

Review article

Transdermal iontophoresis: combination strategies to improve transdermal iontophoretic drug delivery[☆]

Yiping Wang^a, Rashmi Thakur^a, Qiuxi Fan^b, Bozena Michniak^{c,*}

^aErnest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ, USA

^bInterdisciplinary Program for Materials Science and Engineering, New Jersey Institute of Technology, Newark, NJ, USA

^cDepartment of Pharmacology and Physiology, Laboratory for Drug Delivery, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, NJ, USA

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Abstract

For several decades, there has been interest in using the skin as a port of entry into the body for the systemic delivery of therapeutic agents. However, the upper layer of the skin, the stratum corneum, poses a barrier to the entry of many therapeutic entities. Given a compound, passive delivery rate is often dependent on two major physicochemical properties: the partition coefficient and solubility. The use of chemical enhancers and modifications of the thermodynamic activity of the applied drug are two frequently employed strategies to improve transdermal permeation. Chemical enhancers are known to enhance drug permeation by several mechanisms which include disrupting the organized intercellular lipid structure of the stratum corneum [1], ‘fluidizing’ the stratum corneum lipids [2], altering cellular proteins, and in some cases, extracting intercellular lipids [3]. However, the resulting increase in drug permeation using these techniques is rather modest especially for hydrophilic drugs. A number of other physical approaches such as iontophoresis, sonophoresis, ultrasound and the use of microneedles are now being studied to improve permeation of hydrophilic as well as lipophilic drugs. This article presents an overview of the use of iontophoresis alone and in conjunction with other approaches such as chemical enhancement, electroporation, sonophoresis, and use of microneedles and ion-exchange materials.

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1. Skin as a barrier to permeation

Human skin consists of three main layers—the epidermis, dermis and hypodermis. The dermis is vascularised and the thickest of all the layers (3–5 mm thick). It possesses sweat glands, hair follicles, nerve endings and lymph vessels and acts as the systemic absorption site for drugs. The epidermis, however, constitutes the rate-limiting layer

for transdermal absorption of drugs (thickness varies from 0.06 mm on eyelids to 0.8 mm on soles of palms/feet). The major barrier to permeation resides in the uppermost of the five layers of the epidermis, the stratum corneum. It is approximately 10–20 μm thick (depending on hydration level) and acts as a protective membrane preventing water loss from the skin and limiting the ingress of chemicals from the environment. As the cells from the basal layer differentiate upwards towards the upper layers of the skin, they accumulate keratin and become much more flattened [4]. The stratum corneum thus formed has only 20% water and forms a highly lipophilic membrane. The intercellular lipids within this layer are organized into structured bilayers which have to be traversed by the applied drug which in its passage encounters several lipophilic and hydrophilic domains [5].

On average, the skin surface contains 40–70 hair follicles (highest density on the head, neck, shoulders and zero

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* Corresponding author. Department of Pharmacology and Physiology, Laboratory for Drug Delivery, New Jersey Medical School, University of Medicine and Dentistry of New Jersey, Newark, NJ 07103, USA. Tel.: +1 973 972 9720; fax: +1 973 972 9727.

E-mail address: michnibb@umdnj.edu (B. Michniak).

density on plantar and palmer surfaces) and 200–250 sweat ducts in every square centimeter [6]. Together these occupy about 0.1% of total human skin surface and water soluble and ionic compounds (such as peptides and proteins) are known to permeate the skin via such appendages. In addition, some drugs can also penetrate the skin by passive diffusion following either the transcellular or paracellular pathway.

2. Iontophoresis

The highly lipophilic nature of the skin restricts the permeation of hydrophilic, high molecular weight and charged compounds through the stratum corneum into the systemic circulation. However, many therapeutically active drug molecules are hydrophilic and possess high molecular weights for example, peptides.

Iontophoresis simply defined is the application of an electrical potential that maintains a constant electric current across the skin and enhances the delivery of ionized as well as unionized moieties. This technique is capable of expanding the range of compounds that can be delivered transdermally. Along with the benefits of bypassing hepatic first pass effect, and higher patient compliance, the additional advantages that the iontophoretic technique offers can be summarized as follows:

1. Delivery of ionized and unionized drugs
2. Enabling continuous or pulsatile delivery of drug (depending on the current applied)
3. Permitting easier termination of drug delivery
4. Offering better control over the amount of drug delivered since the amount of compound delivered depends on applied current, duration of applied current, and area of skin exposed to the current
5. Restoration of the skin barrier function without producing severe skin irritation
6. Improving the delivery of polar molecules as well as high molecular weight compounds
7. Ability to be used for systemic delivery or local (topical) delivery of drugs
8. Reducing considerably the inter and intra-individual variability since the rate of drug delivery is more dependent on applied current than on stratum corneum characteristics [7,8].

2.1. Principles of iontophoresis

The iontophoretic technique is based on the general principle that like charges repel each other. Thus during iontophoresis, if delivery of a positively charged drug (D^+) is desired, the charged drug is dissolved in the electrolyte surrounding the electrode of similar polarity, i.e. the anode in this example (Fig. 1). On application of an electromotive

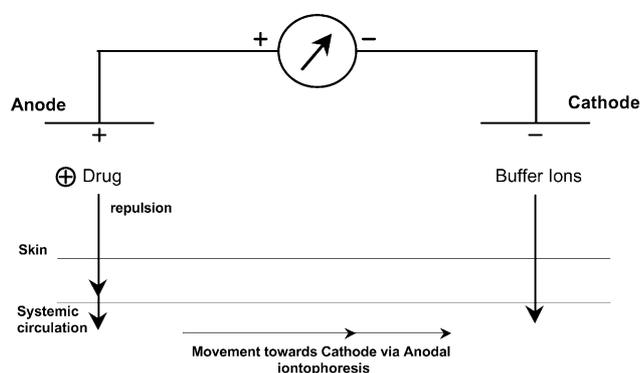


Fig. 1. Diagram of iontophoretic technique: as current is applied the drug cations are repelled into the skin and eventually absorbed in the systemic circulation.

force the drug is repelled and moves across the stratum corneum towards the cathode, which is placed elsewhere on the body. Communication between the electrodes along the surface of the skin has been shown to be negligible [9], i.e. movement of the drug ions between the electrodes occurs through the skin and not on the surface. When the cathode is placed in the donor compartment of a Franz diffusion cell to enhance the flux of an anion, it is termed cathodal iontophoresis and for anodal iontophoresis, the situation would be reversed.

