ELSEVIER

International Journal of Pharmaceutics 179 (1999) 1–19

international journal of pharmaceutics

Review article

Iontophoresis and electroporation: comparisons and contrasts

Ajay K. Banga [a], Sagarika Bose [b], Tapash K. Ghosh [c,*]

[a] *Department of Pharmaceutical Sciences, School of Pharmacy, Mercer University, Atlanta, GA 30341-4155, USA*
[b] *Department of Pharmacal Sciences, School of Pharmacy, Auburn University, Auburn, AL 36849-5503, USA*
[c] *Lavipharm Laboratories Inc., Piscataway, NJ, USA*

Received 24 July 1998; received in revised form 23 October 1998; accepted 23 October 1998

Abstract

The techniques of iontophoresis and electroporation can be used to enhance topical and transdermal drug delivery. Iontophoresis applies a small low voltage (typically 10 V or less) continuous constant current (typically 0.5 mA/cm^2 or less) to push a charged drug into skin or other tissue. In contrast, electroporation applies a high voltage (typically, >100 V) pulse for a very short (μs-ms) duration to permeabilize the skin. This electric assistance of drug delivery across skin will expand the scope of transdermal delivery to hydrophilic macromolecules such as the drugs of biotechnology. These two techniques differ in several aspects such as the mode of application and pathways of transport but can be used together for effective drug delivery. Iontophoresis is already used clinically in physical therapy clinics and is close to commercialization for development of a systemic delivery patch with miniaturized circuits and similar in overall size to a passive patch. The use of electroporation for drug delivery is relatively new and is being actively researched.

Keywords: Iontophoresis; Electroporation; Transdermal; Topical; Skin; Drug delivery

1. Introduction

Use of electricity to increase penetration of electrically charged molecules through different membranes of our body, a process known as iontophoresis, is known for long time. Biomedical applications of iontophoresis have been around for several decades (Chien and Banga, 1989). In contrast, the use of electroporation for transdermal or topical delivery was only suggested about 5 years ago (Prausnitz et al., 1993), though electroporation as a science has about 25 years of history (Tsong, 1991). A recent text provides detailed information on the use of iontophoresis and

* Corresponding author. Tel.: +1-732-572-9660.

0378-5173/99/$ - see front matter
PII: S0378-5173(98)00360-3

electroporation for electrically-assisted delivery of drugs into or across the skin (Banga, 1998). Iontophoresis utilizes a small amount of electric current to push the drug through the skin. In contrast, electroporation, sometimes also termed 'electropermeabilization' involves changes in membranes of cells or artificial planar bilayer membranes which occur when large transmembrane voltages are applied. The changes in the membrane involve structural rearrangement and conductance changes leading to temporary loss of semipermeability of cell membranes suggesting formation of pores. These changes can lead to ion leakage, escape of metabolites, and increased uptake by cells of drugs and DNA (Tsong, 1991; Weaver, 1993). These two electric enhancement techniques expand the scope of transdermal delivery to hydrophilic macromolecules (Banga and Prausnitz, 1998) and are discussed in more details in this review.

2. Iontophoresis

Iontophoresis uses an electrode of the same polarity as the charge on the drug to drive ionic (charged) drugs into the body (Green et al., 1993; Singh and Maibach, 1994; Singh and Bhatia, 1996). The potential of this technique is recently being rediscovered for transdermal systemic delivery of ionic drugs including peptides and oligonucleotides which are normally difficult to administer except by parenteral route. The technique has been observed to enhance the transdermal permeation of ionic drugs several-fold, and this can expand the horizon of transdermal controlled drug delivery for systemic medication. Besides the typical advantages of transdermal delivery, iontophoresis presents a unique opportunity to provide programmable drug delivery. This is because the drug is delivered in proportion to the current, which can be readily adjusted. Such dependance on current may also make drug absorption via iontophoresis less dependent on biological variables, unlike most other drug delivery systems. Also, patient compliance can be improved by including electronic means to remind patients to replace the dose when required. The dose can also be titrated for individual patients by adjusting the current. Iontophoretic devices for systemic delivery are currently under development and they are predicted to be on the market prior to the year 2000 (Green, 1996b). The size of the dosage will be the size of a traditional 'transdermal patch', with miniaturized circuitry and button cells. Prototypes have been developed and may be of the disposable or reusable type. In reusable systems, the drug may be contained in a hydrogel pad, which can be replaced as required. For disposable systems, perhaps the microprocessor can be removed and transferred to another patch, to keep costs low. Commercial development of this technology is discussed later in this review.

3. Electroporation

Electroporation involves the application of a high voltage pulse for a very short duration and could work alone or in conjunction with iontophoresis. Electroporation is best known as a physical transfection method in which cells are exposed to a brief electrical pulse, thereby opening pores in the cell membrane, allowing DNA or other macromolecules to enter the cell (Treco and Selden, 1995; Weaver, 1995). The technique of electroporation is normally used on the unilamellar phospholipid bilayers of cell membranes. However, it has been demonstrated that electroporation of skin is feasible, even though the stratum corneum contains multilamellar, intercellular lipid bilayers with few phospholipids and no living cells (Prausnitz et al., 1993). The electrical behavior of human epidermal membrane as a function of the magnitude and duration of applied voltage closely parallels the electrical breakdown/recovery of bilayer membranes seen during electroporation (Inada et al., 1994). The approximately 100 multilamellar bilayers of the stratum corneum need about 100 V pulses for electroporation, or about 1 V per bilayer (Weaver and Chizmadzhev, 1996). While iontophoresis involves the use of relatively low transdermal voltages ($\ll 100$ V), electroporation of skin takes place at high transdermal voltages (~ 100 V or more). There is considerable indirect evidence that high voltage

pulses cause changes in the skin structure (Edwards et al., 1995; Prausnitz, 1996b). The use of electroporation in conjunction with iontophoresis can expand the scope of transdermal delivery to larger molecules such as therapeutic proteins and oligonucleotides. While iontophoresis acts primarily on the drug, electroporation acts on the skin with some driving force on the drug during a pulse (Prausnitz et al., 1993). It has been shown that application of continuous low voltage resulted in a calcein flux three orders of magnitude smaller than pulsing at high voltage under 'electrophoretically equivalent' conditions, suggesting that structural changes induced in the skin by pulsing contribute more significantly than the direct electrophoretic force acting on the drug (Prausnitz et al., 1993). However, iontophoresis will have secondary effects on the skin just like electroporation would also apply direct electromotive force on the drug during the brief pulse period. This may be particularly true for the thin cell lining of the sweat ducts which might be electroporated with low voltages as are used in iontophoresis. Besides the model compounds calcein, sulforhodamine, and caffeine, other drugs which have been investigated for transdermal delivery by electroporation include fentanyl (Vanbever et al., 1996), metoprolol (Vanbever et al., 1994), flurbiprofen (Cruz et al., 1997), cyclosporin (Wang et al., 1997), heparin (Prausnitz et al., 1995), oligonucleotides (Zewert et al., 1995; Brand et al., 1998), and genes (Zhang et al., 1997). Results with many of these drugs have been less dramatic than those seen with model compounds. While studies with model compounds have given excellent mechanistic insights, the magnitude of flux enhancement observed may not happen for all drugs; thus each drug needs to be studied as a separate case.

4. Delivery of particulates into the skin

'Electroincorporation' is a technique in which a drug encapsulated in vesicles or particles is delivered into the skin by applying a pulse which causes a breakdown of the stratum corneum (Hofmann, 1995c). The technique involves placing particles on the skin and then pulsing with electrodes placed directly on top of the particles. This creates an electric field which breaks down the stratum corneum by a yet unknown mechanism. Slight pressure is applied on the electrode during pulsing. Following breakdown of the stratum corneum, dielectrophoresis and/or pressure is believe to drive the particles into the skin. Pulsing can be accomplished by using a 'meander electrode' made by Genetronics (San Diego, CA) which consists of an array of interweaving electrode fingers (anode and cathode) coated on a plastic film backing allowing easy placement of particles on the skin and under this electrode. This type of electrode configuration is suitable for including in a patch or device. Electrodes shaped like calipers are also available and have been used to pulse the back skin of hairless mouse. The particles do not have to be charged for delivery. Particles of 0.2, 4.0 and 45.0 μm size were shown to be imbedded in hairless mouse skin when pulsed with three exponential decay pulses of amplitude 120 V and pulse length 1.2 ms (Hofmann et al., 1995). Pressured-mediated electroincorporation has also been used to deliver Lupron Depot® (Leuprolide acetate) microspheres into hairless mouse skin and into human skin xenografted on immunodeficient nude mice. For hairless mice, 1.1 million particles were placed on the skin to provide 100% coverage and resulting efficiency of delivery was 36%. For human skin graft, 4 million particles were used to provide 300% coverage which reduced the delivery efficiency to 4%. If the number of particles on the skin graft is reduced to provide only monolayer coverage, then delivery efficiency could possibly be higher (Zhang et al., 1997). Similarly, gold particles have been used to enhance the transdermal delivery of caffeine through full thickness human cadaver skin via pressure-mediated electroincorporation. In this study, 12 exponential decay pulses (120 V, 8 ms) were administered using the meander electrodes (Zhang and Hofmann, 1997).

5. Pathways of transport

Electroporation reversibly permeabilizes lipid bilayers and possibly involves the creation of aqueous pathways during the application of an electric pulse (Fig. 1). The exact mechanism for electroporation is not clear though the pore mechanism is generally believed to be the case (Tomov, 1995). Changes in the behavior of membranes seen following electroporation such as changes in electrical or mechanical behavior or those seen in molecular transport are consistent with the theory of pore formation. However, these pores have not been visualized by any microscopic techniques, presumably due to factors such as their small size and transient nature. Electroporation is considered to be a non-thermal phenomena since pore formation by membrane rearrangement occurs much before any significant temperature rise takes place in the pulsing medium (Weaver, 1993).

