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Review

Enhancement of transdermal drug delivery via synergistic action of chemicals

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ABSTRACT

Transdermal drug delivery is an attractive alternative to conventional techniques for administration of systemic therapeutics. One challenge in designing transdermal drug delivery systems is to overcome the natural transport barrier of the skin. Chemicals offer tremendous potential in overcoming the skin barrier to enhance transport of drug molecules. Individual chemicals are however limited in their efficacy in disrupting the skin barrier at low concentrations and usually cause skin irritation at high concentrations. Multicomponent mixtures of chemicals, however, have been shown to provide high skin permeabilization potency as compared to individual chemicals without necessarily causing irritation. Here we review systems employing synergistic mixtures of chemicals that offer superior skin permeation enhancement. These synergistic systems include solvent mixtures, microemulsions, eutectic mixtures, complex self-assembled vesicles and inclusion complexes. Methods for design and discovery of such synergistic systems are also discussed.

Contents

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1. Introduction

Injections, pills and to some extent topical and mucosal formulations comprise the most commonly used drug delivery methods. Oral delivery is by far the easiest and most convenient way of delivering drugs especially when repeated or routine administration is required [1]. This advantage, however, is offset for protein and peptide-based drugs sensitive to enzymatic degradation in the gastro-intestinal tract. Drugs based on proteins and peptides now form a significant fraction of the therapeutic spectrum, primarily due to accelerated advances in understanding protein chemistry and drug interactions. Currently, needle-based injection is the most frequently used route to administer protein and peptide drugs. Despite their common use, needle-based drug administrations have several limitations. Needle phobia is a significant issue in adults and children alike [2] and makes drug administration stressful [3]. Accidental needle sticks also add to the limitations of needle use in developed and developing countries alike [4,5]. Further, hepatic metabolism results in rapid clearance of drugs from the blood making repeated administration inevitable. This only aggravates the problem of needle pain especially for patients requiring multiple administrations on a daily basis. The next era of health care will demand more accommodating delivery systems for sensitive drug classes. Patient compliant, noninvasive and sustained delivery will become the key feature desirable of any drug delivery system. Several advances to this effect have been made in the last 2–3 decades and novel drug delivery systems have been brought to the forefront [6–8]. A large contribution to these novel systems appeared as modifications of the active drug or use of formulation excepients to modulate drug pharmacokinetics, safety, efficacy and metabolism. A more radical approach has been to explore newer interfaces on the body for introducing therapeutics. One such approach, transdermal drug delivery, makes use of human skin as a port of entry for systemic delivery of drug molecules [9–14].

Transdermal drug delivery (TDD) offers an advantageous mode of drug administration by eliminating first pass hepatic metabolism and providing sustained drug release for a prolonged period of time. It is painless when compared to needles and therefore offers superior patient compatibility. However, skin is the first line of defense of an organism and the last barrier separating the organism from its hostile environment of viruses, pathogens and toxics. Evolved to impede the flux of exogenous molecules into the body, skin naturally offers a very low permeability to the movement of foreign molecules across it. A unique hierarchical structure of lipid-rich matrix with embedded keratinocytes in the upper strata (15 μm) of skin, stratum corneum (SC), is responsible for this barrier [15]. In addition to its role as a barrier, both physical and biological, skin performs a complimentary role; that of a transport regulator. Skin routinely regulates the flux of water molecules into and out of the body. It also permits the influx of a variety of small molecules that are fairly lipophilic (log P>1.5) and have molecular weight less than 500 Da [16]. As a result, there has been a natural bias in design of transdermal delivery systems to take advantage of therapeutics that meet these requirements. Drug molecules currently administered via the transdermal route fall within a narrow range of molecular weight and lipophilicity. They are typically characterized by high Log P (>1.5) and low MW (<500 Da), thereby taking advantage of the natural selectivity of the skin membrane. A large fraction of drug molecules such as protein and peptide-based drugs lie outside these bounds. The biggest challenge in transdermal drug delivery today is to open the skin safely and reversibly to these high molecular weight hydrophilic drugs.

Several technological advances have been made in the past couple of decades to overcome this challenge. These advances can be broadly divided into two categories; physical methods and chemical methods. Physical methods employed for increasing transport of drug molecules across the skin use some form of mechanical, electrical, magnetic or thermal energy source to promote transport of macromolecules by disrupting the skin membrane. Examples of physical approaches include the use of microneedle array [17–22], ballistic liquid jet [23–25], high velocity particles [26], ultrasound [27–32], electric current [33–39], abrasion [17,40], ablation [41,42], lasers [40,43,44], pressure waves [45–47], radiofrequency thermal ablation [48], magnetophoresis of diamagnetic solutes [49,50] and thermophoresis [51–55]. Several excellent reviews discuss the advantages, limitations and opportunities offered by these approaches in greater details and thus will not be covered in this review.

2. Chemical methods for permeation enhancement

2.1. Chemical permeation enhancers

Several chemicals are known to interact with the skin and disrupt the highly ordered lipid bilayer structure that forms the primary barrier to diffusion of exogenous molecules. This observation led to the study of chemical agents to enhance transport across skin. More than 300 chemicals, termed permeation enhancers or penetration enhancers, have been studied for their ability to increase transport of drug molecules across the skin. Permeation enhancers currently represent the most widely studied approach to transdermal drug permeation enhancement on account of the advantages they offer over physical methods. Chemical permeation enhancers are relatively inexpensive and easy to formulate, they offer flexibility in their design, are simple in application and allow the freedom of self-administration to the patient. Finally, chemical enhancers can be formulated with the active therapeutic as a topical cream or gel, or an adhesive skin patch that can be applied anywhere on the body for prolonged systemic delivery of the drug. In general, permeation enhancers may contain a wide variety of different chemical functional groups and act by a variety of different mechanisms in enhancing drug transport. In this review we use the term ‘chemical enhancers' to include a broad range of chemicals (discussed in Section 3) as well as more complex systems arising from combining individual chemicals (with or without the active therapeutic) such as vesicles, colloids, microemulsions, eutectic mixtures and inclusion complexes (discussed in Section 5). Chemical enhancers can also exhibit a wide range of excepient effects in addition to their primary function of participating in skin permeabilization. These may include, but are not limited to, improving drug solubility, improving aesthetic traits such as odor, color and texture, and acting as emulsifiers, preservatives and fillers. For the purpose of this review we focus on the primary role of chemical enhancers as skin permeabilizing agents.

