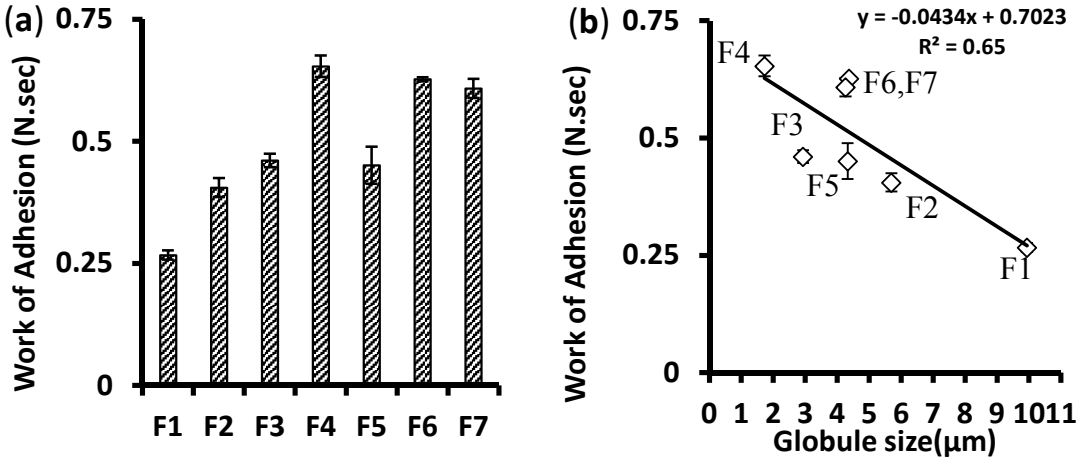


Figure 5:a) Work of adhesion parameter in texture analysis of seven creams; b) The relationship between the work of adhesion and globule size of creams (n=3).

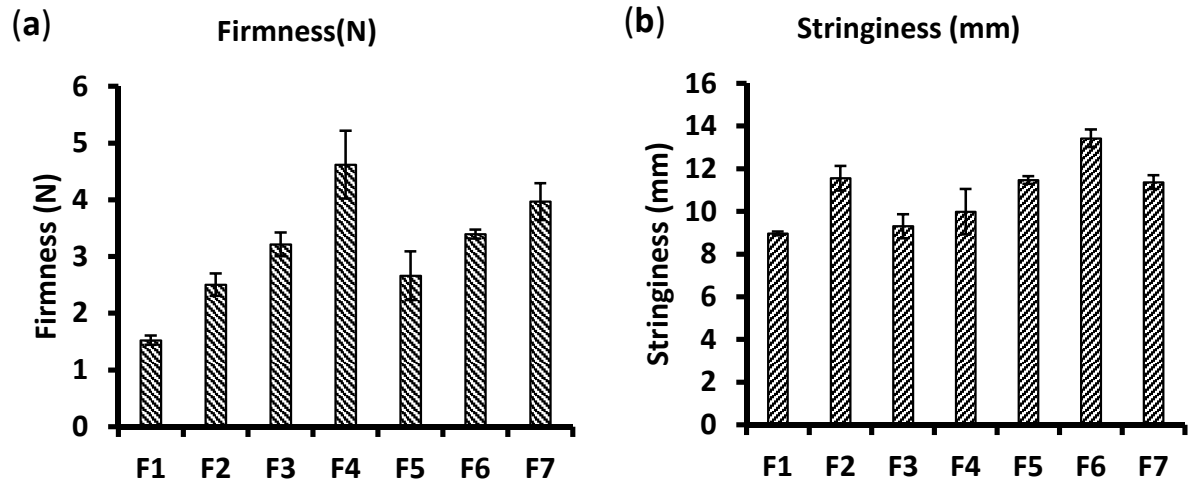


Figure 6:a) Firmness values of seven creams and; b) Stringiness value of seven creams analyzed by Texture Analyzer (TA-XT2i, Texture Tech. Corp., Scarsdale, NY) (n=3).

3.5.Rheology

Figure 7 displays the storage modulus (G') and loss modulus (G") for 7 samples obtained from amplitude sweep tests. G' represents the elastic response of the material, whereas, G" represents the viscous response. For all these samples G' has been found to be higher than G" because of the presence of structure in these materials (Table 5). At low strain, both G' and G" were found to be almost independent of applied strain. At higher strain, a decrease in both G' and G" and a crossover have been observed. Beyond crossover, the samples behave like a liquid, indicating deformation and breakdown of the structure.

Yield stress can be estimated from the amplitude sweep data using tangent analysis method, as shown in Figure 8. The F6 sample has the highest yield stress of ~ 160 Pa, and the F1 sample has the lowest yield stress of ~20 Pa.

The samples were also subjected to thixotropic loop tests. With increasing shear rate (forward) a drop in viscosity (shear-thinning behavior) was observed, whereas, viscosity gradually increases with the decreasing shear rate (backward). All these samples, as shown in Figure 9, display a hysteresis loop, which indicates that the deformed structure in the forward loop did not recover completely in the backward direction. The yield stress was found in inverse trend with globule size ($y = -9.7532x + 128.6$, $R^2 = 0.66$) as shown in Figure 10 (F6 sample was considered an outlier).

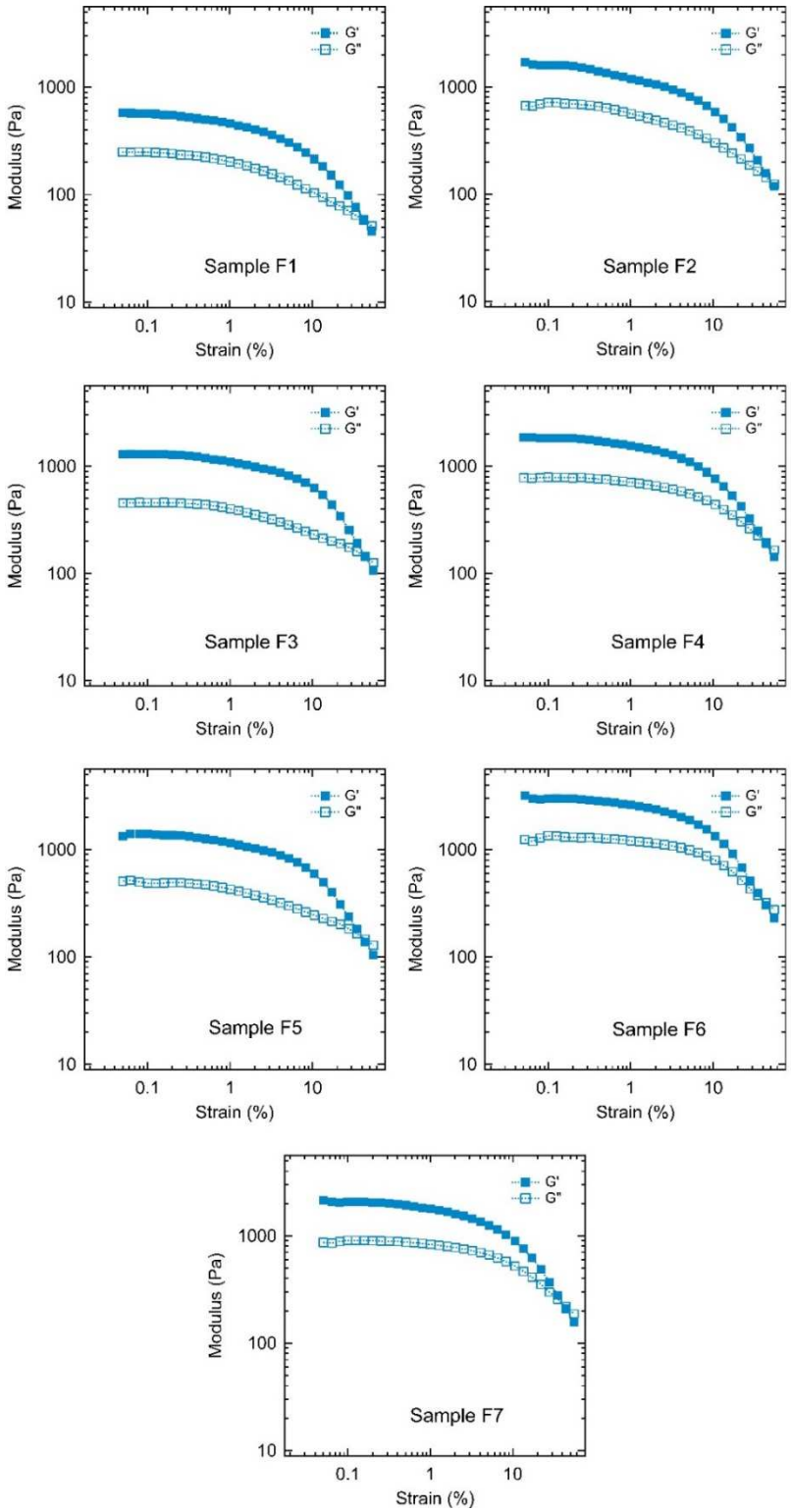


Figure 7: Rheological behavior of the creams as a function of strain amplitude obtained in oscillatory shear experiments at small frequency of 1 Hz.

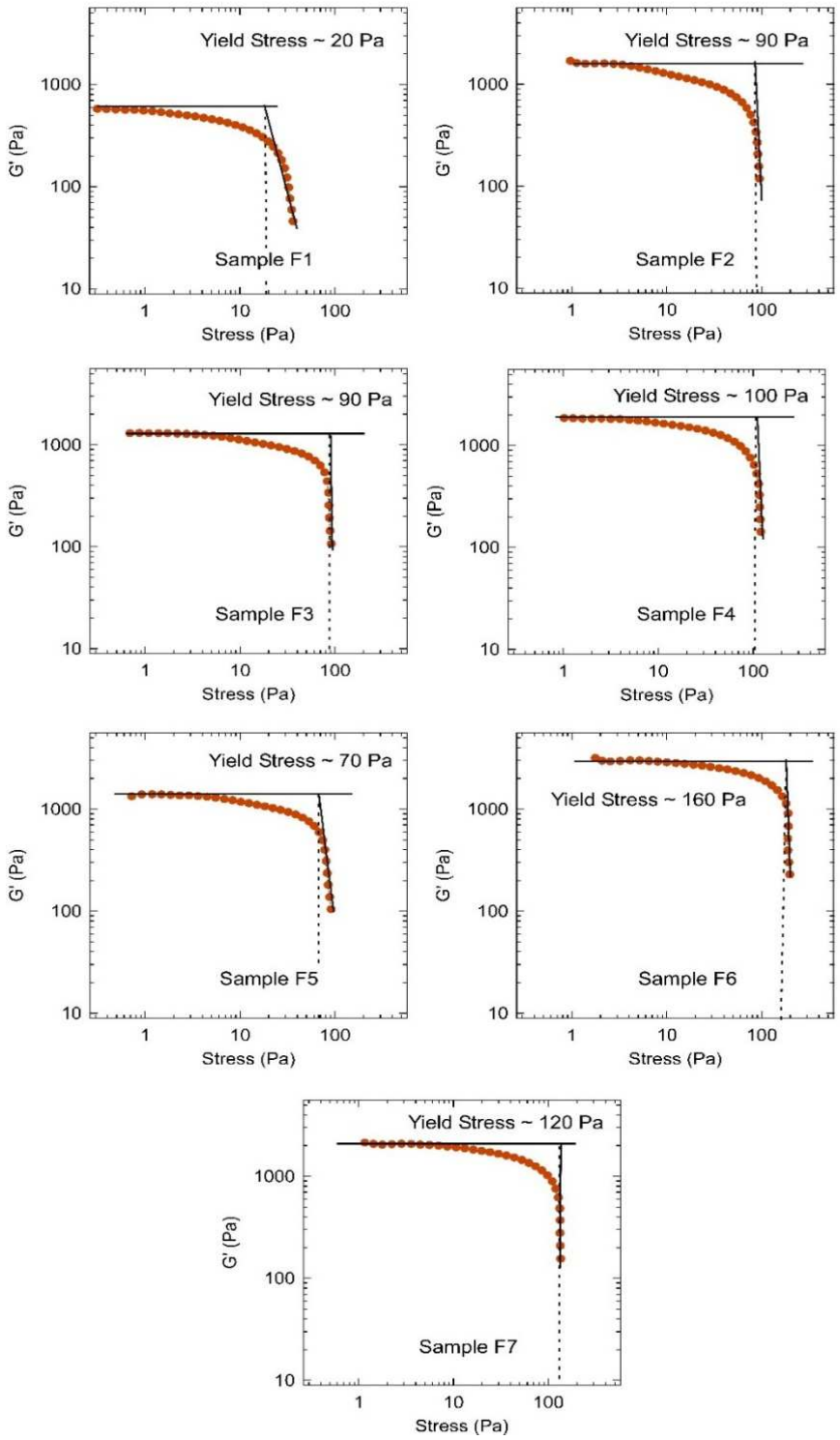


Figure 8: Yield stress of the creams obtained from tangent analysis in oscillatory strain sweep experiment at small frequency of 1 Hz.

