J. Appl. Cosmetol. **14**, 99-109 (July-September 1996)

TRANSDERMAL DRUG DELIVERY BY IONTOPHORESIS. II. TECHNIQUES AND *IN VITRO-IN VIVO* MODELS

Rosario Pignatello[1], Massimo Fresta[1] and Giovanni Puglisi[1,2]

[1] Dipartimento di Scienze Farmaceutiche, Facoltà di Farmacia, Università degli Studi di Catania, Via Andrea Doria, 6 - 95125 Catania (Italy)

[2] Istituto di Scienza del Farmaco, Università "G. D'Annunzio", Chieti (Italy)

Received: *March 15th, 1996*

Key words: *Iontophoresis, transdermal drug delivery, in vitro and in vivo models, iontophoretic devices.*

Synopsis

The recognised validity of iontophoresis in promoting the dermal or transdermal transport of biologically active compounds, mainly ionized species, to gain a systemic effect, is related to an accurately and controlled choice of materials and devices, as well as of the operative conditions used for its realization. Extensive work has been done with *in vitro* experimental models to better define the different factors which can influence the effectiveness and reliability of iontophoresis. In this paper, we took into considerations such parameters, illustrating also the *in vitro* and *in vivo* models and devices, developed during last years.

Riassunto

La iontoforesi rappresenta ormai una tecnica ampiamente considerata per la sua efficacia nel favorire la penetrazione dermica o transdermica di sostanze farmaceutiche ad azione sistemica, altrimenti difficilmente somministrabili per questa via. Comunque, la riproducibilità e l'efficienza di questa metodica è strettamente dipendente da un attento controllo di diversi parametri operativi e dalla scelta dei dispositivi più adatti alle sostanze da somministrare. In questo lavoro, a proseguimento di una precedente rivisitazione dei principi teorici sui quali la iontoforesi si basa, vengono presi in esame alcuni di questi fattori, dei quali è ben nota l'influenza sul trasporto iontoforetico dei farmaci, nonché i diversi modelli sperimentali, *in vitro* e *in vivo*, sviluppati nel corso degli ultimi anni.

Introduction

In a previous part of this review (1), we have described the theoretical considerations governing the technique of iontophoresis. It is defined as the procedure of releasing a drug through the intact skin for an intradermal or a systemic effect, by means of a suitable electric current applied on the skin itself. Transdermal delivery of drugs has a number of advantages over other routes of administration and conventional dermatological systems, in particular that one of increasing the skin penetration of ionized or charged molecules.

After the initial papers which described a number of experimental approaches for optimizing the iontophoresis as a drug delivery device, clinical and therapeutical applications have been referred by many Authors, and iontophoresis is, to date, a valid alternative to the systemic administration of many drugs and biologically active species.

Such a number of works have well evidenced the various factors, whose exact knowledge and definition can influence the effectiveness and reproducibility of this technique. However, many studies are also in progress in such a field and a continuous improvement of materials and operative conditions is expected during next years.

Thus, in this second part of our review on iontophoresis, we have described some of these factors whose effect on iontophoresis are well known; moreover, the *in vitro* and *in vivo* experimental models and devices developed during these years are also reviewed.

Table 1

PRINCIPAL FACTORS INFLUENCING IONTOPHORETIC TRANSPORT OF DRUGS

1. Physico-chemical properties of drugs:

a) Charge
b) Molecular size and weight
c) Solubility and concentration in the donor solution
d) Rate of ionization (also dependent on pH in the donor solution)
e) Hydro-lipophilicity
f) Electro-chemical stability

2. Experimental variables:

a) Density of current applied
b) Nature of electrodes used
c) Duration of treatment
d) Nature of current (costant or pulsatile)
e) Presence of competing ions (i.e. buffer ingredients) (cfr. 15, 21, 37)

3. Physiological factors:

a) site of application (density of appendages)
b) individual variables (sex, age, race)
c) Rate of hydration of skin
d) Integrity of skin surface
e) Utilization of permeation enhancers or delipidizing solvents

Experimental variables affecting iontophoresis

The efficiency of drug delivery by an iontophoretic application depends upon many physico-chemical and technical variables, apart from the same factors which regulate the skin permeation of a drug during its passive diffusion (2). Table I reports a list of factors which can affect iontophoretic transport efficiency and whose contribution is briefly discussed in the following section.

