JOURNAL REVIEW

Transdermal Delivery of Peptide and Protein Drugs: an Overview

B. G. Amsden and M. F. A. Goosen
Dept. of Chemical Engineering, Queen's University, Kingston, Ontario, Canada K7L 3N6

Recently, a large number of new and potentially potent peptide and protein drugs have been developed. Their systemic delivery is difficult because they are rapidly cleared from the bloodstream, are of large molecular size, are vulnerable to proteolytic attack, and tend to undergo aggregation, adsorption and denaturation. Controlled release strategies have many advantages over the current delivery method of injection or intervenous infusion. Controlled release allows for prolonged delivery while maintaining the drug concentration within therapeutic limits. It also improves and increases patient compliance by removing the discomfort of repeated applications. The transdermal route of delivery is particularly attractive because it avoids peptide and protein degradation via the gastrointestinal tract and the hepatic first-pass effect, and delivery can be interrupted by simply removing the device. Its primary problem is that the skin is an excellent barrier to large, hydrophilic, polar compounds. Recent work in the area of transdermal peptide and protein drug delivery is overviewed, including strategies such as prodrugs, chemical enhancement, iontophoresis, electroporation, and ultrasound, with focus on mass-transport mechanisms of different systems. Of the strategies studied, iontophoretic delivery appears the most promising. It is clear, however, that more work remains to be done before transdermal peptide and protein delivery devices come to market. Chemical engineers can contribute significantly to further research in this exciting area.

Introduction

Recent developments in genetic engineering and biotechnology have resulted in an explosion of new and potentially potent peptide and protein drugs. Peptides and proteins range in molecular weight from 300 to greater than 1,000,000. They are amphoteric (that is, they may have either a positive or negative charge) hydrophilic polyelectrolytes that attain their ionic nature from the weakly acidic or basic side chains of their constituent amino acids. As a result, they carry a net charge that can vary significantly (Tanford, 1961). These new drugs tend to be highly specific in their actions and thus have few side effects, are effective at very low doses, and can be endogenous (that is, from human origin) and therefore nonallergenic. A number of commercially available peptide/protein drugs and others undergoing clinical trials are listed in Table 1 (Mackay, 1990).

Developing a drug is only one step in the process. A means of administering the drug effectively must also be found. Peptide and protein drugs have several properties that make their systemic delivery difficult. They are of large molecular size, are vulnerable to proteolytic attack, adhere to surfaces, may be immunogenic and nonbiocompatible, exhibit nonlinear pharmacokinetics, and tend to undergo aggregation, adsorption, and denaturation. These drugs are, therefore, currently administered by injection or intravenous infusion (that is, parenterally). Most peptides, however, have very short half-lives in the bloodstream (Langer, 1985), so that frequent injections are required to maintain the drug concentration in the blood within therapeutic levels. Furthermore, injections or intravenous infusions are costly, painful, and inconvenient, and result in poor patient compliance and an oscillating drug concentration in the blood, which may or may not be appropriate for eliciting the desired response.

Peptide drugs would benefit greatly from controlled release administration technology. Controlled release products provide prolonged delivery of a drug while maintaining its blood concentration within therapeutic limits. They have the

Correspondence concerning this article should be addressed to B. G. Amsden.

Table 1. Examples of Peptide and Protein Drugs and Their Pharmaceutical Use

Drug	Molec. Wt.	Pharmaceutical Use	Transdermal Delivery
Human insulin	6,000	Blood glucose regulation	Possible
Tissue plasminogen activator	70,000	Fibrinolytic	Unlikely
Human growth hormone	22,124	Growth stimulant	Difficult
Interleukin-2	15,000	Antiviral	Possible
Human erythropoietin	30,400	Erythropoiesis stimulant	Unlikely
Factor VIII*	unknown	Antihaemophilic	Unknown
Human interferon*	20,000	Immunoregulation	Difficult
Tumour necrosis factor*	unknown	Treatment of cancer	Unknown
Epidermal growth factor*	6,201	Wound healing	Possible
Calcitonin*	4,500	Calcium regulator	Possible

*Undergoing clinical trials.

advantages of utilizing a drug more efficiently, allowing for local delivery and containment at the site of action, thereby eliminating side effects, requiring less frequent administration, and removing the need for repeated injections by a trained professional, providing protection of drugs such as peptides and proteins that have very short half-lives, and possibly being noninvasive, that is, the drug gains access to the target organ without the need for penetrating the skin. As a result, they improve patient compliance and comfort. A further incentive for controlled release technology is its economic value. Pioneering companies that develop new drugs eventually start to lose their market share to generic drug manufacturers after the patents for these drugs expire. A means of combating this competition, and to prolong patent protection, is to provide longer dosage schedules and safer delivery profiles through controlled release delivery systems. It is estimated that in the United States alone, revenues for the few controlled release products on the market reached $2.66 billion in 1993 (De Young, 1993).

A number of controlled release strategies for peptide and protein drugs have been investigated. These strategies include drug-loaded, biocompatible polymeric membranes that are implanted in the body (Amsden and Cheng, 1994; Saltzman, 1993; Lee, 1988); oral dosage forms, nasal devices, rectal implants, occular inserts or drug-loaded contact lenses, pulmonary tract aerosols, and buccal (that is, the oral mucosa) and vaginal implants (Wearley, 1991; Mackay, 1990; Lee, 1988).

Transdermal, or through the skin, delivery is an attractive alternative for a variety of reasons. All other epithelial routes of administration (that is, nasal, buccal, oral, rectal, vaginal, and pulmonary) exhibit enzymatic activity comparable to that in the small intestine (Lee, 1988), whereas the skin, although it does contain aminopeptidases, exhibits less enzymatic activity (Tauber, 1987). This means that the bioavailability of the peptide delivered is increased. Other advantages of transdermal delivery are it avoids degradation via the gastrointestinal tract and the hepatic first-pass effect (namely degradation of the drug in the liver), which may result from oral administration; it allows for a controlled, sustained systemic or local therapeutic drug concentration over a prolonged period of time; delivery can be stopped simply by removing the device from the skin; and it improves patient compliance. Transdermal delivery is particularly attractive for peptide/protein drugs that require their levels within the bloodstream to be maintained within the therapeutic range over prolonged time periods, and that are effective at very low doses (within the range of $\mu g/(cm^2 \cdot h)$). Examples of these types of drugs include vasoactive peptides (namely blood vessel constrictors or dilators) and peptide hormones (Boddé et al., 1989a). The challenge facing this route of delivery, however, is that the skin, like all epithelia, is designed to be an excellent barrier to large, hydrophilic, polar compounds.

Although not a traditional field, chemical engineers have the potential to contribute significantly to the development of transdermal controlled release technologies. As will be demonstrated, drug transport through the skin requires an understanding of mass transport principles, thermodynamics, organic and electrolytic chemistry, and kinetics as it applies to enzyme action in the body. In particular, the ability to mathematically model the preceding processes as a means of prediction and illuminating possible controlling factors is an area in which chemical engineers can make important advances.

In this article a critical review will be presented of the various modes of drug transport across the skin with emphasis on peptide and protein drugs. In particular, the ability to promote effective transdermal delivery and the potential for commercial use of these delivery strategies will be examined. Areas requiring further research and in which chemical engineers can contribute will be outlined. To be able to more fully understand the factors affecting transdermal drug permeation, however, necessitates a brief review of the structure and function of the skin.

The Skin

The skin is a multicomponent, complex organ that serves a number of functions. It acts as a chemical, physical, and microbial barrier, a site of thermoregulation, and a sensory end organ. The average adult body has a skin surface area of approximately 2 m^2, which receives about one-third of the blood circulation. Human skin is histiologically divided into three main strata: the epidermis, the dermis, and the hypodermis or subcutaneous tissue (Figure 1) (Bissett, 1987; Elias, 1989). The epidermis is the outermost layer of skin in contact with the environment and is thus the first barrier to drug permeation through the skin. It varies in thickness from 0.075–0.15 mm, except for the palms and soles where it can be from 0.4 to 0.6 mm thick. It contains no vasculature or nerve endings, receiving its nutrients from the underlying dermis. The cells in this layer experience a rapid sequence of division, maturation, and shedding that is unique to the skin. It replaces itself on the average of once every two weeks. The epidermis is