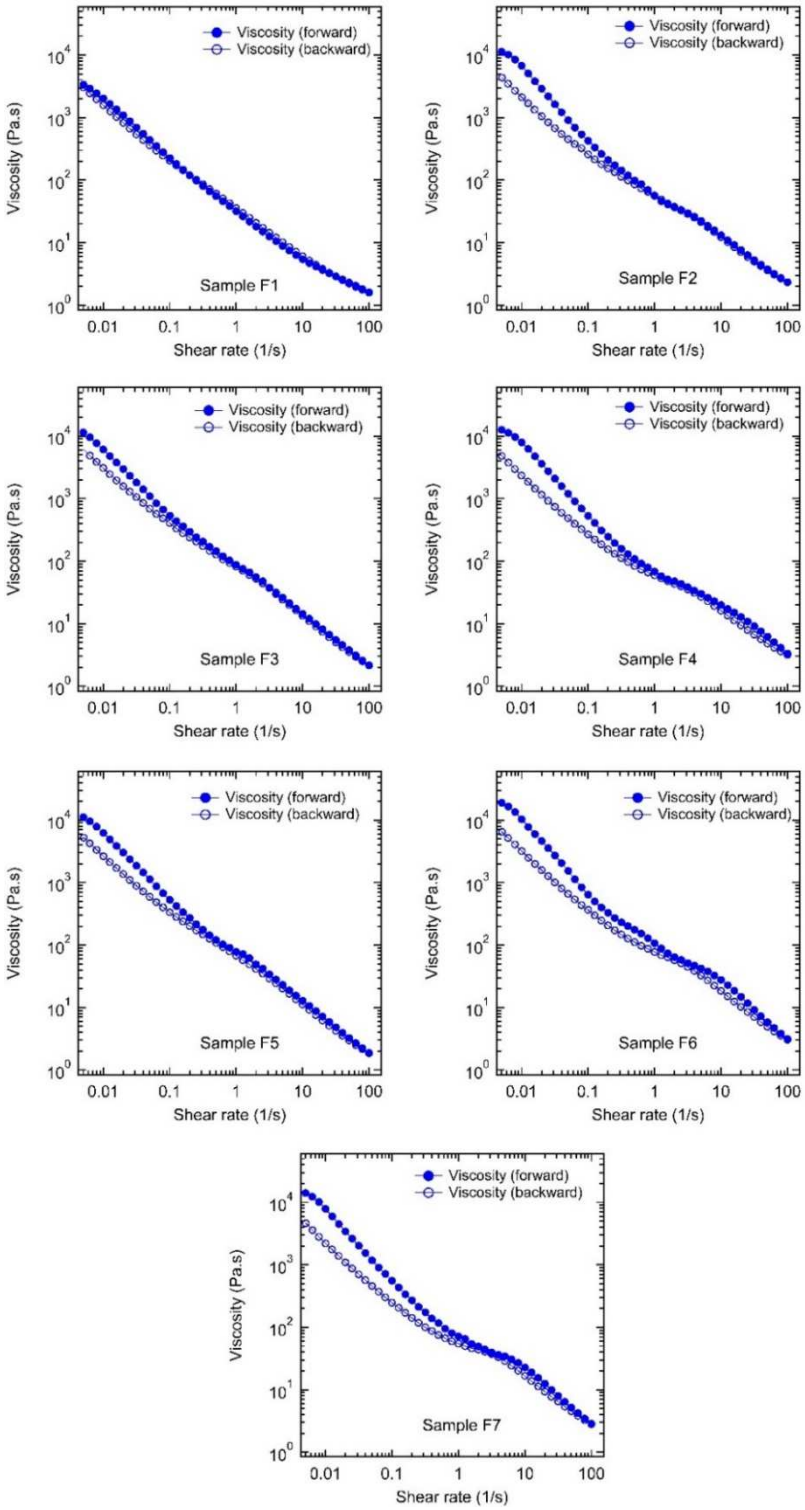


Figure 9: Hysteresis loop associated with thixotropic behavior of the creams in flow sweep experiments. The data obtained in two steps, shearing the creams from low to high shear rate followed by shearing in reverse process from high to low shear rate.

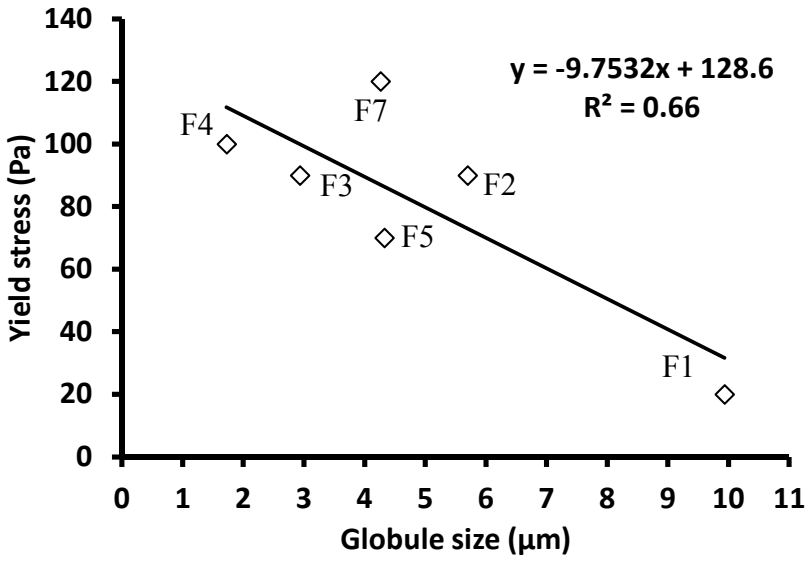


Figure 10: The relationship between the yield stress and globule size of creams.

Formulation	G' at low strain, Pa
F1	550
F2	1550
F3	1300
F4	1800
F5	1350
F6	3000
F7	2000

Table 5: Storage modulus (G') for seven creams obtained from amplitude sweep tests.

3.6.Spreading area

The spreading area (S_{max} values) for creams F1- F7, were found to be 43.3 ± 3.03, 40.9 ± 0.46, 39.6 ± 0.74, 41.8 ± 1.60, 40.2 ± 2.27, 35.9 ± 3.18 and 37.7 ± 1.56 cm^2, respectively. Apparently, the difference in yield stress or in the initial viscosity of the products did not make a notable difference in the spreading area (Figure 11). The average mean deviation of the spreading area was within 7 %.

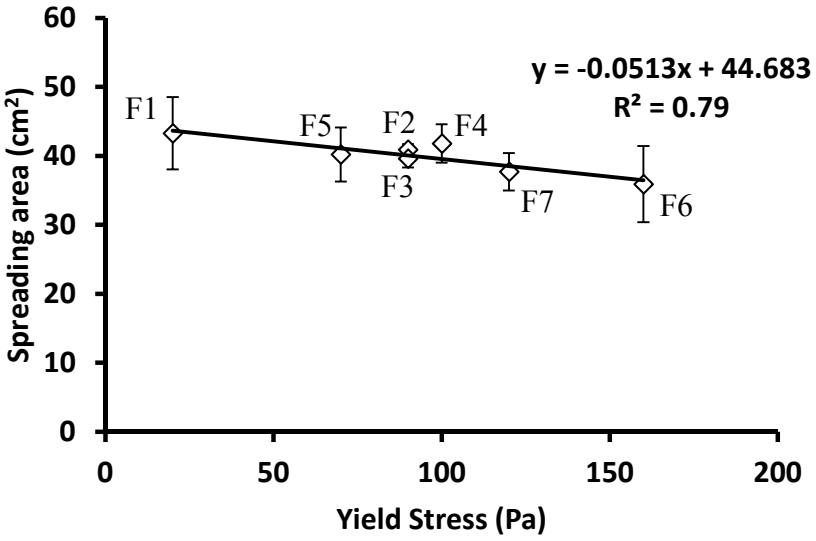
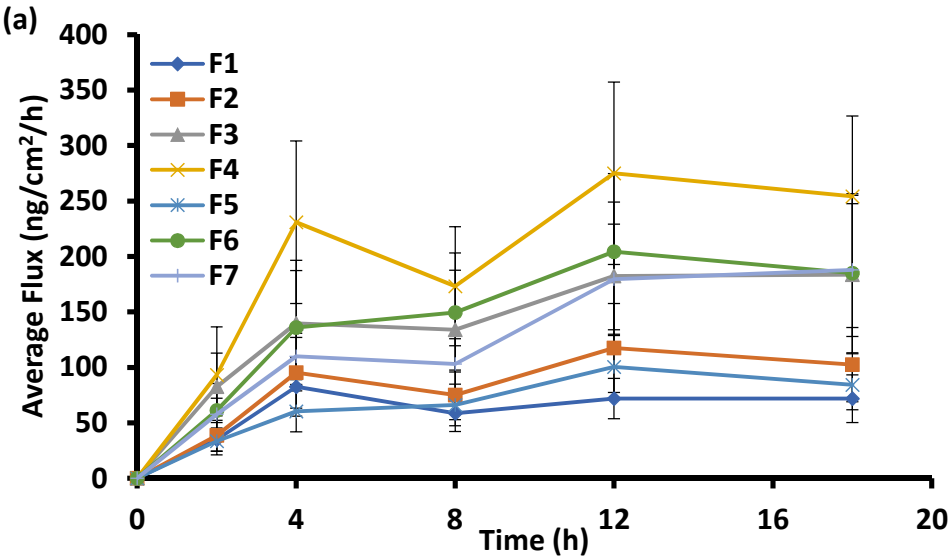


Figure 11: The relationship between spreading area of creams with their yield stress.

3.7.*In vitro* permeation profile

The *in vitro* skin permeation of nile red was evaluated across the porcine epidermis model (Figure 12-a). The results revealed that globule size has a linear correlation with maximum permeation flux. There was a decreasing trend in permeation flux (J_{max}) with an increase in the globule size linearly (y = -0.2111x + 4.0392, R^2 = 0.47) (Figure 12).



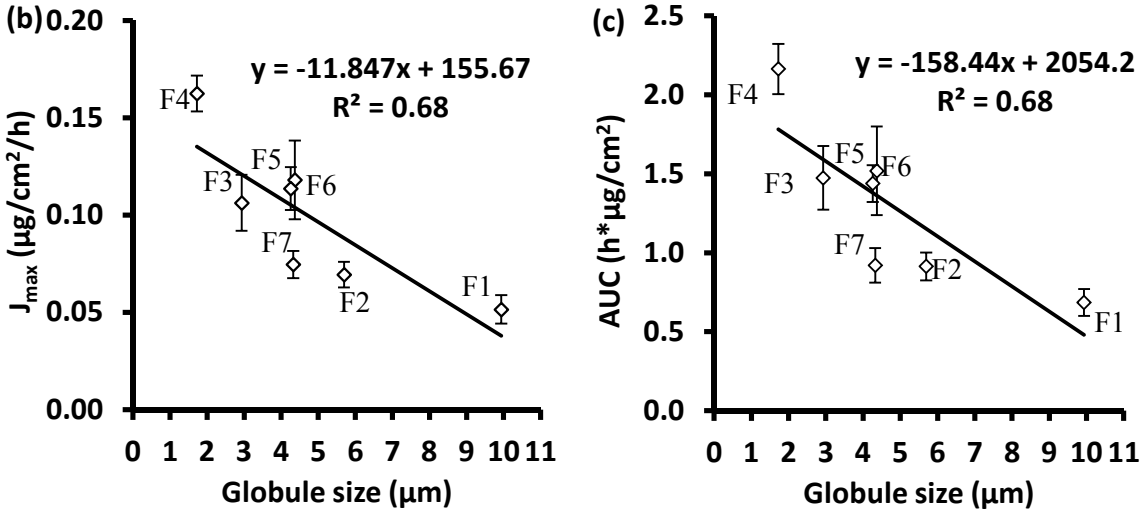


Figure 12:(a) The nile red permeation flux profile for creams; (b) The maximum permeation flux (J_{max}) of different creams depended on the globule size of creams, (c) The relationship between area under curve (AUC) of average flux with the globule size of creams.

3.8.Stability study

Stability studies results were analyzed, and globule size was measured as an indicator of any physical instability. The globule size (d_{10}, d_{50}, d_{90}, µm) of all the formulations were measured in fresh and aged samples (after 90 days storage at 25 °C/65% RH and 40 °C/70 % RH). The globule size range in the fresh samples was; d_{10} was 1.2 ± 0.16-6.7 ± 1.49 µm), d_{50} was 1.8 ± 0.20-16.2 ± 0.37 µm) and d_{90} was 2.3 ± 0.20-26.0 ±2.39 µm for custom made formulations. A significant change in globule size (d_{10}, d_{50}, and d_{90}) was observed for F1 indicating the potential failure of a system with time, as given in Table 6-a, b, and c, respectively. There were no considerable difference in between pre-and post-storage diameters in case of other creams over 90 days.

Stability conditions		Globule size distribution-d_{10} (µm)						
Time	Temperature	F1	F2	F3	F4	F5	F6	F7
0 day	-	6.7±1.49	4.8±0.63	2.2±0.33	1.2±0.16	2.9±0.46	2.8±0.74	2.8±0.58
90 days	25 °C	6.2±0.82	4.1±1.08	2.4±0.74	1.1±0.16	3.1±1.25	2.3±1.34	2.3±0.94
	40 °C	4.8±2.06	4.8±0.71	3.1±0.10	1.0±0.29	3.2±0.89	2.9±0.70	2.7±1.35

(a)

Stability conditions		Globule size distribution-d_{50} (µm)						
Time	Temperature	F1	F2	F3	F4	F5	F6	F7
0 day	-	16.2±0.37	7.8±1.55	4.4±0.25	1.8±0.20	5.1±0.65	4.2±0.70	4.3±0.63
90 days	25 °C	15.1±2.28	8.6±0.40	5.2±1.27	1.7±0.37	6.5±0.89	5.1±1.66	4.5±1.12
	40 °C	20.3±4.98	9.3±1.45	5.1±0.23	1.7±0.11	6.0±1.23	4.8±1.00	5.4±3.04

(b)

Stability conditions		Globule size distribution-d_{90} (µm)						
Time	Temperature	F1	F2	F3	F4	F5	F6	F7
0 day	-	26.0±2.39	10.8±2.71	6.8±0.27	2.3±0.20	7.8±0.46	5.6±0.62	6.0±0.38
90 days	25 °C	49.0±9.51	12.4±1.03	7.2±1.10	2.3±0.51	9.1±0.60	8.7±0.47	7.0±0.81
	40 °C	29.7±5.87	13.7±2.27	7.1±0.23	2.5±0.42	8.8±0.64	7.1±0.67	8.3±3.04

(c)

Table 6: Stability studies data of (a) d_{10} values, (b) d_{50} values, and (c) d_{90} values in the custom made creams.

