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Factors Influencing Percutaneous Absorption:

Effects of Solvents, Solute Physicochemical Properties, and Penetration Enhancer

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ABSTRACT

Skin has been the focus of research as a site of local and systemic drug delivery due to its advantages over other routes of drug delivery. However, the determining factors that could affect the transport of compounds through and into the skin are still unclear. The main purpose of the present dissertation was to understand the determinants affecting skin permeation, including the influence of solvents and solute physicochemical properties on skin permeation and the effects of a penetration enhancer on the skin penetration of highly lipophilic permeants.

The objective of Chapter 3 was to examine the effects of solvents upon the deposition of a model solute, corticosterone (CS) in the stratum corneum (SC) that could influence skin absorption of the solute after topical application. The solvents used in the study had different evaporation rates that were expected to impact skin deposition of CS and its absorption across skin. The results show no correlation between the rate of CS absorption and the rate of solvent evaporation with volatile solvents, suggesting no difference in solvent-induced deposition of CS in the SC. The results of these volatile solvents were different from those of slower evaporating solvents, that a relationship between permeant absorption and solvent evaporation rate was observed.

The study performed in Chapter 4 was a continuing effort to investigate the effects of solvents on skin absorption of lipophilic and polar solutes and examine the relationships between solute physicochemical properties and skin absorption of these solutes. Skin
permeation experiments under the finite and infinite dose conditions were conducted with model solutes and selected solvents. Except for urea, the skin permeation results of the solutes under the finite dose condition of the volatile solvents were in general agreement with the permeability coefficients obtained under the infinite dose condition.

In Chapter 5, we probed the mechanism of the observed high finite dose skin permeation of urea, in contrast to its permeability coefficient. A small hydrophilic solute ethylene glycol (EG) with molecular weight similar to urea was studied for comparison. The results suggest that urea did not have penetration enhancing activity to enhance its permeation across skin under the finite dosing. Tape stripping results are consistent with skin permeation mechanism of solute deposition and diffusion. We hypothesized that the skin permeation behavior of urea could be attributed to the small molecular size of urea. Under the finite dose conditions examined in this study, solutes with molecular sizes similar to or less than urea and EG could lead to high percent of skin absorption independent of solute lipophilicity.

Chapter 6 aimed to examine the skin penetration of petrolatum and soybean oil after skin application with and without a penetration enhancer, glyceryl monooleate (GlyMOle). GlyMOle was found to enhance the penetration of petrolatum into the split-thickness skin and an active dose-dependent effect was observed. However, GlyMOle did not enhance the penetration of soybean oil in any conditions. The results of this study suggest that GlyMOle was a penetration enhancer for petrolatum under the studied condition.
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CHAPTER 1
INTRODUCTION
1.1 Background

1.1.1 Human Skin

Skin is the outermost human organ that provides an efficient barrier against foreign substances and pathogens. There is a current interest in using the skin as a route for delivering ingredients in both cosmetic and pharmaceutical fields. However, the nature of the skin structure limits the penetration of various compounds, which is a challenge in topical and transdermal research. Skin consists of three main layers: the subcutaneous connective tissue (hypodermis), the dermis, and the epidermis as shown in Fig. 1.1.

Figure 1.1: Picture of a histological section of skin (1)

The hypodermis is a layer of fat containing tissue located deepest to the skin surface and serves as insulation for the human body. The hydrophilic dermis, which is 0.5 -5 mm thick depending on the body area: e.g. a thin dermis on the eyelid and a thick dermis on
the back skin (2). The dermis is composed of collagen fibres (account for 70 % of the dry weight of the dermis) and elastic tissue (about 5 % of the dry weight of the dermis), providing strength and elasticity to the skin (1). The upper layer of the dermis (papillary dermis) also contains sensory nerves and a dense network of blood vessels that can be utilized for systemic drug delivery. The reticular dermis is the main part of the dermis and is in contact with the hypodermis. The viable epidermis is a living hydrophilic layer, which contains 70 % of water and is 50 -100 μm thick (3). The highly keratinized epidermis can be divided into several layers based on the state of keratinocyte differentiation. The basal layer, a single layer of keratinocytes is anchored on a basal lamina. This cell layer is related to cell proliferation and the formation of most epidermal cells. Melanocytes are scattered within the basal layer and synthesize melanin, which have an important role in skin pigmentation. Other layers of the epidermis are the stratum spinosum, the stratum granulosum, and the stratum lucidum. The upper most layer of the epidermis is the stratum corneum (SC), a hydrophobic layer consisting of “dead cells.” SC is considered an important barrier to skin permeation; it limits the penetration of external substances into the human body (1, 2)

1.1.2 Stratum Corneum (SC) and Permeation Routes

The SC is the outmost region of the skin and thought to be the major barrier for most penetrants (4). Therefore, efforts have been made to overcome this protective barrier and increase the effectiveness of the delivery of active ingredients though the skin to achieve a desired result. The SC is a thin heterogeneous layer comprised of keratinized epidermal cells (corneocytes) that are embedded within intercellular lipids. The lipid components
(about 14% of the SC by weight) were organized into lamellar bilayers that integrate between the corneocytes called brick and mortar structure. The bricks represent the corneocytes and the mortar is the intercellular mixer of lipids organized in lipid lamella. The intercellular lipid in the stratum corneum was recognized as the most important pathway for solute transport through stratum corneum (5, 6).

There are different potential routes for the permeation of compounds across the SC: the intercellular, transcellular and appendageal (sweat glands or hair follicles) routes (Fig. 1.2)
The transcellular route is a possible pathway for skin permeation of polar compounds but the significant route of most non-polar permeants penetration appears to be the intercellular pathway (7). The appendageal route is not believed to be an important permeation pathway since sweat glands and hair follicles occupy only 0.1% of the total skin surface area. However, very high molecular weight compounds such as nanoparticles or other slowly penetrating substances may be delivered through this route (4).

The diffusional pathlength in the SC has been estimated to be around 500 μm, which is much longer than the SC thickness (approximately 20 μm). The delivery of substances through the intercellular spaces is believed to involve diffusion and partitioning between lipophilic (the alkyl chains) and hydrophilic (the polar head groups) domains of the intercellular lipids before reaching the SC-epidermis junction (8).

### 1.1.3 Effects of Solvents on Percutaneous Absorption

The understanding of the effects of solvents on percutaneous drug absorption is important for topical and transdermal drug delivery systems such as gels, topical aerosols, and sprays that are composed of volatile solvents which evaporate upon application on the skin. These systems were suggested to be more convenient and patient friendly than traditional topical and transdermal systems such as transdermal patches (9). The metered dose topical and transdermal aerosol systems for local and systemic delivery of corticosteroids such as testosterone and estradiol are examples of these systems (10-12).
It is generally believed that a solvent can affect skin permeation of a solute by a combination of factors under the finite dose condition. After the application of the solute in the solvent on skin, the solvent can increase the thermodynamic activity of the solute due to solvent dissipation via evaporation and/or skin absorption. Solvents such as water, and ethanol could also penetrate rapidly into the uppermost 1–3 layers of corneocytes in the SC. The solvent drives the solute into these upper layers via convection and creates an initial concentration profile in the SC for permeation (13, 14). In addition, the solvent can act as a skin penetration enhancer (15) that enhances skin permeation of the solute before the solvent dissipates by evaporation and/or skin absorption. For instance, the solvent can alter the SC barrier and increase the solubility and/or diffusivity of the solute in the SC.

To provide effective topical and transdermal drug delivery, efforts were made to understand the effects of solvents upon percutaneous drug absorption. In a study conducted by Reid et al, the semi-solid matrix formation generated from a drug loaded hydrofluoroalkane (HFA) sprays was characterized in order to investigate how residual phase properties influenced drug localization in human skin. It was shown that the ability of PEG rich residual phase to retard the evaporation of ethanol, a volatile solvent in the HFA spray formulations, could lead to more effective delivery of the drug into the skin. This finding suggested that the longer residence time of ethanol, which has been shown to alter the barrier properties of the SC could enhance the drug transport into the viable
epidermis of the skin (16). Cross et al. studied the relationship between the physical properties of topical solvents (e.g., solubility parameter, hydrogen bonding, and molecular weight) and solvent uptake into a model membrane. It was found that vehicle sorption into silastic membrane could affect both diffusivity and membrane solubility of a solute, impacting the flux of the solute across the membrane (17). Solvent propylene glycol was also found to enhance the maximum fluxes of similarly sized phenolic solutes by increasing the solubilities of the solutes in SC (18, 19). It has been suggested by Twist and Zats that the flux of a drug from saturated solutions would be the same, independent of solvent, when the solvent in the formulation did not alter the SC (20).

In addition to drug delivery, the understanding of solvent effects on percutaneous absorption is important in risk assessment of toxic compound exposure. Ross and Shah attempted to reduce skin permeation of N,N-diethyl-m-toluamide (DEET), a toxic compound that can be rapidly absorbed through skin, and examined the effects of skin/vehicle partition coefficients of DEET in solvents ethanol, propylene glycol, and polyethylene glycol 400 (21). The effects of chemical retardants and modifiers on skin permeation were also investigated in similar studies (22, 23).

### 1.1.4 Solute Physicochemical Property-Skin Permeability Relationships

There are many factors that can influence the penetration rate of a compound into and through human skin. This includes the mode, frequency, and duration of application, temperature and conditions of the skin, local blood flow, binding effects, influence of
vehicle, and concentration and physicochemical properties of the permeant (24-26). The last of aforementioned factors was found to exert the greatest influence on skin absorption, especially in dermal toxicology (27). The solute physicochemical properties have been an interest in many studies of skin permeability. In early studies, researchers correlated skin permeability (K_p) to the lipophilic property and molecular weight (MW) of the solutes (28-30). It was suggested that absorption through the skin is optimal when the water/lipid partition coefficient is near unity (31). Sloan et al. proposed that oil and water solubility should be optimized to give maximum flux from any vehicle (32). Blank showed that while K_p values of alcohols increased with an increase in carbon number (or lipophilicity) for aqueous solutions, they decreased with an increase in carbon number (or lipophilicity) for isopropyl palmitate, olive oil and mineral oil. The author concluded that the alcohols from in which they are less soluble lead to more rapid skin penetration (33). It was argued that the solute lipophilicity alone cannot explain the relative permeabilities of structurally diverse groups of compounds. Kasting et al. (34) proposed that transport across the skin depended on molecular size to a greater extent than previously thought. This finding was supported by Anderson and Raykar (35), who concluded that the human stratum corneum K_p of cresols and hydrocortisone esters with similar lipophilicities possessed a steep dependency on molecular size. Employing a wide range of compounds, Guy and Potts (29, 36) suggested that the nonlinearity between K_p and lipophilicity results from the confounding effect of the larger size solutes.

Furthermore, Abraham et al. (37) and Potts and Guy (38) proposed that hydrogen (H)-bonding is an important factor in diffusion through skin and silicone, respectively. Pugh
and co-workers showed an inverse relationship between the number of hydrogen bonding groups and diffusion coefficient, $D$ for human skin (39, 40). They found that with each additional H-bonding group, $D$ decreased by an order of magnitude. This was confirmed by Plessis et al. (41) who revealed that solute hydrogen bonding may impede diffusion in the stratum corneum. Magnusson et al. (42) developed a model to predict the overall solute maximum flux ($J_{\text{max}}$) from aqueous solution by correlating $J_{\text{max}}$ values with solute physicochemical properties. While MW was the main parameter predicting maximum flux, $J_{\text{max}}$, the model prediction could be slightly improved by inclusion of experimental temperature, H-bonding, melting point, and octanol solubility of the solute.

1.1.5 Chemical Penetration Enhancers

The identification of chemicals to increase skin permeability has been an area of great interest in the last several decades (4, 43-45). Many skin penetration enhancers are found in patents (46) as well as pharmaceutical science literature (47). Examples of penetration enhancers include water, hydrocarbons, DMSO and their analogs, pyrrolidones, fatty acids, esters and alcohols, azone and its derivatives, surfactants (anionic, cationic, and non-ionic), urea and its derivatives, polyols, essential oils, terpenes and derivatives, oxazolidines, epidermal enzymes, polymers, lipid synthesis inhibitors, bio-degradable enhancers, and synergistic mixtures (48).

Even though many chemical entities have been identified as chemical penetration enhancers, only a limited number of these chemical enhancers were used in the market
due to their toxic effects on the skin and economic feasibility (49). Dimethylsulphoxide (DMSO), often used as a “universal solvent” is one of the earliest and most extensively studied penetration enhancers. DMSO has been shown to enhance the permeation of, for example, antiviral agents, steroids and antibiotics. However, at relatively high concentrations, it can cause skin irritation and production of a malodogenous metabolite in the breath, resulting in limited use in commercial topical products (4).

Fatty acids and fatty acid esters have been known to increase skin penetration (45). Oleic acid has been shown to have penetration enhancing activities for many drugs. For example, it increased the fluxes of salicylic acid by 28-fold and 5-flourouracil flux by 56-fold across the human skin in vitro (50). It has been also revealed that only the cis form of oleic acid is effective and the site of unsaturation in the molecule is important for its activity as a penetration enhancer (4). Many of the compounds in this group are classified as Generally Recognized As Safe (GRAS), making these chemicals a popular penetration enhancer in many commercial products. For instance, a combination of glyceryl monooleate and lauryl lactate is used by Theratech Inc. to improve the delivery of testosterone across human skin. However, these compounds could cause skin irritation, leading to the suggestion of the application of triamcinolone acetonide before the product use (51).

The incorporation of a chemical penetration enhancer into a formulation to aid dermal absorption may also possess some difficulties. The solubility and chemical potential of the agents are critical factors and need to be optimized for effective topical preparations. For example, it was shown that the inclusion of laurocapram (Azone) in a commercially
available cream containing fluocinolone acetonide did not enhance vasoconstrictor activity; however, the activity of the steroid was enhanced when it was dissolved in 2% laurocapram in ethanol. The presence of enhancers, which often will have good solvent properties, could decrease the chemical potential (52).
CHAPTER 2
RESEARCH SIGNIFICANCE, HYPOTHESIS,
AND SPECIFIC AIMS
2.1 Significance of the Research

There is a considerable interest in using the skin as a site of drug administration both for local and systemic drug delivery. Compared with the oral administration route and hypodermic injections, transdermal delivery has a number of advantages, including reduction of first pass metabolism, noninvasive method, and ease of use (53). However, the skin, in particular the stratum corneum (SC), with a complex structure composed of various lipid and protein domains is a remarkable barrier that hinders topical and transdermal delivery. One method to overcome this problem is the incorporation of solvents, which have two modes of action. One is to promote a high drug concentration in the stratum corneum by increasing the thermodynamic activity of the drug in the vehicle and thereby enhancing the interfacial drug transfer into the stratum corneum. The other is to select solvents that penetrate the stratum corneum themselves and alter the barrier integrity (54). Understanding the effects of solvents upon percutaneous absorption can improve drug delivery across skin, enhance formulation design of skin care products, and allow better risk assessment of toxic compound exposure (21, 54). It has been suggested that the rate of solute delivery through the skin barrier depends not only on the vehicle in the system but also on the nature of the solute. Molecular size, lipophilicity, ionisation, melting point and H-bonding ability of the solute have been recognized as determinants of solute penetration in previous studies (24, 25, 55). Another approach to promote skin permeation of a compound is to employ chemical penetration enhancers in topical or transdermal formulations (56-58). Modes of action for skin penetration enhancers have been identified. For instance, the enhancers may disrupt the highly ordered structure of the intercellular lipid matrix, interact with the
intracellular keratin domains, or increase partitioning of the solute, solvent, or co-enhancer into the stratum corneum (45). Although an enormous number of studies have evaluated the effects of chemical penetration enhancers and these studies have led to insights into their effects on skin permeation (4, 45), the interplay between the penetration of enhancers, permeants, and solvents into and across skin is not completely understood.

The overall goal of our research was to develop a fundamental understanding of the role of solvents and physicochemical properties of the solutes in percutaneous absorption. The effects of a chemical penetration enhancer on skin penetration and distribution of highly lipophilic permeants after topical application were also investigated. This information could lead to more rational approaches in optimizing cosmetic and pharmaceutical skin formulations.

2.2 Hypothesis

The following hypotheses were tested:

The evaporation and skin penetration rates of a solvent are related to percutaneous absorption of the permeant due to solvent influence on permeant deposition. Different solvent effects on skin absorption would be observed when the solutes with varied physicochemical properties are applied topically in the solvents that have different evaporation rates. The skin penetration of highly lipophilic compounds, petrolatum and soybean oil widely used in topical skin formulations could be enhanced by glyceryl monooleate (GlyMOle) to provide skin health benefits.
2.3 Specific Aims

Aim 1: To investigate the effects of solvents upon skin absorption of a solute after topical application

1.1 Determine the evaporation rates of the solvents used in the study

1.2 Conduct the finite-dose skin permeation study of a moderately lipophilic solute in vitro

1.3 Examine the effect of deposition depth in the SC on skin absorption using computer simulation

Aim 2: To investigate the relationship between the physicochemical properties of both lipophilic and hydrophilic solutes and their skin absorptions under the finite dose condition

2.1 Conduct the finite-dose skin permeation study of solutes in vitro

2.2 Conduct the infinite-dose skin permeation study of solutes in vitro

2.3 Compare the finite-dose and infinite-dose skin permeation results

Aim 3: To probe the unique characteristic of urea permeation through the skin

3.1 Investigate skin penetration enhancing effect of urea

3.2 Examine degraded product permeation

3.3 Investigate the SC penetration depth of permeants using the tape tripping technique

3.4 Evaluate permeation of a small solute, ethylene glycol through the skin
Aim 4: To examine the penetration and distribution of petrolatum and soybean oil in the
skin after topical skin application with and without glyceryl monooleate (GlyMOle)

4.1 Determine skin penetration of petrolatum component and model compounds with and
without GlyMOle in skin studies in vitro.

4.2 Determine skin penetration of soybean oil component and model compounds with
and without GlyMOle in skin studies in vitro.
CHAPTER 3
EFFECTS OF SOLVENT ON PERCUTANEOUS ABSORPTION OF NONVOLATILE LIPOPHILIC SOLUTE
3.1 Introduction

The effects of solvents on the permeation of solutes through human skin are relevant to a wide range of applications including the development of transdermal drug delivery systems, formulation design of skin care products and risk assessment of occupational, environmental, or consumer exposure. Among the different effects of solvents, an aspect that has not been extensively studied is the effect of volatile solvents on the deposition of a solute in the SC that could affect percutaneous absorption via solvent influence on the initial depth of deposition of the solute and its concentration in the SC. This condition is commonly encountered in practice when a small amount of volatile solvent with a permeant is applied on the skin that the solvent is quickly dissipated from the skin, leaving the permeant in the SC for absorption. Under this condition, it is reasonable to hypothesize that the evaporation and skin penetration rates of a solvent are related to percutaneous absorption of the permeant due to solvent influence on permeant deposition.

In the present study, the permeation of a moderately lipophilic solute corticosterone (CS) from solvents with different evaporation and skin penetration rates was determined under finite dosing to examine this hypothesis. The properties of CS and of the solvents employed in the present study are shown in Table 3.1. These solvents were expected to have different effects on skin permeation of CS due to their influence on skin deposition of CS after topical skin application. Computer simulation was also used to investigate the effects of initial depth of permeant deposition in the membrane on membrane transport. Other solvent effects such as skin hydration, skin permeation enhancement, and area of skin exposure to the solvents were also investigated.
Table 3.1. Properties of the solute and the solvents used in the study.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Molecular Mass (Da)</th>
<th>Density (g/cm³)</th>
<th>Vapor Pressure (mmHg)</th>
<th>Boiling Point (°C)</th>
<th>log K&lt;sub&gt;ow&lt;/sub&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosterone (CS) †</td>
<td>346.5</td>
<td>---</td>
<td>8.19 × 10⁻¹²</td>
<td>476</td>
<td>1.94</td>
</tr>
<tr>
<td>Ethanol</td>
<td>46.1</td>
<td>0.785</td>
<td>59.3</td>
<td>78.2</td>
<td>-0.31</td>
</tr>
<tr>
<td>Hexane</td>
<td>86.2</td>
<td>0.659</td>
<td>151</td>
<td>68.7</td>
<td>3.9</td>
</tr>
<tr>
<td>Propylene glycol (PG)</td>
<td>76.1</td>
<td>1.04</td>
<td>0.129</td>
<td>187.6</td>
<td>-0.92</td>
</tr>
<tr>
<td>Polyethylene glycol 400 (PEG 400)</td>
<td>380-420</td>
<td>1.126</td>
<td>&lt;0.01</td>
<td>&gt; 250</td>
<td>-3.05 e</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>60.1</td>
<td>0.785</td>
<td>45.4</td>
<td>82.3</td>
<td>0.05</td>
</tr>
<tr>
<td>Butanol</td>
<td>74.1</td>
<td>0.81</td>
<td>6.7</td>
<td>118</td>
<td>0.88</td>
</tr>
<tr>
<td>Water</td>
<td>18.0</td>
<td>0.997</td>
<td>20.6</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

a  at 25 °C unless indicated otherwise.

b  Vapor pressure and Log K<sub>ow</sub> values obtained from the US Environmental Protection Agency’s EPI Suite™ unless indicated otherwise.

c  Permeant. Solubility of CS at room temperature is 0.14 mg/mL in water (EPI Suite™).

d  Not applicable.

e  from (59)
3.2 Materials and Methods

3.2.1 Materials

\(^3\)H-Corticosterone (CS), specific activity of 70 Ci/mmol, was purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA). \(^3\)H-water, specific activity of 1 mCi/g, was from Moravek Biochemicals (Brea, CA). Non-radiolabeled CS was purchased from Sigma-Aldrich (St. Louis, MO). Ethanol was purchased from Pharmco-AAPER (Brookfield, CT and Shelbyville, KY respectively). Hexane, 1-butanol, propylene glycol (PG), and polyethylene glycol 400 (PEG 400) were purchased from Fisher Scientific (Fair Lawn, NJ). Isopropyl alcohol was from MP Biomedicals, LLC (Solon, OH). Polysorbate 20 (Tween 20) was purchased from Uniqema (Wilmington, DE). Sodium azide (NaN\(_3\)) was from Acros Organics (Morris Plains, NJ). Phosphate buffered saline (PBS: 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride) pH 7.4, was prepared using PBS tablets as described by the manufacturer (MP Biomedicals, LLC, Solon, OH) and preserved using 0.02% NaN\(_3\). Anterior and posterior torso split-thickness cadaver skin was obtained from the New York Firefighters Skin Bank (New York, NY).

