2020

Chemical permeation enhancers and their use in transdermal drug delivery

BOSTON UNIVERSITY

SCHOOL OF MEDICINE

Thesis

CHEMICAL PERMEATION ENHANCERS AND THEIR USE IN TRANSDERMAL DRUG DELIVERY

by

FARIHA KHAN

B.S., Stony Brook University, 2016

Submitted in partial fulfillment of the

requirements for the degree of

Master of Science

2020

Approved by

First Reader _____

Stephanie M. Oberhaus, Ph.D.
Assistant Professor of Microbiology

Second Reader _____

Eden Tanner, Ph.D.
Postdoctoral Fellow at Harvard University, SEAS

ACKNOWLEDGMENTS

I wish to thank all the people whose assistance was a milestone in the completion of this thesis. My mentor, Dr. Eden Tanner, guided me throughout my research and writing of this paper. She remained ever so patient with me while helping me through any obstacles I faced. I'd also like to thank my parents and my little sister whose unwavering support allowed me to reach my ultimate goal.

CHEMICAL PERMEATION ENHANCERS

AND THEIR USE IN TRANSDERMAL DRUG DELIVERY

FARIHA KHAN

ABSTRACT

The field of medicine has advanced such that we are now capable of combating many disorders through the use of carefully studied, well-developed therapeutics. There are a number of ways to administer such therapeutics including via inhalation, intravenously, orally, or transdermally. Of the aforementioned delivery pathways, transdermal drug delivery systems have gained much recognition for their ability to deter the premature metabolism of therapeutics as well as avoid potentially harmful side effects. But transdermal delivery systems come with their own set of challenges. For example, the topmost layer of the skin, the stratum corneum, serves as a sturdy rate limiting barrier to molecular permeation. Researchers have therefore sought out formulations that are able to temporarily and reversibly modify such barriers so that we may take advantage of the skin as a delivery route for necessary therapeutics. A number of chemicals, referred to as chemical permeation enhancers (CPE), have shown great promise in being able to do just that. CPEs have been proven to enhance drug flux without causing irreversible damage to the integrity of our skin's natural barrier. This thesis will therefore explore the mechanisms of action behind various chemical permeation enhancers and their use in transdermal drug delivery today.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

5-FU 5-Fluorouracil

ATR-FTIR ..... Attenuated Total Reflectance-Fourier Transform Infrared

Azone® 1-dodecylazacycloheptan-2-one

AZT ..... Zidovudine

BKC ..... Benzalkonium Chloride

CAGE ..... Choline:Geranic Acid (CAGE)

CG-MD ..... Coarse-Grained Molecular Dynamic

CPE ..... Chemical Permeation Enhancer

DDAB ..... Didecyldimethylammonium Bromide

DEGEE ..... Diethylene Glycol Monoethyl Ether

DMSO ..... Dimethyl Sulfoxide

DPPC 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine

DSC ..... Differential Scanning Calorimetry

DTA ..... Differential Thermal Analysis

DTAB ..... Didecyltrimethylammonium Bromide

ED ..... Electron Diffraction

EPR ..... Electron Paramagnetic Resonance

ER ..... Enhancement Ratio

EtOH ..... Ethanol

FA ..... Fatty Acid

FD-4 ..... Fluorescein Isothiocyanate-Dextran

FT ..... Fourier Transform

FTIR ..... Fourier Transform Infrared

GI ..... Gastrointestinal

HEX ..... Hexagonal

IL ..... Ionic Liquid

INSIGHT ..... In Vitro Skin Impedance Guided High-Throughput

IP ..... Irritation Potential

IPA ..... Isopropyl Alcohol

IV ..... Intravenous

LIQ ..... Liquid-Crystalline

MW ..... Molecular Weight

NMR ..... Nuclear Magnetic Resonance

OA ..... Oleic Acid

OE ..... Oestradiol

OR ..... Orthorhombic

PG ..... Propylene Glycol

SAFL ..... Saturated Fatty Alcohols

SAXD ..... Small-Angle X-Ray Diffraction

SAXS ..... Small-Angle X-Ray Scattering

SC ..... Stratum Corneum

SCOPE ..... Synergistic Combinations of Penetration Enhancers

SER ..... Skin Electrical Resistance

SLS ..... Sodium Lauryl Sulfate

TDDS ..... Transdermal Drug Delivery System

TOF-SIMS ..... Time-of-Flight Secondary Ion Mass Spectrometry

TQ ..... Thymoquinone

TRP ..... Transient Receptor Potential

USDAL ..... Unsaturated Fatty Alcohols

UV ..... Ultraviolet

WAXD ..... Wide-Angle X-Ray Diffraction

WAXS ..... Wide-Angle X-Ray Scattering

INTRODUCTION

Despite significant advances in the field of medicine with respect to the development of potent therapeutics, one of the largest challenges faced by clinicians today is the efficient delivery of such medication with minimal side effects. The most common routes of drug delivery include intravenous (I.V.) injection, inhalation, oral delivery, and transdermal absorption. Each of the aforementioned delivery systems have their respective flaws and benefits. I.V. injections can deliver precise doses of medication quickly and effectively, which may be critical in emergency situations. But with such delivery systems, it becomes increasingly difficult to reverse or cease administration of a drug should an adverse reaction occur. I.V. delivery systems also lead to the systemic distribution of therapeutics making the likelihood of off-target side effects much larger than with other methods of delivery [1]. And yet the oft-quoted, largest downside to I.V. administration is the aspect of patient compliance. Patients that require repeat injections over the course of long periods of time may be deterred by the inconvenience and pain associated with I.V. administration, especially those with needle phobia. Inhalation shares the benefits of the I.V. route in that it can allow the efficient delivery of therapeutics, but it is limited in the number of diseases for which it can effectively administer medication. The oral route of delivery is most commonly employed for patients that require regular doses of medication. Though patient compliance is higher with oral delivery than it is with the I.V. route, there are still significant disadvantages to this system such as the first pass effect. When drugs are administered orally, they must transit the gastrointestinal (GI) tract before reaching

their target site. Throughout this transit, the drug is pre-maturely metabolized at multiple points leading to the delivery of a dose far less than what was initially administered. This decreased bioavailability forces clinicians to administer larger doses than would otherwise be required to reach efficacious plasma concentrations of the given therapeutic [2]. This, coupled with the fact that oral administration leads to systemic distribution of drugs, only heightens the likelihood of a patient experiencing the side effects associated with a given medication. And so, one is left to consider the use of transdermal drug delivery systems (TDDS).

Since the beginning of time, people have applied balms and salves to their skin in an attempt to treat disease processes. In recent years, the science and technology behind the development of TDDS have improved significantly. Today, there are a number of drugs on the market that employ the use of transdermal absorption for both locally and systemic delivery of therapeutics. Such delivery systems hold significant advantages over alternative mechanisms of drug administration. First and foremost, it allows evasion of the first pass effect. Clinicians are therefore able to administer lower doses of a given therapeutic leading to the reduced potential for side effects. Patient compliance may also be higher with TDDS as application is non-invasive, painless and can be self-administered [3]. But as with any other delivery system, transdermal delivery has its fair share of disadvantages, the most significant of which is the difficulty associated with bypassing the natural barriers presented by the layers of the skin.

Structure and Function of the Layers of the Skin

The skin is the largest organ within the human body. It serves an array of functions but first and foremost, it acts as a line of defense against exogenous dangers. The skin is able to block harmful ultraviolet (UV) radiation, pathogen infiltration, and chemical or mechanical injury. But it is this very defense-oriented functionality that makes the use of skin in transdermal delivery systems exceedingly difficult [4].

The structure of the skin can be divided into three layers – the subcutaneous tissue (hypodermis) being the deepest layer, followed by the dermis, then the outermost epidermis (Figure 1) [5].

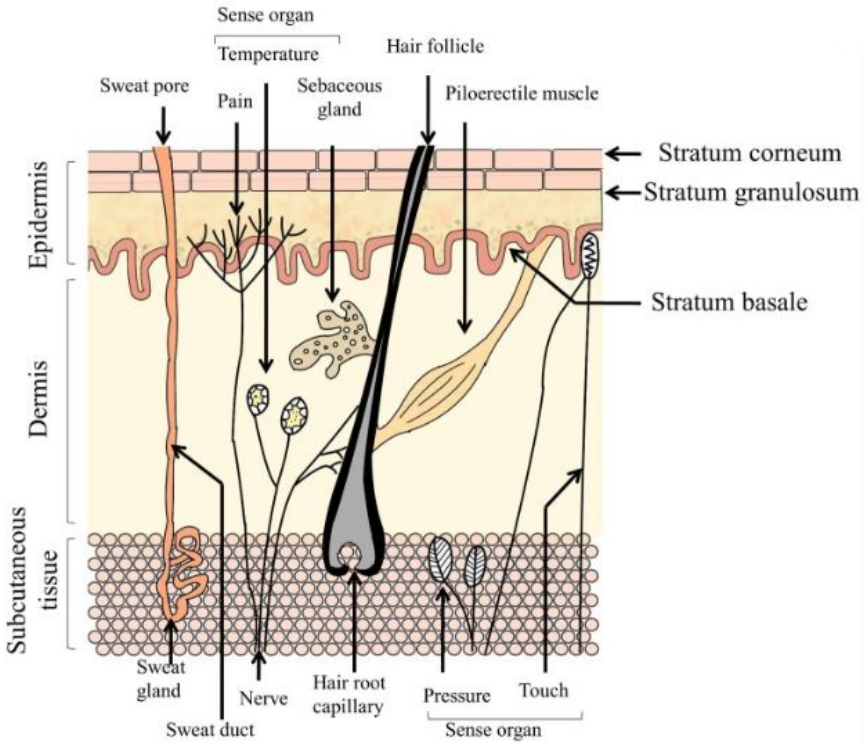

Figure 1: Layers of the Skin. Adapted from Haque et al. 2018. [5]

The hypodermis connects the skin to the underlying fibrous tissue of the bones and muscles. It is well vascularized and mainly composed of fatty adipose and connective tissue. The function of this layer of the skin is to store fat and provide insulation. This latter function is inherently important as the body heavily relies on the skin for its role in thermal regulation [4].

Following the hypodermis is the dermis which is composed of two distinct layers. The hypodermis is directly adjacent to the reticular layer of the dermis, which is composed of well-vascularized, thick connective tissue. This layer contains elastin fibers that contribute to the flexibility of skin and collagen fibers that bind water and allow the skin to remain hydrated. The reticular layer also houses a rich network of sensory nerves. The papillary layer adjacent to the reticular layer is composed of loose connective tissue, small blood vessels, fibroblasts, and a small amount of adipose tissue. The papillary layer also contains phagocytes which are defensive cells that aid in maintaining the immunological barrier the skin presents to external pathogens [4].

The connective tissue of the papillary layer projects into the epidermis which is composed of five layers. The layers, from innermost to outermost, include the stratum basale, the stratum spinosum, the stratum granulosum, the stratum lucidum, and the stratum corneum (SC). The stratum basale contains active stem cells that undergo constant mitotic division. This layer produces new keratinocytes which are the building blocks of the skin. The keratinocytes continuously differentiate as they push up towards the outer layers of the epidermis. By the time the keratinocytes reach the SC they have already transformed into

dead, anucleated cells [6]. Despite this transformation, keratinocytes remain immunologically active and therefore play a role in the initial barrier our skin presents to any foreign substances. Secondary to cell injury, keratinocytes release pre-formed interleukin-1, a potent cytokine capable of setting off a proinflammatory response. Keratinocytes also increase the expression of cell adhesion molecules that ease the movement of other immunological cells such as Langerhans cells. Langerhans cells are the major antigen presenting cells of the epidermis and play a key role in subsequent T-cell migration and proliferation [7]. Together, the aforementioned cells, amongst others, maintain an immunological barrier to TDDS as they are prone to react to any foreign substances, including therapeutics. And yet, an immunological response is not the primary obstacle clinicians must overcome to deliver drugs transdermally.

The primary barrier to transdermal drug delivery is of a structural nature. The composition of the outermost layer of the epidermis is comparable to a wall made of brick and mortar. The keratinocytes, referred to as corneocytes within the SC, resemble bricks that are filled with keratin bundles and surrounded by a protein-lipid cellular envelope. Transmembrane proteins, such as desmosomes and corneodesmosomes, strongly adhere adjacent corneocytes to one another. The mortar is represented by multiple lamellar layers composed of long chain ceramides, cholesterol, esters, and free fatty acids. This brick and mortar structure of the SC is developed over time as newly formed keratinocytes transition into corneocytes from the stratum basale to the stratum corneum of the epidermis [8], [9]. From a functional perspective, this structure is imperative to the SC's ability to prevent water loss and defend the body against foreign substances. But like the immunological

barrier, this structural barrier hinders the absorption of potential therapeutics, especially those that are hydrophilic and of larger molecular weights.

In order to surpass the barriers posed by the epidermis, researchers had to first understand potential paths of diffusion through the SC. There are three main forms of absorption via the SC: intercellular, intracellular, and transappendageal (Figure 2) [10].