2.2. Mechanism of action of chemical permeation enhancers

Permeation enhancers enhance transport of drugs across skin by a variety of complex mechanisms. They can directly exert their effect on skin structure by acting on intercellular lipids or corneocytes, the dead cells of the stratum corneum. Permeation enhancers can be divided broadly in two categories based on their action on intercellular lipids of the skin [56]. Chemical enhancers can either extract lipids from the skin thereby creating diffusion pathways for the drug to permeate through or they can partition themselves into the lipid bilayers thereby disrupting the highly ordered lipid lamellae and causing their fluidization [56–58]. Lipid extraction or fluidization in presence of chemical enhancers could occur through a variety of different mechanisms [57]. Alternately, chemical enhancers can increase skin transport of a drug by enhancing its thermodynamic activity in the formulation, for e.g., by causing supersaturation of the drug in the formulation. The modes of action of various penetration enhancers and some scientific perspectives have been extensively discussed in prior literature [59,60]. Additionally, discussion on individual enhancers below is annotated with relevant references on their mechanism of action.

3. Categories of chemical permeation enhancers

Chemical permeation enhancers have traditionally been classified based on their chemical structures rather than their mechanisms of action on skin. Such a classification is purely out of practical benefits since permeation enhancers can act on skin by a variety of different mechanisms which may not always be straightforward to elucidate. Chemicals belonging to the same group can act on skin by different mechanisms depending on their individual physico-chemical properties. Below, we briefly discuss the most widely accepted grouping of permeation enhancers based on their chemical structures.

3.1. Water

Water is the most natural penetration enhancer [61]. Hydration state of the stratum corneum is important in determining penetration enhancement of a given drug. Usually, increased hydration of the stratum corneum enhances transdermal flux of a variety of drugs. The role of water in promoting transdermal delivery has been extensively reviewed elsewhere [61].

3.2. Hydrocarbons

Several hydrocarbons including alkanes, alkenes, halogenated alkanes, squalane, squalene and mineral oil have been used as vehicles or penetration enhancers to increase permeation of a variety of drugs across the skin [62]. These permeation enhancers generally work by partitioning into the stratum corneum and disrupting the ordered lipid bilayer structure. In a series of experiments on skin permeation using alkanes of varying chain length (9–18 atoms), it was demonstrated that alkanes with 9–10 carbon atoms showed highest skin permeation enhancement of propranolol and diazepam while shorter alkanes (5–6 carbon atoms) showed highest permeation enhancement of caffeine [62,63]. Squalane and squalene improved permeation of sodium diclofenac; mineral oil was effective for methyl nicotinate and chlorododecane enhanced permeation of timolol maleate [64]. Several other hydrocarbons and their effect on skin permeation of a variety of drugs are reviewed in Buyuktimkin et al. [60].

3.3. Alcohols

Alcohols are frequently used as vehicles, solvents or penetration enhancers in improving transdermal delivery of drugs. These include alkanols, alkenols, glycols, polyglycols and glycerols. Alcohols can enhance skin permeation by a variety of mechanisms such as extraction of lipids and proteins, swelling of the stratum corneum or improving drug partitioning into the skin or solubility of the drug in the formulation [60,65–69].

3.4. Acids

The most commonly studied chemicals in this category are fatty acids [59,66–68]. These chemicals enhance transport of drug molecules across the skin by a variety of mechanisms such as partitioning into the lipid bilayers and disrupting their ordered domains, improving drug partitioning into the stratum corneum and forming lipophilic complexes with drugs [70,71]. Acids are typically used as solvents or vehicles but can also be used as permeation enhancers in a solvent or vehicle system [60]. Oleic acid is an example in this category that is extensively studied as a permeation enhancer [72–74].

3.5. Amines

Primary, secondary and tertiary, cyclic and acyclic amines have been used successfully in enhancing skin permeation of a variety of drugs. Amines may enhance skin permeation by partitioning into the lipid bilayers or improving drug partitioning into the skin [56,60,68].

3.6. Amides

Cyclic and acyclic amides form another large class of chemicals studied as permeation enhancers [60]. Azone, the first synthetic permeation enhancer and its analogues along with pyrrolidones are the most extensively studied amides [75,76]. Typically, amides are used as solvents and can act by enhancing the activity of the drug in the solvent or improving drug partitioning in the skin. Urea and its analogues, which fall under this category, are usually used as permeation enhancers in solvents where they can have different effect on the skin based on the solvent system chosen, but generally act by disrupting the skin lipids [77,78].

3.7. Esters

Esters of fatty acids have been used in several studies and show skin permeation enhancement of a wide variety of drugs [56,60,68,79]. Isopropyl myristate is the most widely studied ester along with several other esters of fatty acids. These chemicals generally work by partitioning themselves in the ordered lipid domains of the stratum corneum [60,65].

3.8. Surfactants

A wide variety of surfactants have been actively pursued as skin permeation enhancers [60,80,81]. These include anionic, cationic, zwitterionic and non-ionic surfactants. Surfactants are usually used with a vehicle or solvent system and their activity depends upon the hydrophilic to lipophilic balance, charge and lipid tail length [56]. Anionic and non-ionic surfactants are relatively more widely studied compared to others within this category [82,83].