Neutral molecules have been observed to move by convective flow as a result of electro-osmotic and osmotic forces on application of electric current [10]. Electromigration of ions during iontophoresis causes convective solvent motion and this solvent motion in turn ‘drags’ neutral or even charged molecules along with it. This process is termed as electro-osmosis. At pH values above 4, the skin is negatively charged [11], implying that positively charged moieties like Na^+ molecules will be more easily transported as they attempt to neutralize the charge in the skin to maintain electroneutrality [11]. Thus the movement of ions under physiological conditions is from the anode to the cathode. For loss of each cation (sodium ion in this case) from the electrode in this process, a counterion, i.e. an anion, Cl^- moves in the opposite direction from the cathode to the anode. It is the transport number of each ion, which describes the fraction of the total current transferred by the ion and depends on the physicochemical properties of the respective ions. t_{Na^+} is greater than t_{Cl^-} and also the skin facilitates movement of Na^+ more than Cl^- , hence there is a net increase in the $NaCl$ in the cathodal compartment and net decrease in $NaCl$ on the anodal side. Due to this electrochemical gradient, osmotic flow of water is induced from the anode to the cathode. If any neutral drug molecules are present at the anode at this time they can be transported through the skin along with the water. Such water movement often results in pore shrinkage at the anode and pore swelling at the cathode [12].

The basic mechanisms of ionic/molecular transport across the skin by iontophoresis can be summarized as follows:

Like charges repel. Hence the charged ion is repelled into the skin from a similar charged electrode. The skin being negatively charged at physiological pH acts as a cation-selective membrane and favors movement of cations through anodal iontophoresis. Anodal iontophoresis also causes convective motion of the solvent occurring in response to movement of counterions. This process of electro-osmosis is involved in the motion of neutral compounds as well as positively charged ions.

Due to the complex nature of iontophoretic delivery, a number of attempts have been made to define the rate of iontophoretic delivery. The Nernst-Planck equation has been used with modifications to predict iontophoretic enhancement ratios (ratio of steady state flux in presence of electric potential and in absence of potential) as the original equation lacks a term for convective electro-osmotic flow [13]. Srinivasan and Higuchi [13] and Pikal and Shah [14–16] studied the contributions of osmotic flow and incorporated this fact into several equations.

The increased flux during iontophoresis would include [17]:

1. Flux due to the electrochemical potential gradient across the skin; 2. Change in the skin permeability due to the electric field applied; and 3. Electro-osmotic water flow and the resultant solvent drag.

$$j_{\text{ionto}} = j^{\text{electric}} + j^{\text{passive}} + j^{\text{convective}}$$

j^{electric} is the flux due to electric current application; j^{passive} is the flux due to passive delivery through the skin; and $j^{\text{convective}}$ is the flux due to convective transport due to electro osmosis.

2.2. Pathways of molecular transport in iontophoresis

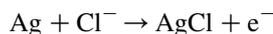
Skin appendages which include sweat glands and hair follicles are postulated to be involved in the major pathways of drug transport during iontophoresis [4]. Evidence from studies comparing iontophoretic delivery in hairless and regular rats suggests a much larger contribution of the sweat glands and ducts as opposed to hair follicles in permeation [18]. Other pathways which have been shown to be involved in iontophoretic delivery include paracellular transport especially for water and uncharged polar solutes [19], artificial shunts due to temporary disruption of the organized structure of the stratum corneum [20], potential-dependent pore formation has also been observed [17].

2.3. Factors affecting iontophoresis

One important factor is pH and this affects iontophoresis in two ways. The pH of the donor solution influences the pH of the skin and thus makes the skin a permselective

membrane especially if the pH of the skin rises above 4. This causes the carboxylic acid moieties in the skin to become ionized and then the anodal iontophoresis promotes the permeation of cationic drugs. The pH of the donor solution also affects the ionization of the drug itself. Thus a weakly basic drug will be ionized to a lower extent at pH higher than its pK_a and will not permeate by electromigration in presence of iontophoresis. The drug will be more dependent on electro-osmosis to travel across the skin.

The type of electrodes used also affect the iontophoretic delivery. Electrodes Ag/AgCl are the most preferred as they resist the changes in pH which are generally seen during the use of platinum or zinc/zinc chloride electrodes. The following reactions typically occur at the anode [21]



The electron is released to the circuit and insoluble AgCl precipitates at the anode surface. In the case of other metals like platinum, the chloride ion at the anode will be converted to Cl_2 which will in turn react with water to generate hydronium ions. These then migrate to the donor solution and compete with similar-charged drug ions and being highly mobile enter the skin thus reducing drug transport and simultaneously causing skin irritation.

Other important factors affecting iontophoretic delivery include concentration of co-ions (buffers), current strength, type of current used, type of skin used, concentration of solute in the donor, temperature of acceptor phase, the charge on the drug and the type of vehicle used. Meidan et al. have been performing delivery studies using buspirone hydrochloride (BH) which is a structurally and pharmacologically unique anxiolytic used to treat a variety of conditions [22]. The in vitro iontophoretic delivery of BH through human skin was investigated using Franz diffusion cells. It was observed that as the concentration of buspirone hydrochloride in the donor compartment was increased from 2 to 3%, the transdermal flux using water as a vehicle increased from 341 ± 18 to $357 \pm 17 \mu\text{g}/\text{cm}^2 \text{ h}$ whereas when the vehicle used was 50% ethanol, the flux of the drug across the human cadaver skin increased from 351 ± 8 to $363 \pm 39 \mu\text{g}/\text{cm}^2 \text{ h}$ thus failing to show any significant influence of vehicle or drug concentration on BH flux. However, the flux increased linearly (following application of iontophoresis) with increasing current density. Thus by applying iontophoresis at $0.5 \text{ mA}/\text{cm}^2$, it was possible to achieve a BH steady state flux of approximately $350 \mu\text{g}/\text{cm}^2 \text{ h}$, which would be therapeutically effective if clinically duplicated. Though the maximum tolerable current increases with increase in electrode area, an upper limiting value of current has been suggested to be $0.5 \text{ mA}/\text{cm}^2$ for iontophoresis [17].

