TECHNOLOGIES FOR SKIN DELIVERY OF HYDROPHILIC MOLECULES AND MACROMOLECULES

by

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DEDICATION

To my beloved father Mr. Jun Song and mother Mrs. Xiaowen Zheng for their unconditional love. They supported all my life choices and were invaluable in giving support.

I also would like to dedicate this dissertation to my aunt Mrs. Evelyn Ding, she is always my inspiration to pursue my dreams and is always by my side to help me in all things great and small.

I love them very much.
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ABSTRACT

TECHNOLOGIES FOR SKIN DELIVERY OF HYDROPHILIC MOLECULES AND MACROMOLECULES

Under the direction of Dr. Ajay K. Banga, Professor and Chair of the Department of Pharmaceutical Sciences, Mercer University

Transdermal delivery can bypass first pass metabolism, increase the bioavailability of drug and usually it is a non-invasive administration method and shows better patient compliance. While the transdermal delivery has some limitations, which hinders its wide application. Skin only allow the passage of drug molecules, which should ideally be moderately lipophilic (log P between 1-3) with a molecular weight <500 Da. Stratum corneum as a lipid barrier, it blocks the delivery of drug through the skin, especially for large and hydrophilic molecules such as peptide or protein. As a result, different strategies or devices have been developed to compromise the stratum corneum and enhance the drug permeation. In our studies, we investigated the delivery of hydrophilic small molecules and large molecules in/through the skin by optimizing the formulation, developing drug delivery systems or using physical enhancement techniques, such as maltose microneedle and ablative laser. The first aim was to optimize a gel formulation of cimetidine to maximize its transdermal delivery across microporated skin.
Specifically, the effect of extent of ionization in formulation on permeation of cimetidine across microporated skin was studied. Results suggest that 0.8% w/w pH 5 gel showed highest permeation through microchannels compared with other formulations. In order to assess the skin irritation potential of cimetidine carbopol gel, skin irritation test had been performed using Epiderm™-200-SIT kit and results showed 0.8% pH 5 gel is not irritate to the skin. In the second aim, we investigated the feasibility of transdermal delivery of human growth hormone (hGH) through laser-microporated dermatomed porcine ear skin. Laser-assisted microporation creates microchannels in the skin that result in the enhancement of drug delivery. The effect of fluence of laser (34.1, 45.4 and 68.1 J/cm²) and micropores density (5%, 10% and 15%) on the permeation of hGH through laser-treated skin was studied. Permeation study results showed after 48 h, application of fluence of 68.1 J/cm² laser resulted in significantly higher permeation of drug (90.94±3.93 µg/cm²) than that from 34.1 J/cm² group (53.13±1.75 µg/cm², p<0.05), but not as compared to the 45.4 J/cm² group (p>0.05). With the increase in density of micropores from 5% to 15%, permeation of hGH increased significantly from 7.1±2.63 µg/cm² to 95.89±13.43 µg/cm² after 48 h study. In the third aim, we developed a controlled release system using Eudragit® RS100 and polyvinyl alcohol polymer. This microsponge based drug delivery technique can incorporate both hydrophilic and hydrophobic molecules and drug release follows the mechanism of process of diffusion. Salicylic acid microsponge was prepared by quasi emulsion solvent diffusion method and dispersed into 2.0 % carbopol gel. The structure of microsponge was checked under bright microscopy and scanning electron microscopy. Characterization studies of salicylic acid microsponge including loading efficiency, production yield and entrapment
efficiency was performed. We tested the drug release profiles and permeation performance from the formulation with/without microsponge using dissolution tester and vertical Franz cell. Release study and permeation study results showed incorporation of salicylic acid in the microsponge did slow down the release rate from the formulation. Microsponge system plays a very important role in controlling the release as well as the permeation of salicylic acid topically. Microsponge was proved to be a potential carrier for salicylic acid in topical acne therapy to prolong drug release, minimize skin irritation and side effects. In the fourth aim, we evaluated the deactivation efficiency of a drug disposal pouch containing granular activated carbon. This activated carbon based drug deactivation system offers a unique disposal method. The deactivation study and desorption study were carried out using diazepam, lorazepam tablets and suboxone® sublingual film. Deactivation study results showed this drug disposal pouch successfully deactivated the drug within 28 days; more than 99% of drug had been adsorbed by the activated carbon. In the desorption study, only about 1% of drug leached out from the activated carbon when it exposed to the large volume of water and organic solvent. This activated carbon based drug deactivation system may provide a convenient way for the patients to dispose their unused medications.
CHAPTER 1

INTRODUCTION

Topical and transdermal delivery offers an alternative for the conventional routes of drug administration for various drug molecules. It avoids the limitations associated with intravenous injections, oral and parenteral administration of drugs (1). Skin is the largest organ of the human body with a surface area about 2 m² and it acts as a protective barrier against the ingress of chemicals, bacteria and any other microbes (2). Skin is a multilayered organ, which consists of two main layers, the epidermis and the dermis. The stratum corneum, outmost layer of epidermis, is interdispersed within a lipid rich matrix; primarily provides a rate-limiting barrier to transport of molecules into and across skin (3). Due to the nature of stratum corneum, skin only allows relatively lipophilic molecules to permeate into the lower layers (4). The second layer beneath epidermis is dermis, which consists of fewer cells and more fibers than epidermis. It houses blood vessels and nerves, so the dermis layer can take up permeated drug molecules into systemic circulation (5).

Transdermal delivery avoids first pass metabolism, so it is beneficial for drugs, which undergo high first pass metabolism with short half-lives. In recent years, the topical and transdermal field has gained popularity, been well explored and more and more topical and transdermal drugs have been marketed, both as over the counter (OCT) and prescription products. Like many other drug administration routes, skin also has its benefits and limitations. Conventionally, topical and transdermal delivery
systems are based on the passive diffusion of drug molecules through the skin layers. As mentioned above, only small (<500 Da), relatively lipophilic molecules are ideal candidates for this route of delivery (6). Novel formulations and technologies have been developed to assist delivery of drug molecules into and across skin. Permeation enhancers, supersaturated systems, and vesicles like liposomes have been investigated to enhance drug passive permeation through the skin. Permeation enhancers such as oleic acid typically increase the skin permeability by disruption of the lipid structure of stratum corneum, interaction with intracellular proteins and increased partitioning of drug through the skin (7). Since the efficiency of permeation enhancers mainly depends on the concentration of the enhancers used in the formulation, high concentration of chemical enhancers may have high potential to cause skin irritation (8). The amount of drug that can be delivered using these enhancement methods across the skin is still limited, especially for hydrophilic molecules due to existence of permeation barrier, stratum corneum. In order to administer a hydrophilic large molecule for systemic circulation, the resistance of the stratum corneum should be compromised and circumvented using appropriate enhancement techniques, which should be non invasive or minimally invasive, and painless in nature. Pain is one of most significant healthcare crisis in USA. Minimally invasive and painless permeation enhancement devices can provide better patient compliance and less infection potential. The passive permeation of hydrophilic molecules through the skin is very less or not achievable due to the lipid nature and tight structure of skin. Our present study is a comprehensive investigation about the topical/transdermal delivery of hydrophilic molecules, which involves the development of topical/transdermal delivery systems, optimization of topical/transdermal
formulations and investigation of physical enhancement techniques, such as microneedles and ablative laser. Transdermal administration is one of the most effective routes to achieve controlled delivery of drug substances into the blood circulation. A goal of our studies was to develop controlled release vehicle for both hydrophobic and relatively hydrophilic molecules. Microspounge delivery is being investigated as a part of rapid developing field of drug release systems. Microspounge drug delivery system is a controlled release system that can be used not only in topical formulations but also in oral drugs (9). Microspounge delivery system can control the delivery rate of active ingredients into the epidermis with reduced skin irritation potential. It is a polymeric system, which consists of macroporous beads, typically 10 to 50 microns in diameter (10). This microspounge-based drug delivery system can provide better drug efficacy, reduced skin irritation, prolonged drug release and extended formulation stability (11). Salicylic acid is a hydrophilic molecule and used as a key ingredient in topical anti-acne products. As a beta-hydroxy acid, salicylic acid can penetrate and break down fats and lipids in skin and cause skin irritation due to the moderate chemical burns (12). Incorporating salicylic acid in microspounge can reduce the potential of skin irritation and provide a prolonged release of drug from the formulation. In this study, we developed salicylic acid microspounge, formulated in hydrogel and investigated the effect of drug/polymer ratios on the drug release from the microspounge. In addition to drug release system development, topical/transdermal delivery of hydrophilic molecules by using physical enhancement techniques was also investigated. Skin microporation by microneedles is expected to open up hydrophilic pathways for drug permeation across the skin (13). Microneedle treatment appears to be a cost-effective and minimally invasive way to physically disrupt
the superficial layers of skin and circumvent the skin barrier. Cimetidine was used as a model drug in this study. It is a relatively hydrophilic drug, which is a base with a octanol-water partition coefficient of 0.4 and pKa value of 6.8 (14) (15). The novelty of our study is to provide a good way to increase the permeation of drug through the microchannels by adjusting the pH of formulation to change the extent of drug ionization, instead of using the chemical enhancers that may potentially cause skin irritation. We also investigated the feasibility of transdermal delivery of macromolecule, human growth hormone (hGH) through laser-porated human skin. Traditionally, two kinds of lasers are available for skin treatment: non-ablative laser and ablative laser. Non-ablative laser does not ablate the stratum corneum. It works by heating up the skin tissue and forms the microthermal zones in skin. The non-ablative laser can enhance the transdermal delivery of small molecules, such as sumatriptan succinate (16). Since non-ablative laser does not ablate the stratum corneum, permeation of macromolecules through the skin may not be possible. We used ablative laser in our studies. The laser device used in this study, P.L.E.A.S.E.® (Precise Laser Epidermal System), is an ablative laser that emits laser light with the wavelength of 2940 nm, which corresponds to the water absorption peak (17)(18). It consists of a diode-pumped fractional Er:YAG (Erbium-Yttrium-Aluminium-Garnet) laser, that efficiently forms a matrix of identical micropores or microchannels with less damage of skin (19). It can provide an alternative administration route of peptide or protein that can avoid first pass metabolism and provide ease of application. hGH is produced in the pituitary gland at the base of the brain and its key function is to regulate protein metabolism and stimulate growth. Though there are numerous applications of hGH, the administration route of hGH is restricted to
subcutaneous and intramuscular injections (20). These modes have resulted in poor patient compliance. Inconvenience with the daily use of injections may result in withdrawal or discontinuation of treatment by patients. As a macromolecule, hGH cannot be delivered efficiently across the skin due to the large molecular weight (22 kDa) and hydrophilic nature (log P of -5.2) (21). In this study, we investigated the feasibility of transdermal delivery of hGH with P.L.E.A.S.E. ® ablative laser.

Apart from the investigations of technologies for topical/transdermal delivery of hydrophilic molecules, we also performed studies about drug disposal. Psychoactive drugs increasingly being prescribed for the treatment of antidepressants, insomnia or pain relief and there is a potential for misuse, abuse or dependency of the drug. In 2014, about 6.5 million illicit drug users in US had reported nonmedical use of psychoactive drugs including tranquilizers, stimulants and sedatives in their lifetime (22). The Food and Drug Administration (FDA) has suggested mixing unused drugs with coffee ground or cat litter which can then be disposed in regular trash instead of flushing medications directly in sewers. However, these disposal techniques cannot effectively deactivate the drug and drug can still be extracted and has high abuse potential. The objective of this study was to test the deactivation efficiency of a drug disposal pouch, which contains 15 grams activated carbon granules. It is an activated carbon based drug disposal system. The psychoactive medications, such as lorazepam and diazepam tablets were used in the deactivation and desorption studies.
CHAPTER 2
LITERATURE REVIEW
Topical and transdermal delivery

Skin, as the largest organ in the human body, receives about one-third of the blood circulation through the body. The thickness of human skin varies from different individuals ranging from 2 to 5 mm with stratum corneum being around 10 to 40 μm thick (23). It is a readily accessible surface for drug delivery, especially for small molecules. It is important to understand the structural aspects of skin in order to better understand the drug diffusion pathways through the skin.

Skin consists of outer epidermis, followed by viable dermis and subcutaneous tissue. Epidermis is separated from dermis by a basement membrane and composed of five cell layers, stratum corneum (SC) being the outermost layer. The SC, also called horny layer, is the outmost layer of epidermis and contains 10 to 25 layers of flattened, stacked and hexagonal dead and keratinized cells, corneocytes. Usually the thickness of SC is about 10 to 40 μm; epidermis can be about 60 μm on eyelids and about 800 μm on palms and soles, which acts as the major permeation barrier in topical and transdermal drug delivery (24). The cornified cells are embedded in the lipid bilayers of fatty acids, ceramides, cholesterol and cholesterol esters. These multilameller lipid bilayers filling the extracellular space provide stratum corneum its barrier properties. The viable epidermis consists of various layers as stratum lucidum, stratum granulosum, stratum basal and stratum spinosum (25). Under epidermis layer is dermis, which is about 100 to 500 μm
thick and composed of collagenous fibers and elastic connective tissue in a matrix of mucopolysaccharides; it contains blood vessels, lymph vessels and nerves (23). This makes dermis layer relatively hydrophilic as compare to the SC. The blood vessels in dermis layer, with a blood flow rate of 0.05 mL/min/cc can regulate skin temperature; provide nutrition and oxygen to the skin. They can also remove toxins and are responsible for systemic absorption of drug delivered through skin (26).

The permeation of drug molecules from the formulation into systemic circulation is a multistep process that begins with the partitioning of drug substances from the formulation into the SC, diffusion of drug through the layers of SC, then pass the epidermis and viable dermis into local capillary network and finally diffuse into systemic blood circulation (27). There are three potential routes for drugs to cross the SC, intercellular route, transcellular route and transappendageal route. For the intercellular pathway, the drug substances follow a tortuous pathway within the intercellular lipids, mainly along the multilamellar bilayers (28). Transcellular pathway involves the direct transport of the drug molecule through the SC cells. The third pathway involves the movement of drug molecule along the skin appendages, such as hair follicles or sweat glands (29). Due to the low surface area of hair follicles or sweat glands, which only accounts for 0.1% area of skin, the appendageal route is considered not very significant for skin delivery. However, it may be critical for the delivery of ionic and large hydrophilic molecules, as they don’t easily diffuse through the skin surface, and appendages can provide shunt pathways that bypass the stratum corneum barrier (30). Essentially, the main function of skin is to protect the bulk of body mass from a relatively hostile environment, maintaining the homeostasis of body and also serve as a sensory
organ. Hence, skin must possess barrier properties, be tough and flexible in order to perform its functions (31). As we mentioned earlier, SC is the main barrier for drug permeation; due to the lipid nature of this layer, only lipophilic small drugs with optimal solubility, good partition coefficient and diffusion coefficient can penetrate into the skin efficiently (32). Hydrophobic drugs can successfully pass the epidermal barrier so that they can be taken up into the blood vessels existing in the dermis layer and diffuse into the systemic circulation.

Use of plant extracts by people for the potential treatment of a variety of topical disorders has been known for centuries and relatively recently, people start to use skin as an alternative route for systemic drug administration. There are literature reports of using anti-infective agents for systemic effects in the early 1960s (33). In the past two to three decades, more and more topical and transdermal products have been developed and marketed. This drug administration route is beneficial for the treatment of localized skin conditions, which can bypass gastric and hepatic metabolism, increase drug efficiency and improve patient compliance. Transdermal delivery also implies delivery of drugs through skin into blood circulation. Compared with other administration routes such as oral or IV injection, major advantages associated with transdermal delivery include bypass the first pass hepatic metabolism, improved therapeutic efficacy, reduced risk of dose dumping, better patient compliance, reduced variability in the absorption and metabolism relative to oral administration, ease of initiation, termination, and provide continuous and controlled drug delivery (34). Transdermal delivery is also beneficial for drugs which cause gastrointestinal irritation, such as some of the NSAIDS.
Transdermal delivery can effectively deliver lipophilic small molecules; nowadays several drug molecules, such as fentanyl, estradiol, selegiline, steroidal hormone, nicotine, and scopolamine are currently formulated and marketed as transdermal patches in US market. Active transdermal delivery is the enhanced permeation of drug molecules through the skin layers by electrical or physical enhancement techniques such as iontophoresis, sonophoresis, laser, microneedles and microdermabrasion. The use of these techniques can assist delivery of both hydrophilic and lipophilic molecules as well as macromolecules such as peptides and proteins through the skin (35). Microneedles and ablative laser will be discussed in more details.

Factors affecting transdermal delivery

Physicochemical Factors

The transport mechanism of drug transport through skin is considered to be passive diffusion and can be given by Fick’s first law of diffusion as:

\[ J = DK\Delta C/h \]

Where \( J \) = Flux per unit area
\( D \) = Diffusion coefficient in the skin
\( K \) = Skin – vehicle partition coefficient
\( \Delta C \) = Concentration gradient across the skin
\( h \) = Diffusional pathlength

Based upon the Fick’s first law, the flux of drug permeation can be influenced by concentration gradient, diffusion coefficient, partition coefficient and length of diffusional pathway.

Concentration gradient
A major driving force for drug diffusion through the skin is the concentration differences in various skin layers. There should be a steep concentration gradient, i.e. the concentration of drug is higher in the outmost layers of stratum corneum than in the lower layers. In order to keep the minimum amount of drug in the lowest layers of stratum corneum, the viable epidermis should act as a sink. When drug has high aqueous solubility and good partitioning from the formulation into top layers of stratum corneum, it is easier to achieve sink condition for the drug permeation. Since these two conditions are somewhat contradictory, except for only a few compounds, most active ingredients do not have optimum solubility in both lipid as well as aqueous phase.

An increase of drug concentration in the formulation can improve thermodynamic activity of the drug in the formulation and increase transdermal flux. It is important that permeant should have the same thermodynamic activity in different vehicles when comparing transdermal formulations. This can be achieved by ensuring the same level of saturation, usually around 90% saturation in different formulations. Even though the concentration of drug could be different, the thermodynamic activity would be the same. Amount of drug applied on the skin could be finite or infinite dose. Finite dosing implies that the drug flux would deplete from the formulation during the period of permeation. Infinite dosing implies the change in drug concentration or thermodynamic activity in the formulation during permeation is negligible. Most topical or transdermal products in the market contain infinite doses of drug in order to obtain steady state fluxes in transdermal delivery.

Partition coefficient
Drug molecule has to be partition from the vehicle into the superficial layers of skin and then into the underlying hydrophilic layers of skin in order to permeate across skin and reach the blood circulation. Partition coefficient is the relative concentration of the substance in the oil phase and aqueous phase at equilibrium (36).

\[
\text{Log } P = \text{Log} \left( \frac{\text{solute in octanol}}{\text{unionized solute in water}} \right)
\]

Hydrophilic molecules have lower partition coefficient, therefore the partitioning in the stratum corneum is also low, which presents a major barrier for the polar drug permeation. On the other hand, lipophilic drug should have high partition coefficient, which at the same time have very limited penetration in the dermis layer and drug could form reservoir in the stratum corneum (37). Drug should have an optimum solubility in both oily and aqueous phases for systemic delivery through the skin (38). The ideal candidate compounds for permeation should have log P values ranging between 1 and 3.5 (39). The value of partition coefficient of drug could determine the predominant pathway of permeation through the skin. High lipophilic molecule with high log P value usually permeates through lipid rich regions, and drug with relatively lower log P values diffuse transcellularly through hydrated keratinocytes (40).

The charge of molecule could also affect the partition coefficient of drug. The drug ionization constant and pH of the vehicle or formulation can determine the concentrations of ionized and non-ionized drug molecules. Maximizing the transdermal flux of the drug compound can be achieved by adjusting the pH of the vehicle or formulation.

The non-ionized drug forms moves faster through the skin than the ionized molecules. This could be attributed to the drug substance pathway; non-ionized molecule diffuses through intercellular pathway, while ionized forms move through the intracellular
pathways. The extent of drug ionization at a certain pH can be calculated using the Henderson-Hasselbalch equation for weak acids and weak bases. The equations are as follows:

For a weak acid,

\[ pH = pK_a - \log\left(\frac{[HA]}{[A^-]}\right) \]

[HA] is the concentration of unionized species and [A\textsuperscript{-}] is the concentration of ionized species at that particular pH.

For a weak base,

\[ pH = pK_a - \log\left(\frac{[BH^+]}{[B]}\right) \]

[BH\textsuperscript{+}] is the concentration of ionized species and [B] is the concentration of unionized species at that particular pH.

Usually, buffers can be used to control the degree of drug ionization. For weak acids, when pH of buffer is 2-3 pH units below the pKa of drug, it ensures negligible drug ionization. For weak base, the use of buffer 2-3 pH units above the pKa of drug also ensures negligible ionization.