Type of iontophoretic devices and electrodes

The quality and nature of the instrument may have a great influence on the resulting iontophoretic flux, in terms of both efficiency and predictability-reproducibility. Basic systems have been reviewed by Tyle (3); in particular, they can be chosen under the light of safety, economical convenience and pa-

tient compliance considerations.
Portable 9-V battery-operated devices which can be easily transported by the patient have been realized. The electrodes are fixed by an adesive pad or membrane at few centimeters from each other on the skin surface and a continuous delivery of the active agent is ensured while the patient can attend his common daily activity. An example of such a device is the Phoreser of Motion Control Inc. (Salt Lake City, U.S.A.) (4). A control system is of great importance to ensure that a constant current (5-50 mA) is supplied to the patient during time. In some cases, it is also possible to choose the polarity of electrodes, in order to achieve the best delivery of a cationic or anionic molecule (1). The drugs are usually placed as an aqueous solution in a refillable plastic chamber limited by a diffusion polymeric membrane, or simply absorbed on a gauze pad. In other systems, the drug-loaded patch consisted of a hydrogel matrix, fitted with a metallic wire or lamina to serve as the donor electrode. The hydrogel reservoir is usually contained in a circular plastic holder.

A pencil-shaped iontophoretic systems has been described by Groning for the transdermal administration of antihistamine agents, in the localized treatment of acute skin irritations, such as insect bites (5). Deeper informations on the *in vivo* iontophoretic delivery devices commercially produced, mainly in the U.S.A., can be found in the review work of Singh and Singh (2).

The type of electrodes can, in turn, deeply modify the transport rate of ionic drugs. Two kinds of electrodes are usually used, inert and reversible ones (6). Inert electrodes, made of platinum, tin, some stainless steels or nickel, do not participate to the electrochemical reaction and then are not consumed during it; however, they induce a bubbling of gas into the solution and a large electrolysis of water into H^+ and OH^- ions, which are responsible for the changes in the pH of the skin surface underneath the electrodes. Apart from the possible consequences for skin integrity (1), modifications in the ionization rate of drugs, and hence in their electrical mobility can result. Risks of burns can be prevented by covering the electrode with a cellophane or plastic pad or sponge, which in the meantime avoids the direct contact with the body and possess a flexible conformation enough for adaptation to irregular skin surfaces. A further adhesive coating (e.g., medical silicon) allows a better permanence of the electrode to the site of application. To avoid pH variations during the treatment a buffered solution can be used as the drug donor and receptor solutions; however, the presence of extraneous ions in the buffer, basing on their specific conductivity (1) and charge (they can be co-ions or counter-ions with respect to the drug), could deeply influence (both by competing or assisting) the transdermal flux of the ionic species to be delivered (7).
Among reversible electrodes, silver/silver chloride ones are the most diffused. They are prepared electrochemically: for example, Oh and Guy (8) used a 3 cm silver wire (1 mm diameter) which was accurately washed and cleaned with a warmed 1 *M* HCl solution. After rinsing with water, the Ag wire was anodically plated with AgCl (using a platinum cathode) by placing it in 0.5 *M* KCl and passing a 0.1 mA current for 20 min (sensing electrodes) or 5 h (signal electrodes). Oldenburg et al. (9) prepared a cathode electrode by placing a 0.1 mm diameter Ag wire in 0.1 *M* HCl and passing a charge of 18-20 coulombs for 18-24 h. The redox potential for the Ag/AgCl system (1.22 V) is lower than the oxidation potential of water, thus avoiding the degradation of the latter. The occurring reactions at the cathode and anode are, respectively, the dissociation of solid silver chloride ($AgCl + e^- \rightarrow Ag + Cl^-$) and the association of chloride ions with silver ($Ag + Cl^- \rightarrow AgCl + e^-$). This kind of electrodes has a particular advantage when the drug to be iontophoresized is a hydrohalide salt, e.g., lidocaine hydrochloride (6). In solution, these salts dissociate into the drug cation and the halide counterion, thus directly providing one of the species needed for the above electrode reaction. Moreover, the product of the reaction, AgCl, is insoluble and precipitates on the anode, without generation of other ions which could compete with the drug for the

current flux. The most evident limitation to the use of Ag/AgCl electrodes, is their precipitation effect on proteins and peptides (10).

To reduce the consumption of the AgCl electrode during the work of the system, a periodically switch of the polarity between the two electrodes has been proposed (11,12): e.g., in the experiment of Su et al. (11), the patch containing the Ag electrode was initially settled as the anode while the other patch containing Ag/AgCl was the cathode. After the first 6 h and then periodically every 4 h, the polarity was reversed. This switching allows to regenerate the Ag/AgCl electrodes during the reverse cycle and to reduce the pH changes. Moreover, the drug (tetraethylammonium bromide) was delivered alternatively from both patches (the one acting as the anode at that time), thus reducing the depletion of solute from a patch.