In contrast, iontophoresis is believed to primarily transport drugs through preexisting pathways (Cullander, 1992). However, it has also been shown that changes in the barrier properties of human epidermal membrane during low to moderate voltage iontophoresis are consistent with the induction of new pores or new pathways (Inada et al., 1994). It has long been known that sweat glands play a role for transport during iontophoresis. An early study showed that pore patterns developed on the skin following iontophoretic transfer of basic and acidic dyes and metallic ions (Abramson and Gorin, 1940). For example, thorough rubbing and washing of the skin following the iontophoretic delivery of methylene blue revealed a remarkable pattern of channels traversed by the dye. The blue dots observed on the skin were found to be the sites of the pores of the skin which are the orifices of the coils of sweat glands, suggesting that the dye enters the skin via these pores. The pore patterns persisted for several weeks in many cases. Similarly, fluorescein dye has been shown to penetrate excised human skin upon applying a current density of 0.16 mA/cm^2 and appeared on the dermal surface as spots at pore sites (Burnette and Ongpipattanakul, 1988). Using special electrodes, it has been suggested that the dominant pathway for flow of electric current through skin is through the sweat ducts. This study used a very thin (0.15 mm diameter) wire which was thin enough to distinguish between most pores and a very thin (0.1 μm) metal film electrode. The film electrode was placed on the skin and was permanently marked by the pathways of current flow so that dots developed after some seconds at places with sweat duct units (Grimnes, 1984). It should be noted that the pore pathway for delivery does not necessarily imply skin appendages only. Even with follicular transport, it should be noted that the final pathway is still intercellular between hair follicles and epidermal cells (Monteiro-Riviere et al., 1994).

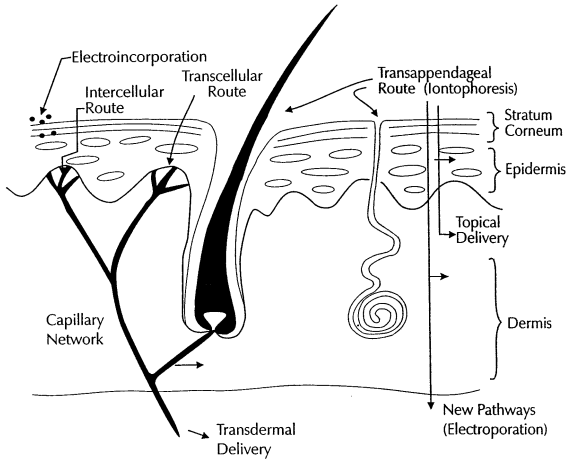

Fig. 1. A schematic showing the pathways of topical and transdermal delivery, including electrically assisted delivery by iontophoresis, electroporation, or electroincorporation (reproduced from Banga, 1998 with permission from Taylor & Francis).

It has been suggested that even low voltage iontophoresis may electroporate the epithelial layers of the skin appendages which in turn allows transport from appendages to epidermal cells. At low voltages (< 30 V), the drop in resistance of skin may be attributed to electroporation of appendageal ducts. At higher voltages (> 30 V), electroporation of the skin itself leads to a further drop in resistance (Chizmadzhev et al., 1998; Hui, 1998). A physical mechanism of activation of skin appendage macropores under the influence of an electric field has been considered theoretically and

described in the literature (Kuzmin et al., 1996). A direct comparison between the pathways of transport during iontophoresis and electroporation has been done recently using human cadaver skin or shed snake skin as the latter does not have any hair follicles. Using several fluorescent tracers, 'localized transport regions' were observed in both human and shed snake skin by electroporation but only in human skin by iontophoresis. This confirms that the mechanism of transport is different for these two electrical enhancement techniques (Chen et al., 1998) though electroporation may be happening at the level of epithelium of appendages even during iontophoresis as discussed earlier.

6. Theoretical basis of electrotransport

Techniques for electrical enhancement of percutaneous absorption, in realistic terms, constitute a rather complex area with a large number of operating variables and the results depend on the drug candidate being studied. Many of the theoretical equations have been derived based on experimentation with simple ions such as sodium transport studies, or with model compounds. These equations may not be applicable to many drugs, especially to drugs which bind to the skin or for macromolecules. The situation may be further complicated for peptide drugs which have the potential to undergo enzymatic degradation during transport through the skin and also undergo other losses such as by adsorption and self-aggregation. A recent publication (Chizmadzhev et al., 1998) bridges the gap between theories of iontophoresis and electroporation.

For iontophoresis, the flux (J_i) of an ionic species, i, is given as:

$$J_i = -D_i \, dC_i/dx - z_i \, m \, F \, C_i \, dE/dx$$

where z_i is the valence of the species 'i', m is the mobility of species 'i', F is Faradays constant and E is the electrostatic potential, and D_i is the diffusivity. This is a fundamental relationship, called the Nernst–Planck equation, and is widely used to describe the membrane transport of ions (Finkelstein and Mauro, 1977). Several more rigorous theoretical models have been developed for iontophoretic delivery, which have been reviewed and discussed for those interested in a more detailed treatment (Kasting, 1992; Kontturi and Murtomaki, 1996). A better appreciation of the meaning of this equation may be achieved by considering the case of a non-electrolyte, in which the charge, $z = 0$. In this case, the Nernst–Planck equation is reduced to:

$$J = -D \, dC/dx$$

which is Fick's first law of diffusion. On the other hand, for an ion with an uniform concentration throughout the system ($dC_i/dx = 0$), the Nernst–Planck equation becomes:

$$J = -z_i \, m \, F \, C_i \, dE/dx$$

which is the equation for electrophoresis. The Nernst–Planck equation may be thus interpreted as implying that when concentration gradient and an electric field both exist, the ionic flux is a linear sum of the fluxes that would arise from each effect alone. Though the permeability of ionized drugs through skin is low, it cannot be assumed to be negligible and thus the potential contribution of passive flux needs to be considered. If a voltage difference is applied across a charged porous membrane, bulk fluid flow, or volume flow, called electroosmosis occurs in the same direction as flow of counterions. This flow is not diffusion and involves a motion of the fluid without concentration gradients (Pikal, 1992). Thus, a modification to the Nernst–Planck equation is necessary as follows:

$$J_i = -D_i \, dC_i/dx - z_i \, m \, F \, C_i \, dE/dx \pm C_i \, J_v$$

where J_v is the velocity of convective flow, i.e. volume flow per unit time per unit area.

Recently, a single unifying equation has been described based on an ionic mobility-pore model which describes a range of determinants of iontophoretic flux (Roberts et al., 1998). For electroporation, the steady-state, time-average, one dimensional solute flux across the stratum corneum has been expressed by Edwards et al. (1995) as:

$$\langle J \rangle = 1/T \, {}_{0-}{}^{T} \, J \, dt \quad \approx \quad K_1 \, \langle U^* \rangle C_1$$

where J is the solute flux normal to the skin surface, T is the time period of any oscillatory process, K_1 is the skin/donor-solution equilibrium partition coefficient, U^* the mean solute velocity component normal to the skin surface (with $\langle U^* \rangle$ its time average), and C_1 the time-independent solute concentration in the donor compartment. The equation which characterizes an electroporation pulse is discussed separately in this review.

7. In-vitro electrotransport studies

The factors affecting electrically assisted transdermal drug delivery are summarized in Table 1. Horizontal or vertical configuration diffusion cells are typically used for in vitro transdermal electrotransport studies. The horizontal cell set-up, sometimes called the two-chambered cell, represents the so-called infinite dose technique, and vertical diffusion cells represent the finite dose technique as small amounts of drug can be placed on the skin. A commonly used vertical cell is the Franz cell though other cells similar in design are also available. The placement of the return electrode is usually in the receptor compartment for both the designs and may be considered as being 'inside' the skin. Any actual device, on the other hand, will place both electrodes 'on' the skin. Thus, it may be preferable to design cells which place both electrodes on the same side of the skin to simulate the in vivo situation. The feasibility of such a cell design was demonstrated by delivering morphine and clonidine across full-thickness hairless mouse skin. It was shown that significant lateral transport does not take place in this cell design (Glikfeld et al., 1988). For evaluation of commercially available iontophoresis electrodes in clinical use, the use of a donor chamber is not required. For such studies, a cell design has been used which uses two pieces of skin, placing one at either end of a central receptor compartment. The reservoir-type electrode filled with drug can then be placed on one side and a dispersive electrode on the other side, and samples can be taken from the central compartment (Bellantone et al., 1986; Petelenz et al., 1992). This design can potentially be used for electroporation studies as well.

In vitro release studies may also be required to assure batch to batch uniformity of product. These studies may not predict the in vivo delivery and are only intended to detect any problems or variations in the product during routine production. For instance, crystallization of drug from solution, its non-homogeneous distribution in polymer matrix or electronic malfunctions are likely to be detected by such studies. Synthetic membranes can be used for such studies. In addition to in vitro release studies, current profiles from the controller may be monitored by instrumentation such as oscilloscopes to detect any electronic malfunctions (Green, 1996a).