3.9. Terpenes, terpenoids and essential oils

Terpenes are a popular choice for permeation enhancers in transdermal drug delivery studies [84–86]. This category includes a heterogeneous range of members and the effect of a specific terpene on skin depends upon its exact physicochemical properties, in particular its lipophilicity. In general, smaller terpenes with non polar groups are better skin permeation enhancers [60].

3.10. Sulfoxides

Dimethyl sulfoxide was the first chemical to be studied in depth as a permeation enhancer. It was originally used as a solvent to improve drug partitioning into the skin; however several studies have reported the use of dimethyl sulfoxide and its derivatives as enhancers in other solvent systems [60,66,68].

3.11. Lipids

Phospholipids have been successfully used as permeation enhancers in the form of vesicles, microemulsions and micellar systems [87,88]. Phospholipids do not have an appreciable effect when interacting with the stratum corneum as individual molecules. However, in the form of self-assembled structures such as vesicles or micelles, they can fuse with the lipid bilayers of the stratum corneum thereby enhancing partitioning of encapsulated drug as well as disruption of the ordered bilayers structure [60,66].

3.12. Miscellaneous

In addition to the classical chemical permeation enhancers discussed above several other chemical groups have been studied for their ability to enhance drug transport across the skin. Cyclic oligosaccharides such as cyclodextrins form inclusion complexes with a variety of hydrophobic drugs thereby increasing their partitioning and solubility in the stratum corneum [89–97]. Amino acids and thioacyl derivatives of amino acids have been shown to enhance transdermal permeation of drugs. Alkyl amino esters and oxazolidinones have also been used successfully as permeation enhancers [60]. Enzymes are a relatively new class of chemicals studied as permeation enhancers. Papain and medicinal leech enzymes have been shown to successfully enhance the transdermal delivery of drugs [60,98]. Ketones have been shown in some studies to enhance skin permeation of steroidal drugs. In general, macrocyclic ketones with 12 carbon atoms or more have also been successful in enhancing transdermal delivery of a wide variety of drugs [60]. Finally, metabolic intervention schemes that affect the synthesis of the stratum corneum components and hence its homeostasis have also been proposed for skin permeation enhancement [66].

4. Limitations of chemical permeation enhancers

4.1. Efficacy

An important limitation of chemical enhancers is that most of the enhancers that have been studied in the transdermal literature for their ability to increase transport across skin do not achieve the desired skin disruption [81]. They show poor permeation across the stratum corneum themselves and hence their activity is limited to the top few layers of the stratum corneum. As their concentration across the stratum corneum decreases, so do their activities. As a result, these chemicals offer poor transdermal delivery of candidate drug molecules. In general, chemical enhancers disrupt skin by a variety of different mechanisms. Several investigators have attempted to identify the physicochemical forces that determine the activity of the permeation enhancers in skin [99–103]. Correlating physicochemical parameters such as charge, hydrogen bonding ability, polar forces, partition coefficient, solubility, etc. allows one to develop quantitative structure activity correlations (QSAR) that relate chemical structure of the permeation enhancer to its skin disrupting potential. Based on such relations, one can design new permeation enhancers that are significantly more potent in their skin permeabilizing ability as compared to conventional chemicals. Azone (1-dodecylazacycloheptan-2-one) and SEPA (2-n-nonyl-1,3dioxolane) are examples of such "synthetic" permeation enhancers that have been designed through knowledge of molecular properties relevant for skin permeabilization [104]. We have used quantitative correlations to design potent enhancers that work as lipid extractors or lipid fluidizers using partition coefficient, hydrogen bonding, polar and dispersion forces [56]. Specifically, we found that it is fundamentally impossible to design lipid extracting permeation enhancers that are both potent and safe since similar molecular properties are responsible for lipid extraction as well as protein denaturation which is related to skin irritation. Permeation enhancers with a very high partition coefficient were found to work as potent lipid fluidizers but their poor solubility in aqueous formulations limits their practical use [56].

4.2. Safety

Yet another challenge in using chemicals in enhancing transdermal transport is their potential to cause skin irritation. In general, the potency of penetration enhancers in causing skin irritation scales proportionally with their ability to cause skin disruption [56]. The term irritation is used to describe any adverse effects caused by interaction of chemicals with skin constituents and may include local inflammation, erythema, swelling, dermatitis or other deleterious reactions. This general limitation of chemical enhancers stems from their mechanism of action on skin. Potent permeation enhancers are very good at disrupting the corneocytes or highly ordered lipid bilayers of the stratum corneum. The stratum corneum represents the largest transport barrier to diffusion of drug molecules but is physiologically dead. Potent permeation enhancers while good at disrupting the stratum corneum cannot limit their activity to this superficial layer and eventually diffuse into the viable epidermis that is directly below the stratum corneum. In the viable epidermis, the penetration enhancers can interact with the keratinocytes, the living cells of the epidermis, and cause cytotoxicity. It is challenging to design permeation enhancers that exert their effect exclusively in the stratum corneum. As a result it is challenging to strike an optimum balance between the safety and potency of chemical enhancers [56].

4.3. Opportunities offered by chemical mixtures

Chemical mixtures offer several opportunities to overcome the limitations of single chemical enhancers. Mixtures of chemicals can offer superior potency as compared to single chemicals through a variety of different ways. Individual components of a mixture can cause skin disruption through similar or complementary mechanisms thereby resulting in additive or synergistic effects on permeation enhancement. For example, a combination of two chemical enhancers, one of which acts on lipids and the other on corneocytes can open up intercellular hydrophobic as well as intracellular hydrophilic pathways for permeation of a drug molecule. Similarly, one component of a mixture can increase the partitioning of drug molecule in the stratum corneum whereas the other component can create diffusion pathways by disrupting the lipid bilayers or corneocytes. Yet another example in which enhancers can show additive or synergistic behavior is when one enhancer stabilizes the drug or prevents it from metabolism in the skin and the other enhancer creates diffusion pathways for permeation of the drug.