Diffusion coefficient

According to Fick’s first law of diffusion, diffusion coefficient is proportional to the flux per unit area. Transdermal delivery of drug can be affected by the interaction between the drug molecule and stratum corneum. Diffusion coefficient can be mainly affected by molecular weight (41). Higher drug molecular weight usually results in lower absorption rate. One reason could be as the carbon length increases; the retention of compounds in the lipid layers of skin will also increase.
Diffusional pathlength

The diffusional pathlength represents the tortuosity of the intercellular pathway and it is difficult to estimate its magnitude precisely (42). Several factors could affect the diffusional pathlength including age of the skin, skin conditions, and regional differences in the skin thickness. Different body sites have different skin thickness and permeability of different body parts can vary. The blood flow to the dermal tissue decreases with aging, which could be attributed to the loss in skin moisture content and as a result the permeability of drug molecule through the skin reduced (43). On the other hand, increasing skin hydration can increase skin permeability.

Enhancement of transdermal delivery

Though the global transdermal products market was valued at $13.5 billion in 2013 and is expected to reach $21.7 billion by 2018, there are only limited products available in the market (44). Due to existence of stratum corneum as permeation barrier, only those drug molecules with proper solubility, partition coefficient and molecule weight can be good candidates for topical and transdermal formulations. Usually hydrophilic charged ionic species or macromolecules such as peptides and proteins with molecular weight larger than 500 Daltons cannot be formulated into topical/transdermal products. Hence, there is a need for using new techniques to improve skin permeability and increase the number of drugs that can be delivered transdermally. Penetration enhancement technology is a promising development that would increase the number of drugs available for transdermal administration.

Enhancement techniques can be broadly divided into physical, electrical, ablation and chemical categories. Chemical enhancers, iontophoresis, sonophoresis, microneedles and
laser have been widely investigated and used to facilitate transdermal delivery. Ablative laser is a relatively new technique used for the transdermal drug delivery. The use of lasers for compromising this outermost layer of epidermis is gaining popularity and seems a promising physical enhancement technique (45)(46)(47). Unlike non-ablative laser, ablative laser can efficiently break the stratum corneum and porate the skin surface. Non-ablative lasers work by heating up the skin tissue and have short recovery times, as they don’t ablate the stratum corneum. These lasers have been shown to mainly enhance the transdermal permeation of small drug molecules due to the formation of microthermal zones of thermal injury in skin (16). However, as the stratum corneum remains intact, permeation of macromolecules through the skin may not be possible with the use of non-ablative lasers.

In the present work, the effect of microneedle and ablative laser on the drug permeation, especially for large and hydrophilic molecules will be discussed.

Chemical permeation enhancers

Ideally, chemical permeation enhancers can interact with skin constituents to promote drug flux and reversibly reduce the barrier resistance of epidermis without damaging viable cells (48). Chemical penetration enhancers should be non-toxic, non-irritating and non-allergic for skin. The effect of enhancers on the skin structure and duration should be predictable and reproducible. There should be no pharmacological activity of penetration enhancers within the body and no irreversible effect on the skin barrier. The penetration enhancers can disrupt the structure of highly ordered stratum corneum which is the major barrier for skin permeation, interact with intercellular protein and improve drug partition into the skin surface. Enhancers act by at least one of these three mechanisms (49) and
cause protein conformational change or solvent swelling. No chemical penetration enhancer has been discovered so far to possess all of the above properties. These agents act by acting either with the polar heads or the nonpolar tail regions of the lipid bilayers and thereby distorting or loosening the barrier for drug permeation (50).

There are several types of permeation enhancers widely used in the market, including sulphoxides, azone, pyrrolidones, fatty acids, essential oil, terpenes, terpenoids, glycols, alcohols, and surfactants. Dimethylsulphoxide (DMSO) is one of the most popular penetration enhancer from sulphoxides group. It is a powerful aprotic solvent, colorless, odorless and widely used not only in skin research, but also in many areas of pharmaceutical and biological sciences. DMSO has been incorporated as co-solvent in a vehicle for the preparation of idoxuridine, which is used for the treatment of severe skin herpetic infections. In the past, DMSO alone has been applied topically to treat systemic inflammation; currently it is used only to treat animals (48). A vast array of literature has reported the penetration enhancing activity of DMSO; it can be used to enhance the permeability of both hydrophilic and lipophilic drug molecules. It has been formulated into different marketed topical and transdermal drug products, including antiviral drug, steroids and antibiotics. The effect of DMSO as a permeation enhancer is concentration dependent; higher concentration of DMSO usually results in the faster permeation of drug molecules and most of the time, more than 60% of DMSO is needed in order to efficiently increase the drug penetration. High concentration of DMSO can cause skin erythema, induce wheals on the stratum corneum, and may denature some of the skin proteins. The mechanism of DMSO as penetration enhancer is that it can change the intercellular keratin conformation from α helical to β sheet (51) (52). There was one
study that investigated the effect of high concentration of DMSO on the skin; healthy volunteers had been applied with 90% DMSO twice a day for 3 weeks which resulted in erythema, scaling, contact uticaria, stinging and burning sensations. Some volunteers even developed systemic symptoms (53). Another problem associated with DMSO is metabolite dimethulsulphide produces a foul odor on the breath. Due to the above drawbacks with DMSO, dimethylacetamide (DMAC) and dimethylformamide (DMF) have been investigated as accelerants, which are similarly powerful aprotic solvents with structures akin to that of DMSO.

A wide variety of long chain fatty acids have been used as penetration enhancers for percutaneous absorption and the most popular one is oleic acid. Aungst et. al. compared the structure of a range of penetration enhancers including fatty acids, alcohols, sulphoxides, surfactants and amides (54). The study showed that saturated alkyl chain lengths of around C_{10} – C_{12} attached to a polar head group yields a potent enhancer; while for penetration enhancers containing unsaturated alkyl chains, then C_{18} appears near optimum. For this kind of unsaturated compounds, the bent \textit{cis} configuration is expected to disturb intercellular lipid order more than \textit{trans} arrangement, which differs little from the saturated analogue. Long chain fatty acid, like oleic acid has been used to enhance the transdermal delivery of estradiol, progesterone, acyclovir, 5-fluorouracil and salicylic acid, indicating that it works for not only lipophilic drugs, but also hydrophilic ones. Compared with DMSO, oleic acid can be effective at relatively low concentrations (< 10%) and can work as co-solvent with propylene glycol (55). Oleic acid interacts with lipid domains of stratum corneum and when oleic acid is at higher concentration, it can exist as a separate phase within the lipid bilayers (56). Another commonly used
permeation enhancer in topical and transdermal products is ethanol. Usually it works as a co-solvent with other solvents to increase the solubility of drug in the formulation and ensure the sink condition during the permeation process. Ethanol has been reported to act as co-solvent with water to enhance the flux of levonorgestrel, estradiol and hydrocortisone through the skin (57). The effect of ethanol as permeation enhancer is also concentration dependent, but not always. There is one study that reported salicylate ion diffusion through human skin increased up to 0.63 ratio of ethanol: water, but higher levels of ethanol decreased permeation (58). This might be because higher concentration of ethanol dehydrated the biological membrane and decreased permeability of the tissue. As we know, the water content in human stratum corneum is about 15% to 20% of the tissue dry weight. Increasing the humidity of skin can increase the permeability of skin for both hydrophilic and lipophilic drugs. Many transdermal patches deliver drugs much higher than expected; this is because patches are occlusive. Application of patches modified water content in the skin and therefore dehydration of skin surfaces by high concentration of ethanol resulted in the reduced skin permeability.

Surfactant is another penetration enhancer that can be found in many existing therapeutic and cosmetic products. Typically, it is composed of a lipophilic alkyl or aryl fatty chain, together with a hydrophilic head group and it can be classified as anionic, cationic and non-ionic surfactants. Anionic and cationic surfactants may damage the skin surface and increase epidermal water loss in human skin. They also have the potential to interact with intercellular keratin. Non-ionic surfactants are relatively safer, such as tween 80. Some studies showed that the effect of surfactants as penetration enhancer is time dependent. Watkinson et al. showed that about 0.5% of the applied dose of
nonoxynol surfactant materials traversed human skin after 48 h application in vitro (59). Even though non-ionic surfactants are safer, they may have very limited enhancement effect in human skin whereas anionic surfactants usually have a more pronounced effect. The above discussion of different penetration enhancers illustrates that potential mechanism of action of enhancers can be varied, and ranges from direct effects on the skin to modification of the formulation. They can act on the stratum corneum intracellular keratin, denature it or modify its conformation causing swelling and increased hydration or affect the desmosomes that maintain cohesion between corneocytes. Penetration enhancers modify the intercellular lipid domains to reduce the barrier resistance of the bilayer lipids. If penetration enhancers could evenly distribute within the complex bilayer lipids, they would homogenously disrupt the stratum corneum. However more likely, they heterogeneously accelerate the skin permeability.

There are many permeation enhancers that can be selected and incorporated into the formulation and optimize permeation. However, the amount of drug that can be delivered using penetration enhancers is still limited because the barrier properties of the skin are not fundamentally changed. With the development of biotechnology in the latter half of the 20th century, more therapeutically active, large hydrophilic molecules, mostly peptides and proteins were generated. Peptides and proteins tend to be extensively degraded by the enzymes in the liver after oral administration, which is first pass metabolism. Hence, there is a need for better and more suitable routes for hydrophilic macromolecules. Penetration enhancers are incapable of enhancing permeation of such large hydrophilic drug substance, which has led to the invention of alternative strategies, such as microneedles, sonophoresis, and iontophoresis.
Iontophoresis

Transdermal iontophoresis is a technique that delivers charged molecules across the skin using mild electric current (60). Iontophoresis involves the delivery of ionic drug molecules under electric fields. The drug molecules will be taking the least resistance pathways such as hair follicles. Though the idea of using electric current to increase drug permeation through the skin has been known for a few centuries, its feasibility has been demonstrated in literature and in clinic recently. There are two components contributing to the drug delivery with iontophoretic delivery, electrorepulsion and electroosmosis (61).

\[ J_{\text{iontophoresis}} = J_{\text{electrorepulsion}} \pm J_{\text{electroosmosis}} \]

Electrorepulsion is the simple repulsive force between similar charges and electroosmosis is the convective solvent flow in the direction from anode to cathode because of the current passage. The efficiency of electroosmosis and electrorepulsion depends on the physicochemical and electrical properties of the membrane. The ideal candidate of drug molecules using iontophoresis to increase permeation should possess some aqueous solubility.

When solution of an ionic drug is placed under an electrode which has the same charge as that of the drug, and then placed under the influence of electric current, the drug gets repelled away from drug formulation into and through the skin (62). In case of anodal iontophoresis, the positively charged drug is placed in the anode chamber at the desired site of application and cathode, which is the receiving chamber, is place at another site. When current is initiated, positively charged ions are pushed across the skin barrier. Ions prefer to pass across the pathways that offer lower diffusional resistance. Three major routes of drug transport through skin include intercellular, intracellular and
transappendageal routes. Appendageal pathways usually offer low resistance such as sweat glands, sebaceous glands and hair follicles (63). Appendageal routes are predominantly responsible for the iontophoretic transport, even though intracellular or intercellular transport is later required for drug to leave the follicular appendage. It has been reported that under the influence of electric current, the lipid bilayer of skin surface becomes more permeable to ions.

Electroosmosis can be described as the movement of hydrophilic molecules from anode to cathode, when electric current is applied to skin (61). Due to ionization of carboxylic acid groups, skin acquires a net negative charge at physiological pH, which is around pH 4 to 4.5. In this case, when current is applied on the skin, ions passing through the skin carry water of hydration along with them. This action results in the bulk movement of water from anode to cathode. In this case, neutral molecules can also be delivered under anode, as electroosmosis and cations will get a second driving force in addition to electrorepulsion. For the highly lipophilic molecules, they are not ideal candidates for iontophoretic delivery; this is because at neutral pH, these lipophilic cations are able to strongly associated with negative charges on the skin and neutralizing them. This reduces the charge on the skin, which results in reducing or stopping the electroosmotic flow (64).

Iontophoretic transport of cations and anions can be predicted by the Nerst-Planck model, which is the fundamental model that describes the transport of ions under the influence of electric current. According to this model, the ionic flux can be calculated as:

$$ J = -D \left( \frac{dc}{dx} \right) + \frac{DZFCE}{kT} $$

Where
J = ionic flux
D = diffusion coefficient
Z = valence of the charged species
F = Faraday’s constant
C = concentration of the ionic species
E = electric field potential
K = Boltzmann’s constant
T = absolute temperature

The sum of ionic flux can be influenced by the concentration gradient of ionic species and electric field. In order to account for iontophoretic delivery of neutral molecules, additional term need to be added to the Nernst – Planck equation. The modified equation is given as

\[
J = -D \left( \frac{dc}{dx} \right) + \frac{DZFCE}{kT} + VC
\]

Where V is the convective flow velocity and is proportional to the applied current.

There are several physicochemical and electronic factors that can influence the effect of iontophoresis on drug delivery across skin. In the Nerst-Plenck model, the iontophoretic flux is directly proportional to the charge of drug molecules. The nature of drug charge can determine the electrode used in delivery. Uncharged molecules can be delivered by electroosmotic flow. Charge on any molecule or membrane is a function of pH of the environment. Formulation pH will affect the degree of drug ionization and also affect the skin charge. Drug ionization can be calculated by Henderson-Hasselbalch equation. When pH equals to the drug pKa, drug will be 50% ionized. In case of proteins and peptides, the charge will be dictated by the isoelectric point (PI) and pH of formulation.
Extreme pH values of drug formulation can produce hydronium or hydroxide ions with high electrical mobility and hamper the iontophoretic flux of drug molecules. When the pH of formulation is high, skin will become negatively charged and acts as a selective permeation barrier giving rise to electroosmotic flow from anode to cathode. Therefore, in case of weakly basic drugs, higher pH of formulation can suppress drug ionization and decrease electrorepulsion. In this case, electroosmosis may become the major driving force to facilitate drug permeation. Marro et al. found that the direction of electroosmotic flow can be affected by changing the electrical properties of the membrane (65). Their study showed that when pH value is lower than pI of skin (around 4.8), skin assumes a positive charge and acts as anion-selective, net positively charged membrane. Another factor can significantly influence the iontophoretic flow is drug concentration. According to the equation, there is a direct relation between iontophoretic flux and drug concentration. Different from conventional passive transdermal delivery, iontophoretic flux generally increases with the increase in the drug concentration only up to a certain level and then it reaches a plateau. This can be attributed to a lack of increase of drug molecules in the membrane by increasing amount of drug in the formulation. There was one study that investigated the effect of drug concentration in the formulation on the iontophoretic flux in the presence and absence of background electrolyte. Lidocaine, propranolol and quinine were used in the study (61). Results showed that in the presence of background electrolyte, the lidocaine flux was linearly increased with donor concentration, while propranolol showed opposite results. Quinine flux was not linearly increased with donor concentration. Lidocaine has more competing tendency with the background ion to transport the current with increasing concentration. In the absence of
background electrolyte, there was no relationship between donor concentration and iontophoretic flux of any of the three drugs used. Compared with electroosmosis, electorepulsion contributes around 90% of the iontophoretic flux which is mainly determined by the current applied. Increasing current will increase the electromigration, but like donor concentration, there is also a plateau called saturation point at a certain current density. Electric current is one of electronic factors that can significantly influence the electrophoretic flux; another factor is electrode material. For iontophoresis to work, the electric current must be efficiently converted to ionic current which happens at the interface of electrode and the aqueous solution (66). The electrodes can be made of platinum, nickel, stainless steel or carbon graphite which are inert electrodes. The limitation of metal electrodes is that they can bring about electrolysis of water and results in the production of protons. Protons can compete with drug molecules for carrying the charge and lower the skin pH, which has a high potential to cause skin irritation. This problem can be overcome by using reversible electrodes such as silver/silver chloride electrodes. Silver/silver chloride electrodes is one of the most commonly used electrodes both in lab and in clinic. They have good conductance and not bring about changes in pH (67).

There are several iontophoretic transdermal system based products have been approved by FDA. IONSYS was launched by ALZA Corporation, Mountain View, CA. This product was developed for the treatment of moderate to severe pain in a medically supervised setting. FDA and European Medicines Agency (EMA) approved IONSYS in 2006 and in 2015; it has been approved for the management of acute post operative pain for adult patients in hospital. This device is a patient controlled drug application system
and it uses low intensity direct current provided by 3V lithium battery to drive fentanyl HCl from hydrogel formulation through skin (68). A hydrogel containing fentanyl HCl located at the anode and an inert hydrogel was placed at the cathode. A pressure sensitive adhesive helps maintain contact of the device to skin. The amount of fentanyl delivered to the skin is directly proportional to the applied current. Since IONSYS has drug abuse, addiction and misuse potential, even at recommended doses; it is only for use in patients who are alert enough and have adequate cognitive ability to understand use directions. IONSYS should only be used in hospital, not for home use.

Sonophoresis

Ultrasound has been used for medical purpose for several decades and can be classified into three categories: higher frequency (2-10 MHz), medium frequency (0.7-2 MHz) and low frequency (20-100 KHz). Ultrasound can be used as a diagnostic tool or for physical therapy. Sonophoresis is a process that significantly increases the absorption of topical compounds into the epidermis, dermis and skin appendages. Transdermal drug delivery offers an attractive alternative to the conventional drug delivery methods of oral administration and injection. However, the stratum corneum acts as a barrier that limits the penetration of substrates through the skin (1). Application of ultrasound (sonophoresis) to the skin increases its permeability and enables the delivery of various substances into and through the skin. Ultrasound, as a physical enhancer for systemic drug delivery, can cause the structural alteration in the surrounding tissue (2). It leads to the disordering of the lipid bilayers and formation of aqueous channels in the skin through which drug can penetrate (3) (4). Sonophoresis operates at frequencies in the range of 20 kHz – 16 MHz and intensities up to 14 W/cm².
The proposed mechanisms of sonophoresis include thermal effects by absorption of ultrasound energy, cavitation effects caused by collapse and oscillation of cavitation bubbles in the ultrasound field, convective transport and mechanical effects. When ultrasound passes through the skin, ultrasound energy can be absorbed by the tissue and causes a local temperature increase. The temperature enhancement depends on the ultrasound frequency, intensity, area of ultrasound beam, duration of exposure and the rate of heat removal by body fluid (69). The diffusivity of skin increases with temperature. Merino et al. reported increasing skin temperature by 20 °C with low frequency ultrasound (20 kHz), the permeation of mannitol was enhanced about 35 folds (70). However if the skin was heated to a similar temperature without ultrasound, the delivery of mannitol only increased about 8 folds, which is much less than treated with ultrasound. This study suggests that thermal effect may play a secondary role in skin permeability enhancement.

Cavitation is the formation of gaseous cavities in a medium exposed to ultrasound; it involves either rapid growth and collapse of a bubble, or the slow oscillatory motion of a bubble in an ultrasound field. Stable and inertial cavitation have been evaluated for their function in sonophoresis. Stable cavitation corresponds to periodic growth and oscillations of bubble around the equilibrium radium in response to relatively lower acoustic pressures in an acoustic field (71). The radius of the cavitation bubbles is related to the frequency and acoustic pressure amplitude; the maximum bubble radius is estimated to be between 10 and 100 μm when the frequency of sonophoresis ranging from 20-199 kHz. Inertial cavitation corresponds to the violent growth and collapse of bubbles that can occur within a period of cycles and is dependent on acoustic pressure as
well as frequency and the size of bubble (72) (73) (74). Collapse of spherical cavitation bubbles results in the formation of a shock wave and this shock wave can disrupt the lipid bilayers to some extent. There are three possible modes through which inertial cavitation may disrupt the stratum corneum and enhance skin permeability. Spherical collapse can either emit shock waves, which may disrupt the stratum corneum or acoustic microjets impacts on the stratum corneum surface. Microjets may physically penetrate the lipid layer and enhance skin permeability.

Applications include the medical diagnostics and skin permeability enhancement. Recently sonophoresis has been used as a noninvasive method to extract clinically useful analytes from human skin (5), such as glucose. Kost et al. reported that by using low frequency sonophoresis as pretreatment with vacuum application, glucose can transport from the interstitial compartment through the skin layers and diffuse into a reservoir filled with water, which was placed on the top of sonophoresis treated skin. This approach allowed to perform continuous glucose monitoring for patients. Correlation between glucose extraction by sonophoresis and blood glucose levels detected by blood sampling has been assessed in rats (75). Infusing insulin at a rate of 10 mU/min for 2 h, the glucose level in blood was varied. The relationship between the predicted and measured glucose values in the blood was linear with $R^2$ value at 0.97. This study illustrated that glucose level in blood circulation can be measured with sonophoresis.