4. Conclusion

The results of the study revealed that the Q1/Q2 similar semisolid topical cream formulation was influenced by critical process parameters leads to a significant difference in Q3 characteristics. The performance attributes are dependent on microstructural attributes of the creams. The difference in Q3 characteristics can influence the performance of the creams.

5. Acknowledgement

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nor does any mention of trade names, commercial practices, or organization imply endorsement by the United States Government.

CHAPTER III

EFFECT OF VARIATION IN THE AMOUNT OF EXCIPIENT ON PERFORMANCE OF TOPICAL PRODUCTS

Abstract

Surfactants are the most common inactive ingredient used in the topical drug products. Surfactants in topical products play many functional roles such as emulsifiers, permeation enhancers and solubilizers. This study was aimed to evaluate the influence of incremental change in the concentration of a surfactant (tween 80) on the quality attributes and performance of semisolid topical products. Four creams were prepared using same protocol and were similar in composition except the concentration of tween 80, which increased by 5% w/w across SF1 to SF4. The quality attributes like globule size distribution, pH, drying rate, texture properties, phase distribution and in-vitro permeation profile was characterized. The critical quality attributes did not differ significantly across the set of products. However, there was a significant difference in the permeation flux-time profile of the products. The maximum permeation flux (J_{max}) varied from SF1 to SF4 (51.25 ± 35.29 to 307.98 ± 138.89 ng/cm2/h, respectively). The reason for difference in performance of products despite having almost consistent quality attributes was systematically investigated. One of the major reasons was found to be due to the differences in thermodynamic activity and the rate of change of thermodynamic activity during the process of evaporative metamorphosis. This study confirms that the rate of change of thermodynamic activity of drug is

a critical quality attribute that can influence bioavailability and performance of a topical drug product.

Key words: Generic drug product; semisolid dosage forms; quality attribute; thermodynamic activity; topical products

1. Introduction

To ensure the pharmaceutical and therapeutic equivalency it is prudent for topical industries to use several functional excipients in their semisolid dosage form [27-31]. Every excipient performs different functions. The presence of surfactants plays multiple roles and often determines the quality attributes of the final products. In semisolid dosage form like creams and lotions, the surfactants are used as emulsifier to develop the desired type of emulsion (O/W or W/O) [28, 30, 31]. Non-ionic surfactants are most preferred for use in development of topical products. The presence of surfactants in the system can impact the thermodynamic state of API and can influence the performance of the dosage form [32]. In the literature, it has been reported that a non-ionic surfactant would change its behavior at critical micellar concentration (CMC) and may also affect the properties of other excipients in a semisolid preparation [33, 34]. Saturation of API provides a greater driving force for the penetration into the skin than a drug product with a lower saturation of API, as reported earlier by Chang et al., 2013 (e.g., highly solubilized system), which may be influenced by small change in surfactant concentration [27, 35-37]. The goal of this project was to explore and evaluate the influence of the incremental differences in the concentration of non-ionic surfactants on the quality attributes of semisolid dosage form (creams) and the performance. The incremental changes in the surfactant concentration (within 5%) between two products that meet the definition of sameness in composition (Q1/Q2) also could have significantly different quality attributes and thus performance as well.

In the present study, the concentration of tween 80 was varied at 5% in creams and the impact on the in vitro permeation testing was assessed. The quality attributes of creams were evaluated across the set of products. It was found that the thermodynamic activity of drug due to differences in the surfactant concentration was significantly different.

2. Material and Methods

2.1. Materials

Metronidazole was purchased from A K scientific, Union city, CA, USA. The formulation excipients Kolliwax CSA 70 (Cetostearyl alcohol), Cremophor A25, Cremophor A6, Kollicream IPM, Kollisolv PEG 400G, Kolliphor TPGS, Kolliphor PS 80 (Tween 80) and propylene glycol were from BASF Corporation, NJ, USA. Tefose 63, Labrafil M 1944 CS was gifted from Gattefosse, Saint-Priest, France. Mineral oil was purchased from Spectrum Chemicals, CA, USA. Span 60 was purchased from Sigma-Aldrich, St. Louis, MO, USA. Purified water was used in all the preparations.

2.2. Preparation of Creams

Topical creams were prepared with metronidazole as a model drug which predominantly localizes in the solvent phase of an o/w emulsion. The composition of the cream formulation is given in table 7. To investigate the effect of tween 80 on the characteristics and performance of creams, they were prepared in progressive change of NMT ± 5% from SF1 and SF4. Creams were prepared such that all formulation ingredients remain same except tween 80, wherein the variation imparted. Metronidazole was weighed (0.75 % w/w of cream weight) and dissolved in propylene glycol while preparing the formulations. First, the aqueous phase components excluding propylene glycol were melted by geometric mixing in a thermostated glass vessel maintained at 80 ºC. Simultaneously, in another glass vessel, lipid phase components were heated to 80 ºC. Both the phases were mixed together in a thermostated vessel at 80 ºC using a Silverson® homogenizer (Model: L5M-A, Silverson Machines, Inc., MA, USA). Once phases were homogenized for 10 minutes it followed addition of propylene glycol. The homogenization variables were optimized for 3000 rpm speed and 20 min time, post addition of propylene glycol. After homogenization for

a specified time, a controlled gradual cooling protocol was followed until the temperature of formulation reached 25 ºC in 25 mins.

Ingredients	SF1 (% w/w)	SF2 (% w/w)	SF3 (% w/w)	SF4 (% w/w)
Mineral oil	15	15	15	15
Cremophor® A6	1.5	1.5	1.5	1.5
Cremophor® A25	1.5	1.5	1.5	1.5
Kolliwax® CSA 70	7	7	7	7
Kollicream® IPM	3	3	3	3
Tefose® 63	1	1	1	1
Labrafil® M 1944 CS	1	1	1	1
Kollisolv® PEG 400 G	5	5	5	5
Kolliphor® TPGS	1.2	1.2	1.2	1.2
Tween 80	**1.21**	**1.27**	**1.33**	**1.39**
Span 60	2	2	2	2
Propylene glycol	10	10	10	10
Drug (Metronidazole)	0.75	0.75	0.75	0.75
Water (q.s.to 100%)	49.84	49.77	49.71	49.65

Table 7: Composition of cream formulations.

2.3. Critical quality attributes:

2.3.1. Microscopy and globule size

For microstructural observation under a microscope, 10 mg of cream was applied on a clean glass slide. A thin film was prepared using a film applicator with the gap adjusted to 10 µm between applicator blade (Gardco® Microm II applicator blade, Japan) and glass slide. With the application of slight pressure on the slide, the applicator was pulled slowly to form a uniform film on the slide. The slide was observed under an Olympus IX inverted research microscope using a 10X objective. The images were obtained and processed using Zen Lite software. For each cream, we considered the mean diameter of randomly distributed 50 globules of each image (n=3/cream), measured using the Zen Lite software. The d10, d50, and d90 are commonly used to represent the midpoint and range of the globule sizes of a given sample [12, 13]. The 10%, 50%, and 90%, ‘d’ values were

determined from the S-curve of cumulative distribution.

2.3.2. pH measurement

In-Lab Micro® pH probe was calibrated using buffered pH standards of pH 4, 7 and 10. 1 g of cream was filled in a container and tapped to remove any entrapped air. A standard buffer solution of pH 4 and of pH 10 was used to check the accuracy of the probe before each measurement. After each measurement, the probe was washed with DI water and 70 v/v % ethanol, alternatively in three cycles. Similarly, the pH of the aqueous phase was measured after phase separation by ultra-centrifugation (TLA55 rotor, Beckman Coulter Inc., USA), at 55000 rpm for 4 h. The pH of each cream and its respective aqueous phase was recorded as an average of three replicate measurements.

2.3.3. Water activity

Water activity was measured using Aqualab® water activity meter (Series 3E, USA). 1 g of cream was dispensed into the sample cup using a positive displacement pipette. Creams were spread uniformly on the sample cup holder evenly using a glass rod. The sample cup holder was placed in the sample chamber until an equilibration value was attained. The sample was later replaced with a cup holder containing activated charcoal in the sample chamber to clear any residual humidity in the chamber.

2.3.4. Rate of drying

A known amount (120 mg) of cream sample was dispensed and spread uniformly in a plastic cup having surface area 8.0 cm^2 (15 mg/cm^2). These cups were placed an incubator oven (Fisher Scientific Isotemp 550D Incubator Oven) at 32 °C for 360 mins and change in mass was recorded at every 10 minute interval during the first 60 mins followed by every 30 mins till the weight of the residual content become constant. Thus, the loss on drying at each time point was estimated as

percent loss by the weight (% loss of weight = [(initial weight−dried weight)/initial weight] x100).

2.3.5. Texture analysis

Texture analysis was conducted at room temperature (25 ± 1 °C) using a Texture Analyzer (TA-XT2i, Texture Tech. Corp., Scarsdale, NY) equipped with a TA-3 acrylate cylindrical probe. The test was conducted in compression mode. The conditions for analysis and set of parameters used are given in table 8. The texture analyzer is equipped with an analytical probe connected to a motor that enables vertical probe displacements to be measured. The cream was filled in the base plate having aperture of 30 mm diameter fixed to the base plate. The probe was allowed to penetrate into the cream and retracted to measure different texture parameters (work of adhesion, firmness, and stringiness) from the curve. Detectors recorded sensor position and resistance to its displacements. All measurements were recorded in triplicate every time in compression mode [32].

2.3.6. Rheology

Rheological studies were conducted using a TA HR2 rheometer (TA Instruments, New Castle, DE) equipped with 20 mm parallel plate. The experiments were conducted at 32 °C and the temperature was controlled using a Peltier stage. To avoid sample slippage at the rheometer plates, the plates were covered with adhesive backed 600 grit sand papers. A gap of 500 μm between the parallel plates was used for the rheological investigations. After loading the samples in between the plates, the samples were allowed to relax for 5 minutes. The relaxation process was captured using time sweep experiment (low frequency and low strain). Amplitude sweep test and thixotropic loop test were conducted on each sample. The samples were allowed to relax for 5 minutes in between two experiments [33-35].

S.No	Parameters 1	Compression mode
1	Pre-test speed (mm/sec)	2
2	Test speed (mm/sec)	2
3	Post-test speed (mm/sec)	10
4	Trigger type	Auto force
5	Trigger force (g)	5.0
6	Target mode	Distance
7	Distance (mm)	2
8	Return distance (mm)	5
9	Points per second	500
10	Probe	TA-3 soft matter acrylic

Table 8: Texture analysis parameter used to evaluate work of adhesion.

2.3.7.1. HPLC Instrumentation and Conditions

A Shimadzu (Kyoto, Japan) UFLC (LC-20AD) system consisting of SIL-20AC autosampler connected with a UV/VIS PDA detector (SPD-M20) and LC-20 AD solvent delivery module was used for detection and separation. Chromatographic separations were performed using reverse-phase chromatography using a waters symmetry C18 5 µm, 150 mm × 4.6 mm (Waters, Milford, CA, USA). The flow rate was 0.5 mL/min and detection wavelength was 319 nm and sensitivity was set at 0.1 a.u.f.s (absorbance units full scale). The mobile phase used was composed of 0.01 M phosphate solution (KH_2PO_4) and acetonitrile (85:15 % v/v, pH 4.7) degassed and filtered through a 0.45 µm filter (Millipore, Saint-Quentin, Yvelines, France) prior to use. The injection volume was 25 µL and the run time was 8 min.

2.3.7.2. *In vitro* release profile

Drug release study across the dialysis membrane (25 kD) was performed using vertical Franz cell apparatus (Logan Instruments, Somerset, NJ). Dialysis membrane was sandwiched between the

two chambers (donor and receiver) of a Franz diffusion cell with an active diffusion area of 0.64 cm2 with the temperature maintained at 32 ± 1 °C using a water circulator. The receiver compartment was filled with 5 mL of freshly prepared phosphate buffer saline (PBS) (pH 7.4). Infinite dose (120 mg/cm^2) of cream was applied uniformly across the active diffusion area of the membrane. Release study was carried out for a period of 24 h and samples were analyzed using Shimadzu UFLC 20A series system.

2.3.8. *In vitro* permeation profile

2.3.8.1. Preparation of porcine epidermis

Freshly excised porcine skin was washed with saline and subcutaneous fat was removed. Epidermis was isolated by heating the skin for 2 min at 60 °C. The isolated epidermis was dried and stored at 4 °C, until further use. Before mounting the epidermis for permeation study, it was thawed for 1 h at room temperature. The epidermal barrier integrity was tested by measuring Trans-epidermal electrical resistance (TEER). The epidermis having a TEER value ≥ 20 k Ω cm^2 and without physical damage was used for in vitro drug permeation studies.