Beyond exhibiting synergistic effects in improving drug transport, mixtures of chemicals can also show a synergy between potency and safety [81]. One way to design chemical permeation enhancers that are both safe and potent is to decouple their activities in the stratum corneum and the epidermis. High potency requires the permeation enhancers to have a very high disruptive activity in the stratum corneum whereas safety demands that the permeation enhancers have little to no activity in the viable epidermis. Achieving such a decoupling of mechanisms for individual chemicals diffusing through the skin is extremely difficult if not impossible. However, combinations of chemicals offer a unique opportunity in achieving such a decoupling. A mixture of chemicals can be designed such that it has a very high cell and lipid

bilayer disrupting activity in the dead stratum corneum. As the components of this mixture diffuse across the stratum corneum into the epidermis, the composition as well as concentration of the mixture changes due to difference in the partition coefficients as well as diffusion rates of the two components from the stratum corneum into the epidermis. The resulting mixture in the epidermis can be expected to be safe if it has a very low disruption potential. It is thus theoretically possible to design a combination to show one concentration and composition in the stratum corneum while a completely different concentration and composition in the epidermis. Since activity of the mixture is related to the concentration and composition of its components, the activities of the mixture in the two layers of the skin can be decoupled. A formulation can thus be designed to have a very high disruption potential in the stratum corneum making it potent and a very low disruption potential in the epidermis thereby making it safe [81].

5. Synergistic mixtures of chemical permeation enhancers

5.1. Synergy

A number of studies have shown that certain chemicals in a mixture interact synergistically and induce skin permeation enhancements higher than that induced by the individual components [68,79,81]. Synergies between chemicals can be exploited to design potent permeation enhancers that overcome the efficacy limitations of single enhancers. Synergy can be quantified objectively by a mathematical parameter, *S*, indicative of the "extent of interaction" between the two penetration enhancers, as the ratio of permeation enhancement obtained by the mixture to the weighted sum average of the permeation enhancements obtained from the individual components of the mixture as follows [81]:

$$S = \frac{E_{A+B}^{X,Y}}{X.E_A^Y + (1-X).E_B^Y}.$$

$E_{A+B}^{X,Y}$ is the enhancement ratio obtained with a formulation containing two permeation enhancers A and B at a total concentration of *Y*% wt/vol and *X* weight fraction of A. E_A^Y and E_B^Y are the enhancement ratios obtained with pure components *A* and *B* respectively at the same total concentration *Y*. Alternately, synergy may be defined with respect to the permeation enhancement obtained from either of the individual components of the mixture. Based on experimental studies on 5000 different chemical enhancer mixtures, we have previously reported that the enhancement induced by formulations is related to synergistic interactions between chemical enhancers [105]. Synergy, *S*, can assume a value greater than 1, indicating positive synergy and superior skin permeabilization, a value of 1 indicating no synergy or no change in permeabilization potential due to mixing of individual enhancers and a value less than 1 indicating negative synergy or reduction of skin permeabilization potential on mixing of individual enhancers.

While mixtures with $S>1$ have clear applications for transdermal drug delivery, formulations with $S<1$ imply reduced interaction with skin. Such formulations have important roles in therapeutic or cosmetic formulations. Skin barrier reduction and sensitization are undesired results of several personal care formulations such as sunscreens, fragrances and cleansers. Such formulations can be potentially designed to have $S\leq 1$ to improve their safety.

This review will focus only on chemical mixtures that have $S>1$ for applications in transdermal drug delivery.

5.2. Types of synergistic chemical mixtures

Chemical mixtures can induce skin permeation enhancement by a variety of complex mechanisms that are not always straightforward to elucidate. A simple way to classify synergistic mixtures is based on the way these mixtures are formulated from their individual components. In some cases, for example vesicles, the individual chemicals in the mixture may self-assemble to form well-defined complex secondary structures that permeabilize the skin. Alternately, the chemicals may individually exert their effect on the skin structure. Below we review several different types of chemical mixtures that have been used to enhance skin permeability to a wide range of drugs.

5.2.1. Solvent mixtures

Many classical permeation enhancers include solvents such as water, fatty acids, alcohols, glycols and fatty esters used in their pure state. A mixture of two or more solvents is one of the most widely studied formulation strategies to facilitate drug transport across the skin. The mechanisms by which such systems increase transdermal flux may include: (a) change in the thermodynamic activity (e.g., by increasing the degree of saturation in the solvent and, hence, increasing the escaping tendency) or (b) specific interaction with the stratum corneum, either by increasing the drug solubility in the stratum corneum (i.e., facilitate partitioning of drug from the vehicle into the skin) or by altering the various transport pathways (i.e., the polar and nonpolar pathways) in the stratum corneum [79,106].

Propylene glycol has been studied extensively as a co-solvent in numerous studies [79]. Several reports have documented the synergistic enhancements obtained from a mixture of propylene glycol with fatty acids [107,108], alcohols [108] and esters of fatty acids or alcohols [109,110]. Rhee et al. [111] observed that the skin permeability of clebopride from a binary mixture of diethylene glycol monoethyl ether:isopropyl myristate (40:60) was 80-fold higher as compared to that from isopropyl myristate alone. Krishnaiah et al. [112] studied the effect of various water:ethanol solutions on skin permeation of ondansetron hydrochloride and found that a synergistic mixture of 60% v/v ethanol:water showed highest skin permeation *in vitro*. Panchagnula et al. [113] studied binary combinations of water, ethanol and propylene glycol for their ability to enhance transdermal permeation enhancement of naloxone and found that well above therapeutically relevant concentrations of naloxone could be obtained by a mixture of propylene glycol:ethanol (33:67). A solvent combination of isopropyl myristate:glyceryl monocaprylate (90:10) showed synergistic enhancement in permeation of pentazocine where the flux obtained from the combined solvent system was 4-fold higher as compared to isopropyl myristate alone [114]. Transdermal flux of highly lipophilic drugs such as antiestrogens, can be enhanced extraordinarily by using a solvent combination of propylene glycol:lauric acid (90:10) [115]. The extraordinary permeation enhancement by this formulation is due to mutual permeation enhancement of these two enhancers and their synergistic lipid-fluidizing activity in the stratum corneum. Binary combinations of isopropyl myristate and short chain alkanols show transdermal flux enhancement of estradiol when compared to alkanols alone [116]. A 1:1 combination of isopropyl alcohol and isopropyl myristate improved estradiol flux by 35-fold when compared to aqueous formulations. Permeation of tegafur across excised hairless mouse skin was significantly enhanced by a binary combination of ethanol: tricaprylin (40:60) [117]. A binary combination of isopropyl myristate:n-methyl pyrrolidone (25:75) significantly improved lidocaine flux across human skin showing an enhancement of 25-fold over 100% isopropyl myristate and 4-fold over 100% n-methyl pyrrolidone [118]. Menthol:n-methyl pyrrolidone and isopropyl myristate:n-methyl pyrrolidone mixed solvent systems have also been documented to show synergistic enhancement of transdermal delivery of formoterol fumarate [119]. A binary system of triethylene glycol monomethyl ether:isopropyl palmitate can improve estradiol delivery by 60-fold when compared to the individual components [120].