Sonophoresis can enhance drug permeation over passive transport, which allows strict control of transdermal diffusion rates. Skin remains intact with low risk of introducing infection, and infection potential is therefore low. The disadvantage of sonophoresis is that this is a time consuming process and stratum corneum must be intact for effective
drug permeation. High frequency ultrasound sometimes may cause minor tingling, irritation and burning on the skin.

Sonophoresis is a promising method of enhancing topical and transdermal delivery of drugs. Since the skin characteristics of each body part and the physiochemical properties of the drug affects efficiency of drug delivery, the specific protocols and ultrasound parameters will differ according to application site and drug used. Sonophoresis is a useful tool for both diagnosis and treatment of diseases.

**Microneedles**

In order to overcome the barrier properties of the skin, hypodermic needles are commonly used for the administration of small and large drug molecules, including peptides and proteins. However, they cause pain, trauma and have high possibility of infection at the site of injection. Microporation is in close comparison to the usage of hypodermic needles for parenteral delivery of drugs and it is a better alternative method to compromise the skin barrier, stratum corneum. Microporation can be achieved by thermal and microneedle technologies. Thermal microporation involves the creation of micropores in the stratum corneum by high pulsed thermal energy and some literature already showed successfully delivery of macromolecules such as insulin both in vitro and in vivo conditions (76).

Compared to thermal microporation, microneedle technology is more widely used in both research and clinic. Microneedle device is composed of arrays of micron-sized needles. The length of microneedle usually is not more than 1000 μm. Application of microneedle to the skin surface can bypass the stratum corneum without stimulating
dermal nerves due to the short length of needles (77). Microneedles are considered pain-free, minimally invasive technique to compromise the skin barrier. As a micro-scale device, microneedle should be long enough to deliver large drug molecules across skin, but still be short enough to avoid nerves in the skin to avoid pain in order to achieve much better patient compliance. Microneedle allows precise tissue localization of drug delivery, such as within the skin and the suprachoroidal space of the eye.

Fabrication material of microneedles can vary. The first microneedle was made from silicon and developed for intracellular delivery in vitro. Hashmi et al. reported this microneedle arrays were inserted into cells and nematodes to increase molecular uptake and gene transfection. Due to brittle characteristics of silicon, it is not an ideal material for microneedle fabrication. It can potentially break off and be embedded in the epidermis. More materials are now being used for the fabrication of microneedles such as sugar, glass, metal and polymer (78). Glass microneedle has similar drawbacks as silicon microneedle; it also has potential to break off in the skin. Therefore, it would be more desirable to use sugars and polymers; both of them can biodegrade in the skin with time. Since sugars and polymers usually are not as strong as glass, the fabrication procedure is more complex. Drug can be preloaded in the microneedle and no sharps waste is generated.

There are two basic forms of microneedle: in-plane and out-of-plane. In-plane microneedle has better control of design as all lithography is on the same plane and it can be more easily integrated with sensors and pumps. However, it is more difficult to develop an array of parallel microneedles. Out-of-plane microneedle is easier to fabricate arrays. Microneedles can be further classified into solid microneedles, dissolving
microneedles, coated microneedles and hollow microneedles.

Solid microneedle can pretreat skin to create micropores or microchannel array on the skin surface before applying drug formulation. Drug can be applied over micropores array and usually microneedle created micropores will take about 72 h to close (13). Drug can be loaded in a semi-solid topical formulation, such as ointment, cream, gel or lotion or it can also be made in a patch. In order to provide sufficient mechanical strength and reduce the force needed to insert microneedles into tissue, selection of microneedle material is very important. As we discussed above, solid microneedle can be made of non-degradable materials, such as polymers including polycarbonate, polymethylmethacrylate (PMMA) or photolithographic epoxy and malei anhydrid (PMVE/MA) and metals like titanium, tantalum or nickel. It also can be made using biodegradable polymers such as poly-lactic-co-glycolic acid (PLGA), polyglycolic acid (PGA) and polyactic acid (PLA) or maltose, which is water-soluble substance; this kind of microneedle also called dissolving microneedle (79).

Oh et al. investigated the effect of various microneedle height (200 and 500 μm) and microneedle densities (45, 99 and 154 MN/cm²) on the delivery of a model hydrophilic drug molecule, calcein through the rat skin (80). The whole permeation study has three groups. For the first group, skin was pretreated with microneedle array 30 min before the permeation study, then applied calcein gel. For the second group, calcein gel was applied on rat skin directly and the microneedle array was inserted into the skin for 30 min. Or the microneedle was inserted into the skin simultaneously with calcein gel application, which was the third group. Results showed that third group achieved the highest drug permeation and with the increase of microneedle height and density, the permeation
amount of calcein also increased. Therefore, these various parameters are of integral importance when designing a microneedle in order to optimize the penetration and permeation performance.

Microfabrication technology was first used for the preparation of silicon microneedles. After that, more silicon-based microneedles with different shape, length and density were made with this fabrication method. Even though microfabrication technology can be feasible for high throughput microneedle manufacturing, the drawbacks are also obvious. This method is expensive, high specialized and manufacture procedure is complex (81) (82). For metal microneedles, fabrication methods can vary, such as laser cutting, wet etching, photochemical etching, metal electroplating and three-dimensional laser ablation (83)(84)(85)(86)(87)(88). Polymer microneedles have been made by photolithography using optically curable polymers, which are then typically employed as master structures for replication by molding. Compared to silicon and metal microneedles, these UV-curable polymers made microneedles usually have weaker mechanical strength. Primarily, these microneedles are used as master structure for making molds. Polyvinyl alcohol (PVA), silicon and aluminum can be used as mold materials to replicate polymer microneedles (89)(90). Then, mold can be filled with softened material such as polycarbonate or PMMA, then forming microneedles after cooling and solidification process.

Maltose microneedles have been developed to completely dissolve in the skin after insertion and thereby leave behind no biohazardous sharps waste after use. Drugs can be preloaded in the microneedles for release into skin. As we discussed, maltose microneedles as dissolving microneedles can be fabricated using micromolds filled by
solvent casting such as water, filled with melted maltose in the mold and allowed to solidify. Drawing methods of fabrication also have been used for maltose microneedle manufacture. Melted maltose has been filled in the cavities of a mold and solidified upon cooling (91). Multi-needle arrays can also be formed using a controlled, stepwise drawing technique enabled by a microfabricated device. These fabrication techniques have been widely used in other dissolving microneedle manufacture, including CMC, dextran, PVP, PLGA microneedles (92)(93)(94)(95)(96)(97). Compared with other dissolving microneedles, which may take at least 5 min to fully dissolve, maltose microneedle only need less than 2 min to evenly dissolve all the microneedles in the skin. Literature reported dissolving microneedles could enhance transdermal delivery of numerous hydrophilic and hydrophobic drug substances including insulin, human growth hormone, ovalbumin, sulforhodamine B and a variety of vaccine antigens (47)(98)(99). Dissolving microneedle is good for heat sensitive compounds like proteins and antigens; the manufacture procedure will not damage the protein activity.

Solid microneedle can be used not only as skin pretreatment technique, it can also be used as vehicle to carry drug substance through the skin barrier and deliver to the skin, when used as coated microneedle. Microneedles can be coated with drugs or antigens for vaccination and left in place for slow release drug delivery and facilitate better drug targeting in skin (100). Small molecules such as fluorescein, calcein, pilocarpine and vitamin B and macromolecules such as insulin, desmopressin, ovalbumin and Alex Flour 488 conjugated goat-anti-mouse IgG have been coated on microneedles and reported in literature (101)(102)(103)(104). Coated microneedle can also be used for vaccination. Hepatitis B surface antigen, inactivated influenza virus, recombinant trimeric soluble
influenza hemagglutinin, inactivated chikungunya virus and influenza virus-like particle have been incorporated in coated microneedle for vaccination.

Hollow microneedles containing a hollow bore offer the possibility of transporting drugs through the interior of well-defined needles by diffusion or for more rapid rates of delivery, by pressure-driven flow (105). It can be used for infusions, fluid extraction for analysis and integration with microchips for infusion. Flow rate can be controlled by pressure for a rapid, slow injection or time-varying drug administration. Hollow microneedles have been manufactured using microelectromechanical systems (MEMS) techniques such as laser micromachining (85) and wet chemical etching. It can be made of glass, polymer and metal like solid microneedle. Most techniques used for solid microneedle fabrication can be used for hollow microneedle production, including drawing lithography, metal electroplating and ultraviolet excimer laser beam technique. Hollow microneedles are either used with a syringe to inject liquid drug formulation or integrated with an actuator and attached to a drug reservoir. Flow rate can also be controlled by \( \text{CO}_2 \) gas pressure, a piezo-electric micropump, a piezoelectric linear servo motor and a micro-gear pump (106)(107)(108). Unlike other microneedles, hollow microneedles made of glass or stainless steel, have been developed to extract body fluid from human, such as taking blood samples from body (109)(110).

Limitations associated with hollow microneedles are also obvious. During microneedle insertion, skin tissue may block the needle bore opening and insertion can be hindered. This issue can be solved by locating the bore opening at the side of microneedle tip. This approach can also increase the area of drug exposure to the tissue and retain tip sharpness. Another limitation associated with the hollow microneedle is that dermal
tissue with high density can resist drug flow from the hollow microneedle through the underlying skin. Wang et al. reported that partial needle retraction following insertion improved the fluid infusion due to relaxation of the compresses tissue and an increase in flow conductivity of skin beneath the microneedle tips (111). Davis et al. (2005) did a study with hollow microneedles to deliver hydrophilic macromolecule, insulin to diabetic rats. The hollow microneedle array was made of metal and height of needles was about 500 μm (85). A glass chamber filled with insulin solution was attached to the base of microneedle array to serve as a drug reservoir. The results showed that after 4 h, blood glucose level decreased by 53% and remained constant in a 4 h post-delivery period. Wang et al. also did investigation with hollow microneedle on the transportation of insulin both in vitro and in vivo (112). For in vivo studies, 5 μL FITC-insulin was successfully injected into diabetic rat skin through the hollows and infused for half an hour elicited a drop in blood glucose levels by 25% below the pre-treatment values. When microneedles were retracted back by same length, increasing injected insulin dose to about 30μL and blood glucose level dropped by 70% below pretreatment values. The authors also used hollow microneedles to delivery Caco-2 human intestinal epithelial cells into hairless rat skin in vivo.

There are several microneedle products available in the market for cosmetic purposes. Dermaroller® is one of the widely used products in clinic which was initially sold in Germany in 1999 (113). The device was designed for home use to improve skin texture or to reduce skin scar in clinic. Other worldwide microneedle products including LiteClear® used for the treatment of acne and other skin conditions and MicroHyala® designed to reduce the appearance of skin wrinkles near eyes (114). Beckton-Dickinson’s
soluvia®️, which is a microinjection device, is the only microneedle-based product used for therapeutic application. Soluvia®️ is currently commercialized worldwide as IDflu®, Intanza®️ and Fluzone Intradermal®️ for intradermal vaccination.

As technological advances continue, microneedle arrays and microneedle based products may well become the pharmaceutical dosage forms and more and more companies work on the microneedle investigation including Zosano Pharma, Corium, 3M and Nanopass Technology. The barriers associated with microneedle application need be addressed in order for microneedle technology to progress.

Laser

The first laser was developed in 1959 by Maniman (115) using a ruby crystal to produce red light at a wavelength of 694 nm. The term GUlaser is an acronym for light amplification by the stimulated emission of radiation (116). A photon of electromagnetic energy could stimulate the emission of another identical photon from atoms that are in an excited state. In 1963, the first time, laser was used for the treatment of cutaneous pathologies by promoting ruby laser. During the next two decades, cutaneous laser research focused on argon laser and carbon dioxide (CO₂) laser development. The argon laser was used to treat benign vascular birthmarks; it can significantly lighten most port-wine stains and hemangiomas. However, the side effect of argon laser is unacceptable which can cause hypertrophic scar formation. Carbon dioxide laser which emits at the wavelength of 10,600 nm was used for tissue vaporization and destruction of various epidermal and dermal lesion (116); unfortunately long term use of carbon dioxide laser can result in excessive thermal injury to the skin and cause hypertrophic scarring as argon laser (117)(118). Invention of the theory of selective photothermolysis improved the laser
therapy; selective photothermolysis can specifically target the skin in localized areas with minimal unwanted thermal injury. With the application of laser on the skin, the light could be absorbed, reflected, transmitted or scattered. Only light which has been absorbed by the skin can affect tissue structure; reflected and transmitted light has no effect. The amount of light absorption by skin is mainly determined by the chromophore present in the skin; the endogenous chromophores in the skin include water, melanin and hemoglobin. Laser has three basic effects on the skin: photothermal, photochemical and photomechanical. Photothermal plays a major role of laser therapy, which only occurs when a chromophore absorbs the corresponding wavelength of energy, and destructs the target tissue by converting absorbed energy to heat (119). Photomechanical related to the extremely rapid thermal expansion, which lead to acoustic waves and destruct the absorbing tissue. To achieve the minimal unwanted thermal injury, a proper wavelength should be absorbed preferentially by the intended tissue target. The skin exposure duration to the laser must be shorter than the chromophore’s thermal relaxation time and the energy density must be high enough to destruct the target tissue. The depth of laser penetration can be influenced by the light absorption by skin and light scattering in the skin layers (120). Scattering is less in the epidermis than that in dermis; this is because there are more collagen fibers present in the dermis layer. The depth of laser penetration increased with wavelength until the mid-infrared region of the electromagnetic spectrum. When the wavelength of laser is between 300 to 400 nm, the effect of laser can be limited by strong scattering in the dermis layer and when laser wavelength is between 1000 to 1200 nm, light will show better penetration into the skin (121).

Non-ablative laser
Vascular lasers mainly target intravascular oxyhemoglobin to destruct various congenital and acquired vascular lesions. The wavelength range of vascular lasers is usually within electromagnetic spectrum and the three primary absorption peaks are 418, 542 and 577 nm.

Different types of lasers including argon (488-514 nm), APTD (577 and 585 nm), KTP (532 nm) and Nd:YAG (532 and 1064 nm) have been used to treat vascular lesions. As we discussed before, argon laser has high rate of causing dyspigmentation and scarring, it is no longer commonly used in clinic. APTD laser and KTP laser belong to the CW system, which can be mechanically shuttered to deliver pulses as short as 20 ns to treat facial telangiectasia. APTD laser also has been used for the treatment of rosacea or port-wine stains, however the short interval does not allow skin to cool down adequately after laser treatment and it can cause hypertrophic scarring (122)(123). For facial telangiectasia treatment, KTP laser using a Nd:YAG crystal at wavelength of 532 nm showed better result. The most common side effects related to KTP laser treatment including edema, transient crusting and mild erythema. Another concern of KTP laser treatment of facial telangiectasia is the short wavelength of laser can decrease skin penetration capability and diminish absorption by deeper vessels (124). The flash-pumped PDL laser was specifically designed to treat vascular lesions, especially for the acne scars and wrinkle reduction. Originally the wavelength of PDL was 577 nm (125)(126)(127)(128)(129)(130) and later modified to 585 nm to effect deeper tissue penetration while maintaining vascular specificity (131)(132)(133). Clinical studies showed by increasing the wavelength to 585 nm, 75% to 90% mild to moderate wrinkles had been improved (134). Since the pulse duration (450 μs) is shorter than the thermal
relaxation of small to medium sized blood vessels, PDL can cause selective vascular injury without unwanted thermal damage to the surrounding tissue. In general, the fluence of PDL ranges from 3 to 10 J/cm$^2$ and the spot size is around 2 to 10 mm. Clinically PDL has been used to treat various vascular lesions including port-wine statins, hemangiomas, hypertrophic, keloid scars, warts, angiofibromas, Goltz’s syndrome, inflammatory linear verrucous epidermal nevus, multiple eccrine hidrocystoma, lupus pernio, granuloma faciale, necrobiosis lipoidica diabeticorum and molluscum contagiosum (135). Postoperative purpura is one of the most common side effects of PDL, which may last one to two weeks after the treatment; transient dyspigmentation, vesiculation, crusting, textural change and scarring are rarely seen. In order to target deeper tissue, PDL with long wavelengths (585-600 nm) and extended pulse duration with fluence ranging from 5 to 15 J/cm$^2$ have been developed. Recently the application of long pulsed PDL in combination with the precursor photosensitizer aminolevulinic acid (ALA) has been used to treat photoaging, which showed better results than using PDL alone for the treatment (136). The wavelength of long pulsed PDL is 595 nm, which has been reported an 18% improvement for the treatment of photodamage in one clinic study (137). The photosensitizer protoporphyrin IX can be activated by long pulsed PDL and preferentially accumulates in photodamaged cells, resulting in their destruction either by apoptosis or an immune-mediated response (138).

The infrared laser firstly came up as infrared Nd:YAG laser at the wavelength of 1320 nm with a pulse duration of 200 microseconds and recently the diode laser at 1450 μm and erbium:glass laser were developed (139). Clinical studies have shown that infrared laser did mild to moderate improvement in rhytides and scars after six months.
treatment. Similar results have been obtained by 1320 nm Nd:YAG laser and 1450 nm diode laser; both of them showed mild improvement of periorbital and perioral rhytides (140). Treatment of rhytides with a 1540 nm erbium doped phosphate glass laser showed moderate improvement, which was better than that treated by 1320 nm and 1450 nm infrared laser. Histology studies also showed an increase in dermal collagen after two months treatment (141). There was another study that investigated the effect of 1540 erbium doped phosphate glass laser on the patients with periorbital and perioral rhytides; results demonstrated a mean increase in dermal thickness with good patient compliance. Since infrared laser only induces the dermal changes, therefore it has very limited benefit for the patients with photoaging who have both epidermal and dermal damages. The 1450 nm laser has been shown to result in fibrosis of the upper dermis and the 1540 nm erbium doped phosphate glass laser to result in an increase in dermal thickness (141)(142).

Recently, there is an improved infrared laser device has been introduced for the treatment of skin laxity. The laser wavelength of this device can emit from 1100 to 1800 nm and is postulated to function by causing volumetric heating of the dermis and followed by skin tissue contraction. This device demonstrated moderate degree of efficacy in treating rhytides and laxity.

Non-ablative lasers such as vascular laser and infrared laser have been commonly used to tighten the skin, improve the appearance of wrinkles, brown spots and scar without or with minimal harming the surrounding tissue. However, the major problem related to the non-ablative lasers is their limited efficacy compared to ablative laser; most vascular laser and infrared laser can only mild and moderate improve the skin condition.
Fractional laser was recently developed to address the drawbacks of traditional ablative lasers with its limited efficacy of dermal remodeling. It is based on the theory of selective photothermolysis, which delivers the narrow beams of high-energy light to the skin in a pixilated pattern. The fractional laser light is mainly absorbed by aqueous tissue and creates microscopic treatment zones of controlled width, depth and densities (143). The microscopic treatment zones was first described as “microscopic thermal wounds”(143); the laser beam precisely penetrate the stratum corneum, confine epidermal and dermal coagulation without compromising the adjacent tissue. The microscopic treatment zones contain thermally damaged cells around the microchannels; these thermally damaged epidermal and dermal cells including elastin and melanin. The undamaged surrounding tissues allows faster epidermal repair through migration of the untreated viable tissue compared with ablative laser; the target chromophore of fractional laser is water containing tissue. In clinic, the 1550 nm erbium fiber laser is one of the most commonly used fractional lasers for skin disease treatment including melisma, rhytides, acne scarring and photoaging. The laser energy, density and number of passes should be set at various values for different applications. The original version of fractional laser is Fraxel SR, which was the aforementioned 1550 nm erbium fiber laser with the fluence of 40 J/cm² and used for the treatment of soft tissue coagulation periorbital rhytides and pigmented lesions. This technique had been approved by Food and Drug Administration (FDA) in 2003; in 2006, the applications have been extended to skin resurfacing, melisma, acne and surgical scarring. FDA approved the new Fraxel SR1500 in 2007; this device emits laser light at the fluence of 70 mJ/cm², which allows the depth of penetration up to 1.4 mm. The original Fraxel SR can only penetrate 300 to
800 micron depth, which showed limited efficacy of treating deeper rhytides. Another commercially available fractional laser is Lux 1540 fractional laser, which was developed by Palomar Medical Technologies. Even though laser light can penetrate up to 1 mm, it is painless. Other versions of fractional resurfacing by the same manufacturer are LuxIR Fractional infrared laser handpiece and Lux2940 fractional laser handpiece. All the laser devices contain a handpiece that can divide pulsed light into microbeams or delivers an array of small beams that create a periodic lattice of isolated hyperthermic columns. Compared with traditional non-ablative laser, increasing the energy delivered and density of coverage, greater depth of dermal penetration is achieved by fractional laser. It has proven to be safer for skin resurfacing with fewer side effects; one of the most common side effects of fractional laser is herpes simplex virus (HSV) infection. The reported incidence of HSV infection is up to 2%; while the incidence of bacterial infection is extremely low (144). The incidence depends on the laser systems, parameter programmed and patient skin type.