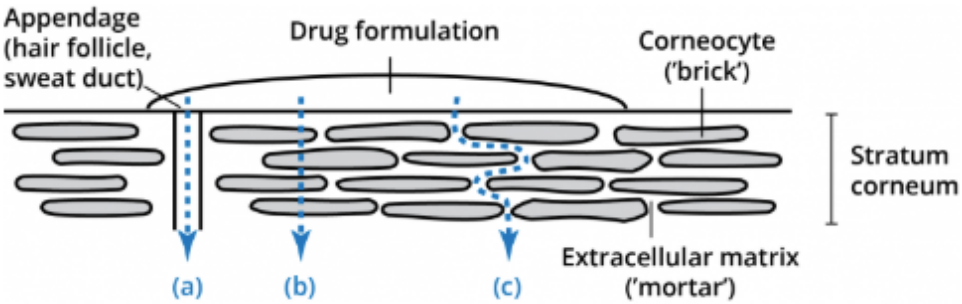

Figure 2: Visual representation of possible routes for surpassing the stratum corneum [10]

Transappendageal transport includes passage through hair follicles, sweat glands, apocrine glands and nails. This form of transdermal delivery is appealing because it allows us to bypass the barriers presented by the SC while also providing the shortest pathway for drug delivery. The downside to this approach is that the surface area available for appendageal delivery is extremely limited, amounting to about 0.1% of the skin's overall surface. An alternative pathway of diffusion would be transcellularly, straight through the SC. This pathway also poses difficulties due to the cellular envelope and lipid lamellae surrounding each corneocyte. Permeants utilizing this pathway would have to undergo multiple steps of partitioning while constantly alternating between the hydrophilic corneocytes and hydrophobic lipids. A third pathway available for transdermal delivery is

the intercellular route via the continuous lipid-rich matrix that surrounds the corneocytes [10], [11].

The intracellular route is often considered the polar route of transdermal delivery as the corneocytes that make up a majority of this pathway are composed of hydrated keratin that provide an aqueous path for hydrophilic drugs. On the hand, the intercellular route, composed primary of lipids, is utilized for the diffusion of hydrophobic drugs through the SC [11]. Between these two pathways, the intracellular route is not considered the preferred path for dermal diffusion due to the very low permeability seen while passing through corneocytes and the obligation of the permeant to partition multiple times [12].

Despite being the major route of diffusion for most compounds, intercellular transport poses significant difficulties. The lipid lamellae that compose this pathway have alternating hydrophilic and hydrophobic components. The transport of large molecular weight drugs, especially those that are hydrophilic, becomes inherently difficult if the lipid bilayers remain intact [13]. In order to make transdermal diffusion a more viable route for drug administration researchers have developed a variety of mechanisms that allow the temporary and reversible alteration of the SC.

Methods Used to Enhance Transdermal Absorption

In recent years, multiple mechanisms have been developed to enhance the delivery of therapeutics through the SC. These include both physical and chemical methods of permeation enhancement. Chemical adjuvants include penetrating enhancers such as hyaluronic acid, peptides, and chemical permeation enhancers (CPEs), as well as

nanocarriers such as liposomes, biphasic vesicles, and microemulsions. Physical enhancement can be electrically driven with iontophoresis, electroporation, or ultrasound or attempt to bypass the SC all together via microneedles or jet needles. Bypassing the SC allows for increased delivery of macromolecular proteins which has proven to be difficult when using chemical adjuvants alone. Microneedles, for example, have become well established in clinical settings for the delivery of influenza vaccines and insulin. But one of the reasons transdermal delivery is so sought out is because it brings with it the promise of non-invasive drug delivery and thereby increased patient compliance. Despite its ability to delivery larger molecules, procedures such as those involving microneedles are still considered invasive [14].

Noninvasive physical enhancers, such as ultrasound, are also effective in the delivery of macromolecules, but the extent of their efficacy is linked to the physiochemical properties of the molecule being delivered. For example, iontophoresis relies on the principles of electrical repulsion to push drugs through the SC. Therefore, this mechanism is far more effective with molecules that are electrically charged than those that are neutral [14]. Non-invasive physical enhancers also require complex, expensive machinery and experienced personnel to operate those machines. Such methods of enhancement therefore cannot be used to self-administer medication, another sought out advantage of TDDS.

In comparison, chemical adjuvants do not require any machinery in order to carry out their effects. These formulations can be self-administered in a noninvasive, painless manner which may contribute to increased patient compliance [14]. The composition and

utility of chemical adjuvants are also comparatively cheaper than those of physical enhancers [3]. Of the aforementioned chemical adjuvants, chemical permeation enhancers in particular have been extensively researched and utilized for decades due to their ease of compositions and fairly low cost of production.

CPE's are mainly classified by their chemical structures. They can be used alone or in conjunction with one another. The mechanism of action of chemical enhancers mainly involves the temporary, reversible disruption of the SC. Ideally, a CPE will have the following characteristics [15]:

1. nontoxic, non-allergenic, and non-irritating,
2. pharmacologically inert,
3. rapid-acting with predictable and reproducible activity,
4. unidirectional,
5. chemically compatible and easily formulated into a variety of systems, and
6. cosmetically acceptable with suitable skin feel.

There is not yet any one chemical enhancer with all six of the aforementioned characteristics. More so, the efficacy of most CPEs is almost always linked to worsening irritation of the skin [16]. A deeper understanding of the mechanistic behavior of such CPEs may allow researchers to find ways to overcome these undesirable side effects. In doing so, we may be able to get closer to creating the ideal CPE as described above.

SPECIFIC AIMS

1. To discuss the mechanisms of action by which various chemical permeation enhancers act to increase the diffusion of drug molecules including: the push effect, the pull effect, fluidization of the lipid lamellae, and lipid extraction.
2. To discuss the most prevalent chemical enhancers used in various transdermal delivery systems today including water, alcohol, fatty acids, Azone®, Transcutol®, terpenes, surfactants, etc.
3. To discuss the possible side effects associated with chemical permeation enhancer formulations as well as the synergistic effect of using a combination of permeation enhancers.

CHEMICAL PERMEATION ENHANCERS – MECHANISMS OF ACTION

Molecular permeation through the SC barrier can be considered in terms of diffusion through a passive membrane. The steady-state flux of a drug through the SC can be estimated by Fick's second law of diffusion. Barry et al. derived the following simplified equation from Fick's law:

$$\frac{dm}{dt} = \frac{DC_0' P}{h} \quad \text{(Equation 1)}$$

where m is the mass of the permeant (the drug) that passes through the membrane in time, t; D is the diffusion coefficient of the permeant; C'_0 is the concentration of the permeant in the formulation that coats the membrane; P is the partition coefficient of the permeant between the formulation and the membrane; h is the membrane thickness. Based on this equation, permeation enhancers may modify the flux of a drug through the stratum corneum in the following ways [17]:

- By increasing the diffusion coefficient of the drug which can be achieved by disrupting the brick and mortar structure of the SC
- By increasing the concentration of the drug in the formulation which can be achieved by incorporating an anti-solvent
- By enhancing the partition coefficient between the formulation and the SC which can be achieved by improving the solvent nature of the SC
- By thinning the SC barrier which would be unlikely.

Barry et al. proposed the Lipid-Protein Partitioning theory to classify the mechanisms by which chemical permeation enhancers can achieve the aforementioned modifications. These mechanisms fall under the following categories: disruption of the intercellular lipid lamellae, accumulation of CPE within the SC leading to increased partitioning of the drug formulation, or modification of the intracellular proteins within the corneocytes of the SC. Most chemical permeation enhancers act through a combination of these mechanisms in order to increase drug flux through the SC [11].

Modification of Intercellular Lipid Lamellae

CPEs that act through modification of the lipid structure of the SC can increase drug flux by disrupting the lamellar and lateral organization of intercellular lipids. This mechanism subsequently increases the diffusion coefficient, D, of the permeant (Equation 1) [17]. In terms of the lamellar organization of the SC lipids, Lane et al. describes three primary sites of disruption: interaction with the polar head groups of ceramide (A), interaction with the hydrophilic region of the lipid bilayer (B), and interaction with the hydrophobic region containing lipid alkyl chains (C) (Figure 3) [18].

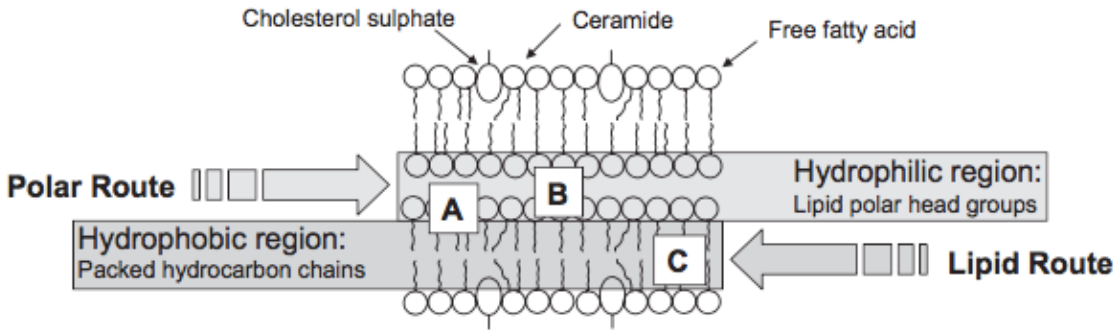

Figure 3: Potential sites of action within the lipid bilayer of the SC. Adapted from Lane et al 2012 [18]

In terms of lateral organization of the SC, intercellular lipids can exist in three phases: orthorhombic (OR), hexagonal (HEX) or liquid-crystalline (LIQ). In the orthorhombic phase the lipid chains exist in an all-trans conformation and are organized in a rectangular, crystalline lattice. This is the densest of the three phases. In the hexagonal phase the lipid chain conformation is tilted and organized in a hexagonal lattice. This phase is less dense compared to the OR phase. In the liquid-crystalline phase the lipid chains exhibit gauche isomerization and show no lateral organization (Figure 4) [19].

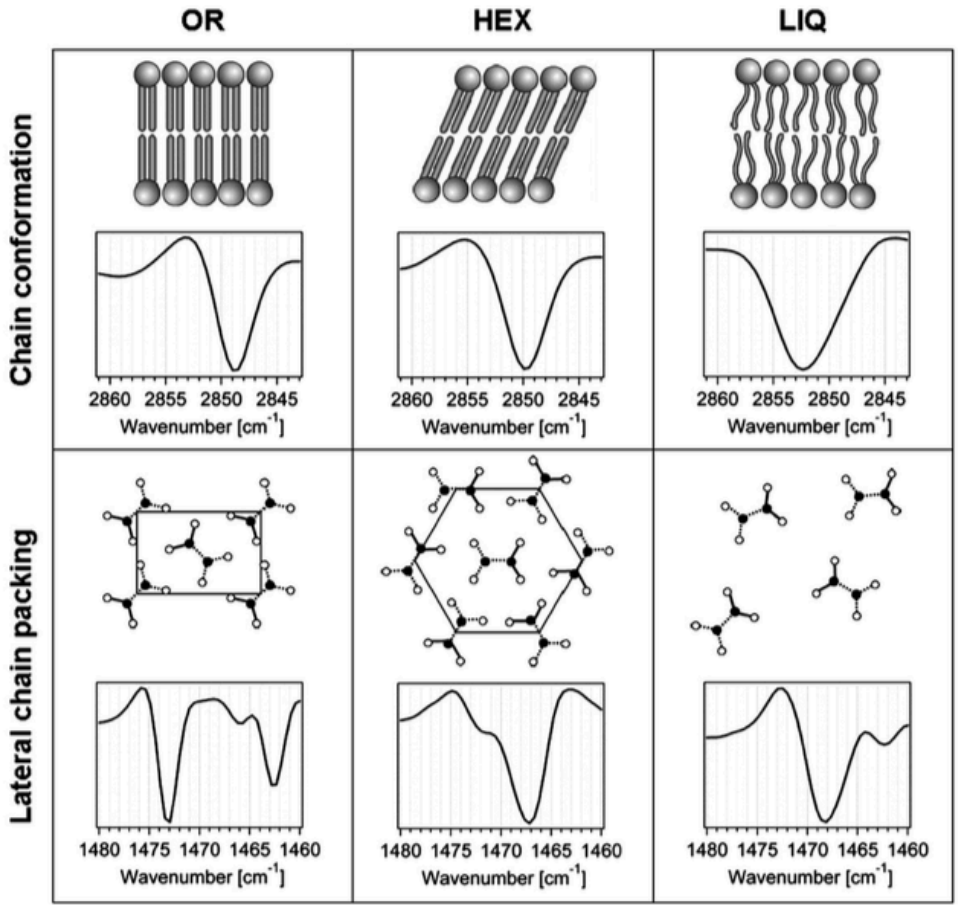

Figure 4: Molecular organization of the SC lipids in the OR, HEX, and LIQ phases. Characteristic IR features of the three phases are shown in the CH_2 symmetric stretching (top row) and the CH_2 scissoring (bottom row) regions of their second-derivative spectra. Adopted from Boncheva et al. 2008. [19].

A study using Attenuated Total Reflectance - Fourier Transform Infrared (ATR-FTIR) spectroscopy has shown that human SC lipids are organized predominantly in the OR phase, although all three phases coexist. The results of this study showed that CPE's can shift the lateral organization of the SC from an ordered OR phase to a highly disordered LIQ phase by incorporating into the lipid bilayer. The resulting phase transition leads to increased drug flux [19]. The transition from one phase to another has also been observed to occur in a temperature dependent manner. Higher temperatures correspond to an increase in the LIQ phase of lipid organization [20]. Some CPEs have been seen to act by shifting the temperature at which phase transitions may occur. These CPEs enhance drug flux by promoting phase transitions at lower temperatures [21].