2.3.8.2. Experimental setup

Permeation study across the epidermis was performed using vertical Franz diffusion apparatus (Logan Instruments, Somerset, NJ). The epidermis was sandwiched between the two chambers (donor and receiver) of a Franz diffusion cell with an active diffusion area of 0.64 cm^2. The skin temperature was maintained at 32 ± 1 °C using a water circulator. Receiving compartment was filled with 5 mL of freshly prepared phosphate buffered saline (PBS) (pH 7.4). Finite dose of cream was applied (15 mg/cm^2) uniformly across the active diffusion area of the epidermis. The permeation studies were carried out for a period of 12 h and samples were analyzed using Shimadzu UFLC 20A series system.

2.3.9. Solubility study

Distribution of solubilized metronidazole in aqueous phase was evaluated by phase separation study using ultra-centrifugation (TLA55 rotor, Beckman Coulter Inc., USA) at 55000 rpm for 4 h. Separated aqueous phase and lipid phase was isolated and subjected to HPLC analysis by Shimadzu UFLC 20A series system. The super-saturation solubility of metronidazole was evaluated by dissolving excess amount of metronidazole in separated aqueous phase of individual formulations followed by analysis.

2.4. Thermodynamic activity

Thermodynamic activity was evaluated for SF1 to SF4 creams considering saturation solubility of separated aqueous phase and drying rate. The change in the metamorphosis resulted due to loss of content by drying can cause saturation solubility. Thermodynamic activity was calculated using formula given below:

Activity coefficient (a)= Caq / Cs …. equation 1

Percentage increase in activity coefficient $= [(a_t - a_o)/ a_o] *100$ …. equation 2

Where, Caq is concentration of API in the aqueous compartment (remaining at the site of application); Cs is saturation solubility of API in aqueous phase (isolated from the formulation); a_o is initial activity coefficient in formulation and a_t is activity coefficient at time 't'.

3. Results and Discussion

3.1. Critical quality attributes of products

The four cream formulations were prepared from SF1 to SF4 using composition shown in table 7 were evaluated to determine the quality attributes.

The microscopy was used to image the globules in the cream products (Figure 13). The globule size distribution d10, d50, d90 values of all cream formulations are given in table 9. The average

globule size, d10, d50 and d90 values were found to have no statistically significant difference across all the products.

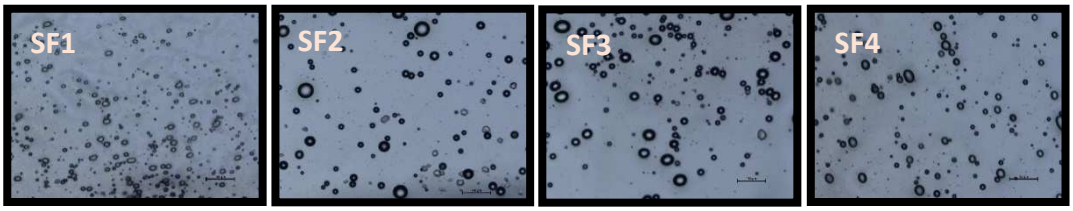

Figure 13: Images of creams at 10X magnification using an Axiovert 200 M Inverted Research microscope.

Formulation	Average Size (µm) (Mean ± SD)	d10 (µm) (Mean ± SD)	d50 (µm) (Mean ± SD)	d90 (µm) (Mean ± SD)
SF1	20.7±16.54	9.5±3.59	32.8±13.73	56.3±26.18
SF2	19.3±14.81	10.5±2.33	30.4±1.94	49.1±11.47
SF3	19.1±16.79	8.6±0.30	31.5±3.12	57.8±12.24
SF4	14.4±10.98	6.4±1.32	21.6±2.73	38.7±2.00

Table 9: Globule size distribution of all cream formulations.

The pH of all creams was in the range of 5.49-5.60 and the separated aqueous phase had pH value in the range of 5.29-5.60 (table 10). Therefore, the percent of ionized and unionized fraction of API in all the aqueous phase would be comparable across all products. The drug being hydrophilic in nature, dissolved drug would be present in the aqueous phase predominantly.

Water activity represents the thermodynamic energy status of water present in a pharmaceutical system. Water activity would influence the rate of evaporation of water from the site of application leading to rapid metamorphosis changes in the cream. The water activity of all the creams was found to be in the range of 0.897 to 0.917 (table 10). Incremental increase in tween 80 concentration did not seem to impact the water activity of creams, likely because they all contained

a profound amount of water in the continuous phase. The drying profile is a function of water activity. The difference in drying curve profile across all the products was within about 10% difference although the profiles were discriminatory statistically (figure 14). The loss of content might lead to change in the metamorphosis resulting in saturation of API in the aqueous phase.

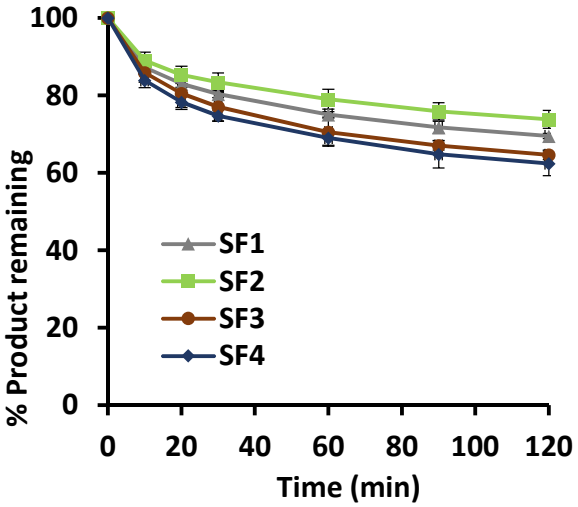

Figure 14: Graph representing drying rate of four creams (n=3).

Code	Amount of surfactant in creams (%, w/w)	Amount in aqueous phase (mg/g)	pH		Water activity	IVRR slope (µg/cm^2/h)	Work of adhesion (g.sec)	Viscosity (Pa.s) at Shear rate 100 1/s	Yield stress (Pa)
			Cream	Aqueous phase					
SF1	1.21	5.17±0.05	5.60±0.15	5.60±0.16	0.912±0.007	245.17±36.25	214.37±14.45	3.37	190
SF2	1.27	5.55±0.10	5.49±0.08	5.47±0.04	0.899±0.008	258.12±32.93	217.12±13.03	3.40	132
SF3	1.33	6.10±0.07	5.54±0.04	5.55±0.14	0.897±0.003	266.13±40.94	221.84±13.90	2.95	132
SF4	1.40	4.43±0.20	5.57±0.04	5.29±0.07	0.917±0.007	233.78±27.39	244.30±26.48	3.56	122

Table 10: Characterization and quality attributes of four creams (n=3).

Work of adhesion represents the total amount of energy involved in withdrawal of the probe from the surface and determined by the area under the force versus time curve (table 10). The work of adhesion showed an increasing trend as the surfactant concentration increased. However, there was no statistically significant difference in work of adhesion values across the products. Rheological evaluation (figure 15-a and b) displayed in figure the stress (Pa) and shear rate (1/s) along with storage modulus (G') with stress (Pa) for cream samples obtained from amplitude sweep tests. G' represents the elastic response of the material all these samples. Yield stress can be estimated from the amplitude sweep data using tangent analysis method. The SF1 sample has the highest yield stress of ~ 190 Pa, and the SF4 sample has the lowest yield stress of ~122 Pa (table 10). It is less likely that this difference in yield stress would have any impact on the in vitro permeation profile of drug from these formulations. The samples were also subjected to thixotropic analysis. With increasing shear rate (forward) a drop-in viscosity (shear-thinning behavior) was observed indicating that all were shear thinning systems.

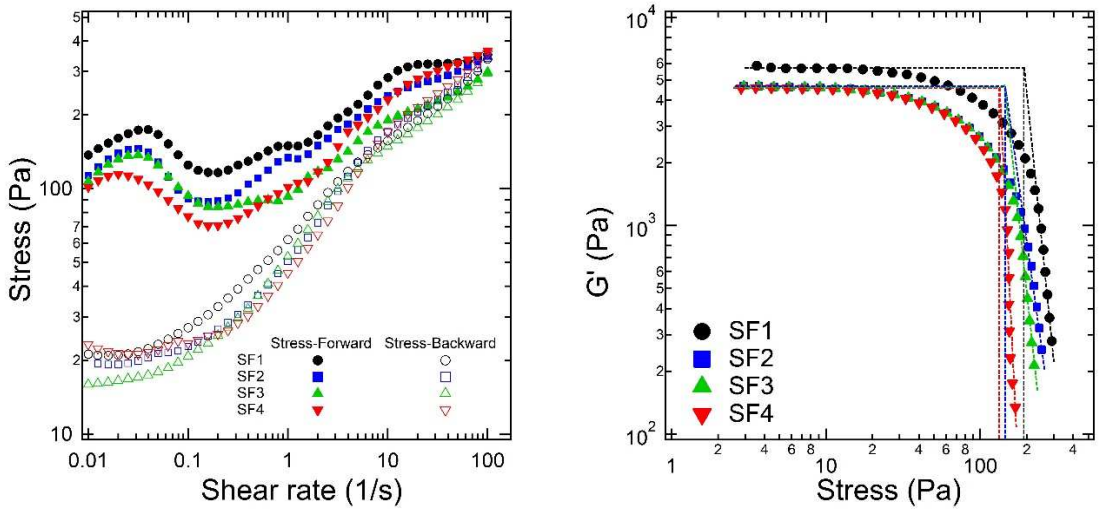

Figure 15: Rheology of creams a) Graph representing the stress vs shear rate of five cream formulation; b) Graph representing storage modulus with stress of four creams.

The in vitro release rate did not differ significantly across SF1-SF4 due to the reason that the formulations did not differ in viscosity significantly (figure 16-a). For the sake of comparison, the viscosity at 100/s is tabulated (table 10). The amount of drug in aqueous phase plays a major role in determining the drug permeation from o/w cream products, especially when the drug is hydrophilic in nature. In the present case, the amount of drug present in the aqueous phase of the creams was not different significantly. Incremental changes in the surfactant concentration did not alter the distribution of drug between aqueous and oil phase of the cream product either.

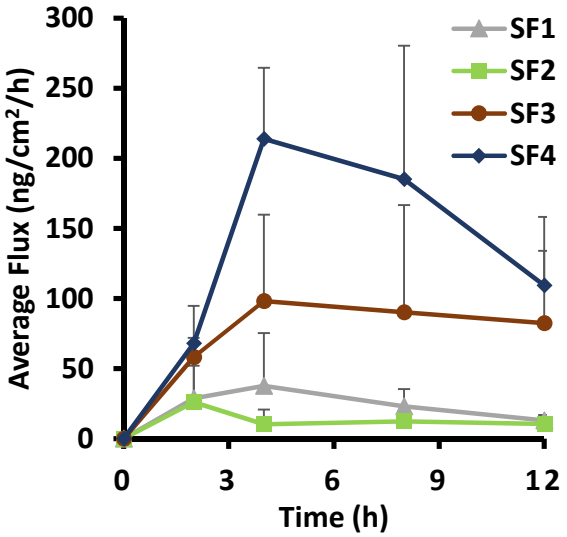

Figure 16: *In vitro* permeation profile (IVPT) of four creams

3.2. In vitro permeation profile

The in vitro permeation profile of all creams between two donors (n=3) showed difference in maximum permeation flux (J_{max}) and cumulative amount permeated (AUC). The Jmax (0-12h) varied with the change in tween 80 significantly (figure 16-b). Particularly if one compares the flux profile of SF2 and SF3 (table 11), there was almost an about 6-fold increase in the bioavailability (t-test, $p<0.05$) of drug from these two products. This was highly intriguing due to the fact that the two products which are compositionally similar (in agreement with Q1 and

Q2 definition by FDA) and prepared using exactly same protocol. Despite having all the quality attributes extremely overlapping, differed significantly in the in vitro permeation profile which lead to a significant different permeation profile of the drug.

Code	Permeation flux (J_{max}, ng/cm^2/h)	Area under curve (AUC, h*ng/cm^2)
SF1	51.25±35.29	289.81±228.87
SF2	41.20±22.33	154.53±86.38
SF3	149.44±93.96	936.78±669.55
SF4	307.98±138.89	1736.50±636.14

Table 11: *In vitro* permeation flux and area under the curve bioavailability data of four creams (two donors, n=3).

3.3. Exploring the reason for differences in performance of SF2 and SF3

When the composition between different products do not differ significantly and when quality attributes are identical, the differences in performance could be attributed to the differences in microstructural arrangement of matter between the products. However, the microstructural characteristics are challenging to quantify. If there are any microstructural differences existing across different products, that would perhaps lead to differences in drug distribution between different phases coexisting in the product. Metronidazole being an aqueous soluble compound, it is likely that the amount of drug in the aqueous phase would influence the extent of drug permeation across the skin. Therefore, it was decided to separate the aqueous phase and quantify the amount of drug.