Ablative laser

Carbon dioxide (CO₂) laser was the gold standard laser treatment for facial rhytides and acne scars by the end of 1990s; it removes the whole epidermis and parts of dermis. It usually takes several weeks for complete recovery from erythema. Common side effects can be skin infections and pigmentary changes, which observed as a consequence of the extended recovery period. The CO₂ laser emits light at the wavelength of 10,600 nm and can be strongly absorbed by water containing tissue. The penetration depth of CO₂ laser is not dependent on the chromophores in skin such as melanin or hemoglobin; it depends on the water content. The pulse duration of CO₂ laser is less than millisecond;
it can penetrate about 20 to 30 μm depth into skin tissue and treated area can be confined to a thin layer of 100 to 150 μm of the surrounding tissue. UltraPulse CO₂ laser system and continuous wave (CW) CO₂ are two basic carbon oxidize laser systems have been used in cutaneous resurfacing. The duration of each UltraPulse CO₂ laser pulse is 600 μs and produces energy up to 500 mJ of energy in each pulse. The CO₂ laser produces the most dramatic improvement for photodamaged and scarred facial skin (145)(146) due to the collagen shrinkage and remodeling after long term clinical treatment. The application of CO₂ laser increases dermal temperature exceed 55 °C which disrupts the interpeptide bonds leading to conformational changes within collagen’s triple helical structure. CO₂ laser demonstrated at least a 50% improvement in overall skin tone, rhytides severity, and atrophic scar depth (116). The significant postoperative morbidity experienced by patients after the CO₂ laser treatment renders it a difficult prospect for many to consider (145)(147)(148).

Er:YAG lasers is another laser system developed and in widespread use since the mid-1990s; it was developed subsequent to the CO₂ laser in an attempt to treat photodamaged facial skin and limit the side effects and morbidity. The absorption coefficient of Er:YAG laser is 12,800 cm⁻¹, which is much higher than that of CO₂ laser (800 cm⁻¹); it renders the erbium energy more efficiently absorbed by water containing tissues (149)(150)(151)(152). The penetration depth of Er:YAG laser can be controlled by the laser fluence, which provides more precise ablation of skin with minimal thermal damage to the surrounding tissues. The penetration depth of Er:YAG laser is limited to 1 to 3 μm of tissue per J/cm² with zones of thermal necrosis extending another 10 to 15 μm (153)(154). Compared with CO₂ laser, the overall efficacy of Er:YAG is similar, while it
demonstrated less tissue tightening and contraction which may impact the long term outcome in photodamaged skin (155). Long term application of Er:YAG laser results in less severe side effects of discomfort, erythema, edema and less healing time than CO₂ laser. Less aggressive ablative laser techniques such as single-pass CO₂ laser and long pulse duration Er:YAG laser have been developed to offer modest clinical improvements in rhytides and atrophic facial scars with reduced postoperative morbidity and less recovery time (156)(157)(158)(159)(160). Despite the side effects and prolonged recovery of ablative lasers, no other techniques can match the clinical results they can achieve when used properly.

As we discussed before, traditionally, two kinds of lasers are available for skin treatment: non-ablative and ablative. Non-ablative lasers, such as vascular laser or infrared laser, work by heating up the skin tissue and have short recovery times, as they don’t ablate the stratum corneum, these lasers have been shown to enhance the transdermal permeation of small drug molecules, such as diclofenac sodium and sumatriptan succinate due to the formation of microthermal zones of thermal injury in skin (16). However, as the stratum corneum remains intact, permeation of macromolecules through the skin may not be possible with the use of these lasers. Therefore, ablative lasers, that generate high temperature to vaporize skin tissue and disrupt the skin barrier, are more useful for the purpose of transdermal delivery of macromolecules (161). The laser device used in this work, P.L.E.A.S.E.® (Precise Laser Epidermal System), is an ablative laser that emits laser light with wavelength of 2940 nm, which corresponds to the water absorption peak (17)(18). The laser system consists of a diode-pumped fractional Er:YAG (Erbium-Yttrium-Aluminium-Garnet) laser, that forms
a matrix of identical micropores or channels with a few seconds, where the pores are at a
distance of 100–150 μm from each other. Unlike the conventional thermal techniques,
P.L.E.A.S.E.® selectively creates micropores with reduced risk to the target skin tissue.
The micropores formed by laser without any significant trauma allowing the active
ingredients to permeate lateral and downwards to the tissue. Microbeams are directed
from the laser that enables efficient and fractional ablation of skin, with less damage. The
full re-epithelization of ablated area happens with 24 to 48 hours; there is no infections
observed in clinical trials. The application of P.L.E.A.S.E.® laser demonstrates less tissue
carbonization, minimal coagulation, shorter healing time and a significantly lower risk of
hypopigmentation. It also creates a highly effective passage for drug delivery. The depth
and number of micropores created by P.L.E.A.S.E.® laser can be controlled by laser
fluence, density and array size.

Overall, P.L.E.A.S.E.® is a patient-compliant technology that has been suitably
programmed to adjust the parameters such as pulse length, energy, number, and repetition
rate, in order to control the extent of stratum corneum ablation (17).
CHAPTER 3

Transdermal delivery of cimetidine across microneedle-treated skin: Effect of extent of drug ionization on the permeation

Abstract

The objective of this work was to optimize a gel formulation of cimetidine to maximize its transdermal delivery across microporated skin. Specifically, the effect of extent of ionization in formulation on permeation of cimetidine across microporated skin was studied. Cimetidine was formulated into a gel using propylene glycol, water and carbopol 980NF. Three strengths of gels (0.1% w/w, 0.5% w/w and 0.8% w/w) were made and Tris base was used to adjust the pH of formulations to pH 5, pH 6.8 and pH 7.5. In vitro permeation testing was performed on vertical Franz cells with dermatomed porcine ear skin. Permeation studies suggested that pH 5 gels showed highest permeation through microchannels. This trend was more prominent with an increase in drug loading. The total amount of cimetidine delivered from 0.8% w/w gel at pH 5 at 24 h was 28.20 ± 4.63 μg, which was significantly higher than that from pH 6.8 (16.89 ± 3.56 μg) and pH 7.5 (12.03 ± 1.66 μg) gels. Cimetidine permeation across microporated skin was found to be pH dependent, with lower pH/ highest ionization resulting in greatest permeation. The effect of ionization contributing to faster release was more pronounced when drug concentration was increased.
Introduction

Cimetidine is a histamine H₂ receptor blocker, which can inhibit the release of extra stomach acid. It has been widely used in the treatment of heartburn and gastric acid reflux. Topical application of cimetidine has been proven to be a valuable treatment for dermatological diseases, such as inflammatory conditions and skin warts (162)(163).

In this paper, cimetidine has been used as a model drug in order to investigate the effect of ionization of drug molecules on permeation through microneedle treated skin. Chemically, cimetidine is a base with a water-octanol partition coefficient (log p) of 0.4, pKa value of 6.8, and is relatively hydrophilic (14)(15). Because of it hydrophilic nature, effective delivery of cimetidine across intact skin is difficult to achieve. The passive permeation of hydrophilic drug molecules can be inhibited by the lipid-rich membranes of skin. Treatment of skin with microneedles is an effective way to enhance transdermal permeation of hydrophilic molecules (164). Our objective was to optimize a formulation of cimetidine to maximize its delivery across microporated skin. Specifically we investigated the effect of extent of ionization of cimetidine on its permeation. The pKa of cimetidine was also appropriate for our investigation. Since the pKa of cimetidine is 6.8, a formulation pH range of 5 to 7.5 would result in 98.4% to 16.6% ionization of cimetidine, respectively. This formulation pH range is not likely to cause skin irritation and at the same time lead to a different extent of drug ionization. In this study, cimetidine was formulated into a carbopol gel, consisting of cimetidine, propylene glycol, deionized water and carbopol 980NF polymer. Tris base was used as neutralizer to adjust the pH of the gel. Permeation studies were performed on pH 5, pH 6.8 and pH 7.5 gels using vertical Franz diffusion cells. These studies were also performed as a function of
concentration by increasing drug loading from 0.1% w/w to 0.8% w/w. Skin accumulation was also studied using a skin extraction method.

Materials and Methods

Materials:

Cimetidine was purchased from Sigma-Aldrich (St. Louis, MO, USA). Propylene Glycol was purchased from EK Industries (Joliet, IL, USA). Maltose microneedle was purchased from Elegaphy, Inc (Tokyo, Japan), which was solid, 500 μm in length, sharp tipped and tetrahedron shaped. Triethanolamine was purchased from Acros Organic (New Jersey, USA). Tris Base, potassium phosphate monobasic, phosphate buffer saline, acetonitrile, ortho-phosphoric acid were purchased from Fisher Scientific (New Jersey, USA). Carbopol 980NF polymer was obtained from Lubrizol Advanced Materials, Inc. (Cleveland, OH, USA). 3M-transpore tapes for tape stripping process were purchased from 3M (St. Paul, MN, USA). EpiDerm™ skin irritation test kit was purchased from MatTek Corporation (Ashland, MA, USA). Centrifuge tubes used for the skin extraction study were purchased from MedSupply Partners (Atlanta, GA, USA). Porcine ear skin and porcine abdominal skin were obtained from slaughter house (Atlanta, GA, USA). De-ionized water was used to dilute phosphate buffer saline and prepare the solution required in the HPLC analysis.

Methods

Preparation of cimetidine carbopol gel

In comparison with various hydrogel bases, the carbopol polymers are known for possessing properties like high clarity, low toxicity and are widely used as thickeners or suspending agents. They can also be used as efficient rheology modifiers to provide good
suspension and stabilization benefits. Patel et al. stated that aceclofenac carbopol gel showed much higher drug release, approximately 36% in 6 hours, which was more than that from CMC, HPMC, and sodium alginate gels (165).

In order to investigate the effect of ionization of cimetidine on the permeation across microporated skin, cimetidine was formulated into transdermal gels having different pH values - pH 5, pH 6.8 and pH 7.5. At these pH values, cimetidine would be 98.4%, 50% and 16.6% ionized respectively. To prepare the gels, propylene glycol and de-ionized water were first mixed in a ratio of 1:3. Appropriate amounts of cimetidine were then dissolved in the mixture. The polymer (Carbopol 980NF) was then slowly added into the cimetidine-water-propylene glycol mixture under constant magnetic stirring. Mixing was continued until all ingredients were mixed to form a homogenous swollen mass. In order to achieve the desired pH, carbopol polymer was neutralized using Tris base. Tris was added to the dispersion and stirred thoroughly to obtain a pH of 5, 6.8 and 7.5. The final product was a clear, homogenous gel. In this study, three strengths of cimetidine gel (0.1% w/w, 0.5% w/w and 0.8% w/w) were made. The components of these three strength gels are shown in table 1.

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Cimetidine</th>
<th>Propylene Glycol</th>
<th>Carbopol 980NF</th>
<th>DI Water</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.1% w/w</td>
<td>0.1%</td>
<td>24.7%</td>
<td>1%</td>
<td>72.4%</td>
</tr>
<tr>
<td>0.5% w/w</td>
<td>0.5%</td>
<td>24.6%</td>
<td>1%</td>
<td>73.9%</td>
</tr>
<tr>
<td>0.8% w/w</td>
<td>0.8%</td>
<td>24.3%</td>
<td>2%</td>
<td>72.9%</td>
</tr>
</tbody>
</table>

Table 1: Composition of cimetidine carbopol gel

Physical examination of formulation

The clarity, color and homogeneity of carbopol gels were evaluated by visual inspection. Cimetidine gels were visually inspected against a black and white background. The homogeneity was used to confirm all the components were mixed
completely. All the gels were visually inspected for their appearance and to ensure they were free of aggregates.

pH evaluation and adjustment

The pH of carbopol gels was tested by a digital pH meter by bringing the pH meter in contact with cimetidine gel and allowing it to equilibrate with the gel for approximately 2 or 3 minutes. 40% Tris was used to adjust the pH of the gel to the appropriate value. pH evaluation was performed for all the formulations before and after the experiment. Formulation pH was kept within ± 0.2 units of the desired pH.

Characterization of maltose microneedle

Histology studies

Histological sectioning was performed to confirm the creation of microchannels by maltose microneedles. The untreated porcine ear skin and microporated porcine ear skin were placed flat in Tissue-Tek® optical coherence tomography (OCT) compound medium (Sakura Finetek USA, Inc., Torrance, CA, USA). The skin sample blocks were stored in the -80 °C for at least 1 hour to solidify before sectioning using Microm HM 505 E (Southeast Scientific, Inc., GA, USA). The skin samples were sectioned to skin pieces with a thickness of 10 μm and stained with hematoxylin and eosin (H & E staining). The stained transverse sections were then mounted on glass slides (Globe Scientific, Inc., NJ, USA) and imaged under a Leica DM 750 microscope.

Scanning electron microscopy

The Phenom™ filed emission scanning electron microscopy (Nanoscience Instruments, Inc., Phoenix, AZ, USA) was performed to investigate the shape and dimension of maltose microneedle and the Denton Vacuum Desk V sputter coater was
used to coat the microneedle. A single line of microneedle was carefully separated from the microneedle array and mounted on SEM Pin stub mount (Ted Pella, Inc., Redding, CA, USA). By analyzing SEM images, the dimension of microneedle, such as needle length, width and tip-to-tip distance can be obtained.

Pore uniformity studies

The uniformity of microchannels created by maltose microneedle was investigated by calcein imaging. Calcein solution was carefully applied to the skin portion treated with microneedles for one minute in a dark room. The excess calcein solution was cleaned with Kim wipes and alcohol swabs. A two-dimensional fluorescent image was obtained using a digital camera (Canon, USA) fitted with a micro-lens having a 525 nm long pass filter (166).

Confocal microscopy studies

Confocal microscopy was used to measure the depth of microchannels created by maltose microneedles. Maltose microneedles were vertically applied on porcine ear skin for one minute to form microchannels. Fluoresoft® was applied on the microchannels array for one minute and excess calcein was wiped off by Kim wipes and alcohol swabs. Next, the maltose microneedle treated porcine ear skin was placed on a microscope slide and the depth of microchannels was measured by X-Z sectioning using a computerized Leica SP8 confocal laser microscope with 10X objective at an excitation wavelength of 495 nm.

Skin preparation

Porcine skin was obtained from a local slaughter house. Hair on the stratum corneum side of the porcine skin and adhering subcutaneous fat were carefully removed. Skin was
dermatomed to the thickness of 0.5 mm to 0.7 mm. Dermatomed porcine skin was cut into small pieces of appropriate size. For microporation studies, dissolving maltose microneedles were vertically inserted in the skin samples to create microchannels. Microneedles were kept inserted for a period of 1 minute. During this time, the needles completely dissolved in the skin (167). In order to confirm the integrity of untreated and microporated skin, skin resistance was tested before the permeation study. The resistances of all the skin pieces were higher than 4 kΩcm (163)(168).

Release study

Release study (n=3) was performed using dissolution apparatus 5 to certificate pH of formulation did not influence the release of cimetidine from the carbopol gel. The 0.8% w/w pH 5, 6.8 and 7.5 carbopol gel (1 gram) was used in the release study. The release study was carried out at 32 °C and 900 mL 10 mM pH 7.4 phosphate buffer was filled into each vessel. Samples (1 mL) were withdrawn at predetermined time points and were replaced with fresh dissolution media. All the samples were filtered with 0.45 μm nylon filter and analyzed by HPLC.

Permeation studies

In vitro studies (n≥3) were carried out on dermatomed porcine ear skin using vertical diffusion cells. The receptor compartment was surrounded by a water jacket to maintain the temperature at 37±1 °C. All of the receptor compartments were cleaned properly and filled with receptor medium (Phosphate buffered saline pH 7.4) before the experiment. For the passive transdermal permeation study, skin was mounted on the surface of the receptor compartment. Donor compartments were placed on the skin and the entire assembly was held together using clamps. For microporation studies, skin samples were
microporated as mentioned in the previous section and then mounted onto the surface of the Franz cell receptor compartment in a manner similar to passive permeation. Donor formulation (500 mg) was placed on the skin surface. Samples of receptor solution (300 μL) were withdrawn at predetermined time points and were replenished with the same amount of fresh receptor medium. The obtained samples were analyzed using a HPLC method.

Skin extraction

Cimetidine amounts present in the different layers of skin was quantified with a skin extraction study. After the permeation study, dermatomed porcine skin samples were removed from vertical Franz cells, cleaned with receptor medium and dried with Kim wipes. The skin samples were tape stripped with 3M Transpore tapes twenty times to remove the stratum corneum layer (169). The tape strips were placed into 6 well plates. The first strip was placed separately in one well and strips 2-5, 6-10, 11-15 and 16-20 were pooled together into subsequent wells. Tape stripped skin samples were then minced with a pair of scissors and also placed into 6 well plates. Extraction solvent (2 ml phosphate buffer saline) was added to each well and shaken overnight. The extraction solvents were then filtered on the following day and analyzed using a HPLC method.

Quantitative and statistical analysis

The concentration of cimetidine in the samples was quantified using HPLC. Analysis was performed with a UV detector at 219 nm. The column was Gemini 5u C18 (250x4.60 mm), which was obtained from Phenomenex. The mobile phase was acetonitrile and pH 3 phosphate buffer (11:89). Triathenolamine was used to improve the shape of cimetidine
peaks. Isocratic elution was performed at flow rate of 1.2 ml/min after injecting 10 µL of sample. Under these conditions, the retention time of cimetidine was around 4 minutes. The outcomes of permeation studies using different formulations were analyzed statistically. Statistical significance was determined using ANOVA test. The correlation coefficient and p value were calculated by the statistical software (Graph PAD Instat, CA, USA). The significance level was considered at p<0.05.

Results

Physical examination of formulation

All the formulated gels were characterized for their color and homogeneity. The cimetidine carbopol gels were transparent with a smooth homogeneous appearance and texture. They were easily spreadable and no aggregates were observed in the formulations.

pH evaluation and adjustment

The pH values of all the pH 5, pH 6.8 and pH 7.5 cimetidine carbopol gels were 5 ± 0.21, 6.8 ± 0.18 and 7.5 ± 0.11 respectively.

Characterization of maltose microneedle

Histology studies

Histological sectioning images of untreated and microporated porcine ear skin (Figure 1) shows the morphology of microchannels in the skin. The application of microneedle disrupted the stratum corneum and epidermis layer and formed microchannels.
Fig. 1. Cross-sectional images of histological skin sections of (A) intact porcine ear skin and (B) maltose microneedle treated porcine ear skin

Scanning electron microscopy studies

The SEM image (Fig. 2) shows dimension and morphology of maltose microneedle. Each tetrahedron shaped microneedle was about 500 μm in length, about 200 μm in width, and had a sharp tip.

Fig. 2. SEM image of maltose microneedle

Pore uniformity studies

The pore uniformity of microchannels was investigated by calcein imaging. Fig. 3 shows three lines of microchannels formed on the skin surface after maltose microneedle treatment. There were 81 microchannels in total, with each line having 27 microchannels.
Fig. 3. Microchannels array created by maltose microneedle

Confocal microscopy studies

The depth of microchannels was measured by confocal laser microscopy. After application of calcein on the treated porcine skin, calcein flowed down through the breached stratum corneum, along the microchannels and into lower epidermal tissue. A z-stack was used to capture a sequence of images at the same horizontal position (x, y axis) but different depths (z axis). The z-stack was conducted from skin surface to the point where the signal of calcein visually disappeared. Each step of z-stack was 10 μm in this study. Results in Fig. 4 indicated the depth of microchannels created by maltose microneedle was approximately 180 μm.
Fig. 4. Confocal microscopy z-stack of microchannels created by maltose microneedle

3.4 Release studies

Release studies were performed on 0.8% w/w pH 5, 6.8 and 7.5 carbopol gels and results showed cumulative amount of cimetidine from different pH of formulation was similar (Fig. 5). Statistic study (One-way ANOVA) was performed and there was no significant difference (p<0.05) among three groups. Results illustrate pH of formulation did not influence the release of cimetidine from the carbopol gel.