The skin permeation enhancement of binary solvent mixtures can be further improved by including a third component in the mixture. For example, the permeation enhancement of estradiol and acyclovir from a ternary mixture of oleic acid, lauroylcholine and propylene glycol was much greater than the sum of the corresponding binary mixtures [107]. Fang et al. [121] studied the effect of a ternary solvent system of triethanolamine, ethanol and isopropyl myristate (IPM) on the skin permeation of acidic, basic and neutral drugs *in vitro* using excised hairless rat skin. The binary enhancer system consisting of isopropyl myristate and ethanol produced marked improvement on the penetration of all the drugs tested. When triethanol amine was added to the binary system, a greater enhancing effect was found on acidic drugs. On addition of another amine, mefenamic acid to the binary system of ethanol and isopropyl myristate, the flux improved approximately 14–180 fold. A judiciously selected ternary solvent system of propylene glycol, cis-oleic acid and dimethyl isosorbide was also proved effective in improving the flux of nifepidine across hairless mouse skin [122].

5.2.2. Mixtures of permeation enhancers in a vehicle

Using permeation enhancers at high concentrations in the form of solvents provides the opportunity to exert strong effects on skin structure, thereby improving the transdermal flux of drug molecules. While such systems may have particular benefits for certain drugs that show poor solubility in aqueous formulations, they can cause safety concerns due to the irritation induced in deeper, living layers of the skin. High levels of very potent solvents may have drastic effects on skin. They may damage desmosomes and protein-like bridges, leading to fissuring of the intercellular lipid and splitting of the stratum corneum squames. Solvents may also enter the corneocyte, drastically disrupting the keratin and even forming vacuoles [106]. Also, solvents or solvent mixtures are practically difficult to employ in transdermal patches or topical formulations. Instead chemical permeation enhancers can be formulated in a vehicle that could be a passive co-solvent, cream or gel base. In such systems the contribution of the vehicle to skin permeabilization is usually very small or negligible.

A large number of studies have now accumulated that convincingly point to superior permeation potential of permeation enhancer mixtures as compared to individual chemicals. Examples exist on permeation enhancer combinations between chemicals belonging to different groups or from within the same group [60,67,68,79]. The combination of cineole and oleic acid synergistically enhanced transdermal flux of zidovudine across rat skin [123]. The combination of two ester derivatives, dibutyl adipate and isopropyl myristate shows a synergetic effect of increased transdermal delivery [60]. N-(2-mercaptopropionyl) glycine enhances delivery of prazosin in presence of some esters and alcohols [60]. Aqueous solutions of n-lauroyl sarcosine and ethanol enhanced the flux of fluorescein across human cadaver skin by 47-fold [58]. Menthol and ethanol work synergistically to significantly enhance the flux of tetracaine across mouse skin *in vitro* and showed the shortest anesthesia onset time, the longest anesthesia duration and the strongest anesthesia efficacy in human volunteer studies [124]. The *in vitro* permeation rate of dapiprazole base (DAP-B) through hairless mouse skin was significantly enhanced by a mixture of linoleic, linolenic and arachidonic acid [125]. The *in vitro* skin delivery of furosemide was significantly improved by using a combination of oleyl alcohol and azone as permeation enhancers [126]. Combination of azone and propylene glycol was able to increase clonazepam and lorazepam percutaneous fluxes through excised human skin [127]. In the past we have undertaken several studies on exploring synergistic behavior of chemical mixtures in increasing skin permeability [80,81,105,128]. Combination of an anionic surfactant, sodium lauryl sulfate, and a cationic surfactant, dodecyl pyridinium chloride, was 2- to 3-fold better as compared to individual surfactants alone in increasing skin electrical conductivity—a measure of skin permeability [128]. Similarly, a combination of sodium laureth sulfate and phenyl piperazine was 4- to 6-fold more potent in increasing skin permeability as compared to the individual components alone [81]. This combination was effective in increasing the *in vitro* skin permeability of drug candidates such as methotrexate, low molecular weight heparin, leutinizing hormone releasing hormone (LHRH) and oligonucleotides [81]. Another combination, sodium lauroyl sarcosinate and span 20 was capable of delivering therapeutically significant doses of leuprolide acetate, a synthetic analogue of LHRH *in vivo* in a rat model [81].

