Critical Quality Attributes of Topical Semi-solid Dosage Forms: Solvent Activity

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CRITICAL QUALITY ATTRIBUTES OF TOPICAL SEMI-SOLID DOSAGE FORMS:

SOLVENT ACTIVITY

DISSERTATION

A Dissertation
presented in partial fulfillment of requirements
for the degree of Doctor of Philosophy
in Pharmaceutical Sciences with an emphasis in
Pharmaceutics & Drug Delivery
The University of Mississippi

by
MURALIKRISHNAN ANGAMUTHU

December 2016
ABSTRACT

Critical quality attribute has been defined as “a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality”. Deviations from preset quality metrics may potentially result in drug products with inferior therapeutic efficacy and unacceptable clinical safety profile. Topical cream formulations may possess numerous quality attributes that can affect the permeation of the drug through skin and consequently therapeutic efficacy. CQAs of topical formulations are often aimed at characterizing either the physicochemical properties of APIs or bulk characteristics of the formulation itself. Solvent systems constitute a major proportion of semisolid cream type formulations and its inclusion is critical to overall product performance (drug delivery to skin). A majority of the commercially available dermatological formulations contain solvent systems (water, ethanol, propylene glycol, glycerol and liquid chemical permeation enhancers) but the significance of solvent activity (thermodynamic solvent properties) on product performance has not been investigated so far, to be translated into a quality metric system (CQA). Water was utilized as a model solvent in order to link thermodynamic solvent activity and drug delivery performance of topical semi-solid cream systems. The overall aim of the research investigation is to evaluate water activity as a critical quality attribute of topical semi-solid dosage forms. Primary goal of this study was to investigate the mechanistic effects of water activity on drug release properties of formulation and drug permeation across the skin. A related objective was to investigate the mechanistic effects of
water activity on skin hydration and barrier properties. For mechanistic investigation, a simple topical vehicle of varied $a_w$ (0.97 - 0.42) was formulated using deionized water with caffeine (model drug). Drug transport studies were performed using Franz diffusion cells across cellulose and silicone membrane (porous-hydrophilic and non-porous-hydrophobic membrane models, respectively) in order to elucidate the effects of water activity on drug release from formulation vehicles. Caffeine flux was found to decrease with decreasing water activity of the vehicle. The same trend was observed for drug release studies across all synthetic membranes tested. Series of dye diffusion study was designed to investigate bulk diffusion/release properties of caffeine (model solute) from water activity modulated topical formulations. Sucrose solution was utilized as viscosity control to decouple/delineate water activity effects on bulk solute diffusion. Caffeine diffusion was relatively slow in low water activity formulation vehicles versus to respective viscosity controls of same water activity (sucrose solution). *In-vitro* permeation studies were performed across porcine epidermis to investigate water activity effects on drug permeation across model skin membrane. Utilizing drugs of varied skin affinity (caffeine, acyclovir and nicotine) IVPT was performed across porcine epidermis under finite and infinite dosing conditions. Under both dosing conditions, caffeine, acyclovir and nicotine flux were found to reduce with lowering water activity of formulation vehicle. Also, the lag time for drug permeation increased with a decrease in water activity of solution formulation. Effects of low water activity on skin hydration were studied by placing vehicles of varied water activity on porcine skin equilibrated to ambient conditions (22°C/50% RH) and monitoring the changes gravimetrically. The hydration level measurement following exposure to different water activity formulations showed that the moisture content increases in the epidermis when hydrated with
water, as expected. Notably, when exposed to lower water activity solutions, the epidermis would lose existing water, exhibiting an apparent dehydration effect. The histological evaluation revealed mild perturbation in the stratum corneum layer of the skin samples treated with lower water activity vehicles. This was potentially caused by osmotic shrinkage of corneocytes, which would be in agreement with the data from the hydration experiment. Role of hydrating agents (propylene glycol) on preservation of skin hydration, barrier properties and drug permeation was also investigated. Increasing levels of propylene glycol in low water activity vehicle (aw-0.78) enhanced drug flux across porcine epidermis. Notably, porcine skin treated with propylene glycol-aw 0.78 vehicle mixture (1:1) reverted stratum corneum perturbation caused by low water activity vehicle. Manufacturing process studies were undertaken to investigate effects of manufacturing process on water activity of topical cream (w/o type). Process parameters had profound impact on modulating water activity of a Q1/Q2 similar w/o type cream. Dynamic drying measurement revealed same water content in process prototypes. Based upon the results, water activity appears to be a potentially critical quality attribute for topical semisolid dosage forms, and may have the potential to influence the drug release from formulation as well as the permeation of the drug across the skin.
DEDICATION

This Dissertation is Dedicated to my Wife Swetha Ainampudi
ACKNOWLEDGEMENT

I would like to express my deep and sincere gratitude to my advisor, Dr. S. Narasimha Murthy for his support, patience and encouragement throughout my research work. His motivation and support helped me a lot to learn many aspects required to achieve high standards in research.

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CHAPTER 1

INTRODUCTION

Topical semi-solid dosage forms are traditionally constituted in the form of creams (oil-in-water & water-in-oil type based), gels (aqueous & hydro-alcohol based), ointments (hydrocarbon & polyethylene glycol based) or pastes\(^{1,2}\). Most important advantage of topical formulations is the allowance for localized treatment of number of several dermatological conditions with minimal systemic exposure. Other relevant advantages include possible application of therapeutically relevant higher drug (at the site of action), reduced risk of systemic side effects and better patient compliance.

Choice of topical semi-solid formulation vehicle depends upon several factors but predominantly the type of disease, pathological condition of skin type to be treated and biopharmaceutical considerations\(^{3-5}\). Generally, lipophilic or hydrocarbon based vehicles are employed to treat conditions associated with dry skin. Ointment formulations are very effective as they provide moisturizing effect on dry/flaky skin owing to occlusive properties which leads to potential hydration of skin and may enhance percutaneous drug absorption. Ointments may possess less irritation potential but may have a greasy feel and patients dislike. On contrary, emulsion based cream formulations (contain both oil and water) are preferred for their cosmetic appeal and may be less viscous and greasy. Depending on the physicochemical properties of drug, it is feasible to formulate both hydrophilic and lipophilic drugs (based on the dispersion medium: oil or water) to achieve certain degree of solubilization in the dermatological vehicle.
Topical semi-solid dosage forms may be either simple or complex in nature and may contain few or several excipients formulated as a thermodynamically stable/unstable multi-component system. The components of a topical semi-solid drug product will depend on the choice of formulation type and could range from simple solution (containing drug and solvent) to inherently complex polymeric gels and thermodynamically unstable emulsion based cream systems. A simple topical solution may constitute active pharmaceutical ingredient dissolved in suitable solvent together with additives like pH modifiers, co-solvents and preservatives. A topical gel formulation may contain viscosity modifiers like cellulose (natural polymer) or acrylic (semi-synthetic) polymers in aqueous or hydro-alcohol systems with preferable rheological properties for consistent delivery of active ingredients. An ointment may contain active ingredients dissolved or dispersed in monolithic hydrocarbon or water washable PEG based vehicles. A cream formulation is inherently complex and are predominantly emulsion based systems containing two immiscible liquids dispersed (micron scale), one within the other, leading to the formation of thermodynamically unstable system (but kinetically stabilized to achieve suitable shelf life) with predetermined physical and chemical stability. The stability of emulsion can be potentially improved by inclusion of viscosity enhancing agents acting at liquid interface. Additional excipients like pH modifiers, humectants, penetration enhancers, drug co-solvents and preservatives may be employed to arrive at a therapeutically viable dermatological base/vehicle for drug delivery.
<table>
<thead>
<tr>
<th>S. No.</th>
<th>Functional Component</th>
<th>Description</th>
<th>Example</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Ointment Base</td>
<td>Bulking material for semi-solid product</td>
<td>Lanolin, Lanolin alcohols, Paraffin, Petrolatum, White wax, Stearic acid, Stearyl alcohol</td>
</tr>
<tr>
<td>2.</td>
<td>Emulsifying Agent</td>
<td>Surface acting agents to reduce interfacial tension and improve contact/wetting between hydrophilic and hydrophobic medium</td>
<td>Polysorbate, Poloxamer, Cetostearyl alcohol, Sodium lauryl sulfate</td>
</tr>
<tr>
<td>3.</td>
<td>Humectant</td>
<td>Improves retention of water in the formulation</td>
<td>Glycerin, Propylene glycol, Polyethylene glycol, Sorbitol</td>
</tr>
<tr>
<td>4.</td>
<td>Thickening Agent</td>
<td>Increases viscosity of the formulation Bulking material for formulation</td>
<td>Carbomer (acrylic polymers), Cellulose polymers, Polyethylene oxide, Sodium alginate, Gelatin</td>
</tr>
<tr>
<td>5.</td>
<td>Preservative</td>
<td>Improves microbial stability of the product</td>
<td>Parabens, Benzoic acid, Benzalkonium chloride, Phenoxyethanol</td>
</tr>
<tr>
<td>6.</td>
<td>Permeation Enhancer</td>
<td>Improves percutaneous permeation of active ingredients</td>
<td>Propylene glycol, Oleic acid, Polyethylene glycol</td>
</tr>
<tr>
<td>7.</td>
<td>Antioxidant</td>
<td>Prevent oxidative degradation</td>
<td>Butylated hydroxytoluene, Butylated hydroxyanisole</td>
</tr>
<tr>
<td>8.</td>
<td>Acidifying/Alkalizing Agent</td>
<td>Maintain appropriate pH of the formulation</td>
<td>Citric acid, Phosphoric acid, Trolamine, Sodium hydroxide</td>
</tr>
<tr>
<td>9.</td>
<td>Vehicle/ solvent</td>
<td>Medium for the formulation</td>
<td>Purified water, mineral oil, Propylene glycol</td>
</tr>
</tbody>
</table>