As discussed above, an alternate mechanism of lipid modification is via interactions with the polar head groups of ceramides (site A from Fig. 3). Under normal conditions, the ceramides found in the lipid lamellae are closely interconnected via strong hydrogen bonds between amide I groups from adjacent ceramides. These hydrogen bonds strengthen and stabilize the lipid bilayer, largely contributing to the barrier property of the SC. Jain et al. showed, using Fourier Transform Infrared (FTIR) studies, that CPEs with more electronegative groups than the amide I group of the ceramide, i.e. an alcoholic -OH group, can compete with the ceramide head for hydrogen bond formation. When such CPEs enter the lipid bilayer, they disrupt the existing hydrogen bonding amongst the polar ceramide heads. This destabilizes the lipid bilayer leading to lipid fluidization and increased drug flux [21]. More recently, Kontogiannidou et al. studied the effects of sesquiterpenes on SC lipids. Their differential scanning calorimetry (DSC) and ATR-FTIR studies showed that

sesquiterpenes also caused lipid detachment which resulted in lipid fluidization and increased permeation through the SC lipid bilayer [22].

"Pooling" of CPEs in the hydrophobic and hydrophilic regions (sites B and C in Figure 3) of the SC lipid bilayer has been considered another common mechanism of increasing drug flux. Janusova et al. used IR spectroscopy to study the interactions between CPEs and SC lipids. They found that the CPE L-Pro2 acted by incorporating into the SC and forming a separate liquid ordered domain within the lipid bilayer. This "pooling" of L-Pro2 led to a temporary defect in the skin lipid barrier, opening up a more permeable pathway for molecular diffusion [23]. Naik et al. reported similar findings of phase separation when studying the effects of oleic acid (OA) on SC intercellular lipids using ATR-IR. This study showed that OA exists in a fluid state in the SC while the surrounding endogenous lipids exist in a disordered state within the superficial layers and in an ordered state within the deeper layers of the SC. They attributed the permeation enhancement of OA to the formation of this fluid state amidst the solid and fluid lipid domains of the SC [24]. This mechanism of incorporation by CPEs has a two-fold effect. It increases the drug partition coefficient (P in Eq. 1) and it modifies the solvent properties of the SC such that increased drug partitioning into the SC is favored [25].

Lipid extraction by CPEs may also increase drug permeation. Sugibayashi et al. used ATR-FTIR to study the effects of solvents like ethanol (EtOH) on SC lipids. This study showed that EtOH pretreatment of skin led to the extraction of lipids decreasing the permeation pathway for lipophilic drugs. This mechanism decreased resistance to

molecular permeation [26]. Mendenha et al. used electron paramagnetic resonance (EPR) spectroscopy to show similar effects of terpenes on 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) model membranes. Their studies showed that terpenes led to the extraction of spin-labeled lipids of the SC [27]. In previous studies using FTIR, this mechanism of lipid disruption was attributed to the subsequent increase in permeation of luteinizing hormone releasing hormone through porcine skin [28]. According to Karande et al., the potency of a molecule to utilize lipid extraction as a mechanism of action is intrinsically linked to its ability to form intermolecular ionic interactions, especially hydrogen bonds. The ability to form hydrogen bonds originates from highly electronegative atoms such as sulfur, nitrogen, or oxygen. Therefore, CPEs with such components will be able to compete with the polar head groups of intercellular lipids to form hydrogen bonds. Formation of hydrogen bonds by CPEs can then weaken the lipid bilayer through lipid extraction leading to a subsequent increase in drug flux [29].

Modification of Intracellular Corneocytes

Multiple studies have shown that most drugs applied to the skin diffuse across the intercellular lipid pathway of the SC [30], [31]. But the bulk composition of the SC (70-95%) is made up of intracellular proteins found within corneocytes, so the role of the intracellular pathway in transdermal drug delivery must also be considered [31]. Crosslinked soft keratin constitutes a majority of the protein content found within corneocytes. The keratin bundles that fill corneocytes are highly hydrated and exist mainly in an α-helix conformation. Certain CPEs, especially those that retain H-bonding capabilities, have been seen to interact with intracellular keratin. Anigbogu et al. studied

the effects of dimethyl sulfoxide (DMSO) on SC via Fourier Transform (FT) Raman spectroscopy. They found that DMSO tends to absorb into corneocytes and alters the conformation of intracellular keratin from α-helices to β-sheets. They postulated that this resulted in a more permeable structure of the SC, leading to increased drug flux [25]. More recent studies have also reported interactions amongst CPE's and intracellular keratin. Hatta et al. used small-angle and wide-angle x-ray diffraction (SAXD and WAXD, respectively) to study the uptake of ethanol into the SC. They found that ethanol penetrates into corneocytes and partially disrupts the structure of the soft keratin found within it. In this way ethanol is able to form routes through which hydrophilic molecules can permeate the SC [32]. Water is a commonly used CPE. Though its exact mechanism of action is unclear, it has been noted that an increase in the hydration level of the SC will generally lead to an increase in drug flux [30]. A study by Bouwstra et al. showed that at hydration levels of 57 %-87% wt/wt, water accumulation is only observed in the intracellular, central regions of the SC [33]. The fact that water applied to the SC is mainly taken up by corneocytes again suggests that being able to affect the intracellular pathway may also contribute to the mechanism of CPEs.

Indirect Mechanisms of Enhanced Diffusion

There are notable ways in which CPEs have been seen to increase drug flux that are not directly correlated with modification of the SC structure. Two such mechanisms are the "push" effect and the "pull" or "drag" effect which are depicted in Figure 5 [5].

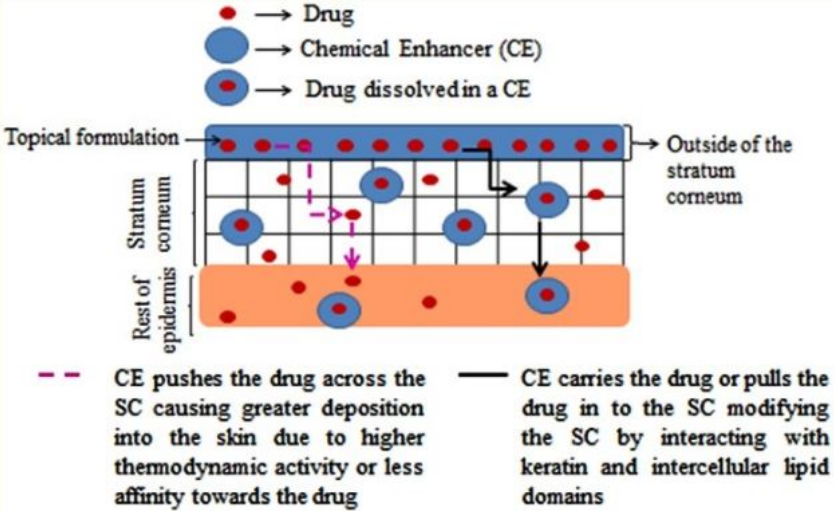

Figure 5: CPE "Push" and "Pull" Mechanisms of Action. Adapted from Haque et al. 2018. [5]

The pull effect is considered as a mechanism of action when a close relationship is noted between the rate of excipient and the rate of solute permeation. According to this effect, some CPEs have the ability to pull molecules with them as they cross the SC barrier. One example of this mechanism in action was studied by Dai et al. through the use of coarse-grained molecular dynamic (CG-MD) simulations. Borneol, utilized as the CPE, was seen to first directly weaken the lipid bilayer by destroying the hydrogen bond network of the polar heads. It was then seen to pull the osthole molecules (the drug) with it as it permeated into the hydrophobic tail regions of the lipid bilayer. According to this study, the pull effect was the main mechanism behind increased permeation of osthole into the SC during the first 100 ns of the study [34]. Similar findings were previously reported by Heard et al. who showed the ability of ethanol and 1,8-cineole to pull molecules of mefenamic acid with them as they permeated the SC barrier. Because there was found to be a close connection between the rates of enhancer and solute permeation, the authors

stated that, in this case, the pull effect sufficiently accounted for the permeation enhancement mechanism of ethanol and 1,8-cineole [35].

There are two mechanisms of action by which a formulation can act to "push" a drug into the skin. The first would be to increase the thermodynamic activity of the drug such that it is more likely to permeate the SC. This can be achieved by choosing a CPE that will itself quickly diffuse into the SC. This will increase the concentration of the solute in the donor phase, leaving it with excess free energy. The excess free energy will then push the drug down a favorable concentration gradient into the SC [36]. The push effect may also be achieved by choosing a drug and CPE combination that have minimal affinity for one another. If the solubility parameters between the drug and the CPE are sufficiently different, the attraction of the drug towards the CPE will be low. This will allow the drug to easily escape the formulation and diffuse into the skin [5].

A third indirect mechanism by which CPEs can increase drug flux is by solubilizing the drug within the formulation. Surfactants are examples of potent CPEs that act via this mechanism. They are able to solubilize a permeant by forming micelles which in turn eases the diffusion of the permeant into the SC [37]. This mechanism is especially important for drugs that would otherwise exhibit low solubility within the layers of the SC. The ability to solubilize a permeant has been associated with the mechanisms of both the push effect and the pull effect. Some studies have found that when a CPE solubilizes drug molecules within a formulation, it increases the concentration of the drug in the donor phase. This creates a concentration gradient that favors the movement of the drug into the SC. This

description aligns closely with the push effect and is described as such by Mura et al. [38]. A previous study by Kadir et al. attributed the solubilization of the permeant in the formulation to the pull effect. This study proposed that solubilizing the drug within the formulation allowed the CPE to drag the permeant with it as it diffused into the SC [36]. Despite which umbrella term the solubilization of a permeant falls under, it has been well established as a potent mechanism for increasing drug flux.

Though examples of specific CPEs have been associated with each mechanism described in this section, most studies have found that CPEs rarely act through just one mechanism. Rather, most CPEs employ a combination of multiple mechanisms in order to carry out their effects. For this reason, CPEs are usually categorized by their distinct structural properties rather than their mechanisms of action [30]. The following section will follow this trend in summarizing the most prevalent CPE's used in transdermal delivery today while organizing them based on their unique structural properties.

CHEMICAL CLASSES OF PERMEATION ENHANCERS

There are a large number of chemicals to date that have been identified as potent transdermal permeation enhancers. Due to their varied mechanisms of action, these CPEs are categorized based on similarities in their chemical structures. Although it is far from a complete list, a few of the major chemical classes of CPEs that will be discussed throughout this paper are listed in Table 1 [30].

Chemical Class	Enhancer
Alcohols	*Short-chain alcohols* Ethanol Isopropyl alcohol *Long-chain alcohols* Decanol Octanol *Glycols* Propylene glycol (PG)
Amides	*Cyclic amides* Azone® (1-dodecylazacycloheptan-2one or laurocapram
Fatty Acids	Lauric acid Oleic acid Linoleic acid
Ether alcohols	Transcutol® (diethylene glycol monoethyl ether)
Surfactants	*Anionic surfactants* Sodium lauryl sulphate (SLS) *Cationic surfactants* Benzalkonium chloride Cetylpyridinium chloride *Nonionic surfactants* Polysorbates (Tween® 20, Tween® 80) *Zwitterionic surfactants* Dodecyl betaine
Terpenes & Essential Oils	D-Limonene L-Menthol 1,8-Cineole Eucalyptus Ylang ylang Chenopodium

Table 1: Chemical class categories of CPE's with respective examples. Adapted from Dragicevic et al. 2015 [30].

Water

Although it is not listed as one of the classes of CPEs, water is the most common and safest way to increase the transport of drugs across the SC. At baseline, the water content of the SC is usually 5-10% but this can be increased beyond 50% in occlusive conditions [5]. Alonso et al. used electron spin resonance to study the effects of different hydration states on the permeability of the SC. Their results showed that membrane fluidity increased with increased water content in neonatal rat SC. The increase in fluidity was noted to be larger near the polar head groups of the lipid lamellae. The proposed mechanism behind this lipid fluidization was the formation of small hydration shells through hydrogen bonding by water molecules. This in turn enlarged the free space for segmental motion of the first carbons of the lipid acyl chains. Due to the low fluidity of this area, penetration through the region of the first carbon of the acyl chain is otherwise considered the rate limiting step of water transport [39]. The results of this study were complemented by those of Denda et al. who analyzed changes in the SC of hairless mice maintained at a high relative humidity (RH > 80%) vs low humidity (RH < 10%) environment for two weeks. The study found that when kept in a low humidity environment, the number of layers of the SC, the total concentration of SC lipids, and the overall thickness of the epidermis increased significantly [40]. The morphological changes to the SC seen in low hydration circumstances may further explain the potency of water as a CPE.

Later studies found that occlusion by water molecules increases drug flux by diffusing into intracellular corneocytes and causing them to swell. According to Zhai et al., increasing skin hydration also led to increased skin temperature (from 32°C to 37°C). The

same study detailed a ‘reservoir effect’ of hydration. Under occlusive conditions, drug flux into the SC was increased. Once the occlusive dressing was removed, the SC dehydrated and movement of the drug slowed, thereby creating the reservoir of the permeant in the SC [41]. Though the exact mechanism of action of water as a CPE is still debated, it remains one of the oldest and safest ways to increase drug flux through the SC.

Alcohols

Alcohols are commonly used by transdermal drug delivery systems to enhance drug flux. They are organic molecules characterized by the presence of one or more hydroxyl groups (-OH) attached to a hydrocarbon chain [42]. There are three main chemical variations amongst alcohols utilized as CPEs. They can be classified as long-chained/fatty alcohols (containing a longer hydrocarbon chain), short-chained alcohols, or glycols (containing two hydroxyl groups). Alcohols can act through various mechanisms of action. The highly electronegative oxygen atom found in hydroxyl groups allows alcohols to act via lipid fluidization and lipid extraction. Alcohols have also been recognized as potent solvents, which may allow them to increase drug partitioning by changing the solubility of the drug in the SC or increase the thermodynamic activity of the drug in the donor phase [30].