Any difference in drug distribution profile between aqueous phase and oil phase, could significantly contribute to variation in the drug permeation profile. Therefore, the drug distribution between the aqueous and oil phase was measured. The drug concentration in the

aqueous phase was found not to differ significantly across all formulations, especially between SF2 and SF3. Although concentration is important in determining the drug permeation, the driving factor would be the thermodynamic activity of the drug in aqueous phase. Thermodynamic activity for a molecule is generally considered as the ratio of concentration of drug to solubility in the respective medium. As the pH of the formulation and the aqueous phase was not significantly different between the two products SF2 and SF3, extent of ionization of drug would be comparable. Therefore, to calculate the thermodynamic activity of the drug, the saturation solubility of aqueous phase separated from SF2 and SF3 by centrifugation was determined (table 6). The initial thermodynamic activity or activity potential of the drug in the aqueous phases of these two formulations is given in table 12.

Critical Quality Attributes	**SF1**	**SF2**	**SF3**	**SF4**
Amount of drug in the aqueous phase (mg/g)	5.17±0.05	5.55±0.10	6.10±0.07	4.42±0.20
Saturation solubility of drug in the aqueous phase (mg/g)	6.05±0.08	5.64±0.23	10.48±0.23	9.19±0.25
Initial thermodynamic activity	0.855	0.984	0.582	0.482

Table 12: Critical quality attributes observed in four creams (n=3).

Considering the activity potential of drug in the aqueous phases, one would expect SF2 to perform better over SF3. However, SF3 was found to outperform the SF2 despite the initial thermodynamic activity of drug in SF2 was significantly higher than SF3 (figure 17). Evaporative metamorphosis is a phenomenon that occurs at the site of application of the formulation. The solvent and volatile contents will evaporate leading to change in concentration of the drug in the remnant vehicle. The rate at which the thermodynamic activity of the drug changes with time depends on the initial thermodynamic activity potential of the drug in the formulation vehicle and drying rate of the formulation. One can calculate the activity potential

of drug at different time points using the solvent fraction remaining in the formulation and concentration of drug in the remnant vehicle (figure 17). It appears that the difference in performance between SF2 and SF3 could be emanating from the differences in the rate of change of activity potential of the drug in the formulation at the applied region. Figure 17 shows the profile of change of thermodynamic activity with time in SF2 and SF3 formulation. From the figure it is evident that although the SF3 had a lesser thermodynamic activity of drug to start with, the rate of change of activity potential of drug in the formulation was relatively more drastic in SF2 nearing saturation compared to SF3. Although at this point it is not clear what is the optimal rate of change of thermodynamic activity for achieving optimal performance of the product and it requires systematic investigation using APIs of different physicochemical characteristics and different solvent systems.

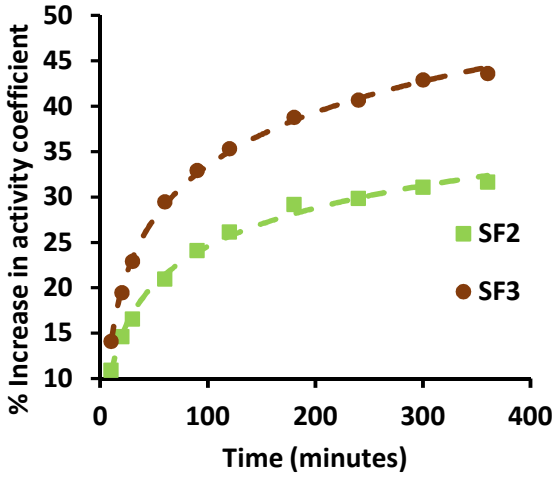

Figure 17: Graph representing initial change in thermodynamic activity of SF3 and SF4 creams with time.

4. Conclusion

The results from this study revealed that the systematic variations in surfactant concentration in semisolid topical creams can influence the performance attributes. This study illustrated that

within 5% variations in tween 80 can progressively influence the thermodynamic activity of drug products. It was evident that SF3 had a significant rapid increase in the thermodynamic activity coefficient as compared to SF2 with evaporative metamorphosis. The results also showed that variations in tween 80 can alter the texture of a cream, which may influence a patient's perception of product quality. In this study, the change in thermodynamic potential during metamorphosis due to slight variations in surfactant concentration has shown that it could potentially alter the rate and extent of bioavailability (permeation flux and AUC). Our limited results are thereby suggestive of a possible mechanism whereby 5 % changes of surfactant quantity could potentially influence the performance of a hydrophilic compound in a topical drug product.

5. Acknowledgement

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CHAPTER IV

BUFFER CAPACITY AN IMPORTANT QUALTIY ATTRIBUTE OF TOPICAL SEMISOLID DOSAGE FORMS

Abstract

The natural pH on the skin surface lies in the acidic range (pH 4 - 6) as its inherent property due to the skin's buffering capacity. Topical products such as creams are made with certain pH to favor the permeation of drugs. Any change in the pH of topical product could significantly affect the extent of permeation of acidic or basic drugs. In this study, the influence of buffering capacity of skin on the change in pH of topical semisolid products (creams) at the site of application was investigated. Six custom-made O/W creams were formulated with qualitatively and quantitative (Q1/Q2) similarity by varying the pH of aqueous phase (deionized water: F1-F3) and using three buffers having pH range from 3.9 to 8.9 (F4-F6). Creams were prepared with a consistent manufacturing protocol and a controlled cooling protocol. Colorimetric evaluation was performed to study the change in the pH over time profile of creams. *In vivo* studies in human volunteers revealed that the pH of the formulations prepared using DI water having pH 7.0 and 8.5 changed significantly just in one hour after application on subject's volar forearm skin, whereas creams prepared using buffers resisted the change in pH. It is also evident from the data that the change in pH is driven not by the formulation's interaction with the atmosphere but rather due to the physiological response of the skin tissue. In the case of

formulations having DI water with pH 7.0 and 8.5 in visual colorimetric analysis, the color change was distinct. Creams prepared imparted with buffer capacity has the potential to overcome the skin physiological response by resisting any change in the pH of topical product. It is evident that the buffer capacity is one of the most critical quality attributes that determine the performance of topical products.

Key words: Topical creams; pH; Buffer capacity, Quality attribute, Skin

1. Introduction

The pH values of skin range in the acidic value due to the acid mantle present on the skin as its inherent property [42]. Many reports demonstrated how the barrier function is compromised with the change in skin pH, as indicated by a change in the biophysical parameters of barrier function (TEWL and TEER) [43-48]. It has been reported that several key enzymes present in the skin are involved in the development and maintenance of its competent barrier property which can be largely influenced by skin pH [48, 49]. The acidic pH also favors the adhesion of resident skin microflora and the formation of stratum corneum. Overall, the importance of skin pH in relation to its function and integrity of the skin is significant [49-50]. The internal body's environment maintains an approximately neutral pH (ranging between 7–9), which creates a pH gradient of 2–3 units between the stratum corneum and underlying epidermal and dermal layer [45,49, 51]. The role of stratum corneum as a formidable barrier balance between its hydrophobic character, distribution of lipids, and organization of lipids into a series of lamellar bilayers [48, 51]. In addition, the skin has been known to be associated with excellent buffering potential to resist the change in pH of skin against external pH influencing factors. The pH of the skin was found to recover to its baseline after exposure to strong acid or alkali showing the existence of strong tissue buffer in the skin [46, 47]. There can be a range of factors contributing to this buffering capacity which include, proteins, keratin, sweat glands, epidermal water-soluble constituents, the thickness of stratum corneum, etc. All these factors have been reported to establish the skins buffering capacity. Buffering capacity of skin can impact the performance of topical products. Topical products are designed and prepared to enhance the permeation potential of active molecules on the skin surface. Physio-chemical properties of active molecules are critical for topical formulations to enhance its performance, which is reported to

follow pH partition theory. pH-partition theory is well accepted in concept in pharmaceutics and evidently most of the drugs in the unionized form are relatively more permeable compared to the ionized form of drugs across the biological barriers [51, 52]. Therefore, the pH of the topical formulations is often adjusted to render most of the drug in the unionized form for the successful permeation of drugs. Any change in the formulation pH could significantly affect the extent of permeation of acidic or basic drugs. The primary objective of this project was to explore if the skin has the ability to bring the formulation pH in homeostasis with the skin. The secondary objective was to investigate the significance of considering buffer capacity as an important quality attribute in the topical products.

2. Experimental

2.1. Method for pH measurement of creams

The pH of creams was measured using three pH probes (InLab Science Pro®, InLab Viscous®, and InLab Micro®) and skin was measured using surface probe (InLab Surface Pro-ISM) attached to Mettler Toledo (Model: S 220, Seven compact pH/Ion, Mettler-Toledo GmbH, Analytical, Switzerland) [27]. The pH meter was calibrated each time before use with 3-point calibration using buffers (pH 4, 7 and 10). The built-in temperature probe was maintained at 25 °C. The slope of 95 % - 105 %, offset -15 to +15 mV and drift less than 3 mV was considered during the studies while calibration and cleaning of each electrode.

2.2. Preparation of creams

a. Composition and process

Topical creams were prepared with composition as described in table 13(a and b). Creams were prepared such that all formulation ingredients remained same except the aqueous component (either water or buffer was used). Briefly, the aqueous phase components

excluding propylene glycol were heated by geometric mixing in a thermostated glass vessel maintained at 80 °C. Simultaneously, in another glass vessel, lipid phase components were heated to 80 °C. Both the phases were mixed together in a thermostated vessel at 80 °C using a Silverson ® homogenizer (Model: L5M-A, Silverson Machines, Inc., MA, USA). Once the phases were homogenized for 10 minutes, it was followed by the addition of propylene glycol. The homogenization variables were optimized for 3000 rpm speed and 20 min time, post addition of propylene glycol. After homogenization for a specified time, a controlled gradual cooling protocol was followed until the temperature of the formulation reached 25 °C in 25 mins.

b. pH adjusted creams

i. **DI water creams**: In these creams pH of DI water was adjusted to pH 4, 7 and 9 using 0.1 N HCl and 0.1 N NaOH. The preparation of creams followed the procedure as described above.

ii. **Buffered creams**: In these creams, different buffers were selected as aqueous phase. The citrate buffer was used to prepare creams of pH 4, phosphate buffer was used to prepare creams of pH 7.4 and borate buffer was used to prepare creams of pH 9. The preparation on creams was followed a similar procedure as described above.

Ingredient	Quantity (% w/w)
Cetostearyl alcohol	7
Mineral oil	12
Cremophor A25	1.5
Cremophor A6	1.5
Propylene glycol	8
DI Water / Buffer	70

(a)

Formulations	pH of Aqueous phase	
	Phosphate Buffer	DI water
F1	-	3.6
F2	-	7.7
F3	-	10.1
F4 (Citrate buffer)	3.9	-
F5 (Phosphate buffer)	7.6	-
F6 (Borate buffer)	8.9	-

(b)

Table 13: (a) Basic composition of cream formulation; (b) Formulation prepared with variations in the pH of buffer and DI water as the aqueous phase

2.3. pH measurement of creams

Measuring cream pH has been found to be critical as it offer lots of variability due to its complex matrix. We developed and validated a measurement method used for the pH of creams. pH of creams was measured by allowing cream to equilibrate around the probe and pH was measured. The probe was immersed in the cream, with care to ensure that the tip of the probe was completely covered and reach equilibrium by the samples.

Three pH probes (InLab Science Pro®, InLab Viscous®, and InLab Micro®) were initially identified from reports in the literature and recommendations by the manufacturer for this study. The probes were calibrated using buffers of pH standard 4, 7 and 10. Enough quantity of cream was filled in a container and tapped to remove any entrapped air. The probe was immersed in the cream-filled in the container, with care to ensure that the tip of the probe was completely covered in the cream. After measuring the pH, the cream was wiped off and the probe was rinsed sequentially with 70 % (v/v) ethanol and deionized (DI) water, and its performance was

verified with standard buffers. If the confirmatory pH measurement of the pH standard buffers differed by 0.5 pH units or more, the probe was cleaned again using the protocol above, as represented in figure 18. While measuring the pH of the creams, each serial measurement was alternated with a probe cleansing and a verification of pH measurement using standard buffers of pH 4 after one measurement and of pH 10 after the next. The pH of each cream was recorded as an average of three replicate measurements.