Influence of pH

The degree of ionization of a weak electrolyte is known to depend upon pH. Thereby, changes of pH of the fluid under the releasing electrode have been indicated as responsible for significant variations in iontophoretic transport of some drugs: e.g., lignocaine (13), sulfamidics (14), verapamil (15), thyrotropin-releasing hormone (16), and other solutes (17). As pH determines the charge of the drug ions, it can modify the fractional contribution of these species to the total current, namely their transport number (1). Noteworthy, the pH range of solutions that can be applied to the skin is usually between 3 and 8, since at outer values skin damage and irritation may compare (18). This implies that for most acidic drugs, with low p*K*a values, which are in a neutral form or carry negative charge(s) at most experimental pHs, the iontophoretic flux will be quite negligible. Of course, for peptides, proteins and other substances which show an isoelectric point, the pH of the vehicle or in the donor solution is of extremely great importance, since it will determine the charge of solutes.

The pH variations at the releasing electrode can also induce a rapid depletion of the drug from the donor compartment (11, 12).Sanderson et al. (19) have payed their attention on the possibility of limiting pH variations in the subcutaneous layer during iontophoresis. Their objective was that to optimize the delivery of cationic drugs which request a treatment for extended periods of time (also for 24 hs), so that to minimize the effects (i.e., skin trauma) of the large amount of current required to delivery the drug.

Four approaches have then been described to control skin pH changes near to the electrodes: i) to use a salt of the (cationic) drug with a weak acid (e.g., acetate or succinate), instead of the hydrochloride. This would result in a reduction of pH lowering at skin surface under the donor electrode; ii) to change the drug with a charged form of it (e.g., a quateruary ammonium salt for the corresponding free base); iii) to increase the concentration of the drug in the donor solution or, even better, to enhance its solubility by choosing a suitable solvent (e.g., by replacing aqueous buffer solutions with an ethanol-water mixture); iv) finally, these investigators suggested to reduce the permselectivity of the skin to cations by reducing the presence of anions in the receptor compartment, i.e., by using a polyacrylic acid solution instead of Cl^--containing saline, or by a skin pretreatment with a surfactant, like sodium lauryl sulfate, which neutralizes the fixed positive charges on the skin surface and enhances the iontophoretic flux of cationic species. Apart from the practical utility of such approaches, the Authors demonstrated the possibility of a real enhancement of drug delivery, by reducing the amount of current required and thus its side effects on the skin (19).

Duration and intensity of the current applied

From Faraday's law, it is clear that in an electrolytic solution the amount of electricity conducted depends on the strength of the current applied and the duration of its passage:

$$M_D = \frac{i_D}{Z_D} \frac{t}{\mathcal{F}}$$

where M_D is the moles of the ionic drug and Z_D is its valence (number of charge per drug molecule), t is the time (in seconds), i_D is the current carried by the drug, and $\mathcal{F}$ is a proportionality factor (Faraday's constant).

A linear relationship between the flux of many drugs and current density has been reported under different experimental conditions, both for cathodal and anodal iontophoresis (16, 20-33). For example, studies on a model peptide (TRH) (23) showed that flux is proportional to the applied electric field and this linearity was observed both at pH 4 and 8, that is, either when the drug was in a neutral or charged state in the donor solution. Such a behaviour can obviously be related to the appearance of an electroosmotic flow from the anode to the cathode, involved in the transdermal transport of large molecules with a different charge than positive one (1).

The positive effect of increasing applied current densities on drug flux, is mainly related to the parallel reduction of skin resistance (34). However, for application to human, the used current can not be extremely high, and an optimal intensity between 40 µA and 10 mA, a limit which was found to not cause perceptible physical discomfort or pain (35), has been individuated; it corresponds to a maximum current density of 500 µA/cm2 (36) [a current of one ampere (A) corresponds to one coulomb per mole (C/mol)]. At higher densities or/and for exposure times longer than 10-30 min, an irreversible modification of skin conducibility could begin, as a consequence of serious hystological alterations of the skin itself (1).