Table 1: Typical Formulation Components of Topical Drug Products
Quality by Design (QbD) in Topical Semi-solid Cream Products

Pharmaceutical product quality is instrumental in ensuring patient safety and thus it has been realized of late that the quality should be built in the product rather than being finally tested. In order to encourage continuous improvement in the pharmaceutical quality control, US FDA recently outlined a QbD framework for product development in the ‘Pharmaceutical Current Good Manufacturing Practices (cGMPs) for the 21st Century’ initiative to achieve a desired state in quality pharmaceutical manufacturing\(^8\). In conjunction, the International Conference on Harmonization (ICH) developed guidelines to globally harmonize technical requirements for the manufacture and use of pharmaceutical ingredients and drug products. The ICH guidelines have helped to transform the conventional univariate-, trial-and-error based product development approach to a multivariate-, science- and risk-based practice governed by the principles of Quality by Design (QbD).

The concepts of QbD are well described in several regulatory guidelines including ‘PAT (Process Analytical Technology) – A Framework for Innovative Pharmaceutical Manufacturing and Quality Assurance’, ICH Q8 (R2) entitled ‘Pharmaceutical Development’, ICH Q9 entitled ‘Quality Risk Management’ and ICH Q10 entitled ‘Pharmaceutical Quality System’\(^9,10\). The ICH Q8 guidelines are applicable to Product design, Process design and Scale-up and Transfer while ICH Q10 guidelines are relevant from process design through to commercial manufacturing. On the other hand, the ICH Q 9 guidelines are known to regulate all the stages of the product life cycle. QbD begins with outlining the quality target product profile (QTPP) as well as critical quality attributes (CQAs) for the target drug product.
QTPP can be defined as “prospective summary of the quality characteristics of a drug product that ideally needs be achieved to ensure the desired quality, taking into account safety and efficacy of the drug product”.

Critical quality attribute has been defined as “a physical, chemical, biological or microbiological properties or characteristics that should be within an appropriate limit, range, or distribution to ensure the desired product quality”. Deviations from preset quality metrics may potentially result in drug products with inferior therapeutic efficacy and unacceptable clinical safety profile\textsuperscript{11}.

Topical cream products may possess numerous quality attributes that can affect the permeation of the drug through skin, skin retention of cream and patient acceptability which can further potentially impact therapeutic efficacy and safety\textsuperscript{14}. Few of the critical quality attributes are direct result of microstructural arrangement (Q3 characteristics) of the semi-solid cream products. Microstructural (Q3) properties of cream products are significantly affected by manufacturing process to an extent that even cream lots of Q1/Q2 (qualitative and quantitative) similarity result in varying degree of microstructural (Q3) arrangement when processed at different conditions. Table 2 contains a comprehensive list of critical quality attributes (inclusive of microstructural properties) for a semi-solid cream product.
Table 2: Comprehensive list of potential critical quality attributes of a topical semi-solid cream product

<table>
<thead>
<tr>
<th>Critical Quality Attributes of Topical Semi-solid Creams</th>
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</thead>
<tbody>
<tr>
<td>Organoleptic properties</td>
</tr>
<tr>
<td>Drug identification</td>
</tr>
<tr>
<td>Drug Assay</td>
</tr>
<tr>
<td>Content uniformity/homogeneity</td>
</tr>
<tr>
<td>pH</td>
</tr>
<tr>
<td>Particle size distribution</td>
</tr>
<tr>
<td>Polymorphism</td>
</tr>
<tr>
<td>Globule size distribution</td>
</tr>
<tr>
<td>Rheology</td>
</tr>
<tr>
<td>Water content</td>
</tr>
</tbody>
</table>

1. **Organoleptic properties:**

   A qualitative description of organoleptic properties of the proposed drug product is provided as a test specification which include, color, smell, texture etc. For example, if color of the product is critical for patient acceptability and is susceptible to change upon storage, adequate quantitative tests should be developed to set the acceptable criteria for the color change.

2. **Drug identification and assay:**

   Drug assay affects clinical efficacy of the drug product and the acceptable limits are between 90-110%.
3. **Content uniformity and homogeneity:**

Visual investigation of the drug product may be a useful tool to detect phase separation, syneresis (extrusion of water from gel) and no foreign matter and product homogeneity. Also, drug content uniformity and homogeneity may critically affect the clinical efficacy of the drug product and should be assessed using validated analytical techniques.

4. **pH of the formulation:**

pH is a potential critical quality attribute of topical cream formulations. pH may affect drug stability and physicochemical properties of semi-solid products like emulsion stability and rheological properties. Also, pH affects the product performance by the way of influencing;

With respect to product performance and therapeutic outcome, solubilized drug content in aqueous compartment is the most relevant parameter for emulsion based cream formulations. Drugs with pH dependant solubility are greatly affected by pH changes within the formulation. Also, it is a well known fact that unionized drug species traverse the stratum corneum barrier more effectively compared to the ionized drug species. The ionic equilibrium (ionized-unionized fraction) of solubilized drug species can also be potentially affected by pH conditions built within the formulation.

Skin membrane acts as a pH dependant perm-selective barrier to permeation of drug molecules. It was reported that drug permeability through skin barrier membrane is a function of pH of formulation vehicle applied. Inclusion of pH modifiers in formulation has the potential to alter baseline pH conditions of the skin membrane therefore eventually altering its permeability status. On the other hand, it was reported that the skin membrane was shown to resist acid/alkaline regression owing to its buffer capacity and transiently regain its baseline pH. Hence, pH appears to be one of the critical quality attribute of topical cream formulation and
needs to be controlled after thorough review of the physicochemical properties of drug and its permeability characteristics through skin membrane.

5. **Particle size distribution and drug polymorphism:**

   If the topical product contains suspended drug particles, then particle size distribution and crystal habit of the drug may potentially become critical to intended performance of the formulation. Particle size distribution and polymorphic nature may affect drug dissolution rate in aqueous component of cream formulation thereby influencing the clinical performance\(^{15}\).

6. **Drug concentration in aqueous phase:**

   Drug solubilization in formulation vehicle is one of the prerequisites for percutaneous absorption of drugs\(^{15}\). Hence, the amount of drug dissolved in the aqueous component of the drug product becomes the most relevant parameter to gauge the clinical efficacy\(^{11}\).

7. **Globule size distribution:**

   In case of emulsion based cream formulations (o/w & w/o type), process parameters (homogenization temperature, speed and time) have high impact on globule size distribution of dispersed phase and may potentially affect both macro and microstructure level quality attributes like organoleptic, sensory, rheological characteristics and drug solubility in formulation vehicle.

8. **Rheology:**

   Understanding the rheological properties of topical cream formulations is critical to drug delivery across skin, formulation stability and ease of application on skin. Rheological properties of semi-solid products are extensively influenced by microstructural (Q3) properties, which may be affected by manufacturing process parameters. Ease of spreading of formulation on skin is inversely related to its viscosity and may limit the total area of application thereby limiting the area available for percutaneous drug absorption. Also, viscosity inversely affects the rate of drug
release from the formulation vehicle, at microstructure level, thereby hindering the rate and extent of drug availability for percutaneous absorption.

9. **Specific gravity:**

Specific gravity of cream formulations is very sensitive to manufacturing process due to potential for air entrapment in the product. Air entrapment in the product may lead to variation in assay value of the product and most importantly the desired dose may not be delivered during ‘in-use’ conditions.

**Problem statement**

Aforementioned critical quality attributes are aimed at characterizing either the physicochemical properties of APIs or bulk characteristics of the formulation system itself. Solvent systems constitute a major proportion of semisolid cream type formulations and its inclusion is critical to overall product performance (drug delivery to skin). A majority of the commercially available dermatological formulations contain solvents like water, ethanol, propylene glycol, glycerol and liquid chemical permeation enhancers. However, the significance of solvent activity (physicochemical properties of solvent) over product performance has not been investigated so far to be translated into a quality metric system (CQA).

Critical quality attribute has been defined as “a physical, chemical, biological or microbiological property or characteristic that should be within an appropriate limit, range, or distribution to ensure the desired product quality”. Deviations from preset quality metrics may potentially result in drug products with inferior therapeutic efficacy and unacceptable clinical safety profile. Topical semi-solid cream formulations may possess numerous quality attributes that can affect permeation of drug through skin, skin retention of cream and patient acceptability
which can further potentially impact therapeutic efficacy\(^9\). A comprehensive list of critical quality attributes for a topical cream product is given in table 2.