Chandra et al. studied the effects of multiple alcohols (ethanol, n-propanol, isopropyl alcohol (IPA), n-pentanol, n-butanol, and propylene glycol (PG)) on the permeation of ketorolac through rat skin. The study found that smaller chained alcohols, such as IPA or ethanol, were more efficient at enhancing dermal permeation. It was

theorized that as solvents, alcohols extracted lipids from the SC which resulted in reduced lag time of diffusion and overall increased diffusivity of the permeant [43]. Many other studies have also described the efficacy of ethanol as a solvent or cosolvent in transdermal delivery systems. Haq et al. found that ethanol solvents, when used as a vehicle for Thymoquinone (TQ) diffusion, increased the capacity of the SC for TQ uptake. They stated that via the "pull" effect, ethanol was more effective than Tween 80, N-M ethyl pyrrolidone, Azone®, Transcutol®, and oleic acid in solubilizing and dragging TQ molecules with it into the skin membrane [44]. Megrab et al. studied the efficacy of ethanol as a cosolvent used to increase the permeation of Oestradiol (OE) into the SC from a drug-saturated ethanol/water system. They found that the effects of ethanol on OE permeation were concentration dependent. At first, OE flux increased with increased concentrations of ethanol. At low concentrations of ethanol, the mechanism of increased flux was correlated to ethanol increasing the solubility of OE within the SC. At higher concentrations of ethanol, lipid fluidization was said to be its main mechanism of action. Interestingly, there was also found to be a concentration dependent limit to the efficacy of ethanol. Ethanol's optimal concentration range was found to be between 40% – 60% wt / wt. Once ethanol reached concentrations greater than 60% wt / wt, a decrease in OE flux was observed. The study postulated that at concentrations above 60%, ethanol may remove water from the SC. Because increased flux has been correlated with increased hydration of the SC, this dehydration was postulated to cause the decreased flux of OE [45].

Long chained fatty alcohols have also been shown to be effective permeation enhancers for multiple drugs [46]. Kandimalla et al., studied the effects of saturated and

unsaturated long chained, fatty alcohols in increasing melatonin diffusion through the SC. A biphasic flux of melatonin was observed secondary to the addition of both saturated and unsaturated fatty alcohols (SAFL and USFAL, respectively). For both fatty alcohols, melatonin flux was seen to increase with increased chain length in the first phase of permeation but the mechanism behind this increased flux varied. For SFAL, the mechanism was mediated by accumulation within the SC lipid bilayer leading to increased solubilization and lipid extraction. Interactions between USFAL and SC lipids were more difficult because most of the lipids in the SC are saturated. Therefore, USFAL increased permeation by inserting itself into the SC lipid packing and disrupting its order and stability due to its kinked structure. In the second phase, a decrease in melatonin flux was noted with increased chain length. It was theorized that drastic modifications of the SC due to ongoing effects of fatty alcohols led to the diffusion of polar components from the applied formulation. These components then brought the polarity of the skin closer to that of the formulation leading to reduced formulation/skin partitioning of melatonin [47]. Kanikkannan et al. studied the effects of SFAL on melatonin diffusion across hairless rat skin. Their study found that maximum efficacy of SFAL was dependent on its chain length with decreased permeation seen at chain lengths increased beyond ten carbons [46]. These findings were supported by similar correlations between chain length and enhancer efficacy seen when studying the effects of SFAL on the diffusion of naloxone through human skin. The difference with this study was that greatest efficacy was seen with twelve carbon chain lengths [48]. Effective chain lengths of 10-12 carbons correspond to the chain length of the steroid nucleus of cholesterol. It was therefore suggested that the link between carbon chain

length and enhancer efficacy was due to CPE disruption of ceramide-cholesterol or cholesterol-cholesterol interactions within the SC [46]. The efficacy of long chained fatty alcohols is therefore considered to be dependent on both carbon chain length and timespan of application.

The most common glycol used as a CPE in TDDS is propylene glycol [30]. PG has been used in transdermal formulations since 1932, both individually as a chemical enhancer and as a cosolvent or vehicle for increased permeation. Though multiple mechanisms have been proposed, the exact mechanism of action of PG is still unclear [12]. The effects of PG on the diffusion of estradiol and metronidazole through excised full thickness human skin was studied [49]. Here, PG was seen to enhance permeation of both drugs by acting as a solvent vehicle, i.e. by carrying the drug with it as it diffused into the SC. This mechanism closely resembles what is now considered the "pull" effect. The effects of PG were also studied via small-angle X-ray scattering (SAXS) and differential thermal analysis (DTA). According to the results of this study, PG was able to enhance drug flux by incorporating into the polar head regions of the intercellular lipid bilayer and increasing its interfacial area. Additionally, this study found that application of PG led to a decrease in the transition temperature required for changes in the lateral organization of intercellular lipids [50]. Pudney et al. monitored the penetration of *trans*-retinol secondary to the addition of PG in vivo using confocal Raman microspectroscopy. They found that the depth of permeation of *trans*-retinol was directly correlated with the depth of penetration of PG. They stated that PG may decrease the polarity of the aqueous regions of the SC to insert itself within the lipid bilayer. Accumulation of PG within the SC may then lead to increased partitioning

of the permeant [51]. A study employing the use of ATR-FTIR found that PG could successfully enhance the diffusion of phenols across the SC. The mechanisms of action observed in this study included dehydration of keratin within corneocytes and lipid extraction leading to a change in the solubility properties of the SC. Notably, this study disputed previous claims that PG enhanced diffusion through a carrier mediated effect. This claim was made secondary to findings that showed that the amount of phenol carried into the SC by PG was minute compared to the amount that diffused independently. Rather, they stated that the main mechanism behind the increased flux by PG was due to its ability to change the solubility profile of the SC towards one more favorable to the diffusion of phenols within intercellular lipids of the SC [52]. Carrer et al. more recently studied the effects of PG on the permeation of hydrophilic and hydrophobic drugs through pig skin using Franz diffusion cells. They found that although PG could increase the permeation of all permeants tested, it showed greatest efficacy in increasing flux of hydrophilic molecules. They attributed this effect to PG's ability to modify the skin lipidic barrier. Their study also analyzed the effects of PG via μFTIR. Their results supported previous findings reporting PG's ability to alter the lipidic order of the bilayer to a more disordered lateral organization. Interestingly, they found this effect of PG stretched beyond the epidermis to the dermal layers of the skin [12]. Although the exact mechanism of PG is remains unclear, it is evident that PG, like other alcohols, acts as a potent CPE.

Amides

Azone®

Azone (1-dodecylazacycloheptan-2-one or laurocapram) was the first synthetically prepared transdermal penetration enhancer, initially patented in 1976. It falls under the chemical category of amides and is composed of a polar head group joined to a twelve-carbon aliphatic chain. This hydrocarbon chain makes Azone highly lipophilic. Despite this attribute, Azone has proven to be effective at enhancing the penetration of both hydrophobic and hydrophilic drugs through the SC. Similarly, the molecular weight of a drug does not seem to hinder the efficacy of Azone with enhancements seen of both small and large molecular weight molecules. At room temperature, Azone exists as a clear, odorless liquid. When applied to the skin, Azone appears smooth and oily without leaving behind a greasy residue [53], [54]. Such properties make this CPE especially favorable for use in topical formulations.

Stoughton et al. found that, like alcohol, the effects of Azone are also concentration dependent. Interestingly, Azone was found to be most effective when used in lower concentrations with decreasing efficacy as concentrations were increased [54]. Initial studies found the optimal concentration of Azone to be between 2-10%, but more recent studies have found that Azone works best between concentrations as low as 0.1-5%. The concentration of Azone required for optimal enhancement of the permeant varies from drug to drug. The efficacy of Azone is also dependent on the formulation within which it is used. Azone was found to be insoluble in water, but soluble in most organic solvents with the simplest formulations best promoting the efficacy of Azone [54].

Although Azone has been studied extensively since its initial discovery, its exact mechanism of action is still under investigation. Various mechanisms have been proposed to understand the efficacy of Azone as a CPE. The most supported theory is that Azone exerts its effect by interacting with the lipid domain of the SC. Hadgraft, et al. proposed that, because of its large polar head group and lipophilic chain, Azone is able to heterogeneously partition into the lipid bilayer and disrupt its lamellar packing arrangement [55]. This theory is supported by the idea that Azone can exist in a bent "soup spoon" conformation with the ring in its head group arranged at an angle to its lipophilic chain (Figure 6).

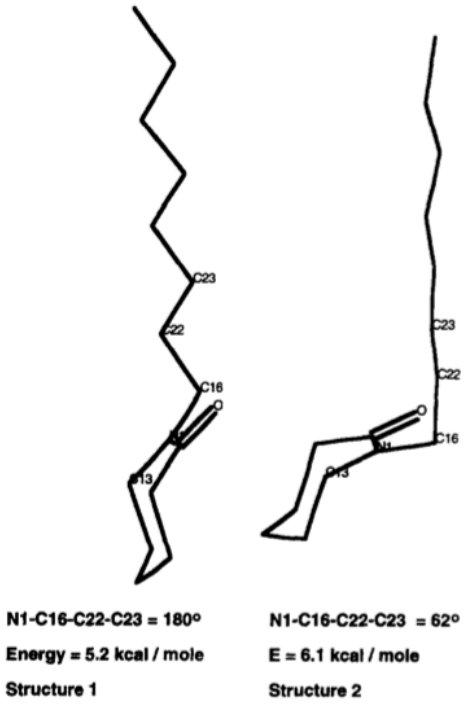

Figure 6: Conformations of Azone. Only 8 chain hydrocarbons shown. Adapted from Hadgraft et al. 1996 [55]

As can be seen in Figure 6, the bent spoon conformation has a higher energy of 6.1 kcal in comparison to the non-bent conformation energy of 5.2 kcal. This energy difference of about 1 kcal corresponds to a high probability of 0.2 for the existence of the "bent spoon." It was proposed that the increased energy of this bent conformation is compensated by the removal of hydrophobic ring methylene groups from the polar head region of the lipid bilayer [55]. Hadgraft et al. argued that the ceramide molecules packed into the bilayer structure would resist the existence of this higher energy formation of Azone. Alternatively, they proposed that after partitioning into the lipid bilayer, Azone competes for the hydrogen bonding sites between ceramide head groups. Because it is widely accepted that the hydrogen bonds between ceramide molecules stabilize the lipid structure of the SC, it was concluded that disrupting these bonds allowed Azone to enhance partitioning of drugs within the SC [55]. Pham et al. studied the effects of Azone on intact porcine SC organization via natural abundance ^{13}C polarization transfer solid-state nuclear magnetic resonance (NMR). This method allowed them to detect small changes in the mobility of the minor fluid and major solid components of the SC under various hydration levels. They found that with high water content, Azone led to increased mobility in the ceramide headgroups of the SC. This is consistent with the idea that despite what the exact interactions may be, Azone likely partially acts through modifications of the polar region of the SC lipids [56].

Pilgram et al. suggested that the mechanism of action behind Azone's efficacy lies in its ability to intercalate within the lateral arrangement of the SC lipids. They studied the interactions between Azone and lipids isolated from human SC via electron diffraction

(ED). Their results showed that upon addition of Azone, separate fluid domains formed between SC lipids, while the orthorhombic lipid organization surrounding these domains remained locally present. It was suggested that between these separate fluid domains, new penetration pathways may arise which then result in increased permeability [57]. Additionally, via NMR studies, Pham et al. found that these segregated fluids domains dissolved small amounts of SC lipids further contributing to increased molecular mobility in the headgroups and chains of SC lipids. Though it is clear that Azone acts by disrupting the lipophilic region of the SC, it was not found to exert an effect on the protein structures within the SC [56].

Terpenes and Essential Oils

Terpenes are non-aromatic compounds composed only of carbon, hydrogen, and oxygen atoms. They are naturally occurring substances that are primarily extracted from vegetable oils, but also found in a number of well-known essential oils such as eucalyptus, chenopodium, and ylang ylang. Due to the aforementioned properties terpenes have long been used as flavorings, perfumes, and medications [17], [27].

The chemical structure of terpenes is based on repeated isoprene units (C_5H_8) and depending on the number of these units, they can be categorized as either monoterpenes (C_{10}), sesquiterpenes (C_{15}) or diterpenes (C_{20}). Furthermore, they can be classified by the chemical groups attached to them such hydrocarbons, alcohols, oxides, ketones, or esters. The ability of a terpene to effectively enhance permeation depends on its chemical structure and on the drug being applied [30]. Barry et al. studied the effects of seventeen simple

cyclic terpenes (varied based on the chemical groups attached to them i.e., hydrocarbons, alcohols, ketones, oxides) in enhancing permeation of the model hydrophilic drug, 5-fluorouracil (5-FU). The permeation experiments performed on excised human epidermal membranes found that alcohol and ketone terpenes were more effective than hydrocarbon terpenes in enhancing penetration of 5-FU. Within the subgroup of oxides, the study found that cyclic ethers were more effective that epoxides in enhancing diffusion [58]. Zhu et al. used skin electrical resistance (SER) to investigate the effects of seven oxygen-containing terpenes on the SC structure. Their studies showed that cyclic terpenes such as 1,8-cineole, terpinen-4-ol, menthol and alpha-terpineol possess higher enhancement ratio (ER) values compared with linear terpenes such as linalool, geraniol and citral. These results were supported by ATR-FTIR analysis which revealed that the effect of cyclic terpenes on the SC lipid arrangement was stronger than that of linear terpenes [59].