Chemical structure of solutes

Yoshida and Roberts (37) have extensively described the relationships existing between the molecular size and structure of many drugs and their iontophoretic behaviour. In general, it has been demonstrated that the logarithm of iontophoretic flux is inversely proportional to solute molecular weight (27, 38, 39). Two different theories have been advocated to explain such results: the 'free volume' theory predicts that a molecule diffuses only when a hole or free volume into which it can move is present near to it (Fig. 1a) (40); this approach better fits with the assumption that solutes move through the lipid domains of the SC. According to that theory, the enhancement of iontophoretic flux sometimes observed by using a penetration "enhancer" (e.g., ethanol, DMSO, DMA) (37) can be explained by considering that these solvents dissolve some lipids from the stratum corneum matrix, thus allowing more "void" volumes to become available for the diffusion of solutes through the epidermis.

The second model is based on the hypothesis that when a compound has to pass through skin pores, dimensions of these latter lead to the exclusion of molecules with too large sizes (Fig. 1b) (41). Scheuplein (42) suggested that in human skin the radii of outer and inner sweat ducts are 7 and 2.35

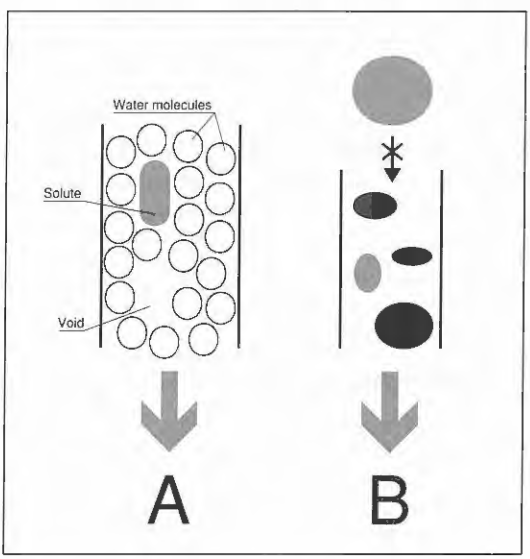

Fig. 1: Schematic representation of the "free volume" model (A) (40) and "size exclusion" theory (B) (41) to explain the influence of solute molecole size on its transport through skin pores (adapted from ref. 37)

μm, respectively, and those of surface opening of sweat ducts and hair follicles of about 35 μm. Lower values (0.675-2.7 nm) were reported for hairless mouse skin pores (43). Moreover, the movement of the solute within skin pores is also affected by its friction with pore walls. However, at present none of the two models seems to completely explain the experimental data collected.

Oldenburg et al. (9) have studied the effects of composition of some oligonucleotides on their iontophoretic transport efficiency. Working with 15-mer homopolymers, they concluded that the base composition can strongly influence the flux of compounds; in particular, bases that can form more hydrogen bonds, as pyrimidine ones, may better interact with skin structures and therefore slow the transdermal migration of oligomers.

Drug concentration

Increased concentrations of the ionic drug to be released in the donor electrode generally are related to an enhancement of drug flux across the skin. Findings in such direction have been reported for benzoates (21), verapamil (22), morphine hydrochloride (24), and many other inorganic salts and ionic drugs (28).

However, the positive effect of such a parameter on a drug iontophoretic flux is limited by the solubility of the drug itself in the donor medium (water or buffer solution), along with the possible variation of solubility linked to the eventually occurring pH variations during iontophoresis (see above).

In vitro models

The simplest apparatus for *in vitro* iontophoresis studies is illustrated in Fig. 2. It basically consists of a source of electric power (a battery) connected to a couple of electrodes immersed in the donor and receptor compartments; they acts as the anode and the cathode and operates by transforming the electron current to ionic current. The donor reservoir (i.e., skin surface) is an aqueous (or buffered) solution of the ionic drug; however, a drug-containing gel formulation can be used, e.g., when the experiment has to be carried out on a living animal. The receptor compartment commonly is an isotonic solution of sodium chloride (normal saline), which simulates the dermis. The driving system maintains the current field constant during the operating time.

A diffusion membrane, either natural (human or animal) or artificial (cellophane or cellulose) separates the two compartments. Human skin samples generally derive from plastic surgery or from cadavers (44). For permeation studies, either full skin (excised or dermatomed) or its isolated layers are employed. Intact epidermis is obtained by bathing the skin in water at 60°C for 1-2 min, then peeling the epidermis from the other tissues, while full thickness skin was prepared by removal of the subcutaneous fat. Stripped skin can be obtained by removing the stratum corneum by repeated stripping (25-30 times) with an adhesive tape. Finally, isolated stratum corneum can be prepared by one of the known methods, as the heat separation described by Kligman and Cristophers (45).