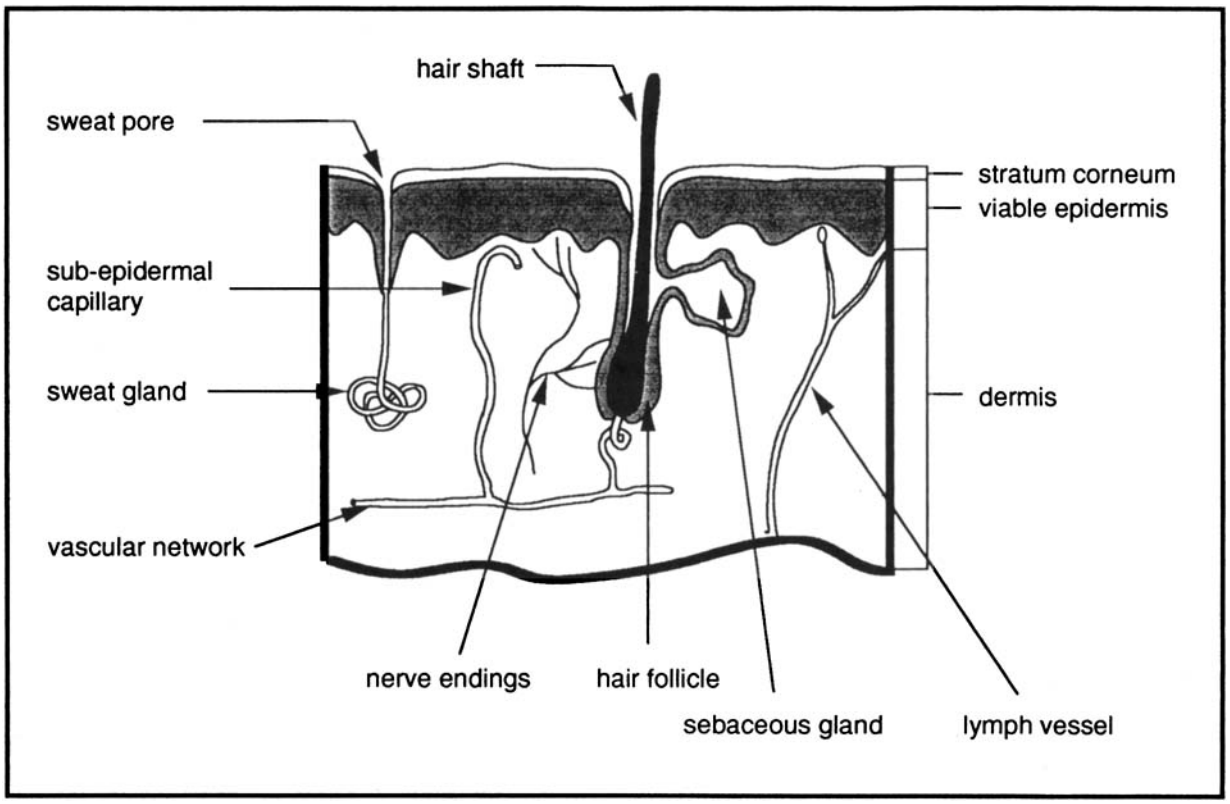


Figure 1. Skin structure.

further divided into the aqueous viable epidermis and the lipoidal stratum corneum (Figure 1).

The viable epidermis is divided into three layers: basal, spinous, and granular (Figure 2). All the cells in these layers, and in the stratum corneum, are attached by protein fibers called desmosomes. Cells are generated in the basal layer and migrate upwards through the spinous and granular layers. During their migration their shape changes from rounded to flattened, lipids are extruded into the extracellular space, the organelles, and nucleus are degraded, and the cytoplasm becomes almost completely filled with keratin (a protein) filaments enclosed with keratohyalin (a protein) forming a dense, amorphous interior. These morphological changes and their end products increase the mass-transfer resistance of a permeating drug. Ultimately, the cells become the keratinocytes of the stratum corneum.

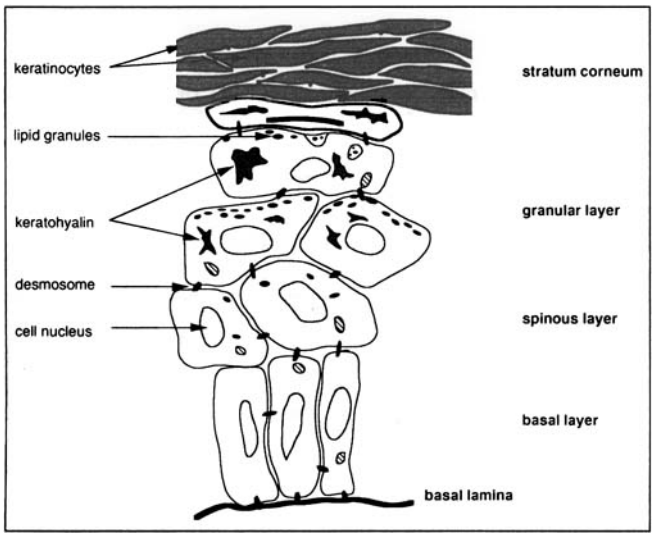


Figure 2. Epidermis.

The stratum corneum is the outermost layer of the epidermis and is between 15 and 20 cell layers thick. It presents the first and primary mass-transfer barrier to topically administered drugs. It is a heterogeneous structure containing about 20% lipids, 40% keratinized protein, and between 15 and 20% water in comparison to the viable epidermis, which is roughly 70% water (Anderson and Cassidy, 1973). Its cells are roughly hexagonal and flat and are usually organized into a closely fitted, interdigitating structure. The cells, called *keratinocytes*, are filled with a semicrystalline, polar protein called *keratin*, which exists as tightly compacted bundles of fibrils. There is some question as to whether lipids are associated with the fiber bundles (Elias and Friend, 1975). The keratinocyte membranes have a dense lipoprotein cell envelope about 12 nm thick. They become progressively more physiologically inactive as they are pushed toward the surface of the skin. The interior of a mature keratinocyte is so structurally dense and crystalline that just a small fraction of it appears to be available for diffusion (Flynn et al., 1987). The extracellular space (approximately 30% by volume of the stratum corneum) is filled with broad, multiple, lipid lamellae. The lipids are composed primarily of cholesterol, fatty acids, and ceramides (Wertz and Downing, 1989). Both the lipid and protein domains of the stratum corneum have a degree of crystallinity

(Van Duzee, 1975). The lamellae are thought to consist of bands of lipid and aqueous phases cemented together by the polar groups of fatty acids and cholesterol (Elias, 1981). At the skin surface the lipid layer is disassembled and the desmosomes degraded or detached to allow for shedding. The compactness and specific organization of the keratinocytes is pivotal to the large resistance to diffusion of drugs through the skin.

Diffusing drugs must also pass at least partly through the dermis in order to reach the bloodstream or the lymphatic system. The dermis is a moderately dense network of fibroelastic connective tissue composed of collagen fibers and elastic fibers. It varies from 1 mm thick on the scalp to 4 mm on the back. These fibers reside in a gel of glycosaminoglycans, salts, and water. Diffusion through this layer is analogous to diffusion through hydrogels. Embedded in the dermis are blood vessels, lymphatic vessels, nerve endings, hairs, sebaceous glands, and sweat glands. It is the blood and lymphatic vessels that are the target of systemic transdermal drug delivery. These vessels and glands create a complex solute transport network. Particulate and liquid matter are collected by the lymphatic system, which arises as terminal bulbs in the upper dermis. The lymphatic system transports inflammatory cells, proteins, and possibly topically delivered molecules that bind to particulates or proteins. The system in which the drug ultimately becomes transported depends on drug size and molecular weight. Drugs of molecular weight less than 5,000, such as peptides and polypeptides, enter the systemic circulation principally by capillary penetration. For drugs of molecular weight greater than 20,000 (proteins), the predominant route of entry is via the lymphatic system (Charman and Stella, 1992).

The skin is punctuated by hair follicles, sebaceous glands, and sweat glands, which are generally collectively referred to as the skin appendages. Hair follicles extend through the epidermis into the dermis. At its base the hair follicle is well vascularized. Sebaceous glands are attached to the sides of the hair follicles and secrete a lipid mixture, sebum, into the space between the hair and the external root sheath. The majority of the sweat glands exist as a coiled tube in the lower dermis and subcutaneous tissue. The tubes empty a mixture of water salts and small organic molecules (sweat) onto the skin surface to assist in thermoregulation. These appendages are also possible routes of entry for topically administered drugs.

Between the dermis and epidermis is an undulating tissue layer. This junction contains a 500-Å-thick structure called the *basal lamina*. It is composed of a network of filamentous glycoproteins with anchoring fibrils extending into the dermis. The functions of the basal lamina are to attach the epidermis to the dermis, to supply mechanical support to the epidermis, and to act as a semipermeable filter to the transport of materials/cells across the interface. It is not a barrier to molecules less than about 40,000 molecular weight, but larger molecules are excluded (Bissett, 1987). This filtering function limits the size of drugs that can be delivered transdermally.

One of the advantages of transdermal drug delivery is reduced drug enzymatic degradation. However the skin is capable of metabolizing a large number of compounds. In fact the skin is capable of undertaking most of the biotransformations occurring in the liver, such as oxidation, reduction, hydrolysation, and conjugation. The total biotransformation capacity of the skin has been estimated at less than one percent of that of the liver (Tauber, 1987), although some enzymes have been found to have greater activities in the skin than in the liver (Noonan and Wester, 1989). During percutaneous transport, therefore, metabolism of the drug may occur. If metabolism does occur the metabolites may be either active or inactive and more or less toxic than the parent drug. Skin metabolization occurs primarily in the viable epidermis. Therefore the activity of proteolytic enzymes in the skin was thought to be insufficient to affect transdermal peptide delivery significantly (Pannatier et al., 1978), as most proteases and peptidases are intracellular. In a recent study of the transdermal permeation of the opioid peptide *des*-enkaphalin-γ-endorphin, however, it was found that some degradation of the peptide occurred (Boddé et al., 1989a). The degradation rate was considered too slow in comparison to the plasma elimination rate to be an obstacle in delivering the compound transdermally. Choi et al. (1990), however, determined that substantial degradation of leucine-enkaphalin, a pentapeptide opioid, occurred during permeation through hairless mouse skin. This degradation could be reduced by decreasing the pH of the donor solution and by simultaneously permeating enzyme inhibitors. Furthermore, Pikal and Shah (1990b) found evidence of bovine serum albumin degradation, probably in the viable epidermis or dermis, during iontophoretic delivery across hairless mouse skin. It is possible, therefore, that peptide degradation will occur in human skin during transdermal delivery. This degradation will alter the delivery profile and pharmacological effects of the administered peptide. It should be noted, though, that the metabolic activity of human skin differs from that of the animal skin in which many of these studies were performed, so that conclusive results need to be found from human skin.

The properties of the skin also vary with region on the human body. Stratum corneum thickness, keratinocyte size, and lipid composition, for example, are known to vary from body site to body site (Bissett, 1987; Lampe et al., 1983; Elias et al., 1981). A recent study found that the percutaneous permeation of a melanotropic peptide, which induces pigment change in the skin, was dependent on the site from which the skin was excised, with mass-transfer rates higher in skin from the face and scalp as compared to skin from the leg and trunk (Dawson et al., 1990). Sebum production and the concentration of hair follicles and glands also exhibit regional differences. All these parameters will affect drug permeation through the stratum corneum. It is therefore important to consider the site of application carefully when investigating transdermal delivery.