3.2.2 Solvent evaporation study

Evaporation experiments were conducted to determine the evaporation rates of the solvents used in the study at room temperature. 10 \(\mu\)L of each solvent was pipetted individually into a deposable weighing boat of known weight (10 \(\mu\)L was the volume used in the permeation study). The weight of the solvent and weighing boat was then measured on an analytical balance at different time intervals. Room temperature was 21
21°C and the relative humidity was approximately 23%. The evaporation of CS was also checked by pipetting a mixture of 0.4 µCi $^3$H-CS and 10 µL ethanol into scintillation vials and leaving the vials open in a fume-hood. Scintillation cocktail, 10 mL, (Ultima Gold, Boston, MA) was then added into the vials at different time points (0, 8, 24, and 48 h), and the amounts of CS in the vials were determined using a liquid scintillation counter (Beckman Coulter LS 6500 Multipurpose Scintillation Counter, Fullerton, CA).

3.2.3 Preparation of Skin Samples

Human epidermal membrane (HEM) was prepared by the removal of the dermis from split-thickness skin via heat separation (60, 61). After heat separation, HEM was placed on an aluminum foil, sealed, and stored at -20°C until use. Prior to use, HEM was cut into 1.5 cm × 1.5 cm pieces and allowed to equilibrate in a Petri dish filled with PBS at room temperature for 2 hours; this procedure allowed easier separation of the HEM from the aluminum foil and easier handling of the tissue for tissue mounting on the diffusion cell.

3.2.4 HEM Permeation

Each hydrated HEM sample was supported by a Millipore filter (Durapore PVDF membrane filters, 0.22 µm pore size) and mounted onto a Franz diffusion cell system that had a diffusional area of 1.4 cm². The SC side of HEM was exposed to the environment while the epidermis side was in contact with 5 mL PBS as the receptor medium. The diffusion cell was placed on a thermostated heating and stirring module and maintained at
37 °C, resulting in SC temperature of 34 ± 1°C. A micro magnetic stir bar was used in the receptor compartment to ensure stirring throughout the experiment. The humidity level in the room during the permeation experiment was approximately 30%. The HEM sample in the diffusion cell was allowed to equilibrate for 2 h before checking the integrity of the membrane. The integrity of HEM was checked by a prescreening water permeability assay similar to that described previously (62). Briefly, 150 μL of ³H-water was pipetted onto each HEM in the diffusion cell. Five minutes post-dosing, a cotton swab was used to remove excess ³H-water from the donor chamber. After 1 h, 2 mL-sample from the receptor chamber was collected in a scintillation vial, and the solution was mixed with 10 mL scintillation cocktail (Ultima Gold, Boston, MA) and analyzed using the liquid scintillation counter described in Section 3.2.2. HEM samples with water permeation values greater than 1.6 μL/cm² were discarded. Following the HEM prescreening, the receptor solution was replaced with fresh PBS three times once every 2 h for 6 h to remove the residual radioactivity. HEM was then allowed to equilibrate in the heating and stirring module overnight. Before the start of the HEM permeation experiments, samples were taken from the receptor to check for residual ³H-water in the system.

The test solutions (donor solutions) were prepared by mixing 4 μCi ³H-CS with 100 μL of the solvents. The concentration of ³H-CS in the solutions (~0.2 μg/mL) was below its solubility for all solvents. The same amount of CS was used in these solvents for the direct comparison of solvent effects. Although the percentage of a solute delivered across skin is a function of the applied dose (load) in topical delivery when the dose is not significantly depleted over the duration of the experiment (63), this influencing factor
was not investigated in the present study. After the integrity test and equilibration, 10 μL of each test solution was pipetted onto the SC side of HEM. All test solutions except water, PG, and PEG 400 spread well on HEM and completely covered the SC surface immediately after dosing. The permeation study was conducted with the donor chambers open to the environment without occlusion. 2-mL samples were collected at predetermined time points (i.e., 2, 4, 6, 10, 24, 48, and 72 h) and fresh PBS was added back to the receptor chambers to maintain a constant volume. The samples were then mixed with scintillation cocktail and analyzed using the liquid scintillation counter as described above. After the experiments, the donor chambers were rinsed with 4 × 500 μL (20-30 s for each rinse) PBS to remove the remaining permeant on the HEM surface. The rinses were collected and mixed with scintillation cocktail and analyzed to determine the amount of permeant remaining on the SC surface at the end of the experiments. The diffusion cells were then disassembled and the HEM samples were dissolved in scintillation vials containing 1 mL Solvable (Perkin Elmer Life and Analytical Sciences, Boston, MA) and kept in an oven at 50 °C overnight. Dissolved HEM samples were then mixed with 11 mL of scintillation cocktail and analyzed to determine the amount of permeant remaining in HEM at the end of the experiments.

The average fluxes \( J \) of CS across HEM were calculated from the changes in the cumulative amount \( \Delta Q \) of CS permeated into the receptor chamber over time \( t \) divided by the effective diffusion area \( A_D \) for every two consecutive time points:

\[
J = \frac{1}{A_D} \frac{\Delta Q}{\Delta t} \quad (3.1)
\]
The fluxes were then divided by the applied doses and presented as the flux normalized by dose values in the analyses.

3.2.5 Effect of PG Pretreatment on HEM Permeation

Possible enhancer effect of PG on CS permeation was investigated in skin permeation study with PG pretreatment. In these experiments, HEM samples were mounted in the Franz diffusion cells and prescreened with $^3$H-water as described in “HEM Permeation” above. After prescreening, 10 $\mu$L PG (without $^3$H-CS) were pipetted onto the SC side of HEM. HEM was then allowed to equilibrate with PG for 24 h before the permeation study with the test solution containing $^3$H-CS in PG. The experimental condition, sampling procedure, and analytical method of the permeation study were the same as stated in “HEM Permeation.”

3.2.6 Effect of Occlusion on HEM Permeation

The effect of skin hydration on CS permeation was investigated under occluded condition that prevented water evaporation from the donor chamber. Permeation experiments of CS were performed as described in “HEM Permeation” except that the skin was occluded by sealing the donor chambers with Parafilms (Pechiney Plastic Packaging Inc, Chicago, IL) after dosing. 10 $\mu$L of $^3$H-CS in water and PEG 400 were the test solutions in this study.
3.2.7 Effect of Skin Area Exposure

The effect of skin area exposure upon CS permeation was investigated in two different sets of experiments to identify the impacts of solvent dosing area and lateral spread on the results of the present study. The first set of permeation experiments was similar to those described in “HEM Permeation” but used a Franz diffusion cell system that had a diffusional area of 0.71 cm² (instead of diffusional area of 1.4 cm²). In the second set of experiments, CS permeation study of solvent water was performed in the presence of 1% Tween 20. The addition of Tween 20, a non-ionic surfactant, served as a wetting agent in the test solution of water to reduce the surface tension of water, allowing the water to spread on HEM and cover the entire HEM surface, and thus increased the skin area exposure for CS permeation.

3.2.8 Computer Simulation

Computer simulation was used to examine membrane transport under the condition when a permeant in a small amount of volatile solvent was applied on the membrane that the solvent quickly dissipated from the system. In this situation, the permeant diffused from the upper section of the membrane, which was loaded with the permeant without the solvent, to the receptor chamber. This simulation was to illustrate the effects of the initial depth of permeant deposition in the membrane and the diffusion coefficient of the permeant upon the flux in finite dose transport similar to the situation of skin permeation with the volatile solvents in the present study. The simulations were performed using a time-dependent diffusion model and COMSOL Multiphysics software (version 4.2,
Comsol, Inc.; Burlington, MA) based on the Fick's second law:

\[ \frac{\partial C_m}{\partial t} = D_m \nabla^2 C_m \]  \quad (3.2)

where \( \nabla \) is the vector differential operator, and \( C_m \) and \( D_m \) are the concentration and diffusion coefficient of CS in the membrane, respectively. Due to the complex heterogeneous structure of the SC and the objective of the computer simulation study, which was to examine the effects of initial deposition depth and diffusion coefficient, the assumption of a homogeneous membrane was employed. In the model, the thickness of the membrane was the SC thickness \( (h_{SC}) \). The diffusion coefficient \( (D_m) \) examined was estimated using the permeability coefficient of CS \( (P_{HEM}) \), \( h_{SC} \), and CS water-to-skin partition coefficient \( (K_{mw}) \):

\[ D_m = \frac{P_{HEM} h_{SC}}{K_{mw}} \]  \quad (3.3)

where \( P_{HEM} \) of CS ranges from 2 to \( 4 \times 10^{-7} \) cm/s \( (64, 65) \), \( h_{SC} \) is 0.0013 cm \( (66, 67) \), and \( K_{mw} \) is related to the octanol/water partition coefficient of CS \( (K_{ow}) \) \( (68) \):

\[ K_{mw} = K_{ow}^{0.71} \]  \quad (3.4)

where \( \log K_{ow} = 1.94 \) \( (69) \). In the computer simulation, the concentration profiles of the permeant in the membrane over time were generated within a 1-D geometry representing the thickness (length) of the membrane. The initial drug concentration \( (t = 0) \) in the membrane was a constant value from the membrane surface \( (x = 0) \) to a certain depth in the membrane \( (x = \text{depth}) \) and was zero from \( x = \text{depth} \) to the membrane/receptor interface \( (x = h_{SC}) \). This initial concentration from \( x = 0 \) to \( x = \text{depth} \) was calculated by
dividing the applied dose by the volume of the membrane from $x = 0$ to $x = \text{depth}$. The fluxes of the drug across the interface at $x = h_{\text{SC}}$ over time were obtained as outputs from the computer software. The transient flux versus time profiles were determined under conditions of diffusion coefficients from $2 \times 10^{-12}$ to $2 \times 10^{-10}$ cm$^2$/s, membrane thickness of 0.0013 - 0.01 cm, and initial deposition depth of the permeant at 10-50% of the membrane thickness in the simulations for comparison.

### 3.3 Results

#### 3.3.1 Solvent evaporation

The time for complete evaporation of 10mL solvent at room condition was determined for the solvents used in the permeation experiments (Table 3.2). The evaporation rates of the solvents from the fastest to the slowest are: hexane > ethanol % isopropyl alcohol>butanol>water>PG>PEG 400. For comparison, no CS evaporation was detected from the CS dose in 48 h. It should be emphasized that the time for a solvent to dissipate from skin surface after topical application would be different from the results of the present solvent evaporation study. The present study only provided a measure of the solvent evaporation rate for comparison among the solvents. Despite the difference between solvent evaporation from skin and a plastic surface, the results indicate that the small amounts of volatile solvents (10 $\mu$L hexane, ethanol, isopropyl alcohol, and butanol) could quickly dissipate from the skin at the beginning of the 72-h skin permeation experiments in the present study. Hence, these solvents likely did not remain in the system to affect CS permeation after their initial effect—the initial effect of
solvents on the deposition of CS in the skin that created the initial CS concentration profile in the SC. For PG and PEG 400, these less volatile solvents would remain on the skin over a major portion of the skin permeation experiments after dosing.

Table 3.2. The time for complete evaporation of 10 μl solvent under normal room condition.

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Time for Complete Evaporation a</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hexane</td>
<td>&lt; 1 min</td>
</tr>
<tr>
<td>Ethanol</td>
<td>&lt; 6 min</td>
</tr>
<tr>
<td>Isopropyl alcohol</td>
<td>&lt; 6 min</td>
</tr>
<tr>
<td>Butanol</td>
<td>&lt; 30 min</td>
</tr>
<tr>
<td>Water</td>
<td>&lt; 1 h</td>
</tr>
<tr>
<td>PG</td>
<td>&lt; 3 days</td>
</tr>
<tr>
<td>PEG 400</td>
<td>&gt; 2 weeks</td>
</tr>
</tbody>
</table>

a Results obtained from experiments in triplicate.
### 3.3.2 HEM permeation of CS

Fig. 3.1 shows the cumulative amounts of CS transported across HEM as percent of the applied dose. The fluxes of CS were calculated using Eq. (3.1), and the normalized fluxes (fluxes divided by the applied doses) are presented in Fig. 3.2. The fluxes of CS across HEM from organic solvents hexane, ethanol, isopropyl alcohol, and butanol increased initially from the start of the experiments, and then gradually decreased over time. There is no statistical difference between the amounts of CS permeated across HEM for these solvents, and no correlation between the flux profiles of CS from these solvents and the evaporation rates of the solvents can be observed. However, the CS cumulative amount versus time and normalized flux versus time profiles of polar solvents water, PG, and PEG 400 were different from those of ethanol, hexane, isopropyl alcohol, and butanol. Among these polar solvents, the flux of CS in water was the highest. It had the highest initial flux in the first 2 h after topical application and then the flux dramatically decreased to the value similar to those of the organic solvents. The flux of CS from PEG 400 was the lowest over the entire 72 h of the experiments. For PG, the initial flux was low and the flux began to increase around 20 h after dosing and then decreased at the end of the experiment.

At the end of the 72-h permeation experiments, the amounts of CS on the skin surface recovered from the wash and those remained in the HEM were quantified (Table 3.3). The total recovery of CS in the experiments was calculated as the sum of CS in these compartments and those transported across HEM into the receptor chamber. Except for PEG 400, approximately 50–75% of the permeant was found in the wash, and the
amounts of CS extracted from HEM ranged from 11 to 24%. For PEG 400, around 85% of the permeant was found in the wash and 1% was detected in HEM. The amounts of CS permeated across HEM constituted a small fraction of the total dose (0.4% for PEG 400 and between 7 and 15% for the other solvents). Overall, the total amounts of CS recovered in all permeation experiments were >80%. 
Table 3.3 CS recovered in the different compartments 72 h after the application of CS in different solvents (ethanol, hexane, isopropyl alcohol, butanol, water, PG, and PEG 400) on HEM. The percent of drug recovery relative to the total dose applied is presented (mean ± SD, water n = 8, the rest n = 4).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Wash (%)</th>
<th>HEM Extraction (%)</th>
<th>Total Permeated into Receptor (%)</th>
<th>Total (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>50 ± 5</td>
<td>20 ± 6</td>
<td>14 ± 3</td>
<td>84 ± 7</td>
</tr>
<tr>
<td>Hexane</td>
<td>50 ± 10</td>
<td>18 ± 7</td>
<td>15 ± 5</td>
<td>83 ± 14</td>
</tr>
<tr>
<td>Isopropanol</td>
<td>47 ± 4</td>
<td>24 ± 7</td>
<td>12 ± 5</td>
<td>82 ± 3</td>
</tr>
<tr>
<td>Butanol</td>
<td>58 ± 6</td>
<td>15 ± 4</td>
<td>7 ± 4</td>
<td>81 ± 5</td>
</tr>
<tr>
<td>Water</td>
<td>74 ± 7</td>
<td>11 ± 1</td>
<td>7 ± 2</td>
<td>92 ± 6</td>
</tr>
<tr>
<td>PG</td>
<td>57 ± 10</td>
<td>18 ± 4</td>
<td>12 ± 3</td>
<td>88 ± 8</td>
</tr>
<tr>
<td>PEG 400</td>
<td>85 ± 11</td>
<td>1 ± 1</td>
<td>0.4 ± 0.2</td>
<td>86 ± 11</td>
</tr>
</tbody>
</table>
Figure 3.1: Permeation profiles of CS across HEM as percent of the applied dose from solutions of hexane (Δ), ethanol (X), isopropyl alcohol (○), butanol (□), water (†), PG (■), and PEG 400 (◆) without occlusion (mean ± SD; water n=8, the rest n=4).
Figure 3.2: Flux profiles of CS across HEM as flux normalized by the applied dose from solutions of hexane (Δ), ethanol (X), isopropyl alcohol (○), butanol (□), water (♦), PG (■), and PEG 400 (♦) without occlusion (mean ± SD; water n=8, the rest n=4).
3.3.3 Effect of PG pretreatment on HEM

The effect of PG on CS permeation was further investigated in the PG pretreatment study to examine whether the changes in the CS flux profile from 20 to 48 h for PG in Fig. 3.2 was due to possible enhancement effect of PG on skin permeation. Fig. 3.3 presents the results of the PG pretreatment study. In the figure, the normalized flux of CS across HEM after PG pretreatment was 3 times larger than those in Fig. 3.2 at the first time point (the 2-h time point). The normalized flux then decreased to the baseline level from 4 to 10 h, and then increased again at the later time points. This flux profile after PG pretreatment suggests that (a) PG only provides a transient enhancement effect of skin permeation in the first 2 h after dosing and (b) the increase in CS flux observed from 20 to 48 h is likely not related to PG permeation enhancement effect.

![Figure 3.3](image)

**Figure 3.3:** Flux profiles of CS across HEM as flux normalized by the applied dose from PG solution with PG pretreatment (■) and without PG pretreatment (◆). The asterisk denotes significant differences (Student’s t-test; \( p < 0.05 \)) \( (n=4) \).
3.3.4 Effect of occlusion on HEM

The effect of skin hydration on CS permeation was investigated to examine whether the higher initial CS flux with solvent water in Fig. 3.2 was related to solvent hydration effect. Figs. 3.4 and 3.5 compare the normalized flux profiles of CS across HEM with water and PEG 400 as the solvents, respectively, with and without occlusion. For water, the results show that CS flux under occluded condition was more than 2 times higher than that without occlusion. The initial decrease in flux after the first time point in the experiments without occlusion can be attributed to the drying of HEM due to water evaporation when water was the solvent. For PEG 400, similar results of higher normalized fluxes under occlusion compared to those without occlusion were observed. In general, the fluxes of CS under occlusion were higher than those without occlusion.

Figure 3.4: Flux profiles of CS across HEM as flux normalized by the applied dose from solution of water under non-occluded condition (♦) (n=8) and occluded condition (■) (n=4).
Figure 3.5: Flux profiles of CS across HEM as flux normalized by the applied dose from PEG 400 solution under non-occluded condition (♦) and occluded condition (■) (n=4).

3.3.5 Effect of skin area exposure

The effect of skin area exposure upon CS permeation was investigated to examine whether the diffusional areas of Franz diffusion cells and solvent dosing areas would affect the conclusion in the present study. Fig. 3.6A shows that the CS permeation data in the Franz diffusion cell experiments of 0.71 cm$^2$ were not statistically different from those of 1.4 cm$^2$ after normalization for the diffusional areas (percent of CS divided by the area) when hexane was the solvent (Student’s t-test; $p > 0.05$), albeit that the values in the 0.71 cm$^2$ experiments were slightly higher than those of 1.4 cm$^2$. The profiles of fluxes normalized by dose under these two diffusion area conditions were also not significantly different (Fig. 3.6B). This suggests minimal effects of the Franz diffusion cell area on the permeation results and the conclusion in the present study. In addition, when Tween 20 was added to improve spreading of water on the skin surface, similar cumulative amount and normalized flux profiles of CS were observed with and without Tween 20.
(Fig. 3.7). The results with Tween 20 suggest that (a) there was no interaction between Tween 20 and SC that would impact CS permeation across HEM and (b) the increase in skin area exposure due to better water spreading did not affect CS permeation. In summary, skin area exposure to the solvents did not have any significant effects on the permeation results under the conditions in these experiments.
Figure 3.6: (A) Permeation profiles of CS across HEM as percent of the applied dose after normalization for the diffusional areas and (B) flux profiles of CS across HEM after normalization for the applied doses with diffusional areas of 0.71 cm$^2$ (■) and 1.4 cm$^2$ (◆) under non-occluded condition (mean ± SD; n=3). Hexane was the test solution.
Figure 3.7: (A) Permeation profiles of CS across HEM as percent of the applied dose and (B) flux profiles of CS across HEM as flux normalized by the applied dose from solutions of water with Tween 20 (■) and without Tween 20 (◆) under non-occluded condition (mean ± SD; n=4).
3.3.6 Effect of deposition depth in SC on skin absorption

Fig. 3.8 shows the representative flux versus time profiles to evaluate the effect of permeant deposition depth upon membrane transport using the computer simulation. The parameters of diffusion coefficient and membrane thickness of $1 \times 10^{-11}$ cm$^2$/s and 0.0013 cm and those of $8 \times 10^{-11}$ cm$^2$/s and 0.01 cm, respectively, correspond to P$_{HEM}$ of $2 \times 10^{-7}$ cm/s when $K_{mw}$ is assumed to be 26 according to Eq. (3.3). As expected, the initial depth of permeant deposition in a membrane could affect permeant flux during membrane absorption. The initial deposition depth was also related to the delay in flux increase after topical application (i.e., transport lag time). Deeper permeant deposition could result in higher maximum flux and shorter delay in flux increase. Permeant diffusion coefficients in the membrane and the thickness of the membrane also affect the flux versus time profiles. More pronounced deposition depth effect (percentage-wise) is observed when the permeant has lower diffusion coefficient in the membrane (relative difference between black dash-dot line and solid line versus that between black dotted line and dashed line in Fig. 3.8) and when the membrane is thicker (relative difference between black dash-dot line and solid line versus that between gray dash-dot line and solid line).

In the present skin permeation experiments, the shapes of the flux versus time profiles of CS with the volatile organic solvents (hexane, ethanol, isopropyl alcohol, and butanol) were similar to that with diffusion coefficient of $2.5 \times 10^{-12}$ cm$^2$/s and membrane thickness of 0.0013 cm or $8 \times 10^{-11}$ cm$^2$/s and 0.01 cm in Fig. 3.8 although the delays in flux increase in the simulated profiles in the figure were longer and the fluxes were higher than those in the permeation experiments. Possible explanations for the discrepancies between the
experimental and simulated flux profiles include the uncertainties related to (a) the assumptions of constant initial concentration of CS in the deposition layer in SC and constant diffusion coefficient across SC, (b) the length of the permeation pathway of SC for CS, and (c) the assumption of homogeneous membrane transport that ignores possible binding and spatially changing diffusion and partition coefficients. For example, some CS might deposit deeper in the SC than the deposition depth parameter of 40%, leading to the shorter lag time in the permeation experiment than that in the model simulation. In addition, the CS on the skin surface (approximately half of CS was found on the skin surface at the end of the permeation experiment) perhaps did not contribute to the flux, and the inclusion of the skin surface CS in the concentration calculation would result in higher SC deposition concentration and hence higher calculated CS flux in the simulation than that in the experiment. Another possible explanation is the higher initial CS concentration in the SC permeation pathway than that in the model due to the preference of CS deposition into the SC lipids initially (i.e., into a fraction of the membrane volume) and the subsequent redistribution of CS from the lipoidal pathway to the other SC components via partitioning and binding. This would result in a shorter lag time and higher initial flux than that obtained in the model.