Studies have shown that the effects of terpenes are also highly dependent on their lipophilicities. For example, oxygen-containing polar terpenes (carvacrol, menthol) have been found to be more potent at increasing permeation of hydrophilic drugs and hydrocarbon monoterpenes (limonene, p-methane) have been found to be more potent at increasing permeation of lipophilic drugs [15]. The aforementioned studies conducted by Barry et al. support these findings in that permeation of hydrophilic 5-FU was found to be most enhanced by hydrophilic alcoholic and ketone terpenes [58]. Tas et al. studied the effects of various terpenes (anethole, carvacrol, and menthol) on the permeation of the highly lipophilic anti-inflammatory drug, Etodolac. Their ex vivo permeation studies on excised rat skin revealed that the hydrophobic terpene, anethole, most significantly

enhanced the absorption of etodolac. In contrast, hydrophilic terpenes, menthol and carvacrol, did not enhance the absorption of etodolac [60].

Like most CPEs, various mechanisms of action have been associated with terpenes. Barry et al. referred to the LPP theory in describing the mechanism of action behind the terpenes studied throughout their experiments. It was proposed that terpenes act in part by modifying intercellular lipids while having little effect on intracellular proteins found within corneocytes. Furthermore, terpenes were not seen to increase partitioning of 5-FU within the SC as expected due to the poor solubility of 5-FU in most terpenes [58]. Narishetty et al. found similar results in studying ex vivo permeation of hydrophilic zidovudine (AZT) across rat skin secondary to the addition of terpenes. Terpenes were not seen to alter the SC/vehicle partition coefficient of AZT or the thermodynamic activity of the drug in the donor formulation. Alternatively, a significant reduction in activation energy for AZT permeation was noted secondary to the addition of cineole. This reduced energy indicated disruption of the intercellular lipid bilayer. It was proposed that the molecular mechanism of this disruption may be due to the preferential hydrogen bonding of monoterpenes with ceramide head groups. These bonds break the lateral/transverse hydrogen bond networks of the lipid bilayer creating new polar pathways within interlamellar regions of the SC. This effect was further substantiated by molecular modeling studies using Sybil software, the results of which are displayed in Figure 7 [61].

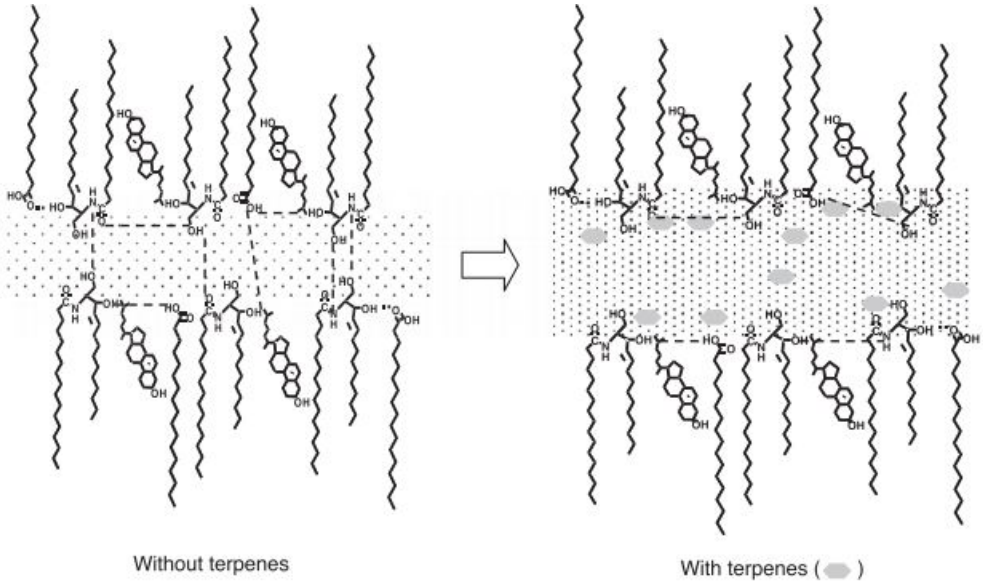

Figure 7: Representation of the effect of terpenes on the SC lipid bilayer. Terpenes are shown to break transverse hydrogen bonds leading to wider aqueous regions near polar head groups of adjacent lipid lamellae. Adopted from Narisheety et al. 2004 [61].

Later studies by Narishetty et al. employed the use of ATR-FTIR and DSC to assess the effects of 1,8-cineole and L-menthol on phase behavior of SC lipids and permeation of AZT across human cadaver skin. Their results showed that both terpenes enhanced permeation of AZT by increasing the hydration levels of the SC lipid system through the formation of new aqueous channels. A reduction of lipid phase transition temperatures was also noted which led to a transformation of the lateral organization of SC lipids from its highly ordered OR packing to a less ordered HEX packing [62].

Kontogiannidou et al. studied the effect of chemically synthesized sesquiterpenes on the permeation of macromolecule fluorescein isothiocyanate-dextran 4 kDa (FD-4) across full thickness human skin. Their ATR-FTIR study showed that increased permeation secondary to the addition of sesquiterpenes could be attributed to lipid extraction. These

results were supported by subsequent DSC studies where shifts to lower endothermic peak temperatures signified lipid disruption and detachment of lipids from the SC [22]. Lan et al. found similar results when studying the effects of Z. bungeanum oil (an essential oil), limonene, terpinen-4-ol, and 1,8-cineole on permeation through rat abdominal skin. Their FTIR studies showed that the tested CPEs increased drug flux by perturbing and extracting SC lipids [63].

Despite initial claims against interactions between terpenes and intracellular proteins, recent studies have shown that such interactions may exist depending on the terpene applied. DSC studies by Kontogiannidou et al. revealed detachment of proteins from the SC secondary to the addition of sesquiterpenes [22]. Molecular modeling and ATR-FTIR studies done by Zhu et al. provide further evidence of interactions between terpenes and skin proteins. Their results show that terpenes such as 1,8-cineole interact with intracellular keratin through van der Waals forces and hydrophobic interactions subsequently allowing terpenes to interfere with the interactions between water and keratin. But despite these findings, Zhu et al. maintained that the main mechanism promoting the actions of terpenes were those mediated by disruption of the SC lipid organization [59].

Joshi et al. provides a rather unique mechanistic effect of the terpene, menthol. Desmosomal adhesion proteins are known to restrict paracellular transport through the SC by promoting the close packing of corneocytes. In order to function, desmosomes require calcium-dependent cadherin as high extracellular calcium induces cadherin mediated cell-cell adhesion. Menthol is known to trigger the Transient Receptor Potential channel (TRP)

TRPM8 which mediates calcium entry into the cell, lowering extracellular calcium concentrations. In-vivo microdialysis studies showed that menthol's ability to increase drug permeation was significantly hindered when a calcium channel blocker was added to the formulation. Based on these results, Joshi et al. proposed that menthol physiologically increased percutaneous penetration by decreasing cadherin modulated cell-cell adhesion through interactions with the TRPM8 channel. They stated that these results may serve to complement the accepted concept that menthol acts by interfering with the lipid arrangement of the SC in order to increase its fluidity and thereby drug flux [9].

Fatty Acids

Fatty acids (FA) are characterized as amphiphilic molecules due to their nonpolar hydrocarbon chain and a polar terminal carboxylic group. The general formula for a fatty acid is $–CH_3(CH_2)_nCOOH$. FAs may exist in a saturated (containing only single bonds) or unsaturated form (containing double or triple bonds). Depending on the arrangement of their double bonds, unsaturated FAs may exist in a *cis* or *trans* configuration. The effectiveness of FAs as CPEs has been found to depend on their degree of saturation, chain length, and structure [64].

By studying permeation profiles of melatonin through rat skin, Kandimalla et al. found that saturated FA's showed continued increases in permeation of melatonin when the chain lengths were increased from 9 to 10 and from 10 up to 12 carbons. Decreased permeation of melatonin was seen when chain lengths were increased beyond 12 carbons. These finding were in close correlation with the previous reports stating the optimal chain

length for saturated FAs to be between 10-12 carbons. It was proposed that increasing the chain length of the hydrocarbon group beyond 11 carbons may increase the lipophilicity of the molecule which would then increase their affinity towards SC lipids. This increased affinity may slow the permeation of FAs and subsequently the flux of melatonin. Alternatively, FAs with less than 10 carbon atoms are not lipophilic enough to sufficiently partition through the skin which then slows the permeation of melatonin. For unsaturated fatty acids, an 18-carbon chain length and *cis* double bond conformation was found to be optimal for increasing drug flux. It was also found that increasing the number of double bonds in unsaturated fatty acids generally increased the diffusion of melatonin through rat skin. But contradicting results were seen in studies utilizing porcine skin where an increase in the number of double bonds in unsaturated FAs lead to a decrease in permeation. These findings are note-worthy as permeation through porcine skin is said to be a closer representation of permeation through human skin [30], [65].

Within the chemical category of FAs, oleic acid is one of the most widely studied chemical permeation enhancers. Francoeur et al. studied the effects of oleic acid on porcine SC via DSC analysis. Their results showed that OA markedly decreased the phase transition temperatures of SC lipids. They suggested that OA must mainly carry out its effect by disrupting SC lipids since no keratin denaturation was observed within these studies [66]. Past studies have suggested that OA is able to act by incorporating itself into the lipid lamellae of the SC with its polar end aligning with the polar heads of the lipids. It is thereby able to break associations between lipid polar groups and disrupt cholesterol stiffened regions leading to lipid fluidization [11]. Ex vivo penetration studies were

recently conducted on human skin using time-of-flight secondary ion mass spectrometry (TOF-SIMS) bioimaging to visualize and analyze distribution of OA in skin sections. This study suggested that OA may participate in the formation of regions of increased fluidity within the SC. Interestingly, OA was also seen to permeate into both the epidermal and dermal layers of the skin [67].

The mechanism of action behind the enhanced permeation seen with the addition of fatty acids is generally thought to be caused by a disruption of the densely packed SC lipids leading to fluidization. For saturated FAs, optimal chain lengths of 10-12 carbons also correspond to the dimensions of the cholesterol skeleton found within the intercellular space of the SC. It was therefore proposed that disruption of ceremide-cholesterol or cholesterol-cholesterol interactions may play an important role in the mechanistic action of such FAs [65]. The double bonds found within unsaturated FAs have been shown to form kinks in the SC lipid structure. These kinks allow such FAs to create separate domains within the intercellular lipid lamellae. This structural change may either decrease the diffusional path length of the permeant or decrease resistance to permeation through the SC.

Surfactants

Surfactants are amphipathic molecules that contain a hydrophobic (lipophilic alkyl or aryl fatty chain) tail connected to a hydrophilic (polar) head group. Surfactants are classified according to their polar head groups. Possible categories include anionic surfactants (negatively charged head group), cationic surfactants (positively charged head

group), non-ionic surfactants (uncharged head group), and zwitterionic surfactants (both positively and negatively charged head group) (Figure 8) [68].

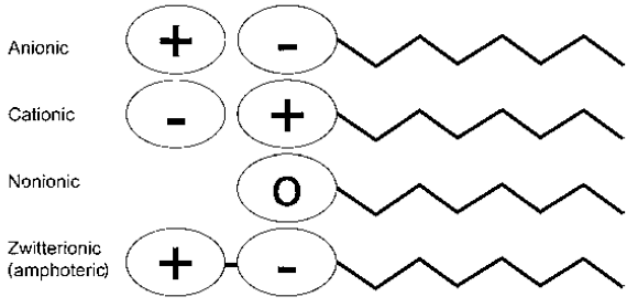

Figure 8: Schematic of various types of surfactants. Adapted from Malmsten M. 2002. [68]

Depending on the size of each portion, surfactants may present different solubility profiles. For example, if the hydrophobic segment is large, the surfactant will not be water-soluble. If the hydrophobic segment is comparatively smaller, the surfactant will be soluble but contact between the hydrophobic tail and the aqueous medium will still be energetically unfavorable. Therefore, surfactant molecules will tend to accumulate at various interfaces such that water contact may be reduced. Surfactants also exhibit the ability to self-assemble into structures that allow reduced water contact. In such situations, the hydrophobic domains of the surfactant molecules associate to form structures such as micelles or microemulsions. The formation of such structures give rise to a number of advantages i.e., through solubilization in the core of micelles the effective solubility of a hydrophobic drug may be increased, its hydrolytic degradation decreased, and its bioavailability improved [68].

Anionic surfactants include carboxylates, sulfates, sulfonates, and phosphate esters. Such surfactants tend to permeate slowly through the SC but permeation is seen to increase

with extended exposure [69]. One of the most well-studied anionic surfactants is the alkyl sulfate sodium lauryl sulfate (SLS). To explain their mechanism of action, Ribaud et al. studied the effects of SLS on the organization of the SC. Their DSC results showed that SLS led to a decrease in the phase transition temperature of the SC lipid lamellae when used above a critical micellar concentration. They found that these effects were reversible after prolongated rinsing and therefore concluded the SLS disorganized but did not extract SC lipids [70]. Shokri et al. studied the effects of SLS in enhancing the permeation of diazepam through rat skin. They proposed that hydrophobic interactions between the SLS alkyl chain and the SC would leave the polar sulfate head group of the surfactant exposed. This would create additional water binding sites in the membrane leading to increased skin hydration and thereby increased drug flux [71]. It has been noted though that extended treatment of skin with anionic surfactants such as SLS leads to irreversible damage including protein denaturation, membrane expansion, and loss of water binding capacity [69].