Aforementioned critical quality attributes are aimed at characterizing either the physicochemical properties of API or bulk characteristics of the formulation system itself. Solvent systems constitute a major proportion of semisolid cream type formulations and its inclusion is critical to overall product performance (drug delivery to skin). A majority of the commercially available dermatological formulations contain solvents like water, ethanol, propylene glycol, glycerol and liquid chemical permeation enhancers. However, the significance of solvent activity (physicochemical properties of solvent) on product performance has not been investigated by far in order to construct a quality metric system (CQA).

Water is the predominantly used solvent type in most cream and hydrogel type semisolid formulations (containing as high as 70% w/w and 95% w/w water content, respectively). Hence, water was utilized as a model solvent system in the present research work and we propose to evaluate water activity as a potential critical quality attribute of topical semi-solid cream formulations.

**Water Activity**

Water activity \((a_w)\) is thermodynamic energy status of solvent water, which is mathematically represented as ratio of partial vapor pressure of water (\(\rho\)) to vapor pressure of pure water (\(\rho_o\)), at equilibrium temperature. Water activity does not essentially depend on absolute water content in a system, but is the measure of thermodynamic energy status. Solvent water has potential to interact with chemical species like salts, sugars, amino acids, glycols and polymers via surface interaction (dipole-dipole), ionic bonds, hydrogen bonds and Van der Walls
forces and as a consequence could alter its thermodynamic activity. Water activity can measurably range between 0.0 to 1.0 (pure water)\textsuperscript{16}.

In pharmaceutical applications, water activity has been linked to stability (micro-biological/physical/chemical), thermodynamic/kinetic activity (drug dissolution) and processability (powder flow and compaction) of several pharmaceutical systems. However, it is the goal of proposed research study to investigate the effects of water activity of topical semisolid formulations on its skin drug delivery attributes. To bridge the knowledge gap, it is the objective of the proposed research study to investigate the effects of solvent activity of topical semisolid formulations on its performance attributes.

**Research Objectives**

Main objective of the research study is to perform mechanistic investigation to outline the effects of water activity on individual factors controlling the topical drug delivery (variables governing formulation, skin and formulation-skin interface).

In the first objective, effects of water activity on formulation related variables *(physicochemical properties)* governing topical drug delivery was investigated in detail. Utilizing Franz diffusion cells, drug transport studies were performed across synthetic membranes (cellulosic and silastic) to evaluate the effects of water activity on *in-vitro* drug release from model topical vehicles of diverse water activity. In addition, physicochemical characterization, mathematical modeling and qualitative dye diffusion test were performed to mechanistically understand the effects of water activity on drug diffusion/release from water activity vehicles.
In the second objective, effect of water activity on skin related variables (*physiological properties*) governing topical drug delivery was investigated in details. Utilizing Franz diffusion cells, drug transport studies were performed to evaluate the effects of water activity on *in-vitro* drug delivery and drug permeation across model biological membrane (porcine skin). In addition, effects of water activity on topical drug delivery under finite/infinite dosing conditions, drugs of various physicochemical properties, skin hydrodynamics and barrier properties were also investigated. *In-vitro* permeation studies were undertaken to investigate the role of hydrating agents on topical drug delivery upon inclusion in low water activity vehicles. Histological investigation was undertaken to study the role of hydrating agents on barrier property restoration of porcine skin (model biological membrane).

In the third objective, model water-in-oil type creams were prepared under various process parameters (*manufacturing variables*) to study its’ effects on resultant water activity of the formulation base.
CHAPTER 2
MECHANISTIC INVESTIGATION OF WATER ACTIVITY EFFECTS AT DRUG-FORMULATION VEHICLE INTERFACE AND INFLUENCE ON DRUG RELEASE

1. Objective

Topical drug delivery is a net result of complex interaction between the following: drug-vehicle interaction, vehicle-skin interaction and drug-skin interaction. In order to investigate the effects of water activity at drug-vehicle interface, as well as, to delineate other skin and drug related variables, simple aqueous based topical vehicles were prepared using caffeine as a model drug. Mechanistic investigation by the way of qualitative dye diffusion test and mathematical modeling is undertaken to characterize in-vitro drug release from various water activity vehicles.

2. Materials

Caffeine, methylene blue and lithium chloride were purchased from Sigma Aldrich (St. Louis, MO). Sucrose NF was purchased from Spectrum chemical manufacturing Co., (New Brunswick, NJ). Cuprophan membrane was purchased from Agilent technologies (Santa Clara, CA) and silicone membrane was obtained from SMI medical device manufacturer (Saginaw, MI). All solutions and dilutions were prepared using purified deionized water (resistivity ≥18.2 mΩ.cm, Barnstead Nanopure Diamond™, Barrington, IL).
3. **Methods**

3.1. **Formulation of topical vehicle with modulated water activity (a\textsubscript{w})**

To delineate other formulation effects, simple topical solution vehicle was prepared by mixing drug in deionized water to yield a homogenous solution. Water activity of topical solution vehicle was modified by adding predetermined quantity of lithium chloride to yield a wide range of water activity (0.98 - 0.42). Water activity was experimentally determined by dew point technique using AquaLab Series 3 (Decagon Devices Inc.). Drug content in topical vehicles is maintained as follows (within parentheses is the saturation solubility in water at 25° C): caffeine - 15mg/mL (21.6 mg/mL), nicotine – 50 mg/mL (1g/mL) and acyclovir – 1 mg/mL (1.6 mg/mL) (KRISTL et al., 1993, Yalkowsky et al., 2010, Budavari and Windholz, 1989).

3.2. **Determination of viscosity of topical drug solution**

Viscosity of all topical solution vehicles were determined using Ostwald viscometer. Density of the topical solution vehicles was experimentally determined at 22° C using calibrated pipette and weighing balance. Viscosity (0.8990 centipoises) and density (0.9982 g/mL) reference values for deionized water (standard reference) at 22° C, was used for the calculation. Briefly, time taken for the passage of reference and test solution between the upper and lower mark in the viscometer was determined and relative viscosity of the test solution was calculated using the formula shown below.

\[
\frac{\eta}{\eta_{ref}} = \frac{t * \rho}{t_{ref} * \rho_{ref}}
\]

where, \(\eta\), \(\rho\) and \(t\) are viscosity, density and time taken for passage for the standard reference (water) and \(\eta_{ref}\), \(t_{ref}\) and \(\rho_{ref}\) are viscosity, density and time taken for passage for the test solution. Stokes-Einstein equation was used to theoretically calculate drug diffusion coefficient as a function of vehicle viscosity,
\[ D = \frac{kT}{6\pi\eta r} \]

where, \( D \) is the diffusion coefficient, \( k \) is the Boltzmann constant, \( T \) is absolute temperature, \( \eta \) is the solution viscosity and \( r \) is the hydrodynamic radius of solute/drug.

### 3.3. Qualitative dye diffusion test

Dye diffusion test was performed in control (deionized water, \( a_w \)-0.98) and test vehicles (modulated water activity between \( a_w \)-0.78 and \( a_w \)-0.42) using methylene blue as a model solute (mol. wt. 319.85 g/mol). Control and test vehicles (3 mL) were taken in scintillation glass vials and 50 \( \mu \)L of 0.05% w/v methylene blue solution (prepared in deionized water) was carefully added along the side of the vial using a pipette. As a result, methylene blue solution forms a distinct layer or ring on top of the bulk medium (control and test vehicles). Followed by, time lapse observation was performed and extent of bulk dye diffusivity was pictorially recorded using a digital camera.

### 3.4. Analytical methods

All chemical analysis and drug quantification in transport studies were performed using HPLC analytical method. The analytical conditions utilized for all model drugs are as follows. Reversed phase chromatography was performed for quantification of model drug caffeine. HPLC system consists of Waters® 1525 binary pump (Waters® Corporation, Milford, MA) equipped with Waters® 717 plus autosampler equipped with UV absorbance detector. Chromatographic separation was achieved under isocratic mode at a flow rate 1 mL/min on Symmetry® C18 (4.6 x 150 mm; 5\( \mu \)) reversed phase analytical column. Caffeine was eluted using mobile phase consisting of 50% v/v methanol and 50% v/v water system (50:50 v/v) at analytical wavelength 254 nm. The chromatogram was analyzed using Breeze® software system.
3.5. Statistical treatment

GraphPad InStat 3 software was used for statistical analysis. One-way ANOVA was used to determine the level of significance for correlation between parameters and $P < 0.05$ was considered as the acceptable level of significance.

4. Results and Discussion

4.1. Effect of water activity on in vitro drug release from formulation vehicles

![Graph showing cumulative caffeine permeation over time for different water activities](image)

**Figure 1: Effect of water activity of the vehicle on caffeine diffusion across Cuprophan membrane.** Each data point represents mean ± standard deviation ($n=6$).

*In vitro* drug release studies were performed across Cuprophan and silicone membrane to systematically evaluate the effects of topical vehicles’ water activity on its drug release properties. Synthetic membranes are devoid of heterogeneity and have been extensively used to investigate formulation variables and associated drug release phenomena (Houk, 1988).
Release studies were performed utilizing Cuprophan membrane (semi-permeable hydrophilic porous membrane). In order to avoid differences in transmembrane osmotic pressure gradient, receptor compartment was filled with either control or test vehicles with same water activity as caffeine solution taken in donor compartment. This precaution was undertaken to avoid the osmotic pressure difference across donor and receptor to act as a driving force for drug diffusion across Cuprophan membrane.