However, the uneasy availability of good samples, generally makes the animal models more suitable for basic studies, both from hairless (mouse, nude rat, guinea pig) and furry animals (mouse, rat, rhesus monkey) (46). Hairless mouse skin (47) or shed snake skin [the latter completely lacking of any appendageal structure (47, 48)], have been particularly proposed as models for human skin in the *in vitro* assessment of transdermal iontophoretic drug delivery.

A comparison of the electrokinetic behaviour affecting the iontophoretic transport of drugs between excised human skin and hairless mouse skin has been reported by Pikal (49). While many of the flux characteristics [e.g., the different effects of cathodal or anodal delivery of neutral or charged compounds (1) as well as skin damage effects] after iontophoresis are similar between the two skin models, however human skin showed a worse correlation between the calculated electroosmotic flow and the permeability (flux) measured for neu-

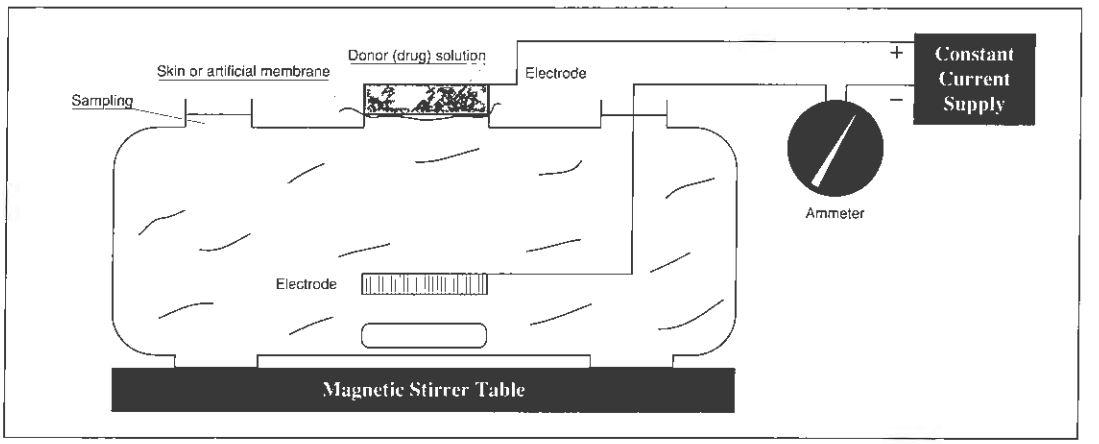

Fig. 2: A basic esperimental device for in vitro *iontophoretic investigations.*

tral species, in particular when low current densities are used.

More recently, Hager et al. (50) have described a cultured skin system, obtained from cultures of human cells and matrix normally present in the skin and known as "living skin equivalent" (LES). It well simulates the human skin, showing differentiated stratum corneum, epidermis and dermis layers, and lack the typical skin appendages (hair follicles, glands), thus allowing to better define the permeability properties of the skin. Studies on LSE indicated that such a membrane is an accurate model for *in vitro* experiments on iontophoretic transport through the skin and permeation results of different model drugs are in good agreement with those obtained by using guinea pig skin (50).

When skin was used as the diffusion membrane, the stratum corneum is obviously oriented toward the donor compartment. Normally, subcutaneous fat layer was removed before experiments: in fact, it is generally not involved in permeation and absorption phenomena through the skin, since it is placed under the blood circulation within the dermis.

To study the particular influence of iontophoresis on cutaneous penetration of model or drug molecules, modified skin or isolated skin layers are often used. For example, isolated epidermis can be obtained by placing the full skin in water at 60°C for about 90 sec (51) and then peeling off the epidermis. In the model of "stripped skin", stratum corneum was removed by means of an adesive tape applied on the skin; when these samples are used, they are mounted vertically in the diffusion cell with the dermal side bathing the receptor fluid. This latter can be constituted by an isotonic buffer, like pH 7.4 phosphate-buffered saline.

Masada et al. (52) have described a four-electrode system for a two-chamber diffusion cell. In such a system, a couple of reference electrodes are placed close to the two sides of the diffusion membrane, while a constant voltage difference is maintained between them by a potentiostat. Another couple of counter electrodes are placed into the donor and receiver-containing cell, respectively, in order to maintain the required current flow through the cell itself. The main advantage of such a system, is the possibility to know and measure simultaneously the voltage difference and the current across the membrane.

To develop a cathodal iontophoresis (i.e., the release of negatively charged species) (1), the cathode electrode was placed in the donor compartment (an aqueous solution of the drug to be administered) and the anode in the receiver compartment. Their position is reversed in the case of an anodal iontophoresis (with cationic drugs or ions).