Cationic surfactants are known to be more effective permeation enhancers than anionic surfactants, but they are also known to be more damaging towards skin [30], [72]. Cationic surfactants have been shown to interact with skin via both polar interactions and hydrophobic binding [69]. Fang et al. studied the effects of various surfactants on the transdermal delivery of enoxacin. Their results demonstrated that cationic surfactants, such as benzalkonium chloride (BKC), increased the permeation of enoxacin by disrupting intracellular keratin proteins [72]. Moghadam et al. found that cationic surfactants, such as didecyldimethylammonium bromide (DDAB), didecyltrimethylammonium bromide

(DTAB), and BKC severely disrupted the lateral organization of the SC. Their wide-angle X-ray scattering (WAXS) results showed decreased peak intensity suggesting that such surfactants destroyed OR packing of SC lipids [73].

Non-ionic surfactants are less damaging towards skin, but they have also been found to be less effective as CPEs. Examples of such CPEs include Tween 20, Tween 80, and polyoxyethylene alkyl ethers [30]. Three mechanisms of action have been proposed for non-ionic surfactants. They may penetrate into the intercellular region of the SC thereby increasing its fluidity. This can lead to the eventual solubilization and extraction of lipid components. Such surfactants may also interact with intracellular keratin filaments and their associated water molecules making such areas more aqueous. According to Shokri et al., Tween 80 is thought to enhance the penetration of diazepam via both of the aforementioned mechanisms [71]. A third mechanism proposed for non-ionic surfactants is by changing the thermodynamic activity of the drug through emulsification of sebum. [69].

Zwitterionic surfactants include examples such as dodecylbetaine, hexadecylbetaine, hexadecylsulfobetaine, N, N–dimethyl–N-dodecyl amine oxide, dodecyltrimethylammonium bromide. Such surfactants are known to increase permeation of drugs through the SC by promoting lipid fluidization [30].

Ester Alcohols

Transcutol®

Transcutol is a diethylene glycol monoethyl ether (DEGEE) that exists as a hygroscopic liquid. It is able mix readily with both polar and non-polar solvents. This excipient offers advantages over other enhancers as it is clear (transparent), non-volatile, and nearly odorless. There are currently three brands of Transcutol on the market today: Transcutol CG, Transcutol P, and Transcutol HP. Transcutol has been shown to enhance the permeation of a number of drugs including clonazepam, ketoprofen, dorzolamide hydrochloride, piroxicam, indomethacin, amlodipine, nitrendipine, and quercetin [74], [75].

Godwin et al. studied the effects of Transcutol CG on the transdermal permeation of two UV absorbers, oxybenzone and cinnamate. Their in vitro permeation studies on hairless mice skin showed the Transcutol increased skin accumulation of oxybenzone and cinnamate while decreasing overall transdermal permeation. Similar accumulation results were seen with hydrocortisone and dexamethasone when mixed with saturated Transcutol solutions. The mechanism behind this accumulation is termed “intracutaneous depot.” It was proposed that Transcutol caused swelling of the SC intercellular lipids without altering its multi-lamellar structure. The swollen lipids were then able to retain drugs (especially lipophilic compounds), forming a depot. The data from Godwin et al., study are consistent with the aforementioned theory in that application of increasing concentrations of Transcutol lead to increased accumulation of the UV absorbers without a simultaneous increase in their transdermal diffusion [76].

Arora et al. studied the effects of Transcutol P as a cosurfactant in nanoemulsion-based hydrogels for the transdermal delivery of ketoprofen. Contrary to the study done by Godwin et al., the results of this ex vivo permeation study on rat skin showed that a *lack* of Transcutol P within the formulation led to increased skin retention and lag time of ketoprofen within the SC. The addition of Transcutol P rather played a significant role in enhancing the permeation of ketoprofen. The CPE's mechanism of action was thought to result from the solvation of intracellular α-keratin and occupation of proteinaceous hydrogen binding both of which led to a cumulative increase in the permeation of ketoprofen [77].

In a recent review, Osborne and Musakhanian proposed an explanation for the discrepancies seen across studies regarding Transcutol's ability to enhance vs retard drug flux. They suggested that as a solvent, Transcutol can significantly increase a drug's solubility within the SC which subsequently influences the drug's thermodynamic driving force. It is when this thermodynamic activity is not considered that Transcutol may appear to retard rather than enhance skin penetration [75]. In support of this claim, Bialik et al. showed that ibuprofen, when fully dissolved in Transcutol, did not produce measurable flux across human skin [78]. But when the formulation was diluted with water an increase in permeation was noted. Ibuprofen solubility in Transcutol is significantly higher than in water. The author of the study explained that with the addition of water, an increasing amount of ibuprofen falls out solution, creating a higher thermodynamic activity which translates to increased skin permeation.

The ability of Transcutol to act as a powerful solubilizing agent was also suggested by multiple studies to be due to its primary mechanism of enhancing drug flux. Mura et al. studied the effects of Transcutol on the flux of clonazepam from hydrophilic gel formulations. The results of in vitro permeation experiments showed that Transcutol primarily acted as a cosolvent, increasing permeation of clonazepam by increasing its solubility and therefore its concentration gradient in solution. A similar mechanism was proposed for the Transcutol-enhanced permeation for thymoquinone across human cadaver skin. [38], [44]. It is worth noting that by solubilizing drug molecules, Transcutol is also able to decrease the charge on ionized drugs, a mechanism known as the 'solvent effect'. According to this effect, anytime a liquid with a dielectric constant lower than the dielectric constant of water ($\varepsilon = 78$) is added to an aqueous solution of ionized drug, the solvent can preferentially solvate the drug and prevent it from completely ionizing by forming ion pairs in the low dielectric medium. This decrease in drug charge can significantly increase the amount of unionized drug partitioning into and through the skin [75].

An alternative mechanism of action was proposed by Moghadam et al. who studied the effects of Transcutol P on permeation of interferon-alpha. Their SAXS and WAXS studies suggested that Transcutol created a disordering effect within the SC membrane. This overall change in membrane disorder generally correlates to a disturbance of the crystalline packed lateral structures of the SC lipids, i.e., changes from their natural OR arrangement to a HEX or LIQ phase. These shifts have previously been reported to improve the permeability of molecules and was therefore attributed to Transcutol's mechanism of action in this study [73].

FUTURE OUTLOOK ON CHEMICAL PERMEATION ENHANCERS

As can be seen from the previous section, most CPEs exert their effects by disrupting the SC barrier in one way or another. Ideally, these effects would be reversible and localized to the non-viable layers of the SC but that is not always the case. CPEs run the risk of eventually affecting viable epidermal cells which may cause irritation by triggering the interstitial release of cytokines or other inflammatory processes [79].

With fatty acids, erythema and edema have been reported after cutaneous administration of lauric acid, palmitic acid, myristic acid, stearic acid, or oleic acid in 5 % (w/v) alcohol solutions. Follicular epidermal hyperplasia due to oleic acid and thinning of collagen fibers by myristic acid have also been seen. [80]. With respect to fatty alcohols, Kanikkannan et al. found that lauryl alcohol caused worsened irritation, as indicated by erythema of skin, than myristyl alcohol, tridecanol, decanol, undecanol, nonanol, and octanol [46]. The use of ethanol in topical formulations has been seen to cause skin irritation or contact dermatitis. But it is worth noting that ethanol is usually used in combination with other chemicals and it has therefore been difficult to conclusively attribute the irritation seen to ethanol alone [81]. Alternatively, PG has not been found to be a skin sensitizer, causing little to no skin irritation upon application [82]. Application of Azone has been seen to increase the number of neutrophils and lymphocytes within the epidermis indicating the triggering of an inflammatory response in the superficial layers of the skin [83]. Azone has also been found to cause erythematous plaques upon application to the skin, although the same study concluded that the effects were minimal and that Azone

was generally well tolerated amongst patients [84]. Surfactants are considered one of the most damaging CPEs with respect to the skin irritation they have been shown to invoke. Ionic surfactants are much more irritating that nonionic surfactants which are generally recognized as safe. The level of irritation with surfactants has also been shown to worsen with increased time-span of application [69].

Despite the array of studies showing skin irritation as a likely side effect for most CPEs, they are still valued due to their ease of use and affordable production. For this reason, researchers have been keen on finding ways to work around the potential for skin irritation. One proposed method for doing so is to combine multiple CPEs. Whereas individual CPEs may cause irritation to the skin, such effects have been seen to be reduced with synergistic use of CPEs. For example, Narishetty et al., conducted histological evaluations of human skin after exposing it to oleic acid and menthol. With application of 5% w/w menthol or 5% wt/wt oleic acid, skin irritation was indicated by slight to marked sub-epidermal oedema and swelling in collagen fibers. But the observed irritation was found to be much less with formulations combining menthol and oleic acid at 2.5% wt/wt [85].

Karande et al. tested 32 individual CPEs and did not find anyone that achieved therapeutic levels of macromolecule skin permeability without causing irritation. They therefore set out on a search for a new family of permeation enhancers which they designated synergistic combinations of penetration enhancers (SCOPE). In order to create a library of SCOPE candidates, the researchers randomly paired CPEs from various

categories (surfactants, fatty acids, fatty esters, Azone, etc.) which led to the creation 496 binary CPE pairs. For each pair, 44 distinct chemical compositions were created with the concentration of each CPE ranging from 0–2% (wt/vol), yielding a library of ~25,000 candidate SCOPE formulations. They then went on to test 20% (5,040 formulations) of this library through *in vitro* skin impedance guided high-throughput (INSIGHT) screening. Formulations were screened for enhancement potential measured in terms of enhancement ratios (ER) as well as for irritation potential (IP). The results of the INSIGHT screening are shown in Figure 9 where ER-IP data are plotted across four quadrants [79].

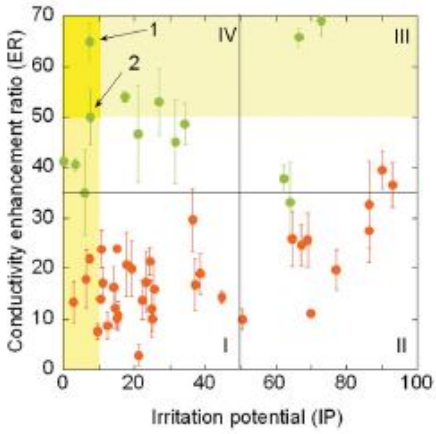

Figure 9: A plot of the IP versus ER for selected enhancers (red circle, individual enhancers; green circle, enhancer mixtures). Adapted from Karande et al. [79]

In Figure 9, quadrant I corresponds to safe but weak enhancers, quadrant II corresponds to weak and irritating enhancers, quadrant III corresponds to potent and irritating enhancers and quadrant IV corresponds to potent and safe enhancers. Most single CPEs were located in quadrants I or II (red circles). However, a substantial number of leading hits from the INSIGHT screening (green circles) were located in quadrant IV, meaning they were found to be both potent and safe! It is also worth noting that for single enhancers, IP increased

proportionally with ER. This gives credence to the fact that it is very difficult, if not impossible, to design a potent yet non-irritating formulation for TDDS using a single enhancer [79].

TDDS centered around the use CPEs have long been sought out due to their affordable nature and simplicity of use. Many chemical classes of enhancers have been developed and thoroughly studied. While almost every class of CPE has been shown to modify the SC such that drug flux can be increased, two significant disadvantages persist. First, side effects such as skin irritation seem to be unavoidable with increased potency of a given enhancer. And second, the upper limit of a CPEs efficacy remains intrinsically linked to the molecular weight (MW) of the drug being delivered i.e., most successful CPEs have been known to deliver drugs with MWs < 500 Da [86]. The future of CPEs therefore relies on the ability to tune their characteristics such that these disadvantages may be addressed.

As discussed, the synergistic activity of CPE pairs may be the answer to overcoming the limitation of increased skin irritation seen with effective enhancers. In the past, the design of synergistic systems was limited by the low throughput of experimental screening methods [87]. Now, through advanced screening systems like INSIGHT, we may be able to find a new category of chemical enhancers that are increasingly potent without a subsequent increase of skin irritation. The need for formulations capable of enhancing the diffusion of large MW drugs may also be addressed by a relatively new chemical class of enhancers called ionic liquids (IL). ILs are organic salts composed of large asymmetric

cations paired to anions. Studies by Qi and Mitragotri have shown that ILs such as 1:2 Choline:Geranic acid (CAGE) can enhance the transport of macromolecules up to 150 Da. Past studies have also observed an increase in the flux of proteins, siRNA, and polysaccharides secondary to the addition of ILs [86]. Additionally, according to studies by Tanner et al., slight modifications to the ion pairs comprising ILs can lead to significant changes in the chemical properties of such enhancers making them extremely tunable [88]. This allows ILs to be modified to present favorable characteristics such as minimal skin irritation while retaining maximal efficacy [88].

A thorough understanding of the mechanistic behavior of both synergistic CPE pairs and ILs is still in its infancy. Thus, we are only in the shadows of the full the potential of TDDS. With the use of novel permeation enhancers, such as those discussed, we may soon find ourselves with a drug delivery system that affords all the benefits of transdermal delivery without the undesirable side effects that remain today.

REFERENCES

[1] G. R. Doyle and J. A. McCutcheon, "7.5 Intravenous Medications by Direct IV Route," in *Clinical Procedures for Safer Patient Care*, BCcampus, 2015.