Despite the discrepancies between the experimental and simulation results, the model simulation has demonstrated the impacts of initial deposition depth and diffusion coefficient on permeant flux across the membrane. The lack of an observable difference between the flux versus time profiles of CS among the fast-evaporating solvents in the present experiments suggests that (a) the solvents provide essentially the same CS deposition in the SC (i.e., same deposition depth) or (b) the permeation experiments in
the present study were not sensitive enough to detect the small differences among the CS flux versus time profiles, which can be due to higher than anticipated CS diffusion coefficient in the membrane.

**Figure 3.8:** Flux profiles as flux normalized by the applied dose of a permeant in model simulations of initial deposition depth of 10 and 40% in the membrane, membrane diffusion coefficient of 2.5 x 10^{-12}, 1 x 10^{-11}, and 8 x 10^{-11} cm²/s, and membrane thickness of 0.0013, 0.005, and 0.01 cm. Profiles generated by deposition depth, diffusion coefficient, and membrane thickness: 10%, 2.5 x 10^{-12} cm²/s, 0.0013 cm (black, dashed line); 40%, 2.5 x 10^{-12} cm²/s, 0.0013 cm (black, dotted line); 10%, 1 x 10^{-11} cm²/s, 0.0013 cm (black, solid line); 40%, 1 x 10^{-11} cm²/s, 0.0013 cm (black, dash-dot line); 10%, 1 x 10^{-11} cm²/s, 0.005 cm (gray, solid line); 40%, 1 x 10^{-11} cm²/s, 0.005 cm (gray, dash-dot line); 10%, 8 x 10^{-11} cm²/s, 0.01 cm (gray, dashed line); 40%, 8 x 10^{-11} cm²/s, 0.01 cm (gray, dotted line), respectively.
3.4 Discussion

3.4.1 Mechanisms of Solvent Effects

For the solvents investigated in the present study, the permeation of CS could be affected by a combination of factors. First, the rate of skin absorption of CS is proportional to its fraction of saturation in the system. After the application of CS in the solvent on skin, the thermodynamic activity of CS in the solvent increases, mainly due to solvent dissipation via evaporation and/or skin absorption. This can promote CS permeation. Second, the solvent can penetrate rapidly into the uppermost 1-3 layers of corneocytes in the SC. The solvent drives CS into these upper layers via convection and creates an initial CS concentration profile in the SC for permeation (13, 14). Third, the solvent can act as a skin penetration enhancer (15) that enhances skin permeation of CS before the solvent dissipates by evaporation and/or skin absorption. For example, the solvent can alter the SC barrier and increase the solubility and/or diffusivity of CS in the SC. Fig. 3.9 provides a schematic diagram illustrating the possible mechanisms of skin absorption influenced by the solvents under these conditions. According to these mechanisms, the different evaporation and skin permeation rates of the solvents in the present study could lead to differences in CS absorption into and across HEM.

From the results in the solvent evaporation and CS permeation experiments, the effects of the solvents in the present study can be divided into two categories: fast-evaporating solvents (complete evaporation in < 30 min) and slow-evaporating solvents (complete evaporation in > 30 min). The fast-evaporating solvents are hexane, ethanol, isopropanol,
and butanol that have the characteristics to dissipate almost completely from the skin within a few hours (13, 70). The slow-evaporating solvents are water, PG, and PEG 400. The evaporation rates of the solvents are in the following order: hexane > ethanol ≈ isopropyl alcohol > butanol > water > PG > PEG 400. The skin penetration rates of the solvents are related to the diffusivity of the solvents in SC. The diffusivity of the solvents in SC can be compared based on the molecular weight of the solvents (68, 71), and the diffusion coefficients of the solvents in SC are expected to be in the following order: water > ethanol > isopropanol > butanol > PG > hexane > PEG 400. The different results of CS permeation with the fast and slow evaporating solvents in the present study are consistent with the relationship between CS permeation and solvent evaporation. However, there was no identifiable relationship between the CS permeation results and solvent diffusion rates in SC. This suggests that (a) the initial penetration of CS into the SC due to solvent convection in the deposition process was not significantly different among the studied solvents and/or (b) solvent convection was not a major mechanism for CS deposition and skin permeation after topical application of CS in the solvents. The discussion in this paper is therefore focused on the relationship between skin permeation of CS and solvent evaporation. Further studies are required to understand the interplay between percutaneous drug absorption and solvent penetration into SC.
Figure 3.9: Schematic diagram of possible effects of solvents upon skin absorption under the conditions in the present study.
3.4.2 Effect of Solvent Evaporation on Skin Permeation

In general, after the application of a drug solution on skin, the drug delivery rate changes as the composition of the vehicle changes on the skin surface. During solvent evaporation and absorption, drug concentration in the solvent increases, resulting in an increase in the absorption rate of the drug into and across the skin. After the solvent is completely dissipated and when only the drug is left, a drug reservoir is formed within the SC and/or the drug precipitates on the skin surface. Drug permeation during this period is related to its solubility in the SC lipids (or concentration of free drug in SC), and as the drug reservoir slowly depletes, the rate of drug absorption decreases over time. For the drug that precipitates on the skin surface, it is absorbed slowly into the SC (72). For example, a previous study on skin permeation of oxybutynin has shown that after the depletion of the solvent used in the formulation, oxybutynin was left on the skin resulting in slower drug permeation (73). In the present study, the CS flux profiles from solvents PG and PEG versus those of the other solvents are in general agreement with the effect of solvent evaporation as described above.

Since the solvents used in the present study have different evaporation rates, this could result in different CS permeation profiles across skin from these solvents. For organic solvents ethanol, hexane, isopropanol, and butanol, they all have fast evaporation rates (complete evaporation of 10 µl solvent in < 30 min). The small amount of these volatile solvents (10 µL) is expected to completely dissipate from the skin before the majority of CS absorption has occurred in the 72-h permeation study. When the solvents evaporate and/or are absorbed, CS permeation decreases due to the precipitation of CS on the skin.
surface. Further decrease in CS permeation can be attributed to the depletion of CS in the skin when CS diffuses into the receptor chamber. The solvents act mainly as a vehicle for the deposition of CS into SC creating the initial CS concentration profiles in SC for percutaneous absorption. However, the results of solvents ethanol, hexane, isopropanol, and butanol show no correlation between the rates of CS permeation and solvent evaporation. Possible explanations of the lack of correlation include that (a) the fast-evaporating organic solvents all dissipated before the first sampling time point in the experiment, making it difficult to detect the effect of the solvents before complete solvent evaporation, (b) the limitation of the diffusion cell technique and skin-to-skin variability masked the effects of the solvents, (c) there was little or no effect of solvent upon skin deposition of lipophilic compounds such as CS and its permeation across skin, and/or (d) the physicochemical properties of the solvents were not different enough to induce distinctive skin deposition and permeation profiles of CS.

For polar solvents water, PG, and PEG 400, which all have slower evaporation rates, the permeation profiles of CS are different from those of the fast-evaporating organic solvents. Unlike the organic solvents, different solvent effects were observed with water, PG, and PEG 400. For these solvents, the slower the evaporation rate of the solvent, the slower was CS permeation. This relationship is believed to be a result of the increase in thermodynamic activity of CS when the solvents evaporate and/or are absorbed and is illustrated in the CS flux profile with PG as the solvent (see Section 3.4.5).
3.4.3 Volatile Solvents: Ethanol, Hexane, Isopropanol, or Butanol

Among the organic solvents examined in the present study for their effects on skin deposition after solvent evaporation, ethanol and isopropanol are solvents commonly used in topical and transdermal formulations. Particularly, these two solvents are useful in topical and transdermal aerosols, sprays, and gels. Although hexane is not a common pharmaceutical ingredient, it could provide insights into risk assessment of toxic compound exposure when hexane is the solvent of a toxic compound. Butanol is a model solvent for comparison because of its higher lipophilicity and slightly longer evaporation time than ethanol and isopropanol. Previous studies have mainly focused on the percutaneous absorption of solvents (74-77) and data on nonvolatile solute absorption after the application of a volatile solvent containing the solute on human skin are lacking.

In the present study, despite the different physicochemical properties of these organic volatile solvents, there was no significant difference between the skin permeation profiles of CS when compared under the same condition (i.e., same amount of CS applied on the skin in the solvents without occlusion). The amounts of CS recovered in the skin (skin extraction) or skin surface (wash) at the end of the experiments for these solvents were also essentially the same. The cumulative permeation profiles of CS have similar shapes as those observed in previous finite-dose skin absorption studies of other solutes (13, 78)—an initial increase followed by a gradual decrease of solute flux after topical skin application—and are consistent with the mechanisms as discussed in Sections 3.4.1 and 3.4.2. After the application of CS and solvent evaporation within the first hour, skin absorption of CS continued over the duration of the permeation study with a total of
approximately 8-15% CS permeated across HEM into the receptor. In addition, approximately 15-25% CS was found in HEM at 72 h. These results suggest that lipophilic drugs such as corticosteroids can be delivered into and across skin using these organic solvents in topical and transdermal applications. No identifiable relationship between skin permeation of CS and the characteristics of these volatile solvents (e.g., vapor pressure and molecular size) and no observable effect on skin permeation of CS as a result of possible differences in solvent-induced deposition of CS in the SC were found.

### 3.4.4 Water as Solvent

Water is considered a universal solvent and is a common ingredient in topical and transdermal formulations. The effects of water on skin deposition and percutaneous absorption of a model lipophilic solute were evaluated in the present study, which can provide insights into (a) topical and transdermal delivery of lipophilic drugs in aqueous formulations and (b) risk assessment of cleaning the skin with water after exposure to toxic lipophilic compounds. Water could enhance skin penetration of both hydrophilic and lipophilic permeants (43) as water could alter the SC barrier by swelling the corneocytes and increasing the free volume and fluidity of the SC intercellular lipids (79). This would result in an increase in the partitioning of a solute from the vehicle into the SC and faster diffusion of the solute in the SC enhancing solute penetration (80). Skin permeation enhancement due to hydration was also observed under skin occlusion that prevented water loss from the skin surface and maintained skin hydration (81, 82). When water was the solvent in the present study, the higher flux of CS at the first time point could be a result of water penetration into the SC and elevated skin hydration that
promoted CS permeation in the upper layers of the SC during that period. To examine this hypothesis, skin permeation experiments were conducted with occlusion over the duration of the experiments. Under the occluded condition, a significant increase in CS permeation was observed. In addition, the extent of flux increase under occlusion was similar to that of the first time point in the water solvent experiment without occlusion. This result therefore suggests that skin hydration could contribute to the higher initial flux of CS immediately after dosing when water was used as the solvent (relative to other solvents) without occlusion. After this transient effect, CS permeation with water as the solvent under the non-occluded condition was not significantly different from those of the other volatile solvents, suggesting that skin deposition of CS was likely not to be different between water and the other solvents.

3.4.5 Nonvolatile Solvents: PEG 400 and PG

PEG 400 is a low molecular weight polymer and has been suggested to be a skin penetration retardant (83). Because the evaporation of PEG 400 was slow, the concentration (activity) of CS for permeation was low compared to the other solvents, resulting in low CS flux from PEG 400 throughout the duration of the present permeation study. At the end of the 72-h study, the majority of PEG 400 still remained on the surface of the skin. The effect of PEG 400 upon skin deposition of CS is minimal as most of the drug remained in the solvent after topical application. This result is consistent with the findings in previous studies that showed PEG 400 could hinder skin permeation (56, 84, 85). In addition, it has been hypothesized that PEG 400 is a poor vehicle for percutaneous absorption because of its high viscosity and hygroscopic nature
that tends to dehydrate the SC (86). This could also lead to the slow absorption of CS observed in the present PEG 400 study.

PG is an organic solvent commonly used in topical formulations and has been shown to increase or decrease skin permeation of various chemical agents. For example, the incorporation of PG in an aqueous polyacrylate gel retarded skin penetration of lipophilic drug flufenamic acid (87), but the increase in the volume fraction of PG in a PG:oleyl alcohol (OA) mixture enhanced the permeation of ketoprofen (88). In the present PG study, the results suggest that the relatively low CS flux in the first 20 h after dosing and the increase in flux after 20 h could be attributed to the slow evaporation of PG. Based on the results in the evaporation study, around one third of the applied PG evaporated at 24 h, two thirds evaporated at 48 h, and more than 90% evaporated at 72 h. The evaporation of PG would increase the thermodynamic activity of CS, resulting in faster CS permeation from around 20 to 48 h. After that, the flux of CS started to decrease, similar to the decreases in fluxes in the flux versus time profiles observed with the organic solvents in the present study; this could be due to CS precipitation in the upper skin layer and then CS depletion in the skin reservoir. This result is in agreement with the findings of Trottet et al., which suggested that the depletion of PG is an important factor for the permeation of drug across the skin (89).

PG could also act as a penetration enhancer for the permeation of CS in the present study. Previous studies have investigated the interactions of PG with SC and hypothesized that PG could interact with the polar head group regions of the intercellular lipids, solvate the
alpha-keratin structures in SC, alter the thermodynamic activity and solubility of the drug, and act as a solvent carrier to provide convective transport or solvent drag effect when PG diffuses across the skin (79, 80, 90-93). In the present study, the permeation enhancing effect of PG was investigated in the PG pretreatment experiments. It was expected that if PG had interacted with SC, sustained higher fluxes than the baseline (from the beginning of the experiment) after PG pretreatment similar to the levels around 20 – 48 h without PG pretreatment would be observed. The results of the pretreatment study suggest that PG could provide a transient enhancement effect of skin permeation around the time of CS dosing but did not lead to the increase in CS flux around 20 h in the permeation study without pretreatment. Particularly, the initial higher flux in the pretreatment study is consistent with the hypothesis that PG might affect the barrier function of the skin, but such increase in CS flux was approximately two times smaller than that observed around 40 h in the experiments without the pretreatment. In summary, the CS flux profile with PG around 24 – 48 h in Fig. 3.2 is likely a result of the changes in thermodynamic activity rather than PG being a penetration enhancer; when a significant amount of PG evaporated from the skin surface, the thermodynamic activity of CS was altered leading to the changes in CS permeation. The small permeation enhancement effect of PG observed in the present study is consistent with previous evaluation of PG as chemical penetration enhancers (83, 94).

3.5. Conclusion

Skin absorption of a moderately lipophilic permeant CS after topical application was investigated to determine the effect of CS deposition in SC during and after solvent
dissipation from the skin. No identifiable relationship between skin permeation of CS from the solvents deposited on the skin and solvent diffusion rates in SC was observed, i.e., skin permeation due to solvent convection. For the solvent evaporation effect, there was no relationship between the rates of solvent evaporation and skin absorption of CS with organic solvents ethanol, hexane, isopropanol, and butanol; all of these solvents have fast evaporation rates (complete evaporation of 10 μl in < 30 min). Conversely, the results of the computer simulation illustrated an impact of permeant initial deposition depth in a membrane on permeant flux across the membrane. Together, this suggests that the volatile organic solvents did not introduce different deposition of CS into the SC that would significantly affect percutaneous absorption of CS after the solvent dissipated despite the different physicochemical properties of the solvents (i.e., vapor pressure and molecular size that are related to solvent evaporation rate from skin and diffusion rate across skin). These organic solvents act only primarily as a vehicle for CS dosing in topical skin application. Different solvent effects were observed with polar solvents water, PG, and PEG 400 that have slower evaporation rates compared with the organic solvents. The slower evaporating solvents led to slower CS permeation across skin. To provide insights into the mechanisms that lead to the different permeation profiles between solvents water and PG, selected permeation experiments were also conducted under occlusion and with PG pretreatment. The increase in skin hydration under occluded condition enhanced CS permeation across skin, which could contribute to the higher initial flux of CS when water was used as the solvent without occlusion. For solvent PG, the changes in the CS flux profile could be attributed to the alteration of thermodynamic activity of CS in the solvent although PG could act as a penetration
enhancer resulting in higher initial flux of CS. In general, the permeation profiles of CS in the present study are in agreement with the effect of solvent dissipation leading to a change in the thermodynamic activity of the permeant during skin absorption for the slow evaporating solvents and indistinguishable CS deposition in the SC and CS percutaneous absorption for the fast evaporating solvents.
CHAPTER 4
EFFECTS OF SOLVENTS ON SKIN
ABSORPTION OF NONVOLATILE LIPOPHILIC
AND POLAR SOLUTES UNDER FINITE DOSE
CONDITION
4.1 Introduction

Understanding of the relationships between physicochemical properties of solutes in a vehicle and their skin permeation is important to predict the absorption of solutes through the skin and is crucial for both the design and choice of chemical agents for transdermal and topical delivery (55). Although our understanding of skin absorption based on factors such as solute lipophilicity and molecular size, as well as the interactions between the solute, vehicle, and skin has improved in recent years (24), particularly for steady-state skin permeation, the interplay between these factors on skin absorption under the finite dose conditions remains elusive. For example, while the maximum flux ($J_{\text{max}}$) of a solute across skin from an aqueous vehicle under steady state and infinite dose conditions generally can be predicted from the physicochemical properties of the solute (42), the relationships between solute properties and skin penetration from different solvents under finite dose conditions are not well defined. In the previous chapter, we investigated the effects of solvents upon skin absorption of a moderately lipophilic solute corticosterone (CS) after topical application (95). The findings indicated no correlation between the rate of solvent evaporation and the rate of CS absorption for the volatile organic solvents studied. As solute deposition behavior in the SC would affect percutaneous absorption, which was demonstrated in a computer model simulation in the same study, these results suggest that the volatile organic solvents did not introduce different SC deposition of CS that significantly affected skin absorption of CS. These solvents act primarily as a vehicle for CS dosing in topical skin application. For the nonvolatile polar solvents studied, different solvent effects were observed and the effects of these solvents were discussed.
As a continuing effort of our investigation, the present study investigated solvent effects on skin permeation using solutes of varied physicochemical properties and solvents of different evaporation rates. Skin permeation experiments were conducted using human epidermal membrane (HEM) to determine the relationships between the physicochemical properties of lipophilic and polar solutes and their skin permeation under the finite dose condition. Estradiol and triamcinolone acetonide (TA) were selected as model lipophilic solutes. Urea, glycerol, and mannitol were chosen as model hydrophilic compounds. Volatile solvents ethanol and butanol (complete evaporation of 10 μl solvent in < 30 min) and slower evaporating solvents water and propylene glycol (PG) were selected as the model solvents. To determine the skin permeability coefficients of the solutes, steady state skin permeation experiments with infinite dosing were conducted. The effects of solvents on skin permeation of the lipophilic and polar solutes were examined and compared with the results of CS from our previous study (95).
4.2 Materials and Methods

4.2.1 Materials

$^{3}$H-CS (specific activity: 70 Ci/mmol), $^{14}$C-glycerol (specific activity: 161 mCi/mm mol), and $^{14}$C-mannitol (specific activity: 51 mCi/mmol) were purchased from Perkin Elmer Life and Analytical Sciences (Boston, MA). $^{3}$H-water (specific activity: 1 mCi/g), $^{3}$H-estradiol (specific activity: 24.5 Ci/mmol), $^{3}$H-TA (specific activity: 0.8 Ci/mmol), and $^{14}$C-urea (specific activity: 55 mCi/mmol) were from Moravek Biochemicals (Brea, CA). Ethanol was purchased from Pharmco-AAPER (Shelbyville, KY). Butanol and propylene glycol (PG) were purchased from Fisher Scientific (Fair Lawn, NJ). Sodium azide (NaN$_3$) was purchased from Acros Organics (Morris Plains, NJ). Glycerol was USP grade. Phosphate buffered saline (PBS: 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride) pH7.4, was prepared using PBS tablets as described by the manufacturer (MP Biomedicals, LLC, Solon, OH) and preserved using 0.02% NaN$_3$.

4.2.2 Preparation of skin samples

Anterior and posterior torso split-thickness cadaver skin was obtained from the New York Firefighters Skin Bank (New York, NY). Human epidermal membrane (HEM) was prepared by the removal of dermis from the cadaver skin via heat separation (60, 61). HEM was placed on aluminum foil sheets and stored at -20 °C until use. Prior to use, HEM was cut into 1.5 cm X 1.5 cm pieces, and allowed to thaw in a Petri dish filled with PBS for 2 h. A total of six skin donors were used to prepare the HEM. For each solute,
the same group of HEM samples (one HEM sample from each skin donor) was used in the experiments to examine the effects of solvents. For the experiments among different solutes, different groups of HEM samples were used (due to the limited skin from each skin donor) but the skin donors in these groups overlapped that all these groups had at least two common skin donors.

4.2.3 HEM permeation under finite dose condition

Fully hydrated HEM sample was supported by a Millipore filter (Durapore PVDF membrane filters, 0.22 μm pore size) and mounted onto a Franz diffusion cell with diffusional area of 0.71 cm$^2$. The SC side of HEM was exposed to the environment while the viable epidermis side was in contact with 5 mL PBS as the receptor medium. Each diffusion cell was placed on a thermostated heating and stirring module and maintained at 37°C, resulting in SC temperature of 34 ± 1°C. Micro magnetic stirring bars were placed in the receptor compartments to ensure stirring throughout the experiments. The humidity level in the room during the transport experiments was about 30%. HEM samples were allowed to equilibrate for 2 h before the start of the transport experiments. The integrity of HEM samples was checked by a prescreening water permeability assay. Briefly, 0.15 mL of $^3$H-water (specific activity of 0.4 μCi/mL) was pipetted onto each HEM in the diffusion cells. Five minutes post-dosing, cotton swabs were used to remove excess $^3$H-water from the donor chambers. After 1 h, 2 mL-samples from the receptor chamber were collected in scintillation vials, and the solutions were mixed with 10 mL scintillation cocktail (Ultima Gold, Boston, MA) and analyzed using a liquid scintillation counter (Beckman Coulter LS 6500 Multipurpose Scintillation 177 Counter, Fullerton,
CA). HEM samples with water permeation values greater than 1.6 μl/cm² were discarded. Following the skin integrity test, the receptor solution was replaced with fresh PBS twice to remove the residual radioactivity. HEM was then allowed to equilibrate in the heating and stirring module overnight. After this procedure, no significant amount of $^3$H-water remained that would interfere the skin permeation study.