In observation, steady state flux of caffeine was found to decrease with reduction in water activity of test vehicles. Lowering of solution water activity by one unit (from $a_w$-0.98 to $a_w$-0.87) resulted in ~3 fold reduction in the steady state flux of caffeine). Further, reduction in water activity of control vehicle to $a_w$-0.78, $a_w$-0.54 and $a_w$-0.42, drastically reduced the steady state flux by ~11.5, ~38.3 and ~115 fold, respectively. Drug concentration (or chemical potential) and diffusivity in formulation vehicle are two major rate controlling factors that greatly influence drug release from formulation vehicles (Al-Khamis et al., 1986). Caffeine concentration (or drug chemical potential) was found to be very similar in control and all test vehicles utilized for drug release studies (table 2)]. However, it is very intriguing that caffeine release flux (across Cuprophan membrane) from test vehicle of low water activity ($a_w$-0.78) was several fold (12 fold) lower than control vehicle ($a_w$-0.98) Graphical plot figure 1 depicts drug release across Cuprophan membrane, from control and test vehicles of different water activity.
Further, release studies were performed across a non-porous hydrophobic membrane (silicone membrane) in order to validate the drug release phenomenon observed in case of Cuprophan membrane. Here, the rate of transmembrane drug transport was dependant on water activity of the vehicle. Although, similar phenomenon was observed in case of release studies across Cuprophan membrane, there was a significant difference in the magnitude in the amenability. In observation, when water activity of formulation vehicle was lowered, the drug diffusion flux across silicone membrane was found to diminish (figure 2).

Drug diffusivity in a topical vehicle is inversely proportional to medium viscosity (could be modeled using Stokes-Einstein equation). An increase in viscosity was observed when water activity...
activity of test vehicles was lowered, due to incorporation of lithium chloride (table 3). According to Stokes-Einstein equation, diffusion coefficient of caffeine was expected to reduce only by a factor of 2 fold, in case of a\textsubscript{w}-0.78 test vehicle (3.26 x 10^{-6} \text{ cm}^2/\text{s}) versus control (a\textsubscript{w}-0.98) (7.15 x 10^{-6} \text{ cm}^2/\text{s}). However, from diffusion studies across Cuprophan membrane, a resultant 12 fold reductions in caffeine release/diffusive flux was observed for a\textsubscript{w}-0.78 group versus control. Thus, it can be inferred here that lowering water activity of the formulation vehicle had profoundly affected the rate of drug release/diffusion from formulation vehicles through other mechanisms besides lowering drug diffusion coefficient formulation viscosity related effects.
4.2. Mathematical Modeling

<table>
<thead>
<tr>
<th>Water activity vehicle ($a_w$) (22°C)</th>
<th>Caffeine concentration (molar) (22°C)</th>
<th>Viscosity (centipoises) (22°C)</th>
<th>Theoretical modeling (Stokes-Einstein) of caffeine diffusion coefficient in water activity vehicles (cm²/s)</th>
<th>Experimental determination of caffeine diffusive flux across Cuprophan membrane (mg/0.6 cm²/hour)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>0.98 (control)</td>
<td>~0.08</td>
<td>0.89</td>
<td>7.15 x 10⁻⁶</td>
<td>1.15 ± 0.03</td>
</tr>
<tr>
<td>0.87</td>
<td>~0.08</td>
<td>1.23</td>
<td>5.17 x 10⁻⁶</td>
<td>0.42 ± 0.08</td>
</tr>
<tr>
<td>0.78</td>
<td>~0.08</td>
<td>1.95</td>
<td>3.26 x 10⁻⁶</td>
<td>0.10 ± 0.01</td>
</tr>
<tr>
<td>0.54</td>
<td>~0.09</td>
<td>2.75</td>
<td>2.13 x 10⁻⁶</td>
<td>0.02 ± 0.01</td>
</tr>
<tr>
<td>0.42</td>
<td>~0.10</td>
<td>3.64</td>
<td>1.75 x 10⁻⁶</td>
<td>0.01 ± 0.001</td>
</tr>
</tbody>
</table>

Table 3: Physicochemical properties of test vehicles of varied water activity

Each data represents mean ± standard deviation (n=6)
Aforementioned results had invoked extreme curiosity and in order to provide functional and more direct evidence, qualitative dye diffusion study was designed to visually characterize the influence of water activity on solute diffusivity in water activity vehicles. Methylene blue was used as a model dye and a time lapse observation in bulk diffusivity in control, a\(_w\)-0.78 and a\(_w\)-0.42 vehicles are shown in figure 3a, 3b and 3c. Dye diffusivity in control vehicle (a\(_w\)-0.98) was found to be instantaneous and rapid, yielding a homogenous solution at the end of 2 hours. However, in case of a\(_w\)-0.78 and a\(_w\)-0.42 test vehicles, rate of dye diffusion was observed to be very slow.

Viscosity of the vehicle medium is expected to slow down solute diffusivity. From Stokes-Einstein relationship, diffusion coefficient of methylene blue would be expected to reduce only by 2 fold between control (a\(_w\)-0.98) and a\(_w\)-0.78 test vehicle. However, it took longer than 12 hours for the dye to diffuse down the medium in a\(_w\)-0.78 test vehicle but only 2 hours in case of a\(_w\)-0.98 vehicle.

### 4.3. Qualitative Dye Diffusion Study

In conjunction, separate set of dye diffusion studies were performed utilizing sucrose solution which acted as viscosity controls for the test vehicles. The latter set of dye diffusion studies presumed to be an efficient tool to decouple viscosity related effects from water activity in governing the bulk diffusivity of methylene blue. Sucrose solution was prepared at two concentration levels 20% and 31% w/v in deionized water to match the viscosity of a\(_w\)-0.78 and a\(_w\)-0.42 test vehicles, respectively. It was very interesting to observe that methylene blue dye took comparatively lesser time for complete diffusion in case of 20% w/v sucrose solution (viscosity control) versus a\(_w\)-0.78 test vehicle. In case of a\(_w\)-0.78 test vehicle (1.9 cP), it took ~16 hours (picture not shown) to achieve complete dye diffusion whereas in its viscosity control
(sucrose solution – 20% w/v, viscosity - 1.9 cP) complete diffusion was achieved in just ~10 hours. Similar trend was observed in case of a\textsubscript{w}-0.42 test vehicle (3.6 cP) and its viscosity control. It took ~44 hours for complete dye diffusion in case of a\textsubscript{w}-0.42 test vehicle (3.6 cP) versus ~24 hours for sucrose solution (31% w/v, viscosity - 3.6 cP).

It was evident that water activity of solution had overwhelming control over solute (methylene blue dye) diffusivity compared to viscosity. Owing to relatively low free water content in case of a\textsubscript{w}-0.78 and a\textsubscript{w}-0.42 test vehicles, methylene blue molecules (methylene blue dye) had to endure a more tortuous pathway in order to diffuse through the medium. Hence, the bulk diffusion of methylene blue assumed longer time than anticipated (Stokes-Einstein equation) in case of low water activity vehicles (a\textsubscript{w}-0.78 and a\textsubscript{w}-0.42) compared to respective viscosity controls.

In retrospect, water activity was the predominant reason for anomaly observed in caffeine release behavior from various water activity vehicles. Diminishing water activity of formulation vehicles had apparent rate-controlling like (non-linear) effects on its drug releasing properties. Water activity could be a critical physicochemical property of formulation vehicles that can detrimentally affect the drug release characteristics if not controlled appropriately.
Figure 3a: Time lapse observation of effect of water activity on solute (methylene blue) diffusivity in deionized water (a<sub>w</sub>-0.98)

Figure 3b: Left: Time lapse observation of effect of water activity on solute (methylene blue) diffusivity in formulation vehicle (a<sub>w</sub>-0.78; viscosity – 1.9 cP). Right: Time lapse observation of solute diffusion in viscosity control for the a<sub>w</sub>-0.78 formulation vehicle (20% w/v sucrose solution in deionized water, viscosity – 1.9 cP)

Figure 3c: Left: Time lapse observation of effect of water activity on solute (methylene blue) diffusivity in formulation vehicle (a<sub>w</sub>-0.42; viscosity – 3.6 cP). Right: Time lapse observation of solute diffusion in viscosity control for the a<sub>w</sub>-0.78 formulation vehicle (31% w/v sucrose solution in deionized water, viscosity – 3.6 cP)
5. Conclusion

Drug diffusion/release across model synthetic barriers (cellulosic and silastic) from a topically applied vehicle was influenced by water activity of the solution. Drug transport studies reveal that caffeine diffusive flux is significantly affected by low water activity of formulation vehicles. Mathematical modeling suggest that water activity, in addition to viscosity, exerts apparent rate controlling properties on drug diffusion within formulation vehicles and consequently affecting drug release. Qualitative dye diffusion studies validate that drug diffusion is relatively slow in low water activity vehicles and support the findings from drug transport study and mathematical modeling.
CHAPTER 3

MECHANISTIC INVESTIGATION OF WATER ACTIVITY EFFECTS AT FORMULATION VEHICLE-SKIN INTERFACE AND INFLUENCE ON SKIN DRUG DELIVERY

1. Objective

In this objective, effects of water activity on topical drug delivery at vehicle-skin interface were studied using simple topical vehicles of diverse water activity. Utilizing Franz diffusion cells, drug transport studies were performed to evaluate the effects of water activity on \textit{in-vitro} drug delivery and drug permeation using porcine skin model. For a broad spectrum investigation of water activity effects at vehicle-skin interface, \textit{in-vitro} permeation studies were performed using model drugs of varied physicochemical properties [log P ranging between (-) 1.56, (-) 0.79 and 1.17]. In addition, effects of water activity on topical drug delivery under finite/infinite dosing conditions, skin hydrodynamics and barrier properties were also investigated. Also, \textit{in-vitro} permeation studies were undertaken to investigate the role of hydrating agents on topical drug delivery upon inclusion in low water activity vehicles. Histological investigation was undertaken to study the role of hydrating agents on barrier property restoration in porcine skin model.