[2] S. M. Pond and T. N. Tozer, "First-Pass Elimination Basic Concepts and Clinical Consequences," *Clinical Pharmacokinetics*, vol. 9, no. 1, pp. 1–25, Feb. 1984, doi: 10.2165/00003088-198409010-00001.

[3] M. R. Prausnitz and R. Langer, "Transdermal drug delivery," *Nature Biotechnology*, vol. 26, no. 11, pp. 1261–1268, Nov. 2008, doi: 10.1038/nbt.1504.

[4] OpenStax, "5.1 Layers of the Skin," in *Anatomy and Physiology*, OpenStax, 2013.

[5] T. Haque and M. M. U. Talukder, "Chemical Enhancer: A Simplistic Way to Modulate Barrier Function of the Stratum Corneum," *Advanced Pharmaceutical Bulletin*, vol. 8, no. 2, pp. 169–179, Jun. 2018, doi: 10.15171/apb.2018.021.

[6] H. Yousef, M. Alhajj, and S. Sharma, "Anatomy, Skin (Integument), Epidermis," in *StatPearls*, Treasure Island (FL): StatPearls Publishing, 2020.

[7] J. K. Salmon, C. A. Armstrong, and J. C. Ansel, "The skin as an immune organ." *Western Journal of Medicine*, vol. 160, no. 2, pp. 146–152, Feb. 1994.

[8] Z. Nemes and P. M. Steinert, "Bricks and mortar of the epidermal barrier," *Experimental & Molecular Medicine*, pp. 5–19, 1999.

[9] A. Joshi, A. Joshi, H. Patel, D. Ponnoth, and G. Stagni, "Cutaneous Penetration–Enhancing Effect of Menthol: Calcium Involvement," *Journal of Pharmaceutical Sciences*, vol. 106, no. 7, pp. 1923–1932, Jul. 2017, doi: 10.1016/j.xphs.2017.03.041.

[10] "The Difference Between Topical and Transdermal Medications," *Gensco Pharma*, Mar. 2017, Accessed: Feb. 04, 2020. [Online]. Available: https://genscopharma.com/difference-topical-transdermal-medications/.

[11] B. W. Barry, "Action of skin penetration enhancers? The Lipid Protein Partitioning theory," *International Journal of Cosmetic Science*, vol. 10, no. 6, pp. 281–293, Dec. 1988, doi: 10.1111/j.1467-2494.1988.tb00028.x.

[12] V. Carrer *et al.*, "Effect of propylene glycol on the skin penetration of drugs," *Archives for Dermatological Research*, Nov. 2019, doi: 10.1007/s00403-019-02017-5.

[13] V. Jhawat, V. Saini, S. Kamboj, and N. Maggon, "Transdermal Drug Delivery Systems: Approaches and Advancements in Drug Absorption through Skin,"

International Journal of Pharmaceutical Sciences Review and Research, vol. 20, no. 1, pp. 47–56, 2013.

[14] Y.-H. An *et al.*, "Recent Advances in the Transdermal Delivery of Protein Therapeutics with a Combinatorial System of Chemical Adjuvants and Physical Penetration Enhancements," *Advanced Therapeutics*, vol. 3, no. 2, p. 1900116, Feb. 2020. doi: 10.1002/adtp.201900116.

[15] U. K. Patil and R. Saraogi, "Natural products as potential drug permeation enhancer in transdermal drug delivery system," *Archives of Dermatological Research*, vol. 306, no. 5, pp. 419–426, Jul. 2014, doi: 10.1007/s00403-014-1445-y.

[16] *Percutaneous penetration enhancers chemical methods in percutaneous penetration enhancement: modification of the stratum corneum*. New York, NY: Springer Berlin Heidelberg, 2015.

[17] A. C. Williams and B. W. Barry, "Penetration enhancers," *Advanced Drug Delivery Reviews*, vol. 64, pp. 128–137, Dec. 2012, doi: 10.1016/j.addr.2012.09.032.

[18] M. E. Lane, P. Santos, A. C. Watkinson, and J. Hadgraft, "Passive Skin Permeation Enhancement," in *Topical and Transdermal Drug Delivery*, H. A. E. Benson and A. C. Watkinson, Eds. Hoboken, NJ, USA: John Wiley & Sons, Inc., 2012, pp. 23–42.

[19] M. Boncheva, F. Damien, and V. Normand, "Molecular organization of the lipid matrix in intact Stratum corneum using ATR-FTIR spectroscopy," *Biochimica et Biophysica Acta BBA - Biomembranes*, vol. 1778, no. 5, pp. 1344–1355, May 2008, doi: 10.1016/j.bbamem.2008.01.022.

[20] J. M. Ruysschaert, A. Tenenbaum, and C. Berliner, "Correlation between lateral lipid phase separation and immunological recognition in sensitized liposomes," *FEBS Letters*, vol. 81, no. 2, pp. 406–410, Sep. 1977, doi: 10.1016/0014-5793(77)80565-6.

[21] A. Jain, "Transdermal drug delivery of imipramine hydrochloride. I. Effect of terpenes," *Journal of Controlled Release*, vol. 79, no. 1–3, pp. 93–101, Feb. 2002, doi: 10.1016/S0168-3659(01)00524-7.

[22] E. Kontogiannidou *et al.*, "Evaluation of sesquiterpenes as permeation enhancers for a model macromolecule across human skin in vitro," *Journal of Drug Delivery Science and Technology*, vol. 41, pp. 384–389, Oct. 2017, doi: 10.1016/j.jddst.2017.08.004.

[23] B. Jan, "Amino acid derivatives as transdermal permeation enhancers," *Journal of Controlled Release*, vol. 165, no. 2, pp. 91–100, 2013.

[24] A. Naik, L. A. R. M. Pechtold, R. O. Potts, and R. H. Guy, “Mechanism of oleic acid-induced skin penetration enhancement in vivo in humans,” *Journal of Controlled Release*, vol. 37, no. 3, pp. 299–306, Dec. 1995, doi: 10.1016/0168-3659(95)00088- 7.

[25] A. N. C. Anigbogu, A. C. Williams, B. W. Barry, and H. G. M. Edwards, “Fourier transform raman spectroscopy of interactions between the penetration enhancer dimethyl sulfoxide and human stratum corneum,” *International Journal of Pharmaceutics*, vol. 125, no. 2, pp. 265–282, Oct. 1995, doi: 10.1016/0378-5173(95)00141-5.

[26] K. Sugibayashi, S. Nakayama, T. Seki, K. Hosoya, and Y. Morimoto, “Mechanism of Skin Penetration-Enhancing Effect by Laurocapram,” *Journal of Pharmaceutical Sciences*, vol. 81, no. 1, pp. 58–64, Jan. 1992, doi: 10.1002/jps.2600810112.

[27] S. A. Mendanha and A. Alonso, “Effects of terpenes on fluidity and lipid extraction in phospholipid membranes,” *Biophysical Chemistry*, vol. 198, pp. 45–54, Mar. 2015, doi: 10.1016/j.bpc.2015.02.001.

[28] K. S. Bhatia and J. Singh, “Mechanism of transport enhancement of LHRH through porcine epidermis by terpenes and iontophoresis: permeability and lipid extraction studies.,” *Pharmaceutical Research*, vol. 15, no. 12, pp. 1857–1862, 1998, doi: 10.1023/A:1011906022903.

[29] P. Karande, A. Jain, K. Ergun, V. Kispersky, and S. Mitragotri, “Design principles of chemical penetration enhancers for transdermal drug delivery,” *Proceedings of the National Academy of Sciences of the United States of America*, vol. 102, no. 13, pp. 4688–4693, Mar. 2005, doi: 10.1073/pnas.0501176102.

[30] N. Dragicevic, J. P. Atkinson, and H. I. Maibach, “Chemical Penetration Enhancers: Classification and Mode of Action,” in *Percutaneous Penetration Enhancers Chemical Methods in Penetration Enhancement: Modification of the Stratum Corneum*, N. Dragicevic and H. I. Maibach, Eds. Berlin, Heidelberg: Springer, 2015, pp. 11–27.

[31] P. A. Cornwell, B. W. Barry, J. A. Bouwstra, and G. S. Gooris, “Modes of action of terpene penetration enhancers in human skin; Differential scanning calorimetry, small-angle X-ray diffraction and enhancer uptake studies,” *International Journal of Pharmaceutics*, vol. 127, no. 1, pp. 9–26, Jan. 1996, doi: 10.1016/0378-5173(95)04108-7.

[32] I. Hatta, H. Nakazawa, Y. Obata, N. Ohta, K. Inoue, and N. Yagi, “Novel method to observe subtle structural modulation of stratum corneum on applying chemical agents,” *Chemistry and Physics of Lipids*, vol. 163, no. 4–5, pp. 381–389, May 2010, doi: 10.1016/j.chemphyslip.2010.02.005.

[33] J. A. Bouwstra, A. de Graaff, G. S. Gooris, J. Nijsse, J. W. Wiechers, and A. C. van Aelst, "Water Distribution and Related Morphology in Human Stratum Corneum at Different Hydration Levels," *Journal of Investigative Dermatology*, vol. 120, no. 5, pp. 750–758, May 2003, doi: 10.1046/j.1523-1747.2003.12128.x.

[34] X. Dai, Q. Yin, G. Wan, R. Wang, X. Shi, and Y. Qiao, "Effects of Concentrations on the Transdermal Permeation Enhancing Mechanisms of Borneol: A Coarse-Grained Molecular Dynamics Simulation on Mixed-Bilayer Membranes," *International Journal of Molecular Sciences*, vol. 17, no. 8, p. 1349, Aug. 2016, doi: 10.3390/ijms17081349.

[35] C. M. Heard, D. Kung, and C. P. Thomas, "Skin penetration enhancement of mefenamic acid by ethanol and 1,8-cineole can be explained by the 'pull' effect," *International Journal of Pharmaceutics,* vol. 321, no. 1–2, pp. 167–170, Sep. 2006, doi: 10.1016/j.ijpharm.2006.05.018.

[36] R. Kadir, D. Stempler, Z. Liron, and S. Cohen, "Delivery of Theophylline into Excised Human Skin from Alkanoic Acid Solutions: A 'Push-Pull' Mechanism," *Journal of Pharmaceutical Sciences*, vol. 76, no. 10, pp. 774–779, Oct. 1987, doi: 10.1002/jps.2600761004.

[37] Mohd. Yasir, I. Som, and K. Bhatia, "Status of surfactants as penetration enhancers in transdermal drug delivery," *Journal of Pharmacy & Bioallied Sciences*, vol. 4, no. 1, p. 2, 2012, doi: 10.4103/0975-7406.92724.

[38] P. Mura, M. T. Faucci, G. Bramanti, and P. Corti, "Evaluation of transcutol as a clonazepam transdermal permeation enhancer from hydrophilic gel formulations," *European Journal of Pharmaceutical Sciences*, vol. 9, no. 4, pp. 365–372, Feb. 2000, doi: 10.1016/S0928- 0987(99)00075-5.

[39] A. Alonso, N. C. Meirelles, V. E. Yushmanov, and M. Tabak, "Water Increases the Fluidity of Intercellular Membranes of Stratum Corneum: Correlation with Water Permeability, Elastic, and Electrical Resistance Properties," *Journal of Investigative Dermatology*, vol. 106, no. 5, pp. 1058–1063, May 1996, doi: 10.1111/1523-1747.ep12338682.

[40] M. Denda *et al.*, "Exposure to a Dry Environment Enhances Epidermal Permeability Barrier Function," *Journal of Investigative Dermatology*, vol. 111, no. 5, pp. 858–863, Nov. 1998, doi: 10.1046/j.1523-1747.1998.00333.x.

[41] H. Zhai and H. I. Maibach, "Effects of Skin Occlusion on Percutaneous Absorption: An Overview," *Skin Pharmacology and Physiology*, vol. 14, no. 1, pp. 1–10, 2001, doi: 10.1159/000056328.

[42] "What is Alcohol? – The Alcohol Pharmacology Education Partnership," Accessed: Feb. 12, 2020. [Online]. Available: https://sites.duke.edu/apep/module-1-gender-matters/content/content-what-is-alcohol/.

[43] A. Chandra, P. K. Sharma, and R. Irchhiaya, "Effect of alcohols and enhancers on permeation enhancement of ketorolac," *Asian Journal of Pharmaceutics*, vol. 3, no. 1, 2009, doi: 10.22377/ajp.v3i1.239.

[44] A. Haq and B. Michniak-Kohn, "Effects of solvents and penetration enhancers on transdermal delivery of thymoquinone: permeability and skin deposition study," *Drug Delivery*, vol. 25, no. 1, pp. 1943–1949, Nov. 2018, doi: 10.1080/10717544.2018.1523256.

[45] N. A. Megrab, A. C. Williams, and B. W. Barry, "Oestradiol permeation across human skin, silastic and snake skin membranes: The effects of ethanol/water co-solvent systems," *International Journal of Pharmaceutics*, vol. 116, no. 1, pp. 101–112, Mar. 1995, doi: 10.1016/0378-5173(94)00321-U.

[46] N. Kanikkannan and M. Singh, "Skin permeation enhancement effect and skin irritation of saturated fatty alcohols," *International Journal of Pharmaceutics*, vol. 248, no. 1–2, pp. 219–228, Nov. 2002, doi: 10.1016/S0378-5173(02)00454-4.