The presence of a co-ion (ion with the similar charge as the drug) results in competition between the drug and the co-ion, a reduction of the fraction of the current carried by the drug and thus a reduction in the transdermal iontophoretic flux of the drug. A most common source of co-ions is the buffer

added to control the pH of the donor medium. Nugroho et al. compared the transdermal iontophoretic permeation of rotigotine in presence of three different co-ions: Na^+ , tetra ethyl ammonium (TEA^+) or tetra butyl ammonium (TBA^+) at pH 5 and 6 [23]. The iontophoretic flux of rotigotine was lower in presence of Na^+ as compared to TEA^+ and TBA^+ which can be attributed to the higher mobility of the sodium ion due to its lower molecular weight. Replacing Na^+ by the larger co-ion TEA^+ resulted in an increase of the rotigotine flux both at pH 5 and 6.

The pattern in which the current is applied also affects the permeation profile. Use of continuous direct current may result in skin polarization, which in turn reduces the efficiency of iontophoresis. To overcome this build-up, pulsed direct current is used which delivers direct current periodically allowing the skin to return in between to its original condition. The flux obtained by both methods is comparable and any polarization induced skin damage is also prevented [24].

2.4. Applications of iontophoresis

2.4.1. Treatment of hyperhidrosis

Hyperhidrosis (also called hyperhidrosis) is a condition that most often results in excessive sweating in the hands and feet. Tap water iontophoresis is one of the most popular treatments used in this condition. The procedure uses a mild electrical current that is passed through tap water to temporarily shut off sweat glands. A hand and foot is each placed in a different water basin and the electric current is gradually increased to the required level and maintained for 20 min followed by a gradual decrease. The underlying mechanism of how iontophoresis helps treat this ailment is not fully understood. According to one hypothesis, iontophoresis may induce hyperkeratosis of the sweat pores and obstruct sweat flow and secretion (although no plugging of the pores has been found) [25]. Other proposed mechanisms include impairment of the electrochemical gradient of sweat secretion and a biofeedback mechanism [26]. Successful induction of hypohidrosis by tap-water iontophoresis requires the application of 15–20 mA to each palm or sole for 30 min per session for 10 consecutive days, followed by one or two maintenance sessions per week [25]. The advantage of using tap water iontophoresis is that the patient can conduct the procedure at home.

2.4.2. Topical delivery

The ability to control the delivery rates of drugs by changes in current makes iontophoresis an attractive technique to use. Yamashita et al. studied the efficacy of iontophoretic delivery of calcium for treating hydrofluoric acid-induced burns [27]. The authors conducted the experiments using rats as *in vivo* models. Hydrofluoric acid burns were induced by dispensing 50% hydrofluoric acid on the backs of the rats under anesthesia and the rats were divided into five groups: control group (untreated), one

group treated with 2.5% calcium gluconate jelly applied once for the duration of the experiment on the burn area on the back of the rat, third group treated with intradermal and subcutaneous calcium gluconate injection and the last group was subjected to calcium chloride iontophoresis at 1.5 V. Burn areas were used as a measure to assess the efficacy of treatment and pathologic findings were classified microscopically into five stages at 1 week: stage 1, epidermal burn; stage 2, superficial dermal burn; stage 3, deep dermal burn; stage 4, full-thickness burn; and stage 5, burn affecting the skeletal muscle. They observed that burn areas were significantly reduced by iontophoresis more than any other mode of calcium administration, and iontophoresis was more efficacious than topical or injection therapy for experimental hydrofluoric acid burns.

Topical delivery of anesthetics during dermal surgery remains the most common topical application of iontophoresis. Hydrochloride salts of anesthetics of the amide type like lidocaine [28], bupivacaine [29], etidocaine [29], mepivacaine [29], prilocaine [29] and ropivacaine [29] have been widely studied. Lidocaine been successfully formulated in an iontophoretic patch for dermal anesthesia (Vyteris, Inc., Fairlawn, NJ, USA).

2.4.3. Non-invasive monitoring of glucose

Electro osmotic flow generated by application of low-level current has been used for extraction of glucose through the skin. As the direction of glucose flow is in the opposite direction (in outward direction in skin) to conventional iontophoresis, it is called reverse iontophoresis. This property in combination with *in situ* glucose sensors has been used in GlucoWatch[®] Biographer (Cygnus Inc., Redwood City, CA, USA) [30]. This device allows noninvasive extraction glucose across the skin, allowing a diabetic's glycemia to be evaluated every 10 min over several hours. The Biographer is constituted of a small wristwatch device containing sampling and detection devices, electronic circuitry, and a digital display. As the negatively charged skin at physiological pH is subject to iontophoresis by the electrodes in the device, the sodium ions move from the anode towards the cathode and create a convective flow. Glucose thus gets transported to the cathode with the solvent where it is oxidized by glucose oxidase to release hydrogen peroxide. This is then detected by the custom designed biosensor in the system [31]. Research in the near future could link the detection level to release of insulin as per the needs of the patient which would be another substantial step towards creating a 'closed loop biofeedback' drug delivery system. Another study by Merino et al. has demonstrated sampling of phenylalanine by reverse iontophoresis [32]. The limitations of these non-invasive biological sampling techniques would be in their ability to measure reliably and accurately low levels of analytes.

2.4.4. Delivery of antisense oligonucleotides

Antisense oligonucleotides bind to the mRNA of the disease-causing genes and inhibit their expression so as to block synthesis of disease related proteins. These oligonucleotides are usually delivered by injection and hence an alternative route for systemic delivery is desirable. The transdermal delivery route is attractive because it may enable the localized delivery of the oligonucleotide into skin layers, which is desirable in conditions such as dermatitis and psoriasis. IL-10 over-expression for example, is one of the important pathogenic factors in skin lesions resulting from atopic dermatitis (AD). Thus, the regulation of IL-10 production is a potential solution for immunotherapeutic intervention in AD. A study has been conducted by Sakamoto et al. [33] which included the topical delivery of an antisense oligonucleotide for mouse IL-10 and the observation of the therapeutic effect on the AD skin lesions of mice. By using iontophoresis the authors were able to deliver 30% of the applied dose locally to the dermis and the epidermis. Topically delivered oligonucleotide decreased the levels of mRNA and protein of IL-10 in the lesions of mice and the dorsal lesions disappeared with repeated topical application. It was concluded that this delivery system offered potential therapy for established dermatitis patients.