In the skin permeation experiments, the test solutions were prepared by mixing 1 μCi $^{14}$C-urea, 1 μCi $^{14}$C-mannitol, 1 μCi $^{14}$C-glycerol, 2 μCi $^3$H-estradiol, or 4 μCi $^3$H-TA with 100 μL of each solvent. 10 μL of the test solutions (containing approximately 0.1 μg urea, 0.36 μg mannitol, 0.03 μg glycerol, 0.002 μg estradiol, or 0.2 μg TA) were pipetted onto the SC side of HEM. The permeation study was conducted with the donor chamber open to the environment without occlusion. 2-mL samples were collected at predetermined time points (2, 4, 6, 10, 24, 48, and 72 h) and fresh PBS was added back to the receptor chambers to maintain a constant volume. The samples were then mixed with scintillation cocktail and analyzed using the liquid scintillation counter as described earlier. After the experiments, the donor chambers were rinsed with 4 X 0.5 mL (20-30 s for each rinse) PBS to remove the remaining solute on the HEM surface. The rinses were collected and mixed with scintillation cocktail and analyzed to determine the amount of solute remaining on the HEM surface at the end of the experiments. The diffusion cells were then disassembled and the HEM samples were dissolved in scintillation vials containing 1 mL Solvable (Perkin Elmer Life and Analytical Sciences, Boston, MA) and kept in an oven at 50°C overnight. Dissolved HEM samples were then mixed with 11 mL of scintillation cocktail and analyzed to determine the amounts of solute remaining in
HEM at the end of the experiments.

4.2.4 Steady state permeation experiment under infinite dose condition

Steady state permeation experiments were performed in a similar manner as the method described in the finite dose permeation experiments except that an infinite dose condition was used and the donor chamber was sealed with Parafils (Pechiney Plastic Packaging Inc, Chicago, IL). The donor solution was 5 μCi $^{14}$C-urea, 1 μCi $^{14}$C-mannitol, 1 μCi $^{14}$C-glycerol, 0.4 μCi $^{3}$H-CS, 0.4 μCi $^{3}$H-estradiol, or 0.2 μCi $^{3}$H-TA (corresponding to approximately 4.9, 3.5, 0.28, 0.002, 0.004, and 0.1 μg, respectively) in 0.5 mL PBS. The permeability coefficient ($P$) of the skin for the solute was determined using the steady state flux of the solute across the skin ($J_{SS}$) normalized by the solute concentration ($C_d$) in PBS in the donor compartment:

$$P = \frac{J_{SS}}{C_d}$$  \hspace{1cm} (4.1)

4.2.5 Data analysis

The means ± standard deviations (SD) of the data are presented. Analyses and statistical tests (Student’s t-test and ANOVA) were performed using Microsoft Excel (Redmond, WA).
4.3 Results and Discussion

4.3.1 HEM permeation under the infinite dose condition

The permeability coefficients of HEM for the solutes were determined in the infinite dose experiments and are shown in Table 4.1. The highly lipophilic solute estradiol had the highest $\log P (-5.91)$, followed by moderately lipophilic CS ($-6.74$) and TA ($-6.99$), hydrophilic urea ($-7.59$) and the more hydrophilic glycerol and mannitol (both $\log P$ around -8). The $\log P$ values of estradiol, CS, urea, and mannitol are consistent with those observed in previous human skin studies (64, 96, 97) and serve to validate our Franz diffusion cell approach and the quality of the radiolabeled compounds used in the present study. The trend of $\log P$ values of estradiol, CS, TA, urea, glycerol, and mannitol can be explained by the lipophilicities and molecular sizes of the solutes. The permeability coefficient of estradiol is larger than those of CS and TA because estradiol ($\log K_{ow} = 4$) is more lipophilic than CS ($\log K_{ow} = 1.9$) and TA ($\log K_{ow} = 2.5$), and they have similar MW. However, the different lipophilicities of mannitol and glycerol (more than two orders of magnitude lower $K_{ow}$ of mannitol compared to glycerol) did not have a significant effect on their permeability coefficients. Mannitol is also larger than glycerol (two times higher MW than glycerol). Urea is more polar and has lower MW than glycerol. The comparable permeability coefficients of mannitol, glycerol, and urea could be attributed to the polar pathway being the dominant skin transport mechanism for these solutes when the skin was fully hydrated in the infinite dose experiments. The permeability of the skin polar pathway is independent of solute lipophilicity, and the small differences in the permeability coefficients of the polar solutes (i.e., urea vs. mannitol and glycerol) are likely related to their MW for diffusion across this permeation
pathway. Overall, the observed permeability coefficients are in agreement with the skin permeation model of parallel lipoidal and polar pathways (29, 98).

**Table 4.1.** Permeability coefficient (P) values of the permeants

<table>
<thead>
<tr>
<th>Permeant</th>
<th>P (cm/s) 1.9 × 10⁻⁷ (± 0.6 × 10⁻⁷)</th>
<th>Log P (cm/s) - 6.7</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corticosterone (CS)</td>
<td>1.9 × 10⁻⁷ (± 0.6 × 10⁻⁷)</td>
<td>- 6.7</td>
</tr>
<tr>
<td>Urea</td>
<td>3.1 × 10⁻⁸ (± 2.4 × 10⁻⁸)</td>
<td>- 7.6</td>
</tr>
<tr>
<td>Mannitol</td>
<td>9.7 × 10⁻⁹ (± 9.4 × 10⁻⁹)</td>
<td>- 8.2</td>
</tr>
<tr>
<td>Glycerol</td>
<td>7.0 × 10⁻⁹ (± 3.5 × 10⁻⁹)</td>
<td>- 8.2</td>
</tr>
<tr>
<td>Triamcinolone acetonide (TA)</td>
<td>1.1 × 10⁻⁷ (± 0.4 × 10⁻⁷)</td>
<td>- 7.0</td>
</tr>
<tr>
<td>Estradiol</td>
<td>1.2 × 10⁻⁶ (± 0.1 × 10⁻⁶)</td>
<td>- 5.9</td>
</tr>
</tbody>
</table>

*Mean (± SD), n=4

### 4.3.2 HEM permeation under the finite dose condition

Figs. 4.1-4.5 show the cumulative amounts of urea, mannitol, glycerol, estradiol, and TA transported across HEM as percent of the applied dose from solutions ethanol, butanol, PG, and water under the finite dose condition. Data of CS from a previous chapter (95) are also presented for comparison (Fig. 4.6). The total recovery of each solute at the end of the 72-h permeation experiments is shown in Table 4.2. Total recovery was between ~80-100%, and significant percent of the solutes was found to remain on the skin surface.
at the end of the experiments: >50% for CS, glycerol, and mannitol and ~30-70% for urea, TA, and estradiol.

The data in the figures show that the cumulative amounts of urea permeated across HEM at 72 h were the highest in percent applied dose (33 – 55%) among the studied solutes for all solvents in the present study including CS from the previous study, except when water was the solvent (Fig. 4.1); cumulative amount of urea permeated (22%) was not larger than that of estradiol when water was the solvent (Fig. 4.4). For the other solutes, mannitol (Fig. 4.2) showed the lowest overall percent dose permeated at 72 h (< 3%), followed by glycerol (Fig. 4.3). Among the lipophilic solutes, estradiol showed the highest permeation in percent dose at 72 h (21 – 39%) followed by TA that was comparable to CS (Figs. 4.4-4.6).

The interpretation of the skin permeation data under the finite dose condition is more complex than those of the permeability coefficients. The results of the finite dose study of estradiol, CS, TA, glycerol, and mannitol are generally consistent with their steady state permeability coefficients (estradiol > CS ~ TA > glycerol ~ mannitol). However, urea showed unexpectedly high percent dose permeation compared to the other solutes for all the solvents (except for water) studied under the finite dose condition in the present study. The inconsistency between the steady-state skin transport and finite dose permeation results of urea (in which the steady-state permeability coefficient of urea was about 40x lower than that of estradiol) suggests that different skin absorption mechanisms are in play for these solutes under the finite and infinite dose conditions. Possible causes
of this discrepancy can be related to the different skin conditions in the infinite and finite
dose experiments (e.g., fully hydrated skin vs. partially hydrated skin), contribution of the
polar pathway to skin permeation in the permeability coefficient measurements that may
not be accessible during finite dose permeation, and lack of a descriptor in the
permeability coefficient expression for solute dissolution on the skin surface. These
possible causes will be discussed later in this chapter.
Table 4.2. The amount of the solutes (% of applied dose) recovered in the different compartments 72 h after application in the finite dose permeation study\textsuperscript{a}

<table>
<thead>
<tr>
<th>Solvent</th>
<th>CS</th>
<th>Urea</th>
<th>Mannitol</th>
<th>Glycerol</th>
<th>TA</th>
<th>Estradiol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% of applied dose</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ethanol</td>
<td>Wash\textsuperscript{b}</td>
<td>50 ± 5</td>
<td>46 ± 15</td>
<td>84 ± 9</td>
<td>81 ± 4</td>
<td>41 ± 10</td>
</tr>
<tr>
<td></td>
<td>HEM\textsuperscript{c}</td>
<td>20 ± 6</td>
<td>18 ± 13</td>
<td>17 ± 9</td>
<td>9 ± 4</td>
<td>30 ± 13</td>
</tr>
<tr>
<td></td>
<td>Permeation</td>
<td>14 ± 3</td>
<td>33 ± 13</td>
<td>1.1 ± 0.5</td>
<td>2.0 ± 1.3</td>
<td>12 ± 2</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>84 ± 7</td>
<td>96 ± 4</td>
<td>102 ± 1</td>
<td>92 ± 3</td>
<td>83 ± 15</td>
</tr>
<tr>
<td>Butanol</td>
<td>Wash\textsuperscript{b}</td>
<td>58 ± 6</td>
<td>33 ± 16</td>
<td>61 ± 8</td>
<td>60 ± 7</td>
<td>28 ± 7</td>
</tr>
<tr>
<td></td>
<td>HEM\textsuperscript{c}</td>
<td>15 ± 4</td>
<td>15 ± 9</td>
<td>25 ± 11</td>
<td>26 ± 3</td>
<td>21 ± 5</td>
</tr>
<tr>
<td></td>
<td>Permeation</td>
<td>8.2 ± 3.5</td>
<td>38 ± 11</td>
<td>3.1 ± 1.6</td>
<td>7.6 ± 4.0</td>
<td>15 ± 5</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>81 ± 5</td>
<td>86 ± 7</td>
<td>89 ± 2</td>
<td>94 ± 4</td>
<td>64 ± 5</td>
</tr>
<tr>
<td>PG</td>
<td>Wash\textsuperscript{b}</td>
<td>57 ± 10</td>
<td>30 ± 14</td>
<td>70 ± 5</td>
<td>59 ± 6</td>
<td>54 ± 21</td>
</tr>
<tr>
<td></td>
<td>HEM\textsuperscript{c}</td>
<td>18 ± 4</td>
<td>17 ± 9</td>
<td>38 ± 6</td>
<td>33 ± 3</td>
<td>12 ± 3</td>
</tr>
<tr>
<td></td>
<td>Permeation</td>
<td>12 ± 3</td>
<td>55 ± 8</td>
<td>3.1 ± 3.0</td>
<td>5.8 ± 2.7</td>
<td>41 ± 26</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>88 ± 8</td>
<td>102 ± 11</td>
<td>111 ± 4</td>
<td>98 ± 6</td>
<td>106 ± 8</td>
</tr>
<tr>
<td>Water</td>
<td>Wash\textsuperscript{b}</td>
<td>74 ± 7</td>
<td>48 ± 11</td>
<td>89 ± 6</td>
<td>86 ± 7</td>
<td>74 ± 15</td>
</tr>
<tr>
<td></td>
<td>HEM\textsuperscript{c}</td>
<td>11 ± 1</td>
<td>17 ± 13</td>
<td>3 ± 1</td>
<td>4 ± 1</td>
<td>15 ± 10</td>
</tr>
<tr>
<td></td>
<td>Permeation</td>
<td>6.6 ± 2.1</td>
<td>22 ± 7</td>
<td>1.3 ± 0.8</td>
<td>2.1 ± 2.2</td>
<td>3.5 ± 1.4</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>92 ± 6</td>
<td>83 ± 14</td>
<td>93 ± 4</td>
<td>92 ± 5</td>
<td>92 ± 7</td>
</tr>
</tbody>
</table>

\textsuperscript{a} Mean ± SD, n=4 (except for CS in water, n=8)

\textsuperscript{b} Amount of solute on the HEM surface recovered from the donor chamber rinse at the end of the 72-h permeation experiments
Amount of solute in the HEM recovered from dissolved HEM samples at the end of the 72-h permeation experiments

**Figure 4.1.** Permeation profiles of urea across HEM as percent of the applied dose from solutions of ethanol (X), butanol (☐), water ( ), and PG (◆). Mean ± SD (n=4).
**Figure 4.2.** Permeation profiles of mannitol across HEM as percent of the applied dose from solutions of ethanol (X), butanol (☐), water ( ), and PG (◆). Mean ± SD (n=4).
Figure 4.3. Permeation profiles of glycerol across HEM as percent of the applied dose from solutions of ethanol (X), butanol (□), water ( ), and PG (♦). Mean ± SD (n=4).
**Figure 4.4.** Permeation profiles of estradiol across HEM as percent of the applied dose from solutions of ethanol (X), butanol (☐), water ( ), and PG (◆). Mean ± SD (n=4).

**Figure 4.5.** Permeation profiles of TA across HEM as percent of the applied dose from solutions of ethanol (X), butanol (☐), water ( ), and PG (◆). Mean ± SD (n=4).
Figure 4.6. Permeation profiles of CS across HEM as percent of the applied dose from solutions of ethanol (X), butanol ( ), water ( ), and PG ( ). Mean ± SD (water n=8, the rest n=4).

4.3.3 Solvent effects on skin absorption

The permeation profiles of the solutes with solvent PG were different from those of solvents ethanol, butanol, and water when they were applied on the skin (Figs. 4.1-4.6). For PG, similar shapes of the skin permeation profiles were observed for all the studied solutes, including CS in the previous study; the permeation profiles of solutes from PG solvent possess a unique shape compared to the other solvents that the initial amounts of solute permeated were low and then began to increase at later sampling time points. The permeation rates started to increase at ~ 10 h for glycerol, TA, and estradiol and ~1 day for the other studied solutes after dosing (i.e., initial low fluxes and then higher fluxes
after ~ 10 h or 1 day). The small difference between the permeation profiles of these solutes could be due to interactions of PG with the solutes. However, the overall solute permeation profile could be a result of the alteration in thermodynamic activity of the solutes when PG gradually evaporated (and was absorbed) from the skin surface and/or (b) the initial low skin permeability due to skin dehydration by PG before it dissipated, which are both phenomena relatively independent of solute properties. In addition, PG could interact with the SC due to its slow evaporation from the skin surface and enhance solute permeation ~1 day after dosing compared to the other solvents. PG solvent provided the highest amount of TA permeated (41% applied dose) compared to the other solvents for all skin donors studied. This result suggests that PG might have specific effect on TA permeation and synergism when combined with solute TA, leading to the high skin permeation.

When water was used as the solvent, the percent of solute permeation was among the lowest of the solvents studied except when estradiol was the solute. Particularly, the percent of urea and TA permeation across skin at 72 h was the lowest with water compared to solvents ethanol, butanol, and PG. Estradiol showed the highest skin permeation (39% of the applied dose) with water compared to the other studied solvents for all skin donors studied. Water also leads to high initial solute permeation of polar solutes glycerol and mannitol, but the percent of permeation at 72 h for glycerol and mannitol with water are similar to those from ethanol (Figs. 4.2-4.3). These solvent effects on skin permeation under the finite dose condition could be related to the polar nature of water compared to the other solvents and its slower evaporation rate (longer
solvent contact time on the skin) compared to ethanol and butanol. Water could also hydrate the skin immediately after dosing, resulting in faster initial skin permeation, especially for the polar solutes.

Ethanol and butanol were the fast evaporating solvents examined in the present study. In a previous study, CS was found to have similar skin permeation behaviors in volatile solvents hexane, isopropanol, ethanol, and butanol (95). It was suggested that these fast evaporating solvents did not introduce significantly different skin deposition of CS. However, the present results indicate possible different effects of ethanol and butanol on solute deposition and/or permeation across the skin (different total amounts of solutes permeated), which was not observed in the previous study with a single permeant, CS, and there were no general patterns in the permeation profiles of these solutes. The lack of a general pattern suggests no simple relationship such as solvent evaporation rates and skin permeation of these solutes (as butanol has a slower evaporation rate than ethanol). Particularly, significantly higher percent dose of glycerol and mannitol was observed to permeate the skin with butanol compared to that of ethanol ($p < 0.05$ for glycerol and $p < 0.1$ for mannitol). This suggests possible influence of the solvent during the initial solute deposition phase of skin permeation before the solvents dissipated (evaporated and were absorbed) from the skin surface after dosing. The solvents might not simply act as a vehicle for spreading the solute on the skin surface during dosing. The modes of skin absorption of the solutes with the volatile solvents and the influence of solute physiochemical properties on skin absorption are discussed in the following sections.
4.3.4 Mechanism of skin absorption under finite dose conditions

Skin absorption of a solute after solvent deposition of the solute on the skin surface could be related to a number of factors under the finite dose conditions in the present study. For simplicity, the present study divided the finite dose absorption results into three categories: Case I, the solvent dissipates quickly due to solvent evaporation and/or absorption, and the solute is completely dissolved in the superficial SC layers at below solute saturation; Case II, the solvent dissipates quickly, leaving the solute saturated in the superficial SC layers and on the SC surface; and Case III, the solvent is dissipated slowly from the SC surface and the solute diffuses from the slowly dissipating solvent layer into the skin. A previous chapter (95) has investigated the effects of solvents on skin permeation of CS after the deposition of CS on the skin with solvents hexane, ethanol, isopropanol, butanol, water, PG and PEG400. In this previous study, solvents hexane, ethanol, isopropanol, butanol, and to some extent water were examples of either Case I or Case II and solvents PG and PEG400 were examples of Case III. The present study examined the effects of solute physiochemical properties on skin permeation based on these categories.

In Case I, when the concentration of the solute in the first few layers of intercellular lipids in the SC is below saturation after solvent deposition (i.e., the solute exists as free molecules dissolved in the SC immediately after deposition), solute diffusion across the skin is the main transport mechanism and solute solubility is not a major factor on the rate of skin permeation. In this case, the flux of the solute is a function of its concentration and diffusion coefficient in the SC. When the flux is normalized by the
dose, solute diffusion coefficient becomes the only major factor for skin permeation. An
equation with a simple expression for Case I is not available, but the relationship can be
evaluated by computer model analyses such as those described in a previous chapter (95).
In the present study, the hypothesis of Case I was examined by comparing the percent
dose permeated and MW of the solutes under the finite dose condition in Fig. 4.7. No
apparent relationship was observed between the percent dose of solute permeation and
solute MW for the volatile solvents studied. The result does not support the solute
permeation mechanism described in Case I for all the studied solutes, indicating that
these solutes could not be completely solubilized in the SC after deposition and solute
diffusion across the skin is not the only transport mechanism that dictates the rate of
solute permeation. This is also consistent with the data of large portions of solutes
remaining on the skin surface at the end of the experiments.

In Case II, when the concentration of the solute in the superficial SC layers is above its
solubility after solvent deposition, the solute is at saturation in those layers. Skin
permeation from either the pure solute phase on the skin surface or in the SC is related to
both solute dissolution and its diffusion across the SC (99). As a result, the flux of the
solute is a function of solute lipid solubility and diffusion coefficient in the SC. When a
significant portion of the solute remains on the skin surface throughout the skin
permeation study (i.e., no significant solute depletion), the cumulative amount \(Q\) of
solute permeated the SC is proportional to its flux \(J\): \(Q = A_D \cdot \int_0^t J_{solv} dt\), where \(A_D\) is the
diffusional area and \(t\) is time. The percent dose permeated (cumulative amount of solute
permeated normalized by the applied dose) is therefore also a function of solute lipid
solubility and diffusion coefficient in the SC.

Case III is for solute permeation after skin deposition with a solvent that is not volatile.
Under this condition, skin permeation is related to solute concentration in the solvent,
solute partitioning tendency from the solvent into the SC, and the diffusion coefficient of
the solute in the SC. The solvent can also interact with the skin and affect skin
permeability. Skin absorption is therefore related to the evaporation of the solvent that
leads to the increase in the concentration of the solute in the solvent on the skin surface
and the interactions between the solvent and the skin. An example is solute absorption
from PG in the present study. The quantitative relationships of skin permeation in Case
III could be complicated and were not to be investigated in the present study.