The skin is a complex biological tissue and thus permeability measurements across skin tend to have a high variation. Furthermore, the application of electric current can change skin permeability, thus making delivery more unpredictable in some cases. In order to avoid these problems, various synthetic membranes have been tried in iontophoresis research. However, these membranes may not be predictive of what to expect with skin precisely because skin is a complex biological tissue and these membranes are not. Nevertheless, these membranes could be of some use in iontophoresis research as the electromotive force is acting primarily and continuously on the drug. In contrast, electroporation depends on permeabilizing the skin, with direct electromotive force on the drug being of secondary importance. Thus, the use of membranes is unlikely to be predictive of electroporation induced skin permeation. The ideal membrane should be hydrophobic to mimic the lipophilic skin barrier and prevent excessive flow of water. At the same time, the membrane should also be conductive. This combination of characteristics is hard to find but several membranes have been investigated (Banga, 1998). Nucleopore® (Nucleopore, Pleasanton, CA, USA) is a synthetic membrane with essentially cylindrical, aqueous filled pores (pore radius 75 A; porosity 0.001). It has a polyvinylpyrrolidone coated polycarbonate backbone with a net negative charge and a nominal thickness of 6 μm. It has been used in iontophoresis research by stacking 50 such membranes together to form a net negatively charged, random pore network for dif-

Table 1
Factors affecting electrically assisted drug delivery

Factor	Iontophoresis	Electroporation
Electric input	Constant current (low voltage); Current density (<0.5 mA/cm^2).	High voltage pulses. Pulse voltage($\geq$100 V); pulse duration (ms-μs); number of pulses given (one-many); spacing between pulses given (all at once or in certain interval).
Electrode material	The electrode must avoid electrolysis of water (e.g. Silver–silver chloride).	The electrode must avoid electrolysis of water and must withstand high instantaneous current
Physicochemical properties of drug	Charge of drug (high charge density better); size of drug (smaller ions are better); structure of drug (compact structure); lipophilicity (drug must be water soluble); molecular weight of the drug (maximum about 12 000).	Charge of drug (not as critical as in iontophoresis); molecular weight of drug (upper limit not known).
Formulation factors	Concentration of drug in solution pH (adjust to keep drug charged); ionic strength (just adequate buffer capacity).	Concentration of drug
Driving mechanism	Electric force on the drug. No new pathway (transappendageal route).	Electric force on the drug and permeabilization of skin. New pathways created
Reversibility of skin	Reversible at low current density.	Reversible at low pulse voltage
Electrosmosis	Significant electroosmotic flow accompanies with iontophoresis.	Insignificant

fusion with a resistance of about 1.5 kΩ which is of the same order of magnitude as skin (Sims et al., 1991; Hoogstraate et al., 1994; Peck et al., 1996; Li et al., 1997). The enhanced transport of cations and anions across nucleopore porous membranes under an applied electric field was found to be asymmetric, possibly due to the direct effect of the field and convective solvent flow (Sims et al., 1991). Thus, it seems the membrane is somewhat representative of what to expect with skin since iontophoresis is accompanied by electroosmosis.

Power supplies commonly used for in vitro iontophoresis studies can typically power several transdermal diffusion cells at one time, allowing several triplicate experiments to run simultaneously. Many researchers have used custom built power supplies and some have published the circuit diagrams required to build one (Kumar et al., 1992; Jaw et al., 1995). A constant (D.C.) current of 0.5 mA/cm^2 or less should be typically used, as higher currents are usually not acceptable for human studies. In constant current iontophoresis, the voltage drop across skin adjusts to keep the current constant using a two-electrode system. A four-electrode system is also described in the literature and is useful to test the predictions of the Nernst–Planck equation. In this four-electrode potentiostat system, the voltage drop across the skin or membrane is maintained constant and the current flowing through the skin is monitored. Since the enhancement due to applied electric field is directly proportional to the voltage drop, this system provides additional fundamental information for investigations of factors that control iontophoresis (Masada et al., 1989; Li et al., 1997).

Power supplies for electroporation are very different from those designed for iontophoresis. Electroporation equipment is typically manufactured for genetic manipulation of living cells, and has been used as such or adapted for transdermal studies. Commercially available equipment will come with a chamber which typically is a sealable cylinder with an electrode plate at each end. These chambers are typically disposable, presterilized civets with molded-in aluminum electrodes and come in different gap sizes, depending on whether bacteria or mammalian cells need to be electroporated (Hofmann, 1995a; Jones et al., 1996). These chambers are not required for electroporation of tissues and will not be discussed here. In such studies, the nominal field strength, E is related to the applied voltage, V and the interelectrode gap, d($E = V$/d). Field strengths vary from a few hundred V/cm for mammalian cells to many kV/cm for bacteria. A capacitor is connected to a power supply to build up an electric charge and is then discharged after isolating from its charging source. The current and pulse length obtained depends on the speed at which the stored energy is released, which in turn can be controlled by using resistors (Jones et al., 1996). The voltage output from electroporation equipment may be in the form of exponential decay or square wave pulses. The exponential decay waveform represents complete discharge of the capacitor into a resistor while square wave pulse is often produced by a partial discharge of a large capacitor. For an exponential wave form generator, the voltage of a capacitor C discharging into a resistor R follows an exponential decay law:

$$V = V_0.\exp\ (-t/RC)$$

the pulse length will be characterized by the $1/e$ time constant, which is the time required for the initial voltage to decay to $1/e \approx 1/3$rd of the initial value. This time constant is a product of R and C, where C is the capacitance of the storage capacitor in the generator and R is the total resistance into which the capacitor discharges (Hofmann, 1995a). Both waveforms have been successfully used for electroporation of skin, though it has been suggested that exponential decay pulses may be somewhat more effective than square wave pulses at the same applied energy (Vanbever et al., 1996). However, not enough studies have been done to unequivocally establish the superiority of one pulse waveform over the other. The waveforms are determined by the principles of electrical engineering used by the pulser which generally have to be designed for one waveform only. Unlike capacitive discharge devices, a microprocessor-controlled logic-driven unit can produce reproducible DC pulse lengths independent of the conductivity of the solutions bathing the cells (Jones et al., 1996). Commercially available elec-

troporation equipment has been discussed recently (Banga, 1998).

8. Chemical enhancement of electrically assisted delivery

Combined use of chemical and electrical enhancement may offer some additional advantages over using each mode separately (Ganga et al., 1996). A combination of several penetration enhancers with iontophoresis has been investigated in several studies (Hager et al., 1993; Hirvonen et al., 1993; Fang et al., 1997). The combined use of iontophoresis and penetration enhancers may allow greater amounts of drug to be delivered than either technique alone. In a study on transport of LHRH through porcine epidermis, iontophoresis was found to synergize with enhancers, such as 10% oleic acid in combination with ethanol and 10% oleic acid in combination with propylene glycol. FT-IR spectroscopic study showed that the combination of enhancer and iontophoresis increased the lipid fluidity, suggesting that the synergism in enhancement could be due to the greater fluidization of the stratum corneum lipids (Bhatia et al., 1997b). A somewhat similar observation was made for the in vitro transport of cholecystokinin-8 through porcine epidermis. Iontophoresis was found to further increase the permeability of the drug through enhancer-pretreated porcine epidermis in comparison to the control (Bhatia et al., 1997a).

A chemical enhancer effect for transdermal electroporation has been shown by high voltage pulsing in presence of macromolecules. Heparin has been shown to alter its own transport during electroporation (Prausnitz et al., 1995) as well as the transport of other molecules (Vanbever et al., 1997; Weaver et al., 1997). It has been hypothesized that heparin or other linear macromolecules (such as DNA) can enter an aqueous pathway between corneocytes, get trapped, and keep the pathways open. This resulted in a persistent low post-pulse electrical resistance of the skin in presence of heparin ($\sim 300\ \Omega$). In absence of heparin, the post pulse recovery was from the initial post pulse 400 Ω to about 800 Ω within 2 h (pre-pulse skin resistance was about 82 kΩ). As a result of these open pathways, transport of sulforhodamine (charge $= -1$) was increased in presence of heparin. However, the transport of highly charged (charge $= -4$) calcein was decreased, presumably because of the electrostatic repulsion between the highly negatively charged calcein and the negatively charged heparin trapped in the aqueous pathways (Weaver et al., 1997). In a recent study, several macromolecules such as dextran sulfate, neutral dextran and poly-lysine were shown to have an enhancer effect on the electroporation-induced transdermal delivery of mannitol across freshly excised abdominal hairless rat skin. Macromolecules with a greater charge and size were found to be more effective enhancers. The effect of macromolecules added at the time of pulsing lasted for several hours after pulsing. It has been suggested that these enhancers do not disrupt lipids but rather stabilize the transient disruptions already induced by electroporation of skin due to their flexible linear structures entering the skin. This hypothesis was supported by the observation that enhancer effect was seen only for electroporation, and not for passive diffusion or iontophoresis. Small ions were also used but provided no enhancement for electroporation (Vanbever et al., 1997).