Transdermal Permeation

The aim of transdermal drug delivery is usually for the drug to reach the systemic circulation, either vascular or lymphatic. For passive delivery this means that there are a number of solubility and diffusional barriers to be overcome (Figure 3). For most drugs, and particularly peptides, the rate-limiting steps are partitioning into and diffusion through the stratum corneum (Scheuplein, 1965, 1967). Before discussing the routes of permeation further, it would be helpful to out-

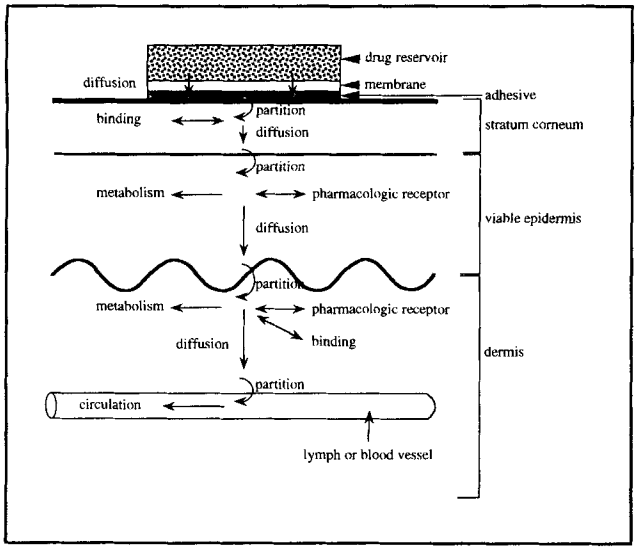


Figure 3. Stages in the percutaneous transport of a peptide drug.

line the thermodynamics and mathematics of solute mass transfer through the stratum corneum.

Thermodynamic considerations

The driving force for mass transfer is the activity gradient between the permeant in contact with the stratum corneum and the permeant in the relatively aqueous viable epidermis. Although heterogeneous in nature, the skin has been modeled as a trilaminate membrane, the laminates consisting of the stratum corneum, the viable epidermis, and the dermis (Scheuplein, 1965, 1967). Each layer is assumed to be homogeneous, and permeation through these layers is assumed to occur by Fickian diffusion. Since the dominant diffusional barrier for water-soluble drugs, which include peptides and proteins, resides in the stratum corneum, the gradient in permeant activity across the skin is localized within this layer. The steady-state flux, J, is then given by

$$J = -D_{sc}\frac{dC_{sc}}{dx}, \tag{1}$$

in which D_{sc} is the permeant diffusivity in, dC_{sc} is the gradient in permeant concentration across, and dx is the distance through, the stratum corneum. It should be noted that D_{sc} is the apparent permeant diffusivity, as it includes the effects of binding and/or interaction with the stratum corneum. If the permeant concentration in the receiving media is kept at zero, then Eq. 1 becomes

$$J \cong \frac{D_{sc}C_{sc,1}}{h}, \tag{2}$$

in which $C_{sc,1}$ is permeant concentration in the stratum corneum in contact with the solution or the adhesive of the device. As it is usually very difficult to determine $C_{sc,1}$, Eq. 2 is often expressed as

$$J = P\left(\frac{C}{h}\right), \tag{3}$$

in which P is the specific permeability of the stratum corneum to the permeant, and C is the permeant concentration in the phase contacting the stratum corneum. In terms of flux comparisons for different delivery protocols, it is useful to define the normalized flux, $\bar{J}$, as

$$\bar{J} = \frac{J}{C} = \frac{P}{h}, \tag{4}$$

where P can be determined from,

$$P = D_{sc}\left(\frac{C_{sc,1}}{C}\right) = k_{sc}D_{sc}, \tag{5}$$

where k_{sc} is the partition coefficient of the permeant between the stratum corneum and the contacting phase. The partition coefficient is defined as the ratio of the solubility of the drug in the stratum corneum to the solubility of the drug in the donor medium. Solubility in the donor medium is easily determined. The difficulty lies in determining the permeant solubility in the stratum corneum. For solution, the change in free energy, ΔG, given by

$$\Delta G = \Delta H - T\Delta S, \tag{6}$$

in which ΔH is the enthalpy change and ΔS the entropy change on mixing must be less than zero. Since the entropy change is always positive, ΔH must be very small in order for solution to occur. Here ΔH is a function of the volume of the mixture, V_m, the volume fractions of the permeant, ϕ_p, and the oil phase of the stratum corneum, ϕ_{sc}, and the solubility parameters of the permeant and the stratum corneum oil phase, δ_p and δ_{sc}, respectively. ΔH is given as follows (Munk, 1989):

$$\Delta H = V_m(\delta_{sc} - \delta_p)^2\phi_{sc}\phi_p. \tag{7}$$

It is therefore necessary for the solubility parameters to be very close in magnitude. Since the stratum corneum lipid phase is a complex medium, an oil phase that has a similar solubility parameter is often used to estimate k_{sc}, namely octanol. There is some correlation between k_{sc} and k(octanol/water) for *in vivo* permeation, but k(octanol/water) cannot be considered predictive, simply indicative of probable high and low permeation rates (Guy and Hadgraft, 1987c).

As the stratum corneum is relatively impermeable to the penetration of water-soluble compounds, a long time is required to reach steady state. Non-steady-state diffusion equations can be derived from Fick's second law,

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2}. \tag{8}$$

The solutions to this equation depend on the boundary conditions imposed on the situation. For the typical case in which

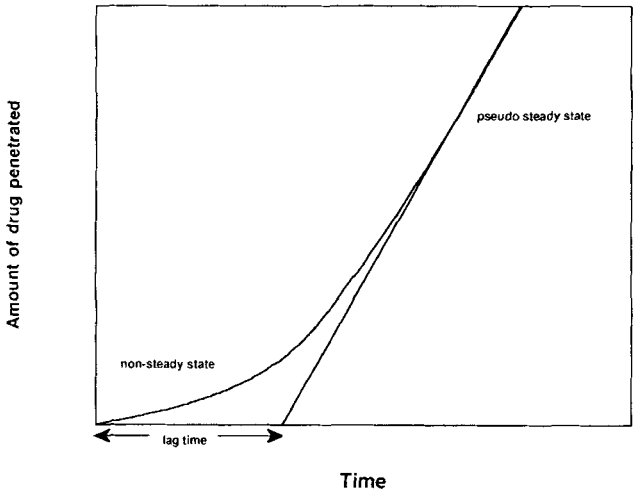


Figure 4. Typical Fickian membrane permeation profile.

the concentration of the permeant in the donor phase (that is, in contact with the stratum corneum) is kept relatively constant and the concentration in the receiving phase is maintained at or close to zero, then the solution to Eq. 8 is (Baker and Lonsdale, 1974)

$$Q_t = \frac{DCt}{h} - \frac{hC}{6} - \left(\frac{2hC}{\pi^2}\right)\sum_{n=1}^{\infty}\frac{(-1)^n}{h^2}\exp\left(\frac{-Dn^2\pi^2 t}{h^2}\right), \tag{9}$$

where Q_t is the cumulative amount of permeant in the receiving phase, h is the thickness of the stratum corneum, and t is time. At long times, Eq. 9 approaches steady state (Figure 4). The lag time is determined by extrapolating the steady state to the time axis. From the lag time, L, the diffusivity can be determined from (Baker and Lonsdale, 1974),

$$D = \frac{h^2}{6L}. \tag{10}$$

This analysis is analogous to solute diffusion through polymeric materials.

Routes of entry

Based on its physiology, there are three possible pathways for nonfacilitated, or passive, transport through the heterogeneous stratum corneum: transcellular, paracellular, and via the skin appendages (that is, the follicles and sweat glands) (Scheuplein, 1965; Scheuplein and Blank, 1971). In the transcellular route the permeant dissolves into and diffuses through the lipid lamellae and the more hydrophilic keratinocytes in series. The paracellular route consists of the drug permeating through the continuous lipid lamellar region surrounding the keratinocytes, while permeation through the appendages involves the drug moving through the restricted sebum environment along the hair shaft or sweat gland (Figure 5).

Transcellular permeation has the advantages of being the most direct route and having the greatest surface area. This route requires that the permeant possess a strong tendency to partition into both the lipid phase and the protein phase of the keratinocytes (Michaels et al., 1975). However, there is no direct experimental evidence to illustrate the existence of this route. The paracellular route for transdermal drug permeation has more experimental support. The interstitial lipid lamellae in the stratum corneum are generally acknowledged to be the primary barrier to the penetration of water and other polar compounds, and the route of penetration for lipophilic compounds (Scheuplein and Blank, 1971; Elias and Friend, 1975; Michaels et al., 1975; Grayson and Elias, 1982; Golden et al., 1987; Flynn, 1989). When the lipids of the stratum corneum of excised human abdominal skin were removed by solvent extraction, the permeability of water and large molecules increased by four to six orders of magnitude (Scheuplein and Blank, 1971). Increasing skin temperature resulted in an increase in stratum corneum permeability, which was suggested to be due to melting of the interstitial lipids (Blank et al., 1984; Al-Saidan et al., 1987). However, these results have also been explained by noting that the intracellular keratin fibrils become unraveled and more hydrated when heated or contacted with solvents, thereby increasing the permeation of small water-soluble compounds transcellularly (Flynn, 1989). This observation illustrates a problem. As it is impossible to affect the lipid phase without affecting the keratinocytes, it is not easy to separate the effects of solvent and temperature on lipid and cellular diffusional resistance. Regional variability in skin permeation of water and salicylic acid was found to be a function of regional lipid content rather than stratum corneum thickness or cell number (Elias et al., 1981; Lampe et al., 1983), which suggests that penetrant partitioning and diffusivity is a function of the lipid composition. The most telling experimental support for the paracellular route of transport comes from the work of Boddé et al. (1989b, 1991). Mercuric chloride was allowed to permeate through dermatomed excised human skin from a phosphate buffered saline solution. After a period of time, the mercury was precipitated as mercuric sulfide using ammonia sulfide vapor. Visualization of the sections using transmission electron microscopy revealed that the mercuric sulfide was found predominantly in the extracellular space. Considering all the available evidence, the inference is strong

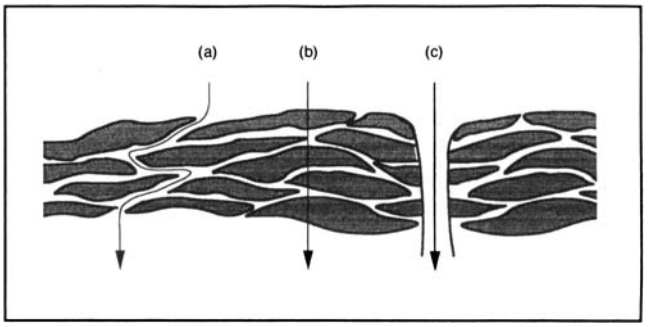


Figure 5. Routes of drug permeation through the skin.