2. Materials

Caffeine, nicotine, acyclovir, and sodium fluorescein were purchased from Sigma Aldrich (St. Louis, MO). Propylene glycol was acquired from BASF chemical company (Tarrytown, NY). Porcine skin was acquired from a local slaughter house and epidermis was
harvested using heat separation technique. All solutions and dilutions were prepared using purified deionized water (resistivity ≥18.2 mΩ.cm, Barnstead Nanopure Diamond™, Barrington, IL).

3. Methods

3.1. In vitro drug permeation studies across porcine epidermis

In vitro permeation studies were performed to investigate the effects of water activity of topical vehicles on drug permeation across model biological membrane (heat separated porcine epidermis). IVPT was performed using vertical Franz diffusion cells and the experimental setup remained similar to in vitro release studies involving synthetic membranes. Caffeine (log P: -0.79; mol. wt.: 194.19 g/mol) was utilized as a model drug and 15 mg/mL drug solution was prepared in control (a_w-0.98) and test vehicles (a_w-0.78). The active diffusion area for drug permeation was maintained at 0.64 cm². Permeation studies were performed under both infinite and finite dosing conditions. Two hundred microliter and 20 µL of formulation vehicles were utilized for infinite and finite dose studies, respectively.

Utilizing similar experimental conditions, in-vitro permeation studies were also extended for model drugs like acyclovir (log P: -1.56; mol. wt.: 225.21 g/mol) and nicotine (log P: 1.17; mol. wt.: 162.23 g/mol). Model drugs caffeine, acyclovir and nicotine were chosen based on their broad spectrum physicochemical properties. Drug solution was prepared in control (a_w-0.98) and test water activity vehicles (a_w-0.78) and employed for permeation studies at following concentration: Nicotine – 50 mg/mL (1g/mL) and acyclovir – 1 mg/mL (1.5 mg/mL).
3.2. Effect of varying levels of propylene glycol (humectant) on skin permeation of model drug from low water activity vehicle \((a_w-0.78)\)

*In vitro* permeation testing, under infinite dosing condition, was performed in order to investigate the effects of propylene glycol (humectant) on skin permeation of drugs from low water activity vehicle \((a_w-0.78)\). Porcine epidermis was used a model biological membrane and the active diffusion area for drug permeation was maintained at 0.64 cm\(^2\). Sodium fluorescein is utilized as a model drug in this study owing to its analytical sensitivity. Two hundred microliter of sodium fluorescein drug solution (1mg/mL) was prepared in \(a_w\)-0.78 vehicle and added to donor compartment which served as control group. Similarly, three different sets of sodium fluorescein drug solution (1mg/mL) prepared in \(a_w\)-0.78 vehicle containing 10, 20 and 30% v/v propylene glycol, which served as test group. Water activity was retested for test vehicles after inclusion of propylene glycol. Receiver compartment was filled with solution of same water activity as the donor (without drug) in order to maintain constant osmotic pressure across donor and receiver compartments. At predetermined time points, 0.2 ml samples were withdrawn from receiver compartment to analyze the amount of sodium fluorescein permeated across porcine epidermis from low water activity vehicles \((a_w\)-0.78) containing propylene glycol.

3.3. Determination of effect of water activity of vehicle on dermal penetration of topically applied drugs

Franz cell setup was utilized to investigate the effect of water activity of topical vehicle on dermal penetration of model drug caffeine. Porcine epidermis was utilized as a model biological membrane and was sandwiched between donor and receiver compartment in Franz diffusion cell apparatus. Donor compartment was filled with 500 µL control and test vehicles containing 15 mg/mL caffeine. The receiver compartment was maintained empty. The donor
compartment and sampling port of the receiver compartment were covered with paraffin film. After 8 hours of treatment, the epidermis was rinsed with deionized water to remove excess drug solution off the surface. Followed by, the epidermis was digested in 1N sodium hydroxide solution using a tissue homogenizer, solution filtered and drug content analyzed using a suitable analytical method. The active diffusion area was maintained at 0.64 cm$^2$ and absolute amount of caffeine loaded in epidermis was recorded as the extent of dermal penetration of drug.

3.4. Determination of skin hydrodynamics

The effect of water activity of formulation vehicle on skin hydration status was determined using porcine epidermis. Porcine epidermis was equilibrated to ambient conditions (22°C/60% RH) for 2 hours, as a prerequisite. Followed by, pre-weighed strip of porcine epidermis (2 cm x 2 cm) was mounted on Franz diffusion cell apparatus with stratum corneum side facing the donor compartment. Donor compartment was filled with test vehicles ($a_w$-0.78 and $a_w$-0.54) and receiver compartment was kept empty. Donor compartment was then covered with paraffin film to prevent water escaping from formulation vehicles. After 6 hours of equilibration, epidermis was blotted with Kimwipes® to remove excess solution sticking on the skin surface and weighed again. The difference in weight of epidermis pre- and post-treatment with test vehicles was recorded as resultant percent water content (test group). In order to determine the baseline hydration status (control), pre-weighed epidermis strip (2 cm x 2 cm) was equilibrated to ambient conditions (22°C/60% RH) and subsequently stored in hot air oven (40°C/25%RH) for an extended period. Followed by, weight of dried epidermis strip was determined. Difference in pre-and post-drying weight was recorded as the baseline percent water content of porcine epidermis.
3.5. Histology

Histological investigation was undertaken to investigate the potential structural changes in full thickness human cadaver skin following exposure to formulation vehicle of low water activity (a_w-0.78). A strip of skin (2 x 2 cm) was mounted on Franz diffusion cell apparatus with the stratum corneum side facing donor compartment. Topical formulation vehicle of low water activity (a_w-0.78) was taken in the donor compartment while receiver compartment was maintained empty. In addition, low water activity vehicle (a_w-0.78) containing propylene glycol (1:1) was also investigated as one of the test formulations and deionized water (a_w-0.98) served as control. Skin was treated with formulation vehicles for a period of 6 hours followed by cross-sections were prepared for histological investigation. Briefly, skin cross-sections were flash frozen in liquid nitrogen and were subjected to freeze drying. Freeze dried skin sections were coated with platinum using a sputter coater under argon gas for observation under JSM-7800F PRIME field emission scanning electron microscopy (Jeol, Tokyo, Japan).

3.6. Analytical methods

All chemical analysis and drug quantification in transport studies were performed using HPLC analytical method. The analytical conditions utilized for all model drugs are as follows. Reversed phase chromatography was performed for quantification of model drug caffeine. HPLC system consists of Waters® 1525 binary pump (Waters® Corporation, Milford, MA) equipped with Waters® 717 plus autosampler equipped with UV absorbance detector. Chromatographic separation was achieved under isocratic mode at a flow rate 1 mL/min on Symmetry® C18 (4.6 x 150 mm; 5µ) reversed phase analytical column. Caffeine was eluted using mobile phase consisting of 50% v/v methanol and 50% v/v water system (50:50 v/v) at analytical wavelength 254 nm. The chromatogram was analyzed using Breeze® software system.
3.7. Statistical treatment

GraphPad InStat 3 software was used for statistical analysis. One-way ANOVA was used to determine the level of significance for correlation between parameters and P < 0.05 was considered as the acceptable level of significance.

4. Results and Discussion

4.1. Effect of water activity of the vehicle on \textit{in vitro} drug permeation across the porcine epidermis

![Cumulative caffeine permeation](image)

\textbf{Figure 4:} Effect of water activity of the vehicle on caffeine permeation flux across porcine epidermis under infinite dosing conditions. Each data point represents mean ± standard deviation (n=6)

Porcine epidermis (heat separated) model was utilized to study the effects of topical vehicles’ water activity on drug permeation behavior in a biological membrane. Porcine epidermis was elected as a model membrane owing to the fact that its stratum corneum lipid composition and SC layer thickness closely resembles with that of human skin (Simon and Maibach, 2000). Figure 4 represents cumulative caffeine permeation with time under infinite
dosing conditions across porcine epidermis. Cumulative permeation of caffeine was found to decrease when water activity of formulation vehicle was lowered. The steady state flux of caffeine in case of control vehicle was relatively higher than lower water activity vehicles (1.071 and 0.219 µg/0.6cm²/hr for control and a_w-0.78 vehicle, respectively. P < 0.05)

![Figure 5: Effect of water activity of the vehicle on caffeine permeation flux across porcine epidermis under finite dosing conditions. Each data point represents mean ± standard deviation (n=6)](image)

Traditionally, infinite dose technique has been most frequently used to study percutaneous absorption events such as drug diffusion properties and penetration enhancement mechanisms. During infinite dose studies in vitro, the skin is completely bathed by donor and receptor solution and such an incidence is unrealistic under clinical or “in use” conditions. Apparently, transient changes in drug concentration are negligible hence changes in permeation profile due to vehicle
drying or metamorphosis of the formulation cannot be effectively studied, under infinite dose condition.