9. Biological and safety issues of electrically assisted delivery

Application of current will typically reduce the resistance of the skin as a function of time and current density. This decreased skin resistance often reflects increased permeability of the skin due to changes in the skin caused by current flow. For excised skin, such changes are partially irreversible and the resulting permeability change has been termed as damage factor in the literature. The post-iontophoresis passive permeability is thus generally higher than the passive permeability before iontophoresis. Since electroporation involves the use of very short pulses, much higher voltages (as compared to iontophoresis) can be used without causing sensation. The minimum current which is required to be applied to the skin

to evoke a sensation is termed perception threshold while the minimum to cause a painful sensation is called pain threshold. For single pulses with about 90 V across human stratum corneum, the recovery of the skin resistance has been shown to be very complete, returning to about 90% of the pre-pulse value. For higher voltage pulse (>130 V), the recovery was typically less than 50% (Pliquett et al., 1995). The recovery time for these conditions may be quicker if full thickness skin is used. Using excised full-thickness porcine skin, the recovery was shown to take place almost instantaneously or within the time required to switch to measuring instrument. This was claimed to be due to the stratum corneum being still attached to epidermis and underlying tissue, and being relaxed unlike the former study where the stratum corneum was heat stripped, hydrated, and mounted in a chamber. The permeabilization was found to depend on the electrical exposure dose which is the product of the pulse voltage and cumulative pulsing exposure time. Skin resistance was observed to drop to 20% of its pre-pulsing value when pulsed beyond a critical dosage of 0.4 V-s but recovered rapidly. When the dose exceeded 200 V-s, the recovery was slow and incomplete (Gallo et al., 1997).

As the stratum corneum has a much higher electrical resistance than other parts of the skin, an electric field applied to the skin will concentrate in the non-viable stratum corneum to induce electroporation. In contrast, the field will be much lower in the viable tissues, thereby protecting the already permeable viable parts of the skin (part of epidermis and all of dermis) and deeper tissues. The reversibility of permeation following electroporation suggests that the technique is not damaging to the skin. The skin toxicology of electroporation relative to iontophoresis has been studied using 14 pigs. In the first study using eight pigs, exponential voltage pulses were applied followed by constant current anodal iontophoresis. The erythema, edema and petechiae were observed under study conditions. Pulses of 0, 250, 500 and 1000 V were applied followed by iontophoresis of 0, 0.2 and 2.0 mA/cm^2 for 30 min or 10 mA/cm^2 for 10 min. The results of gross evaluation immediately after or 4 h after treatment are recorded. The data showed that the erythema increases immediately after treatment with increasing pulse voltage, but was absent or minimal after 4 h. It was reported that it disappeared or reduced within 5 min. The pulse voltage had no effect on edema or petechiae. Erythema, edema, and petechiae all increased with increasing current though the application of a pulse did not increase the irritation induced by iontophoresis. These changes were comparable to those seen with iontophoresis alone. In the second study with six pigs, it was found that at both the gross and light microscopic level, electroporation does not result in any skin changes not previously seen with iontophoresis alone (Riviere et al., 1995).

The skin will respond to any damage to its barrier by initiating a series of biochemical events designed to repair the damage and the resulting level of irritation is reflective of the extent of perturbation caused by the enhancement mechanism (Guy, 1996). While several isolated literature reports exist on the adverse effects of application of current, it should be realized that many of these early studies were not well controlled in the sense that the electrochemical changes at electrodes were not controlled to prevent pH shifts or the pH of the drug formulation itself may be low. However, transient erythema under the skin would be considered normal. In human studies done by us in collaboration with coworkers, an erythema under the electrodes was noted which resolved within a few hours. A mild tingling sensation was reported as the current was being ramped up (Panus et al., 1997). Another report also describes a mild tingling sensation to the subjects as the current was ramped up to 0.25 mA/cm^2, with more tingling perceived under the anode. The sensation diminished with time of current application and disappeared in less than 30 min. Recent studies have also shown that the type and concentration of electrolyte in the formulation affects the irritation response of the skin to iontophoresis (Kalia and Guy, 1995; Anigbogu et al., 1997). In a comprehensive study on 30 pigs in vivo and 112 IPPSFs, alterations in skin were seen following iontophoretic delivery of lidocaine hydrochloride. The change was characterized by light microscopy as the appearance of dark

basophilic staining nuclei oriented parallel to the stratum corneum in the stratum granulosum and spinosum layers. However, the dose-dependent non-immune mediated epidermal alteration was considered to have minimum toxicological significance (Monteiro-Riviere, 1990). While allergic contact dermatitis is rare in clinical applications of iontophoresis, it could develop as all drugs can potentially be allergenic and the iontophoresis patch has a relatively complex construction (Ledger, 1992). A recent case report describes a cutaneous allergic reaction to iontophoresis of 5-fluorouracil which was reactivated at a distant site upon a subsequent treatment with iontophoresis (Anderson et al., 1997). Another potential concern may be dose dumping. However, solid-state circuits are quite reliable and can be set to avoid the discharge of excessive current in the remote chance that a circuit failure should happen. In the event of an adverse reaction, the device can just be removed since the remaining dose resides in the patch outside the body (Sage, 1993). Safety features can also be easily built into the circuitry of iontophoresis devices as is evident from the numerous patents issued in this area (Banga, 1998).

10. Clinical applications of electrotransport

Iontophoresis has been widely used for clinical dermatological applications for drug delivery (Sloan and Soltani, 1986; Kassan et al., 1996). However, electroporation has also been used in physical therapy, though mostly for 'transcutaneous electrical nerve stimulation' (TENS). TENS is commonly used to treat chronic conditions such as low back pain, arthritis and pain caused by a variety of neurologic disorders (Prausnitz, 1996a). The potential use of electroporation for dermatological drug delivery has not been fully exploited at this point and some applications may be found as research continues. The use of electroporation for cancer chemotherapy (electrochemotherapy) is discussed elsewhere in this review. The rest of this section briefly discusses the clinical applications of iontophoresis in physical therapy clinics.

Most of the clinical applications of iontophoresis currently used in physical therapy involve the use of lidocaine and dexamethasone (Costello and Jeske, 1995). Iontophoresis of dexamethasone, often in combination with lidocaine is commonly performed in physical therapy clinics to treat local inflammatory musculoskeletal conditions such as bursitis, tendinitis, arthritis, carpal tunnel syndrome, tennis elbow, and temporomandibular joint dysfunction (Bertolucci, 1982; Hasson et al., 1992; Banta, 1994; Bogner and Banga, 1994; Pellecchia et al., 1994; Liss and Liss, 1995; Li et al., 1996). Other steroids which have been tested include hydrocortisone (Wang et al., 1993; Seth et al., 1994), and prednisolone (James et al., 1986). However, dexamethasone remains the drug of choice due to its much greater anti-inflammatory effect.

The successful use of iontophoresis for local anesthesia, using lidocaine as the drug of choice, has been reported in the dental, ENT, ophthalmic, and physical medicine literature (Henley, 1991). Iontophoresis of lidocaine has been used for anesthesia in several regions, including external ear (Comeau and Brummett, 1978), dermatology (Russo et al., 1980), painless injections (Petelenz et al., 1984), and painless venipuncture (Zeltzer et al., 1991). Potential use of iontophoretic delivery of lidocaine for acute wound healing has also been investigated (Ernst et al., 1995). A combination of 2% lidocaine HCl and 1:100 000 epinephrine is also approved (Iontocaine®) for dermal iontophoresis with the Phoresor System (Iomed, UT, USA). This is the first drug-iontophoresis device combination to be approved by the FDA (Guy, 1996; Merino et al., 1997). This combination can achieve dermal anesthesia to depths of up to 1 cm in about 10 min (NDA No. 20-530 filed by Iomed). The formulation is produced for Iomed by Abbott Laboratories (IL, USA). Epinephrine is a vasoconstrictor and as it is positively charged, it may be administered along with lidocaine under a positive electrode to prolong the anesthetic activity of lidocaine (Costello and Jeske, 1995). Iontophoretic delivery of non steroidal anti-inflammatory agents (NSAIDs) have also been investigated to avoid adverse GI effects which

occur in about 10–30% of patients receiving oral NSAIDs (Panus et al., 1997).

Another clinical use of iontophoresis is in the treatment of hyperhidrosis, which is a condition of excessive or abnormal sweating. Iontophoresis of just tap water has been reported to be effective for hyperhidrosis of palms, soles, and axillae (Akins et al., 1987; Elgart and Fuchs, 1987). Iontophoretic devices are approved for the diagnosis of cystic fibrosis by iontophoresis of pilocarpine. Iontophoresis of pilocarpine for about 15 min results in profuse sweating which continues for about 30 min following the treatment. Sweat is collected during this period and analyzed for chloride. The finding of a high sweat chloride value (above 60 mEq/l), on at least two occasions along with the presence of clinical features of cystic fibrosis is consistent with the diagnosis of the disease. Commercial devices are now available following several studies which established the usefulness of the technique (Yeung et al., 1984; Warwick et al., 1986).

11. Delivery of proteins

The skin, with its accessibility, enormous surface area and possibility for site targeting, offers a potential means of non-invasive delivery. However, peptide and protein drugs, being hydrophilic and macromolecular in nature, do not readily permeate the skin. Transdermal delivery of peptides may become feasible if assisted by phonophoresis, chemical enhancers, or electrically. Iontophoretic enhancement provides another benefit for peptide delivery because, theoretically speaking, the rate of peptide delivery can be initiated, terminated or accurately controlled/modulated merely by switching the current on and off or adjusting the current application parameters, respectively (Chien et al., 1989; Parasrampuria and Parasrampuria, 1991; Cullander and Guy, 1992; Banga, 1996). This would be especially useful since a pulsatile delivery may be required for some peptides, as opposed to constant delivery. Also, the skin is relatively low in proteolytic activity, as compared to other mucosal routes, thereby reducing degradation at the site of administration.