In addition, a number of studies have demonstrated measurable concentrations of oligonucleotides with *in vitro* and *in vivo* delivery [34,35]. However, the question still unanswered is whether an iontophoretic patch of reasonable size and current strength is able to deliver a useful dose of pharmacologically active oligonucleotide [36]. The need currently is for more studies in this area and additional *in vivo* studies to support the *in vitro* data.

2.4.5. Peptide delivery

This seems to be one of the most promising applications of iontophoretic transdermal delivery. Transdermal delivery itself offers the advantages of bypassing first pass metabolism and gastrointestinal degradation as well as patient compliance over the existing oral and parenteral routes of administration for peptide delivery. An additional advantage that it offers specifically for proteins and peptides is the avoidance of strong proteolytic conditions as found in the gastrointestinal tract [37]. Chien et al. [4] have studied the delivery of oligopeptide, vasopressin, with transdermal periodic iontophoretic system (TPIS). The TPIS procedure delivered a d.c. pulse with various combinations of waveforms, frequency, on/off ratio and current intensity for specified application time. The results suggested that in the absence of TPIS, the rate of skin permeation of vasopressin was negligible but in the presence of TPIS, not only did the flux increase 190-fold but the lag time was also reduced by almost 9 h. Over the years a wide range of proteins and peptides such as LHRH [38], salmon calcitonin [39], and human parathyroid hormone [40] have been studied for transdermal delivery via iontophoresis.

3. Iontophoresis in conjunction with electroporation

3.1. Electroporation

Transdermal electroporation is the application of short (< 1 s), high voltage (50–500 V) pulses to the skin to cause disorganization the stratum corneum lipid structure and hence to enhance drug delivery. Electroporation is also used for DNA transfection of mammalian cells [41,42].

Electrical studies have shown that membrane resistance can drop orders of magnitude on a time scale of milliseconds or faster upon the application of electroporation and there is indirect evidence that high voltage pulses cause changes in the skin structure [42,43]. The creation and/or the enlargement of aqueous pathways during electroporation has been proposed and observed in many studies. In an X-ray scattering analysis, disordering of lipid lamellar stacking and of the lipid lateral packing was observed after application of long electroporation pulses [44]. Freeze-fracture electron microscopy showed that high voltage pulses induced a general perturbation of the intercellular lipid materials [45]. A light microscopy study found increased detachment in the stratum corneum cell layers with increasing electroporation voltages from 100 to 300 V [45].

Voltage, pulse length, number of pulses, and physico-chemical properties of drugs are among the factors affecting drug permeation in electroporation. Two different types of electrical pulsing protocols have been used: exponentially decaying pulses (ED) and square wave pulses (SW). A study performed by Vanbever et al. [46] indicated that the intermittent application of short (~1 ms) high-voltage (~100 across skin) ED pulses and a few applications of long (= 100 ms) medium-voltage (> 30 V across skin) ED pulses had similar alterations and recovery processes of skin electrical resistance, while long pulses of medium-voltage appeared to be more efficient in transporting sulforhodamine (molecular weight 607) across skin. For the same total transported charge, long pulses induced faster and greater molecular transport across skin than short pulses.

However, in another study [47], the application of ED (10 pulses with a pulse time ± 150 ms separated by 30 s) and SW (10 pulsed with a pulse time 150 ms separated by 30 s or 891 pulses with a pulse time of 1.68 ms separated by 0.125 s), which transferred the same amount of charge (0.63 C) and had the same voltage (150 V), induced the same transport of FITC-dextran (molecular weight 12 kDa).

Pulse length could be the second most important parameter next to the voltage determining electroporative drug penetration. In the Sharma et al. study [48], 20 voltage pulses were used with lengths of 10, 20, 30, and 40 ms to enhance the *in vitro* delivery of terazosin hydrochloride through hairless rat skin. The results showed a fairly linear relationship ($r^2=0.94$) between terazosin hydrochloride delivered and the pulse lengths. The Sharma et al. study also illustrated the importance of pulse number. With the same

pulse length, an increase in the number of pulses, resulted in a marked increase in the amount of terazosin delivered to the skin.

Electroporation has been demonstrated to increase transport across and into the skin of hydrophilic molecules, neutral or highly charged compounds, and macromolecules. FITC and a series of FITC-dextrans of increasing molecular weight (4.4, 12 and 39 kDa) were used as model macromolecules to assess the influence of molecular weight on electroporation-induced transdermal delivery [47]. The transdermal delivery of FITC and FITC-dextran 4.4 kDa was equivalent whereas the fluxes of the two higher molecular weight FITC-dextrans were one order of magnitude lower.

Although some sensation may be caused by direct excitation of nerves by the applied electric field, electroporation is normally thought to be safe. Riviere et al. performed an in vivo evaluation of porcine skin, using histological scores and by scaling the degree of erythema, edema and recording the presence of petechia after electroporative applications [49]. The only skin alterations observed with electroporation were mild intraepidermal vacuolization and transient erythema.

3.2. Iontophoresis in conjunction with electroporation

Iontophoresis and electroporation are both methods of electrically assisted transdermal drug delivery. Iontophoresis is more commonly used to deliver lipophilic small molecular weight drugs, while electroporation seems more effective for the delivery of some macromolecules such as antisense oligonucleotides, peptides and proteins. Drug delivery with iontophoresis and electroporation are thought to utilize different penetration pathways (Fig. 2). Fluorescent microscopy and laser scanning confocal microscopy were used to visualize the FITC labeled phosphorothioate oligonucleotides transport at the tissue and cell level respectively in hairless rat skin after iontophoresis or electroporation [50]. In the SC the transportation pathways for FITC labeled phosphorothioate oligonucleotides were more transcellular during electroporation and paracellular during iontophoresis. Another study performed by Piquett

et al. showed at low trans-SC voltages (<5 V) electrically driven transport of charged species occurs predominantly via pre-existing aqueous pathways. In contrast, high voltage, (>50 V) has been hypothesized to involve electroporation within the multilamellar bilayer membranes of the SC, creating new aqueous pathways that contribute to a rapid, large increase in drug transport [51].