[47] K. K. Kandimalla, R. J. Babu, and M. Singh, "Biphasic flux profiles of melatonin: The Yin–Yang of transdermal permeation enhancement mediated by fatty alcohol enhancers**'Yin' and 'Yang' describe opposing qualities in a phenomenon, which are in a dynamic equilibrium. Each advance (Yang) is followed by a retreat (Yin), and every fall (Yin) transforms into a rise (Yang). Any mutable phenomenon is a consequence of Yin and Yang.," *Journal of Pharmaceutical Sciences*, vol. 99, no. 1, pp. 209–218, Jan. 2010, doi: 10.1002/jps.21812.

[48] B. J. Aungst, N. J. Rogers, and E. Shefter, "Enhancement of naloxone penetration through human skin in vitro using fatty acids, fatty alcohols, surfactants, sulfoxides and amides," *International Journal of Pharmaceutics*, vol. 33, no. 1–3, pp. 225–234, Nov. 1986, doi: 10.1016/0378-5173(86)90057-8.

[49] A. Hoelgaard and B. Møllgaard, "Dermal drug delivery — Improvement by choice of vehicle or drug derivative," *Journal of Controlled Release*, vol. 2, pp. 111–120, Nov. 1985, doi: 10.1016/0168-3659(85)90037-9.

[50] J. A. Bouwstra, M. A. de Vries, G. S. Gooris, W. Bras, J. Brussee, and M. Ponec, "Thermodynamic and structural aspects of the skin barrier," *Journal of Controlled Release*, vol. 15, no. 3, pp. 209–219, Jun. 1991, doi: 10.1016/0168-3659(91)90112-Q.

[51] P. D. A. Pudney, M. Mélot, P. J. Caspers, A. van der Pol, and G. J. Puppels, “An In Vivo Confocal Raman Study of the Delivery of Trans-Retinol to the Skin,” *Applied Spectroscopy*, vol. 61, no. 8, pp. 804–811, Aug. 2007, doi: 10.1366/000370207781540042.

[52] Q. Zhang, P. Li, and M. S. Roberts, “Maximum transepidermal flux for similar size phenolic compounds is enhanced by solvent uptake into the skin,” *Journal of Controlled Release*, vol. 154, no. 1, pp. 50–57, Aug. 2011, doi: 10.1016/j.jconrel.2011.04.018.

[53] J. Jampilek and K. Brychtova, “Azone analogues: classification, design, and transdermal penetration principles,” *Medicinal Research Reviews*, vol. 32, no. 5, pp. 907–947, 2012, doi: 10.1002/med.20227.

[54] R. R. Stoughton and W. O. McClure, “Azone®: A New Non-Toxic Enhancer of Cu’Taneous Penetration,” *Drug Development and Industrial Pharmacy*, vol. 9, no. 4, pp. 725–744, Jan. 1983, doi: 10.3109/03639048309044701.

[55] J. Hadgraft, J. Peck, D. G. Williams, W. J. Pugh, and G. Allan, “Mechanisms of action of skin penetration enhancers/retarders: Azone and analogues,” *International Journal of Pharmaceutics*, vol. 141, no. 1, pp. 17–25, Sep. 1996, doi: 10.1016/0378-5173(96)04609-1.

[56] Q. D. Pham, S. Björklund, J. Engblom, D. Topgaard, and E. Sparr, “Chemical penetration enhancers in stratum corneum — Relation between molecular effects and barrier function,” *Journal of Controlled Release*, vol. 232, pp. 175–187, Jun. 2016, doi: 10.1016/j.jconrel.2016.04.030.

[57] G. S. K. Pilgram, J. van der Meulen, G. S. Gooris, H. K. Koerten, and J. A. Bouwstra, “The influence of two azones and sebaceous lipids on the lateral organization of lipids isolated from human stratum corneum,” *Biochimica et Biophysica Acta BBA - Biomembranes*, vol. 1511, no. 2, pp. 244–254, Apr. 2001, doi: 10.1016/S0005-2736(01)00271-1.

[58] A. C. Williams and B. W. Barry, “Terpenes and the Lipid-Protein-Partitioning Theory of Skin Penetration Enhancement.,” *Pharmaceutical Research*, vol. 08, no. 1, pp. 17–24, 1991, doi: 10.1023/A:1015813803205.

[59] X. Zhu *et al.*, “Skin Electrical Resistance Measurement of Oxygen-Containing Terpenes as Penetration Enhancers: Role of Stratum Corneum Lipids,” *Molecules*, vol. 24, no. 3, p. 523, Feb. 2019, doi: 10.3390/molecules24030523.

[60] C. Tas, Y. Ozkan, A. Okyar, and A. Savaser, “In Vitro and Ex Vivo permeation studies of etodolac from hydrophilic gels and effect of Terpenes as enhancers,”

Drug Delivery, vol. 14, no. 7, pp. 453–459, Oct. 2007, doi: 10.1080/10717540701603746.

[61] S. T. K. Narishetty and R. Panchagnula, "Transdermal delivery of zidovudine: effect of terpenes and their mechanism of action," *Journal of Controlled Release*, vol. 95, no. 3, pp. 367–379, Mar. 2004, doi: 10.1016/j.jconrel.2003.11.022.

[62] S. T. K. Narishetty and R. Panchagnula, "Effect of l-menthol and 1,8-cineole on phase behavior and molecular organization of SC lipids and skin permeation of zidovudine," *Journal of Controlled Release*, vol. 102, no. 1, pp. 59–70, Jan. 2005, doi: 10.1016/j.jconrel.2004.09.016.

[63] Y. Lan *et al.*, "Essential oil from Zanthoxylum bungeanum Maxim. and its main components used as transdermal penetration enhancers: a comparative study," *Journal of Zhejiang University. Science. B*, vol. 15, no. 11, pp. 940–952, Nov. 2014, doi: 10.1631/jzus.B1400158.

[64] L. Kumar, S. Verma, S. Kumar, D. N. Prasad, and A. K. Jain, "Fatty acid vesicles acting as expanding horizon for transdermal delivery," *Artificial Cells, Nanomedicine, and Biotechnology*, vol. 45, no. 2, pp. 251–260, Feb. 2017, doi: 10.3109/21691401.2016.1146729.

[65] K. Kandimalla, N. Kanikkannan, S. Andega, and M. Singh, "Effect of Fatty acids on the Permeation of Melatonin across Rat and Pig Skin In-vitro and on the Transepidermal Water Loss in Rats In-vivo," *Journal of Pharmacy and Pharmacology*, vol. 51, no. 7, pp. 783–790, Jul. 1999, doi: 10.1211/0022357991773140.

[66] M. L. Francoeur, G. M. Golden, and R. O. Potts, "Oleic Acid: Its Effects on Stratum Corneum in Relation to (Trans)Dermal Drug Delivery," *Pharmaceutical Research*, vol. 07, no. 6, pp. 621–627, 1990, doi: 10.1023/A:1015822312426.

[67] V. Čižinauskas, N. Elie, A. Brunelle, and V. Briedis, "Fatty acids penetration into human skin *ex vivo* : A TOF-SIMS analysis approach," *Biointerphases*, vol. 12, no. 1, p. 011003, Mar. 2017, doi: 10.1116/1.4977941.

[68] M. Malmsten, *Surfactants and Polymers in Drug Delivery*, p. 45. Routledge.

[69] I. Som, K. Bhatia, and Mohd. Yasir, "Status of surfactants as penetration enhancers in transdermal drug delivery," *Journal of Pharmacy & Bioallied Sciences*, vol. 4, no. 1, pp. 2–9, 2012, doi: 10.4103/0975-7406.92724.

[70] C. Ribaud, J. Garson, J. Doucet, and J. Lévêque, "Organization of Stratum Corneum Lipids in Relation to Permeability: Influence of Sodium Lauryl Sulfate and

Preheating," *Pharmaceutical Research*, vol. 11, no. 10, pp. 1414–1418, 1994, doi: 10.1023/A:1018987721531.

[71] J. Shokri, A. Nokhodchi, A. Dashbolaghi, D. Hassan-Zadeh, T. Ghafourian, and M. Barzegar Jalali, "The effect of surfactants on the skin penetration of diazepam," *International Journal of Pharmaceutics*, vol. 228, no. 1–2, pp. 99–107, Oct. 2001, doi: 10.1016/S0378- 5173(01)00805-5.

[72] J.-Y. Fang, H.-H. Lin, H.-I. Chen, and Y.-H. Tsai, "Development and evaluation on transdermal delivery of enoxacin via chemical enhancers and physical iontophoresis," *Journal of Controlled Release*, vol. 54, no. 3, pp. 293–304, Aug. 1998, doi: 10.1016/S0168-3659(98)00010-8.

[73] S. H. Moghadam *et al.*, "Effect of Chemical Permeation Enhancers on Stratum Corneum Barrier Lipid Organizational Structure and Interferon Alpha Permeability," *Molecular Pharmacology*, vol. 10, no. 6, pp. 2248–2260, Jun. 2013, doi: 10.1021/mp300441c.

[74] O. G. Strusovskaya, S. V. Poroiskii, and A. G. Strusovskaya, "Chemical Enhancers or Transcutaneous Conductors: Transcutol," *Pharmaceutical Chemistry Journal*, vol. 52, no. 11, pp. 879–884, Feb. 2019, doi: 10.1007/s11094-019-01920-5.

[75] D. W. Osborne and J. Musakhanian, "Skin Penetration and Permeation Properties of Transcutol®—Neat or Diluted Mixtures," *AAPS PharmSciTech*, vol. 19, no. 8, pp. 3512–3533, Nov. 2018, doi: 10.1208/s12249-018-1196-8.

[76] D. A. Godwin, N.-H. Kim, and L. A. Felton, "Influence of Transcutol® CG on the skin accumulation and transdermal permeation of ultraviolet absorbers," *European Journal of Pharmaceutics and Biopharmaceutics*, vol. 53, no. 1, pp. 23–27, Jan. 2002, doi: 10.1016/S0939- 6411(01)00215-6.

[77] R. Arora, G. Aggarwal, S. L. Harikumar, and K. Kaur, "Nanoemulsion Based Hydrogel for Enhanced Transdermal Delivery of Ketoprofen," *Advances in Pharmaceutics*, 2014, doi: https://doi.org/10.1155/2014/468456.

[78] W. Bialik, K. Walters, K. Brain, and J. Hadgraft, "Some factors affecting the in vitro penetration of ibuprofen through human skin," *International Journal of Pharmaceutics*, vol. 92, no. 1–3, pp. 219–223, May 1993, doi: 10.1016/0378-5173(93)90283-L.

[79] P. Karande, A. Jain, and S. Mitragotri, "Discovery of transdermal penetration enhancers by high-throughput screening," *Nature Biotechnology*, vol. 22, no. 2, pp. 192–197, Feb. 2004, doi: 10.1038/nbt928.

[80] "3: Final Report on the Safety Assessment of Oleic Acid, Lauric Acid, Palmitic Acid, Myristic Acid, and Stearic Acid," *Journal of the American College of Toxicology*, vol. 6, no. 3, pp. 321– 401, May 1987, doi: 10.3109/10915818709098563.

[81] D. W. Lachenmeier, "Safety evaluation of topical applications of ethanol on the skin and inside the oral cavity," *Journal of Occupational Medicine and Toxicology*, vol. 3, no. 1, p. 26, 2008, doi: 10.1186/1745-6673-3-26.

[82] "Final Report on the Safety Assessment of Propylene Glycol and Polypropylene Glycols," *Journal of the American College of Toxicology*, vol. 13, no. 6, pp. 437–491, Dec. 1994, doi: 10.3109/10915819409141005.

[83] J.-Y. Fang, T.-L. Hwang, C.-L. Fang, and H.-C. Chiu, "In vitro and in vivo evaluations of the efficacy and safety of skin permeation enhancers using flurbiprofen as a model drug," *International Journal of Pharmaceutics*, vol. 255, no. 1–2, pp. 153–166, Apr. 2003, doi: 10.1016/S0378-5173(03)00086-3.

[84] M.-F. Demierre, L. Vachon, V. Ho, L. Sutton, A. Cato, and B. Leyland-Jones, "Phase 1/2 Pilot Study of Methotrexate-Laurocapram Topical Gel for the Treatment of Patients With Early-Stage Mycosis Fungoides," *Archives of Dermatology*, vol. 139, no. 5, May 2003, doi: 10.1001/archderm.139.5.624.

[85] S. T. K. Narishetty and R. Panchagnula, "Transdermal delivery system for zidovudine: *in vitro*, *ex vivo* and *in vivo* evaluation," *Biopharmaceutics and Drug Disposition*, vol. 25, no. 1, pp. 9–20, Jan. 2004, doi: 10.1002/bdd.381.

[86] Q. M. Qi and S. Mitragotri, "Mechanistic study of transdermal delivery of macromolecules assisted by ionic liquids," *Journal of Controlled Release*, vol. 311–312, pp. 162–169, Oct. 2019, doi: 10.1016/j.jconrel.2019.08.029.

[87] P. Karande and S. Mitragotri, "Enhancement of transdermal drug delivery via synergistic action of chemicals," *Biochimica et Biophysica Acta BBA - Biomembranes*, vol. 1788, no. 11, pp. 2362–2373, Nov. 2009, doi: 10.1016/j.bbamem.2009.08.015.

[88] E. E. L. Tanner *et al.*, "Design Principles of Ionic Liquids for Transdermal Drug Delivery," *Advanced Materials*, vol. 31, no. 27, p. 1901103, Jul. 2019, doi: 10.1002/adma.201901103.

CURRICULUM VITAE