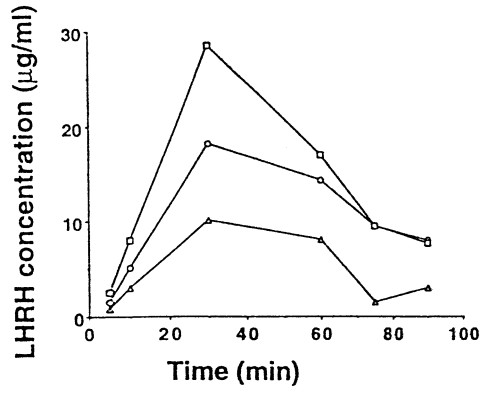

Fig. 2. The mean LHRH concentration in the IPPSF perfusate resulting from (○) one 500 V pulse followed by 30 min of iontophoresis (0.2 mA/cm^2), (□) three 500-V pulses, each followed by 10 min of iontophoresis, or (△) 30 min of iontophoresis alone (reproduced from Riviere et al., 1995 with permission from Elsevier).

Peptides which have been investigated for iontophoretic delivery include thyrotropin releasing hormone (Huang and Wu, 1996a), vasopressin (Banga et al., 1995), and calcitonin (Santi et al., 1997). The delivery of another peptide, luteinizing hormone-releasing hormone (LHRH), also known as gonadotropin-releasing hormone (GnRH), has been investigated by using both iontophoresis and electroporation. Electroporation has been shown to significantly and reversibly increase the flux of LHRH through human skin. The iontophoretic delivery flux of LHRH across epidermis separated from human cadaver skin has been shown to be enhanced by five- to ten-fold following a single electroporation pulse. The pulse was an exponentially decaying type with an initial amplitude of 1000 V and a time constant of 5 ± 1 ms. At a current of 0.5 mA/cm^2, the flux was 0.27 ± 0.08 without the pulse and increased to 1.62 ± 0.05 μg/h per cm^2 with the pulse (Bommannan et al., 1994). The usefulness of electroporation to enhance the iontophoretic flux of LHRH has been verified using the isolated perfused porcine skin flap (IPPSF) model, a model which closely resembles human clinical use. It was found (Fig. 2) that the application of a single pulse (500 V, 5 ms) immediately prior to 30 min of iontophoresis increased the LHRH concentration in the IPPSF

perfusate by nearly two-fold while application of a pulse every 10 min resulted in a three-fold increase (Riviere et al., 1995). By using repeated applications of the pulse/iontophoresis protocol, it was shown that electroporation is able to repeatedly enhance LHRH transport in a pulsatile manner relative to iontophoresis thus allowing pulsatile delivery of therapeutic peptides.

12. Iontophoretic delivery of insulin: fact or fiction? Can electroporation help?

Transdermal iontophoretic delivery of insulin for systemic effect has been extensively investigated over the last decade or so (Stephen et al., 1984; Kari, 1986; Banga and Chien, 1993; Shin and Lee, 1994; Huang and Wu, 1996b). However, these studies have not yet conclusively established the feasibility of delivering intact insulin across human skin in therapeutically meaningful amounts. This is because different investigators have used different methods and there are several variables to be considered before data can be interpreted. First and foremost, the molecular weight of the polypeptide being administered is not certain. At a molecular weight of about 6000, insulin would be expected to have sufficient permeability under iontophoresis to make the technology feasible. However, most commercially available insulin products actually exist in hexameric form, so that we may really be trying to deliver a protein with a molecular weight of about 36 kDa, which is most likely too high to be within the scope of iontophoretic delivery. Furthermore, the p*I* of insulin (5.3) falls in the region of skin p*I* (4.0 to 6.0). This poses major hurdles to its delivery. While there is no evidence for precipitation of insulin in the skin, the formation of a depot upon iontophoresis of insulin has been suggested by several investigators. However, the depot effect could also result simply due to accumulation of insulin in the less accessible regions of the skin and its subsequent slow leaching from those regions. The self-association behavior of insulin may also be implicated in its depot effect. While insulin circulates in blood in low concentrations (10^{-8}–10^{-11} M) and brings about its biological effects as a monomer. Thus, insulin exists as a hexamer as commonly used but its absorption may require a monomeric form. Using analogs of insulin or formulations in which insulin exists as monomer could lead to better absorption from subcutaneous site. The use of other electrical assistance mechanisms such as electroporation, has not been investigated to enhance the transdermal delivery of insulin. Electroporation may have a potential use to minimize some of the several problems facing the iontophoretic delivery of insulin. The depot effect observed by several investigators could be a disadvantage for modulation of delivery or development of a biofeedback system. However, iontophoresis would still be useful to load skin tissues with insulin in a non-invasive manner, for a relatively prolonged release similar to the long-acting preparations on market. The combined use of iontophoresis and electroporation may also result in some interesting results.

13. Commercial development

Iontophoresis devices are already commercially available on the market for delivery of local anesthetics and corticosteroids such as Phoresor®II (Iomed), Empi® Dupel (Empi, St. Paul, MN, USA), Life-Tech® Iontophor (Houston, TX, USA) and Henley Intl. Dynaphor® (Houston, TX, USA). In addition, devices for iontophoresis of pilocarpine for diagnosis of cystic fibrosis are on the market, and these include CF Indicator® (Scandipharm, Birmingham, AL, USA) and the system based on Webster sweat inducer with pilogel® discs and Macroduct® collector (Wescor, UT, USA). Several companies are now actively trying to commercialize miniature patch systems and are close to the market. A partial list includes Alza Corporation (USA), Becton Dickinson (USA), Fournier (France), Hisamitsu (Japan), and Cygnus (USA). Also, some companies like Pharma Peptides (France), Sanofi Recherche (France), and Novartis (Europe/USA) have done some iontophoresis work. Dermion, a relatively new subsidiary of IOMED Inc., is also working on the development of wearable iontophoresis patches through contracts with pharmaceutical

companies. IOMED also recently bought the iontophoresis technology of Elan Corporation (Ireland). There are hundreds of patents on iontophoresis between these companies and some individuals, with majority belonging to Alza and Becton Dickinson. Much of the activity in the area is proprietary and will unfold over the next few years (Banga, 1998).

Alza currently has a E-TRANS^{SM} fentanyl product under development with Janssen Pharmaceutica. Currently in Phase III clinical trials, the product is an on-demand delivery system intended to allow a patient to manage acute pain by self-titrating the level of fentanyl administered according to his or her need. With this system, the onset for control of pain is almost instantaneous and research investigations have been published (Ashburn et al., 1995). Alza is also working with its Therapeutic Discovery Corporation (TDC) to develop E-TRANS^{SM} electrotransport delivery of insulin (Alza 1996 Annual Report). Becton Dickinson is developing reusable controllers which can be connected and removed from the disposable housing. The controller monitors and controls the power supplied during use, thus permitting safer and more reliable operations. It also has the ability to detect the number of times the patch has been used, records the date and time of use, and its microprocessor can detect when drug supply is exhausted. Once the drug is exhausted, the controller can be rendered unusable to avoid abuse (Flower, 1996; Flower et al., 1996). The reusable controller will allow to keep costs down since the more expensive part of the device can be reused. Based on the principles of reverse iontophoresis, a glucose monitoring device (GlucoWatch™) is under development by Cygnus (Redwood City, CA, USA). The device consists of collection reservoirs, iontophoresis electrodes and sensor, and will be useful for continuous glucose monitoring, such as in neonates or subjects requiring frequent testing (Azimi et al., 1996). Glucose is extracted from the body due to electroosmosis induced by reverse iontophoresis and using a electrochemical reaction linked with a sensor and a control module, blood glucose levels can be continuously monitored and displayed at the push of a button. The system can sound an alarm in the event of hypo- or hyperglycemia. Glucowatch is currently in development and clinical trials are expected to commence shortly (ID Weekly Highlights, July 2, 1997, Current Drugs Ltd., London).

In terms of progress towards commercial development of electroporation for transdermal delivery of drugs, the field is in its infancy compared to the progress made for commercial development of iontophoresis. Nevertheless electroporation can expand the scope of iontophoresis to deliver peptides in greater quantities (Potts et al., 1997). More importantly, electroporation can expand the scope of transdermal delivery to larger molecules than what can be delivered by iontophoresis. If 'electroincorporation' can be commercialized to successfully deliver particulates in skin, then no molecular size limitations would exist to what can be delivered through the skin. The miniaturization of technology to develop wearable patches is currently not feasible for electroporation due to the need for a capacitor. However, Genetronics has developed palm sized generator which can deliver pulses to a medication patch attached to the skin (Shaw, 1997). Patent activity by several universities or companies such as the Massachusetts Institute of Technology (Weaver et al., 1991), Genetronics (Hofmann, 1995b,c), and others shows the developing interest in this area. Cygnus has a patent on the application of a driving force to assist the delivery of drugs through skin or tissue which has been electroporated. If iontophoresis and electroporation are compared at the same total charge delivered, electroporation appears to be better based on some literature reports but the safety of these two protocols is not likely to be same, with iontophoresis conditions being more acceptable. In a study which compared the transport efficiency of electroporation and iontophoresis, the transport numbers were in the same range and were a function of voltage and current. They did not show any dependance on pulse length, rate, energy, waveform, or total charge transferred. The area fraction of skin available to transport was larger during high voltage pulsing than during iontophoresis (Prausnitz et al., 1996). Unlike iontophoresis, very few human studies have been done with electroporation. In one recently reported double blind study,

garlic juice was used as a simple model compound to demonstrate the feasibility of using electroporation for transdermal delivery. Six pulses (100 V, 10 ms) were delivered on the volar side of each arm, with some pressure applied during and after pulsing. The intensity of tongue sensation and taste was scored by the volunteers, and it was shown that pulses and some pressure were required to obtain a positive response (Zhang and Hofmann, 1997). The electric fields developed in the tissue during pulsing will have to be characterized. Thus, the use of electroporation pulses to permeabilize the skin and then followed by low current iontophoresis could be a very useful technique to achieve high flux levels of a drug without the skin irritation which would result if high iontophoresis current is used instead. Also, iontophoresis alone will not be able to deliver large molecular weight drugs, even if high current is used.