Drug permeation behavior under finite dosing technique was reported to closely mimic clinical/realistic conditions as the amount of vehicles applied is typically in the range of few milligrams per square centimeter of skin. (Franz, 1977, Franz, 1975, Seta et al., 1992, Kubota and Yamada, 1990).

Consequently, \textit{in vitro} permeation studies were performed under finite dosing conditions as well, to ascertain drug permeation behavior from low water activity vehicles, to closely mimic “in-use” conditions. Twenty microliter of drug solution (300 µg caffeine content) was utilized for finite dose study versus 200 µL (3 mg caffeine content) utilized for infinite dosing. Cumulative caffeine flux was found to decrease with lowering water activity of formulation vehicles and was found to be at least 3 fold higher for control vehicle versus test vehicle with a \(a_w\)-0.78 (figure 5). In summary, low water activity of topical vehicles conclusively affected drug permeation behavior across porcine epidermis, a model biological membrane.
Figure 6: Effect of vehicle water activity on acyclovir permeation \([\log P (-) 1.56]\) across porcine epidermis under infinite dosing conditions. Each data point represents mean ± standard deviation (n=6)
Figure 7: Effect of vehicle water activity on nicotine permeation [log P 1.17] across porcine epidermis under infinite dosing conditions. Each data point represents mean ± standard deviation (n=6).

In addition to caffeine (log P: -0.79; mol. wt.: 194.19 g/mol), infinite dose IVPT studies were extended for model drugs acyclovir (log P: -1.56; mol. wt.: 225.21 g/mol) and nicotine (log P: 1.17; mol. wt.: 162.23 g/mol). In observation, cumulative permeation flux of all model drugs from test formulation (a<sub>w</sub>-0.78) was several folds low (almost infinitesimal) compared with that of their respective controls (Figures 6 and 7). It is noteworthy that low water activity of formulation vehicle (a<sub>w</sub>-0.78) had affected permeation of drugs with broad spectrum physicochemical properties.
4.2. Effect of water activity of the vehicle on skin hydrodynamics

Figure 8 represents the resultant water content in porcine epidermis post 6 hours treatment with topical vehicles of varied water activity. The resultant hydration levels (exhibited as % values compared to baseline) of porcine epidermis post treatment with control vehicle ($a_w$-0.98) was found to be 50 ± 3%. Rise in hydration levels here was an expected behavior. However, porcine epidermis treated with low water activity vehicle groups ($a_w$-0.78 and $a_w$-0.54) lowered the degree of hydration and resulted in < 20% water content (which was lower than the baseline values ~37±6%).

The hydration level measurement following exposure to different water activity formulations showed that hydration levels increased when hydrated with water (control vehicle $a_w$-0.98), as expected. Notably, when exposed to lower water activity test solutions, epidermis would lose baseline water, leading to an apparent dehydration effect. The dehydration phenomenon observed here could be attributed to osmotic induced drying effects exerted by low
water activity of formulation vehicles. The extent of skin water loss depends on how low the water activity is and water activity gradient established between the outer layer of skin and formulation vehicle.

It was well established that aqueous based solution systems with relatively low water activity values exhibits strong osmotic dehydration effects on cellular materials (Gekas et al., 1998, Rastogi and Raghavarao, 1994, Toledo, 2007, Sereno et al., 2001). From mathematical equation described below, a logarithmic relationship exists between water activity and osmotic pressure for aqueous based systems. By virtue, lowering water activity of a system tends to increase its osmotic pressure multiple fold.

\[
\pi = (-)2.3026 \cdot \frac{RT}{V} \cdot \ln a_w
\]

where, \(\pi\) is the osmotic pressure, \(R\) is the gas constant, \(T\) is the temperature, \(Vm\) molar volume of water in the system and \(aw\) is the water activity of the system.

State of hydration of skin is a critical factor which determines the rate of percutaneous absorption of drug molecules. Enhanced skin hydration levels typically result in net ‘free’ water molecules within the tissue which could act as solvent for predominately polar permeants. Also, free water molecules in the stratum corneum region has potential to alter the permeant solubility leading to enhanced partitioning into the skin membrane (Williams and Barry, 2012). Elevated hydrating levels of skin was also reported to alter lipid bilayer packing resulting in elevated hydrophilic drug fluxes (Alonso et al., 1995, Alonso et al., 1996, Williams and Barry, 2012). Increased skin hydration levels have been shown to clearly enhance the skin flux of both hydrophilic and lipophilic drug moieties (Idson, 1978, Roberts and Walker, 1993). On contrary, osmotic stress induced drying effects were reported to cause structural re-organization of lipid
macromolecular assemblies in the stratum corneum leading to impaired skin permeability (Nowacka et al., 2012).

It was evident that low water activity of formulation vehicles ($a_w$-0.78) exhibited greater tendency to induce dehydration in model biological tissue (porcine epidermis) upon topical application. A similar dehydrating effect in human skin tissue could be anticipated when topical formulations with low water activity were to be utilized for practical drug delivery application. Under such circumstances, low water activity of topical vehicles would undoubtedly limit or affect skin permeability status or flux of therapeutic agents formulated within.

4.3. Effect of water activity of the vehicle on dermal penetration of drugs

![Figure 9: Effect of water activity of the vehicle on caffeine accumulation in porcine epidermis after pretreatment for 8 hours [n=6; ** P < 0.001 compared to control ($a_w$-0.98)]]
Most dermatological disorders manifest in the viable epidermis/dermis region hence topical dermatological preparations are aimed at delivering therapeutic agents regionally to deeper skin layers. This in turn depends on myriad of factors governing nature of drug, formulation properties and complex interplay at vehicle-skin interface. Applying this context to present investigation, we studied the effect of formulation vehicles’ water activity on skin penetration of the model drug caffeine.

After treating porcine epidermis with control and test formulation vehicles (a\textsubscript{w}-0.98, a\textsubscript{w}-0.78 and a\textsubscript{w}-0.54) for 8 hours period, epidermis piece was digested in 1N sodium hydroxide to determine the absolute amount of caffeine loaded in it. From figure 9, it can be found that 32.76 ± 14.60 µg caffeine was found in epidermis treated with control vehicle whereas, 9.40 ± 7.24 µg caffeine was found in epidermis treated with low water activity vehicle (a\textsubscript{w}-0.78) (P < 0.001 at 95% confidence interval). In case of test vehicle a\textsubscript{w}-0.54 the amount of caffeine loading in epidermis was found to be even lower (4.13 ± 1.60 µg) compared to control and a\textsubscript{w}-0.78 test vehicle (P < 0.01).

The data furnished in figure 7 corroborates that drug penetration in skin (porcine epidermis - model biological membrane) is limited by low water activity of test formulation vehicles (a\textsubscript{w}-0.78 and a\textsubscript{w}-0.54). This could be attributed to collective effects of low water activity on (i) drug release and diffusivity from formulation vehicle, (ii) limited drug permeation through skin and (iii) dehydration effects on skin tissue. In retrospect, water activity of formulation vehicles was found to be critical in determining the extent of skin penetration of drugs.
5.4. Histological Evaluation

Figure 10a: Representative SEM picture of human cadaver skin treated with control vehicle ($a_w$ 0.98)

Figure 10b: Representative SEM picture of human cadaver skin treated with test formulation vehicle ($a_w$ 0.78)
Histological evaluation utilizing scanning electron microscopy was performed to investigate water activity led structural changes in the stratum corneum layer in human cadaver skin. Figure 10a and 10b, represents high resolution SEM images obtained for the cadaver skin treated with control (aw-0.98) and test water activity vehicle (aw-0.78). Cadaver skin treated with control (aw-0.98) served as control for cadaver skin treated with low water activity formulation vehicle (aw-0.78).

The stratum corneum in control skin exhibited tightly bound corneocytes layer with minimal or absence of any intercellular space. Opposed to control skin, stratum corneum in cadaver skin treated with test vehicle (aw-0.78) was found to exhibit very loosely bound corneocytes layer. Moreover, the individual corneocytes layer was observed to remain detached from subsequent layers. This trending behavior in SC microstructural modifications was attributed to varying degrees of dehydration effects exerted by low water activity of vehicle (aw-0.78). The drying effects were shown to be associated with significant structural modifications in stratum corneum layer sabotaging the transport pathways available for drug permeation through skin.