Electroporation has the advantages of [1] quick drug effect onset, [2] delivery of macromolecules, and [3] resultant insignificant or minor skin damage. There was also evidence showing greater drug uptake by skin cells during electroporation [50]. Combination of iontophoresis and electroporation could possibly further enhance drug transport, and allow rapid delivery of a bolus dose and precise control of drug delivery modulation and programmability. However, in some cases, lowered combined effects than the effects with each individual treatment were also reported. Electrically assisted delivery of salmon calcitonin (sCT) (molecular weight 3600) was conducted by Chang et al. [52]. Electroporation pulses (six pulses of 120 V, 10 ms each) followed by iontophoresis (0.5 mA/cm²) gave a flux about four times higher than with iontophoresis alone. Lag time of the iontophoretic delivery was shortened significantly as well. However, pulsing at lower voltages (60 and 100 V) followed by iontophoresis did not result in sCT transport increase over iontophoresis alone. Pulsatile transdermal delivery of luteinizing hormone releasing hormone (LHRH) using electroporation followed by iontophoresis was studied by Riviere et al. [49]. The application of a single pulse (500 V, 5 ms as exponential) to initiate the experiment resulted in a nearly two-fold increase in LHRH concentration at the end of 30 min of iontophoresis (0.4 mA/cm²). LHRH transport in a pulsatile manner was achieved by repeated processes of one pulse immediately followed by 30 min iontophoresis. Skin toxicity of electroporation together with iontophoresis was also evaluated in this study. Pulses of 0, 250, 500 and 1000 V were applied followed by constant current anodal iontophoresis of 0, 0.2, and 2.0 mA/cm² for 30 min or 10 mA/cm² for 10 min. At the gross microscopic level, immediately after or 4 h after treatment, erythema increased with increasing pulse voltage. Erythema, edema and petechiae all increased significantly with increased current in the absence of a pulse. The application of an electroporation pulse did not increase the iontophoretic-induced irritation with any current tested. All skin changes tended to decrease within 4 h after the treatments. Denet et al. reported lowered transdermal delivery of the lipophilic drug timolol with iontophoresis and electroporation combination than with iontophoresis alone [53]. The decreased transport was explained as due to an accumulation of positively charged timolol in the SC, which was amplified by electroporation, and a resulting decrease of electro-osmotic flux during iontophoresis.

The practical application of combining electroporation with iontophoresis is still in its initial feasibility stage much

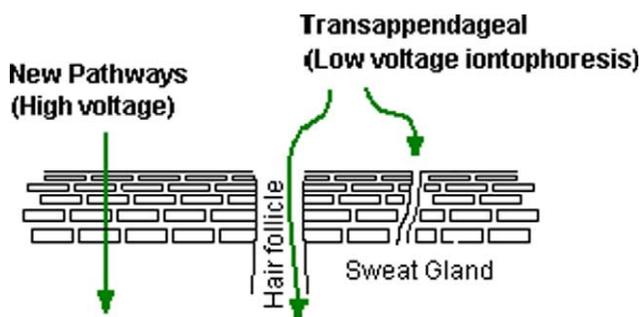


Fig. 2. Drug penetration pathway in low voltage iontophoresis and high voltage electroporation.

like the commercial development of electroporation devices for transdermal delivery of drugs. Iontophoretic studies at least have resulted in some marketed medical device products and some drug-containing ones which are close to FDA approval.

4. Iontophoresis in conjunction with chemical enhancers

4.1. Chemical enhancers

The use of chemical penetration enhancers is one of the more widely studied techniques for increasing transdermal drug transport [54,55]. Many different chemicals are able to modify the penetration characteristics of different drugs into the skin, but only very few have actually been incorporated into marketed products due to safety concerns. Several research groups have evaluated the mechanism of enhancement activity of these compounds [56–59]. It is believed that some of the chemical enhancers can increase permeability of the SC by acting as solvents to dissolve the skin lipid or to denature skin proteins. In other cases, enhancers can modify drug solubility parameters in the vehicle or in the skin to increase drug penetration. In addition, these compounds will affect the partitioning of the drug from the applied formulation.

Enhancers offer several to more than a hundred times higher drug penetration in terms of the flux, depending on the properties of the penetrants and the enhancers as well as the other additional ingredients of the formulation. Based on their chemical structures, chemical enhancers can be classified into several categories.

For most chemical enhancers, the strength of the activity depends on their concentration. Toxicity concerns of these enhancers become the major barrier for enhancer application in transdermal formulations. There are data showing the existence of synergistic effects among some enhancers [60]. In addition, one can reduce the concentration of individual enhancers required to achieve the desired enhancement by combining two or more enhancers within the same formulation.

A technique termed In Vitro Skin Impedance Guided High-Throughput (INSIGHT) screening was recently developed to screen putative enhancer mixtures 100-fold faster than by using conventional Franz diffusion cells [61]. This technique uses skin conductivity measurements to qualify impairment of the lipid bilayers of the skin. For screening purposes, it is assumed that skin conductivity (reciprocal of impedance) is directly related to skin permeability. The assumption was confirmed by a Franz cell study using inulin as a model penetrant. About 5000 formulations with binary combinations of 32 enhancers were tested using porcine skin in the published study. The authors identified two enhancer binary combinations, sodium laureth sulfate (SLA): phenyl piperazine (PP) [total concentration of 0.5 (wt.vol), SLA weight fraction of 0.7] and sodium lauryl

sulfate (NLS):sorbitan monolaurate (S20) [total concentration of 1% (wt/vol), NLS weight fraction of 0.6] as having synergistic enhancement effects and lower toxicity than either enhancer alone. The INSIGHT technique could be useful for enhancer screening in iontophoretic delivery since skin electrical properties produce significant effects on the efficiency of drug transport due to the presence of the electrical field.

4.2. Iontophoresis in conjunction with chemical enhancers

Although the use of iontophoresis results in much higher drug delivery if compared with conventional passive transdermal delivery, it still has limitations as a technique. Chemical enhancers can be used in combination with iontophoresis to achieve even higher drug penetration. In addition to increasing transdermal transport, a combination of chemical enhancers and electrically assisted delivery should also reduce the side effects such as irritation caused by high concentration of enhancers or stronger electric forces. The combined effects of enhancers and electrically assisted delivery depend on the physico-chemical properties of the penetrant, enhancer and their behavior under the influence of an electric field. Occasionally, the use of chemical enhancers was reported to result in reduced flux compared with using iontophoresis alone [58,62]. However, more often synergistic effects have been reported such as those with fatty acids, and terpenes and others.