14. Alternate applications

This review has focussed on electrically assisted topical and transdermal delivery but the applications of iontophoresis and electroporation extend beyond skin delivery. In fact, the main use of electroporation is for the introduction of DNA into cells. Ocular iontophoresis has been widely investigated for delivery of drugs such as gentamicin, cefazolin, fluorescein, tobramycin, lidocaine, epinephrine, timolol maleate, idoxuridine, dexamethasone and others for several potential clinical applications (Hill et al., 1993; Sarraf and Lee, 1994). Iontophoresis of fluoride has been used for tooth densensitization (Brough et al., 1985; Gangarosa, 1986). Electroporation has been used for a novel form of cancer treatment, called electrochemotherapy, which has utilized a combination of electroporation and chemotherapeutic agents (Dev, 1994; Dev and Hofmann, 1994). The technique typically involves the systemic administration of anticancer drug followed by delivery of electric field pulses at the site of the cancer. The rationale for this approach is that many cancer drugs are very poorly permeable into the tumor cells and pulsing the tumor site will increase the uptake of the drug from the systemic circulation into which the drug has previously been injected (Dev and Hofmann, 1996). While iontophoresis can be used to provide baseline levels, electroporation pulses can potentially be applied to provide rapid boluses. This has been shown to be feasible for transdermal transport of calcein across human epidermis. More complex delivery schedules were also achieved by changing pulse voltage (Prausnitz et al., 1994).

15. Conclusion

It should be evident from this review that both iontophoresis and electroporation hold a lot of promise for the future of transdermal drug delivery. These techniques are also likely to expand the scope of transdermal delivery of drugs to drugs of biotechnology origin and other hydrophilic macromolecules. The combined use of iontophoresis and electroporation is likely to yield useful and interesting data which will intensify the efforts to more fully explore electroporation as a means of transdermal drug delivery. The reader should realize that electrically-assisted delivery of drugs involves several chemical, biochemical, and physiological processes and there is overlap in the mechanisms involved in transport via iontophoresis or electroporation. More human clinical data is however required before the techniques can be commercialized. It seems that iontophoresis is close to commercialization while research investigations are intensifying in the electroporation area.

References

Abramson, H.A., Gorin, M.H., 1940. Skin reactions. IX. The electrophoretic demonstration of the patent pores of the living human skin; its relation to the charge of the skin. J. Phys. Chem. 44, 1094–1102.

Akins, D.L., Meisenheimer, J.L., Dobson, R.L., 1987. Efficacy of the drionic unit in the treatment of hyperhidrosis. J. Am. Acad. Dermatol. 16, 828–832.

Anderson, L.L., Welch, M.L., Grabski, W.J., 1997. Allergic contact dermatitis and reactivation phenomenon from iontophoresis of 5-fluorouracil. J. Am. Acad. Dermatol. 36, 478–479.

Anigbogu, A., Singh, P., Liu, P., Dinh, S., Maibach, H., 1997. Effects of iontophoresis on rabbit skin in vivo. Pharm. Res. 14, 308.

Ashburn, M.A., Streisand, J., Zhang, J., Love, G., Rowin, M., Niu, S., Kievit, J.K., Kroep, J.R., Mertens, M.J., 1995. The iontophoresis of fentanyl citrate in humans. Anesthesiology 82, 1146–1153.

Azimi, N.T., Bhayani, B.V., Cao, M., Lee, R.K., Leung, L., Plante, P.J., Tamada, J., Tierney, M.J., Vijayakumar, P., 1996. Iontophoresis sampling appts for transdermal monitoring of target substance—has collection reservoir comprising ionically conductive hydrogel and ionically conductive solution, and two iontophoresis electrodes in contact with collection reservoirs in contact with subject's skin (WO 9600110).

Banga, A.K., 1996. Therapeutic Peptides and Proteins: Formulation, Processing, and Delivery Systems. Technomic, Lancaster, PA.

Banga, A.K., 1998. Electrically-Assisted Transdermal and Topical Drug Delivery. Taylor and Francis, London.

Banga, A.K., Chien, Y.W., 1993. Characterization of in vitro transdermal iontophoretic delivery of insulin. Drug Dev. Ind. Pharm. 19, 2069–2087.

Banga, A.K., Prausnitz, M.R., 1998. Delivery of protein and gene-based drugs by skin electroporation. Trends Biotechnol. 16, 408–412.

Banga, A.K., Katakam, M., Mitra, R., 1995. Transdermal iontophoretic delivery and degradation of vasopressin across human cadaver skin. Int. J. Pharm. 116, 211–216.

Banta, C.A., 1994. A prospective, nonrandomized study of iontophoresis, wrist splinting, and antiinflammatory medication in the treatment of early-mild carpal tunnel syndrome. J. Occup. Med. 36, 166–168.

Bellantone, N.H., Rim, S., Francoeur, M.L., Rasadi, B., 1986. Enhanced percutaneous absorption via iontophoresis I. Evaluation of an in vitro system and transport of model compounds. Int. J. Pharm. 30, 63–72.

Bertolucci, L.E., 1982. Introduction of antiinflammatory drugs by iontophoresis: double blind study. J. Orthop. Sports Phys. Ther. 4, 103–108.

Bhatia, K.S., Gao, S., Freeman, T.P., Singh, J., 1997. Effect of penetration enhancers and iontophoresis on the ultrastructure and cholecystokinin-8 permeability through porcine skin. J. Pharm. Sci. 86, 1011–1015.

Bhatia, K.S., Gao, S., Singh, J., 1997. Effect of penetration enhancers and iontophoresis on the FT-IR spectroscopy and LHRH permeability through porcine skin. J. Control. Release 47, 81–89.

Bogner, R.H., Banga, A.K., 1994. Iontophoresis and-phonophoresis. US Pharmacist 19, H10–H26.

Bommannan, D.B., Tamada, J., Leung, L., Potts, R.O., 1994. Effect of electroporation on transdermal iontophoretic delivery of luteinizing hormone releasing hormone (LHRH) in vitro. Pharm. Res. 11, 1809–1814.

Brand, R.M., Wahl, A., Iverson, P.L., 1998. Effects of size and sequence on the iontophoretic delivery of oligonucleotides. J. Pharm. Sci. 87, 49–52.

Brough, K.M., Anderson, D.M., Love, J., Overman, P.R., 1985. The effectiveness of iontophoresis in reducing dentin hypersensitivity. J. Am. Dent. Assoc. 111, 761–765.

Burnette, R.R., Ongpipattanakul, B., 1988. Characterization of the pore transport properties and tissue alteration of excised human skin during iontophoresis. J. Pharm. Sci. 77, 132–137.

Chen, T., Segall, E.M., Langer, R., Weaver, J.C., 1998. Electroporation of the skin on a per-pulse basis. Proc. Int. Symp.Control. Rel. Bioact. Mater. 25, 22–23.

Chien, Y.W., Banga, A.K., 1989. Iontophoretic (transdermal) delivery of drugs: overview of historical development. J. Pharm. Sci. 78, 353–354.

Chien, Y.W., Siddiqui, O., Shi, W.M., Lelawongs, P., Liu, J.C., 1989. Direct current iontophoretic transdermal delivery of peptide and protein drugs. J. Pharm. Sci. 78, 376–383.

Chizmadzhev, Y.A., Indenbom, A.V., Kuzmin, P.I., Galichenko, S.V., Weaver, J.C., Potts, R.O., 1998. Electrical properties of skin at moderate voltages: contribution of appendageal macropores. Biophys. J. 74, 843–856.

Comeau, M., Brummett, R., 1978. Anesthesia of the human tympanic membrane by iontophoresis of a local anesthetic. Laryngoscope 88, 277–285.

Costello, C.T., Jeske, A.H., 1995. Iontophoresis: applications in transdermal medication delivery. Phys. Ther. 75, 554–563.

Cruz, M.P., Eeckhoudt, S.L., Verbeeck, R.K., Preat, V., 1997. Transdermal delivery of flurbiprofen in the rat by iontophoresis and electroporation. Pharm. Res. 14, 309.

Cullander, C., 1992. What are the pathways of iontophoretic current flow through mammalian skin? Adv. Drug Deliv. Rev. 9, 119–135.

Cullander, C., Guy, R.H., 1992. (D) Routes of delivery: case studies (6) transdermal delivery of peptides and proteins. Adv. Drug Deliv. Rev. 8, 291–329.

Dev, S.B., 1994. Killing cancer cells with a combination of pulsed electric fields and chemotherapeutic agents. Cancer Watch 3, 12–14.

Dev, S.B., Hofmann, G.A., 1994. Electrochemotherapy—a novel method of cancer treatment. Cancer Treat. Rev. 20, 105–115.

Dev, S.B., Hofmann, G.A., 1996. Clinical applications of electroporation. In: Lynch, P.T., Davey, M.R. (Eds.), Electrical Manipulation of Cells. Chapman and Hall, London, pp. 185–199.

Edwards, D.A., Prausnitz, M.R., Langer, R., Weaver, J.C., 1995. Analysis of enhanced transdermal transport by skin electroporation. J. Control. Release 34, 211–221.

Elgart, M.L., Fuchs, G., 1987. Tapwater iontophoresis in the treatment of hyperhidrosis. Int. J. Dermatol. 26, 194–197.