Thus, low water activity of topical formulation could lead to structural modifications in stratum corneum layer and plausibly affect the permeation of topically applied drugs by the way of impairing continuous transport pathways available in skin. This was potentially caused by osmotic shrinkage of corneocytes, which would be in agreement with the data from skin hydrodynamics and skin penetration experiments.
4.4. Investigation of propylene glycol (humectants) in enhancing skin permeability of drugs from low water activity formulation vehicles and associated effects on skin microstructure

From figure 11, it can be observed that incremental inclusion of small molecule humectants, eg. propylene glycol (PG) in low water activity vehicle ($a_w$-0.78) had profound effects on enhancing solute permeation across skin. Sodium fluorescein (SF) was utilized as a model drug to study the humectants effect owing to its analytical sensitivity. From in vitro permeation studies across porcine epidermis, cumulative SF flux from low water activity test vehicle ($a_w$-0.78) was found to be negligible (even after prolonged equilibration). Whereas, SF flux from control vehicle ($a_w$-0.98) was found to be several folds higher, comparatively. The low water activity of test vehicle ($a_w$-0.78) had significantly affected the permeation of sodium fluorescein across porcine epidermis and was in good agreement with permeation data obtained

Figure 11: Effect of propylene glycol on permeation behavior of sodium fluorescein from various water activity vehicles (n=6)
for other model drugs (caffeine, acyclovir and nicotine). However, incremental inclusion of propylene glycol in the test vehicle (a_w-0.78) had led to a dose dependant enhancement in SF permeation flux. The trend for SF permeation flux enhancement with propylene glycol dose, from low water activity test vehicle (a_w-0.78) was as follows: a_w-0.78 < a_w-0.78 (10% PG) < a_w-0.78 (20% PG) < a_w-0.78 (30% PG) < aw-0.98. Utilization of 30% v/v propylene glycol in low water activity vehicle (a_w-0.78) had enhanced the SF permeation flux to the levels observed in case of control vehicle (a_w-0.98). This phenomenon could be attributed to the ability of humectants to transiently increase skin hydration levels and associated permeability status of skin.

On contrary, owing to strong water binding properties, humectants such as propylene glycol can be expected to lower water activity of aqueous systems. This was evident in case of test formulation (a_w-0.78) incorporated with varying levels of propylene glycol. From table 3, it can be observed that addition of propylene glycol had further lowered the water activity of test formulation vehicle (a_w-0.78). In earlier sections, we had demonstrated that relatively low water activity of formulation vehicle could diminish drug permeation across skin. However, it was interesting that SF flux was found to proportionately increase with propylene glycol levels despite a decreasing trend in lowering water activity of the formulation vehicle (a_w-0.78).
<table>
<thead>
<tr>
<th>Formulation Vehicle</th>
<th>Water activity ($a_w$) (22°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control vehicle</td>
<td>0.98</td>
</tr>
<tr>
<td>Test vehicle ($a_w$-0.78)</td>
<td>0.78</td>
</tr>
<tr>
<td>$a_w$-0.78 (10% v/v PG)</td>
<td>0.75</td>
</tr>
<tr>
<td>$a_w$-0.78 (20% v/v PG)</td>
<td>0.73</td>
</tr>
<tr>
<td>$a_w$-0.78 (30% v/v PG)</td>
<td>0.71</td>
</tr>
</tbody>
</table>

**Table 4: Effect of propylene glycol levels on water activity of test formulation vehicle ($a_w$-0.78)**

Pirot et al., had demonstrated that small molecule humectants (like glycerol and propylene glycol) had ability to penetrate into the stratum corneum following topical application. The hygroscopic nature of such humectants resulted in efficient binding of water molecules in the stratum corneum region and increased its overall water holding capacity (Pirot et al., 2003). Nowacka et al. had demonstrated that osmotic drying would cause structural re-organization of lipid macromolecular assemblies in the stratum corneum leading to impaired skin permeability. However, small molecule humectants (like glycerol and urea) had ability to protect lipid bilayer in the stratum corneum against osmotic drying and preserved its fluidity under dry conditions (Nowacka et al., 2012).
In conjunction, histology studies were undertaken to evaluate the hydrating effects of propylene glycol present in low water activity ($a_w$-0.78) vehicle, on structural changes in human cadaver skin. Briefly, the cadaver skin was treated with $a_w$-0.78 vehicle containing propylene glycol (1:1). Followed by, cross sections of the skin were acquired and observed under SEM to evaluate associated structural changes, if any. It can be observed from figure 12 that the stratum corneum layers were found to be intact versus skin treated with $a_w$-0.78 (figure 10b). Presence of propylene glycol in low water activity vehicle ($a_w$-0.78) could plausibly counteract the unfavorable effects of low water activity on skin dehydration and associated structural damage.

![Image](image1.png)

**Figure 12:** Representative SEM picture of human cadaver skin treated with propylene glycol and $a_w$-0.78 vehicle (1:1)

In conclusion, despite the low water activity of topical formulations, inclusion of propylene glycol could increase hydration levels in stratum corneum, maintain structural integrity of skin and promote drug permeability in skin.

5. **Conclusion**

Drug permeation across the porcine skin from a topically applied vehicle was measurably influenced by water activity of topical vehicles. Water activity effects on drug permeation (across biological membrane) were found to be universal for drugs of varied skin affinity.
Vehicles with low water activity altered the hydrodynamics of skin, and this was associated with corresponding impacts on the drug permeation. Inclusion of small molecule humectants like propylene glycol, retained skin hydrodynamics and counteracted water activity led effects on drug permeation.
CHAPTER 4

INVESTIGATION OF MANUFACTURING PROCESS PARAMETERS ON WATER ACTIVITY OF TOPICAL SEMI-SOLID CREAM (WATER-IN-OIL TYPE)

1. Objective

Topical cream products may possess numerous quality attributes that can affect the permeation of the drug through skin, skin retention of cream and patient acceptability which can further potentially impact therapeutic efficacy and safety. Few of the critical quality attributes are direct result of microstructural arrangement (Q3 characteristics) of the semi-solid cream products. Microstructural (Q3) properties of cream products are significantly affected by manufacturing process to an extent that even cream lots of Q1/Q2 (qualitative and quantitative) similarity result in varying degree of microstructural (Q3) arrangement when processed at different conditions. In previous chapters, profound investigation was undertaken to elucidate the mechanisms through which water activity influence topical drug delivery. In retrospect, water activity is a critical microstructural property that plausibly affects drug release and topical drug delivery. In the present chapter, focal point is to evaluate if variation in manufacturing process conditions could influence resultant water activity of a model semi-solid cream formulation. A model water-in-oil type cream formulation is utilized to study the effects process parameters like homogenization speed and time on resultant water activity of topical semi-solid cream.

2. Materials

Mineral oil USP, white wax NF, cetostearyl alcohol NF, propylene glycol NF and sodium borate NF were purchased from Spectrum chemical manufacturing Co., (New Brunswick, NJ).
Cuprophan membrane was purchased from Agilent technologies (Santa Clara, CA) and silicone membrane was obtained from SMI medical device manufacturer (Saginaw, MI). All solutions and dilutions were prepared using purified deionized water (resistivity ≥18.2 mΩ.cm, Barnstead Nanopure Diamond™, Barrington, IL).

3. Methods

3.1. Preparation of water-in-oil type test cream formulations

<table>
<thead>
<tr>
<th>Ingredients</th>
<th>Quantity (%) w/w</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cetostearyl Alcohol</td>
<td>12.5</td>
</tr>
<tr>
<td>White Wax</td>
<td>12</td>
</tr>
<tr>
<td>Mineral Oil</td>
<td>56</td>
</tr>
<tr>
<td>Sodium Borate</td>
<td>0.5</td>
</tr>
<tr>
<td>Water</td>
<td>19</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Table 5: Composition of model w/o type cream formulation used for manufacturing process investigation

Experimental batches of topical semi-solid cream formulation were prepared in order to investigate the effects of processing parameters on modulation of water activity. Two lots (1kg batch size) of qualitative and quantitatively similar water in oil type cream formulations were prepared by mixing oil phase and water phase (maintained at 70° C) using Silverson® L5M-A high shear mixer under varying process conditions (formulation I: mixing at 3500 rpm for 15 minutes; formulation II: mixing at 7000 rpm for 30 minutes). Mixing process was performed in a temperature controlled sealed glass beaker with limited head space in order to avoid loss of water.
from formulation batches. After high shear mixing phase, both cream lots were allowed to cool at a steady rate (5°C/min) until the temperature reaches 45°C while mixing at 1500 rpm then stopped. The creams were then allowed to cool to room temperature in a closed jar and post 24 hour wait period, water content, drying rate (water loss kinetics) and water activity were tested.

3.2. **Determination of water activity of test creams**

Water activity was experimentally determined by dew point technique using AquaLab Series 3 (Decagon Devices Inc.). Briefly, a dollop of test cream was placed in sample cup holder and introduced into the sample chamber and water activity of the cream was directly read from the water activity meter.

3.3. **Determination of total water content in test creams using thermogravimetry**

Thermogravimetric analysis was performed to determine total water content (post process) and rate of water loss for the cream lots using Perkin Elmer® Pyris 1 TGA. To determine total water content, samples were loaded in an aluminum sample pan and heated using temperature ramp program (5°C/min) from 25°C to 150°C (under constant nitrogen purge) until constant weight loss was achieved. The mass loss represents total water content in cream after mixing/homogenization unit operation.