Fig. 5. 0.8% w/w cimetidine carbopol gel release study

Permeation Studies

Effect of formulation pH and drug concentration on the permeation of cimetidine across microporated skin

The permeation profile of cimetidine from 0.1% w/w gel formulations across microneedle treated dermatomed porcine ear skin (Fig. 6a) suggested that pH 5 cimetidine gel resulted in significantly higher permeation of drug across the skin in 8
hours (p<0.05). This data shows a trend that permeation of cimetidine across microporated skin increased with an increase in percent ionization in the formulation. A possible reason for this could be that aqueous microchannels can better assist transdermal permeation of more hydrophilic moieties (170). In this case, pH 5 gel has the highest ionized drug content (98.4%) in comparison to pH 6.8 and pH 7.5 gels having ionized drug content of 50% and 16.6% respectively. Hence, the nature of pH 5 gel is more hydrophilic as compared to pH 6.8 and pH 7.5 gels. Therefore, we hypothesized that cimetidine permeation across microchannels would be higher for pH 5 gel in comparison to pH 6.8 and pH 7.5 gels. However, at the 24 hour time point, the cumulative amount of cimetidine permeated across microporated skin was similar for all three formulations. We suspected that this was possible due to relatively low drug loading in the gels at 0.1% w/w concentration. Therefore, we performed similar studies with higher cimetidine concentrations in the carbopol gels.

Permeation profiles of 0.5% w/w cimetidine gels showed similar trends as the 0.1% w/w cimetidine gels (Fig. 6b). The effect of higher drug ionization contributing to higher permeation of cimetidine was more pronounced when drug concentration was increased to 0.5% w/w. At the 8 h time point, permeation of cimetidine from 0.5% w/w gels showed the same trend as that from 0.1% w/w gels (pH 5> pH 6.8> pH 7.5). However, at 24 hours, permeation of cimetidine from the pH 5 cimetidine gel was significantly higher than that from pH 6.8 and pH 7.5 gels (p<0.05). Similar cumulative delivery of cimetidine was observed for pH 6.8 and pH 7.5 gels at 24 hours.

With a further increase in cimetidine concentration in the gels to 0.8% w/w, the effect of pH/ionization on permeation across microporated skin was further pronounced.
Cimetidine permeation from pH 5 gel across microporated skin was higher throughout 24 hours in comparison to pH 6.8 and pH 7.5 gels. Also, after 4 hours, cimetidine permeation with pH 6.8 gel was higher at all time points as compared to the pH 7.5 gel (Fig. 6c). These permeation profiles suggest that delivery of cimetidine across microporated skin was dependent on both factors—gel pH and drug concentration. Delivery was highest with highest drug concentration and with the pH which resulted in highest ionized drug content in the formulation.

Fig. 6a: Effect of pH on the permeation of 0.1% w/w cimetidine carbopol gel

Fig. 6b: Effect of pH on the permeation of 0.5% w/w cimetidine carbopol gel
Tape stripping and skin extraction study

Tape stripping and skin extraction studies carried out on skin samples after permeation studies indicated a significantly higher accumulation of cimetidine in stratum corneum than in the tripped skin (viable epidermis and dermis) (p<0.05). There was no significant difference seen in topical accumulation of cimetidine gels (p>0.05). For each of the three gel concentrations, 0.1% w/w, 0.5% w/w and 0.8% w/w, the cumulative amount of cimetidine in the stratum corneum and underlying skin from pH 5, pH 6.8 and pH 7.5 formulations was similar. Higher ionization did not result in the greater delivery of cimetidine. The topical accumulation of cimetidine however seemed to increase with increase in drug concentration and significantly higher levels of drug were observed in both stratum corneum and stripped skin when treated with 0.8% w/w gel (Fig. 7)
Fig. 7. Effect of pH on the drug accumulation in stratum corneum and underlying skin (0.1% w/w, 0.5% w/w and 0.8% w/w cimetidine gel)

Passive versus microneedle enhanced permeation of cimetidine

Amongst all formulations tested, 0.8% w/w pH 5 cimetidine gel resulted in the highest permeation across microporated skin. A permeation study was carried out to compare the transdermal delivery of this optimized cimetidine gel from intact (untreated) dermatomed porcine skin versus microneedle treated dermatomed porcine skin (Fig. 8). It was observed that the presence of microchannels significantly increased the permeation amount of cimetidine in 24 hours.
Fig. 8. Comparison of passive and microneedle enhanced drug permeation using 0.8% w/w pH5 cimetidine carbopol gel

**Discussion**

Skin is the largest organ of the human body. Even though the average thickness of skin is only 1 mm (171), the stratum corneum and epidermis act as a barrier for the penetration of drug molecules into or across skin. Usually, only lipophilic drugs with low molecular weight can pass through the skin barrier. Cimetidine is a relatively hydrophilic drug (172) and its passive permeation can be restricted by the stratum corneum. Microneedle treatment appears to be a cost effective and minimally invasive way to physically disrupt the stratum corneum and circumvent the skin barrier. However, even after formation of microchannels in skin, formulation related factors could impact the extent of drug permeation. In this study, we investigated the effect of extent of drug ionization in the formulation on its permeation across microchannels. Carbopol gels with different extents of drug ionization were prepared by maintaining the gels at different pHs. Carbopol is a pH dependent polymer, which will be in solution form at acidic pH and the viscosity of the solution increases to a gel like consistency when pH is increased\(^\text{15}\). The three pH values chosen were pH 5 where cimetidine would be 98.4% ionized, pH 6.8, which is equal to the pKa of cimetidine and thus it would be 50% ionized, and pH 7.5 where cimetidine would be 16.6% ionized (Table 1). The extent of percent drug ionized at each pH was calculated using the Henderson- Hasselbalch equation. Also, topical formulations with these pH values would be well tolerated on skin and would not be irritant. The effect of ionization (pH) on cimetidine permeation across...
microchannels was tested at three different drug loadings. To study this, gels were prepared at three different concentrations of cimetidine, 0.1% w/w, 0.5% w/w and 0.8% w/w.

Swarbrick J et al studied the permeability of ionizable compounds across intact human skin. Permeation properties of compounds of interest (chromone-2-carboxylic acids with pKa values less than 2) were studied in the pH range of 5-7 and as a function of concentration. The authors observed in this study that both ionized and unionized forms permeated across skin. However, the permeability coefficients of the unionized forms were approximately $10^4$ times higher compared to the ionized forms. The authors concluded that the flux of an ionizable compound across skin could thus be optimized by controlling the levels of ionized and unionized species in the formulation, which can be achieved by varying the formulation pH (173). Based on this study, in case of intact skin, the unionized moiety of a compound would be preferred for increased delivery across intact skin.

In our study, however, microporation of skin results in an altered structure of the topmost skin layers. Our hypothesis is that creation of microchannels in skin would favor the more hydrophilic, ionized species to permeate the skin. On similar lines, Banks et al demonstrated several approaches for increasing flux of naltrexone and naltrexol over microneedle treated skin. They compared the permeation of naltrexone hydrochloride salt in comparison to naltrexone base with and without microneedle treatment. The authors observed that naltrexone base showed higher permeation across intact skin as compared to the hydrochloride salt while a reverse trend was observed on microneedles treatment. They also performed a study comparing permeation of naltrexone base and an alcohol
derivative of naltrexone base (NTXOL) at two different pH values - 4.5 and 8.5 (174).

From these studies, the authors concluded that charged (protonated) drug moieties resulted in increased permeation across microneedle treated skin. In our study, we have tested for the first time, the effect of extent of ionization of cimetidine on its permeation across microneedle treated skin. We have also studied this as a function of increasing concentration of cimetidine in the formulation.

The permeation studies of 0.1% w/w, 0.5% w/w and 0.8% w/w gel formulations suggest a trend that pH 5 gels, having highest levels of ionized species of cimetidine, showed higher permeation of cimetidine through microchannels. The permeation of cimetidine from carbopol gels across microporated skin positively correlated with the extent of ionization of drug molecules and the drug concentration in the carbopol gel. With an increase of drug concentration in the formulation from 0.1% w/w, 0.5% w/w to 0.8% w/w, the trend of higher ionization resulting in faster and greater drug delivery was more pronounced. There was no significant difference of drug accumulation in the skin layers among different pH formulations with the same drug concentration. This could be because in the drug concentration and pH range studied, the affinity of ionized and unionized species towards stratum corneum and stripped skin could be similar. In this design space, although the degree of ionization affected permeation of cimetidine across microchannels, it did not affect the retention of this drug in skin.

**Conclusion**

Microneedle treatment is a promising approach to enhance transdermal delivery of hydrophilic molecules. The extent of ionization of cimetidine in the formulation significantly influenced permeation through microchannels. Permeation data for 0.1%
w/w, 0.5% w/w and 0.8% w/w cimetidine carbopol gels suggest a trend that pH 5 gel, which contains highest amount of cimetidine in ionized state showed highest permeation of drug through microchannels. As the concentration of drug incorporated in the carbopol gel increased, the delivery of cimetidine also increased, indicating concentration dependency. The effect of ionization on transdermal permeation was more pronounced with increasing drug concentration in the gels. This study shows that permeation of hydrophilic molecules across skin could be optimized by formulating at appropriate pH and drug concentration.
CHAPTER 4

Transdermal Delivery of Human Growth Hormone via Laser-generated Micropores

Abstract

The epidermal skin barrier plays an important role in protecting underlying structures. It allows the passage of low molecular weight lipophilic molecules, but restricts the passage of hydrophilic molecules and macromolecules. The objective of this study was to investigate the feasibility of transdermal delivery of human growth hormone (hGH) through laser-microporated dermatomed porcine ear skin. The permeation of hGH was evaluated at different laser fluences and micropore densities. In vitro permeation studies were performed on vertical Franz diffusion cells using dermatomed porcine ear skin treated with ablative laser (2940 nm; P.L.E.A.S.E®, Pantec Biosolutions AG). The effect of different fluences (34.1, 45.4, and 68.1 J/cm²) at 10% pore density as well as different densities of micropores (5%, 10%, and 15%) at fluence of 34.1 J/cm², on the permeation of hGH was evaluated. After 48 h, application of fluence of 68.1 J/cm² showed permeation of 90.94±3.93 µg/cm², that was significantly higher than that from 34.1 J/cm² group (53.13±1.75 µg/cm², p<0.05), but not as compared to the 45.4 J/cm² group (p>0.05). With the increase in density of micropores from 5% to 15%, permeation of hGH increased significantly from 7.1±2.63 µg/cm² to 95.89±13.43 µg/cm² after 48 h (p<0.05). Thus, overall, the variations in the fluence as well as micropore density of the
laser was observed to influence hGH permeation, through laser microporated dermatomed porcine skin.
Introduction

Human growth hormone (hGH) or somatotropin, a 191 amino acid protein, is produced in the pituitary gland at the base of the brain. Its key function is to regulate protein metabolism and stimulate growth. Human growth hormone was first isolated from human cadaveric pituitaries and was used for children suffering with hormone deficiency (175). The development of recombinant hGH in 1970s, made large-scale commercial and research use possible. hGH is used in the treatment of children’s growth disorders, turner syndrome, chronic renal insufficiency, dwarfism, and Prader-Willi syndrome (176). It is used with performance enhancing drugs to build muscles (177). The hGH levels decrease with age, and thus hGH supplements are proposed to improve viability in geriatric patients and have found a place in the anti-aging industry (178).

Though numerous applications of hGH exist, the modes of its administration are restricted to subcutaneous and intramuscular injections (20). These modes have resulted in poor patient compliance. Inconvenience with the daily use of injections may also result in withdrawal or discontinuation of treatment by patients. Alternative administration routes such as intranasal, pulmonary, and transdermal have thus, been investigated (176)(179). Transdermal delivery, is one of the most promising delivery methods, that can successfully avoid first pass metabolism and provide ease of application of medications. However, skin only allows the passage of drug substances possessing specific physiochemical properties such as molecular weight of less than 500 Da, log P between 1-3, and melting point of < 250 °C (29). Macromolecules, such as large peptides and proteins drugs, cannot be delivered efficiently across the skin due to their size and hydrophilic nature, including hGH, that has a molecular weight of 22 kDa and log P of -
5.2 (21). The passive permeation of hydrophilic molecules is inhibited by the outermost dead lipophilic layer of skin, the stratum corneum. It is thus, the rate limiting step for transdermal drug delivery.

The use of lasers for compromising this outermost layer of epidermis, is gaining popularity and seems a promising physical enhancement technique (45)(46)(47). Laser-assisted microporation creates microchannels in the skin that result in the enhancement of drug delivery. Traditionally, two kinds of lasers are available for skin treatment: non-ablative and ablative. Non-ablative lasers, work by heating up the skin tissue and have short recovery times, as they don’t ablate the stratum corneum. These lasers have been shown to enhance the transdermal permeation of small drug molecules, such as diclofenac sodium and sumatriptan succinate due to the formation of microthermal zones of thermal injury in skin (16). However, as the stratum corneum remains intact, permeation of macromolecules through the skin may not be possible with the use of these lasers. Therefore, ablative lasers, that generate high temperature to vaporize skin tissue and disrupt the skin barrier, are more useful for the purpose of transdermal delivery of macromolecules (161). The laser device used in this study, P.L.E.A.S.E.® (Precise Laser Epidermal System), is an ablative laser that emits laser light with wavelength of 2940 nm, which corresponds to the water absorption peak (17,18). The laser system consists of a diode-pumped fractional Er:YAG (Erbium-Yttrium-Aluminium-Garnet) laser, that forms a matrix of identical micropores or channels, where the pores are at a distance of 100–150 μm from each other. Microbeams are directed from the laser that enable efficient and fractional ablation of skin, with less damage. Overall, P.L.E.A.S.E.® is a patient-compliant technology, that has been suitably programmed to adjust the parameters such
as pulse length, energy, number, and repetition rate in order to control the extent of stratum corneum ablation (17).

Enhancement in transdermal permeation of drug molecules such as 5-aminolevulinic acid, indomethacin, vitamin C, nalbuphine, 5-fluorouracil, oligonucleotides, and DNA with the use of ablative lasers, has been reported in literature (180)(181)(19). Furthermore, the feasibility of transcutaneous immunotherapy or intradermal vaccination with the treatment of ablative laser, has also been investigated (46)(47)(182)(183).

In the present study, we investigated the feasibility of transdermal delivery of hGH, a hydrophilic macromolecule, through laser-microporated skin. Two important parameters, fluence (energy per unit area) as well as density of micropores (number of pores per unit area), that can be adjusted in the P.L.E.A.S.E.® system, were varied and their effect on transdermal permeation of hGH was evaluated and compared in vitro, using dermatomed porcine ear skin. Furthermore, permeation of hGH through dermatomed human and porcine skin was also compared. Also, the microporation of skin using different parameters of P.L.E.A.S.E.® laser system was characterized by confocal laser scanning microscopy, calcein imaging, and histology studies.

**Materials & Methods**

**Materials**

hGH was obtained from Eli Lilly and Company (Indianapolis, IN, USA). Potassium phosphate monobasic and sodium hydroxide were purchased from Fisher Scientific (Bridgewater, NJ, USA). Human growth hormone ELISA (Enzyme-linked immunosorbent assay) Kit was purchased from DRG International Inc., Springfield, NJ, USA. Tissue-Tek® optical coherence tomography compound medium was procured from
Sakura Finetek USA, Inc. (Torrance, CA, USA). Microscopy glass slides were purchased from Globe Scientific, Inc. (Paramus, NJ, USA). Porcine ear skin was obtained from a local slaughter house (Atlanta, GA, USA) and human skin was purchased from The New York Firefighters Skin Bank (New York, NY, USA).

Skin preparation

Dermatomed porcine ear skin was used to study the effect of laser ablation on the delivery of hGH solution. After porcine ears were obtained from slaughter house, intact full thickness porcine ear skin was removed carefully with a scalpel from outer region of the ear. Skin was then washed with 10 mM phosphate buffered saline (pH 7.4), dried, wrapped with parafilm and aluminium foil and stored at -80°C for about a week.

Dermatomed porcine ear skin was prepared before the permeation study. For preparation of dermatomed skin, hair were first trimmed with a pair of scissors and then Dermatom 75 mm (Nouvag AG, Goldach, Switzerland) was used for dermatoming. The average thickness of dermatomed skin pieces obtained was 0.6 ± 0.18 mm. The dermatomed skin was then wrapped in parafilm and stored overnight at -20 °C, before conducting the permeation study.

Dermatomed human skin was stored at -80°C for about a week before use. The thickness of dermatomed human skin used in the study was 0.5±0.12 mm.

hGH stability

The stability of hGH solution over the study duration was assessed by preparing 0.1 μg/mL solution in pH 7.4 phosphate buffer (25 mM) and keeping in oven at 32 °C, to simulate skin temperature (n=3). Samples were analyzed by ELISA kit (DRG International Inc., Springfield, NJ, USA) after 24 and 48 h of storage.
Laser treatment

Laser parameters, such as fluence, density of pores, pulses/pore, and pore array size were programmed using the device software before the permeation study. In the laser fluence study, the laser parameters were set at 34.1 J/cm² (10% density and 7 mm array size), 45.4 J/cm² (10% density and 7 mm array size), and 68.1 J/cm² (10% density and 7 mm array size). In the pore density study, the laser parameters were set at 5% density (34.1 J/cm² and 7 mm array size), 10% density (34.1 J/cm² and 7 mm array size), and 15% density (34.1 J/cm² and 7 mm array size). All the laser ablation parameters were fixed in order to create reproducible micropores. Dermatomed porcine ear skin or human skin was properly placed at the focal length of laser (24.5 mm). Hair were gently trimmed with a pair of scissors prior to the laser treatment. The laser button was then triggered to create the micropores.

Characterization of laser-treated skin

Histology

Histology studies were carried out to confirm the formation and morphology of micropores in the skin, created with different fluence settings (34.1, 45.4 and 68.1 J/cm²) of the laser. Dermatomed porcine ear skin pieces were treated with ablative laser at different fluences and embedded in the Tissue-Tek® optical coherence tomography compound medium (Sakura Finetek USA, Inc., Torrance, CA, USA). The skin samples were then stored in -80 °C freezer for 1 h and sectioned with Microm HM 505 E (Southeast Scientific Inc., Powder Springs, GA, USA) to obtain sections of 10 μm thickness. All the sectioned skin pieces were stained with hematoxylin and eosin (H&E staining) and imaged under a Leica DM 750 microscope (184).
Confocal microscopy

Dermatomed porcine ear skin piece was treated with laser at fluence of 34.1 J/cm², 45.4 J/cm², and 68.1 J/cm². Confocal microscopy was used to measure and confirm the depth of micropores created by laser. Fluoresoft® was applied on the array of micropores for 1 min and excess dye was wiped off with Kimwipes and alcohol swabs (184). Then, the laser-porated skin was placed on a microscope glass slide and depth of microchannels was measured by X-Z sectioning using a computerized Leica SP8 confocal laser microscope (Leica microsystems, Heerbrugg, CH-9435, Switzerland) with 10x objective at an excitation wavelength of 495 nm. A z-stack was used to capture a sequence of images at the same horizontal position (x, y axis) but at different depths (z axis). The z-stack was conducted from skin surface to the point where the signal of calcein visually disappeared. Each step of z-stack was 10 μm. Width of the micropores (n=3), formed at different fluence settings was measured from the microscopic images using ImageJ 1.41o software (National Institutes of Health, USA).

Calcein imaging

Calcein imaging was performed to characterize the number and uniformity of micropores created by laser machine in a predetermined area (0.64 cm²). Skin samples were treated by laser to create 5%, 10%, and 15% density of micropores, while the fluence was fixed at 34.1 J/cm². Calcein solution was applied on dermatomed porcine skin surface for 1 min in a dark room after which it was cleaned thoroughly with Kimwipes and alcohol swabs. A two-dimensional fluorescent image of laser-porated skin sample was obtained using a digital camera (Canon Inc., Ōta, Tokyo, Japan) fitted with a micro-lens having a 525 nm long pass filter (166).
Effect of P.L.E.A.S.E.® device parameters on the permeation of hGH solution through dermatomed porcine ear skin

Evaluation of skin integrity

Before performing the permeation studies, resistance of the skin pieces was measured to assess the barrier integrity and select suitable pieces for permeation study. For the resistance measurement, an arbitrary waveform generator (Agilent 33220A, 20 MHz Function), a 34410A 6½ digital multimeter (Agilent Technologies, Santa Clara, CA, USA), and silver/silver chloride electrodes were used. Phosphate buffered saline, pH 7.4 (10 mM, 300 µL) was added in the donor chamber and 5 mL in the receptor compartment. Skin was mounted on static vertical Franz diffusion cells and allowed to equilibrate for 30 min. After equilibration, silver chloride and silver wire were placed in the donor and receptor compartment, respectively. Voltage across skin (V_S) and entire circuit (V_O) was displayed on the multimeter (V_S), after a load resistor was added in series with skin. Skin resistance (R_S) was calculated using the formula:

\[ R_S = \frac{V_S}{V_O - V_S} \frac{R_L}{V_O} \]

Where, V_O and R_L were 100 mV and 100 kΩ, respectively (185)

The resistances of all the skin pieces were higher than 3.94 kΩ/cm² (168).