Ernst, A.A., Pomerantz, J., Nick, T.G., Limbaugh, J., Landry, M., 1995. Lidocaine via iontophoresis in laceration repair: a preliminary safety study. Am. J. Emerg. Med. 13, 17–20.

Fang, J.Y., Fang, C.L., Huang, Y.B., Tsai, Y.H., 1997. Transdermal iontophoresis of sodium nonivamide acetate. III. Combined effect of pretreatment by penetration enhancers. Int. J. Pharm. 149, 183–193.

Finkelstein, A., Mauro, A., 1997. Physical principles and formalisms of electrical excitability. In: Anonymous Handbook of Physiology. Am. Phys. Soc. Bethesda, pp.161–213.

Flower, R.J., 1996. Iontophoretic drug delivery device with disposable skin patch and reusable controller -which detects patch compatibility and exhaustion, and the date and time of usage. Pat. WO 9610440. Assignee: Becton Dickinson.

Flower, R.J., Mcarthur, W.A., Stropkay, S.E., Tanner, M.W., 1996. Iontophoretic drug delivery system—has disposable housing with reservoirs and high current power source and removable reusable controller. Pat WO 9610441. Assignee: Becton Dickinson.

Gallo, S.A., Oseroff, A.R., Johnson, P.G., Hui, S.W., 1997. Characterization of electric-pulse-induced permeabilization of porcine skin using surface electrodes. Biophys. J. 72, 2805–2811.

Ganga, S., Ramarao, P., Singh, J., 1996. Effect of azone on the iontophoretic transdermal delivery of metoprolol tartrate through human epidermis in vitro. J. Control. Release 42, 57–64.

Gangarosa, L.P., 1986. Fluoride iontophoresis for tooth desensitization. J. Am. Dent. Assoc. 112, 808–810.

Glikfeld, P., Cullander, C., Hinz, R.S., Guy, R.H., 1988. A new system for in vitro studies of iontophoresis. Pharm. Res. 5, 443–446.

Green, P., 1996a. Iontophoretic transdermal drug delivery: A new commercially feasible technology, Hotel International, Basel, Switzerland, October 17–18 (Organized by A.K. Banga and P.Green through Technomic Publishing).

Green, P., 1996b. Iontophoretic delivery of peptide drugs. J. Control. Release 41, 33–48.

Green, P.G., Flanagan, M., Shroot, B., Guy, R.H., 1993. Iontophoretic drug delivery. In: Walters, K.A., Hadgraft, J. (Eds.), Pharmaceutical Skin Penetration Enhancement. Marcel Dekker, New York, pp. 311–333.

Grimnes, S., 1984. Pathways of ionic flow through human skin in vivo. Acta. Derm. Venereol. (Stockh.) 64, 93–98.

Guy, R.H., 1996. Current status and future prospects of transdermal drug delivery. Pharm. Res. 13, 1765–1769.

Hager, D.F., Laubach, M.J., Sharkey, J.W., Siverly, J.R., 1993. In vitro iontophoretic delivery of CQA 206–291—influence of ethanol. J. Control. Release. 23, 175–182.

Hasson, S.M., Wible, C.L., Reich, M., Barnes, W.S., Williams, J.H., 1992. Dexamethasone iontophoresis: effect on delayed muscle soreness and muscle function. Can. J. Spt. Sci. 17, 8–13.

Henley, E.J., 1991. Transcutaneous drug delivery: iontophoresis, phonophoresis. Crit. Rev. Ther. Drug Carr. Syst. 2, 139–151.

Hill, J.M., O'Callaghan, R.J., Hobden, J.A., 1993. Ocular iontophoresis. In: Mitra, A.K. (Ed.), Ophthalmic Drug Delivery Systems. Marcel Dekker, New York, pp. 331–354.

Hirvonen, J., Kontturi, K., Murtomaki, L., Paronen, P., Urtti, A., 1993. Transdermal iontophoresis of sotalol and salicylate—the effect of skin charge and penetration enhancers. J. Control. Release 26, 109–117.

Hofmann, G.A., 1995. Instrumentation. In: Nickoloff, J.A. (Ed.), Electroporation Protocols for Microorganisms. Humana Press, Totowa, pp. 27–45.

Hofmann, G.A., 1995b. Electroporation system with voltage control feedback for clinical applications. US Patent 5,439,440. Assignee: Genetronics.

Hofmann, G.A., 1995c. Transdermal drug delivery by electroincorporation of vesicles. US Patent 5,464,386. Assignee: Genetronics.

Hofmann, G.A., Rustrum, W.V., Suder, K.S., 1995. Electroincorporation of microcarriers as a method for the transdermal delivery of large molecules. Bioelectrochem. Bioenerg. 38, 209–222.

Hoogstraate, A.J., Srinivasan, V., Sims, S.M., Higuchi, W.I., 1994. Iontophoretic enhancement of peptides: behaviour of leuprolide versus model permeants. J. Control. Release 31, 41–47.

Huang, Y.Y., Wu, S.M., 1996. Transdermal iontophoretic delivery of thyrotropin- releasing hormone across excised rabbit pinna skin. Drug Dev. Ind. Pharm. 22, 1075–1081.

Huang, Y.Y., Wu, S.M., 1996. Stability of peptides during iontophoretic transdermal delivery. Int. J. Pharm. 131, 19–23.

Hui, S.W., 1998. Low voltage electroporation of the skin, or is it iontophoresis? Biophys. J. 74, 679–680.

Inada, H., Ghanem, A.H., Higuchi, W.I., 1994. Studies on the effects of applied voltage and duration on human epidermal membrane alteration/recovery and the resultant effects upon iontophoresis. Pharm. Res. 11, 687–697.

James, M.P., Graham, R.M., English, J., 1986. Percutaneous iontophoresis of prednisolone—a pharmacokinetic study. Clin. Exp. Dermatol. 11, 54–61.

Jaw, F.S., Wang, C.Y., Huang, Y.Y., 1995. Portable current stimulator for transdermal iontophoretic drug delivery. Med. Eng. Phys. 17, 385–386.

Jones, B., Lynch, P.T., Power, J.B., Davey, M.R., 1996. Electrofusion and electroporation equipment. In: Lynch, P.T., Davey, M.K. (Eds.), Electrical Manipulation of Cells. Chapman and Hall, London, pp. 1–14.

Kalia, Y.N., Guy, R.H., 1995. The electrical characteristics of human skin in vivo. Pharm. Res. 12, 1605–1613.

Kari, B., 1986. Control of blood glucose levels in alloxan-diabetic rabbits by iontophoresis of insulin. Diabetes 35, 217–221.

Kassan, D.G., Lynch, A.M., Stiller, M.J., 1996. Physical enhancement of dermatologic drug delivery: iontophoresis and phonophoresis. J. Am. Acad. Dermatol. 34, 657–666.

Kasting, G.B., 1992. Theoretical models for iontophoretic delivery. Adv. Drug Deliv. Rev. 9, 177–199.

Kontturi, K., Murtomaki, L., 1996. Mechanistic model for transdermal transport including iontophoresis. J. Control. Release 41, 177–185.

Kumar, S., Char, H., Patel, S., Piemontese, D., Iqbal, K., Malick, A.W., Neugroschel, E., Behl, C.R., 1992. Effect of iontophoresis on in vitro skin permeation of an analogue

of growth hormone releasing factor in the hairless guinea pig model. J. Pharm. Sci. 81, 635–639.

Kuzmin, P.I., Darmostuk, A.S., Chizmadzhev, Y.A., White, H.S., Potts, R.O., 1996. A mechanism of skin appendage macropores electroactivation during iontophoresis. Membr. Cell Biol. 10, 699–706.

Ledger, P.W., 1992. Skin biological issues in electrically enhanced transdermal delivery. Adv. Drug Deliv. Rev. 9, 289–307.

Li, L.C., Scudds, R.A., Heck, C.S., Harth, M., 1996. The efficacy of dexamethasone iontophoresis for the treatment of rheumatoid arthritic knees: a pilot study. Arthritis Care Res. 9, 126–132.

Li, S.K., Ghanem, A.H., Peck, K.D., Higuchi, W.I., 1997. Iontophoretic transport across a synthetic membrane and human epidermal membrane: a study of the effects of permeant charge. J. Pharm. Sci. 86, 680–689.

Liss, H., Liss, D., 1995. Lateral epicondylitis. New Jersey Rehab. Nov, 8–11.

Masada, T., Higuchi, W.I., Srinivasan, V., Rohr, U., Fox, J., Behl, C., Pons, S., 1989. Examination of iontophoretic transport of ionic drugs across skin: baseline studies with the four-electrode system. Int. J. Pharm. 49, 57–62.

Merino, V., Kalia, Y.N., Guy, R.H., 1997. Transdermal therapy and diagnosis by iontophoresis. Trends Biotechnol. 15, 288–290.

Monteiro-Riviere, N.A., 1990. Altered epidermal morphology secondary to lidocaine iontophoresis: in vivo and in vitro studies in porcine skin. Fundam. Appl. Toxicol. 15, 174–185.

Monteiro-Riviere, N.A., Inman, A.O., Riviere, J.E., 1994. Identification of the pathway of iontophoretic drug delivery: light and ultrastructural studies using mercuric chloride in pigs. Pharm. Res. 11 (2), 251–256.

Panus, P.C., Campbell, J., Kulkarni, S.B., Herrick, R.T., Ravis, W.R., Banga, A.K., 1997. Transdermal iontophoretic delivery of ketoprofen through human cadaver skin and in humans. J. Control. Release 44, 113–121.