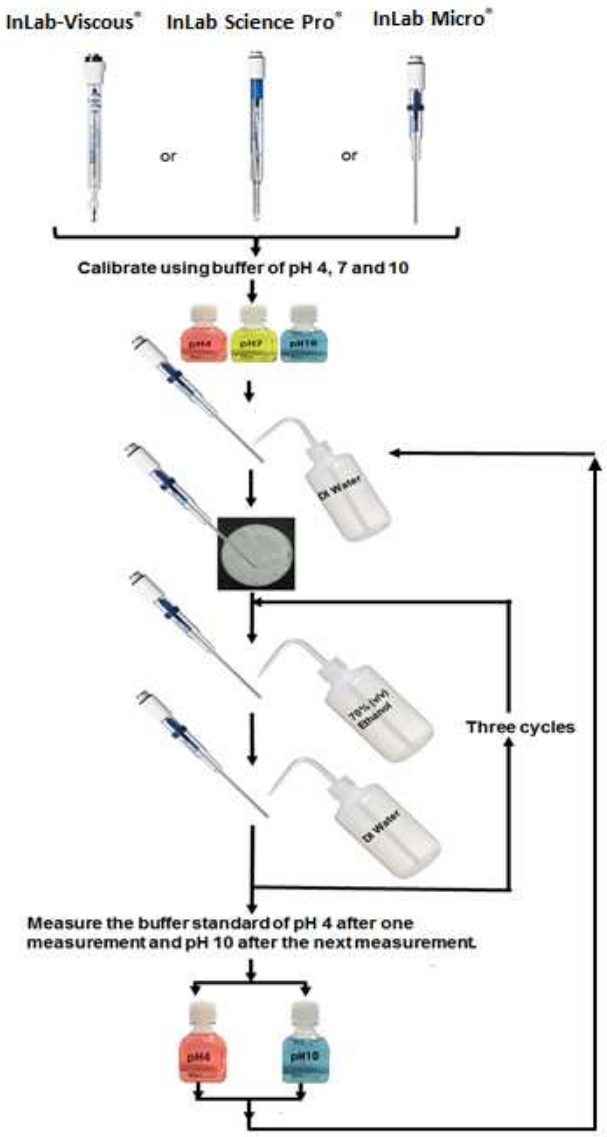


Figure 18: Standardize protocol for the measurement of pH in oil-in-water based cream products

2.4. Buffer capacity measurement

Buffer capacity was measured using the commercially available buffer capacity kit (Topical Products Testing LLC, MS). Briefly pH of each cream was measured prior to experiment. 4 g of cream was added to each glass vial of higher and lower concentration of acid/base buffer capacity testing kit, as mentioned in protocol provided. Cream was mixed and allowed to stand for 24 h for equilibration, followed by mixing the creams again and pH measurement. Buffer capacity was calculated using the following formula.

$$\text{Lower concentration glass vial (A-L/B-L)} = 0.0008/\text{ difference in pH from initial value} \quad \text{..equation 4}$$

$$\text{Higher concentration glass vial (A-H/B-H)} = 0.002/\text{ difference in pH from initial value} \quad \text{..equation 5}$$

2.5. *In vivo* studies

a. Measurement of pH in human subjects

To evaluate the effect of topical products on skin pH, we used custom made creams for in vivo evaluation in human subjects. The human subject study protocol was approved by the Institutional Review Board at The University of Mississippi (16-043). We evaluated the pH of skin using calibrated pH meter attached with surface probe. Six healthy human volunteers were screened and selected for this study. Prior to study the volar forearm of the subjects was cleaned with tap water and area of application was marked. pH of skin on the marked surface was measured after 5 min. A dose of 15 mg/cm^2 was applied to the marked area of skin for 1 h and after one hour pH of creams the skin surface was measured using surface probe.

b. Measurement of pH of topical products before and after application

The pH of topical products was measured using calibrated pH meter attached with InLab Micro® probe using method described above. During this study creams were applied to the volar forearm of healthy volunteers as per study protocol. After 1 h the cream was recovered using clean spatula into a 1 ml eppendorf tube and pH was measured using InLab Micro® probe.

c. Colorimetric pH measurement studies

To visualize the real time change in pH of the creams due to skin buffering capacity, universal indicator solution (Ward's science, ON, Canada) was utilized. In this study, the pH of creams on the volar forearm skin surface was evaluated for every 15 minutes up to 1 h. At each time point two drops (15 µL/drop) of universal indicator was dispensed on to 3.14 cm^2 area of application and images were captured immediately using digital camera (Nikon DSLR, Japan). The cream was recovered after 1 h from the area of application using clean spatula and pH was measured.

d. pH change in topical drug products

The change in pH of topical products present in the market was evaluated using InLab Micro® under different conditions such as ambient room conditions one hour after application on skin in vivo in healthy human volunteers. In this study creams (dose 15 mg/cm^2 over 15 cm^2 area) were applied to the glass slide, cadaver skin and volar forearm of healthy volunteers. One hour after application the cream was recovered using a clean spatula and collected in 1 mL Eppendorf tubes. The pH of the cream recovered from the applied site was measured.

3. Results and Discussion

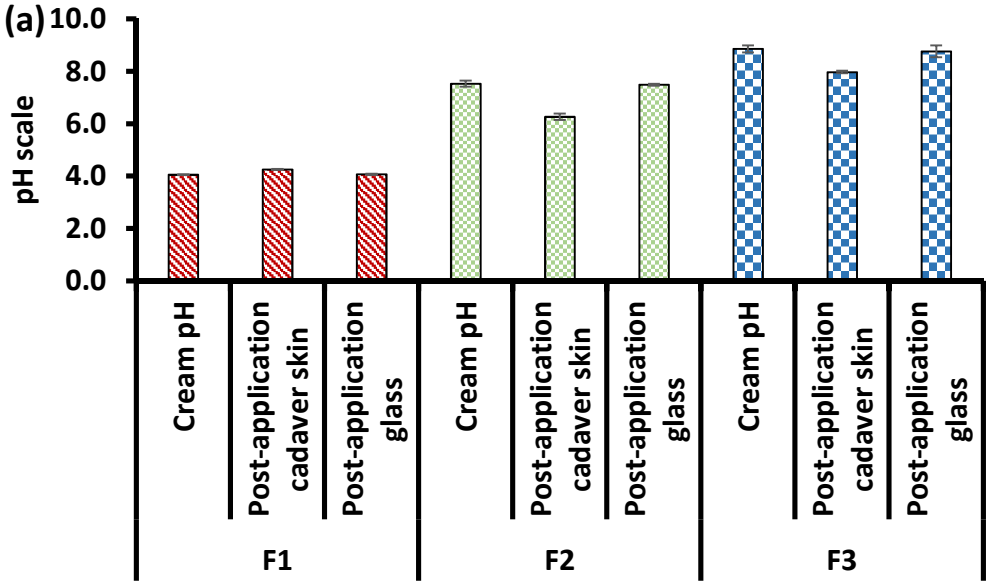
3.1. Does the pH of formulation applied on the skin change?

3.1.1. The creams prepared with deionized water

The creams prepared using deionized water was adjusted to three different pH conditions. The

formulation was applied (dose 15 mg/cm^2 over 15 cm^2 area) on the volar forearm skin in human volunteers. The average pH of skin was 4.6 ± 0.15. The pH of the formulations prepared with distilled water was measured one-hour after application. The pH of formulation adjusted to pH 7.0 ± 0.06 and pH 8.5 ± 0.05 was found to change eventually tending to homeostasis with the skin pH. The formulation with pH 4.3 ± 0.06 did not change, likely because of its closely matching pH with the skin. The control samples that were applied on glass plate and cadaver skin did not undergo relative change in pH (figure 19-a).

It is evident form this data that the skin is associated with a buffering system that would not only acidify the alkaline materials in contact with the skin, but also neutralize acidity to bring the formulation in homeostasis with the skin condition (figure 19-b and c). It is also evident from the data that the change in pH is not driven by the formulations' interaction with the atmosphere and it could be attributed to the physiological response of the skin tissue.



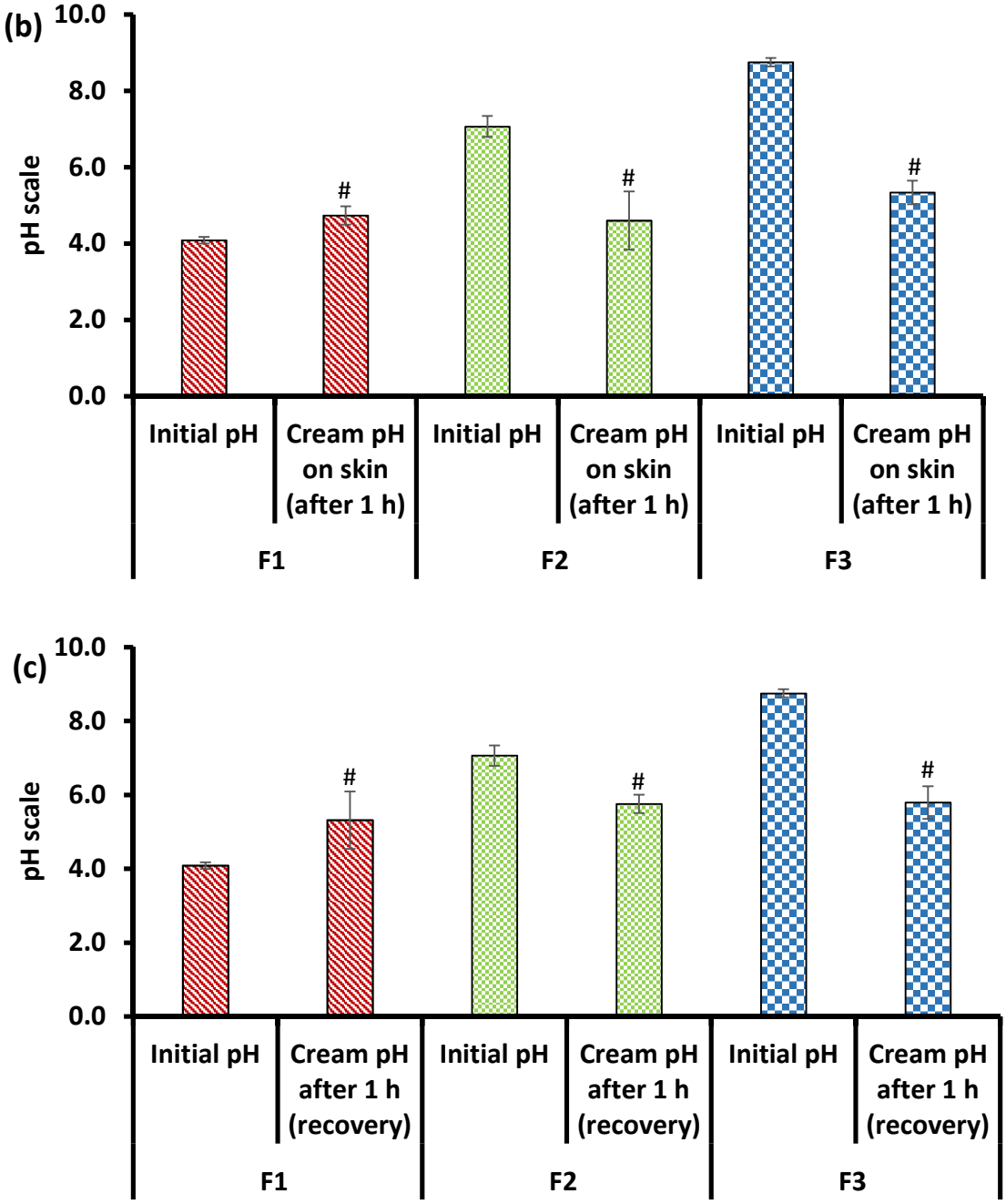


Figure 19: Change in pH of creams prepared by using DI water (a) by environment on cadaver skin and glass plate (n=3) (b) on skin by healthy human subjects (n=6); and (c) pH of cream recovered from healthy human subjects (n=6) (#$p<0.05$ vs control, t test)

3.1.2. The creams prepared with buffers

The same experiment as above was performed using creams prepared with buffers. The pH of

the buffered creams did not change significantly within one hour both in control (figure 20-a) and in vivo studies (figure 20-b and c). This shows that the pH change in the formulations could be conveniently resisted by incorporating buffers in the topical formulations rather than preparing products with mere water and water miscible components (figure 20-b and c). The duration that the buffer would sustain its pH by resisting the physiological driving depends on the buffer capacity of the product.

Colorimetric studies offer a visual proof of concept. The universal indicator used in this study is sensitive to any change in the pH range of 2-10. The buffer will impart a red color at pH less than pH 5 and blue to purple coloration at pH >8. A drop of indicators incorporated in the cream changed the color of the formulation corresponding to its original pH. The formulation was applied on the human subjects and a real time change in the color was recorded. A set of representative pictures are shown in figure 21.

In case of colorimetric study, the color change in formulations prepared using DI water adjusted to pH 7 and pH 9 was distinct (figure 22). The color changed to orange from greenish yellow in pH 7 product and to dark blue to orange in pH 9 formulation. In agreement with the study, product adjusted to pH 4 did not show any noticeable change in the color. The control products on the glass slide and on the cadaver skin did not change in color even after 3 hours.