(a) Paracellular diffusion through the lipid lamellae. (b) Partitioning into and diffusion through both keratinocytes and the lipid lamellae. (c) Appendageal diffusion, that is, via follicles and sweat ducts.

that, for a wide range of molecules, permeation occurs via the paracellular route.

Several researchers have argued that there may be both a polar pathway and a nonpolar pathway for drug transport through the interstitial lipid lamellae of the stratum corneum (Elias, 1981; Berner and Cooper, 1987; Flynn, 1989; Sims and Higuchi, 1990). The polar pathway has been described in terms of aqueous pores in the limited aqueous phase between the lipid lamellae (Elias, 1981). The evidence suggesting such a polar pathway is that polar molecules, such as water, methanol, and small ions, permeate the skin and that the flux of these molecules is independent of their oil/water partition coefficient (Cooper, 1984). As the polarity of the penetrating molecule is decreased, the flux becomes a function of the partition coefficient (Scheuplein and Blank, 1971).

Free-volume theory has been proposed to explain this behavior. In this theory a diffusing molecule cannot move until a free volume, or hole, is formed adjacent to it in the region through which it is diffusing (Cohen and Turnbull, 1959). Solute diffusivities are estimated from free-volume theory as

$$D = D^0 \exp\left(-\frac{M}{aV_{av}}\right), \tag{11}$$

in which D^0 represents the diffusivity of a molecule having zero molecular volume, a is a constant, M is the solute molecular weight, and V_{av} is the average free volume of the continuous phase. Applying this theory to permeation through the stratum corneum, it is assumed that molecules diffuse through the free-volume fluctuations of the polar domain. An alternate suggested explanation is that the small polar molecules permeate via free-volume fluctuations in the lipid domain, similar to living cell membranes (Kasting et al., 1987). Potts and Guy (1992) have used this idea to develop a mathematical model of the permeation of solutes through skin at steady state. Given that flux is dependent on the solubility coefficient (Eq. 5), substituting Eq. 11 for the diffusivity and expressing the relationship between k_{sc} and k_{oct} as,

$$k_{sc} = (k_{oct})^f, \tag{12}$$

they came up with the following expression relating measured permeability, P, to k_{oct}:

$$\log(P) = \log\left(\frac{D^0}{h}\right) + f \cdot \log(k_{oct}) - \frac{M}{aV_{av}} \tag{13}$$

The authors fit this model to literature data to determine its applicability and determined the adequacy of their fit by examining the correlation coefficient. Although it is a useful measure of the extent to which a fitted model agrees with the measured values of a dependent variable, the correlation coefficient should not be taken as an indication of the adequacy of the fitted model because it ignores the magnitude of the random error associated with the measured values of the dependent variable. A better indication of the adequacy of fit of their model would have been given in an F test. Also, as the number of solutes of varying molecular weight to which they fit their model increased, the value of the correlation coefficient decreased, from 0.90 for 23 solutes to 0.67 for 93 solutes. The model suggests the possibility that the transport of small, polar nonelectrolytes through the stratum corneum is determined by the properties of the extracellular lipids, although it is definitely not conclusive. It has also been suggested that the ionic permeability of the stratum corneum is due to inherent, minor, interfacial defects in the lipid lamellae (Potts et al., 1992). To resolve this issue it is apparent that a more thorough knowledge of the morphology of the lipid lamellae is required.

The final possible route for drug transport is through the skin appendages. An average human skin surface contains 40 to 70 hair follicles and 200 to 250 sweat ducts per square centimeter, occupying 0.1% of the total surface area (Scheuplein, 1967). Diffusion through the appendages has been visualized with dyes (Scheuplein, 1978) and electric currents (Grimnes, 1984). In passive diffusion through the skin, these appendages contribute significantly to the initial permeation (Scheuplein and Blank, 1971). For molecules that permeate the skin readily, however, appendageal transport was initially thought to become quickly insignificant because of the small diffusional cross-sectional area fraction of the appendages and the nonlinear nature of transient diffusion (Scheuplein and Blank, 1971; Keister and Kasting, 1986). Recent work illustrated that steady-state fluxes of tritiated hydrocortisone, niflumic acid, caffeine, and p-aminobenzoic acid through a model skin, regrown dorsally on the hairless rat so that it contained no appendages, were 2–4 times lower than in normal hairless rat skin (Illel and Schaeffer, 1988; Illel et al., 1991). These results indicate that appendageal skin penetration is greater in importance than is generally supposed and warrants further attention.

It is apparent from the preceding discussion that the transport mechanisms of drugs across the skin barrier are complex and still not well-defined. Whether the transcellular pathway is significant, whether a continuous polar pathway exists throughout the lipid lamellae, or whether the observed permeation of small polar molecules is due to free-volume transport through the lipid phase, the detailed morphology of the lamellar region, and the degree to which appendageal transport is important are all unresolved issues demanding further study. Nevertheless, it is clear that the stratum corneum represents the main resistance to diffusion. At present, it is considered that, for a large number of molecules, transport through the stratum corneum occurs predominantly in the interstitial lipid phase.

Permeant physicochemical characteristics

The relative importance of the possible routes of permeation through the skin depend on the chemical characteristics of the permeant, such as its water solubility, oil–water partition coefficient, molecular size, ionization state, and possible interactions between the drug and the skin as well as the hydration state of the skin. In general, it is necessary to maximize the activity of the permeant in the external medium to maximize flux; therefore, permeants that have high aqueous solubilities, such as peptides, may have relatively significant transdermal fluxes. For example, Okumara et al. (1989) demonstrated that dopamine hydrochloride, a highly water-soluble drug, had a higher permeation rate than in-

domethacin (458 vs. 1.7 $\mu g/(cm^2 \cdot h)$, respectively) even though its skin permeation coefficient was about 100 times lower.

Polarity of the molecule is also important. Nonionized drugs generally permeate more readily than ionized drugs (Michaels et al., 1975; Parry et al., 1990) because the free-energy change required for an ionized drug to partition into the oil phase is greater than that for a nonionized drug. However, if the water solubility of the ionized form is greater than that of the neutral form, then the ionized drug may display a greater transport rate (Michaels et al., 1975). Peptides and proteins can be either positively or negatively charged, depending on the pH in relation to their isoelectric points. At a pH below the isoelectric point, the amine groups of the peptide are positively charged so that the molecule tends to have a net positive charge, whereas at a pH above the isoelectric point the carboxylic groups are negatively charged so that the molecule has a net negative charge.

If the free-volume mechanism is accepted, then permeation is dependent on the size and shape of the molecule (Crank and Park, 1989). Smaller molecules have been found to permeate faster through the stratum corneum than larger molecules (Scheuplein and Blank, 1971; Okumara et al., 1989). For molecules with a molecular weight less than 750 daltons the effect of size on the diffusivity is small and can be described by a weakly exponential function (Guy and Hadgraft, 1987a). For larger molecules, such as peptides, the size dependency is unknown but larger molecules will permeate much more slowly.

It is also possible that the drug may interact with the skin. Many drugs display a strong chemical bond or van der Waals attraction to components in the skin. As a result the drug becomes bound, perhaps strongly enough to form a drug depot, thereby reducing its permeation rate (Chandrasekaran et al., 1980; Barry, 1986). In fact, peptides and proteins have been found to become reversibly bound while permeating through excised skin (Wearley et al., 1990; Banga and Chien, 1993; Hoogstraate et al., 1994; Delgado-Charro and Guy, 1994; Thysman et al., 1994). There is also the possibility that the drug will irritate the skin (Dalvi and Zatz, 1982).

Since the majority of permeation studies involve the use of aqueous solutions, it is important to consider the effect of hydration on the stratum corneum and permeation through it. The stratum corneum will absorb up to 5–6 times its dry weight of water. Swelling is not immediate, taking about 3 days to reach equilibrium, but maceration may be detected within a few minutes. When fully hydrated, its permeability to water and other low molecular weight penetrants is substantially increased (Scheuplein and Blank, 1971). This effect is discussed below.