Parasrampuria, D., Parasrampuria, J., 1991. Percutaneous delivery of proteins and peptides using iontophoretic techniques. J. Clin. Pharm. Ther. 16, 7–17.

Peck, K.D., Srinivasan, V., Li, S.K., Higuchi, W.I., Ghanem, A.H., 1996. Quantitative description of the effect of molecular size upon electroosmotic flux enhancement during iontophoresis for a synthetic membrane and human epidermal membrane. J. Pharm. Sci. 85, 781–788.

Pellecchia, G.L., Hamel, H., Behnke, P., 1994. Treatment of infrapatellar tendinitis: a combination of modalities and transverse friction message versus iontophoresis. J. Sport Rehab. 3, 135–145.

Petelenz, T., Axenti, I., Petelenz, T.J., Iwinski, J., Dubel, S., 1984. Mini set for iontophoresis for topical analgesia before injection. J. Clin. Pharmacol. Ther. Toxicol. 22, 152–155.

Petelenz, T.J., Buttke, J.A., Bonds, C., Lloyd, L.B., Beck, J.E., Stephen, R.L., Jacobsen, S.C., Rodriguez, P., 1992. Iontophoresis of dexamethasone: laboratory studies. J. Control. Release 20, 55–66.

Pikal, M.J., 1992. The role of electroosmotic flow in transdermal iontophoresis. Adv. Drug Deliv. Rev. 9, 201–237.

Pliquett, U., Langer, R., Weaver, J.C., 1995. Changes in the passive electrical properties of human stratum corneum due to electroporation. Biochim. Biophys. Acta 1239, 111–121.

Potts, R.O., Bommannan, D., Wong, O., Tamada, J.A., Riviere, J.E., Monteiroriviere, N.A., 1997. Transdermal peptide delivery using electroporation. In: Sanders and Hendren (Eds.), Protein Delivery: Physical Systems. Plenum Press, New York, pp. 213–238.

Prausnitz, M.R., 1996. The effects of electric current applied to skin: a review for transdermal drug delivery. Adv. Drug Deliv. Rev. 18, 395–425.

Prausnitz, M.R., 1996. Do high-voltage pulses cause changes in skin structure? J. Control. Release 40, 321–326.

Prausnitz, M.R., Bose, V.G., Langer, R., Weaver, J.C., 1993. Electroporation of mammalian skin—a mechanism to enhance transdermal drug delivery. Proc. Natl. Acad. Sci. 90, 10504–10508.

Prausnitz, M.R., Pliquett, U., Langer, R., Weaver, J.C., 1994. Rapid temporal control of transdermal drug delivery by electroporation. Pharm. Res. 11, 1834–1837.

Prausnitz, M.R., Edelman, E.R., Gimm, J.A., Langer, R., Weaver, J.C., 1995. Transdermal delivery of heparin by skin electroporation. Biotechnology 13, 1205–1209.

Prausnitz, M.R., Lee, C.S., Liu, C.H., Pang, J.C., Singh, T.P., Langer, R., Weaver, J.C., 1996. Transdermal transport efficiency during skin electroporation and iontophoresis. J. Control. Release 38, 205–217.

Riviere, J.E., Monteiroriviere, N.A., Rogers, R.A., Bommannan, D., Tamada, J.A., Potts, R.O., 1995. Pulsatile transdermal delivery of LHRH using electroporation: drug delivery and skin toxicology. J. Control. Release 36, 229–233.

Roberts, M.S., Lai, P.M., Anissimov, Y.G., 1998. Epidermal iontophoreis: I. development of the ionic mobility-pore model. Pharm. Res. 15, 1569–1578.

Russo, J., Lipman, A.G., Comstock, T.J., Page, B.C., Stephen, R.L., 1980. Lidocaine anesthesia: comparison of iontophoresis, injection and swabbing. Am. J. Hosp. Pharm. 37, 843–847.

Sage, B.H., 1993. Iontophoresis. In: Swarbrick, J., Boylan, J.C. (Eds.), Encyclopedia of Pharmaceutical Technology. Marcel Dekker, New York, pp. 217–247.

Santi, P., Colombo, P., Bettini, R., Catellani, P.L., Minutello, A., Volpato, N.M., 1997. Drug reservoir composition and transport of salmon calcitonin in transdermal iontophoresis. Pharm. Res. 14, 63–66.

Sarraf, D., Lee, D.A., 1994. The role of iontophoresis in ocular drug delivery. J. Ocular Pharmacol. 10, 69–81.

Seth, S.C., Allen, L.V., Pinnamaraju, P., 1994. Stability of hydrocortisone salts during iontophoresis. Int. J. Pharm. 106, 7–14.

Shaw, K., 1997. The new transdermal technologies. Pharm. Times July, 38–41.

Shin, B.C., Lee, H.B., 1994. Iontophoretic delivery of insulin: effects of skin treatments. Proc. Intern. Symp. Control. Rel. Bioact. Mater. 21, 373–374.

Sims, S.M., Higuchi, W.I., Srinivasan, V., 1991. Interaction of electric field and electro-osmotic effects in determining iontophoretic enhancement of anions and cations. Int. J. Pharm. 77, 107–118.

Singh, J., Bhatia, K.S., 1996. Topical iontophoretic drug delivery: pathways, principles, factors, and skin irritation. Med. Res. Rev. 16, 285–296.

Singh, P., Maibach, H.I., 1994. Iontophoresis in drug delivery: basic principles and applications. Crit. Rev. Ther. Drug Carr. Syst. 11, 161–213.

Sloan, J.B., Soltani, K., 1986. Iontophoresis in dermatology. J. Am. Acad. Dermatol. 15, 671–684.

Stephen, R.L., Petelenz, T.J., Jacobsen, S.C., 1984. Potential novel methods for insulin administration: I. iontophoresis. Biomed. Biochim. Acta 43, 553–558.

Tomov, T.C., 1995. Quantitative dependence of electroporation on the pulse parameters. Bioelectrochem. Bioenerg. 37, 101–107.

Treco, D.A., Selden, R.F., 1995. Non-viral gene therapy. Mol. Med. Today 1, 314–321.

Tsong, T.Y., 1991. Electroporation of cell membranes. Biophys. J. 60, 297–306.

Vanbever, R., Lecouturier, N., Preat, V., 1994. Transdermal delivery of metoprolol by electroporation. Pharm. Res. 11, 1657–1662.

Vanbever, R., LeBoulenge, E., Preat, V., 1996. Transdermal delivery of fentanyl by electroporation.1. Influence of electrical factors. Pharm. Res. 13, 559–565.

Vanbever, R., Prausnitz, M.R., Preat, V., 1997. Macromolecules as novel transdermal transport enhancers for skin electroporation. Pharm. Res. 14, 638–644.

Wang, S., Kara, M., Krishnan, T.R., 1997. Topical delivery of cyclosporin A coevaporate using electroporation technique. Drug Dev. Ind. Pharm. 23, 657–663.

Wang, Y., Allen, L.V., Li, L.C., Tu, Y.H., 1993. Iontophoresis of hydrocortisone across hairless mouse skin: investigation of skin alteration. J. Pharm. Sci. 82, 1140–1144.

Warwick, W.J., Huang, N.N., Waring, W.W., Cherian, A.G., Brown, I., Stejskal-Lorenz, E., Yeung, W.H., Duhon, G., Hill, J.G., Strominger, D., 1986. Evaluation of a cystic fibrosis screening system incorporating a minature sweat stimulator and disposable chloride sensor. Clin. Chem. 32, 850–853.

Weaver, J.C., 1993. Electroporation: a general phenomenon for manipulating cells and tissues. J. Cell. Biochem. 51, 426–435.

Weaver, J.C., 1995. Electroporation theory—concepts and mechanisms. In: Nickoloff, J.A. (Ed.), Electroporation Protocols for Microorganisms. Humana Press, Totowa, NJ, pp. 1–26.

Weaver, J.C., Chizmadzhev, Y., 1996. Electroporation. In: Polte, C., Postow, E. (Eds.), Biological Effects of Electromagnetic Fields. CRC Press, Boca Raton, NY, pp. 247–274.

Weaver, J.C., Powell, K.T., Langer, R.S. 1991. Control of transport of molecules across tissue using electroporation. US Patent 5,019,034. Assignee: MIT.

Weaver, J.C., Vanbever, R., Vaughan, T.E., Prausnitz, M.R., 1997. Heparin alters transdermal transport associated with electroporation. Biochem. Biophys. Res. Commun. 234, 637–640.

Yeung, W.H., Palmer, J., Schidlow, D., Bye, M.R., Huang, N.N., 1984. Evaluation of a paper-patch test for sweat chloride determination. Clin. Pediatr. 23, 603–607.

Zeltzer, L., Regalado, M., Nichter, L.S., Barton, D., Jennings, S., Pitt, L., 1991. Iontophoresis versus subcutaneous injection: a comparison of two methods of local anesthesia delivery in children. Pain 44, 73–78.

Zewert, T.E., Pliquett, U.F., Langer, R., Weaver, J.C., 1995. Transdermal transport of DNA antisense oligonucleotides by electroporation. Biochem. Biophys. Res. Commun. 212, 286–292.

Zhang, L., Hofmann, G.A., 1997. Electric pulse mediated transdermal drug delivery. Proceed. Int. Symp. Control. Rel. Bioact. Mater. 24, 27–28

Zhang, L., Li, L.N., An, Z.L., Hoffman, R.M., Hofmann, G.A., 1997. In vivo transdermal delivery of large molecules by pressure-mediated electroincorporation and electroporation: a novel method for drug and gene delivery. Bioelectrochem. Bioenerg. 42, 283–292.