4.2.1. Fatty acids

Effects of fatty acids on skin have been extensively investigated in recent years. Unsaturated fatty acids with long carbon chains have been found to be more effective than the analogous saturated fatty acids. C₁₂ and C₁₄ fatty acids have an optimal balance of partition coefficient and affinity to lipids in the SC. Short chain fatty acids have insufficient lipophilicity to penetrate the skin while long chain fatty acids have too much affinity to the lipids in SC and actually retard the penetration of drugs [63]. The permeation-enhancing effects of fatty acids are greatly influenced by the vehicle used. For example, compared with ethanol, PEG 400 and isopropanol, propylene glycol (PG) produces significantly higher enhancing effects. It is believed that PG can 'drag' fatty acids into the skin.

Scanning electron microscope (SEM) was utilized to identify human skin structure change in a study of fatty acids-enhanced iontophoretic delivery of midodrine hydrochloride [64]. Pretreatment with fatty acids in PG was found to cause the opening up of the tightly packed SC cell layers and thereby increased the permeability of the skin to midodrine hydrochloride delivered iontophoretically. The higher the concentration of oleic acid, the more the epidermis swelled and the higher the resulting permeability of the skin. Unsaturated long-chain fatty acids: oleic acid and linoleic acid were more effective as enhancers of midodrine permeation than saturated fatty acids, lauric and

decanoic acid. C₁₂ lauric acid had a slightly higher enhancing effect than decanoic acid. The order of the enhancing effects of the fatty acids used in iontophoretic delivery are similar as in passive diffusion, indicating the enhancement of passive and iontophoretic delivery by fatty acids may be occurring through the same mechanism.

A study was performed by Smyth et al. to determine the effect of oleic acid/propylene glycol (PG) and iontophoresis combination on the permeation enhancement of LHRH through human epidermal membrane [65]. In their study tetraethylammonium bromide (TEAB), a small ionic solute and sucrose, an electroosmotic flow marker, were used to investigate the enhancement mechanism of oleic acid. The effect of combinations of iontophoresis and chemical enhancers were additive suggesting different mechanisms of action. Iontophoretic permeability of sucrose was not promoted by enhancer treatment, suggesting oleic acid/PG treatment did not increase electroosmosis. Although there was evidence showing iontophoresis and oleic acid/PG acted synergistically to increase membrane conductance, the permeation of TEAB was not increased. The postulated hypothesis from the TEAB study was that some skin conductance pathways produced during enhancer treatment, were not available for solute transport other than for very small conducting ions, such as Na⁺ or Cl⁻.

4.2.2. Terpenes

Terpenes, which are volatile and fragrant, are constituents of essential oils and are found mainly in flowers, fruits, and leaves of plants. They have been reported to have high percutaneous enhancement abilities in passive drug delivery [66–71] and low cutaneous irritancy at low concentrations (1–5%) [72].

The effect of limonene/EtOH and iontophoresis on the *in vitro* percutaneous absorption of LHRH and ultrastructure of human epidermis was investigated by transmission electron microscopy (TEM) [73]. Limonene/EtOH at 5% significantly enhanced the passive flux of LHRH through human epidermis. Iontophoresis further increased the flux of LHRH through enhancer treated epidermis. The combination of iontophoresis and enhancer treatment significantly enhanced LHRH delivery in comparison to the one with iontophoresis alone. TEM results revealed that iontophoresis in combination with the enhancer treatment transformed the highly compact cells of the SC into a looser network of filaments, disrupted the keratin pattern, and resulted in swelling of SC cell layers of human epidermis.

4.2.3. Other enhancers

Fatty acids and terpenes are probably the most studied chemical enhancers for promoting iontophoretic drug delivery. However, synergistic effects of iontophoresis of some other enhancers have also been reported.

Dimethyl acetamide (DMA) was found to be able to severely compromise skin barrier properties and enhance

iontophoretic delivery of insulin [74]. The effects of some solvents used [EtOH, ethyl acetate (EtAC), isopropyl myristate (IPM) and PG] in insulin iontophoresis were also investigated. All the solvents produced synergistic enhancement with iontophoresis. FTIR study showed that EtOH and EtAC caused lipid extraction, whereas IPM caused increase in lipid fluidity. Thermogravimetric (TGA) studies showed that EtOH and PG caused dehydration of skin.

Effects of sodium dodecyl sulfate (SDS), an anionic surfactant, on the iontophoretic transport of a neutral drug, hydrocortisone, across hairless mouse skin were investigated by Wang et al. [75]. The effect of SDS on the transport of hydrocortisone was highly concentration-dependent and driving mode-dependent. Below the critical micelle concentration (cmc), increasing the concentration of SDS increased both the passive and the iontophoretic fluxes of hydrocortisone, and the increase was most significant with anodal iontophoresis. Above the cmc, the transport with anodal iontophoresis, however, reached a plateau and then leveled off, suggesting that the transport of micellar-solubilized drug is retarded by anodal iontophoresis, possibly due to electrostatic attraction.

Azone[®] (laurocapram) is regarded as an effective and non-toxic chemical enhancer for passive drug delivery. If used in low concentrations, the compound is non-irritant to the skin and enhances the permeation of both polar and non-polar drugs. Azone[®] also showed enhancement effects in the iontophoretic delivery of buspirone hydrochloride [22]. The application of 2.5% Azone[®] and iontophoresis with the current density of 0.025 mA/cm² increased the delivery the buspirone hydrochloride significantly more than the application of Azone[®] alone or iontophoresis alone, indicating the existence of a synergistic effect. Such synergism often indicates that the two modalities act by using the same pathway or mechanism. This however, will have to be investigated further.

5. Iontophoresis in conjunction with sonophoresis

5.1. Sonophoresis

Sonophoresis, which is also known as phonophoresis, is the movement of drug molecules through the skin under the influence of ultrasound. In this technique, a short application of ultrasound is used to permeabilize skin with effects lasting for a prolonged period of time and this allows drug molecules to traverse the membrane more easily. Ultrasound is a pressure wave having a frequency of more than 20 kHz. It is used extensively in clinical practice for applications in diagnostic imaging, cardiovascular therapy and kidney lithotripsy procedures. Ultrasound conditions used by diagnostic instruments are of typically very high frequency ($\gg 1$ MHz) and low intensity ($\ll 1$ W/cm²) [76].