Mathematical models

Mathematical models of transport through the stratum corneum have been developed in an attempt to elucidate which transstratum corneum route dominates in passive diffusion. By modeling the stratum corneum as "bricks and mortar," with the bricks being the proteinaceous keratinocytes and the mortar the intercellular lipid lamellae, Michaels et al. (1975) developed a mathematical model of transport through the stratum corneum that included permeation both paracellularly and transcellularly. Their model, although simplistic, is important in that it was the first attempt at modeling solute diffusion through the stratum corneum. Their proposed structure of the stratum corneum is supported by experimental evidence (Elias and Brown, 1978; Grayson and Elias, 1982). Tojo (1987) took this model a step further and randomized the order of the keratinocytes within the lipid phase. The steady-state flux of permeant from this model is given by

$$J = \frac{D_p C_p}{h}\left[\frac{2\epsilon(1-\epsilon)(2n+4)}{n+\dfrac{n+4}{\gamma}} + \epsilon^2\gamma + \frac{(1-\epsilon)^2(2n+4)}{2n+\dfrac{4}{\gamma}}\right], \tag{14}$$

in which D_p is the diffusivity of the permeant in the protein phase (keratinocyte); C_p is the surface concentration of the permeant in the protein phase, $\gamma = kD_L/D_p$; k is the permeant lipid–protein partition coefficient; D_L is the permeant diffusivity in the lipid phase; ϵ is the average fraction of diffusion area of the lipid phase on the skin surface; $n = (2\phi_v - 2)/(\epsilon - \phi_v)$; and ϕ_v is the total volume fraction of lipid phase in the stratum corneum. The route taken depends on the partition coefficient and diffusivity of the permeant in both phases. Tojo determined that for $\gamma < 1$ transport through the paracellular route is insignificant, but for $\gamma > 10^4$ transport occurs predominantly paracellularly. Between these values, transport through the stratum corneum occurs by a combination of transcellular and paracellular routes.

Since steady state is not always achieved *in vivo*, Albery and Hadgraft (1979) developed a non-steady-state diffusion model based on an idealized geometry of the brick and mortar model of Michaels et al., in which the cells are not stacked but rather are diagonally continuous throughout the stratum corneum. An exact solution was not possible, but they determined that the route through the stratum corneum depended on the ratio of the number of moles of drug in the cells compared to the number in the channels, β,

$$\beta = \frac{h}{kN\delta} \tag{15}$$

and the diffusion characteristics of the two routes, α,

$$\alpha = \frac{D_L h^2}{D_P h_L^2}, \tag{16}$$

in which δ is the width of the lipid channel separating the cells, N is the number of layers of keratinocytes, and h_L is the total diffusion path of the lipid phase of the stratum corneum. If $\beta > \alpha$, then the diffusion path is transcellular, and if $\beta < \alpha$, then the diffusion path is paracellular. It is easily shown that $\gamma = K(\alpha/\beta)$, where K is a constant determined by the geometric properties of the stratum corneum; thus, the two models have similar results even though they were developed along different lines.

These models are simplistic in their assumed geometry and ignore possible flux through the appendages, which, as indi-

Table 2. Transdermal Passive Steady-State and Normalized Transdermal Fluxes of Peptides from the Literature

Peptide	Molec. Wt.	Skin Source	Flux ($ng/cm^2 \cdot h$)	Normalized Flux ($\times 10^5$ cm/h)	Reference
Vasopressin	1,084	Hairless rat	3.56 ± 0.90	7.0 ± 4.2	Banerjee and Ritschel (1989)
des-Enkaphalin-γ-endorphin	1,300	Human stratum corneum	1.3	1.2 ± 0.4	Boddé et al. (1989a)
(Nle^4, *D*-Phe^7)-α-melanocyte stimulating hormone	1,833	Human	n/a	n/a	Dawson et al. (1990)
Thr-Lys-Pro	344	Hairless rat	7.6	2.0	Green et al. (1992a)
Amino acids	75–204	Hairless mouse	200–500	2–5	Ruland and Kreuter (1991)

cated earlier, may be an important route of mass transport. The simplicity of these models, though, is attractive since the authors indicate possible important parameters involved in transdermal permeant transport, which are the partition coefficient and diffusivity in each phase. However, these values are difficult to obtain with accuracy, reducing the utility of the models as predictive tools.

In the preceding models, the continuous pathway through the stratum corneum was assumed to be paracellular and lipoidal. Another model of the stratum corneum was proposed by Berner and Cooper (1987), in which there are two parallel and continuous pathways through the stratum corneum: polar and lipoidal. The actual geometries of these pathways are unknown. The steady-state flux of a solute through the stratum corneum is a sum of its flux through both the pathways, or,

$$J = C_w(A_p D_p + A_L D_L k_L), \tag{17}$$

in which C_w is the compound's solubility in water, A_p and A_L are the area fractions of the polar and lipoidal routes, respectively; D_p and D_L are the diffusivities in the polar and lipoidal routes, respectively, estimated from free volume theory (Eq. 11); and k_L is the partition coefficient of the solute into the lipid phase. The value of A_p was estimated as 0.10.

By applying the model to the data of Michaels et al. (1975), it was concluded that solute flux could be predicted to within a factor of 4. Although elementary, this model illustrates the difficulty of delivering peptides and proteins through the skin. Due to their high molecular weight, their diffusivity as predicted by Eq. 11 would be quite low, and because they would not partition readily into the lipid region, their route of entry would be almost exclusively through the polar pathway that has the lowest area fraction.

It is obvious that modeling diffusional transport through the stratum corneum is an area requiring additional work. The approaches described assume that the lipid domain is homogeneous, whereas it is in fact a multilamellar lipid bilayer. Improvements in this area will likely result from a better understanding of the morphology of the stratum corneum, in particular the microgeometry of the paracellular lipid lamellae, and the impact of the underlying viable epidermis and dermis on the movement of peptides and proteins through the skin. More sophisticated models are needed that account for possible drug entry via the appendages and for the possibility and geometry of a polar pathway through the paracellular lipid lamellae.

From the previous discussion, the physiology of the skin and the parameters influencing permeation through it have been outlined. With this background, we can now turn our attention to the various approaches that have been taken to deliver peptide and protein drugs transdermally.

Approaches to Percutaneous Peptide/Protein Transport

Passive diffusion

Although diffusion of peptides through the skin is difficult, passive diffusion of some peptides through this barrier has been measured (Table 2). The normalized fluxes of those compounds measured all lie within the same approximate range of 2 to 7 (10^{-5}) cm/h as compared to 5.0 (10^{-4}) for water (Del Terzo et al., 1986), illustrating the lower permeability of peptides through the stratum corneum. Ruland and Kreuter (1991) found that there was no dependence on molecular weight, net charge, or lipophilicity of amino acids diffusing through excised hairless mouse skin. Based on these results, they concluded that permeation occurred through a hydrophilic pathway, although the nature of the pathway was not elucidated. The passive transdermal diffusion of (Nle^4, *D*-Phe^7)-α-melanocyte stimulating hormone, which causes pigment alteration in the skin, however, has been attributed to the amphiphilicity of the compound (that is, the ability of the compound to be both hydrophilic and lipophilic) (Dawson et al., 1990), suggesting a paracellular pathway. Again, the nature of the hydrophilic pathway through the stratum corneum for peptides has not been determined. One possibility is that transport occurs predominantly through the skin appendages or stratum corneum imperfections that lead directly to the viable epidermis.

Therapeutic doses of peptides are generally within the range of μg/day. For example, the recommended dose of vasopressin, one of the few drugs in Table 2 to exhibit a significant passive flux, is 2 to 4 μg/d (*Martindale The Extra Pharmacopoeia*, 1993). Using the result of Banerjee and Ritschel (1989) for the passive permeation of this drug across excised rat skin at a pH of 5 and assuming a large application area of 50 cm^2 and that similar fluxes would occur in human skin, translates into a required delivery time of about 12 h for the minimum required dosage. Add to this the lag time of about 8 h and neglecting any peptide degradation during transit results in an *in vitro* delivery period of approximately 20 h. During this time the blood drug concentration of the vasopressin would probably be below therapeutic levels, making this mode of drug delivery unreasonable.

To achieve therapeutic peptide drug levels it is necessary to facilitate permeation through the skin. Methods that have been studied, such as modifying the peptide by covalently

Table 3. Strategies Used to Facilitate Transdermal Peptide Delivery

Mode	Advantages	Disadvantages
Passive diffusion	• No alteration of skin physical properties	• Ineffective permeation rates
Prodrugs	• Drug protection during permeation	• Molecular size restriction
		• Requires synthesis
	• Increased permeability	• Limited range of applicability
		• Interindividual variance
		• Further clinical testing required
Chemical enhancers	• Increased permeability	• Induce skin irritation
		• Potentially toxic
		• Long lag time
Iontophoresis	• Increased permeability	• Potentially irreversible alteration of skin barrier property
	• Pulsatile delivery	
	• Shortened permeation lag time	• Restricted to short delivery duration
		• Inconsistent rates ($M = 8{,}000$–$12{,}000$)
		• Interindividual variance
		• Small fraction of drug delivered
Electroporation	• Noninvasive	• Restricted to short-term delivery
	• High degree of enhancement	• Requires supervision
		• Interindividual variance
		• Field effects on skin?
Ultrasound	• Painless, noninvasive	• Restricted to short-term delivery
		• Possibly alters skin morphology?
		• Range of applicability?

bonding it with a lipophilic component (creating a prodrug), using chemical permeation enhancers, employing electrostatic repulsion as a driving force (iontophoresis), combining iontophoresis and chemical enhancement, using high-energy sound waves (ultrasound), and utilizing an electric field to increase the permeability of the lipid lamellae (electroporation) (Table 3), will be discussed below.

Prodrugs

One strategy to improve the skin permeability of water-soluble drugs involves modifying the drug by covalently bonding lipophilic groups onto primary and secondary amines, amides, imides, hydroxyls, and other functional groups of the drug to form a prodrug (Bundgaard, 1992). The lipophilic groups impart improved skin partitioning into the extracellular lipid lamellae and are detached *in vivo* by enzymes, rendering the drug free and active. It is important that the prodrug retain some water solubility in order for penetration enhancement to be optimized. Examples of lipophilic groups studied include α-acyloxyalkyl derivatives, aminomethyl-type derivatives, acyl derivatives, and α-(bisalkylhetero)alkyl derivatives. These compounds have been used to enhance the skin permeation of drugs such as hydrocortisone, theophylline, testosterone, and 6-mercaptopurine (Sloan, 1989).