In vitro permeation studies through laser microporated porcine skin

In vitro permeation studies were performed on vertical Franz diffusion cells (0.64 cm²) with dermatomed porcine ear skin. Skin was treated with laser at different fluences as well as different densities of micropores as described earlier (n=3). hGH solution (100 µL of 1% w/v hGH in 25 mM phosphate buffer, pH 7.4) was added to the donor compartment and receptor compartment contained 5 mL of pH 7.4 phosphate buffer (25
Receptor samples (0.3 mL) were withdrawn at 0, 1, 3, 6, 9, 24, and 48 h. Each aliquot was replaced with an equivalent volume of fresh receptor solution. All the samples were analyzed by hGH ELISA Kits (DRG International Inc., Springfield, NJ, USA).

Permeation of hGH solution through laser-microporated human skin

Permeation study of 1% w/v hGH solution was further performed using vertical Franz diffusion cell with dermatomed human skin to compare with the permeation results obtained through dermatomed porcine skin (n=3). The fluence of laser was set at 68.1 J/cm² and density of micropores was 10%. Donor volume and sampling schedule used were similar to the permeation study performed with porcine skin. Samples were analyzed by hGH ELISA Kit (DRG International Inc., Springfield, NJ, USA).

Data analysis

All the results for the in vitro permeation studies were reported as the mean of at least triplicates with standard deviation. Statistical calculations were performed using Microsoft Excel. One-way ANOVA (analysis of variance) followed by Tukey’s HSD (honest significant difference) post hoc test was performed to determine statistical difference between groups, which was depicted by p value less than 0.05.

Results

Stability of hGH formulation

Human growth hormone recovery, after 24 and 48 h of storage at 32 °C, was found to be 100 ±1.6% and 92.1± 0.7%, respectively.

Characterization of micropores

Histology
Images of histological sections of porcine ear skin pieces treated with three different laser fluences are shown in Fig. 9. At low fluence (34.1 J/cm²), laser disrupted stratum corneum and upper layers of epidermis, without affecting the deeper skin layers (Fig. 9a). Increasing the fluence to 45.4 J/cm² (Fig. 9b) and 68.1 J/cm² (Fig. 9c), resulted in the formation of deeper micropores reaching the dermo-epidermal junction.

![Images of histological sections of porcine ear skin pieces treated with different laser fluences](image)

(a) (b) (c)

Fig 9. Haematoxylin & Eosin stained histological sections of porcine ear skin samples after laser treatment at different fluence (J/cm²) (a) 34.1, (b) 45.4 and (c) 68.1 with 20x objective. Scale bar showing in the image was 500 μm.

Confocal microscopy

After application of calcein solution on the treated porcine skin, calcein penetrated through the breached stratum corneum, along the micropores, and reached the lower epidermal tissue. Results (Fig. 10a, 10b, and 10c) indicated that the depth of microchannels created by laser at fluence of 34.1, 45.4 and 68.1 J/cm² was about 140, 170 and 250 μm, respectively. Also, the widths of the micropores were 250 ± 3.46, 253 ± 11.15 and 271 ± 12.5 μm, respectively.
Fig. 10. Confocal microscopy z-stack of micropores created by laser at fluence of (a) 34.1, (b) 45.4 and (c) 68.1 J/cm². Scale bar showing in the image was 250 μm.

Calcein imaging

Fig. 11 shows the array of micropores created by laser treatment with different density settings. Laser treatment with pore densities of 5, 10, and 15% resulted in creation of 50, 80, and 130 micropores, respectively, on 0.64 cm² skin surface.

Fig 11. Micropores array created by laser equipment at (a) 5%, (b) 10% and (c) 15% micropores density while the fluence was fixed at 34.1 J/cm².

Effect of laser ablation parameters on the permeation of hGH solution

Effect of fluence on the permeation of hGH solution through microporated dermatomed porcine ear skin

Treatment with ablative laser generated an array of micropores on dermatomed porcine ear skin. In the comparison of effect of fluence on the hGH permeation, pore density was fixed at 10% and fluence of 34.1, 45.4, and 68.1 J/cm² was applied. After 48 h, 77.12 ± 10.77 μg/cm² hGH was delivered into the receptor with the application of fluence of 45.4 J/cm², which was significantly higher than that observed from 34.1 J/cm² group (53.13±1.75 μg/cm², p<0.05). Furthermore, application of fluence of 68.1 J/cm², showed higher permeation within 48 h (90.94±3.93 μg/cm²) as compared to the other two
groups (Fig. 12). It was significantly higher than the permeation from 34.1 J/cm² group (p<0.05), but not significantly different from the amount of hGH permeated from the 45.4 J/cm² group (p>0.05). Also, greater depth of micropores resulted in shorter lag time of drug permeation. Drug became detectable in the receptor at 1 h from the group with the treatment of fluence at 68.1 J/cm². However, no drug was detected in the receptor from 34.1 and 45.4 J/cm² group until 3 h.

Fig 12. Cumulative hGH permeation across laser treated porcine ear skin at the fluence of 34.1, 45.4 and 68.1 J/cm².

Effect of micropores density on the permeation of hGH solution through microporated dermatomized porcine ear skin

This study compared the effect of density of micropores on the permeation of hGH through laser-treated porcine ear skin. With the increase in density of micropores from 5% to 10% and then 15%, the number of micropores in 0.64 cm² area increased from about 50, 80, to 130, respectively and consequently, the permeation of hGH was also observed to increase. During initial 3 h, the cumulative amount of hGH permeated was similar from the three groups (<1 µg/cm²). However, after 6 h, the amount of hGH permeated
through laser-porated skin from the 15% density group (15.74±1.63 µg/cm²) was significantly higher than that from 5% (0.69±0.32 µg/cm²), and 10% (1.57±0.16 µg/cm²) density group (p<0.05). After 48 h, 95.89±13.43 µg/cm² µg/cm² hGH was delivered through the porcine ear skin from 15% density group, which was significantly higher than the permeation from 5% (7.1±2.63 µg/cm²) as well as 10% density group (53.13±1.75 µg/cm²) (p<0.05) (Fig. 13). Also, the amount of hGH permeation from 10% density group was significantly higher than the 5% density group, after 48 h (p<0.05).

![Graph showing permeation of hGH across different laser treatments](image)

**Fig 13.** Effect of pore density on the permeation of hGH across laser treated porcine ear skin.

**Permeation of hGH solution through laser treated human skin**

Permeation of hGH solution was further performed using dermatomed human skin with the rationale to compare the results with the drug permeation obtained through porcine skin, under similar test conditions. Skin pieces used for the permeation study were treated with laser at the fluence of 68.1 J/cm² and 10% pore density. Figure 14 shows the comparative hGH permeation through laser-porated porcine ear skin and
human skin. Results showed similar permeation profile of hGH through porcine ear skin and human skin. The cumulative amount of drug permeation through human skin, after 24 h and 48 h (83.69 ± 12.63 µg/cm² and 106.29 ± 15.03 µg/cm², respectively) was not significantly different from that through porcine ear skin (p>0.05) (Fig. 14).

Fig 14. Cumulative hGH permeation through laser treated dermatomed porcine ear skin and human skin.

Discussion

Passive permeation of peptides or proteins across intact skin is not feasible due to the lipophilic outer layer of stratum corneum that restricts the penetration of hydrophilic macromolecules such as hGH (log P of -5.2). Ablation of stratum corneum by ablative laser enables the entry of peptides or proteins into the skin and its subsequent diffusion through the more hydrophilic lower epidermis layers, thus enhancing their transdermal permeation (186) (187). Changing the laser ablation parameters can affect the number as well as depth of the micropores created.

Fluence, which is defined as the energy applied per unit area (J/ cm²), can be modified in order to create pores with different depth. Moreover, since it is a scanning
system, each of the micropores of the array are created by the full energy of the laser beam, resulting in uniformity in the depth of the created pores (188). The increase in depth of the micropores with the increase in applied fluence was well-evident in the present study from the results of histological evaluation as well as confocal studies as shown in Figure 1 and 2, respectively. Histology studies showed that lower fluence (34.1 J/cm²) ablated the stratum corneum and upper layers of epidermis, whereas higher fluences (45.4 J/cm² and 68.1 J/cm²) resulted in formation of deeper pores that could reach the dermo-epidermal junction. The results of histological evaluation were in concordance with those reported by Bachhav et al., where the use of P.L.E.A.S.E.® laser device, at a fluence of 45.3 J/cm², was shown to reach the epidermal-dermal junction and created deeper pores up to the dermis (19). Furthermore, the confocal microscopy results also supported the observations of the histology study and the depth values were also close to the expected depths of the micropores by the use of different fluence settings at a fixed pore density, as showed by the P.L.E.A.S.E.® laser device software. Results of the confocal microscopy indicated that the depth of microchannels created by laser at fluence of 34.1, 45.4 and 68.1 J/cm² were about 140, 170, and 250 μm, respectively (Figure 2a, 2b, and 2c). These observed depths were close to the theoretical expected depths of micropores (134, 182 and 273 μm), respectively, that were shown by the laser system software.

With respect to the results of the permeation study, significant enhancement in the cumulative amount of hGH permeation was observed with the increase in fluence from 34.1 J/cm² to 45.4 J/cm² as well as 68.1 J/cm² (p<0.05), which can be attributed to the formation of deeper micropores in case of 45.4 J/cm² and 68.1 J/cm² groups, reaching the
dermo-epidermal junction as compared to the ablation of stratum corneum and super
facial layer of epidermis in the former group. Therefore, as there was less resistance to
the movement of the drug in case of deeper microchannels, permeation was observed to
be higher in the latter two groups as compared to the lowest fluence group. However,
there was no significant difference in the amount of drug permeation between 45.4 J/cm²
and 68.1 J/cm² groups. This would have probably occurred as skin treatment with both
these fluence settings resulted in ablation of stratum corneum as well as epidermis that
eventually led to formation of deeper microchannels, thus providing less resistance to the
permeation of drug in the skin in both the cases. Even though, micropores created by
application of 68.1 J/cm² energy were deeper than those created by 45.4 J/cm², as the
epidermal barrier was overcome in both the cases, ablation of part of dermis did not
significantly affect the amount of drug permeation after 48 h in case of 68.1 J/cm² group.
Thus, overall, it would be hard to generalize that increase in fluence would always
enhance drug permeation as the depth of micropores, extent of ablation, and
physicochemical properties of the drug also play an important role. This has also been
reported in other studies in literature as well. Bachhav et al. investigated the effect of
laser fluence on the permeation of lidocaine through the laser treated skin and results
showed that increasing the fluence did not significantly change lidocaine permeation (19).
At the lowest fluence of 22.65 J/cm², about 1180 ± 448 µg/cm² lidocaine permeated into
the receptor, which was not significantly lower than the amount of drug permeated across
the laser treated skin at the fluence of 135.9 J/cm² (p>0.05) Permeation of lidocaine was
thus, not influenced by the fluence of laser. This may be possible as after the removal of
rate limiting barrier of stratum corneum, lidocaine can easily diffuse through the
epidermis and dermis layer due to its small molecular size and moderately lipophilic nature. In another study, Oni et al. investigated the use of laser to enhance the transdermal absorption of topical lidocaine in an in vivo animal model (189). The depths of micropores that were evaluated included: 25, 50, 250, and 500 µm. Peak levels of lidocaine in the plasma were significantly higher (P=0.0002) at the depth of micropores at 250 µm as compared to 25, 50, and 500 µm. This was explained due to the fact that the vascular network exists between 100 to 300 µm depth of skin. Therefore, pores with depth of 250 µm resulted in better absorption of lidocaine as compared to 25 and 50 µm deep pores. However, the reason of lesser drug permeation through 500 µm deep pores as compared to the 250 µm deep pores, can be due to thicker lining of coagulated cells surrounding the microchannels in case of higher energy settings (500 µm), that would have probably impeded drug permeation. Also, bleeding was observed in case of laser application resulting in formation of 500 µm deep pores, that would also have made the absorption of lidocaine against the oncotic pressure pushing the fluid out of skin, difficult (189). Furthermore, in another study performed by Bachav et al., increase in fluence was found to increase the permeation of FITC labeled bovine serum albumin, but not cytochrome c across laser porated porcine skin (188).

Furthermore, greater depth of micropores resulted in shorter lag time of hGH permeation. Drug became detectable in the receptor at 1 h from the group with the treatment of fluence at 68.1 J/cm². However, no drug was detected in the receptor from 34.1 and 45.4 J/cm² group until 3 h. Faster onset of drug permeation by application of fluence of 135.9 J/cm² (420 pores) was also observed in the study conducted by Bachav
et al., where lidocaine uptake was observed in the skin as well as in the receptor, just after 5 min of treatment with P.L.E.A.S.E.® system.

We also investigated the effect of micropore density on the permeation of hGH solution through laser-treated skin. Pore densities of 5%, 10%, and 15%, at a fixed fluence of 34.1 J/cm², resulted in an array of 50, 80, and 130 pores respectively, in 0.64 cm² area. Therefore, with the increase in pore density, number of pores created increased. With the increase in pore density, the amount of permeation of hGH was also observed to increase. These results, therefore, revealed that the cumulative amount of hGh permeation across the laser-porated skin depends on the number of micropores created in certain area by the laser. This can be attributed to greater area of stratum corneum ablated at higher pore density. Thus, as it is the rate limiting barrier, the more the stratum corneum is breached, the greater would be the drug permeation. Our results of enhancement in drug permeation were in concordance with those reported by Bachav et al. In this study, the authors investigated the effect of pore density on the permeation of diclofenac and observed a linear correlation between cumulative permeation amount of diclofenac in 24 h and number of micropores formed (190). Also, in another study, increase in pore density was found to directly increase the permeation of macromolecular proteins such as cytochrome c and FITC labelled bovine serum albumin across laser porated porcine ear skin (188). However, the results of present study were in contrast with those also reported by Bachhav et al. in another study. It included investigation of delivery of lidocaine through laser-treated skin consisting of 0, 150, 300, 450 and 900 pores in 1.9 cm² diffusion area. Cumulative amount of drug permeation across microporated skin with 300, 450, and 900 micropores showed no significant difference (p>0.05) (19). This was
explained due to the high delivery efficiency of lidocaine that signified faster depletion of
drug from the donor solution and subsequent reduction in the thermodynamic driving
force with greater pore density. Therefore, as the concentration gradient between donor
and receptor compartment decreased, driving force for drug permeation also decreased
(19).

Furthermore, the permeation of hGH through porcine ear skin and human skin, when
investigated under similar test conditions, was not found to be significantly different.
Therefore, the results indicated similarity in the hGH transport kinetics across laser
porated porcine and human skin, which was consistent with the literature reports that
mention porcine ear skin as a good model to substitute human skin for in vitro drug
permeation studies (191)(192).

**Conclusion**

The results showed that transdermal permeation of hGH was feasible across
dermatomed human and porcine skin, microporated with the P.L.E.A.S.E.® technology.
Modifications in the operational parameters of laser, such as fluence and pore density,
can control the depth and extent of ablation of stratum corneum. Increase in fluence and
pore density, results in increase in the depth and number of micropores created in a given
area, respectively. hGH transport was directly affected by the increase in pore number. In
terms of pore depth, fluence settings resulting in formation of pores reaching the dermo-
epidermal junction were more efficient and showed higher drug delivery as compared to
the shallow pores formed as a result of ablation of stratum corneum and upper epidermal
layers. Furthermore, greater depth of micropores also depicted shorter lag time for drug
permeation, signifying faster onset of drug delivery. These preliminary results are very
encouraging and indicate the possibility of transdermal delivery of hGH using microporation with the P.L.E.A.S.E.® technology.
CHAPTER 5

Controlled topical delivery of salicylic acid using microsponge delivery system

Abstract

Various techniques are currently used to provide controlled delivery for drugs. One such technique is microsponge based drug delivery technique where both hydrophilic and hydrophobic polymers are allowed to react and form a tight network with drug entrapped in it. In this research, we developed a microsponge matrix system using polyvinyl alcohol and Eudragit® polymer with salicylic acid. The aim here was to evaluate the controlled topical delivery of salicylic acid using the microsponge. Different ratios of drug to polymer were prepared in order to compare the effect of drug: polymer ratio on the drug release profiles. The prepared formulations were characterized using light microscopy and scanning electron microscopy (SEM). The in vitro drug release was tested in Sotax dissolution apparatus and in vitro permeation was conducted on vertical Franz diffusion cells using dermatomed human skin. Receptor samples were analyzed by HPLC. In vitro release study showed salicylic acid microsponge gels showed similar release rate, which was much slower than the gel without microsponge. The in vitro permeation study showed a cumulative permeation of $88.86 \pm 6.37 \, \mu g/cm^2$, $537.77 \pm 37.02 \, \mu g/cm^2$, and $754.62 \pm 84.98 \, \mu g/cm^2$ and flux of $0.53 \pm 0.05 \, \mu g/cm^2/h$, $4.6 \pm 1.39 \, \mu g/cm^2/h$, and $6.2 \pm 1.74 \, \mu g/cm^2/h$ at 72 h for F2, F4 and F5 respectively. Thus, the developed microsponge
matrix system using polyvinyl alcohol and Eudragit polymer can serve as a controlled delivery system.
Introduction

Salicylic acid is one of the most widely consumed analgesics, antipyretic and anti-inflammatory agent (193) and it is commonly used in topical formulations for acne treatment. Statistics data shows nearly 85% of people aged between 12 to 25 years old, about 8% of adults aged 25 to 34 years and about 3% of adults aged 35 to 44 years have been affected by different degree of acne (194). Due to its keratolytic and bacteriostatic properties, topical application of salicylic acid has been shown to reduce the rate of keratinocyte proliferation (12). The formulation containing 10% to 40% of salicylic acid can be used for the treatment of warts and localized hyperkeratosis; over the counter products for treatment of acne contain concentrations of 5% or less. Even though the drug concentration in the formulations for acne treatment is low, these products are liable to induce skin irritation, dryness, peeling and even transient local edema when used on the sensitive skin type. The severity of skin irritation is related to the amount of drug present in the skin; encapsulation of salicylic acid can reduce the side effect to a great extent (195).

Microsponge is polymeric microsphere composed of porous microsphere; it has myriad inter-connected voids of particle size ranging from 5 to 300 μm. Microsponges are tiny, sponge like microparticles, which can entrap various active ingredients (196). They are inert and do not pass through the skin; they get deposited in the smaller nooks and crannies of skin. Microsponge can slow down the release of drug from formulations in controlled manner, which reduces the skin irritation potential. Microsponge can be prepared by several methods such as emulsion solvent diffusion method or suspension polymerization in a liquid-liquid system (197). Other than controlled release of active
ingredients, microsponge can enhance the drug stability, significantly reduce side effects and prevent excessive drug accumulation within the epidermis and dermis (198).

The purpose of present investigation was to prepare salicylic acid microsponges using Eudragit® RS100 and polyvinyl alcohol (PVA) with different drug:polymer ratios by quasi-emulsion diffusion method. Eudragit® polymers are a group of non-biodegradable polymers which have wide spectrum of applications in controlled drug delivery (199). The factors affecting the morphology of microsponges were explored using scanning electron microscopy (SEM). The study also compared the release rate and flux of salicylic acid from formulations with/without microsponge.

**Materials and methods**

**Materials**

Salicylic acid was purchased from Sigma-Aldrich (St. Louis, MO, USA). Poly vinyl alcohol (PVA) 40-88 was obtained from EMD Millipore Corporation (Billerica, MA, USA) and Eudragit® RS100 was obtained from Evonik Industries AG (Essen, Germany). Carbopol 980NF was provided by Lubrizol Advanced Materials, Inc. (Cleveland, OH, USA). Ethanol, acetonitrile, trifluoroacetic acid, phosphate buffer saline and triethanolamine were purchased from Fisher Scientific (New Jersey, USA). Human skin was purchased from The New York Firefighters Skin Bank (New York, NY, USA). De-ionized water was used to dilute phosphate buffer saline and prepare the solution required in the HPLC analysis.

**Methods**

Preparation of microsponge formulation
The hydrophobic Eudragit® RS100 polymer was dissolved in dichloromethane with addition of 1% salicylic acid. The hydrophilic polymer polyvinyl alcohol was dissolved in deionized water followed by gradual addition of Eudragit® RS100 polymer solution, which has dissolved drug in it. The mixing process was continued for at least 8 h at room temperature until dichloromethane was completely evaporated and this resulted in the formation of a cross linked structure, the microsponge.