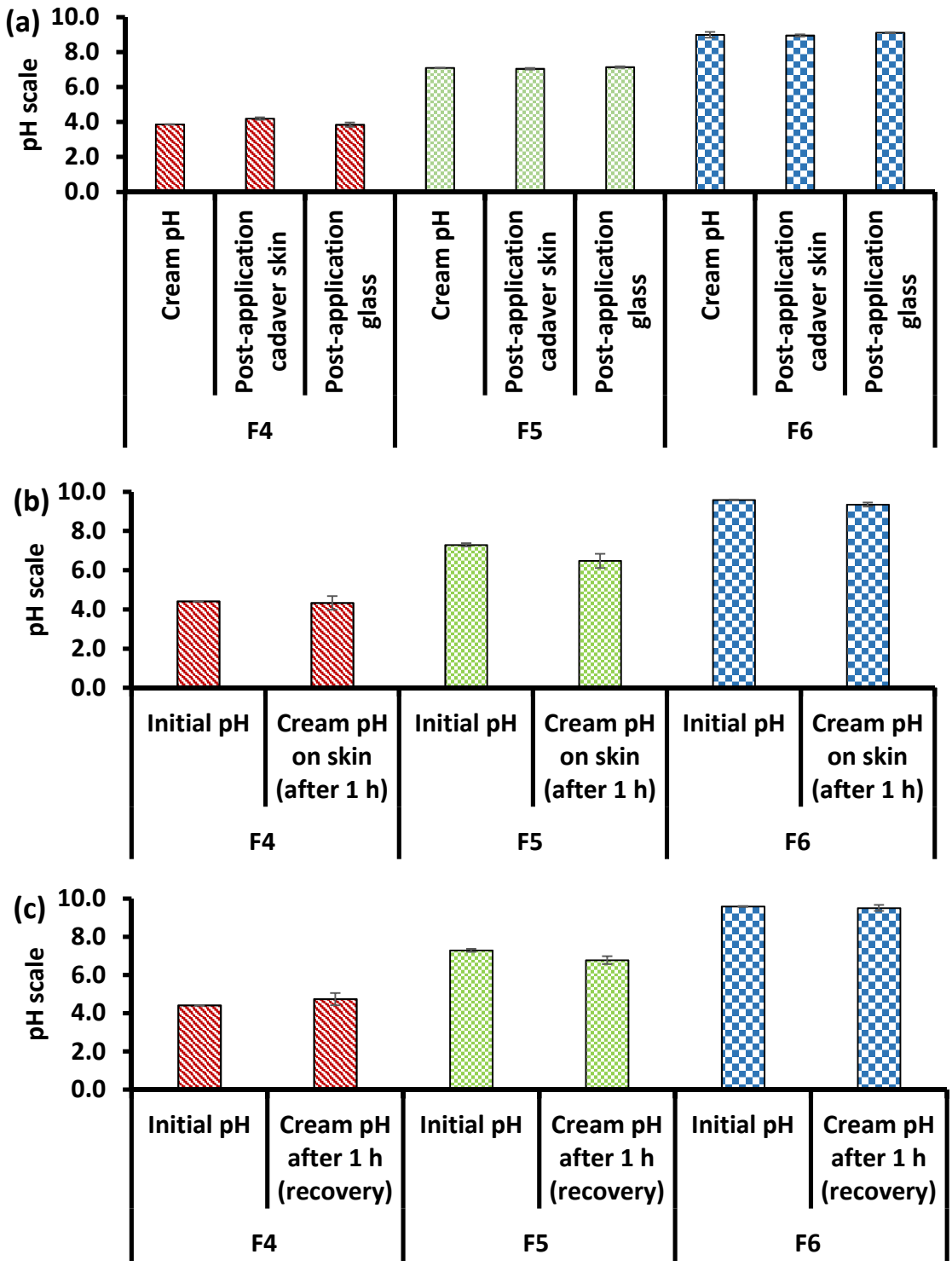


Figure 20: Change in pH of creams prepared by using buffers (a) by environment on cadaver skin and glass plate (n=3); (b) on skin by healthy human subjects (n=6); and (c) pH of cream recovered from healthy human subjects (n=6).

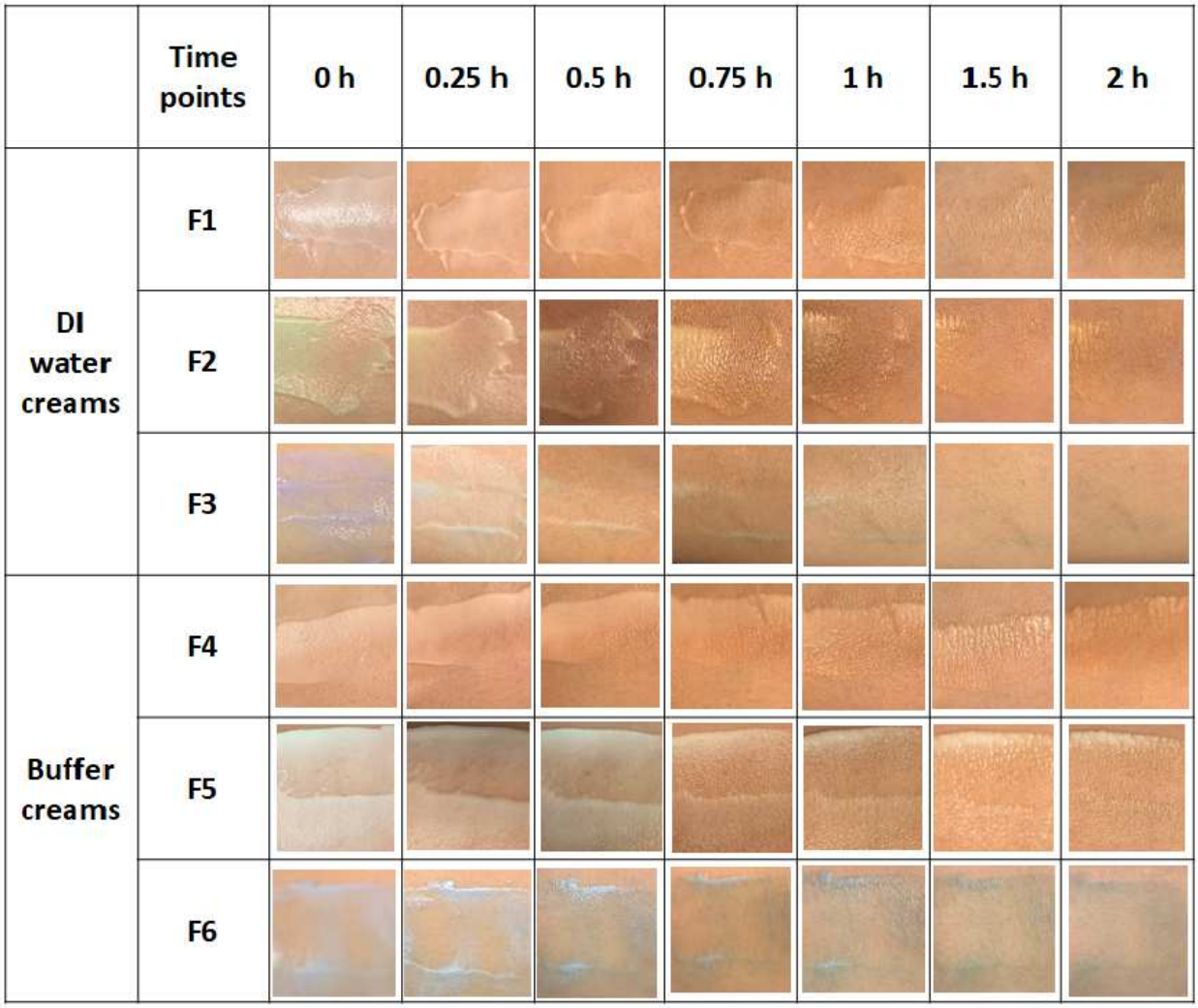


Figure 21: Colorimetric real time kinetic studies to evaluate pH change during clinical studies over period of 2 h.

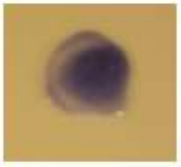
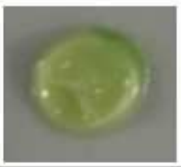
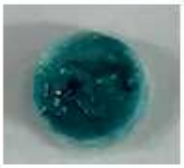
Aqueous phase	Creams pH	Before application	Post 1 h application
DI water	F1		
	F2		
	F3		
Buffers	F4		
	F5		
	F6		

Figure 22: Colorimetric studies to evaluate visual proof of pH change during clinical studies after 1 h application.

3.2. Buffer capacity of the topical products.

Buffer capacity of solutions is generally determined by titration with acid/alkali. The conventional definition of buffer capacity is "the amount of acid or alkali required to increase

the pH of the test product by one unit. Generally, the buffer capacity is determined by titration with acid/alkali. However, in case of topical products, titration would change the composition and microstructure of the product leading to redistribution of contents between phases. Any characterization performed on topical products should be nondestructive to the product microstructure. Therefore, the conventional titration method was modified in which the pH change resulting due to incorporation of small amount of strong acid or base was determined to calculate the buffer capacity. The buffer capacity measurement kit utilized in this project involves incorporation of prescribed amount of semisolid product into the vials precoated with titrant materials. The pH change occurring due to equilibration with a known quantity of titrant material was used to calculate the buffer capacity of the products. The buffer capacity of the products adjusted to specific pH and the creams prepared with buffers are shown in the table 14. The buffer capacity of phosphate and borate buffered creams (F5 and F6 formulation respectively) was 4 to 15 times greater than non-buffered creams. The study also reveals the fact that a buffer capacity of 0.002 for lower strength reagent and 0.005 for higher strength reagent is required to resist any pH change for about an hour due to physiological reaction.

Formulations	Low	High	Average
F1	0.0008±0.00003	0.0019±0.00006	0.001336±0.00005
F2	0.0007±0.00003	0.0008±0.00008	0.000753±0.00006
F3	0.0006±0.00010	0.0007±0.00007	0.000651±0.00009
F4	0.0014±0.00060	0.0010±0.00016	0.001219±0.00032
F5	0.0010±0.00007	0.0022±0.00010	0.001627±0.00009
F6	0.0042±0.00215	0.0061±0.00276	0.005159±0.00245

Table 14: Buffer capacity of creams formulation at low and high concentration of buffer titrant.

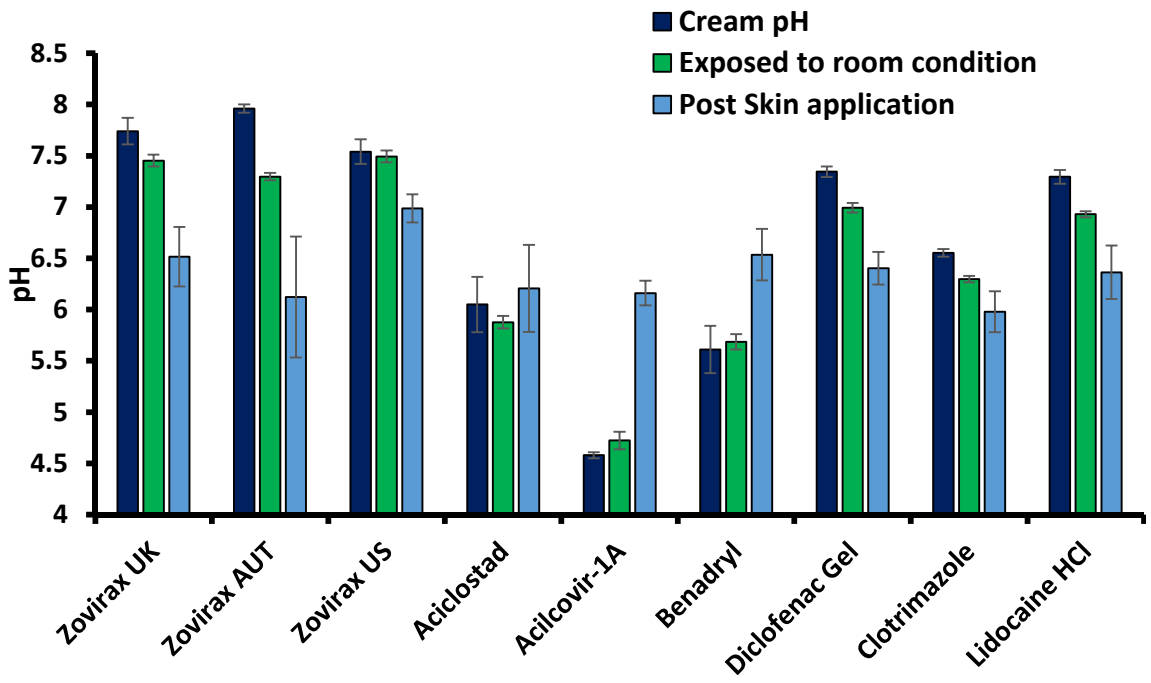


Figure 23: Change in pH of topical products after 1 h under room environment condition (21°C ±2°C, 50%±20% RH) and *in vivo* skin application studies on human volunteers.

3.3. pH change in drug products

A number of topical drug products were tested in human subjects to assess their ability to resist the pH change on skin surface. The products were also subjected for buffer capacity measurements. One of the branded acyclovir cream (zovirax) product has shown higher buffer capacity (table 15), which resists pH change 1 h after application on skin. Generic product of acyclovir at the same time showed a greater pH change 1 h after application on skin which corresponds to its lower buffer capacity (figure 23). The pKa of acyclovir is 9.25 which support the need for basic environment to remain in its unionized form. The buffer capacity when evaluated was found to be higher for zovirax (buffer capacity (low) 0.0019±0.0058) as compared to generic version aciclovir-1A (buffer capacity (low) 0.0003±0.00001). It has been reported that zovirax has better in vitro performance profile than acilcovir-1A, thus in this case buffer capacity of topical products can be a critical parameter and a contributing factor for their

performances. Similarly, other products like lidocaine (pKa 8.98) having higher buffer capacity (buffer capacity (low) 0.0065±0.01622) when applied on skin surface, the pH of formulation unchanged. Lesser strength of buffer capacity might lead to ionization of drug molecules, affecting the product performance, as it has been reported that un-ionized molecules penetrate better than ionized molecules. It was observed that magnitude of change in pH of marketed formulation was higher than the in-house creams. This difference in magnitude can be attributed to complex composition and amount of excipient used in marketed formulations, whereas in-house creams were prepared with simple composition. If the formulation has sufficient buffering capacity as in case of zovirax (figure 23) the performance of the creams can be enhanced. This resistance to change in pH after 1 h application on skin can be attributed to its greater buffer capacity. Similarly, the different marketed creams products such as clotrimazole (pKa 4.1), diclofenac (pKa 4.15) are acidic in nature thus they remain in unionized form on the surface of skin after application causing lesser change in pH on application to skin of volunteers as reported in figure 23.