5.2.3. Eutectic mixtures

Solid drugs transformed into a highly concentrated oily state at ambient temperatures exhibit increased skin permeability due to their high thermodynamic activity in the vehicle [129,130]. Melting point of a drug is inversely proportional to its lipophilicity and solubility in skin lipids. As a consequence, lowering the melting point results in increased transdermal permeation [131]. Several eutectic systems of active drug along with a skin permeation enhancer have been studied in the literature. These systems are interesting since they provide two mechanisms by which skin permeation of an active drug across skin can be enhanced. In the first, they form a low melting mixture with the drug thereby improving its partitioning into the skin. In the second, they act on skin directly to disrupt its structure and further enhance drug permeation. This synergy in mechanism can be exploited by selecting the right permeation enhancer or enhancers to be combined with the drug. Eutectic systems of ibuprofen formed with terpenes and propranolol with fatty acids have been studied successfully for improved transdermal permeation of drugs [132,133]. Kang et al. showed that the lidocaine:menthol eutectic system enhanced permeation of lidocaine across shed snake skin [134]. Kaplun-Frischoff and Touitou [135] showed enhanced permeation of testosterone across human cadaver skin when combined with menthol in a eutectic formulation.

5.2.4. Vesicles

Vesicles are colloidal particles that are composed of concentric bilayers formed from self-assembly of amphiphilic molecules. Vesicles have gained prominence as skin permeation enhancing agents as well as drug carrier agents in transdermal drug delivery [87,88]. Depending on the molecules or group of molecules that constitute the vesicles, they can be grouped in several different categories. The composition of the vesicles influences their physico-chemical characteristics such as, size, charge, thermodynamic phase, lamellarity and bilayer elasticity [136]. These physico-chemical characteristics in turn have a profound effect on the behavior of the vesicles and hence on their effectiveness in enhancing transdermal drug delivery [137–151]. Several mechanisms mediating the vesicle–skin interactions have been proposed in the literature. These interactions can putatively occur either at the skin surface or in the deeper layers of the stratum corneum depending upon the elasticity or deformability of the vesicles [152–158]. Synergistic interactions between the components of the vesicles and between the vesicles and skin constituents are believed to be responsible for the superior skin permeation enhancement of vesicular systems [159,160]. It is quite likely that the plurality of multicomponent vesicle systems interact with the stratum corneum via very similar mechanisms. We have therefore classified these mixed chemicals systems based on their constituents rather than mechanisms of interaction with the skin.

Several different kinds of vesicles have been described in the literature. Liposomes consist of lipids such as cholesterol and phospholipids and typically work by encapsulating drugs in their core and increasing their deposition in the stratum corneum [141,161–163]. Mezei and Gulasekharam [164] showed that triamcinolone acetonide concentrations in skin were observed to be 4- to 5-fold higher when

delivered from liposomes as compared to conventional formulations. Similar observations were made for progesterone and econazole. Several other studies have corroborated these findings of improved skin deposition of drugs from liposomes [165–168]. Dermal delivery with skin-lipid liposomes was shown to be more effective than delivery with phospholipid vesicles. One limitation of liposomes is that they are ineffective in delivering drugs to deeper layers of skin [169], however some studies have claimed that these particles are indeed transported across the skin [146,170].

Niosomes are composed of non-ionic amphiphiles (surfactants) and are similar in function to the liposomes [150,171–173]. Several studies have documented the superiority of niosomes in enhancing permeation of drugs across the stratum corneum [171,174,175]. Recently, Paolino et al. [176] have shown that Niosomes constructed from a new non-ionic surfactant alpha,omega-hexadecyl-bis-(1-aza-18-crown-6) (Bola-surfactant), span 80 and cholesterol show significantly improved percutaneous permeation of ammonium glycyrrhizinate with respect to both the aqueous drug solution and a physical mixture between unloaded Bola-niosomes and the aqueous drug solution. Niosomes constructed from cholesterol, span 60 and dicetylphosphate were effective in increasing skin permeation of frusemide across mouse skin as compared to conventional formulations [177]. In general niosomes are well suited for delivery of hydrophobic drugs as compared to hydrophilic drugs.

Ethosomes are relatively new types of vesicle systems, primarily composed of water, ethanol and phospholipids [160,178,179]. Ethosomes were reported to be effective at delivering molecules to and through the skin to the systemic circulation. Elsayed et al. [169]. have reviewed the use of ethosomes in both *in vivo* and *in vitro* studies for delivery of various drugs across skin including acyclovir, testosterone, cannabidiol, erythromycin, ammonium glycyrrhizinate, sotalol, sodium salicylate, propanolol, trihexyphenidyl, minoxidil, azelaic acid, zidovudine and ketotifen. More recently, Rao et al. [180] demonstrated that the transdermal flux of fenasteride from ethosomal formulations was 2- to 7-fold higher as compared to aqueous formulations. Dubey et al. [181] showed in their work that transdermal flux of methotrexate across human cadaver skin can be enhanced from an optimally designed ethosomal formulation containing 3% phospholipids and 45% ethanol.

Transfersomes are ultradeformable hydrophilic lipid vesicles that putatively cross the skin under the influence of a transepidermal water activity gradient. Transfersomes consist of phospholipids and an edge activator that increases the deformability of the bilayers and is often a single chain surfactant such as sodium cholate, sodium deoxycholate, Span 60, Span 65, Span 80, Tween 20, Tween 60, Tween 80 or dipotassium glycyrrhizinate [182–190]. The effects of different edge activators on transfersome properties have been extensively investigated in several studies [190–193]. Ultradeformable vesicles have been shown to be successful in delivering a range of different dugs across the skin including 5-fluorouracil [194], lidocaine [195], tetracaine [195], cyclosporine A [196], insulin [197, 198], diclofenac [198], triamcinolone acetonide [199,200], hydrocortisone [201], dexamethasone [202], levonorgestrel [202], estradiol [203], low molecular weight heparin [204], methotrexate [189], dipotassium glycyrrhizinate [205] and zidovudine [206].