The prodrug approach has also been used to facilitate the transdermal delivery of the peptide thyrotropin-releasing hormone (TRH) (Møss and Bundgaard, 1990). TRH, which has demonstrated potential for treating neurologic and neuropsychiatric disorders, was combined with *N*-isobutyloxycarbonyl and *N*-octyloxycarbonyl. These derivatives showed measurable permeation capacity through excised human skin (0.045 μmol/(cm^2·h) from an aqueous buffer), whereas the parent drug did not permeate the skin to a measurable extent. Another advantage of using prodrugs is that the added lipophilic group can protect the peptide from degradation in the bloodstream prior to reaching the site of therapeutic action (Møss and Bundgaard, 1990). Nevertheless, this approach is complicated, as knowledge of enzymatic activity and kinetics is needed, requires synthesis, and may not be applicable to a wide range of peptide drugs, as permeation is still restricted by the molecular mass of the peptide-lipophilic group complex. Since the activation of the drug requires enzymatic cleavage of the bond between the lipophilic group and the peptide, bioavailability will vary from individual to individual. Another consideration is that prodrugs are regarded as new drugs by regulatory agencies in most countries, so that animal testing for toxicity, skin irritation in both animals and humans, pharmacokinetic studies, and clinical trials will be required (Higuchi and Yu, 1987). These tests are expensive and may prohibit the use of this technology. Instead of altering the drug's lipophilic properties by chemical means to facilitate transdermal transport, another approach is to weaken the skin barrier using chemical permeation enhancers.

Chemical permeation enhancers

Excellent reviews of permeation enhancers and their modes of action have been provided by Walters (1987) and Goodman and Barry (1989). A chemical permeation enhancer is any compound that alters the skin as a permeability barrier to the diffusive flux of a desired component. Known permeation enhancers include solvents (water, alcohols, pyrrolidones, dimethyl sulfoxide (DMSO), and 1-dodecylazacycloheptan-2-one also called Azone), urea, fatty acids such as oleic acid, sugar esters, and surfactants. This section is intended as a brief overview of the various enhancers used and their mechanism of action.

Barry (1986) outlined the desirable criteria of a chemical permeation enhancer. It should be pharmacologically inert, nontoxic, nonirritating, and nonallergenic; upon application its enhancing effect should be immediate; it should have no irreversible effects on the skin; it should be chemically and physically compatible with a range of drugs and excipients; it should be cosmetically acceptable; and it should not increase

the permeation of excipients and environmental chemicals, nor should it lead to the loss through the skin of endogenous material. In practice, however, an enhancer having all these characteristics has yet to be found. Although many chemical enhancers have been investigated, the physical basis for their methods of enhancement is still not understood. As yet, no overall theory of chemical enhancement has been presented.

Chemical enhancers are assumed to increase permeation by disrupting the structure of the stratum corneum, or by increasing penetrant solubility in the stratum corneum, or by a combination of these effects. The stratum corneum structure can be disrupted either chemically or physically. The disruption may affect both intracellular and extracellular structure (Rhein et al., 1986) and may arise as a result of protein denaturation (Scheuplein and Ross, 1970; Wood and Bettley, 1971; Weiner et al., 1972; Kurihara-Bergstrom et al., 1986), fluidization and disorganization or extraction of the extracellular lipid lamellae (Wood and Bettley, 1971; Emberry and Dugard, 1971; Rhein et al., 1986; Golden et al., 1987; Hoogstraate et al., 1991), or delamination of the extracellular lipids via osmotic pressure (Chandrasekaran et al., 1977).

Permeation is enhanced by increasing the solubility and mobility of the permeant in either the intracellular protein or the extracellular lipid phase, or both. Examples of enhancers that increase permeant solubility are organic solvents such as 2-pyrrolidone, *N*-methyl-2-pyrrolidone, ethanol, and methanol. The extracellular mobility of the drug is enhanced by increasing the fluidity of the lipids in the extracellular lipid lamellae. This is accomplished by either disrupting the packing array of the lipids, or by solubilizing the lipids. Intracellular mobility is increased by expanding the protein structure of the intracellular keratin protein and the proteins within the keratinocyte protein shell (Goodman and Barry, 1989).

Solvents such as water, pyrrolidones, and propylene glycol increase permeability by hydrating the stratum corneum. Hydration results in stratum corneum swelling and increased permeation of both polar and nonpolar compounds (Scheuplein, 1967; Scheuplein and Blank, 1971; Valia and Chien, 1986). Hydration of the stratum corneum results from protein expansion and disruption of the lipid packing of the lipid lamellae by interaction with the polar head groups of the lipids. Disrupting the lipid array increases lipid fluidity by reducing lipid crystallization (Goodman and Barry, 1987). Water is preferentially absorbed by the cellular keratin, causing it to expand. This expansion should increase transcellular penetration. It has been proposed from evidence supplied by differential thermal analysis that water is absorbed into the lipid lamellae, fluidizing the lipids and thereby increasing permeation through this region (Goodman and Barry, 1989). However, small-angle X-ray diffraction data on hydrated stratum corneum collected by Bouwstra et al. (1991) indicates that water and propylene glycol are not inserted into the lipid lamellae to a great extent.

DMSO is the most widely known and the most effective permeation enhancer. It is dipolar, aprotic, and miscible in both water and organic solvents. It enhances permeation by both expanding proteins and solubilizing lipids (Goodman and Barry, 1989). Next to DMSO in effectiveness is Azone. This substance enhances the permeation of both hydrophilic and hydrophobic drugs (Stoughton, 1982; Hoogstraate et al., 1991). As yet, the action of Azone on the stratum corneum has not been resolved. It does not appear to cause any morphological changes at low concentrations (Stoughton, 1982). Its enhancing effect has been suggested to result from interference with the packing arrangement of the extracellular lamellae by insertion into the lipid lamellae (Goodman and Barry, 1985; Hoogstraate et al., 1991). Azone works best when used with a polar cosolvent such as ethanol (Stoughton and McClure, 1983) or polyethylene glycol (Wotton et al., 1985; Hoogstraate et al., 1991). The polar cosolvent appears to be necessary to swell proteins to allow for intracellular permeation (Goodman and Barry, 1989).

Other than DMSO and Azone, the best known enhancers are surfactants. The ability of a surfactant to enhance permeation is associated with its polar head group and hydrocarbon chain length (Scheuplein and Dugard, 1973; Howes, 1975). Hydrophilic polar head groups are very effective at increasing the permeability of polar molecules (Cooper and Berner, 1987) and surfactants alter the permeation of polar molecules to a greater degree than nonpolar molecules. The optimum chain length of an alkylsulfate is 12 carbons (Scheuplein and Dugard, 1973), which has been interpreted as a balance between sufficient lipophilicity to partition into the lipid lamellae and water solubility (Cooper and Berner, 1987; Walters, 1987). Anionic surfactants induce stratum corneum and viable epidermis swelling, possibly by separating the protein matrix and uncoiling the keratin filaments, thereby exposing more hydrophilic sites on the proteins (Rhein et al., 1986). As noted earlier, increased hydration of the stratum corneum results in increased permeabilities, particularly of polar molecules. In contrast, cationic and nonionic surfactants do not induce swelling. These compounds act by partitioning into the lipid lamellae, disrupting their organization to increase lipid fluidity, and by penetrating into the intracellular matrix and interacting and binding with the keratin filaments.

There are several drawbacks to the use of chemical permeation enhancers. They induce irritation (the more effective the enhancer the greater the irritation (Lashmar et al., 1989)) and may have pharmacological, and possibly toxic, actions of their own. For example, DMSO produces a burning sensation as well as localized irritation (Kligman, 1965), and at high concentrations can produce erythema (superficial inflammation of the skin in patches) and weals (Hadgraft, 1984). Since significant enhancement of permeation rates using DMSO are only obtained at concentrations greater than 65% (Chandrasekaran et al., 1977; Kurihara-Bergstrom, 1986) this limitation effectively rules out its use as a transdermal enhancing agent. Anionic surfactants have also been found to cause skin irritation (Wood and Bettley, 1971; Novak and Francom, 1984; Sherertz et al., 1987). Cationic surfactants are reputedly more irritating than anionic surfactants and so have not been widely investigated as skin enhancers (Walters, 1987). Azone shows the most promise as a permeation enhancer because it is almost as effective as DMSO in enhancing permeation and yet it is pharmacologically inert and relatively nonirritating (Vaidyanathan et al., 1987). However, Azone requires hours to elicit enhancement, resists washing out from the stratum corneum (Barry, 1986; Wester et al., 1994), and has been shown to cause skin irritation in nude mice in a 10% solution (Lashmar et al., 1989) and in rabbit pinna skin (Hirvonen et al., 1993a).

Despite these drawbacks, permeation enhancers have been

investigated as a means of increasing the permeation of peptides and polypeptides through the skin. DMSO was found to increase the permeation of arginine vasopressin (an antidiuretic hormone used in treating diabetes) and corticotropin, a hormone, through rat skin (Kastin et al., 1966), although the increase was only slight. Azone of varying hydrocarbon chain length was investigated as a means of enhancing the permeation of desglycinamide arginine vasopressin (DGAVP) (Hoogstraate et al., 1991). Decyl-Azone increased the permeation rate 1.9 times ($\bar{J} = 1.86\ (10^{-11})$ cm/s), dodecyl-Azone 3.5 times ($\bar{J} = 3.53\ (10^{-11})$ cm/s), and tetradecyl-Azone 2.5 times ($\bar{J} = 13\ (10^{-11})$ cm/s) over the passive diffusion rate ($\bar{J} = 5.3\ (10^{-11})$ cm/s). These increases in flux are probably insufficient to have pharmocological effect. A nonionic surfactant, *n*-decyl methyl sulfoxide, was found to enhance the permeability and reduce the lag times of amino acids, dipeptides, and leucine enkaphalin, a pentapeptide endorphin, through hairless mouse skin (Choi et al., 1990) (Figure 6).