To examine the relationships between finite dose skin permeation of the solutes and their
physicochemical properties, Table 4.3 summarizes the percent dose permeated at 72 h
and the properties of the solutes. The percent of applied dose permeated after finite
dosing for the solutes in volatile solvents ethanol and butanol are in the following rank
order: urea > estradiol > CS~TA > glycerol > mannitol. Except for urea, the finite dose
permeation rank order is in agreement with the permeability coefficients obtained in the
steady state permeation experiment under the infinite dose condition, and a general
correlation was observed between the percent dose of solutes permeated and their
permeability coefficients (Fig. 4.8). The inability to predict finite dose absorption of a
solute with its permeability coefficient can be attributed to the differences between
steady-state (i.e., permeability coefficient) and finite dose skin permeation. For example, the skin conditions in infinite dose and finite dose permeation experiments are different due to the different hydration states of the skin in the experiments. In addition, polar solutes can utilize the polar pathway in skin permeation under the steady state skin permeation experiments but this pathway may not be as available in the skin under the finite dose condition. Analyses using the solute permeability coefficients also do not take into account of solute solubility, which can be important in solute permeation involving solute lipid dissolution on the skin surface (Case II).
Table 4.3. Properties of the permeants and % dose permeated at 72 h (%Q) in the finite dose permeation study

<table>
<thead>
<tr>
<th>Name</th>
<th>MW (Da)</th>
<th>log $K_{ow}^a$</th>
<th>SC solubility (g/L)$^b$</th>
<th>%Q$^c$ (Ethanol</th>
<th>Butanol</th>
<th>PG</th>
<th>Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea</td>
<td>60.1</td>
<td>-2.1</td>
<td>4.2</td>
<td>33 ± 14</td>
<td>38 ± 11</td>
<td>55 ± 8</td>
<td>22 ± 7</td>
</tr>
<tr>
<td>Estradiol</td>
<td>272.4</td>
<td>4.0</td>
<td>40</td>
<td>25 ± 14</td>
<td>21 ± 9</td>
<td>23 ± 3</td>
<td>39 ± 25</td>
</tr>
<tr>
<td>TA</td>
<td>434.5</td>
<td>2.5</td>
<td>4.4</td>
<td>12 ± 2</td>
<td>15 ± 5</td>
<td>41 ± 26</td>
<td>3.5 ± 1.4</td>
</tr>
<tr>
<td>CS</td>
<td>346.5</td>
<td>1.9</td>
<td>17</td>
<td>14 ± 3</td>
<td>8.2 ± 3.5</td>
<td>12 ± 3</td>
<td>6.6 ± 2.1</td>
</tr>
<tr>
<td>Glycerol</td>
<td>92.1</td>
<td>-1.8</td>
<td>0.47$^d$</td>
<td>2.0 ± 1.3</td>
<td>7.6 ± 4.0</td>
<td>5.8 ± 2.7</td>
<td>2.1 ± 2.2</td>
</tr>
<tr>
<td>Mannitol</td>
<td>182.2</td>
<td>-3.1</td>
<td>0.17</td>
<td>1.1 ± 0.5</td>
<td>3.1 ± 1.6</td>
<td>3.1 ± 3.0</td>
<td>1.3 ± 0.8</td>
</tr>
</tbody>
</table>

$^a$ Log $K_{ow}$ values obtained from the US Environmental Protection Agency’s EPI Suite™

$^b$ Estimated SC solubility calculated from $K_{ow}$ and water solubility at 25 °C

$^c$ Mean ± SD, n=4 (except for CS in water, n=8)

$^d$ Unpublished octanol solubility determined experimentally in 1-octanol at room temperature
Figure 4.7. Correlation between cumulative amount permeated (as % applied dose) of the solutes in volatile solvents ethanol (■) and butanol (◆) under finite dose condition and solute MW. Mean ± SD (n=4).
Figure 4.8. Correlation between cumulative amount permeated (as % applied dose) of the solutes in volatile solvents ethanol (■) and butanol (◆) under finite dose condition and solute permeability coefficients. Mean ± SD (n=4).

4.4 Conclusions

The effects of solvents on skin permeation of lipophilic and polar solutes were investigated in skin permeation studies after solute deposition on the skin with the solvents (i.e., solvent deposition method). The main findings in the present study could be divided into: (a) the effects of volatile and nonvolatile solvents and (b) the effects of the physicochemical properties of the solutes on skin permeation. Although the effects of volatile solvents ethanol and butanol on skin permeation of the solutes were different, no simple relationship was identified such as solvent evaporation rates and skin absorption of these solutes. This indicates possible influence of the solvents on solute permeation, due to solvent interactions with the solute and/or skin, during the initial solute deposition.
phase before the solvents dissipated from the skin surface. Among the solvents studied, water led to the lowest solute permeation except when estradiol was the solute. The effect of PG on skin permeation of the solutes was relatively independent of solute properties; similar skin permeation profiles of initial low skin absorption followed by a significant increase in absorption at later time points were observed for these solutes with PG. This effect of PG on skin absorption of the solutes could be attributed to the change in thermodynamic activity of the solutes and PG interactions with the SC when PG slowly evaporated (and absorbed) from the skin surface. Together, the solvent effects on these lipophilic and polar solutes illustrated in the present study suggest that these solvents did not act as a simple vehicle for spreading the solutes on the skin surface during dosing, and even for the volatile solvents, these solvents could interact with the skin and solutes in the solvent deposition process to impact skin permeation during and after solvent dissipation. To examine the relationships between solute physicochemical properties and skin permeation, the finite dose permeation results of the solutes were compared to their permeability coefficients obtained under the infinite dose condition.

Except for urea, the results of the finite dose condition with solvents ethanol and butanol were in general agreement with solute permeability coefficients. Particularly, the amounts of solutes permeated the skin (in percent applied dose) under the finite dose condition were in the rank order of urea > estradiol > CS ~ TA > glycerol > mannitol, and the permeability coefficients were in the rank order of estradiol > CS ~ TA > urea > glycerol ~ mannitol. Additional studies are required to fully understand the unique permeation characteristics of urea under the finite dose condition.
CHAPTER 5
MECHANISM OF SKIN PERMEATION OF UREA
UNDER FINITE DOSE CONDITION
5.1 Introduction

The relationships between the solute physicochemical properties and the effects of solvents on skin permeation of solutes after topical application when the solutes were deposited on the skin with volatile solvents were reported in Chapter 4. In this previous chapter, skin permeation experiments under the finite dose and steady state (infinite dose) conditions were conducted with Franz diffusion cells and human epidermal membrane (HEM). The findings indicated a general correlation between the percent applied dose of the solutes permeated under finite dose condition and their permeability coefficients obtained under the infinite dose condition except for solute urea. Skin permeation of urea (22 – 55% of applied dose) was the highest compared to the other model solutes (estradiol, corticosterone, triamcinolone acetonide, mannitol, and glycerol) in the finite dose experiments, but urea has one of the lowest permeability coefficients in the steady state permeation experiments. This observation has raised a number of questions on the mechanism of skin permeation of solutes under the finite dose condition. For example, what was the major cause of the high permeation rates of urea under the finite dose condition? Was the observed discrepancy specific to urea? Did urea act as a penetration enhancer that enhances its own permeation in the finite dose permeation study? Was there a particular experimental condition in the study that could lead to the urea results?

To answer these questions, a multiple study approach would be needed and the mechanism of skin permeation of urea and its skin permeation behavior under the finite dose condition should be investigated.

Urea is a natural moisturizing factor and has been used in skin topical preparation for
several decades to increase the hydration of the skin and improve the skin barrier function (100). The strength of urea in topical preparation ranges from 1% to 50%. Urea acts as a humectant providing cosmetic benefits at lower concentrations of 1 - 20%, while at the higher concentrations, urea is useful for skin debridement and can be used in the treatments of diseases such as onychomycosis, dystrophic toenails, and psoriatic nails (100-102). Urea is also added to many topical skin formulations to treat other skin disorders. Cream with 10% urea has been used to treat dry skin and hand dermatitis (103). The ointment of 40% urea and 1% bifonazole has been found to be effective in the treatment of scalp seborrheic dermatitis and psoriasis (104). A 40% urea foam formulation provided the therapeutic moisturizing benefit to minimize the signs and symptoms of xerosis with improved patient satisfaction and compliance (105). In addition, urea has been found to possess modest skin penetration enhancing activity, which could be attributed to a combination of increasing stratum corneum hydration and through its keratolytic properties (45).

The objective of the study in this chapter was to investigate the mechanism of skin permeation of urea when it was deposited on the skin surface at finite dose with a volatile solvent, which could provide insights into the skin permeation mechanisms of solutes under the finite dose conditions. The skin permeation behavior of urea can be examined for phenomena specific to urea and general to all solutes. To examine possible specific interactions, it was speculated that urea might possess a penetration enhancing effect. The impacts of different applied doses of urea on its permeation and on the permeation of another solute were investigated. Additionally, it was reported that some permeants may
be metabolized when they permeated through the skin (106). Urea, similarly, might 
degrade during its permeation process and the degradation product of urea could 
penetrate the skin more rapidly. This was evaluated in octanol-water partition study 
performed together with the finite dose permeation study. To understand the general skin 
transport phenomena of urea, tape stripping was performed on skin permeation of urea 
and other solutes. In addition, a small hydrophilic compound with molecular size similar 
to urea was investigated under the same experimental conditions to examine the 
hypothesis that the rapid skin permeation of urea was due to its small molecular size.

The approaches in the present study are summarized in Table 5.1.
<table>
<thead>
<tr>
<th>Experiment</th>
<th>Solute used</th>
<th>Dose condition</th>
<th>Question addressed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose-dependent effect of urea permeation</td>
<td>$^{14}$C-urea</td>
<td>Finite dose</td>
<td>Investigate possible penetration enhancer effect of urea that enhances its own permeation and skin permeation behavior of urea in the finite dose permeation study.</td>
</tr>
<tr>
<td>Penetration enhancing effect of urea on skin permeation of glycerol (with and without urea)</td>
<td>$^{14}$C-glycerol</td>
<td>Finite dose</td>
<td></td>
</tr>
<tr>
<td>Examination of skin permeation of degradation product of urea - HEM permeation experiments - octanol/water (o/w) partition study</td>
<td>$^{14}$C-urea</td>
<td>Finite dose</td>
<td>Assess the impact of degradation product (or impurity) in the skin permeation study of urea.</td>
</tr>
<tr>
<td>Tape stripping study</td>
<td>$^3$H-Corticosterone, $^{14}$C-urea, $^{14}$C-mannitol, and</td>
<td>Finite dose</td>
<td>Examine the concentration profile of urea in the stratum corneum and its permeation mechanism compared to</td>
</tr>
<tr>
<td>Permeation of small hydrophilic solute</td>
<td>$^{14}$C-ethylene glycol</td>
<td>Infinite and finite dose</td>
<td>Test hypothesis of fast permeation of low MW nonvolatile solute after finite dose solvent deposition.</td>
</tr>
</tbody>
</table>
5.2 Materials and Methods

5.2.1 Materials

\(^{14}\text{C}\)-urea (specific activity: 55 mCi/mmol), \(^{14}\text{C}\)-ethylene glycol (EG, specific activity: 118 mCi/mmol), and \(^3\text{H}\)-water (specific activity: 1 mCi/g) were purchased from Moravek Biochemicals (Brea, CA). \(^3\text{H}\)-corticosterone (CS, specific activity: 70 Ci/mmol), \(^{14}\text{C}\)-glycerol (specific activity: 161 mCi/mmol), and \(^{14}\text{C}\)-mannitol (specific activity: 51 mCi/mmol) were from Perkin Elmer Life and Analytical Sciences (Boston, MA). Ethanol was purchased from Pharmco-AAPER (Brookfield, CT and Shelbyville, KY respectively). Butanol and propylene glycol (PG) were purchased from Fisher Scientific (Fair Lawn, NJ). Octanol (98%) and sodium azide (NaN\(_3\)) were purchased from Acros Organics (Morris Plains, NJ). Phosphate buffered saline (PBS: 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride) pH 7.4, was prepared using PBS tablets and deionized water as described by the manufacturer (MP Biomedicals, LLC, Solon, OH) and preserved using 0.02% NaN\(_3\).

5.2.2 Preparation of skin samples

Anterior and posterior torso split-thickness cadaver skin was obtained from the New York Firefighters Skin Bank (New York, NY). HEM was prepared by the removal of the dermis from the cadaver skin via heat separation. HEM was stored at -20°C until use. Prior to use, HEM was cut into 1.5 cm X 1.5 cm pieces and was allowed to thaw in a Petri dish filled with PBS for 2 h.
5.2.3 Franz diffusion cell setup in skin permeation study

Fully hydrated HEM was supported by a Millipore filter (Durapore PVDF membrane filters, 0.22 μm pore size) and mounted onto a Franz diffusion cell with diffusional area of 0.71 cm$^2$. The SC side of HEM was exposed to the environment while the viable epidermis side was in contact with 5 mL PBS as the receptor medium. Each diffusion cell was placed on a thermostated heating and stirring module and maintained at 37°C, resulting in SC temperature of 34 ± 1°C. A micro-magnetic stirring bar was placed in the receptor chamber to ensure stirring throughout the experiments. The relative humidity level in the room during the permeation experiments was approximately 30%. HEM samples were allowed to equilibrate for 2 h before the start of the permeation experiments. The integrity of the HEM samples was checked by a prescreening water permeability assay. Briefly, 0.15 mL of $^3$H-water was pipetted onto each HEM in the diffusion cells. Five minutes post-dosing, cotton swabs were used to remove excess $^3$H-water from the donor chambers. After 1 h, 2 mL-samples from the receptor chamber were collected in scintillation vials, and the solutions were mixed with 10 mL scintillation cocktail (Ultima Gold, Boston, MA) and analyzed using a liquid scintillation counter (Beckman Coulter LS 6500 Multipurpose Scintillation 177 Counter, Fullerton, CA). HEM samples with water permeation values greater than 1.6 μL/cm$^2$ were discarded. Following the prescreening procedure, the receptor solution was replaced with fresh PBS twice to remove the residual radioactivity. HEM was then allowed to equilibrate in the heating and stirring module overnight before the skin permeation experiments.
5.2.4 Skin permeation study under finite dose condition

The donor solutions (test solutions) were prepared by mixing radiolabeled solutes with 100 μL of each solvent. After the skin integrity test, 10 μL of the donor solutions (solute in solvent) were pipetted onto the SC side of HEM in the Franz diffusion cell. The permeation study was conducted with the donor chamber open to the environment without occlusion. 2-mL samples were collected at predetermined time points (2, 4, 6, 10, 24, 48, and 72 h) and fresh PBS was added back to the receptor chambers to maintain a constant volume. The samples were then mixed with scintillation cocktail and analyzed using the liquid scintillation counter as described earlier. After the experiments, the donor chambers were rinsed with 4 X 0.5 mL (20-30 s each rinse) PBS to remove the remaining solute on the HEM surface. The rinses were collected and mixed with scintillation cocktail and analyzed to determine the amount of solute remaining on the SC surface at the end of the experiments. The diffusion cells were then disassembled and the HEM samples were dissolved in scintillation vials containing 1 mL Solvable (Perkin Elmer Life and Analytical Sciences, Boston, MA) and kept in an oven at 50°C overnight. Dissolved HEM samples were then mixed with 11 mL of scintillation cocktail and analyzed to determine the amount of solute remaining in HEM at the end of the experiments.

5.2.5 Dose-dependence effect of urea permeation

The possible effect of dose dependence on urea permeation was investigated with three applied doses: 0.01, 0.1, and 0.5 μg $^{14}$C-urea in ethanol with the final volume of 10 μL as
the donor solution. The 0.1 µg dose (corresponding to 0.1 µCi) was the dose used in the
study performed in Chapter 4. The dose-dependence permeation experiments were
performed using Franz diffusion cells and HEM. The experimental condition, sampling
procedure, and analytical method were the same as stated in “Skin permeation study
under finite dose condition.”

5.2.6 Penetration enhancing effect of urea on skin permeation of glycerol

The test solution was prepared by adding 10 mg of non-radioactive urea into a vial
containing 100 mL ethanol. 1 µCi $^{14}$C-glycerol was then mixed with 0.1 mL of the
ethanol solution. The permeation experiments were performed using Franz diffusion
cells and HEM. The donor solution was 10 µL of the prepared test solution composed of
0.1 µCi (0.03 µg) $^{14}$C-glycerol and 1 µg of urea in ethanol. Experiments were also
conducted with donor solution composed of $^{14}$C-glycerol without urea for direct
comparison. The experimental condition, sampling procedure, and analytical method
were the same as stated in “Skin permeation study under finite dose condition.”

5.2.7 Examination of skin permeation of degradation product of urea

The donor solution of urea in ethanol was prepared by mixing 1 µCi $^{14}$C-urea with 0.1
mL of the solvent. The permeation experiments were conducted using Franz diffusion
cells under the finite dose condition (0.1 µg dose in 10 µL solvent) with the same
procedure including the sampling time points as described in “Skin permeation study
under finite dose condition.” At the end of each experiment, octanol/water (o/w) partition study was performed to determine the apparent partition coefficient of $^{14}$C-compound in the receptor samples ($K_{o/w}$). The apparent $K_{o/w}$ value of $^{14}$C-urea in the donor solution was the reference. In the octanol/water partition experiments of the receptor solution, 1 mL receptor sample at each sampling time point was mixed with 1 mL n-octanol in a vial. After the aqueous and octanol phases were mixed and equilibrium was achieved, the two phases were separated and the concentration of $^{14}$C-compound in each phase was measured. In the octanol/water partition experiment of the donor solution, 10 $\mu$L of the donor solution (containing 0.1 $\mu$Ci or 0.1 $\mu$g of $^{14}$C-urea) was mixed with 1 mL PBS and then 1 mL octanol to determine the apparent $K_{o/w}$ value. The $K_{o/w}$ values of the receptor and donor samples were compared to test for degradation product involvement in skin permeation of urea. In addition, the $^{14}$C-radioactivity of the aqueous phase in the octanol/water partition study was also used to calculate the cumulative percent dose of urea permeated across HEM. The permeation results with and without the octanol/water partition treatment were compared.

5.2.8 Tape stripping study

Different skin permeation behaviors were observed for urea and other solutes in the previous skin permeation experiments (Chapter 4). Tape stripping was performed to investigate the concentration profile of urea and its penetration depth in SC compared to the other model solutes that were investigated in the previous study. The same dosing condition as described in “Skin permeation study under finite dose condition” was used. 4 $\mu$Ci $^3$H-CS or 1 $\mu$Ci $^{14}$C-urea, $^{14}$C-mannitol, or $^{14}$C-glycerol was mixed with 0.1 mL
ethanol to prepare the donor solution. 10 μL of donor solution was then applied onto the skin. Tape stripping was started 30 min after dosing and the procedure was completed before 1 h. The mounted skin was then covered with an adhesive tape with a central hole of 1 cm² (the available area for tape stripping). Standard D-Squame® disc Cuderm Corporation, Dallas, TX), diameter of 2.2 cm and area of 3.8 cm², was pressed onto the skin for 5 s and then quickly removed. 20 tapes were used in this procedure consecutively or until the complete SC had been removed. Protein content on each tape (removed from the SC) was quantified by UV absorption measurement at 850 nm with the infrared densitometer Squame-Scan 850 (Heiland Electronic, Germany). The protein content (in concentration, C_protein) was calculated using the following relationship:

\[ C_{\text{protein}} (\mu\text{g/cm}^2) = 1.366 \times \text{Absorption} (\%) - 1.586 \quad (5.1) \]

The stripped disc was then transferred into a scintillation vial and mixed with 10 mL of scintillation cocktail to analyze for the radioactivity of the solute on the disc. Possible interference of D-Squame® disc on the radioactivity measurement of this method was checked in a recovery study and found to be negligible.

5.2.9 Permeation of small hydrophilic solute ethylene glycol (EG) across skin

To determine the permeability coefficient of ethylene glycol, skin permeation study was performed under the infinite dose condition in the Franz diffusion cell setup similar to those described in “Skin permeation study under finite dose condition” except that 0.5 mL donor solution was used and the donor chamber was sealed with Parafilm (Pechiney Plastic Packaging Inc, Chicago, IL). The donor solution was 5 μCi of 14C-ethylene
glycol (EG) in PBS. The permeability coefficient \( P \) was determined using the steady state flux of the solute across the skin \( (J_{SS}) \) normalized by the concentration \( (C_d) \) of the solute in the solution in the donor chamber:

\[
P = \frac{J_{SS}}{C_d}
\]  

(5.2)

In the finite-dose skin permeation study, the donor solutions were prepared by adding 1 \( \mu \)Ci \(^{14}\)C-ethylene glycol into 0.1 mL of ethanol, butanol, water, or PG. The skin permeation experiments were carried out with 10 \( \mu \)L donor solution, corresponding to 0.05 \( \mu \)g EG dose, in Franz diffusion cells as described in “Skin permeation study under finite dose condition.”

5.2.10 Data analysis

The means ± standard deviations (SD) of the data are presented. Statistical tests (Student’s t-test and one-way ANOVA) were performed using Microsoft Excel (Redmond, WA).
5.3 Results and Discussion

5.3.1 Urea as skin penetration enhancer

Fig. 5.1 shows the results of the skin permeation experiments at urea doses of 0.01 to 0.5 µg. As the effects of a penetration enhancer are normally concentration dependent (45, 107), different skin permeation of urea was anticipated under different applied doses if urea had possessed a skin penetration enhancing effect. When the applied dose of urea (approximately 0.1 µg) in the finite dose permeation study was increased 5 times to 0.5 µg and decreased 10 times to 0.01 µg, no significant dose-dependent permeation was observed under these three dosing conditions (p > 0.05). This suggests that the higher permeation rate of urea in the finite dose permeation study was not a result of urea penetration enhancing activity under the conditions in the present study.

Possible penetration enhancing effect of urea was also examined by mixing urea with glycerol donor solution in the skin permeation study of glycerol, and the results are shown in Fig. 5.2. Glycerol was chosen as a test solute because it was a polar solute studied in the previous finite dose permeation study (Chapter 4). The cumulative percent dose result of $^{14}$C-glycerol in the figure showed no significant difference between those with and without urea (p > 0.05). This suggests that urea did not possess any skin penetration enhancing activities under the conditions in the present study, in agreement with the conclusion of the dose dependence study of urea. Other studies have also found that urea was ineffective as skin penetration enhancer (108, 109). It should be noted that the percent dose permeation profile of glycerol in the present study displayed differences
compared to that in the previous study performed in Chapter 4 due to the different skin donors used in the present and previous studies.

**Figure 5.1.** Permeation profiles of urea through HEM with applied doses of 0.01 (◆), 0.1 (■), and 0.5 µg (⊕), from solutions of ethanol under finite dose condition. The results were presented as percent of the applied dose. Mean ± SD (n=8 for 0.1 µg dose, the rest n=4).
Figure 5.2. Permeation profiles of glycerol with (●) and without urea (☐) across HEM as percent of the applied dose, from solutions of ethanol under finite dose condition. Mean ± SD (n=4).