Formulations	Low	High	Average
Clotrimazole	0.0073±0.04719	0.0787±0.22143	0.0430±0.13431
Diclofenac Sodium	0.0041±0.00117	0.0082±0.00183	0.0061±0.00150
Benadryl	0.0335±0.03624	0.0148±0.01173	0.0241±0.02398
Lidocaine	0.0065±0.01622	0.0094±0.00447	0.0014±0.01035
Zovirax (US)	0.0019±0.00581	0.0007±0.00012	0.0006±0.00297
Aciclovir IA Pharma	0.0003±0.00001	0.0005±0.00001	0.0004±0.00001

Table 15: Buffer capacity of topical products at low and high concentration of buffer titrant.

On further analysis of buffer capacity of creams developed and its change in pH on skin after application, a correlation was established between extent of pH change and buffer capacity in one hour (figure 24-a and b). It was found that buffer capacity could be inversely correlated to change in pH of product on skin. Higher buffer capacity has less change in pH 1 h after application as shown in figure 24. This demonstrates the importance of buffering potential in topical products in this case creams, which could impact the performance attribute of product.

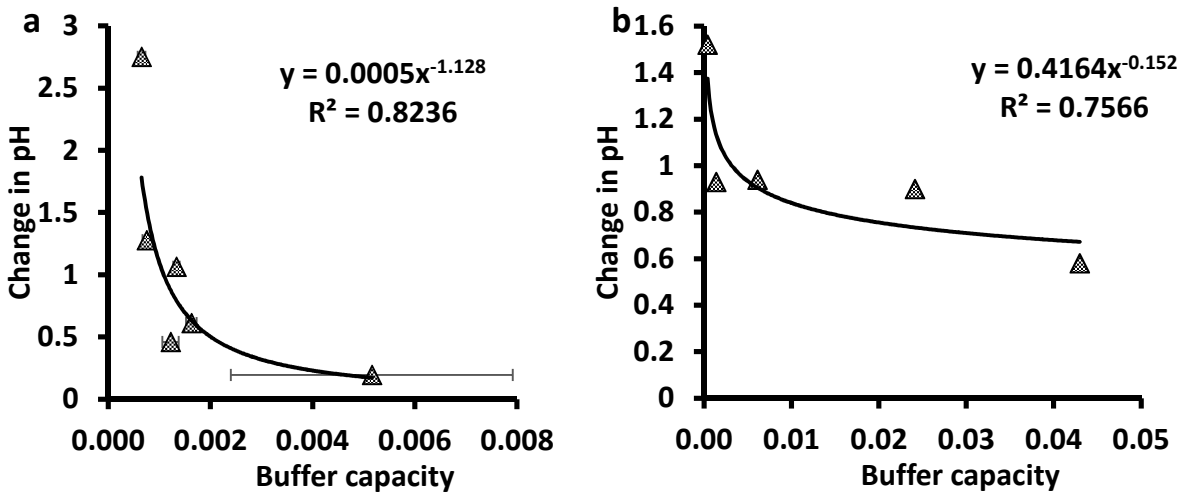


Figure 24: Relationship between change in pH and buffer capacity of a) custom made creams and; b) marketed products.

4. Conclusion

Any change in the pH of aqueous phase in cream or any other topical products at the site of application on skin could potentially alter its efficacy to deliver drugs into the skin. Altering pH of the formulation is a protective mechanism of skin is a mechanism to protect from exposure to extreme pH materials or environment. Often it could impact the delivery of drug form a topical formulation across the skin. The formulation with significant buffering potential could resist this physiological mechanism and sustain the formulation pH over prolonged time. When a product with similar composition as that of reference product is prepared with exact same

composition, it is expected to perform the same as reference product. However, if the reference product has buffer and the generic product is made with deionized water replacing buffer (As it generally does not appear on the package), the pH of the generic product would end up changing within a short span of time , while the reference product sustaining its pH and favoring permeation. This could lead to failure in bioequivalence of generic products. Therefore, the formulations need to be characterized for buffer capacity as one of the critical quality attributes of semisolid product.

CHAPTER V

CONCLUSION

Semisolid dosage forms are most widely used topical formulations, still the knowledge gap in quality attributes of semisolid dosage form has been oversighted. The results of the first project revealed that the Q1/Q2 similar semisolid topical cream formulation was influenced by critical process parameters which can lead to a significant difference in Q3 characteristics. The performance attributes are dependent on microstructural attributes (Q3) of the creams. The difference in Q3 characteristics can influence the performance of the creams.

The results second project revealed that the systematic variations (NMT 5%) in surfactant of Q1/Q2 similar semisolid topical cream formulation has influenced the performance parameter and leads to a difference in efficacy. This study illustrated that within 5% variations in Tween 80 (Q2 same) for an emulsion can progressively influence the thermodynamic activity of drug. The superiority of creams can be due to its increase in thermodynamic activity and rapid change in metamorphosis. The change in thermodynamic activity and higher rate of increase in thermodynamic potential during metamorphosis are thereby suggestive of a possible mechanism whereby even NMT ±5% w/w changes in excipient could potentially alter the rate and extent of bioavailability and performance of a hydrophilic compound in a topical emulsion. Our limited results are thereby suggestive of a mechanism whereby changes within 5% of surfactant quantity

could potentially alter the rate and extent of bioavailability and performance of a hydrophilic compound in a topical emulsion.

The results of third project characterized buffering capacity of topical products as a critical attribute that helps in protecting the products targeted therapeutic efficiency. Low or high buffering capacity of formulation can result in different performance of the product as shown in results of custom-made cream products, wherein the product with high buffering capacity has maintained the pH of the product as compared to product with low buffering capacity. Overcoming the skin physiological pH buffering potential can be beneficial for the efficient therapies to reach its potential through topical route.

CHAPTER VI

SUMMARY

Topical drug delivery such as creams and emulsions, are most widely used in the treatment of skin diseases. It offers advantages such as non-invasiveness, direct drug delivery at the site of action, patient compliance and lower cost of treatment. However, topical formulation development face challenges in market in terms of critical evaluation parameters for understanding the effect of manufacturing process variables, role of excipients and effect of its compositions and skins buffering capacity. The objective of the first project was to investigate the effect of different manufacturing process variables which can influence the microstructure having similar qualitative and quantitative parameters (Q1 / Q2 same), followed by understanding the correlation between these process parameters with performance of these creams. The custom-made formulation with application of nile red for visualization of globule size in o/w creams was used for this study. The results of the study revealed that the Q1/Q2 similar semisolid topical cream formulation was influenced by critical process parameters which can lead to a significant difference in Q3 characteristics. The performance attributes are dependent on microstructural attributes of the creams. The difference in Q3 characteristics can influence the performance of the creams.

In second project, the custom-made cream formulations were prepared by varying the tween 80 quantitively (NMT ± 5 %) having with same process parameters such that they are

compositionally identical. Surfactants are the most common inactive ingredient used in the topical drug products. Surfactants in topical products play many functional roles such as emulsifiers, permeation enhancers and solubilizers. This study was aimed to evaluate the influence of incremental change in the concentration of a surfactant (tween 80) on the quality attributes and performance of semisolid topical products. Four creams were prepared using same protocol and were similar in composition except the concentration of tween 80, which increased by 5% across SF1 to SF4. The quality attributes like globule size distribution, pH, drying rate, texture properties, phase distribution and in-vitro permeation profile was characterized. The critical quality attributes did not differ significantly across the set of products. However, there was a significant difference in the permeation flux-time profile of the products. The permeation flux (J_{max}) varied from SF1 to SF4 (51.25 ± 35.29 to 307.98 ± 138.89 ng/cm2/h, respectively). The reason for difference in performance of products despite having almost consistent quality attributes was systematically investigated. One of the major reasons was found to be due to the differences in thermodynamic activity and the rate of change of thermodynamic activity during the process of evaporative metamorphosis. This study confirms that thermodynamic activity of drug can be a critical quality attribute which can influence bioavailability and performance of a topical drug product.

In third project, we evaluated buffer capacity of topical products on skin as a critical quality attribute and impact of buffer capacity on the performance of topical products. In this project, we investigated the buffer capacity of topical formulations as a critical parameter to improve and develop the topical formulation for its maximum efficacy. Herein we found the effect of buffering capacity of skin, altering the pH of formulation. We prepared creams of different pH using DI water and buffers. We evaluated the pH of these creams after application and changes

that can influence the performance of creams. This application is critical in terms of drug delivery as non-ionized/ un-ionized drug permeate faster as compared to ionized drug on the site of application. The creams which can resist the buffering potential of skin due to its own buffering potential may perform better as may prevent the ionization of drug on skin surface post application. We studied different pH behavior of topical product before and after application on *in vivo* human volunteers. Any change in the pH of aqueous phase in cream or any other topical products at the site of application on skin can alter its efficacy. Altering the pH of the formulation is a protective mechanism of skin to protect our body from exogenous substances. This protective mechanism can challenge the efficiency of drug products on application of product. Inducing a buffering potential in the formulation can lead to resistance for change in the pH of the drug product. The buffering capacity of formulations can be a critical attribute that helps in protecting the products targeted therapeutic efficiency. Low or high buffering capacity of formulation can result in different performance of the product as shown in results of custom-made cream products, wherein the product with high buffering capacity has maintained the pH of the product as compared to product with low buffering capacity. Overcoming the skin physiological pH buffering potential can be beneficial for the efficient therapies to reach its potential through topical route.

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2. Recipient of **2018 ADDF Young Investigator Award** on a project titled "**Effect of Resiniferatoxin, a TrpV1 Receptor Agonist on Neuronal Cells and evaluating its Invitro Neurotrophic Activity for Neurodegenerative Disorders**", Annual Drug Discovery for Neurodegeneration Conference, Washington, D.C.
3. Recipient of **2017 AAPS Graduate Student Research Award** in **Manufacturing Science and Engineering** on a project titled "**The Systematic Influence of Changes in Manufacturing Process Variables on the Microstructure and Performance of Topical Emulsions**", San Diego, CA.
4. **Executive Officer-Treasurer** of **Graduate Student Council** of The University of Mississippi for the year 2017-2018.
5. **Student Representative** of **Graduate Student Body**, School of Pharmacy, The University of Mississippi **in Information Resources and Computing Committee (IRC)** for the year 2016-2018.
6. Recipient of **AAPS Travelship Award 2016** in CPTR section on a project titled "**Formulation development of Resiniferatoxin nanovesicles for topical delivery in neuropathic pain management**", Denver, CO.
7. Recipient of **Pre-Doctoral Neuroscience fellowship grant (R03)** from Center of Biomedical Research Excellence, Center of Research Excellence Natural Products Neuroscience (CORE-NPN)" on "**A novel intravenous formulation of Exendin-4 for treatment of Parkinson's Disease"** 2015-2016 funded by grant number (P20GM104932) and the National Institute of General Medical Sciences (NIGMS), a component of the National Institutes of Health (NIH).
8. Recipient of **Graduate Student Council (GSC) Research Grant** from The University of Mississippi on "**Formulation development of resiniferatoxin nanovesicles for transdermal delivery in neuropathic pain management"** 2015-2016 funded by the Office of Research and Sponsored Programs (ORSP), University of Mississippi.
9. Recipient of **Prime Minister Scholarship (2006-10)** and All India Council Technical Education (AICTE)-**Graduate Pharmacy Aptitude Test (GPAT)** Qualified with **All**

India Rank-797.

- **Work Experience:**

- **Tergus Pharma, Durham, NC, USA**

1. Development and evaluation of topical drug products in *in-vitro* and *ex-vivo* model's for safety and efficacy testing (Target engagement studies, IVPT, irritation testing and toxicity studies).

- **The University of Mississippi, School of Pharmacy, Oxford, MS, USA**

1. Transdermal delivery and pharmacokinetic evaluation of 17-Hydroxyprogesterone caproate using niosomes for minimizing of preterm birth defect".
2. Trans-ungual delivery of a novel antifungal molecule AR-12.
3. Formulation development and pharmacokinetics of resiniferatoxin nano-vesicles for transdermal delivery and *in vitro/ in vivo* evaluation for neuropathic pain management.
4. Critical quality evaluation of process variables (Q1/Q2 similar) on microstructure and performance of semisolid dosage forms (creams).
5. A novel intravenous formulation development of Exendin-4 for treatment of Parkinson's disease.
6. Studying the effect of Resiniferatoxin for its *in vitro* neurotrophic activity in treatment of neuro-degenerative disorders.
7. Critical evaluation of quantitative variation of excipients like surfactants (Q1/Q2/Q3 similar) on quality and performance of semisolid dosage forms (creams).
8. Formulation and development of characterization method for topical foam for treatment of burns.

- **Plex Pharmaceuticals Inc., San Diego, CA, USA** (Internship, May 2017 to August 2017)

1. Drug discovery and formulation development, compound screening in biochemical and cell-based assays, assay development for further characterization of compounds.

- **National Institute of Nutrition- (NIN), Hyderabad, India** (February 2013 to January 2015) - Technical Assistant

1. Assessment of safety and distribution profile of pharmaceuticals, biopharmaceuticals and natural products.
2. *In vitro* and *in vivo* pre-clinical toxicity evaluation of pharmaceuticals, biopharmaceuticals and natural products.
3. Quantitative detection of heavy metals and phthalates in formulation, toys and food products.

- **Sipra Labs, Hyderabad, India** (June 2012 to February 2013)-Project Assistant

1.Pre-Clinical Evaluation, Pharmacokinetics, and Toxicity study of various Drugs, Bio-pharmaceuticals, and genetically modified (GM) products.

- **Industrial Training**

1. Biocon- Syngene International Limited, Bangalore, India (Intern)-Summer 2009
2. Sipra Labs, Hyderabad, India Role: Project Trainee- Summer 2008