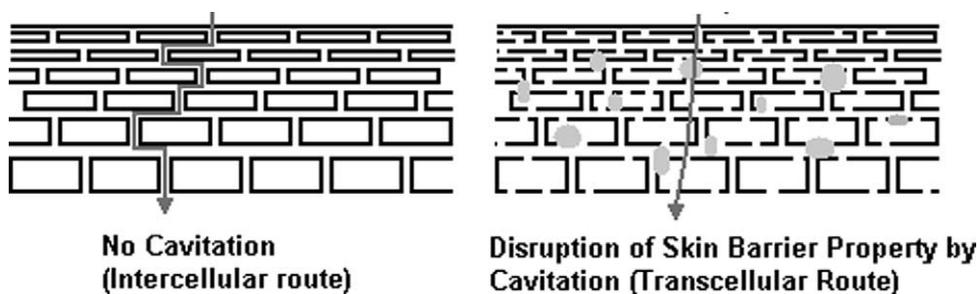


Fig. 3. Permeabilization of the SC by cavitation upon low frequency ultrasound application.

The effects of ultrasound in the body can be classified into two categories: thermal and non-thermal. Skin heating by ultrasound could increase transdermal transport by fluidizing SC lipids and/or increasing convective flow. Cavitation is one of the non-thermal effects ultrasound produces when the frequency is less than 1 MHz and the intensity is higher than 1 W/cm². With low frequency and high intensity conditions, ultrasound can cause extensive growth and oscillation of gas bubbles in biological tissues, which becomes significant if the cellular structures are of comparable dimensions to, or larger than, the wavelength of the ultrasound beam (Fig. 3). Disruption of barrier property of the skin by ultrasound cavitation is believed to be the mechanism of enhanced transdermal delivery in sonophoresis [77–81].

Cavitation of the SC has been demonstrated by a TEM study [82] and a fluorescein bleaching study [83]. Mitragotri et al. determined the dependence of sonophoretic enhancement on ultrasound parameters, including intensity and exposure time [84]. The enhancement varies linearly with ultrasound intensity and exposure time, the higher the intensity and the longer the exposure time, the more significant the enhancement. Another study performed by Tezel et al. [85] showed that for each frequency in the range of 19.6–93.4 kHz, there exists a threshold intensity below which no detectable skin conductivity enhancement can be observed. The threshold intensity increased with frequency and low frequencies (~20 kHz) induced localized transport compared to a more dispersed effect seen with higher frequencies (~58.9 kHz).

Several research groups have successfully performed transdermal delivery of macromolecules with *in vitro* and *in vivo* sonophoresis [86,87]. The safety issues of the technique have also been addressed by Boucaud et al. [88]. Structural modifications of human skin after 20 kHz ultrasound exposures were evaluated using optical and electron microscopy. Human skin samples exposed to intensities lower than 2.5 W/cm² showed no modifications *in vitro*, while 5.2 W/cm² resulted in epidermal detachment and edema of the upper dermis. Obvious histological modifications such as detachment of the epidermis and dermal necrosis could be seen with 4 W/cm² continuous intensity. It is important to note that 7 W/cm² continuous

and 12.3 W/cm² pulsed intensity resulted in second-degree burns.

5.2. Iontophoresis in conjunction with sonophoresis

Synergy between low-frequency ultrasound and iontophoresis would be expected since the techniques both enhance transdermal transport although through different mechanisms [89]. As a matter of fact, the disruption of SC lipid bilayer by the application of ultrasound can be utilized by further use of iontophoresis to increase transdermal drug transport to a greater degree. This combination has been found to enhance transdermal transport better than any of the single treatments alone. Iontophoresis combined with low frequency ultrasound was used in the transdermal delivery of sodium nonivamide acetate (SNA) by Fang et al. [90]. Pretreatment of the skin with low frequency ultrasound (0.2 W/cm², 2 h) alone did not increase the skin permeation of SNA. The combination of iontophoresis (0.5 mA/cm²) and sonophoresis increased transdermal SNA transport more than iontophoresis alone. Another study also performed by Fang et al. suggested that in some cases ultrasound could enhance drug permeation through hair follicles to a greater extent than through the bulk SC [91].

6. Iontophoresis in conjunction with microneedles

6.1. Microneedles

Microfabrication technology used to make integrated circuits is now being utilized to produce microneedle transdermal patches. ALZA Corp. has designed its micro-projection patch, Macroflux[®] with a thin titanium screen with precisely manufactured microprojections. Microstructured Transdermal Systems (MTS) are microneedle patches developed by 3 M. The needles or projections on the surface of patch are sufficiently long to penetrate through the SC, but short enough not to stimulate nerves and hence pain receptors in the deeper tissues. Microneedle patches are suited for delivery of vaccines, proteins or peptide-based drugs. Studies at 3 M have achieved successful delivery of

water soluble, polar, ionic, and large molecules (19,500 Da) with their MTS system.

Microneedles used in transdermal delivery can be classified into two categories: solid and hollow microneedles [92]. Solid microneedles have been successfully used in the delivery of proteins, peptides, oligonucleotides and nanoparticles *in vitro* and *in vivo* [93,94]. Hollow microneedles contain a hollow bore offering the possibility of rapid bolus dose drug delivery by pressure-driven flow.

One example of microneedle patch applications is the delivery of ovalbumin [93]. The performance of Macroflux[®] microprojection array systems (330 μm micro-projection length, 190 microprojections/ cm^2 , 1- and 2- cm^2 area) coated with ovalbumin (OVA) were evaluated in a hairless guinea pig model. It was observed that Macroflux[®] microprojections penetrated into guinea pig skin at an average depth of 100 μm with no projections deeper than 300 μm and the needles were able to reach skin immune cells. The quantity of ovalbumin delivered can be controlled by either the formulation, patch wearing time, or system size.

6.2. Iontophoresis in conjunction with microneedles

Few studies have reported the combination of iontophoresis with microneedle technologies. This combination may provide the possibility of macromolecule transdermal delivery with precise electronic control. Lin et al. designed a Macroflux[®] and iontophoresis combined transdermal delivery system for the delivery of an antisense oligonucleotide ISIS 2302 [94]. The Macroflux[®] array, 2 cm^2 , had a microprojection density of 240/ cm^2 and a needle length of 430 μm . Macroflux[®] and iontophoresis combined system was made by assembling the Macroflux[®] array, a drug reservoir, a membrane, a conductive gel and the iontophoretic electrode. Study results showed the system was capable of delivering therapeutically relevant amounts of ISIS 2302 into and through the stratum corneum. The rate of delivery can be controlled by duration of the patch application, donor drug concentration, current density, and active patch area.