3.4. **Determination of drying rate of test creams using thermogravimetry**

To determine drying rate, samples were thermally equilibrated in an aluminum pan under constant temperature program (37 ± 1°C) for 24 hours. Drying rate under isothermal equilibration determines the water loss kinetics (microstructural differences) between cream formulations processed at varying mixing/homogenization conditions.
3.5. Determination of globule size distribution in test creams

Globule size distribution was determined using optical microscopy technique. Approximately, 10 mg cream formulation was directly weighed on a clean glass slide and thin film (~10 µm) was formed using film applicator (Gardco® Microm II applicator blade, Japan). Samples were viewed under bright field optical microscopy (Axiolab A1, Carl Zeiss, NY) under appropriate magnification and images (containing globules) were acquired using AxioVision software (Carl Zeiss, NY). Projectile diameter was then analyzed using image analysis suite in AxioVision.

3.6. Statistical treatment

GraphPad InStat 3 software was used for statistical analysis. One-way ANOVA was used to determine the level of significance for correlation between parameters and P < 0.05 was considered as the acceptable level of significance.

4. Results and Discussion

4.1. Effect of manufacturing process parameters on water activity of test cream formulations

Emulsion based cream formulations are thermodynamically unstable dispersion of two immiscible materials (water and hydrocarbons), kinetically stabilized using emulsifier/surfactant system. Due to multiple degree of interaction between aqueous–hydrocarbon-emulsifier system, cream formulations were hypothesized to encompass intricate polymeric network predominantly constructed with partly/completely swollen gel structure (emulsifier phase) containing interlamellarly fixed crystalline water (or bound water) and bulk/free water. (Gašperlin et al., 1994, De Vringer et al., 1987, Junginger, 1984). The concept of bound and free water associated with polymeric systems had been around for at least three decades and several researchers had
successfully studied the delineated characteristics between bound and free water fraction utilizing several thermal techniques (Hatakeyama et al., 1988, Nakamura et al., 1981). Owing to the restricted ‘freedom of movement’, bound water exhibits delineated thermodynamic solvent properties compared to free/bulk water fraction and such an anomaly in solvent (water) properties was reported to affect various phenomena like drug dissolution, drug diffusion, drug chemical stability and microbial growth. However, overall thermodynamic energy status of bound and free water can be collectively expressed as water activity ($a_w$) (Caurie, 2011).

Manufacturing process investigation and material characterization was undertaken in this section to understand the influence of process parameters on water activity modulation of certain water-in-oil type cream formulation. Table 4, represents the composition of model water-in-oil type cream formulation and its process parameters. It can be inferred from table 5 that modification of process parameters had significant effects in modulating the water activity of the model cream formulation. The cream formulation exhibited a resultant water activity $a_w$-0.93 when initially processed at relatively lower homogenization speed and time. However, upon doubling the homogenization speed and time, the resultant water activity was significantly lowered to $a_w$-0.87. Further, thermogravimetric investigation (figure 13) had revealed that resultant water content of model creams processed at two difference conditions was almost similar (15 – 16 % w/w).
Table 6: Effect of process parameters on water activity of w/o type cream formulation processed at various conditions

<table>
<thead>
<tr>
<th>Formulation Code</th>
<th>Homogenization Speed (RPM)</th>
<th>Homogenization Time (minutes)</th>
<th>Water Activity ($a_w$) 22°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>3500</td>
<td>15</td>
<td>0.932 ± 0.02</td>
</tr>
<tr>
<td>2.</td>
<td>7000</td>
<td>30</td>
<td>0.871 ± 0.03</td>
</tr>
</tbody>
</table>

Processing investigation on model cream was performed in a completely sealed glass chamber with limited head space. This precaution was necessarily taken to prevent the loss of water to ambience during emulsification process. The resultant difference in cream water activity between creams processed at two set of conditions can be strictly attributed to difference in thermodynamic energy status of water (owing to changes in bound and free water fraction) rather than absolute amount of water present in it. It can be inferred here that changes in manufacturing process conditions could potentially influence the water activity of semi-solid cream formulations and by carefully controlling the process parameters it is feasible to achieve a topical cream product with desired water activity.
Figure 13: Determination of total water content in model w/o type cream formulation using thermogravimetry

Figure 14: Determination of drying rate of model w/o type cream formulation using thermogravimetry
Figure 14 represents the drying rate curve of two cream lots possessing significantly different water activity as a result of varied process conditions. Evidently, cream lot (formulation II) processed at 7000 rpm/30 minutes led to formation of relatively smaller globules (dispersed aqueous phase) compared to the cream lot (formulation I) processed at 3500 rpm/15 minutes (figure 13).

**Figure 15a: Globule size distribution of dispersed phase (aqueous phase) in test cream I (a_w-0.93)**
Figure 15b: Globule size distribution of dispersed phase (aqueous phase) in test cream I ($a_w$-0.87)

Higher percent of relatively smaller globules in formulation II had plausibly facilitated a stronger interfacial interaction leading to an intricate 3D interlamellar gel network containing relatively strongly bound water globules. In retrospect, formulation II (processed at 7000 rpm/30 minutes) was presumed to exhibit relatively higher degree of water binding in interlamellar gel network when compared to formulation I (processed at 3500 rpm/15 minutes) and hence these differences were found to complement the low water activity of formulation II ($a_w$-0.87) and eventually slower drying rate when compared to formulation I ($a_w$-0.93).

Apparently, under in-use conditions (clinical use), dermatological creams with high water activity and higher degree of drying (rate of water loss) could potentially lead to faster precipitation of dissolved drug content and thus affect skin permeation of drugs. On contrary, dermatological creams with low water activity could strongly retain its water content (otherwise
slower rate of water loss) and dissolved drug fraction leading to prolonged skin permeation of drugs.

5. Conclusion

Variation in manufacturing process conditions influenced water activity of model semi-solid cream formulations. Net changes in water activity potentially influenced drying rate of the cream formulations thereby altering dynamics of topical drug delivery by affecting variables governing vehicle-drug and vehicle-skin interactions.
BIBLIOGRAPHY


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Education

**Ph.D. (Pharmaceutics & Drug Delivery)**
University of Mississippi
January 2012 – December 2016
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University of Tennessee
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**M.S. (Pharmaceutics & Industrial Pharmacy)**
Long Island University, New York
August 2008 - December 2011
GPA: 3.45/4.00

**B. Pharmacy**
Tamil Nadu Dr. MGR Medical University
September 2006 - June 2010
GPA: 4.00/4.00

Work Experience

**Amgen Inc., San Francisco, CA**
June 2013 – August 2013
Product Development Intern – Solid Oral Dose Development

- Developed directly compressible extended-release tablet for an investigational drug utilizing swellable-core matrix technology.
- Performed raw material screening, preformulation, excipient compatibility and dosage form design.
- Developed multiple dose strength prototypes with optimized drug release profiles.
- Assisted development team in scale-up efforts, pilot lab tech transfer and optimization of clinical trial batch.
- Presented the research findings at company ‘Tech-ops’ meeting.
- Demonstrated strong analytical thinking and creative problem solving.

**Novartis Consumer Healthcare, Parsippany, NJ**
February 2007 – August 2008
Offshore Drug Safety Associate (CTS, India)

- Collected and documented product quality complaints and responded to medical inquiries for Novartis OTC line of products.
- Collected adverse event complaints from patients and healthcare professionals.
- Classified adverse events and reported to client drug safety team with associated documentation and narrative writing.
- Administered behavior support program and offered smoking cessation counseling.
German Remedies Ltd., Goa, India  
September 2006 – February 2007

Production Assistant

- Acquired working experience in manufacturing equipment including PMA, powder blenders, homogenizer, mechanical mills, fluid bed coater/dryer, and pan-coaters.
- Observed the journey of a formulation from just a chemical to an actual dosage form in cGMP facility.

Achievements and Awards

- US Food and Drug Administration Doctoral Fellowship
- National Institutes of Health Fellowship Award
  - Awarded with $10,000 grant funding for developing competitive research proposal.
  - Developed study objectives, experimental plans, contingency plans and timeline.
  - Drafted a strong technical write up for submission to NIH.
- BASF Travelship Award – Received for best research presentation at 2013 AAPS meeting.
- Academic Excellence Award – Rho Chi Honorary Society
- Newsletter Editor & Steering Committee Member – Dermatopharmaceutics Focus Group, AAPS

Technical Skills

- Solid-state characterization: TGA, DSC, DVS, PXRD, FT-IR
- Microscopy: Optical, SEM, AFM
- Particle size analyses: Horiba laser diffraction analyzer, Malvern Zetasizer
- Texture analyzer (TA XT Plus), Density (Accupyc II gas pycnometer)
- Performance testing: In vitro release study, In vitro permeation study

Publications

Book Chapter

Book title: Percutaneous penetration enhancers - Editor: Howard I. Maibach. (in press)

Chapter title: Therapeutic applications of transdermal electroporation.

Muralikrishnan Angamuthu, S. Narasimha Murthy

Research Publications