However, chemical enhancement does not work in every case. For example, DMSO was unsuccessful in improving the permeation of native collagen ($M = 288{,}000$) and collagen derivatives ($M = 96{,}000$) through intact hairless mouse skin stratum corneum (Coapman et al., 1988). In addition, an anionic surfactant (sodium lauryl sulfate (SDS)) failed to significantly increase the flux of vasopressin through rat skin (Banerjee and Ritschel, 1989). In the former case, the large molecular weight and therefore size of the molecule restricts its permeation through the stratum corneum, whereas for the latter, although its molecular weight is not great (1084), the SDS acts by opening a pathway through the cellular protein and it is likely that this pathway is sufficiently small and limited to restrict the passage of the vasopressin molecule. These results lead to the conclusion that the ability of chemical permeation enhancers to facilitate the delivery of peptides or proteins is limited. A more promising method is electrically assisted transdermal drug delivery, also called iontophoresis.

Iontophoresis

A considerable amount of research has focused on the use of iontophoresis in facilitating the delivery of peptide and protein drugs across the skin. A typical iontophoretic device consists of two electrodes, with each electrode immersed in an electrolyte solution, placed on the skin (Figure 7). Whereas the stratum corneum is fairly nonconductive, the viable epidermis below is conductive. Upon application of an electric current, a potential difference is created across the stratum corneum. It is this electric field that is the driving force for iontophoretic drug delivery. Positively charged ions at the anode and negatively charged ions at the cathode are driven across the skin due to electrostatic repulsion. The donor solution is generally buffered to ensure that most of the solute molecules are present in ionized form and to prevent significant pH changes from occurring during delivery. Iontophoresis removes the hazard of toxicity involved with chemical enhancers and results in increased transdermal fluxes of water-soluble compounds, which makes it a promising strategy for peptide and protein delivery. Another advantage is its ability to deliver drugs in a pulsatile manner, which is critical to obtain the desired pharmacological response of some peptide hormone drugs.

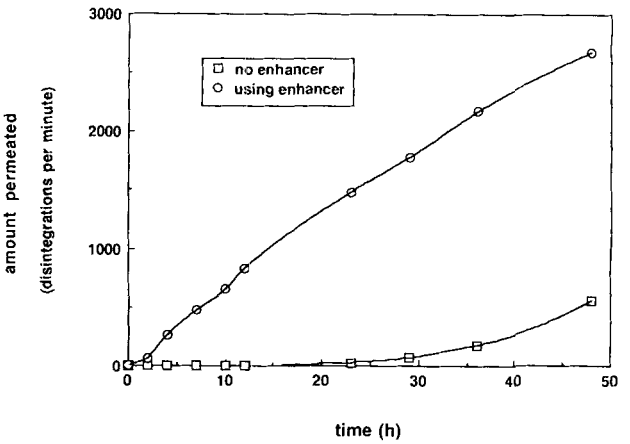


Figure 6. Effect of the penetration enhancer *n*-decyl methyl sulfoxide on the permeability of iodine labeled leucine-enkaphalin. (From Choi et al., 1990.)

The route of ion transport through the skin follows the path of least resistance and appears to be mainly through aqueous pores such as the hair follicles and sweat glands, and possibly skin imperfections (Abramson, 1941; Grimnes, 1984; Burnette and Marrero, 1986; Siddiqui et al., 1987; Burnette and Ongpipattanakul, 1988; Pikal, 1990; Pikal and Shah, 1990a,b; Cullander and Guy, 1991; Cullander, 1992; Turner et al., 1994). For example, Scott et al. (1993), based on studies of the iontophoretic flux of $Fe(CN)_6^{4-}$ across hairless mouse skin, suggest that pores are created in the skin by the activation of endogenous skin structures rather than from current-induced creation of artificial holes. They implicate the hair follicles as the most likely precursors to these pores and suggest that the pores are formed by swelling of the hair follicles in response to the applied current.

However, other pores may also be created by the applied current. For example, Pikal (1990) noted that the calculated number of pores required to explain iontophoretic transport through hairless mouse skin as occurring primarily through aqueous pathways was greater than the number of hair follicles and sweat ducts, and that the calculated radius of the pores was smaller than the radius of a hair follicle. Pikal and Shah (1990a) also found that excised hairless mouse skin was capable of undergoing significant changes when subjected to an electric field. It has been proposed that the applied elec-

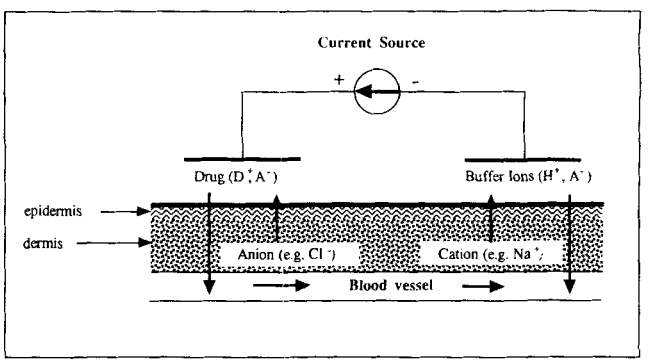


Figure 7. Iontophoretic device.

trical current alters the molecular arrangement of the keratin polypeptide helices such that they form a parallel arrangement. Pores are then formed by the repulsion between neighboring dipoles, with water and ions flowing into the pore channels to neutralize the dipole moments (Chien et al., 1989). However, this mechanism implies a transcellular route of entry for which there is as yet no evidence. Alternatively, the current may disrupt the extracellular lipid lamellae, thereby creating artificial shunts (Siddiqui et al., 1987). There is evidence that the paracellular pathway is the dominant pathway for small solutes. Using mercuric chloride iontophoretically driven across hairless mouse skin (Sharata and Burnette, 1989), porcine skin (Monteiro-Riviere et al., 1994), and human skin (Boddé et al., 1989b, 1991), it was found that, although some of the solute permeated via the appendages, most of the mercury was present in the extracellular space. Similarly, it has been demonstrated using confocal microscopy and hairless mouse skin that a paracellular iontophoretic pathway exists for both charged (calcein) and uncharged (NBD-diethanolamine) solutes (Cullander, 1992). To explain these results, Jadoul et al. (1994) have suggested, by means of X-ray diffraction and attenuated total reflectance–Fourier transform infrared (ATR-FTIR) spectroscopy studies of hairless rat skin submitted to different current densities, that iontophoresis decreases or even partly destroys lipid stacking in the lamellae, particularly at high current density. This effect was found to be reversible. They also determined that lipid fluidity was not affected, so that flux enhancement must be through temporary holes formed in the lipid lamellae. This finding is in direct contradiction to the postulated pathway of Scott et al. (1993). Further research is required to resolve this conflict to determine which pathway (paracellular or transfollicular) dominates in the iontophoretic flux of peptides and proteins across the skin.

A variety of mechanisms is involved in transporting the drug through the skin. Transdermal iontophoretic transport is a result of either electrostatic repulsion, electroosmosis, or an increase in the intrinsic permeability of the skin due to the applied electric field. Electrostatic repulsion is related to the mobility of the solute, its concentration and the magnitude of the applied potential, and is described by the Nernst–Planck equation. Electroosmosis and the increase in skin permeability that results from the application of an electric current will be discussed below.

Electroosmosis. When an electric potential gradient is applied across a porous membrane in which there are fixed charges, bulk fluid flow occurs in the direction of counterion movement. This phenomenon is called electroosmosis. Since the skin has an isoelectric point between pH 3 and 4, at physiological conditions (that is, pH of 7) the skin has a net negative charge. As a result, positively charged molecules pass preferentially through the skin. When an electric field is applied a volume flow is created by the momentum transferred to the solvent by anions moving through the skin and by the induced osmotic pressure. This flow is termed *electroosmosis*. The induced osmotic pressure contributes little to the volume flow, however (Gangarosa et al., 1980). The water flux is in the same direction as the applied electric field and is proportional to the current density (Srinivasan et al., 1989; Burnette, 1989; Pikal, 1990). Therefore, electroosmosis assists the flux of solutes moving in the same direction as the water flux, such as peptides or proteins that move at a slower rate than buffer ions, while transporting with it neutral solutes (Gangarosa et al., 1980; Burnette and Ongpipattanakul, 1988; Pikal and Shah, 1990a). Since flow occurs in pores, it is possible that some solute will be filtered or reflected by a sieving action. A more detailed review of electroosmosis and its effect on the transport of ions through the skin has been summarized by Pikal (1992).

Pikal (1990), to quantitate electroosmotic flux enhancement of neutral molecules through the skin, developed a model in which the skin is a heterogeneous pore system of positively charged, negatively charged, and neutral skin pores, with the larger pores having, on average, a higher distribution of negative charge. A result of this model is that the negatively charged pores dominate electroosmotic flow, and therefore flux enhancement due to electroosmosis should be greatest at the anode. For charged molecules present in relatively high concentrations, however, the contribution of water transport to transdermal permeation is generally lower than the contribution of the applied voltage gradient (Pikal, 1990; Srinivasan et al., 1989). These predictions were supported by the supplied data and by literature data, indicating that the model has merit. Pikal (1990) does not elucidate the nature of these pores, but only demonstrates their probable existence. The model would be strengthened if supported by physical evidence of these pores in the skin. This evidence is as yet forthcoming. It is noteworthy that the reported normalized fluxes of the large molecular weight carboxy inulin and bovine serum albumin are virtually identical (2.4 (10^{-3}) and 2.6 (10^{-3}) cm/h, respectively) (Pikal and Shah, 1990b), suggesting that electroosmosis is the dominating factor in their delivery and that they probably pass through the same distribution of pores. These pores are quite possibly the skin appendages.