5.2.5. Microemulsions

Microemulsions are clear, stable, isotropic mixtures of oil, water, and surfactant, frequently in combination with a cosurfactant [207]. These are easy and inexpensive to formulate, have high thermodynamic stability and improve the solubilization of hydrophilic as well as hydrophobic drugs. Several excellent reviews include examples of a large range of microemulsions used in transdermal drug delivery of a wide variety of drugs [207–211]. The permeation enhancement offered by a microemulsion depends largely on the selection of oil, surfactant and co-surfactant as well as their relative composition and concentration in the mixture. The oil phase is usually represented by acids such as oleic acid or esters such as isopropyl myristate, isopropyl palmitate, isostearylic isostearate, glycerin triacetate or terpenes such as limonene or medium chain triglycerides. The surfactant phase is usually represented by naturally occurring lipids such as phosphatidylcholine, dioleylphosphatidyl ethanolamine and distearoylphosphatidyl choline. Other surfactants such as Tween 20, Tween 80, Span 20, Azone, plurol isostearique and plurol oleique have also been used. Commonly used co-surfactants in microemulsions include long chain alcohols such octanol, decanol and dodecanol [207]. Each of the individual components of the microemulsion may be capable of enhancing the transdermal delivery of a drug but their presence in combination results in synergistic enhancement, significantly increasing the transdermal flux of the drug molecule. Gupta et al. [212] have shown that transdermal flux of 5-Fluorouracil, an antineoplastic drug, increased 2- to 6-fold from a microemulsion of sodium bis(2-ethylhexyl) sulfosuccinate:water:isopropyl myristate as compared to an aqueous solution of the same drug. Changez et al. [213] studied transdermal flux of tetracaine hydrochloride from lecithin:n-propanol:isopropyl myristate:water microemulsions. They showed that microemulsions enhanced mouse skin permeability to tetracaine hydrochloride by 20- to 25-fold depending upon the composition of the microemulsion. Microemulsions may work by enhanced disruption of skin-lipid structure or by improving the stability of the drug in the formulation. Gallarate et al. [214] studied the stability of ascorbic acid in several microemulsions. Isopropyl palmitate or cetearyl octanoate were used as oils, dodecylglucoside and cocoamide propylbetaine were used as surfactants, and 2-ethyl-1,3-hexanediol was chosen as a cosurfactant. Stability of ascorbic acid against oxidation was found to be superior in the microemulsion systems as compared to that in the aqueous formulations. Zhu et al. [215] showed that skin permeation of penciclovir from a microemulsion formulation of oleic acid:Cremorphor EL:ethanol:water was 3.5-fold higher as compared to a commercial cream.

5.2.6. Inclusion complexes

Inclusion complexes are structured molecular cages that encapsulate an active drug molecule in their core. The most extensively studied agents to form inclusion complexes are cyclodextrins [89–94]. In general, cyclodextrin inclusion complexes are believed to improve the drug stability by preventing degradation, oxidation or hydrolysis and improving drug solubility. Many studies have described the action of cyclic oligosaccharides and in particular beta-cyclodextrins in increasing skin permeability of hydrophilic drugs. For example, Masson et al. [95] have shown that cyclodextrins act as permeation enhancers carrying the drug through from the bulk formulation towards the lipophilic surface of biological membranes, where the drug molecules partition from the complex into the lipophilic membrane. Further, De Paula et al. [96] have shown that increased absorption of estradiol in the stratum corneum was a result of increase in drug availability on the skin surface due to inclusion complexation. Jug et al. [97] have proposed that cyclodextrins form inclusion complexes of drugs and deliver the drug molecules to the barrier surface where complex dissociation and drug permeation across the membrane occurs. For example, beta-cyclodextrin was able to maintain the stability of tixoxortol 17-butyrate 21-propionate for 30 days at 40 °C [216]. Beyond providing a stabilizing pocket for the drug, cycoldextrins can work synergistically with permeation enhancers to improve their absorption across the skin. Adichi et al. [217] demonstrated that an inclusion complex of prostaglandin E1 (PGE1) with O-carboxymethyl-O-ethyl-beta-cyclodextrin (CME-beta-CyD) in a fatty alcohol:propylene glycol ointment base supplemented with a permeation enhancer 1-[2-(decylthio)ethyl]azacyclopentane-2-one (HPE-101) improved the transdermal flux of PGE1 across

hairless mouse skin by approximately 100-fold as compared to that of PGE1 alone and approximately 10 times that of PGE1 with HPE-101. Cyclodextrin can complex with enhancers like quaternary ammonium salts and reduce their toxic side effects on skin while still maintaining their skin permeabilization capacity, thereby showing a synergy between safety and potency [218]. Several other studies have reported synergistic behavior between cyclodextrins and conventional permeation enhancers [93,219,220].

6. Design of synergistic mixtures

In the last six decades of extensive research in the area of chemical permeation enhancers, more than 300 chemicals of varying skin permeabilization potential have been identified. New permeation enhancers are being continually added to this pool as our understanding of the interactions between skin constituents and different chemicals advances. Several investigators including us have successfully related structure of chemical enhancers to their safety and potency [56,100,101,106]. These efforts have resulted in design of superior chemical permeation enhancers. However, the field is not yet mature enough to design synergistic combinations of permeation enhancers from this knowledge. Existing theories explaining interactions between permeation enhancers and skin constituents are not always sufficient to explain the interaction between a mixture of permeation enhancers and skin constituents. Another complexity arises from the interactions between the individual enhancers of the combination in the vehicle even before they interact with the skin. Enhancers distributed in various chemical classes can interact with each other in a variety of ways resulting in myriad different species exhibiting polydispersity in concentration, composition and chemical behavior. In an extensive study of approximately 5000 unique binary combinations of chemicals, we [81] have shown that random combinations of chemicals rarely result in highly synergistic behavior. In fact, less than 1% of the entire candidate pool tested in our study showed synergistic behavior. Further, desired synergistic behavior was more likely to occur in a very narrow range of chemical compositions of the involved components. Since it is difficult to determine *a priori* where synergy would be observed in the composition space of the mixture, it is essential to experimentally determine the activity of multicomponent formulations over all possible composition ranges.