Generally, a constant current was applied across

the skin sample (with a current density between 100 and 500 μA/cm^2) for the wished period of time (up to 12 h for permeation studies). Samples from the receptor compartment were withdrawn periodically and analysed by a suitable method (HPLC, UV, radiolabeling, etc.).

Comparison of in vivo studies with in vitro findings

The main aim of all the iontohpretic experimental models, is to predict as better as possible the results which will be obtained when *in vivo* delivery is performed.

Different Authors have reported interesting observations of very good correlations between the *in vivo* iontophoretic behaviour of drugs and findings drawn by experimental models. Sage and Riviere (6) found that the human skin flap is the best model in predicting the *in vivo* delivery of lidocaine. Riviere et al. (53) used an isolated perfused porcine skin flap (IPPSF) model, which correlated well with the *in vivo* iontophoretic permeation of arbutamine. Such a model of skin has the advantage of possessing anatomical and functional properties similar to the viable skin, like a microcirculation system (54).

Metoclopramide (7) and hydromorphone (55) were also studied *in vivo*. Interestingly, results indicated that a better *in vitro* simulation of the *in vivo* flux of many charged drugs can be obtained by using a hypotonic (0.08-0.09 M) NaCl solution, instead of a normal saline (0.15 M) in the receiver compartment, i.e., the conditions probably existing in the epidermis (7).

Conclusions

Studies on the current-assisted delivery of drugs and other compounds to the body through the intact skin, would obviously need to use the ultimate model: humans *in vivo*. In the practice, investigators have described many interesting experimental models, both using synthetic (e.g., permselective, ion-change resins, etc.) or natural skin (human or animal) as the diffusion membranes. As well, ever more efficient *in vitro* devices have been realized to gain a better prediction of the behaviour of a drug under *in vivo* iontophoresis.

However, both types of studies need a deep knowledge and description of the different operative and formulative variables which can influence the results. Apart from the intrinsic properties of the drug to be delivered, many factors can be suitably standardized to obtain an effective and reproducible output of the treatment.

Correspondence: **Prof. Giovanni Puglisi**
Dipartimento di Scienze Farmaceutiche
Città Universitaria,
Viale A. Doria, 6 - 95125 Catania (Italy)
Tel.: +39 95 222 215 - Fax: +39 95 222 239

References:

1. **Pignatello R., Fresta M., Puglisi G. (1996)** Transdermal drug delivery by iontophoresis. I. Fundamental and theoretical aspects. *J. Appl. Cosmetol.*, **14**:59-72.
2. **Singh S., Singh J. (1993)** Transdermal drug delivery by passive diffusion and iontophoresis: a review, *Med. Res. Rev.*, **13**:569-621.
3. **Tyle P. (1986)** Iontophoretic devices for drug delivery, *Pharm. Res.*, **3**:318-326.
4. **Stephen R.L., Petelenz T.J., Jacobsen S.C. (1984)** Potential novel methods for insulin administration: I. *Iontophoresis. Biomed Biochem Acta*, **43**:553-558.
5. **Gröning R. (1987)** Electrophoretically controlled dermal or transdermal application systems with electronic indicators, *Int. J. Pharm.*, **36**:37-40.
6. **Sage B.H. Jr, Riviere J.E. (1992)** Model systems in iontophoresis - transport efficacy, *Adv. Drug. Delivery. Rev.*, **9**:265-287.
7. **Phipps J.B., Gyory J.R. (1992)** Transdermal ion migration, *Adv. Drug. Del. Rev.*, **9**:137-176.
8. **Oh S.Y., Guy R.H. (1995)** Effects of iontophoresis on the electrical properties of human skin *in vivo*, *Int. J. Pharm.*, **124**:137-1424.
9. **Oldenburg K.R., Vo K.T., Smith G.A., Selick H.E. (1995)** Iontophoretic delivery of oligonucleotides across full thickness hairless mouse skin, *J. Pharm. Sci.*, **84**:915-921.
10. **Lelawongs P., Liu J., Siddiqui O., Chien Y.W. (1989)** Transdermal iontophoretic delivery of arginine-vasopressin: I. Physicochemical considerations, *Int. J. Pharm.*, **56**:13-22.
11. **Su M.H., Srinivasan V., Ghanem A.H., Higuchi W.I. (1994)** Quantitative *in vivo* iontophoretic studies, *J. Pharm. Sci.*, **83**:12-17.
12. **Srinivasan V., Su M.H., Higuchi W.I. (1989)** *Pharm. Res.*, **6**:S 80.
13. **Siddiqui O., Roberts M.S., Polack A.Z. (1985)** The effect of iontophoresis and vehicle pH on the *in-vitro* permeation of lignocaine through human stratum corneum. *J. Pharm. Pharmacol.*, **37**:732-735.
14. **Von Sallmann L. (1942)** *Am. J. Ophthal.*, **25**:1292-1300.
15. **Wearley L., Liu L.C., Chien Y.W. (1989)** Iontophoresis-facilitated transdermal delivery of verapamil: I. *In vitro* evaluation and mechanistic studies, *J. Controlled Rel.*, **8**:237-250.
16. **Burnette R.R., Marrero D. (1986)** Comparison between the iontophoretic and passive transport of thyrotropin releasing hormone across excised nude mouse skin, *J. Pharm. Sci.*, **8**:738-743.
17. **Siddiqui O., Roberts M.S., Polack A.W. (1989)** Iontophoretic transport of weak electrolytes through the excised human stratum corneum, *J. Pharm. Pharmacol.*, **41**:430-432.
18. **Allenby A.C., Fletcher J., Schock C., Tees T.F.S. (1969)** The effect of heat, pH and organic solvents on the electrical impedance and permeability of excised human skin, *Br. J. Dermatol.*, **81**:31-39.
19. **Sanderson J.E., De Riel S., Dixon R. (1989)** Iontophoretic delivery of nonpeptide drugs: formulation optimization for maximum skin permeability, *J. Pharm. Sci.*, **78**:361-364.
20. **Del Terzo S., Behl C.R., Nash R.A. (1989)** Iontophoretic transport of a homologous series of ionized and nonionized model compounds: influence of hydrophobicity and mechanostic interpretation, *Pharm. Res.*, **6**:85-90.
21. **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. Pharmacol.*, **30**:63-72.