7. Iontophoresis in conjunction with ion-exchange materials

7.1. Ion-exchange materials

The ion exchange process is a stoichiometric and reversible process wherein an ion from the solution is replaced with a similarly charged ion attached to an immobile solid phase (e.g. ion exchange device) in order to fulfill the electroneutrality requirement [95]. Ion exchange devices will exhibit greater preference for a particular ion. The higher the preference an ion exchanger exhibits for a particular ion, the greater the exchange efficiency in terms of ion exchanger capacity for removal of

that particular ion from a solution. Electrodialysis, a common application of the ion exchange process is carried out by ion exchange membranes in which ionic groups (e.g. NH_3^+ , $-\text{COO}^-$, $-\text{SO}_3^-$) are bound onto the polymeric framework. Other applications include water purification, protein purification, salt recovery from seawater etc. [96].

Novel applications of ion exchange materials include enhancement of drug release and increasing the dissolution rate of the poorly soluble drugs. Ion-exchange materials used for medical and pharmaceutical application are usually in a form of fibers [97–102], membrane [103] and resins [104]. The equilibrium reaction between the ion exchange device and a particular organic ion of significant molecular weight (e.g. typical drugs) is controlled by the environment in which the drug and the ion exchange material are found. Factors controlling the equilibrium constant include the following: molecular weight, pKa of drug and resin, pH of the solvent, ionic strength, hydrophobicity/hydrophilicity and concentration of competing ions. Drug ions, which are attached to the ion exchange materials via electrostatic interactions, provide a more accurate and homogeneous control of the ion exchange process so that drug release rate could be easily adjusted [99].

The preservation of drug stability during shelf life is always a concern for transdermal drug delivery during commercialization. Ion exchange materials attached with the drug before administration will help overcome this hurdle by forming a drug reservoir. The interchange between mobile ions such as Na^+ , Cl^- and drug ions attached onto ion exchange materials would subsequently release the drug ions for the duration of administration. The presence of ion exchange materials would exclude the buffer solution used to stabilize the drug agent. Furthermore, it would prevent the competition of ions present in buffer solutions and maintain constant pH as well [97,100,104].

Jaskari et al. studied the mechanisms of drug binding and release from cation-exchange fibers (Smopex[®]-101, 102, 107 fibers) [98]. Under *in vitro* equilibrium conditions, they analyzed the effect of [1] the lipophilicity of the drug, [2] cation-exchange fiber activated by NaOH, [3] ionic strength of the extracting salt (NaCl) and [4] divalent ions (Ca^{2+}) compared to monovalent ones (Na^+), and together on the drug absorption/release properties thereof. Using salicylate anions as model compound, Hänninen et al. investigated the systematic impacts of compound lipophilicity, valence, aqueous solubility and hydrogen bond on binding/release from an anion-exchange fiber (Smopex[®]-DS-218v) [98,99]. In order to predict drug transportation, a mathematical model was developed based on Nernst-Planck equations for either passive or iontophoretic permeation experiments under quasi-steady state conditions. For the case of passive diffusion of tacrine (cationic drug) through human epidermis, the theoretical flux was 4.1 $\mu\text{g}/\text{cm}^2\text{-h}$, while the corresponding experimental result was $3.0 \pm 0.7 \mu\text{g}/\text{cm}^2\text{-h}$; when current density was 0.1 mA/cm^2 , the calculated and observed fluxes were 45 and $25 \pm 4 \mu\text{g}/\text{cm}^2\text{-h}$;

the corresponding values for 0.25 mA/cm^2 are 107 and $100 \pm 40 \text{ } \mu\text{g/cm}^2\text{-h}$, respectively. It appears that this mathematical model agreed well with the experimental observations under the testing conditions [97,100].

Torresi et al. used polyaniline membrane to store either salicylic acid or dopamine. They applied potential pulses to discharge the stored drug [103]. From the release curves they obtained, the capability and satisfactory performance of such a system for in vitro drug release was confirmed.

7.2. Iontophoresis in conjunction with ion-exchange materials

For this combined technique, experimentally the ion exchange materials were initially immersed into drug solution for 3 h to overnight. Afterward, such a drug-loaded device (e.g. disc, a bundle of ion exchange fibers or hydrogel filled with ion exchange resins) was transferred to the donor part of a diffusion cell for in vitro or in vivo tests [97,98,101,102].

Conaghey et al. studied the in vitro iontophoretic transdermal delivery of nicotine by ion exchange resins in agar hydrogel [104]. Their results showed that these heterogeneous vehicles (i.e., hydrogel filled with resins) had several advantages over comparably simple agar hydrogel vehicles on account of this composite hydrogel's versatility, capacities of drug storage and preventing pH decrease. The lowest pH value the skin experienced during iontophoresis with ion exchange resin was 6.31, whereas using a simple hydrogel system, a lowest observed pH value was 3.0. The successful in vivo delivery of therapeutic dosage of tacrine, an anti-Alzheimer's disease agent, was demonstrated by Kankkunen et al. [102] Smopex[®]-102 ion exchange fibers were used in their iontophoretic device on 10 healthy adult volunteers. The same group also studied the delivery of levodopa and metaraminol. Their results indicated that ion exchange fibers could be a good material to successfully store an easily degradable drug, such as levodopa, which could be easily oxidized in a basic aqueous environment. Drug stability was greatly enhanced by attaching levodopa to ion exchange fibers in an acidic environment [101].

8. Conclusion

Iontophoresis and the combination of this technique with other transdermal enhancement approaches have been widely investigated in recent years. The strides made in the development of electronic, formulation and material technologies has made clinical application of iontophoresis possible. Much success has been reported in the literature concerning the delivery of small chemical compounds as well as oligonucleotides and peptides. Combination of iontophoresis with electroporation, chemical enhancers, sonophoresis, microneedle and ion-exchange material may

provide easier and more accurate delivery of macromolecules and poorly water soluble compounds.

The skin irritation associated with iontophoresis has been addressed by several studies and it is an issue preventing wide application of the technology. However, the combination with other enhancement techniques may result in the need for less intense levels of current to reach therapeutically effective delivery amounts, and this will dramatically reduce the skin irritation problem.

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