The formed microsponge was dried in room temperature and added gradually to the hydro-alcoholic 2.0% w/w carbopol gel. In addition, 1.0% salicylic acid in hydro-alcoholic 2.0% carbopol gel without microsponge was also prepared (F5). For the evaluation of the effect of drug to polymer ratio on the release of salicylic acid from microsponge formulation, three different weight ratios of the drug to Eudragit® RS100 (4:1, 2:1 and 4:3) were employed to F1, F2 and F3 respectively. For F4, 0.25% w/w drug was dissolved in the carbopol gel; 0.75% w/w drug was incorporated in the microsponges and dispersed in the carbopol gel. In all these formulations, the total amount of drug and polymer were kept constant.

Physical examination of formulation

The clarity, color and homogeneity of salicylic acid carbopol gels were evaluated by visual inspection. Gels were visually inspected against a black and white background; the homogeneity was used to confirm all the components were mixed completely. All the gels were visually inspected for their appearance and to ensure that they are free of aggregates. In order to avoid the skin irritation, the pH of salicylic acid formulation was tested by a digital pH meter and pH of gel was adjusted to the appropriate value before formulation application.
Photomicroscopic Analysis

Microsponge sample was applied on the glass slide and examined for morphological characteristics under a bright microscope (Leica DM750, Berlin, Germany).

Scanning electron microscopy

The Phenom™ field emission scanning electron microscopy (SEM) (Nanoscience Instruments, Inc., Phoenix, AZ, USA) was performed to investigate the morphology of microsponge. The dried microsponge particles were mounted on SEM Pin stub mount with double adhesive tape (Ted Pella, Inc., Redding, CA, USA). By analyzing SEM images, the shape and dimensions of microsponges can be obtained.

Determination of loading efficiency and production yield

The loading efficiency (%) was calculated according to the following equation:

\[
\text{Loading efficiency (\%)} = \left( \frac{\text{actual salicylic acid content in microsponge}}{\text{theoretical salicylic acid content}} \right) \times 100
\]

The production yield was calculated based on the initial weight of raw material and the weight of dried microsponge powder (200)

Skin preparation

Porcine skin used in the permeation studies was obtained from slaughter house. Hair on the stratum corneum side of porcine skin and adhering subcutaneous fat were carefully removed by scissor. Skin was dermatomed to the thickness of about 0.5 mm. Dermatomed porcine skin was cut into small pieces of appropriate size to fit the donor compartment. In order to evaluate the integrity of skin pieces, skin resistance was tested before the permeation study. The resistances of all the skin pieces were higher than 4 kΩ cm² (168).
In vitro release study

Release study (n=3) was carried out using dissolution apparatus 5 to compare the drug release from the formulation with/without the microsponge. The release study was performed at 32 °C, which is the same temperature as skin surface and 500 mL 10 mM pH 7.4 phosphate buffer saline was filled into each vessel. Samples (1 mL) were withdrawn at predetermined time points and were replaced with fresh dissolution media. All the samples were filtered with 0.45 mm nylon filter and analyzed by HPLC.

In vitro permeation studies

In vitro studies (n≥3) were carried out on dermatomed porcine ear skin using vertical Franz diffusion cells. The receptor compartment was surrounded by a water jacket to maintain the temperature of skin surface at 32±1 °C. All the receptor compartments were cleaned properly and filled with receptor medium (pH 7.4 10 mM phosphate buffer saline) before the experiment. Skin was mounted on the surface of receptor compartment, donor compartments were placed on the skin and entire assembly was held together using clamps. Salicylic acid formulation with/without microsponge (100 mg) was placed on the human skin surface; sample solution (300 μL) was withdrawn at predetermined time points and was replenished with same amount of fresh receptor medium. The obtained samples were analyzed using a HPLC method.

Results

Characterization of microsponge
The bright microscopy image showed the formation and morphology of microsponge by using emulsion solvent diffusion method. The formed microsponges had regular, spherical shape with roughness on the surface and several pores (Fig. 15).

Fig.15 Photomicrograph of the microsponges prepared by solvent diffusion method

The SEM image (Figure 16) showed dimension and morphology of microsponges, which were prepared in different Eudragit®RS100: PVA ratios. The size of microsponges ranged from 5 μm to 30 μm; with the increase of Eudragit®RS100: PVA ratios from 3:10, 1:10 to 1:30, the size of microsponge increased to some extent. Especially the microsponge showed in Figure 16(a), the average dimensions were much smaller than that shown in Figure 16(b) and Figure 16(c) and there are more debris shown in Figure 16(a).
Figure 16. The effect of Eudragit® RS100: PVA ratios on the morphology of salicylic acid microsponges: (a) 3:10; (b) 1:10; (c) 1:30 which was incorporated in F1, F2 and F3 respectively.

Determination of loading efficiency and production yield

The loading efficiency and production yield of microsponges prepared in different polymer ratios were shown in Table 1. An increase in ratios of Eudragit® RS100: PVA resulted in decreased particle size, increased production yield and increased loading efficiency.

Table 2. Composition of salicylic acid microsponge gel

<table>
<thead>
<tr>
<th>Formulation</th>
<th>Drug content</th>
<th>Drug:Eudragit RS100 ratio</th>
<th>Eudragit RS100:PVA ratio</th>
<th>Loading efficiency (%)</th>
<th>Production yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>F 1</td>
<td>1% w/w</td>
<td>4:1</td>
<td>3:10</td>
<td>61 ±3.3</td>
<td>51 ±4.4</td>
</tr>
<tr>
<td>F 2</td>
<td>1% w/w</td>
<td>2:1</td>
<td>1:10</td>
<td>83 ±2.7</td>
<td>72 ±3.7</td>
</tr>
<tr>
<td>F 3</td>
<td>1% w/w</td>
<td>4:3</td>
<td>1:30</td>
<td>71 ±4.5</td>
<td>69 ±5.3</td>
</tr>
<tr>
<td>F 4</td>
<td>1% w/w</td>
<td>2:1</td>
<td>1:10</td>
<td>83 ±2.7</td>
<td>72 ±3.7</td>
</tr>
<tr>
<td>F 5</td>
<td>1% w/w</td>
<td>---</td>
<td>---</td>
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</tr>
</tbody>
</table>

Physical examination of formulation

All the formulated microsponge gels were characterized for their color and homogeneity. The salicylic acid carbopol gels with/without microsponge were transparent with a smooth homogeneous appearance and texture. They were easily spreadable and no aggregates were observed in all the formulations. The pH values of all the microsponge gels were tested before the permeation study; the pH of all the formulations was about pH 5.

In vitro release study

Release studies were performed using dissolution apparatus 5 to test the salicylic acid
release profiles from carbopol gels with/without microsponge, and results showed since the drug concentration was the same in all the formulations, cumulative amounts of salicylic acid from formulations were similar (Figure 17). The rate of drug release from F4 and F5 over the first hour was much higher compared to that from other three formulations with microsponges. Even though F4 also contains microsponges, the burst release of active ingredient could be attributed to the presence of 0.25% non-encapsulated salicylic acid formulated in the carbopol gel. Results illustrate microsponge did retard the release of salicylic acid from the formulations. Statistical study (1-way analysis of variance) was performed and there was no significant difference (p > 0.05) among formulations with microsponge.

Figure 17. In vitro release study of salicylic acid gels with/without microsponge

In vitro permeation studies

According to the results from release study, the release profiles of all the three salicylic acid gels with microsponge were similar. While F4 showed higher loading efficiency, production yield and more intact structure in SEM images compared with other two formulations and it was selected to further perform the permeation study on
dermatomed human skin. The permeation profile (Figure 18) of salicylic acid from 1% w/w gel with/without microsponge suggested that incorporation of salicylic acid in the microsponge microparticles resulted in significantly lower permeation of drug across the skin in 72 h (p < 0.05). The data shows a trend that the rate of permeation of salicylic acid across skin decreased with an increase in drug incorporation in the microsponge. In this case, salicylic acid gel without microsponge permeated 754.62 ± 84.98 µg/cm² drug through the dermatomed human skin at 72 h, which is much higher than the formulations with microsponge. The *in vitro* permeation study showed a cumulative permeation of 88.86 ± 6.37 µg/cm², 537.77± 37.02 µg/cm², and 754.62 ± 84.98 µg/cm² and flux of 0.53 ± 0.05 µg/cm²/h, 4.6± 1.39 µg/cm²/h and 6.2± 1.74 µg/cm²/h at 72 h of F2, F4 and F5 respectively.

![Figure 18. In vitro permeation study of salicylic acid gels with/without microsponge](image)

**Discussion**
Salicylic acid is used in the treatment of facial acne and produces common effects in the skin due to excessive penetration and accumulation in the skin (12). It was hypothesized that controlled release of salicylic acid to the skin could reduce the side effects by reducing the drug accumulation in the skin and percutaneous absorption. In this study, we utilized Eudragit® RS100 microsponge as carrier for the controlled release of salicylic acid. Instead of using suspension polymerization method in a liquid-liquid system, microsponges were prepared by quasi emulsion solvent diffusion method (197). Salicylic acid and Eudragit® RS100 was dissolved in dichloromethane and PVA was used as emulsifier. Dichloromethane is capable of dissolving both salicylic acid and Eudragit® RS100, which was selected as internal phase (201). The rapid diffusion of dichloromethane into the PVA solution could reduce the solubility of the water-insoluble polymer Eudragit® RS100 in the droplets. The mixing of the dichloromethane and PVA solution at the interface of the droplets induced precipitation of the Eudragit® RS100 and forming a shell enclosing the internal phase. The evaporation of dichloromethane from the internal phase through the shell promoted further formation of the cavities on the surface of microsponge. The formed droplets with tiny cavities on the surface were solidified in the aqueous phase via diffusion of the solvent (202).

The bright microscopy image (Figure 15) showed the spherical shape microparticles with roughness on the surface were formed. The dimensions of microsponges were various. Scanning electron microscopy of the salicylic acid microsponges are shown in Figure 16. It showed microsponges have predominantly spherical shape with orifices and it could be attributed to the diffusion of dichloromethane from the surface of the microparticles. SEM images illustrate that the ratio of drug/polymer changed the size of
microsponges; as the ratio of drug/polymer decreased from 4:1, 2:1 to 4:3, particle size was increased. The size of microsponges in Figure 16(a) was much smaller than that in Figure 16(b) and Figure 16(c) and more debris formed in Figure 16(a). Increasing the polymer concentration in the internal phase can increase the viscosity of solution; when the internal phase with high viscosity was poured into the aqueous phase, bigger droplets were formed and microsponge particle sizes increased. This has been testified by some of the published microsponge studies. Nokhodchi A et al. study showed the mean particle diameters for the drug/polymer ratios of 3:1 and 13:1 were $442 \pm 50 \mu m$ and $310 \pm 34 \mu m$, respectively (196). When the ratio of drug/polymer is high, the amount of Eudragit® RS100 per microsponges will be less; with the evaporation of organic solvent, nearly all of the internal phase is converted to the form of solid microsponges and separated particles formed. Therefore, in high drug/polymer ratios, less amount of polymer surrounded the salicylic acid and the particle size decreased (203). This study also illustrated the stirring rate can also influence the morphology of microsponges. The drug/polymer ratio was fixed at 13:1 and the stirring rate was altered in the range of 1000 to 4000 rpm. Since the dispersion procedure of internal phase into the aqueous phase to form the droplets is controlled by the agitation speed of stirrer. The results showed as the stirring rate increased from 1000 to 4000 rpm, the size of microparticles was reduced from $604 \pm 58$ to $310 \pm 34 \mu m$ (105). Due to the coalescence and aggregation of polymer globules at lower stirring rate, the mean particle size decreased at high stirring rate. At the same time, a vigorous, uniform, increased mechanical shear is imposed at high stirring rate, which resulted in a rapid division of the formed droplets. Other than that the emulsifier, PVA was nonionic molecules, which can associate away from the oil-water
interface at higher concentrations. Bigger microsponges were obtained when the concentration of PVA increased, which can be attributed to the increased viscosity of PVA solution resulting in the formation of larger emulsion droplets and larger microsponge dimensions.

As shown in Table 2, the mean amount of salicylic acid entrapped in the microsponges was found to be less than theoretical value for all the different drug:Eudragit® RS100 polymer ratios employed. This could be attributed to the dissolving of some salicylic acid in aqueous phase. When drug: Eudragit® RS100 ratio was fixed at 2:1, loading efficiency of formed microsponge is higher than that prepared by other drug:polymer ratios. As can be seen from Figure 16, when drug: polymer ratio was set at 4:1, the size of microsponge is much smaller than the other two types of microsponges prepared at drug: polymer ratio at 2:1 and 4:3. The morphology of microsponges at the drug: polymer ratio of 4:1 is not as intact as the microsponges in F2 and F3; there are more debris shown in the Figure 16(a). Besides that when drug: polymer ratio was 4:1, the production yield and loading efficiency were much lower, therefore, in vitro permeation study on dermatomed human skin was not carried out in this ratio. When the ratio of drug: polymer decreased from 2:1 to 4:3, the production yield and loading efficiency also reduced. The reason for increased production yield and loading efficiency at higher drug: polymer ratios could be due to the reduced diffusion rate of dichloromethane from polymer solution into aqueous phase, which provides longer time for droplets formation (204). M. Jelvehgari et al. performed study of benzoyl peroxide microsponges, their results also showed higher drug loading efficiencies were obtained at higher drug: polymer ratios. Higher drug: polymer ratios caused slightly increase of
viscosity of dispersed phase and higher amount of drug in per unit polymer (205). When organic solvent evaporated out, nearly all the dissolved Eudragit® RS100 polymer was cross linked with PVA and converted to the solid microsponges and separated particles formed.

The release study was carried out on dissolution apparatus 5; 1% w/w salicylic acid was formulated into carbopol gels with/without microsponges (Figure 17). Drug release from resultant formulations was studied and results showed that the formulations (F1, F2 and F3) with all the drug incorporated into the microsponges, the rate of drug release was much slower than that from F4 and F5, especially at the beginning one to two hours. Initial burst release observed in case of F4 can be allocated to existence of 0.25% w/w non-encapsulated drug in the carbopol gel. After burst release, drug release rate slowed down which was similar to F1, F2 and F3. Drug release from F5 without microsponges showed in contrast to microsponge-based gel, it exhibited immediate drug release up to 2 h. Sustained drug release from F1, F2, F3 and F4 minimized the side effects, skin irritation and hypersensitivity reactions.

The in vitro permeation study was carried out with F2, F4 and F5 on dermatomed human skin and the cumulative amount of salicylic acid permeated per unit area of skin from carbopol gel with/without microspunge was plotted against time as shown in Fig.5. It has been observed that F5 exhibited much faster and higher drug permeation on completion of 72 h than the other two formulations, especially F2. In case of F4, there was a slight decrease in cumulative amount of drug diffused within 72 h. All the formulations showed relatively faster drug permeation rate for the first 1 to 2 h compared to next 70 h, this was possibly due to the release of non-encapsulated salicylic acid in the
formulations, which affected the flux to some extent. The existence of microsponge did retard the salicylic permeation; drug permeated through the skin in controlled manner.

**Conclusion**

Microsponge was developed using quasi emulsion solvent diffusion method and characterized as an effective carrier for the topical delivery of salicylic acid. The properties of microsponge were significantly affected by the drug: polymer ratios and the amount of emulsifier (PVA). It can be seen from *in vitro* dissolution and permeation experiments that the microsponge system plays a vital role in controlling the release as well as the permeation of salicylic acid topically. Thus, the developed microsponge matrix system using polyvinyl alcohol and Eudragit® polymer can serve as a controlled delivery system. Microsponge was proved to be a potential carrier for salicylic acid in topical acne therapy to prolong drug release, minimize skin irritation and side effects.
CHAPTER 6

Activated Carbon Based System for Disposal of Psychoactive Medications

Abstract:

The misuse and improper disposal of psychoactive medications is a major safety and environmental concern. Hence, the proper disposal of these medications is critically important. The drug deactivation system which contains activated carbon offers a unique disposal method. In the present study, deactivation efficiency of this system was tested by using three model psychoactive drugs. The extent and rate of deactivation of the drugs was determined at several time points. After 28 days in the presence of activated carbon, extent of leaching out of the drugs was evaluated. HPLC validation was performed for each drug to ensure that the analytical method employed was suitable for its intended use. The method was found to be specific, accurate and precise for analyzing the drugs. Deactivation started immediately after addition of the medications into the disposal pouches. Within 8 hours, about 47%, 70% and 97% of diazepam, lorazepam and buprenorphine were adsorbed by the activated carbon respectively. By the end of 28 days, over 99% of all drugs were deactivated. The desorption/leaching study showed that less than 1% of the active ingredients leached out from the activated carbon. Thus, this deactivation system can be successfully used for the disposal of psychoactive medications.
Introduction

Psychoactive drugs are increasingly being prescribed as antidepressants or for the treatment of insomnia and for pain relief. A large share of prescriptions for older adults are for psychoactive, mood-changing drugs that carry the potential for misuse, abuse, or dependency. According to the survey sponsored by the Substance Abuse and Mental Health Services Administration (SAMHSA), the non-medical use of prescription psychotherapeutics surpassed the total illicit use of cocaine, hallucinogens and heroin as the leading drugs of abuse in individuals over 12 years of age (206). In 2014, approximately 6.5 million illicit drug users in the US had reported nonmedical use of psychoactive drugs including pain relievers, tranquilizers, stimulants and sedatives in their lifetime (22). Such widespread drug abuse arises from not only the greater prescribing by medical practitioners, but also from misconceptions about their safety. Since their introduction in the late 1950s, benzodiazepines have become the most widely prescribed anxiolytics and hypnotics in medical practice. Both diazepam and lorazepam belong to the benzodiazepine class of drugs and can be used to treat anxiety disorders, induce sleep or reduce agitation in status epilepticus (207). Diazepam became one of the top selling drugs of all time due to its potency, high bioavailability and onset of action. However, the same factors also resulted in a high risk of dependence and abuse (208). In case of lorazepam, drug abuse can result in tolerance, dependence and some incompletely reversible effects and hence the drug is recommended for short term use due to its physical addiction potential (209). Suboxone® contains buprenorphine and naloxone in a fixed ratio and is widely used for the treatment of opioid addiction (210). Although use of buprenorphine has been increasing in recent years, buprenorphine is around 30 times
stronger than morphine in many of its effects and safety still remains an issue as consumption with other psychoactive drugs has been frequently reported (211). In addition, there are several reports that demonstrate the diversion and misuse of Suboxone® both for self-medication and to produce euphoria (212)(213).

Another serious issue of concern is the disposal of leftover unused or expired psychoactive medications. Several studies have reported factors that lead to the accumulation of unwanted medications (214), the approaches to minimize or reduce this accumulation and the factors that encourage drug disposal in sewers in comparison to recommended means like take-back programs (215). Due to such widespread drug abuse and inadequate disposal, it is relatively common to find these drugs and their metabolites in the sewage system. Most consumers would prefer to throw the unused drugs in the normal trash or just flush them down the toilet. Flushing the drugs without proper deactivation can pollute our water system and contaminate food supplies. The detection of pharmaceuticals was first reported in 1976 in the treated waste water in USA (216). In recent years, many pharmaceutical compounds have been detected in the environment including anti-inflammatory drugs, cancer therapeutics and tranquillizers (217). Even though the concentration of drugs may be low, it can still hurt marine life and also affect human health. This inadequate disposal of misused/expired medications has even led to their detection in surface water as well as tap water. For this purpose, the US Food and Drug Administration (FDA) has suggested mixing unused drugs with cat litter or coffee grounds which can then be disposed in regular trash. But this is not an effective deactivation process and the drug can still be extracted and has high abuse potential. Thus, mixing psychoactive dosage forms with cat litter or coffee ground cannot effectively
prevent drug theft and abuse (218). The United States Drug Enforcement Administration (DEA), the Food and Drug Administration (FDA) and many other agencies recommend medicine take-back programs as the best way to dispose unused or expired psychoactive medicines. Between 2010 and 2014, even though more than 4 million pounds of medications had been taken back, only about 20% people participated in take-back programs due to lack of awareness about these programs (219). On the other hand, these programs utilize incineration to dispose medications which could produce toxic air emission including smog inducing gases, ozone depleting agents, and other by-products, finally resulting in air and environmental pollution. Hence, a safer, more convenient and less expensive drug disposal method is critically required.