5.3.2 Skin permeation of degradation product of urea

It was reported that some solutes may undergo metabolism during the process of skin permeation (106). Urea could degrade during skin permeation in the experiments, and the degradation product of urea (rather than urea itself) could permeate across the skin. A more lipophilic degradation product of urea could lead to a higher apparent skin permeation rate for urea and the high percent dose of urea permeated across HEM observed in the skin permeation study. The octanol-water partition experiments were performed along with the finite-dose permeation study to examine this hypothesis. These experiments were conducted based on the assumption that the degradation product of urea is more lipophilic (i.e., faster skin permeation) than urea and has different $K_{o/w}$ from
urea; a degradation product that was similar or more polar than urea would not result in the fast apparent skin permeation for urea.

Fig. 5.3 shows the results of the octanol/water partition study. In the figure, the apparent $K_{o/w}$ values of the receptor solutions at the early sampling time points in the permeation study (0.1 - 0.2) are higher than the value of the donor solution (0.04 at 0 h) whereas the values at the later time points (e.g., around 0.04 - 0.05 at 48 and 72 h) are similar to that of the donor solution. Although the early time $K_{o/w}$ values could suggest higher initial fluxes due to radiolabeled impurities or degradation products, this did not significantly affect the total amount of urea permeated at 72 h in the permeation study; the amounts of urea permeated at the later time points (> 24 h) were the main contributor to the total amount permeated values. Particularly, the cumulative amount permeated vs. time profile of urea determined using only the radioactivity in the aqueous phase in the octanol/water partition study (the receptor solution treatment) is similar to the permeation profile determined using the total radioactivity in the receptor without the treatment (Fig. 5.4). Together, these results suggest that possible urea degradation product could not be the cause of the high skin permeation rate of urea observed in the finite dose permeation study; the degradation product would not significantly affect the overall permeation results but only important to the initial permeation of urea in the experiment.
Figure 5.3. Apparent $K_{o/w}$ values of $^{14}$C-compound in the receptor at different sampling time points in the urea permeation study. Mean ± SD (n=4).

Figure 5.4. HEM permeation profiles of urea obtained from previous studies without octanol/water partition treatment (☐) and those of urea partitioned into the water phase from the octanol/water partition study (◆). Mean ± SD (n=4).
5.3.3 Tape stripping study

The amounts and concentrations (in percent applied dose) and penetration depths of solutes urea, CS, mannitol, and glycerol in the SC were determined at 0.5-1 h after each of the solute in the volatile solvent ethanol was applied on the skin. The total amounts of the solutes recovered from the tapes were 92% (± 10%), 70% (± 6%), 89% (± 10%), and 67% (± 12%) of the applied dose for urea, CS, mannitol, and glycerol, respectively. To analyze the penetration results obtained by tape stripping, the amount of the topically applied solute removed with each tape was plotted against the number of tape strips (Fig. 5.5). As shown in the figure, the majority of the solutes were deposited on the skin surface within the upper SC layers removed by the first 3 tapes. Smaller amounts of the solutes were detected in the deeper layers. Fig. 5.6 shows the concentration profiles of the solutes in the SC (excluding tape 1-3). The concentrations of the solutes were calculated from the percent dose permeated divided by μg protein content and these values were plotted against the cumulative μg protein content as the position in the SC. The results show a typical SC concentration profile for skin permeation of urea and that the concentrations of urea in the SC were higher than those of CS, mannitol, and glycerol.

Table 5.2 summarizes (a) the total amount of the solute in the SC (beyond first 3 tapes) as percent of the applied dose and (b) solute penetration depth as percent of the total SC thickness from the tape stripping experiments. The deeper layers (beyond the first 3 tapes) correspond to a cumulative amount of SC protein > 70 μg. Solute penetration depth is defined as the thickness of SC (from the skin surface) that corresponds to tape strips with percent applied dose values greater than 10% for the solute. The total SC
thickness was calculated from the cumulative SC protein content when the entire SC had been removed. The total amounts of solutes in the deeper layers of SC and the penetration depth data are indicators of how fast and how far each solute penetrated into the SC although solute partitioning and binding in the SC could also impact the results. The results show a statistically larger amount of urea beyond the three superficial tape-stripped layers in the SC than CS, mannitol, and glycerol (p < 0.05). The amounts of CS, mannitol, and glycerol penetrated beyond the three superficial layers in the SC were not statistically different (p > 0.05). The larger amount of urea in the SC than CS, mannitol, and glycerol is consistent with the results in the Chapter 4’s finite dose permeation study, in which urea showed the highest percent dose permeated across HEM among the solutes studied. The penetration depth data in the table also show that a substantial amount of solutes penetrated into 19-23% of the total thickness of the SC in only 0.5 – 1 h after the application of the test solution. Urea showed slightly greater penetration depth than CS, mannitol, and glycerol but the differences were not significant (p > 0.05).
Table 5.2. Amount of the solute in the SC as the percent of the applied dose and solute penetration depth as the percent of the total SC thickness from the tape stripping study.

<table>
<thead>
<tr>
<th>Solute</th>
<th>Amount in SC (% applied dose)</th>
<th>Penetration depth (% of SC thickness)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS</td>
<td>10 ± 4</td>
<td>19 ± 8</td>
</tr>
<tr>
<td>urea</td>
<td>29 ± 13</td>
<td>23 ± 10</td>
</tr>
<tr>
<td>mannitol</td>
<td>12 ± 8</td>
<td>19 ± 7</td>
</tr>
<tr>
<td>glycerol</td>
<td>9 ± 4</td>
<td>19 ± 8</td>
</tr>
</tbody>
</table>

Figure 5.5. Amount of the solute CS (□), urea (◆), mannitol (◊), and glycerol (X) removed from SC with each tape as percent of the applied dose 0.5-1 h after the solute was applied on the skin in ethanol versus number of tape strips. Mean ± SD (n=4).
Figure 5.6. Concentration of CS (□), urea (♦), mannitol (○), and glycerol (X) in the SC versus cumulative protein content (μg) in the tape stripping experiments (excluding tape 1-3). The concentration (% dose / μg protein content) in the SC was calculated from the percent of applied dose permeated divided by protein content (μg) in each tape strip. Each data point represents data of a single tape strip with n=4 HEM samples for each solute. Insert: enlarged concentration profiles at deeper SC layers (beyond a cumulative amount of SC protein of 175 μg).
5.3.4 Permeation of small hydrophilic solute ethylene glycol across skin

The molecular size of a solute is believed to be a major determinant of skin permeation (42, 110) with an inverse exponential relationship between the diffusion coefficient and molecular size of the solute according to the free volume theory (34, 68). Small molecules like water were shown to readily penetrate the SC despite the polarity of water molecule (111). Among the model hydrophilic solutes used in the Chapter 4, only urea showed higher percent dose permeation than the model lipophilic solutes (CS, estradiol, and triamcinolone acetonide) when the solutes were deposited on the skin with various solvents under the finite dose condition. It was hypothesized in the present study that this observation was not specific to urea but a general skin permeation phenomenon for small nonvolatile hydrophilic molecules: the fast skin permeation observed in the previous study was due to the small molecular size of urea (MW = 60.1 g/mol). As a result, skin permeation of a small hydrophilic solute, ethylene glycol (EG), was investigated under the same experimental condition to compare the results with urea. EG was selected as the model solute because EG is polar and nonvolatile and has small molecular size (MW = 62.1 g/mol) similar to urea.

The permeability coefficient of EG was $9.65 \times 10^{-8} (\pm 7.95 \times 10^{-8})$ cm/s, corresponding to log P value of -7.1 (similar to log P of urea, -7.6). This log P value of EG is consistent with that obtained previously (74). The recovery of EG in each compartment at the end of the 72-h permeation experiments under the finite dose condition is presented in Table 5.3. The total recovery of EG was 68 – 76% of the applied dose. The relatively low total recovery of EG could be due to solute evaporation. To test for possible loss of EG from
evaporation, a separate evaporation study was conducted by pipetting 10 µL donor solution of EG on a weighing dish on the benchtop and determining the amount of EG remained on the dish after 72 h. This evaporation study showed ~ 27% EG loss over 72 h, in agreement with the recovery results in the finite dose permeation study. Other studies have also reported EG volatilization (112, 113).

The cumulative amounts of EG permeated across HEM at 72 h in the finite dose study were 26 – 33% of the applied dose (Fig. 5.7), greater than those of CS, triamcinolone acetonide, estradiol, mannitol, and glycerol in the Chapter 4. The similar permeation results of EG and urea suggest that the high skin permeation rates of EG and urea under the finite dose condition could be attributed to the small molecular sizes of EG and urea. In fact, EG demonstrated higher initial skin permeation rates than urea; the percent applied dose of EG permeated the skin in the first 24 h was larger than that of urea although EG and urea have similar molecular sizes. This could be attributed to the higher solubility of EG in the SC compared to urea. The observed skin permeation behavior of relatively high percent dose permeated at finite dose with low skin permeability coefficient at infinite dose is not unique to urea.
**Table 5.3.** Total recovery of EG in the skin permeation study. Recovery of EG in different compartments 72 h after the application of EG in different solvents (ethanol, butanol, PG, and water) on HEM. Percent recovery based on the total dose applied is presented (mean ± SD, n = 4).

<table>
<thead>
<tr>
<th>Solvent</th>
<th>Wash(^a) (%)</th>
<th>HEM(^b) (%)</th>
<th>Total Permeated into Receptor (%)</th>
<th>Total Recovery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethanol</td>
<td>33 ± 6</td>
<td>4.4 ± 0.2</td>
<td>30 ± 7</td>
<td>68 ± 7</td>
</tr>
<tr>
<td>Butanol</td>
<td>38.4 ± 0.2</td>
<td>4.0 ± 0.4</td>
<td>33 ± 10</td>
<td>76 ± 10</td>
</tr>
<tr>
<td>PG</td>
<td>39 ± 1</td>
<td>5 ± 2</td>
<td>28 ± 10</td>
<td>72 ± 11</td>
</tr>
<tr>
<td>Water</td>
<td>45 ± 2</td>
<td>4.8 ± 0.4</td>
<td>26 ± 9</td>
<td>76 ± 10</td>
</tr>
</tbody>
</table>

\(^a\) amount of solute on the HEM surface recovered from the donor chamber rinse at the end of the 72-h permeation experiments

\(^b\) amount of solute in the HEM recovered from dissolved HEM samples at the end of the 72-h permeation experiments
Figure 5.7. Permeation profiles of EG across HEM as percent of the applied dose from solutions of ethanol (X), butanol (□), water (+), and PG (◆). Mean ± SD (n=4).

5.3.5 Mechanism of skin permeation of urea

Previous studies have investigated the effects of urea on skin permeation (114-116). It was proposed that the hygroscopic nature of urea could increase the water content of the skin. Urea could also act as a mild keratolytic agent and alter the SC corneocytes (108). It has been suggested that urea might affect the integrity of the skin barrier by decreasing the phase transition temperature of the SC lipids and fluidizing the lipids (117). In addition, a study using rat skin has found that urea could increase the permeation of ketoprofen 8–50-fold, and it was hypothesized that urea could form large, hydrophilic diffusion channels through skin (118). The results of these previous studies suggest possible penetration enhancing activity of urea. In the present urea dose dependence
study (Fig. 5.1) and glycerol permeation study with urea (Fig. 5.2), the results suggest that urea is not a skin penetration enhancer. The difference between the present and previous studies is likely attributed to the level of urea used in the present study. The high permeation rate of urea observed is not a result of the penetration enhancing effect of urea.

The tape stripping results provide insights into the mechanism of skin permeation of urea under the finite dose condition in the present study. First, the similar percent dose profiles in the tape stripping study (Fig. 5.5) suggest similar permeation mechanisms for the studied solutes (urea, CS, mannitol, and glycerol) immediately after they were applied on the skin. The percent dose values in the profiles are within a 2x range among the studied solutes despite that the solutes are very different in lipophilicity (log $K_{o/w}$ ranging from -3.1 to 1.9). In addition, although the hydrophilicity of the polar solutes urea, mannitol, and glycerol could lead to low SC permeation, significant amounts of these polar solutes were found in the SC. This could be attributed the binding of the solutes to the polar regions of the SC (i.e. H-bonding between the functional polar groups of the solutes and the polar heads of the SC lipids). Second, the higher concentrations of urea in the deeper SC layers suggest that urea has a shorter time delay to penetrate the SC compared to the other solutes (CS, mannitol, and glycerol). This finding is in agreement with the higher penetration rate of urea due to its high diffusivity compared to the other solutes. This finding is also consistent with the skin permeation results of urea in the Chapter 4’s finite dose study: the percent dose of urea permeated was larger than CS and was more than 5 times larger than those of mannitol and glycerol at 72 h after dosing.
These results suggest the impact of the molecular sizes and corresponding diffusivities of the solutes and the importance of the diffusion process once the solutes are in the SC.

To investigate the factors that lead to the high permeation rate of urea under the finite dose condition (but not the steady state infinite dose condition), the physiochemical properties of urea, glycerol, and CS were compared. Urea (log $K_{o/w} = -2.1$, MW = 60.1 g/mol) is slightly more hydrophilic and has smaller molecular size than glycerol (log $K_{o/w} = -1.8$, MW = 92.1 g/mol). CS (log $K_{o/w} = 1.94$, MW = 346.5 g/mol) is more lipophilic than urea but has larger molecular size than urea. The solubility of urea in n-octanol (an indicator of lipid solubility) is greater than that of glycerol but smaller than that of CS: the estimated solubilities of urea, glycerol, and CS in octanol are 8.4, 0.47, and 17 g/L, respectively. CS has higher lipid solubility than urea but demonstrates slower permeation. Therefore, it was hypothesized that the observed difference in skin permeation of urea and the other solutes was attributed to the smaller molecular size of urea. To examine this hypothesis, skin permeation experiments were performed with another small hydrophilic solute EG in the present study.

The EG permeation study showed significant skin permeation in 72 h (26 – 33% of applied dose permeated) similar to urea. EG is relatively polar (log $K_{o/w} = -1.36$) and has almost identical molecular size (MW = 62.1 g/mol) compared to urea. It has high lipid solubility (miscible in 1-octanol; unpublished data) compared to the other solutes. The high permeation rate of EG across the SC under the finite dose condition is likely to be related to the small molecular size and high lipid solubility of EG. Similar trends have
also been observed in previous studies on skin permeation of small molecules. Driver et al. (113) and Moody et al. (119) reported large skin permeation of EG after application in vitro (18.3% and 43.2% of the applied dose, respectively). Kenyon et al. showed that trimethylamine (MW = 59.1 g/mol, log K_{o/w} = 0.16, log P = -7.3) could readily penetrate the epidermis at three different dose levels (120). Consequently, it is possible that solutes of similar size to urea and EG (MW ≈ 60 g/mol or less) could readily permeate the lipid barrier of the SC under the low finite dose and solvent deposition conditions in the present study despite their unfavorable lipophilicities.

Although the amount of urea remaining on the skin surface at 72 h (30-48%) suggests that urea was saturated on the skin surface during the 72-h skin permeation study, no significant dose-dependence effect was observed in the present urea permeation study; skin saturation should lead to a decrease in percent skin absorption (based on percent applied dose) when the urea dose was increased from 0.01 to 0.5 µg. The lack of dose-dependence effect of urea in the permeation study together with the lack of solute lipophilicity effects in the tape stripping study suggest the following. At the dose level used in the present study, the solvent deposition method (with a volatile solvent) can deliver a significant portion of the solute into the superficial layers of the SC during dosing. The percent of solute delivered into the SC via this mechanism is relatively independent of solute lipophilicity, resulting in a relatively constant percent dose remaining on the skin surface (i.e., not delivered into the SC) after solvent deposition. For a solute with moderate solubility in the SC lipids such as urea and at the low dose level used, the solute is mainly dissolved in the SC lipids during the deposition process,
and skin permeation of the dissolved solute is mainly a function of the diffusivity of the solute in the SC (i.e., molecular size) and not its lipophilicity. Hence, a lack of the deterrent effect of solute hydrophilicity and the high permeation rate of urea under the finite dose condition (different from the steady-state permeation results under the infinite dose conditions) were observed. This skin transport phenomenon is not expected to be limited to urea and can be applicable to solutes of similar physicochemical properties.

5.4 Conclusions

The mechanism of skin permeation of urea was investigated to address the question why urea had demonstrated a high permeation rate across skin compared to other solutes under the finite dose condition in the study performed in Chapter 4 even though urea has a relatively low skin permeability coefficient among these solutes. Based on the results of the dose-dependence study of urea and glycerol permeation in the presence of urea, urea did not act as a penetration enhancer at the level used in these studies. In addition, no significant urea degradation that could result in the observed high skin permeation was found. In the tape stripping study, although no significant difference was found between the amounts of urea, CS, mannitol, and glycerol (based on percent dose) in the superficial layers of the SC, significant higher concentration of urea was observed in the deeper SC layers than the other solutes. This suggests that urea has a shorter time delay to penetrate the SC compared to the other solutes due to its faster penetration rate, which is consistent with the permeation results of urea under the finite dose condition in the previous study (chapter 4). These tape stripping results are also consistent with solute deposition and
diffusion as the main skin permeation mechanism of urea, i.e., relatively independent of solute lipophilicity. In the study of EG, the skin permeation behavior of EG was similar to urea. EG has similar MW as urea (≈ 60 g/mol) and showed high percent of dose permeated under the finite dose condition. Consequently, it was hypothesized that solutes with similar molecular sizes to urea and EG (MW ≈ 60 g/mol or less) could demonstrate the skin permeation behavior observed in the present study: these solutes would have relatively high percent dose permeated when they are applied on the skin under the low finite dose and solvent deposition conditions despite that they are polar solutes with relatively low skin permeability coefficients.
CHAPTER 6
IN VITRO SKIN PENETRATION OF
PETROLATUM AND SOYBEAN OIL
AND EFFECTS OF
GLYCERYL MONOOLEATE
6.1 Introduction

Skin care products usually contain ingredients that could affect the properties of skin and improve skin barrier function (121). A variety of lipids and oils have been incorporated into topical skin formulations for skin protection and moisturization (122). Examples of these ingredients are petrolatum and soybean oil. Petrolatum is a semi-solid mixture of long-chain aliphatic hydrocarbons with carbon range of C12 – C85 (123, 124), derived from the refinement of crude petroleum oil (125). Its composition depends upon the petroleum source and refining process (124). Petrolatum is widely used as a moisturizer in cosmetic skin care and as a skin protectant for skin infections (126). The occlusive property of petrolatum can reduce transepidermal water loss (TEWL) (127). Despite the skin barrier benefits, the penetration of petrolatum into the skin is expected to be limited (124) and was shown not to penetrate into the deeper layers of the stratum corneum due to its high molecular weight (MW) (121). To our knowledge, the penetration kinetics of petrolatum into and across the skin has not been systematically studied. Soybean oil is a vegetable oil consisting of triglycerides of linoleic (54%), oleic (24%), and linolenic (7%), and saturated fatty acids (128, 129). It was found to have antioxidant activities (130) and used in cosmetic and personal care products. Soybean oil was shown to penetrate into the superficial layers of the stratum corneum, which could affect the skin barrier (121). Similar to petrolatum, the kinetics of soybean oil penetration into and across the skin has not been systematically studied.

In the cosmetic and personal care industry, there is an increasing interest in new approaches to effectively deliver chemical agents into the skin. Chemical agents having
skin penetration enhancing activity are important and commonly incorporated into a variety of pharmaceutical formulations to aid dermal absorption of the main drug ingredient (44, 45). A large number of skin penetration enhancers are found in patents (46) as well as pharmaceutical science literature (47). Many studies have shown that penetration enhancers (PE) could perturb the lipid structure of the stratum corneum (SC) (131), interact with the keratin domains, or alter the partitioning of the main ingredients into the stratum corneum (45). Despite the number of penetration enhancers reported in the literature, the mechanisms of action of many enhancers are still not clearly understood. Fatty acids and fatty acid esters have been known to increase skin penetration (45). Oleic acid has been shown to have penetration enhancing activities for many drugs. For example, oleic acid was found to enhance the fluxes of salicylic acid by 28-fold and 5-flourouracil 56-fold across human skin in vitro (50). A combination of glycercyl monooleate and lauryl lactate was used to improve the delivery of testosterone across human skin in vivo (51). Glycercyl monooleate (GlyMOle) has been another skin penetration enhancer of interest (132, 133). It is biodegradable, biocompatible, and nontoxic. For example, GlyMOle/solvent systems were shown to enhance skin penetration of lipophilic and highly polar compounds (134, 135). Understanding the mechanism(s) of skin penetration enhancers such as GlyMOle is crucial for formulation development and identification of new ingredients for skin penetration enhancement and cosmetic skin benefits.

The present study aimed to examine the penetration and distribution of petrolatum and soybean oil in the SC after application using an in vitro diffusion cell model. The effect
of GlyMOle as a potential skin penetration enhancer during application of petrolatum and soybean oil was also investigated. Skin permeation experiments were conducted using the Franz diffusion cell model with split-thickness skin and human epidermal membrane (HEM) under finite-dose conditions. Dotriacontane (C32 alkane) was the probe marker for petrolatum and triolein was the probe for soybean oil. Petrolatum and soybean oil were applied to the skin in the diffusion cell with solvent deposition using hexane or hexane/pentane mixtures. The effect of permeant dose (50-200 µg) and the kinetics of permeant penetration (1-72 h) were examined with and without GlyMOle using the in vitro skin permeation model and subsequent tape-stripping of the samples. It was hypothesized that GlyMOle could enhance the SC penetration of petrolatum or soybean oil compared with the control.
6.2 Materials and Methods

6.2.1 Materials

\(^{14}\text{C}\)-dotriacontane (specific activity 10 mCi/mmol) was purchased from American Radiolabeled Chemicals (Saint Louis, MO). \(^{3}\text{H}\)-triolein (specific activity 108 Ci/mmol) was from Perkin Elmer Life and Analytical Sciences (Boston, MA). \(^{3}\text{H}\)-water (specific activity 1 mCi/g) were purchased from Moravek Biochemicals (Brea, CA). Glyceryl monooleate (GlyMOle) was from BASF Corporation (Florham Park, New Jersey). Petrolatum was purchased from Witco Corporation (Greenwich, CT). Refined soybean oil was from Cargill, Incorporated (Minneapolis, MN). Hexane and bovine serum albumin, fraction V (BSA) were purchased from Fisher Scientific (Fair Lawn, NJ). Ethanol was purchased from Pharmco-AAPER (Brookfield, CT and Shelbyville, KY respectively). Polysorbate 20 (Tween 20) was purchased from Uniqema (Wilmington, DE). Sodium azide (NaN\(_3\)) was purchased from Acros Organics (Morris Plains, NJ). Phosphate buffered saline (PBS: 0.01 M phosphate buffer, 0.0027 M potassium chloride, 0.137 M sodium chloride) pH 7.4, was prepared using PBS tablets and deionized water as described by the manufacturer (MP Biomedicals, LLC, Solon, OH) and preserved using 0.02% NaN\(_3\).