22. **Wearley L., Liu L.C., Chien Y.W. (1989)** Iontophoresis-facilitated transdermal delivery of verapamil: II. Factors affecting the reversibility of skin permeability, *J. Controlled. Rel.*, **9**:231-242.
23. **Burnette R.R., Marrero D. (1986)** Comparison between the iontophoretic and passive transport of thyrotropin releasing hormone across excised nude mouse skin, *J. Pharm. Sci.*, **8**:738-743.
24. **Corish J., Corrigan O.I., Foley D.** (1989) *Chim. Oggi*, **7**:17.
25. **Lelawongs P., Liu J.C., Chien Y.W. (1990)** Transdermal iontophoretic delivery of arginine-vasopressin: II. Evaluation of electrical and operational factors, *Int. J. Pharm.*, **61**:179-188.
26. **Tu Y.H., Allen L.V. (1989)** *In vitro* iontophoretic studies using a synthetic membrane, *J. Pharm. Sci.*, **78**:211-213.
27. **Phipps J.B., Padmanabhan R.V., Lattin G.A. (1989)** Iontophoretic delivery of model inorganic and drug ions, *J. Pharm. Sci.*, **78**:365-369.
28. **Miller L.L., Smith G.A. (1989)** Iontophoretic transport of acetate and carboxylate ions through hairless mouse skin. A cation exchange membrane model, *Int. J. Pharm.*, **49**:15-22.
29. **Nyambi D.A., Dawes V.H., Lawrence M.J. (1990)** Evaluation of a new iontophoretic system using model compounds, *J. Pharm. Pharmacol.*, **42**(Suppl.):83.
30. **Bannon Y.B., Corish J., Corrigan O.I., Masterson J.G. (1988)** Iontophoretically induced transdermal delivery of salbutamol, *Drug. Dev. Ind. Pharm.*, **14**:2151-2166.
31. **De Nuzzio J.D., Berner B. (1990)** Electrochemical and iontophoretic studies of human skin, *J. Controlled Rel.*, **11**:105-112.
32. **D'Emanuele A.D., Staniforth J.N. (1991)** An electrically modulated drug delivery device: I. *Pharm. Res.*, **8**:913-918.
33. **Singh S., Jayaswal S.B., Upadhyay S.N., Singh J. (1992)** Iontophoretic delivery of propanolol hydrochloride through human epidermis, *J. Controlled. Rel.*, **18**:165-170.
34. **Kasting G.B., Bowman L.A. (1990)** Electrical analysis of fresh, excised human skin: a comparison with frozen skin, *Pharm. Res.*, **7**:1141-1146.
35. **Rapperport A.S., Larson D.L., Henges D.F., Lynch J.B., Blocker T.G., Lewis R.S. (1965)** *Plastic Reconstruc. Surg.* **36**:547-552.
36. **Abramson H.A., Gorin M.H. (1941)** Preseasonal treatment of hay fever by electrophoresis of ragweed extract into the skin, preliminary report, *J. Allergy.*, **12**:169-175.
37. **Yoshida N.H., Roberts M.S. (1992)** Structure-transport relationships in transdermal iontophoresis, *Adv. Drug. Delivery. Rev.*, **9**:239-264.
38. **Roberts M.S., Singh J., Yoshida N., Curries K.I. (1990)** Iontophoretic transport of selected solutes through human epidermis. In: Scott R.C., Guy R.H., Hadgraft J. (Eds) Prediction of percutaneous absorption, IBC Technical Services, London, pp. 231-241.
39. **Green P.G., Hinz R.S., Kim A., Scoba F.C., Guy R.H. (1991)** Iontophoretic delivery of a series of tripeptides across the skin *in vitro*, *Pharm. Res.*, 8:1121-1127.
40. **Lieb W.R., Stein W.D. (1986)** Topical review: non-stokesian nature of transverse diffusion within human red cell membranes, *J. Membr. Biol.*, **92**:111-119.
41. **Renkin E.M. (1955)** Filtration, diffusion and molecular sieving through porous cellulose membranes, *J. Gen. Physiol.*, **38**:225.
42. **Scheuplein R.J. (1967)** Mechanism of percutaneous absorption: II. Transient diffusion and the relative importance of various routes of skin penetration, *J. Invest. Dermatol.*, **48**:79-88.
43. **Pikal M.J., Shah S. (1990)** Transport mechanisms in iontophoresis: III. An experimental study of the contributions of electroosmotic flow and permeability change in transport of low and high molecular weight solutes, *Pharm. Res.*, **7**:222-229.