6.1. Experimental methods for discovering synergistic combinations

Usually, synergistic mixtures are designed empirically from individual components that have been shown to enhance flux of a particular drug across the skin. These components are combined in different proportions and their effects tested on skin permeation of the drug. Alternately, chemical mixtures can be designed in a systematic manner by varying the total concentration as well as composition of all the individual components. This however, can result in a large number of potential test formulations. For example, the number of binary mixtures that can be designed from the current pool of ~300 permeation enhancers at 5 different total concentrations and 10 compositions is well over a million. For ternary mixtures the number of formulations would be well over a trillion. Several synergistic mixtures such as vesicles and microemulsions routinely contain more than 3 components and their rational design would require testing a huge pool of potential test formulations. Franz diffusion cells (FDCs) are currently the workhorse of all permeation experiments in transdermal studies. FDCs utilize permeation of a model solute that may be a dye or radioisotope, or the actual drug to evaluate the effect of penetration enhancers on skin permeation. These experiments are cumbersome, have long hold-up times and require manual sampling. Although semi-automated versions of FDCs have been developed to reduce manual effort, their throughput still remains low (10 experiments a day). As a result FDCs become impractical when used for screening a large library of $O(10^6)$ formulations. To overcome the limitations of FDCs, high throughput methodologies such as INSIGHT (IN vitro Skin Impedance Guided High Throughput) were designed to accelerate the discovery of synergistic combinations of permeation enhancers. The high throughput of INSIGHT comes from the use of a surrogate measure, skin conductivity, instead of solute permeation across the skin. Several hitherto undiscovered combinations of permeation enhancers were identified in this study that provided high skin permeation enhancement. These combinations not only demonstrate a synergy in disrupting skin barrier but also a synergy between safety and potency of permeation enhancers [81]. In addition to discovering synergistic mixtures of chemical enhancers, INSIGHT screening has also been used successfully in generating qualitative "rules" for combining various chemicals from different classes to increase the rate of discovery of synergistic chemical enhancer mixtures [105]. For example, equi-molar or equi-mass combinations of chemical enhancers are more likely to show synergistic behavior. Methyl pyrrolidone, a small molecule, shows a high propensity for forming synergistic combinations that are also very potent in increasing skin permeability. Zwitterionic surfactants are more likely to feature in potent combinations whereas cationic surfactants and fatty esters are more likely to form synergistic combinations amongst different chemical classes. Simple but invaluable rules like these will provide guiding principles for designing focused libraries to further speed up the discovery process.

6.2. Statistical models for discovering synergistic combinations

Models based on fundamental interactions between chemical enhancers, and between chemical enhancer mixtures and skin can expedite the process of designing synergistic mixtures of chemical enhancers without the need for extensive brute force experimental efforts. As noted earlier, this is an extremely challenging task given the diverse nature of chemical enhancers as well as the plurality of mechanisms by which chemical enhancers interact with the skin constituents. We and others have started investigations into exploring why certain chemical enhancer mixtures show synergistic behavior [58,80,105,221,222]. We have discovered that equimolar mixtures of chemical enhancers are typically more likely to form synergistic combinations [105]. We have investigated the mechanism of one such synergistic combination, NLS:S20 in details. The high synergy of equimolar formulations of NLS:S20 can be attributed to secondary micellar-like structures that arise from combining NLS and S20 in equimolar proportions [80]. It is quite likely that, in general, equimolar combinations of chemicals are more likely to form secondary structures and thus are more effective in permeabilizing the skin.

Detailed investigations into the mechanisms of interactions of chemical mixtures with skin are laborious and time consuming. Such investigations become even more challenging when the number of components in the mixture exceeds two. One way to simplify complex multicomponent interactions in chemical mixtures is to view them as additive interactions between all possible binary pairs in the mixture. Such a statistical approach to complex systems has been used successfully in the past [223–225]. While such a representation may not be adequate in providing fundamental insights into the synergistic mechanisms of chemical enhancer mixtures, it provides a basis for predicting interesting mixtures (ternary and higher) of chemical enhancers that can be studied in further details experimentally. High throughput screening tools such as INSIGHT can be used to generate data on a large pool of binary mixtures. These data can then be used in conjunction with simple statistical models to develop algorithms that predict the efficacy of higher order mixtures, thereby significantly reducing the 'wet' effort in discovery. The skin permeabilization

efficacy, for example, of a ternary mixture of three individual chemical enhancers A, B and C can be proposed as follows:

$$E_{ABC} = \gamma_A E_A + \gamma_B E_B + \gamma_C E_C + \gamma_{AB} E_{AB} + \gamma_{BC} E_{BC} + \gamma_{CA} E_{CA}$$

Where E_{ABC} represents efficacy of a ternary mixture of A, B and C; E_{AB}, E_{BC} and E_{CA} represent efficacies of binary mixtures of A, B and C; E_A, E_B, E_C represent efficacies of individual components. γ represents a pseudo activity coefficient for a single component or a pseudo interaction coefficient for combinations. One way to determine these activity or interaction coefficients is by using rigorous thermodynamic treatments. For predictive purposes in equations such as those represented above, γ may be determined by pure statistical approaches by mining large data sets, such as those collected by INSIGHT. Whether or not such models are successful in predicting experimental data remains to be seen.

7. Conclusions

Synergistic systems employing chemical mixtures offer a way to overcome some of the limitations of individual chemicals in enhancing transdermal drug delivery. Combinations of chemicals can be used to not only improve the potency of permeation enhancers but also their safety. In the past, design of synergistic systems was limited by the low throughput of experimental screening methods. With the availability of high throughput screening platforms and rational design strategies based on improved understanding of the interactions between skin constituents and chemicals, the design of novel synergistic systems should be accelerated in the future. Further, synergistic chemical systems are no longer limited to simple empirical mixtures of traditional permeation enhancers but they can be designed to include more complex systems such as vesicles, microemulsions and inclusion complexes.

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