6.2.2 Preparation of skin samples

Anterior and posterior torso split-thickness cadaver skin was obtained from the New York Firefighters Skin Bank (New York, NY). For the split-thickness skin study, the split-thickness skin obtained from the skin bank was stored at -20 °C until use. For the
HEM study, HEM was prepared by the removal of the dermis via heat separation (60, 61) and stored at -20 °C after heat separation. In both cases, the skin sample was cut into 1.5 cm X 1.5 cm pieces and was thawed in a Petri dish filled with Phosphate Buffered Saline (PBS) for 2 h prior to use.

6.2.3 Skin permeation study: Franz diffusion cell setup

For the split-thickness skin study, fully hydrated split-thickness skin sample was mounted onto a Franz diffusion cell with diffusional area of 0.71 cm². For the HEM skin study, fully hydrated HEM was sandwiched between a rubber gasket and a Millipore filter (Durapore PVDF membrane filters, 0.22 μm pore size) and mounted onto the Franz diffusion cell. The stratum corneum side of the skin was exposed to the environment while the other side was in contact with 5 mL receptor medium, which consisted of PBS (pH 7.4) incorporating 2% w/v BSA and 0.02% sodium azide. Each diffusion cell was placed on a thermostated heating and stirring module and maintained at 37 °C, resulting in a stratum corneum surface temperature of 34 ± 1°C. A micro-magnetic stirring bar was placed in the receptor chamber to ensure stirring throughout the experiments. The relative humidity level in the room during the permeation experiments was approximately 30%. Skin sample was allowed to equilibrate for 2 h before the start of the permeation experiments. The integrity of the skin samples was checked by a pre-screening water permeability assay. Briefly, 0.15 mL of ³H-water was pipetted onto each skin in the diffusion cells. Five minutes post-dosing, cotton swabs were used to remove excess ³H-water from the donor chambers. After 1 h, 2 mL-samples from the receptor chamber were collected in scintillation vials, and the solutions were mixed with 10 mL
scintillation cocktail (Ultima Gold, Boston, MA) and analyzed using a liquid scintillation counter (Beckman Coulter LS 6500 Multipurpose Scintillation 177 Counter, Fullerton, CA). Samples with water permeation values greater than 1.6 μL/cm² were discarded. Following the prescreening procedure, the receptor solution was replaced with fresh receptor medium twice to remove the residual radioactivity. The skin sample was then allowed to equilibrate in the heating and stirring module overnight.

6.2.4 Finite dose skin permeation study with split-thickness skin samples

14C-dotriacontane (C₃₂ alkane) was used as a model permeant for petrolatum and ³H-triolein was selected as a model permeant for soybean oil. The donor solution was 50 μg dose of the cosmetic ingredients that were composed of 0.03 μCi ¹⁴C-dotriacontane or 0.2 μCi ³H-triolein and 49 μg petrolatum or soybean oil with or without 1 μg GlyMOle in 3 μL hexane. Hexane showed good dispersion of the model compounds and was a fast-evaporating vehicle; 3 μL hexane took 10-15 sec for complete evaporation on a weighing dish. This dose volume also prevented solvent spreading to the side (the circular edge) that was the interface between the skin sample and the glass wall of the donor chamber. The skin permeation experiments were also carried out with 200 μg dose of the cosmetic ingredients. The donor solution consisted of 0.09 μCi ¹⁴C-dotriacontane or 0.2 μCi ³H-triolein and 196 μg petrolatum/soybean oil with or without 4 μg GlyMOle in 3 μL of hexane. The 50 to 200 μg dosage range mimics the amount of cosmetic ingredients/GlyMOle deposited on the skin surface after personal care product rinsing (unpublished data). 2-mL samples were then collected at 4, 6, 24, 48, and 72 h post-application and fresh receptor medium was added back to the receptor chambers to
maintain a constant volume. The samples were then mixed with scintillation cocktail and analyzed using the liquid scintillation counter. After the final receptor collection, the diffusion cells were disassembled and each skin sample was wiped two times with Whatman filter paper soaked with PBS/0.5% Tween 20 and once with 70%/30% ethanol/deionized water to remove remaining permeants on the skin surface.

6.2.5 Tape stripping after permeation study with split-thickness skin samples

After the skin sample was wiped in the 72-h permeation experiment, tape stripping was performed 5 times to remove a portion of the stratum corneum. Briefly, the skin was covered with an adhesive tape with a central hole of 1 cm² (the available area for tape stripping). Standard D-Squame® disc Cuderm Corporation, Dallas, TX), diameter of 2.2 cm and an area of 3.8 cm², was pressed onto the skin for 5 s and then quickly removed. After the tape stripping was performed, the diffusion region of the epidermis was separated from the remaining skin using a cork borer. Each skin section was dissolved separately in Solvable. The donor chamber was washed with hexane to remove the residual cosmetic ingredient. The receptor collections, filter paper wipes, stripping tapes, solubilized skin sections, and donor chamber wash were analyzed separately using the radioactivity method as described earlier.

In addition to the tape stripping study at 72 h after dosing, Franz diffusion cell experiments with 50 μg dose of the petrolatum and soybean oil with and without GlyMOle were performed for the tape stripping study at either 1 or 24 h after dose.
application. These studies were conducted to investigate the effect of GlyMOle on skin penetration of petrolatum and soybean oil. In these experiments, the receptor samples were collected at 1 h for 1-h permeation experiments and at 4, 6, and 24 h for 24-h permeation study. The analytical methods were the same as described above.

6.2.6 Finite dose skin permeation study with HEM

The donor solutions for petrolatum and soybean oil experiments were approximately 100 μg cosmetic ingredients that were composed of 0.05-0.15 μCi (corresponding to 2.1-6.3 μg) of $^{14}$C-dotriacontane and 98 μg petrolatum with or without 2 μg GlyMOle (equivalent to 2% GlyMOle in petrolatum and 2% petrolatum) or 0.15-0.3 μCi (1.2 – 2.4 ng) of $^{3}$H-triolein and 98 μg soybean oil with or without 2 μg GlyMOle in 5 μL solvent. The solvent was a mixture of hexane and pentane (1:1). The solvent and dose volume provided similar solvent spreading and solvent evaporation time as those in the split-thickness skin study. In a separate study, less solvent spreading of a dye (rhodamine B base) with 3 μL dose volume was observed on HEM than on split-thickness skin samples (unpublished data), and therefore, a larger dose volume (5 μL) than the split-thickness skin study was used. With the larger dose volume, hexane:pentane (1:1) mixture was used because this mixture had a faster evaporation rate compared to hexane alone as the solvent. The dosage of approximately 100 μg cosmetic ingredients was used because it was the average value between the 50 and 200 μg doses in the split-thickness skin study. After dosing, 2-mL samples were collected at 4, 6, 24, 48, and 72 h post-application and fresh receptor medium was added back to the receptor chambers to maintain a constant volume. The samples were then mixed with scintillation cocktail and analyzed using the
liquid scintillation counter. After the final receptor collection, the diffusion cells were disassembled and each skin sample was wiped two times with Whatman filter paper soaked with PBS/0.5% Tween 20 and once with 70%/30% ethanol/deionized water to remove remaining permeants on the skin surface. The HEM was then dissolved in scintillation vials containing 1 mL Solvable (Perkin Elmer Life and Analytical Sciences, Boston, MA) and kept in an oven at 50°C overnight. Donor chambers (including rubber gaskets), receiver chambers, filter membranes, stirring bars, and pipette tips were also rinsed with hexane to remove the residual product. The receptor collections, filter paper wipes, solubilized HEM, and hexane rinses were mixed with scintillation cocktail and assayed separately. The resulting radiolabel content of each compartment for a given cell was normalized to the total applied radiolabel for that cell and expressed as a percent of the applied radiolabel dose. Mass balance was calculated.
6.3 Results

6.3.1 Permeation of petrolatum (dotriacontane) across the skin samples

The amounts of dotriacontane measured in the receptor chamber in the 50 μg dose split-thickness skin permeation experiments at 1, 24 and 72 h with and without the presence of GlyMOle were below the detection limit in the present study. Less than 100 dpm of $^{14}$C-dotriacontane was detected in the receptor, indicating no dotriacontane permeated across the split-thickness skin. Similarly, no dotriacontane permeated across the split-thickness skin into the receptor (< 100 dpm in receptor) when the dose was 200 μg in the 72-h split-thickness skin experiments (Fig. 6.1). The average total percent recovery of dotriacontane with 50 μg dose at 1, 24 and 72 h in the split-thickness skin experiments was ≥ 85% of the applied dose (Table 1). The total percent recovery of dotriacontane at 72 h with 200 μg dose was 69 – 119% (Table 6.2).

To investigate the effect of dermis on petrolatum permeation, in vitro skin permeation experiments were performed using HEM (without dermis). Fig. 6.1 also shows the HEM permeation profiles of dotriacontane as a percent of dose applied from the solutions of petrolatum with and without the presence of GlyMOle (control). At 72 h after application, less than 1.5% of the applied dose of the model permeant was found in the receiver and there was no statistical difference between the amounts of dotriacontane permeated across the skin samples with and without GlyMOle. The total recovery of dotriacontane at the end of the 72-h HEM permeation experiments was presented in Table 6.2. In HEM permeation study, the total recovery of dotriacontane was between 16
– 170% of the applied dose, which was more variable than those in the split-thickness skin permeation study. The recovery measurements include the skin surface wipes, filter membrane under the HEM, and hexane rinse for possible dotriacontane binding on the pipette tips, stirring bar, and receptor glass surface. Only small amounts of dotriacontane were found on the skin surface wipes (< 5% of applied dose) and the filter membrane (not more than 1% of applied dose). The amounts of dotriacontane binding to pipette tips, stirring bars, and receptor glass surface were below detection limit (< 100 dpm; data not shown). However, significant and variable percent of dotriacontane were found in the hexane rinses of donor chambers and gaskets.
Table 6.1. The amount of dotriacontane (petrolatum probe) and triolein (soybean oil probe) as percent of the 50 μg applied dose recovered from different compartments at 1, 24, and 72 h after application in split-thickness skin permeation study in vitro. Mean (± SD)

<table>
<thead>
<tr>
<th>Time</th>
<th>Petrolatum</th>
<th>Petrolatum + GlyMOle</th>
<th>Soybean oil</th>
<th>Soybean oil + GlyMOle</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 h</td>
<td>Total in the skin</td>
<td>56.6 (± 11.6)</td>
<td>65.0 (± 17.1)</td>
<td>56.4 (± 1.5)</td>
</tr>
<tr>
<td></td>
<td>Skin surface wipe</td>
<td>14.6 (± 5.5)</td>
<td>14.7 (± 5.2)</td>
<td>18.7 (± 2.9)</td>
</tr>
<tr>
<td></td>
<td>Donor wash</td>
<td>10.4 (± 3.0)</td>
<td>8.0 (± 4.5)</td>
<td>10 (± 0.9)</td>
</tr>
<tr>
<td></td>
<td>Receptor</td>
<td>&lt; 100 dpm</td>
<td>0.06 (± 0)</td>
<td>0.04 (± 0)</td>
</tr>
<tr>
<td></td>
<td><strong>Total Recovery</strong></td>
<td><strong>85 (range 65 - 110)</strong></td>
<td><strong>85 (range 70-105)</strong></td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>Total in the skin</td>
<td>64.9 (± 13.5)</td>
<td>81.0 (± 8.1)</td>
<td>60 (± 1.7)</td>
</tr>
<tr>
<td></td>
<td>Skin surface wipe</td>
<td>6.0 (± 1.7)</td>
<td>6.5 (± 3.2)</td>
<td>19.7 (± 2.1)</td>
</tr>
<tr>
<td></td>
<td>Donor wash</td>
<td>8.3 (± 1.7)</td>
<td>12.3 (± 3.3)</td>
<td>11.6 (± 0.8)</td>
</tr>
<tr>
<td></td>
<td>Receptor</td>
<td>&lt; 100 dpm</td>
<td>0.19 (± 0)</td>
<td>0.18 (± 0)</td>
</tr>
<tr>
<td></td>
<td><strong>Total Recovery</strong></td>
<td><strong>89 (range 60 - 111)</strong></td>
<td><strong>92 (range 74-114)</strong></td>
<td></td>
</tr>
<tr>
<td>72 h</td>
<td>Total in the skin</td>
<td>83.1 (± 8.8)</td>
<td>88.0 (± 8.6)</td>
<td>57.8 (± 1.3)</td>
</tr>
<tr>
<td></td>
<td>Skin surface wipe</td>
<td>3.9 (± 1.5)</td>
<td>4.6 (± 1.1)</td>
<td>20.1 (± 1.2)</td>
</tr>
<tr>
<td></td>
<td>Donor wash</td>
<td>9.6 (± 5.8)</td>
<td>12.8 (± 3.8)</td>
<td>9.9 (± 0.7)</td>
</tr>
<tr>
<td></td>
<td>Receptor</td>
<td>&lt; 100 dpm</td>
<td>1.12 (± 0.4)</td>
<td>0.78 (± 0.2)</td>
</tr>
<tr>
<td></td>
<td><strong>Total Recovery</strong></td>
<td><strong>100 (range 86 - 111)</strong></td>
<td><strong>83 (range 65-106)</strong></td>
<td></td>
</tr>
</tbody>
</table>
Table 6.2 The amount of dotriacontane (petrolatum probe) and triolein (soybean oil probe) as percent of the applied dose, recovered in the different compartments at 72 h after application in split-thickness skin (200 μg dose) and HEM (100 μg dose) permeation studies in vitro. Mean (± SD)

<table>
<thead>
<tr>
<th></th>
<th>Petrolatum</th>
<th>Petrolatum + GlyMOle</th>
<th>Soybean oil</th>
<th>Soybean oil + GlyMOle</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Split-thickness skin</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total in the skin</td>
<td>82 (±12)</td>
<td>74 (±11)</td>
<td>69 (±12)</td>
<td>71 (±10)</td>
</tr>
<tr>
<td>Total in the receptor</td>
<td>&lt;100 dpm</td>
<td>&lt;100 dpm</td>
<td>2.3 (±2.0)</td>
<td>2.0 (±1.9)</td>
</tr>
<tr>
<td>Skin surface wipe</td>
<td>1.3 – 7.1</td>
<td></td>
<td>1.2 – 11.4</td>
<td></td>
</tr>
<tr>
<td>Wash (Donor chamber)</td>
<td>9.2 – 32.3</td>
<td></td>
<td>5.3 – 27.0</td>
<td></td>
</tr>
<tr>
<td>Total % recovery</td>
<td>69 – 119</td>
<td></td>
<td>65 – 115</td>
<td></td>
</tr>
<tr>
<td><strong>HEM</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total in the skin</td>
<td>14.3 (±3.1)</td>
<td>15.6 (±2.6)</td>
<td>11.9 (±3.8)</td>
<td>12.1 (±6.3)</td>
</tr>
<tr>
<td>Total in the receptor</td>
<td>1.3 (±0.9)</td>
<td>1.5 (±0.6)</td>
<td>0.7 (±0.8)</td>
<td>0.8 (±0.3)</td>
</tr>
<tr>
<td>HEM surface wipe</td>
<td>0.8 – 4.3</td>
<td></td>
<td>0.9 – 3.5</td>
<td></td>
</tr>
<tr>
<td>Wash (Donor chambers &amp; gaskets)</td>
<td>0.5 – 156</td>
<td>27.9 – 106.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total % recovery</td>
<td>16 – 170</td>
<td></td>
<td>36 – 124</td>
<td></td>
</tr>
</tbody>
</table>
Figure 6.1. In vitro permeation profiles of the model permeant of petrolatum (dotriacontane) as percent of the applied dose across the skin in (a) 72-h split-thickness skin permeation study at 200 μg dose for petrolatum with GlyMOle (white squares) and without GlyMOle (white triangles) in solvent hexane. Mean ± SD (n=12); and (b) 72-h HEM permeation study for petrolatum with GlyMOle (black squares) and without GlyMOle (black triangles) in hexane and pentane mixture (1:1). Mean ± SD (n=8). Less than 100 dpm of $^{14}$C-dotriacontane was detected in the receptor at 200 μg in the 72-h split-thickness skin, indicating no detectable dotriacontane permeation across the split-thickness skin.
6.3.2 Penetration of petrolatum (dotriacontane) into the SC

The tape stripping profiles of dotriacontane at 50 μg dose of the cosmetic ingredients with and without GlyMOle in the 72-h split-thickness skin permeation study are shown in Fig. 6.2. The majority of the permeant was deposited in tape 1-2 (top layers of the stratum corneum) and lower amount of dotriacontane was found with increasing skin depth. The amount (% of the applied dose) of dotriacontane in Tapes 3-5 (3 tapes) at 50 μg dose with and without GlyMOle and 1, 24, and 72 h after application are summarized in Fig. 6.3. At this dose level, the amounts of dotriacontane found in Tapes 3-5 with GlyMOle were significantly higher than those without GlyMOle (p < 0.05) at all time points. The largest difference of cumulative amount in the 3 tapes between dotriacontane with and without GlyMOle was observed at 24 h. Fig. 6.4 summarizes the amounts of dotriacontane penetrated into different layers of the skin in the presence of GlyMOle at 1 and 24 h after dosing. Fig. 6.2 also shows the 200 μg dose 72-h split-thickness skin permeation data for comparison. At 200 μg dose, there was no significant difference between the tape stripping results of dotriacontane with and without GlyMOle (p > 0.05).
Figure 6.2. Tape stripping profiles of dotriacontane in 72-h split-thickness permeation study in vitro at (a) 50 μg dose for petrolatum with GlyMOle (white squares) and without GlyMOle (white triangles); and (b) 200 μg dose for petrolatum with GlyMOle (black squares) and without GlyMOle (black triangles). Mean ± SD (n = 8 for 50 μg dose and n = 10-11 for 200 μg dose).
Figure 6.3. The amount of dotriacontane as percent of the applied dose in Tape 3-5 (3 tapes) with GlyMOle (black squares) and without GlyMOle, control (white triangles) from 50 μg dose at 1, 24, and 72 h in split-thickness permeation studies in vitro. Mean ± SD (n = 8). Asterisk denotes significant difference from the control (p < 0.05).
Figure 6.4. The amount of dotriacontane penetrated into different layers of the skin as percent of the applied dose at 1 and 24 h in split-thickness permeation study in vitro with GlyMOle. Mean ± SD (n = 8). Asterisk denotes significant difference (p < 0.05).

6.3.3 Permeation of soybean oil (triolein) across the skin samples

The amounts (% applied dose) of triolein permeated through the split-thickness skin at 72 h were 2.0 (± 1.9) with GlyMOle and 2.3 (± 2.0) without GlyMOle at the 200 μg dose (Fig. 6.5) and these values were not statistically different (p > 0.05). The amounts of triolein permeated the split-thickness skin into the receptor at the 50 μg dose (≤ 1.12% of the applied dose) were smaller than those of 200 μg dose and the results were not significantly different between triolein with and without GlyMOle (p > 0.05), similar to the 200 μg dose. The recovery results of triolein penetration with 50 μg dose at 1, 24 and 72 h after application are presented in Table 6.1. The average total recovery at different
time points was \( \geq 83\% \) of the applied dose (Table 6.1). The total percent recovery of triolein with 200 µg dose was \( 65 - 115\% \) (Table 6.2).

Fig. 6.5 also shows the cumulative amount of triolein permeated across the HEM as the percent of the applied dose from the solutions of triolein with and without GlyMOle. The results showed similar permeation profiles of the test solutions with GlyMOle and without GlyMOle \((p < 0.05)\) and the amounts permeated were not more than 0.8% of the applied dose. The total recovery of triolein at the end of the 72-h HEM permeation experiments was between 36 -124% of the applied dose (Table 6.2). Less than 3.5% of the applied dose of triolein was found on the skin surface wipes and the filter membranes. The amounts triolein binding on pipette tips, stirring bars, and receptor glass surface were negligible \(< 100 \text{ dpm, data not shown}\). Large and variable percent of triolein were also found on the hexane wash of donor chambers and gaskets, similar to those observed with the model permeant of petrolatum, dotriacontane.
Figure 6.5. In vitro permeation profiles of triolein as percent of the applied dose across the skin in (a) 72-h split-thickness skin permeation study at 200 μg dose for soybean oil with GlyMOle (white squares) and without GlyMOle (white triangles) in solvent hexane. Mean ± SD (n=12); and (b) 72-h HEM permeation study for soybean oil with GlyMOle (black squares) and without GlyMOle (black triangles) in hexane and pentane mixture (1:1). Mean ± SD (n=8).

6.3.4 Penetration of soybean oil (triolein) into the SC

Fig. 6.6 shows the tape stripping results of triolein at 50 μg dose with and without GlyMOle in the 72-h split-thickness skin permeation study. The amount (% of the applied dose) of triolein in Tapes 3-5 (3 tapes) at 50 μg dose with and without GlyMOle in 1, 24, and 72-h split-thickness skin permeation studies are summarized in Fig. 6.7. There was no significantly difference between the 3 tapes results of triolein at 50 μg dose with GlyMOle and without GlyMOle for all time points (p > 0.05). Similar to
petrolatum, there was no significant difference between the tape stripping results of triolein with and without GlyMOle (p > 0.05) at 200 μg dose in 72-h split-thickness skin permeation study (Fig. 6.6).