The drug deactivation system investigated in this study offers a unique disposal method to deactivate unused, residual or expired medications in such cases. This drug deactivation system is based on MAT\textsuperscript{TM}\textsubscript{12} Molecular Adsorption Technology which can deactivate the active pharmaceutical ingredients and dosage forms by adsorbing and firmly binding with activated carbon (220). For the purpose of this study, the term ‘deactivation’ is used to signify the irreversible adsorption of the psychoactive substances by activated carbon. These activated carbon granules are contained in a water soluble film packet and packaged in a sealable outer pouch. Unused tablets or other dosage forms can be placed in the outer pouch with addition of warm tap water and the pouch can then be sealed. By adding the water into outer pouch, the inner water soluble film dissolves and activated carbon is released to mix with the medications. Once the drug mingles with activated carbon, the efficiency of the deactivation process is dependent on characteristics of active pharmaceutical ingredient and its dosage form. This study aimed at testing the
ability of this drug deactivation system by using psychoactive dosage forms like diazepam and lorazepam tablets and buprenorphine sublingual film in order to determine if this system provides a simple, safe and promising way for consumers to properly dispose their unused, residual or expired medications. Prior to analyzing the drug content in the pouches, validation of the analytical method was performed in order to ensure that this method was relatively simple, accurate and precise for testing the efficiency of this system. A desorption study was also performed to test the possibility of leaching out of adsorbed drug substances from activated carbon in order to simulate the landfill situation where there is a potential of pharmaceutical wastes seeping into groundwater supplies.

**Materials and Methods**

**Materials**

Active pharmaceutical ingredients used in this study were purchased from Sigma-Aldrich (St. Louis, MO, USA), while Suboxone® sublingual films, generic diazepam tablets and generic lorazepam tablets and deactivation pouches were obtained from Verde Environmental Technologies Inc. (Minnetonka, MN, USA). The pouch contains 15 grams activated carbon granules which is sealed by a water soluble film. Once warm tap water has been added into the pouch, the film dissolves and the released carbon will mix with the medications. Acetonitrile, methanol, potassium phosphate monobasic was purchased from Fisher Scientific (Pittsburgh, PA, USA). Potassium phosphate dibasic and ethanol was also purchased from Sigma-Aldrich. Nylon filters (0.22 µm) used for sample filtration were purchased from Medsupply Partners (Atlanta, GA, USA).

**Methods**

HPLC Validation study
A validation study was performed for all the drugs in order to ensure that the analytical method employed was suitable. The methods were conducted using an isocratic and gradient reverse phase technique and the validation criteria including linearity, specificity, accuracy and precision of the method were then tested. Fresh stock (1 mg/mL) was prepared with active ingredient and diluted to make standard samples ranging from 0.1 µg/ml to 50 µg/ml. Three standard samples with concentrations of 1 µg/mL, 2.5 µg/mL and 25 µg/mL (n=3) that are within the calibration levels were used to test the accuracy and precision.

All the samples were analyzed by using a Waters Alliance 2795 system equipped with a Waters 2998 PDA. For analyzing diazepam and buprenorphine, a Kinetex EVO C18 (150x4.6 mm, 5 µm) (Phenomenex, Torrance, CA, USA) was used. The mobile phase for diazepam consisted of acetonitrile and 20mM potassium phosphate buffer (pH 2.5) (40:60 %v/v). The flow rate was 1.2 mL/min and UV wavelength was set at 230 nm. For the HPLC analysis of buprenorphine, acetonitrile and 10 mM potassium phosphate buffer adjusted to pH 6 (83:17 %v/v) was used as mobile phase. The flow rate was 1.0 mL/min and wavelength was 212 nm (221). Lorazepam was detected using an Xbridge BEH Phenyl column (50x4.6 mm, 2.5 µm) (Waters Corp., Milford, MA, USA). Acetonitrile and water were used as mobile phase delivered under a gradient program and the composition is shown in Table 3. The flow rate was 1.0 mL/min and detection wavelength was 229 nm.

<table>
<thead>
<tr>
<th>Time</th>
<th>Flow Rate</th>
<th>Acetonitrile</th>
<th>Water</th>
</tr>
</thead>
</table>

Table 3. HPLC gradient method for Lorazepam
Deactivation of pharmaceutical dosage forms

The deactivation of the different dosage forms was tested using the three model psychoactive medications. Ten diazepam tablets (10 mg), ten Suboxone® sublingual films (8 mg) containing buprenorphine as one of the active ingredients and ten lorazepam tablets (2 mg) were placed into individual pouches separately followed by addition of 50 ml of tap water warm at a temperature of about 43º C. In order to mix the dosage forms, activated carbon and warm water properly, pouches were shaken for 10 seconds at the rate of 1 shake per second, followed by waiting for 30 seconds to release the air bubbles from charcoal. After ensuring that all the medications remained at the bottom of the pouch, the pouches were then sealed, stored upright and undisturbed at room temperature. Separate pouches were set up for each time point and samples were collected from pouches (n=2) at 8 hours, 1, 2, 4, 7, 14, 21 and 28 days. Fig. 19 is a schematic representation of the deactivation procedure followed for dosage forms. Two extra pouches were set up in order to account for any loss during the study. Before taking samples, pouches were shaken mildly from side to side to ensure the medications mixed homogenously in the pouch. Samples were then filtered with 0.22 μm nylon filter and

<table>
<thead>
<tr>
<th>(min)</th>
<th>(mL/min)</th>
<th>5%</th>
<th>95%</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>1.0</td>
<td>5%</td>
<td>95%</td>
</tr>
<tr>
<td>7.2</td>
<td>1.0</td>
<td>60%</td>
<td>40%</td>
</tr>
<tr>
<td>7.3</td>
<td>1.0</td>
<td>5%</td>
<td>95%</td>
</tr>
<tr>
<td>8.5</td>
<td>1.0</td>
<td>5%</td>
<td>95%</td>
</tr>
</tbody>
</table>
analyzed by the validated HPLC methods. The deactivation rate was calculated as follows

\[
\% \text{ Reacted} = \frac{\text{Initial amount of drug in dosage form} - \text{Amount of drug in } 50 \text{ mL water}}{\text{Initial amount of drug in dosage form}} \times 100
\]

Figure 19. Schematic representation of deactivation study of psychoactive medications
[S1: Medications were added to the deactivation pouches. S2: 50mL warm tap water was added to each pouch. S3: Pouches were stored upright and undisturbed at room temperature. S4: Samples were collected at different time points and analyzed by HPLC].

Desorption study

A desorption or washout study was performed following the deactivation study, in order to determine the potential for leaching of the drug from activated carbon. Fig. 20 shows the schematic outline of the desorption study performed after 28 days. The entire content of each pouch was transferred into an individual container followed by addition of 200 mL distilled water. The samples were shaken for 1 hour at 150 rpm, stored upright
for 23 hours at room temperature, then filtered and analyzed by HPLC. The water was then replaced with 250 mL 30% ethanol, rocked for an additional hour and stored for 23 hours at room temperature. After that the samples were taken from the container, filtered and analyzed by HPLC.

Figure 20. Schematic representation of desorption study of psychoactive medications
[S5: On 28th day, contents of each pouch were transferred into container and distilled water was added. S6: Samples were shaken and then stored upright, undisturbed at room temperature for 23 hours. S7: Samples were collected on 29th day. S8: Water was replaced with 30% ethanol. S9: Samples were shaken and stored undisturbed at room temperature for an additional 23 hours. S10: Samples were collected on 30\textsuperscript{th} day and all samples were analyzed by HPLC].

Results

HPLC Validation
Linearity

Linearity of an analytical method demonstrates a proportional relationship of peak area and the concentration of analyte in the samples over a definite range. Linearity of the analytical methods for diazepam, lorazepam and buprenorphine was established within the range of 0.1 μg/mL to 50 μg/mL. Good correlations between peak area and drug concentrations were obtained with $r^2 \geq 0.99$ for all the three drugs (Figure 21, 22 and 23).

![Figure 21. Linearity of HPLC method for analysis of lorazepam](image)
Specificity

Representative chromatograms obtained from the injected drug solutions are presented in Figure 24, 25 and 26. Specificity is to evaluate the ability of analytical methods to detect the analyte of interest. The analytical method should not be affected by the presence of impurities or excipients in the samples (222). No interference peak from excipients or impurities was observed near the retention time for all drug samples which indicated the specificity of the analytical methods.
Figure 24. Representative chromatograms of lorazepam drug sample

Figure 25. Representative chromatograms of buprenorphine drug sample
Accuracy and Precision

The results of the intra and inter-day accuracy and precision are shown in Tables 4 and 5. The precision and accuracy of analytical method was determined by calculating the percentage deviation of the calculated concentration and the theoretical concentration (223)(224). The intra-day accuracy of all the three drugs was within the range of 100% ± 10% while the intra-day precision did not exceed 5%. Assay precision was calculated by using the formula CV% = (S.D./Mean measured concentration) x 100 where S.D. is the standard deviation of mean measured concentration. Table 5 shows the results of the inter-day accuracy and precision, which were also within an acceptable range. These results show a good capability of response of HPLC system to different concentrations of samples.

Table 4. Intra-day accuracy and precision of HPLC methods

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration (µg/mL)</th>
<th>Mean measured</th>
<th>Precision (%CV)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
</table>

Figure 26. Representative chromatograms of diazepam drug sample
<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration</th>
<th>Mean measured Concentration</th>
<th>Precision (%CV)</th>
<th>Accuracy (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µg/mL ± SD)</td>
<td>(µg/mL ± SD)</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Diazepam</strong></td>
<td>1</td>
<td>0.92 ± 0.042</td>
<td>4.6</td>
<td>92.00</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2.47 ± 0.041</td>
<td>1.66</td>
<td>98.66</td>
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<tr>
<td></td>
<td>25</td>
<td>23.96 ± 0.282</td>
<td>1.18</td>
<td>95.82</td>
</tr>
<tr>
<td><strong>Buprenorphine</strong></td>
<td>1</td>
<td>1.19 ± 0.048</td>
<td>4.06</td>
<td>92.00</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2.42 ± 0.134</td>
<td>5.57</td>
<td>96.72</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>25.18 ± 0.776</td>
<td>3.08</td>
<td>100.70</td>
</tr>
<tr>
<td><strong>Lorazepam</strong></td>
<td>1</td>
<td>1.11 ± 0.023</td>
<td>2.11</td>
<td>92.00</td>
</tr>
<tr>
<td></td>
<td>2.5</td>
<td>2.42 ± 0.083</td>
<td>3.43</td>
<td>97.00</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>24.14 ± 1.132</td>
<td>4.69</td>
<td>96.58</td>
</tr>
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</table>

Table 5. Inter-day accuracy and precision of HPLC methods
<table>
<thead>
<tr>
<th></th>
<th>25</th>
<th>24.84 ± 1.21</th>
<th>4.87</th>
<th>99.35</th>
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<tbody>
<tr>
<td>Lorazepam</td>
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<td>1.12 ± 0.024</td>
<td>2.11</td>
<td>92.00</td>
</tr>
<tr>
<td></td>
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<td>5.37</td>
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<tr>
<td></td>
<td>25</td>
<td>24.49 ± 1.151</td>
<td>4.7</td>
<td>97.96</td>
</tr>
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</table>

Deactivation Study

Deactivation of diazepam tablets, lorazepam tablets and buprenorphine containing sublingual films was performed using activated carbon. Each pouch contained 50 grams activated carbon granules and either ten tablets of diazepam or lorazepam or ten suboxone sublingual films were added. After the addition of warm tap water, about 70% of three medications were adsorbed by the activated carbon within 8 hours (Figure 27). In case of diazepam, only around 46% drug was adsorbed and more than 50% diazepam still remained in the water. The amount of lorazepam measured in the pouch at the end of 8 hours was 6mg, which was around 30% of total amount of drug added into the pouch. For diazepam and lorazepam, about 72% and 87.5% drug deactivated respectively after 2 days. More than 96% of buprenorphine was deactivated by the end of 8 hours, which increased to 99% after 2 days of adsorption. Medications continued to be adsorbed over time with an average total adsorption of 96.44% by the end of 14 days. After 28 days, adsorption by the activated charcoal resulted in more than 99% deactivation/adsorption of diazepam, lorazepam and buprenorphine. Low levels of residual drug were observed in the pouch which was less than 1% for all three psychoactive medications.
Desorption Study

The desorption or washout study was carried out in order to simulate a landfill situation and to test for potential desorption of the various drugs from activated carbon. For the washout phase, contents of pouches after 28 days of the adsorption study were emptied into 500 mL container and excess water was added to make up volume to 250 mL. Addition of excess water facilitated complete release of active pharmaceutical ingredient from tablets or films with continuous adsorption by the activated carbon. The percentage of diazepam, lorazepam and buprenorphine that leached out after 1 day of desorption is shown in Figure 28. The water was then replaced with 30% ethanol to determine the leaching potential from the adsorbent. In this case, about 1.6% of diazepam leached out from the activated carbon. For buprenorphine and lorazepam, only 0.11% and 0.25% of drug leached out from the activated charcoal after 24 hours respectively as seen in Fig. 29.
Discussion

The abuse of psychoactive drugs constitutes a growing problem and it is estimated that by 2020, about 2.7 million adults would have used prescription drugs due to dependency or for recreational purposes (225). In addition, the increased prescribing of psychoactive drugs has raised concerns about proper drug disposal once they are no
longer required by patients. Traditional/improper disposal such as flushing medicines down the toilet or pouring them down the drain can increase the harm of accidental exposure especially in children as well as lead to environmental contamination. Scientists have not only detected the presence of these medications in water bodies which can affect marine life but also in sewage water which can potentially affect animal and human life. Methods which have been recommended by FDA or U.S. Drug Enforcement Administration (DEA) to deal with the disposal of these unused drugs such as mixing with ground coffee or cat litter cannot effectively deactivate the drugs (226). Take back programs are regarded as the safest and most environmentally protective way for drug disposal but the problem is the scarcity of collection sites, general unawareness in the public and complications due to transfer of controlled substances (227). After returning the unwanted/expired medications to drop-off locations, these medications are then securely transferred for further incineration. Even though high temperature incineration meets industry standards for safe disposal, it can still cause increased air emissions of pollutants and ozone depleting agents resulting in environmental pollution.

Hence, the drug deactivation system investigated in this study is an excellent alternative and offers a simple and convenient method to safely deactivate and dispose unused or expired psychoactive medications. Activated carbon is obtained by thermal decomposition of carbon based materials such as coal, coconut or wood. The purpose of this activation procedure is to achieve a high internal surface area which is good for the adsorption of the drug from the formulation to the activated carbon. This large surface area is due to the presence of small, low volume pores on the charcoal where the pore size distribution contributes to the efficiency of the activated carbon in the drug
adsorption. Activated carbon has numerous micropores in comparison to charcoal which provides maximum bonding surface area for drug binding. This granular activated carbon is already being used in water treatment processes for removal of micropollutants including pharmaceuticals and endocrine disruptors (228).

This study was performed as per the protocol and guidelines established by Verde Technologies Inc. in association with NIDA which involved the use of duplicate pouches in order to evaluate the deactivation rate of the model psychoactive medications. In addition, as separate pouches were used for individual time points, performing the study in duplicate helped to minimize the handling and use of these controlled substances. Each pouch contained ten dosage forms of psychoactive substances which was found to be adequate to analyze the deactivation and desorption of each dosage form. A previously published study performed in our laboratory had compared the efficacy of various deactivation agents with activated carbon for deactivation of various active pharmaceutical ingredients. Adsorption by activated carbon resulted in a faster deactivation rate for all the drugs used in the study (226). The Molecular Adsorption Technology (MAT\textsuperscript{12}TM) used in the disposal system neutralizes or adsorbs the psychoactive ingredients from different dosage forms. Thus, the activated carbon in the disposal system will bind to the different pharmaceutical formulations, successfully deactivate the psychoactive drugs and can then be safely disposed in regular trash. The adsorption capacity of activated carbon is also related to the molecular weight of the adsorbents. With increasing molecular weight, the adsorbability of a compound will also increase. In this study, the molecular weight of all the active compounds was less than 500 kDa. However, as the molecular weight of buprenorphine was higher than that of
diazepam and lorazepam, the adsorption of buprenorphine was much faster in comparison to the other two drugs. More than 99% of buprenorphine was adsorbed by activated carbon by the end of the second day. Clinically, suboxone sublingual films can dissolve in 6-7 minutes (229) which may also contribute to its faster adsorption as compared to lorazepam and diazepam immediate release tablets which may show a slower dissolution rate. In addition, the non-polar surface of activated carbon preferentially adsorbs hydrophobic compounds which could also potentially explain the faster deactivation rate of buprenorphine (more than 90%) due to its increased hydrophobicity in comparison to the benzodiazepine drugs.

Mixing drugs with the currently recommended cat litter or coffee grounds may still result in availability of drugs to groundwater when placed in landfills. These emerging contaminants from landfill leachate can then contaminate groundwater for decades. Hence, the robustness of the disposal system in holding on the adsorbed psychoactive drugs was tested by exposure to stress conditions by agitating the pouches and thus simulating a landfill situation. The results of the washout study showed that more than 99% of the drug was deactivated in the presence of water. On replacing with ethanol, less than 1% of adsorbed drug leached out from the activated carbon which suggests that as the discarded drug product would be almost fully deactivated and insoluble in water, this should ultimately contribute to a much lower exposure of groundwater to such pharmaceuticals.

Conclusions

The effectiveness of the activated carbon based drug disposal system was examined using three model psychoactive medications. The deactivation system successfully
adsorbed and deactivated about 70% of the psychoactive medications by 8 hours and more than 99% within 28 days and did not release adsorbed drug substances when exposed to large volumes of water or 30% ethanol. Thus, this unique system is simple, safe and user friendly for patients who can deactivate unused or expired psychoactive medications from the comfort of their homes.
CHAPTER 7

SUMMARY AND CONCLUSIONS

The goal of this work was to investigate topical and transdermal delivery of hydrophilic small molecules and macromolecules through skin; physical and chemical enhancement technologies have been assessed to assist hydrophilic molecule delivery through the skin. The deactivation efficiency of a drug disposal system has been tested in this work using psychoactive medications.

Cimetidine was formulated into a carbopol gel and the effect of extent of drug ionization on the permeation through microneedle treated skin was investigated. We formulated cimetidine drug into different pHs of carbopol gel (pH 5, 6.8 and 7.5) and the pH of formulation at 5, 6.8 and 7.5 resulted in 98.4%, 50% and 16.6% ionization of cimetidine in the formulation. Permeation studies showed that higher drug ionization resulted in the higher drug permeation through microchannels created by maltose microneedle. Skin extraction study results showed drug accumulation within different skin layers was not significantly influenced by drug ionization. The extent of drug ionization in the formulation significantly influenced the permeation of cimetidine through the skin. With increase of drug ionization, the permeation of drug also increased. This was more pronounced with increasing drug concentration in the gels. The feasibility of transdermal delivery of hGH though porcine ear skin and human skin using ablative laser was investigated.
Laser device used in this study was an ablative laser from P.L.E.A.S.E®, Pantec Biosolutions AG. Laser parameters, such as fluence, pore Array size and density of pores were programmed using device software. The effect of different fluences (34.1, 45.4, and 68.1 J/cm²) at micropores density (5%, 10% and 15%) on the permeation of hGH through porcine ear skin was evaluated. All the laser parameters were fixed in order to create reproducible micropores. *In vitro* permeation studies showed permeation of hGH through the skin can be adjusted by the laser fluence and micropores density. With increase of laser fluence and micropores density, the permeation amount of hGH also increased. Higher fluence of laser resulted in the formation of deeper depth of micropores and shorter lag time of drug delivery.

Salicylic acid is a lipophilic monohydroxybenzoic acid and is used in the treatment of warts, psoriasis, dandruff and acne (12). Since salicylic acid as a medication is used most commonly to help remove the outer layer of the skin, it has a high potential to cause skin irritation even with low concentration in the formulation. In this study, microspponge delivery system was developed using Eudragit® RS100 and PVA polymer. Microspponge delivery system is a polymeric system that facilitates controlled release of active ingredients into the skin. *In vitro* drug release from resultant formulations was studied and results showed that for formulations with all the drug incorporated into the microsponges, the rate of drug release was much slower than that from formulations without microspponge, especially at the beginning one to two hours. Incorporation of salicylic acid into microspponge avoided burst release. *In vitro* permeation study showed existence of microspponge in the formulation could slow down the drug permeation from
formulation and reduce or avoid the skin irritation. The ratio of drug: polymer can influence the morphology of microspunge and the drug permeation from microsponges. The deactivation efficiency of activated carbon based drug disposal system was evaluated by using psychoactive medications. The deactivation studies were performed using lorazepam, diazepam tablets and buprenorphine sublingual films; samples were taken out at predetermined time points and analyzed by validated HPLC methods. Results showed more than 90% of drug was deactivated within 7 d and almost 100% drug had been adsorbed by the activated carbon after 28 d. Desorption study results show that only less than 1% of drug leached out from the activated carbon into 70% ethanol or water. The disposal system was effective in deactivation of almost 100% of drug content of different dosage forms.
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