44. **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.
45. **Kligman A.M., Cristophers E. (1963)** Preparation of isolated sheets of human stratum corneum, *Arch. Dermatol.*, **88**:702-705.
46. **Behl C.R., Kumar S., Malick A.W., Del Terzo S., Higuchi W.I., Nash R.A. (1989)** Iontophoretic drug delivery: effects of physicochemical factors on the skin uptake of nonpetide drugs, *J. Pharm. Sci.*, **78**:355-359 (and refs. therein).
47. **Rigg P.C., Barry B.W. (1990)** Shed snake skin and hairless mouse skin as model membranes for human skin during permeation studies, *J. Invest. Dermatol.*, **94**:235.
48. **Itoh T., Xia J., Magavi R., Nishihata T., Rytting J.H. (1990)** Use of shed snake skin as a model membrane for *in vitro* percutaneous penetration studies: comparison with human skin, *Pharm. Res.*, **7**:1042.
49. **Pikal M.J. (1992)** The role of electroosmotic flow in transdermal iontophoresis, *Adv. Drug. Del. Rev.*, **9**:201-237.
50. **Hager D.F., Mancuso F.A., Nazareno J.P., Sharkey J.W., Siverly J.R. (1994)** Evaluation of a cultured skin equivalent as a model membrane for iontophoretic transport, *J. Controlled. Rel.*, **30**:117-123.
51. **Singh P, Anliker M., Smith G.A., Zavortink D., Maibach H.I. (1995)** Transdermal iontophoresis and solute penetration across excised human skin, *J. Pharm. Sci.*, **84**:1342-1346.
52. **Masada T., Higuchi W.I., Srinivasan V., Rohr U., Fox J., Behl C., 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.
53. **Riviere J.E., Bowman K.F., Monteiro-Riviere N.A., Dix L.P., Carver M.P. (1986)** The isolated perfused porcine skin flap (IPPSF): I. A novel *in vitro* model for percutaneous absorption and cutaneous toxicology studies, *Fundam. Appl. Toxicol.*, **7**:444-453.
54. **Riviere J.E., Sage B., Williams P.L. (1991)** Effects of vasoactive drugs on transdermal lidocaine iontophoresis, *J. Pharm. Sci.*, **80**:615-620.
55. **Padmanabhan R.V., Phipps J.B., Lattin G.A., Sawchuk R.J. (1990)** *In vitro* and *in vivo* evaluation of transdermal iontophoretic delivery of hydromorphone, *J. Controlled Rel.*, **11**:123-135.