**Figure 6.6.** Tape stripping profiles of triolein in 72-h split-thickness permeation study in vitro at (a) 50 μg dose for petrolatum with GlyMOle (white squares) and without GlyMOle (white triangles); and (b) 200 μg dose for petrolatum with GlyMOle (black squares) and without GlyMOle (black triangles). Mean ± SD (n = 11-12 for 50 μg dose and n = 9-11 for 200 μg dose).
Figure 6.7. The amount of triolein as percent of the applied dose in Tape 3-5 (3 tapes) with GlyMOle (black squares) and without GlyMOle, control (white triangles) from 50 μg dose at 1, 24, and 72 h in split-thickness permeation studies in vitro. Mean ± SD (n = 8).
6.4 Discussion

6.4.1 Methodology and its impact

The in vitro penetration of petrolatum into and across the skin with and without GlyMOle was examined using Franz diffusion cell model in the present study. $^{14}$C-dotriacontane, an alkane with 32 carbons was used as a model permeant for petrolatum since the C$_{32}$ alkane was found to be one of the major components of petrolatum in gas chromatography of the petrolatum employed in the present study (data not shown). The in vitro penetration of soybean oil into and through the skin was also examined with and without GlyMOle under the same conditions as those of petrolatum. Triolein is a triglyceride with three oleic acid carbon tails and radiolabeled triolein ($^3$H-triolein) was selected as a model permeant for soybean oil.

Due to the high lipophilicity of these model permeants, several important methodological factors should be considered. First, when HEM was used in the study, variable and low percent of permeant recovery was found. The large variability and low percent of total recovery in the HEM permeation study could be due to the binding of the permeant to the rubber gasket in the Franz diffusion cell setup. For example, a large amount of permeant could bind to the rubber gasket during dosing that could decrease the percent recovery in an experiment. The rinsing method employed in this study might not be able to remove all permeant bound to the rubber gasket, leading to a large amount of residual permeant in the gasket and donor chamber carried over to the subsequent experiment; this could lead to higher than 100% recovery in the experiment. When the permeation of
petrolatum and soybean oil was studied using the Franz diffusion cell and split-thickness skin samples, the rubber gasket was no longer required, which could eliminate the binding of the permeant to the gasket. This split-thickness skin study therefore could reduce the recovery variability and improve the percent total recovery as observed in the recovery results (Tables 6.1 and 6.2). Second, the type of skin (split-thickness skin and HEM) used in the permeation study could significantly impact the permeation results. No petrolatum was found to penetrate the split-thickness skin into the receptor with and without GlyMOle, contrast to the results of petrolatum penetration across HEM, in which approximately 1.5% of the applied dose of petrolatum was found in the receptor. This could be due to the impact of the dermis on the penetration of the highly lipophilic permeants. It may be possible that permeant binding to the dermis could significantly reduce the in vitro permeation of these highly lipophilic compounds into the receptor. In addition, compounds of high lipophilicity could accumulate mainly in the stratum corneum and be unable to readily permeate through the hydrophilic underlying dermis (1, 27, 136).

6.4.2 Skin penetration of petrolatum and soybean oil with and without GlyMOle in vitro

Both the model permeants of petrolatum and soybean oil were found to permeate into the receptor in the skin permeation study with HEM in vitro. In these skin permeation experiments, the amount of triolein permeated into the receptor was lower than that of dotriacontane. This could be due to the larger molecular size and more bulky structure of triolein (MW = 885.4 g/mol) compared to dotriacontane (MW = 450.9 g/mol). Both
dotriacontane and triolein are highly lipophilic compounds with log $K_{ow}$ of 16 and 23, respectively, and this could lead to low permeation through the skin since the log $K_{ow}$ of between 1 and 3 is required for optimal skin permeation (27, 31). Table 6.3 shows the percent of applied dose permeated across the HEM at 72 h under finite dose condition for petrolatum, soybean oil, other hydrophilic compounds (urea, glycerol, and mannitol) and lipophilic compounds (corticosterone (CS), triamcinolone acetonide (TA), and estradiol), applied with volatile solvents, in the present and previous chapters (95, 137). The lower skin penetration of petrolatum and soybean oil across and into the skin compared to other lipophilic permeants under similar experimental conditions could be attributed to the high lipophilicities, large molecular sizes, and higher applied doses of petrolatum and soybean oil. The permeation profiles of dotriacontane and triolein, which resemble slow linear release of the permeants from the stratum corneum into the receptor, are also different from those observed for the solutes in the previous study. This difference is likely due to the lipophilic nature of the permeants, resulting in the high stratum corneum loading and slow release of the permeants in the present study. In addition to the receptor permeation data, the amounts found in Tape 1-5 in the 1-h split-thickness skin permeation experiment and the corresponding penetration profiles of petrolatum and soybean oil in the stratum corneum were also compared with those of CS, urea, glycerol, and mannitol in Chapter 5 (138). The profiles were similar showing the decreasing amounts of solutes from the surface to the deeper layer of the stratum corneum with significant amounts of the permeants in Tape 1-2 (the superficial layers). This suggests that permeant lipophilicities did not significantly affect the percent dose profiles in the stratum corneum for the highly
lipophilic permeants in the present study and the polar and lipophilic permeants in the previous study.

To understand the impact of the percent dose permeated data in practice, the amounts of petrolatum and soybean oil delivered in the stratum corneum were compared with the mass of the stratum corneum lipids. In order to calculate the percent mass of petrolatum and soybean oil in the stratum corneum lipids, the mass of stratum corneum lipids was first estimated. Based on the assumption of 10% intercellular lipids in the stratum corneum and total stratum corneum mass of approximately 1 mg (area x thickness x density = 0.71 cm² x 0.015 cm x 1 g/mL), the amount of stratum corneum lipids in Tape 3-5, estimated using the protein content measured in Tape 3-5 and the total protein content of the whole stratum corneum, was approximately 35 μg. With ~7-9 μg petrolatum and ~5-7 μg soybean oil found in Tape 3-5, the amounts of petrolatum and soybean oil in the stratum corneum were ~20-25% and 15-20% of the mass of stratum corneum lipids, respectively. This illustrates the significance of the amounts of petrolatum and soybean oil delivered into the stratum corneum under the conditions in the present study.
Table 6.3 Amount of petrolatum, soybean oil, and other hydrophilic and lipophilic permeants (from Chapter 3 and 4) permeated across HEM at 72 h as percent of the applied dose, applied with a volatile solvent. Mean ± SD (n = 8 for petrolatum and soybean oil, and n = 4 for the other permeants).

<table>
<thead>
<tr>
<th>Permeant</th>
<th>Solvent</th>
<th>Amount permeated at 72 h (% applied dose)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrophilic permeants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Urea</td>
<td>Ethanol</td>
<td>33 ± 13</td>
</tr>
<tr>
<td>Mannitol</td>
<td>Ethanol</td>
<td>1.1 ± 0.5</td>
</tr>
<tr>
<td>Glycerol</td>
<td>Ethanol</td>
<td>2.0 ± 1.3</td>
</tr>
<tr>
<td>Lipophilic permeants</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>Ethanol</td>
<td>14 ± 3</td>
</tr>
<tr>
<td></td>
<td>Isopropanol</td>
<td>12 ± 5</td>
</tr>
<tr>
<td></td>
<td>Hexane</td>
<td>15 ± 5</td>
</tr>
<tr>
<td>TA</td>
<td>Ethanol</td>
<td>12 ± 2</td>
</tr>
<tr>
<td>Estradiol</td>
<td>Ethanol</td>
<td>25 ± 14</td>
</tr>
<tr>
<td>Petrolatum</td>
<td>Hexane + Pentane</td>
<td>1.3 ± 0.9</td>
</tr>
<tr>
<td>Soybean oil</td>
<td>Hexane + Pentane</td>
<td>0.7 ± 0.8</td>
</tr>
</tbody>
</table>

6.4.3 Effect of GlyMOle and its penetration enhancing mechanisms

GlyMOle is commonly used as an emulsifying agent and as a food additive (139). Structurally similar to oleic acid (same carbon chain with the substitution of the carboxylic acid polar head group with the glyceryl head group), it has been used as a skin
penetration enhancer for transdermal and topical drug delivery (133, 140). GlyMOle has MW of 356 g/mol and log K\textsubscript{o/w} of 6.4, and thus could penetrate into the skin and interact with the skin barrier (141). In addition, through hydrolysis in the skin, GlyMOle could break down into glycerin and oleic acid (142), providing skin hydration and skin penetration enhancement. It has been proposed that GlyMOle increases skin permeation via the same mechanism as oleic acid. GlyMOle is able to remove ceramides in the stratum corneum (131) and increases skin intercellular lipid fluidity by disturbing the intercellular lipid packing with its unsaturated alkyl chain and/or through the interactions between the hydroxyl group of GlyMOle and the polar head groups of the intercellular lipid lamella (141, 143, 144).

GlyMOle was found to enhance the in vitro penetration of petrolatum into the stratum corneum, but this effect did not translate into enhanced permeation of petrolatum across HEM into the receptor; the small percent values of petrolatum found in the receptor with and without GlyMOle were not significantly different. GlyMOle was not found to enhance the penetration of soybean oil into and across HEM. Two variables were investigated in the present GlyMOle study: (a) permeant dose effect on the penetration of petrolatum and soybean oil into the stratum corneum under the influence of GlyMOle and (b) skin penetration kinetics of petrolatum and soybean oil in the stratum corneum with and without GlyMOle. The effect of permeant dose was studied using 50 and 200 μg doses of the cosmetic ingredients. The kinetics of skin penetration was evaluated by performing tape stripping at 1, 24, or 72 h after dosing.
A comparison of the tape stripping results of petrolatum in the 72 h split-thickness skin permeation experiments at 50 μg dose and those at 200 μg dose (Fig. 6.8) show that GlyMOle significantly enhanced the amount of petrolatum in the stratum corneum (Tapes 3-5) than the control at 50 μg dose while no significant effect was observed at 200 μg dose. This indicates that the effect of GlyMOle on the in vitro penetration of petrolatum into the skin was affected by the dose of the cosmetic ingredients. In the kinetics study, the results of petrolatum with and without GlyMOle in Tapes 3-5 at 1, 24, and 72 h (Fig. 6.3) suggest that GlyMOle could increase both the rate and extent of the penetration of petrolatum into the stratum corneum (Tape 3-5) and the largest effect of GlyMOle on the amount of petrolatum penetration was at 24 h after application. The penetration of petrolatum with GlyMOle followed the profile of initial fast penetration in the first 24 h (slope = 0.1 between 1-24 h in Fig. 6.3) and then slower penetration afterwards (slope = 0.02 between 24-72 h in Fig. 6.3) while the penetration of petrolatum alone demonstrated a linear penetration profile from 1 h to 72 h (R² = 0.97). These results indicate GlyMOle enhanced the amount of petrolatum penetrated into the stratum corneum and altered the skin penetration kinetics of petrolatum. In addition to the results of Tape 3-5, GlyMOle also has a pronounced effect on the penetration of petrolatum into the deeper layers of the epidermis (“remaining epidermis” values in Fig. 6.4). Other data supporting the GlyMOle effect conclusion are the surface wipe and total skin recovery results (Table 6.1). The amount of petrolatum penetrated into the skin (total skin in Table 6.1) increased with increasing the duration of the experiments and the amount found on the skin surface wipe decreased with increasing time. In contrast, the amounts of soybean oil found in the skin surface wipe and the total skin were comparable at different time points.
in the experiments. The total penetration of soybean oil did not increase with time, different from those observed for petrolatum skin penetration. This supports the different GlyMOle effects on petrolatum and soybean oil.

Figure 6.8. The amount of petrolatum with and without GlyMOle (control) in Tape 3-5 (3 tapes) as percent of the applied dose from 50 and 200 μg doses in 72-h split-thickness permeation study in vitro. Mean ± SD (n = 8 for 50 μg dose and n = 10-11 for 200 μg dose). Asterisk denotes significant difference from the control (p < 0.05).

Although the tape stripping profiles of petrolatum and soybean oil at 200 μg dose were similar (Figs. 6.2 and 6.6), the results of soybean oil were different from those of petrolatum at the 50 μg dose. Particularly, GlyMOle did not affect the amounts of soybean oil permeated into the stratum corneum at 50 μg at all time points (1, 24, and 72 h). The different effects of GlyMOle on petrolatum and soybean oil penetration could be
related to the physicochemical properties of these two permeants and other factors. First, the molecular size of the model permeant of petrolatum, dotriacontane, is almost 2x smaller than the model permeant of soybean oil, triolein. The linear hydrocarbon structure of dotriacontane with no polar functional group is less bulky than a symmetrical triglyceride, triolen, with multiple C=C double bonds. The enhancing activities of penetration enhancers depend mainly on their actions on the stratum corneum lipids such as the alteration of the fluidity of the skin intercellular lipids. Although skin penetration enhancers generally provide less skin penetration enhancement for smaller molecules (145, 146), the enhancing action of GlyMOle could lead to an increase in free volume in the intercellular lipids that might not be large enough for the enhanced penetration of triolen that are significantly larger in molecular size and more rigid than dotriacontane. Second, the molecular structure of dotriacontane does not contain any polar functional groups and therefore could preferably penetrate into the hydrophobic center of the lipid bilayers of the stratum corneum intercellular lipids whereas triolen likely penetrates the stratum corneum via the more conventional pathway near the interface region at the intermediate depth of the lipid bilayers; most drugs and permeants are amphiphilic and preferably partition into the interface region close to the polar head groups of the lipid bilayers, which is different from alkanes that preferably stay in the hydrocarbon region of the lipid bilayers. GlyMOle could act differently in these different domains in the stratum corneum and therefore result in different penetration enhancement effects for dotriacontane and triolen. In addition, it is possible that the oleic acid in the triglycerides of soybean oil itself already enhance the SC penetration of soybean oil. The incremental additional oleic acid from GlyMOle therefore may not lead to further increase in the
soybean oil penetration. Further studies are required to investigate the different responses between petrolatum and soybean oil in the presence of GlyMOle.

The significance of the effect of GlyMOle and the increase in the mass of petrolatum in the stratum corneum due to GlyMOle relative to the mass of skin intercellular lipids can be evaluated by comparing the GlyMOle data with those of the control at all time points. At 1 h after application, GlyMOle increased the amount of petrolatum from 6.8 μg to 7.8 μg in Tape 3-5. This amount of petrolatum without GlyMOle corresponds to an increase of total stratum corneum lipid mass of 20%, and GlyMOle enhanced the mass increase to 23%. At 24 h, the mass of petrolatum in the stratum corneum increased from 7.6 μg to 9.2 μg in Tape 3-5 due to GlyMOle, corresponding to the enhancement of total lipid mass increase of 22% with petrolatum alone to total mass increase of 26% in the presence of GlyMOle, and at 72 h, the increase from 8.5 μg to 9.6 μg petrolatum due to GlyMOle corresponds to the enhancement of total lipid mass increase of 24% to 28% in Tape 3-5.

The ~1-2 μg increase in the amount of petrolatum in the stratum corneum as a result of the enhancement effect of GlyMOle on the in vitro penetration of petrolatum into the stratum corneum is therefore significant.
6.5 Conclusions

The in vitro skin penetration of petrolatum and soybean oil was investigated under finite-dose conditions. Significant skin penetration of petrolatum and soybean oil was found in the stratum corneum and epidermis at 1 h after dosing with and without GlyMOle. In the HEM permeation study, petrolatum and soybean oil were found to permeate across HEM into the receptor but no effect of GlyMOle on skin permeation was observed. In the split-thickness skin permeation study, GlyMOle enhanced the amount of petrolatum penetrated into the skin when it was applied on the skin sample at the 50 μg dose, and this effect was found mainly in the deeper SC layers. When the dose of petrolatum was increased to 200 μg, no effect of GlyMOle on the penetration of petrolatum was observed, indicating a dose-dependent effect. In addition, GlyMOle enhanced the penetration rate of petrolatum into the stratum corneum in the penetration kinetics study with the largest amount of petrolatum penetration found at 24 h after dosing. For soybean oil, no significant effect of GlyMOle on the penetration of soybean oil into and across the skin with both 50 and 200 μg doses at any time points. In summary, the results in this study suggest that GlyMOle can enhance the skin penetration of petrolatum in vitro.
CHAPTER 7
SUMMARY AND FUTURE DIRECTIONS
7.1 Summary

The delivery of compounds into and through the skin has been studied for several decades. Still, skin delivery remains a challenge due to the barrier function of the stratum corneum, and this has led to the continuing interest in the studies of determinants affecting skin permeation. The principal aim of this dissertation is to develop a better understanding of factors that impact transdermal and topical skin delivery including the role of solvents and physicochemical properties of the solutes in percutaneous absorption and the effects of a chemical penetration enhancer on skin penetration of highly lipophilic permeants after topical application. The findings in this dissertation could result in more rational approaches in formulating both cosmetic and pharmaceutical skin products and benefit the fields of transdermal and topical skin delivery and risk assessment of toxic compound exposure.

For the studies of the solvent effects on percutaneous absorption in this dissertation, the permeation of a moderately lipophilic solute CS was determined when it was applied on the human epidermal membrane (HEM) with solvents having different evaporation rates under finite dose condition. There was no relationship between the rates of solvent evaporation and skin absorption of CS for fast evaporating organic solvents. The results of the computer simulation suggest that after these volatile solvents dissipated, they did not introduce different SC deposition of CS that significantly affected percutaneous absorption of CS. These volatile organic solvents act only as a vehicle for CS dosing in topical skin application. Conversely, different solvent effects were observed with polar solvents that have slower evaporation rates compared with the organic solvents. The
slower evaporating solvents led to slower CS permeation across the skin. In general, the permeation profiles of CS are in agreement with (a) CS deposition in the SC that did not significantly affect CS percutaneous absorption for the fast evaporating solvents and (b) the effect of solvent dissipation leading to a change in the thermodynamic activity of the permeant during skin absorption for the slow evaporating solvents.

The rates of solute delivery through the skin barrier depend not only on the vehicle in the system but also on the nature of the solute. Molecular size, lipophilicity, and solubility of the solute have been considered as determinants of the penetration of the solutes. However, the relationships between solute properties and skin penetration from different solvents under finite dose conditions have not been extensively studied. The present dissertation investigated the effects of solvents on skin permeation using solutes of varied physicochemical properties and solvents of different evaporation rates. No simple relationship was identified between solvent evaporation rates and skin absorption of the studied solutes, suggesting possible solvent interactions with the solute and/or skin before solvent dissipation that would impact skin permeation. The relationship between solute physicochemical properties and skin permeation was also determined by comparing the finite dose permeation results of the solutes to their permeability coefficients obtained under infinite dose condition. In general, the results of the finite dose condition with selected volatile solvents were in agreement with solute permeability coefficients, except for a small hydrophilic permeant urea. Skin permeation of urea was the highest compared to other model solutes in the finite dose experiments, in contrast to its relative low permeability coefficients. The unique findings of urea permeation led us to conduct
additional studies to better understand the permeation characteristics of urea under the finite dose condition.

As a continuing effort of our investigation, multiple studies were performed to determine the mechanism of skin permeation of urea and its skin permeation behavior under finite dosing. Our studies showed that urea did not have penetration enhancing activity at the studied dose level based on the results of the dose-dependence study of urea and the study of the effect of urea on glycerol permeation. The significant higher concentration of urea observed in the deeper SC layers suggested that urea had faster penetration rate compared to the other model solutes in the study. In addition, the permeation of a small hydrophilic compound with molecular size ($\approx 60$ g/mol) similar to urea was investigated under the same experimental conditions to test if the rapid skin permeation of urea was due to its small molecular size. The similar permeation behaviors of the model small solute and urea supported our hypothesis that solutes with molecular sizes similar to or less than urea would demonstrate high percent dose permeated independent of solute hydrophilicity under the finite dose conditions examined in our study. This skin permeation phenomenon is likely not specific to urea and could be applied to other solutes with similar physicochemical properties.

This dissertation also investigated the approach of incorporating chemical penetration enhancers into topical or transdermal formulations to enhance skin permeation. An enormous number of penetration enhancers have been reported in literature but the
mechanisms of action and the effects of many enhancers on skin permeation of various compounds are still not clearly understood. The present dissertation investigated the skin penetration of highly lipophilic permeants and the effect of a skin penetration enhancer on the penetration of these permeants into and through the skin. Skin permeation studies of petrolatum and soybean oil were conducted using split-thickness skin and HEM with and without the presence of a potential penetration enhancer, GlyMOle. Our study found that GlyMOle enhanced the amount of petrolatum penetrated into the skin while no effect of GlyMOle on skin penetration of soybean oil was observed. The effect of permeant dose (50 and 200 µg) and the kinetics of permeant penetration (1-72 h) were also examined with and without GlyMOle in skin permeation study and tape-stripping. The results indicated that the largest amount of petrolatum penetration was found at 24 h after dosing and the effect of GlyMOle was dose-dependent with no observed GlyMOle effect when the dose of petrolatum increased to 200 µg. The results of this study also revealed the impact of the dermis binding on the penetration of the highly lipophilic permeants that significantly reduced the amount of these compounds permeated through the skin into the receptor in the Franz diffusion cell. The findings of this study are important for skin formulation development and the identification of new ingredients for skin penetration enhancement.
**7.2 Future Directions**

Additional studies are needed for the comprehensive understanding of solute permeation mechanisms and the interplay between the penetration of solutes and solvents into and across the skin. In vivo skin imaging study using confocal microscopy can be conducted to examine penetration depth and distribution of solutes and solvents in the skin after topical application. In addition, additional studies of solutes with various physicochemical properties can be carried out to increase the sample size to evaluate the influence of these properties on skin permeation. Model analyses can be performed to identify the relationships between the skin permeation of solutes and these physicochemical properties, which will improve our ability to predict the penetration of other solutes in general. More diverse groups of compounds can also be examined under the same conditions in this study to fully understand solute physicochemical property-skin permeability relationships.

According to the findings in Chapter 6, GlyMOle could enhance the penetration of petrolatum in the skin. Hence, the effect of GlyMOle concentration on skin penetration of petrolatum can be examined to test the hypothesis of a GlyMOle concentration effect on petrolatum skin penetration. In addition, the effects of the metabolites (glycerin and oleic acid) can be evaluated and their effects will be compared with GlyMOle. Effect of other monoglycerides with different hydrocarbon tail groups on the penetration of the model compounds may also be investigated to assess the structure-function relationship that can be used to identify new monoglyceride entities similar to GlyMOle.
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