Recent evidence suggests that the permselectivity of the skin that gives rise to the electroosmotic effect can be neutralized or even reversed by transporting a cationic peptide (that is, leuprolide and Nafarelin, which are LHRH analogues) iontophoretically into the skin (Delgado-Charro and Guy, 1994; Hoogstraate et al., 1994). The mechanism proposed to explain this behavior is that the positively charged peptide is adsorbed onto the negatively charged pore walls, causing a change in the net membrane charge and thereby altering the direction of electroosmotic flow (Hoogstraate et al., 1994). This effect increases as the concentration of the peptide in the donor solution is increased and appears to be determined by the lipophilicity of the peptide, as both Nafarelin and leuprolide exhibit this behavior while the less lipophilic parent peptide LHRH does not (Delgado-Charro and Guy, 1994). These findings illustrate a possible limitation of iontophoretic peptide transdermal drug delivery.

Enhanced diffusivity. It is also possible that the applied current increases drug diffusion in the skin. Passive diffusion through excised skin after iontophoresis has been found to be approximately an order of magnitude greater than the preiontophoretic passive flux, suggesting that the intrinisic permeability of the skin had increased by the applied current (Bellantone et al., 1986; Pikal and Shah, 1990a,b; Green et al., 1992a). This enhancement arises from changes in the skin structure caused by current flow. It has been suggested that it results from the accumulation of anions in the aqueous pores

of the skin. The electrostatic repulsion of the like charges would increase the pore radius (Tojo, 1989), a viable explanation but one lacking concrete proof. Alternatively, the hydration of the skin that occurs during iontophoresis may account for the increased flux. Another possible explanation is the formation of transient pores in the lipid lamellae caused by the applied electric field. This effect is called *electroporation* and is discussed later in this review.

Mathematical modeling. All of the mechanisms of flux enhancement described are potentially important. Most of these effects have been explained by the Nernst–Planck equation with a factor added to account for convective flow (Pikal, 1992),

$$J_i = -D_i\frac{\partial C_i}{\partial x} - \frac{D_i C_i z_i F}{RT}\frac{\partial E}{\partial x} \pm C_i v_s(1-\sigma), \qquad (18)$$

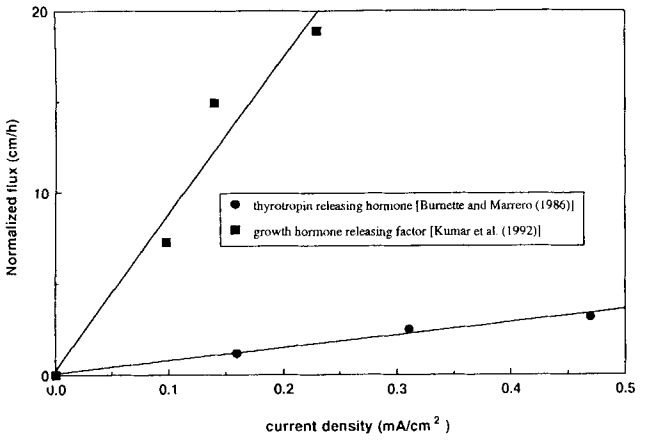
in which J_i is the flux of a given species, D_i is its diffusivity, C_i its concentration, z_i its charge, F is Faraday's constant, R is the gas constant, T is temperature, x is the distance over which the transport occurs, E is the applied electrical potential, v_s is the velocity of the convective flow, and σ is the reflection coefficient of the solute moving into the skin pore. In Eq. 18 the first term accounts for flux due to passive diffusion, the second term accounts for the flux resulting from electrostatic repulsion, and the third term describes the contribution of convection.

For small ions, flux occurs primarily by passive diffusion and electrostatic repulsion, while for larger ions, such as peptides and proteins, flux is dominated by electroosmotic flow. This is particularly important for neutral peptides (Burnette and Marrero, 1986; Srinivasan et al., 1989). The reflection coefficient places a limit on the extent to which convective flow will enhance flux. If the solute is too large and cannot pass through the pore freely ($\sigma = 1$), there is no contribution due to convective flow. However, electroosmotic flux of bovine serum albumin (molecular weight 69,000, Stokes radius 35 Å) has been demonstrated in hairless mouse skin (Pikal and Shah, 1990b), which suggests that the upper Stokes radius limit for electroosmotic flux enhancement, in this skin, is at least 35 Å.

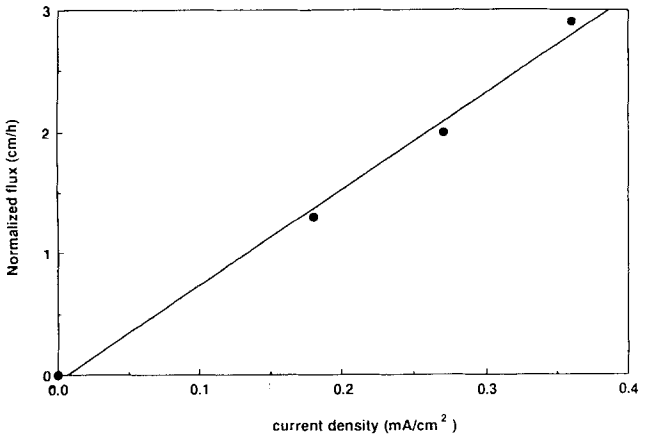
Factors affecting peptide and protein transport. Electrically facilitated peptide transport depends on the pH, ionic strength, and peptide concentration of the donor solution, the size of the solute, and the current density and duration of electric field application. The pH affects the charge carried by the peptide based on its isoelectric point and preferably should be such that the peptide is positively charged in order to maximize its transdermal flux. For example, insulin has a greater skin permeability below its isoelectric pH and almost no permeability at its isoelectric point, at which it exhibits a minimum in aqueous solubility (Siddiqui et al., 1987). The solution pH also affects the charge distribution on the skin surface (Burnette and Ongpipattanakul, 1987; Lelawongs et al., 1989). Above pH 3 the skin is negatively charged and favors the passage of cations, while below pH 3 it is weakly positively charged, thereby favoring the passage of anions. As the pH increases, the skin's negative fixed charge density increases, and electroosmotic flux increases (Burnette and Marrero, 1986). This may influence the transdermal flux of a peptide, regardless of its charge state, if it carries only a small fraction of the applied charge (Burnette and Marrero, 1986; Pikal, 1990). Also, if the donor solution pH differs from the aqueous pore pH, the charge and the electronegativity of the peptide may change during transport. This change may cause the peptide to precipitate or degrade, and will alter its iontophoretic transdermal flux (Burnette and Marrero, 1986). It should also be noted that donor solution pH can change during iontophoresis if reactions occur at an electrode (Kari, 1986), possibly altering the permeation rate of the peptide.

Solute flux is determined by the amount of the applied charge carried by the solute. For charged solutes of relatively high concentration in the donor solution, increasing ionic strength by adding more buffer ions increases competition for the applied current. The smaller, and therefore more mobile, buffer ions will carry more charge and be transported into the skin preferentially, reducing delivery efficiency. Increasing the buffer ion concentration of the donor solution, thereby increasing its ionic strength, or decreasing the concentration of solute, have been found to decrease the iontophoretic flux of both charged (Burnette and Marrero, 1986; Bellantone et al., 1986; Lelawongs et al., 1989; Green et al., 1991) and neutral (Burnette and Marrero, 1986; Burnette and Ongpipattanakul, 1987; Pikal and Shah, 1990a,b) solutes at relatively high concentrations (0.05–0.5 M), while increasing flux at low concentrations (0.01–0.05 M) (Kumar et al., 1992). Removing buffer ions results in a steadily increasing delivery rate of peptide with time (Green et al., 1991). Depending on the required pharmacology of the peptide, this could be a disadvantage. For neutral solutes or charged solutes present in relatively small concentrations, buffer ions are often necessary to induce electroosmotic flux in order to facilitate transdermal drug transport. However, as the buffer ion concentration increases, electroosmotic flux decreases (Burnette, 1989; Pikal and Shah, 1990a). There thus appears to be an optimum donor solution ionic strength for peptide flux enhancement, beyond which increasing ionic strength retards peptide flux. Since the majority of the applied current is carried by the buffer ions, delivery efficiency is generally low. To increase the efficiency of iontophoretic peptide delivery, Sarpotdar and Daniels (1990) used large molecular weight buffers in transporting thyrotropin releasing hormone and aminotyrosyl-lysyl-prolineamide across excised human skin. The use of a cationic buffer (M = 70,000) resulted in a twofold increase in permeation, whereas the use of an anionic buffer (M = 41,000) resulted in five- to eightfold increases in permeation at pH 4 where both drugs are positively charged.

The ionic strength or peptide concentration of the donor solution affects the fraction of applied current carried by the peptide (Lelawongs et al., 1989; Pikal, 1990; Pikal and Shah, 1990a,b; Burnette, 1989), and the volume flow due to electroosmosis (Pikal, 1990; Pikal and Shah, 1990a,b; Burnette, 1989). Electroosmotic flux has a significant influence on the delivery rate only if the total charge carried by the peptide is small compared to the charge carried by the buffer ions (Burnette and Marrero, 1986; Pikal and Shah, 1990b). This may explain the finding of Lelawongs et al. (1989), who determined that the delivery rate of arginine vasopressin, which has an isoelectric point of 10.9, was relatively unaffected as the pH was changed from 5 to 7.4 at constant ionic strength. At the higher pH, the skin would be more permselective and therefore a higher electroosmotic flux would be expected. In



(a)



(b)

Figure 8. Effect of current density on normalized iontophoretic flux.

(a) Data from Kumar et al. (1992) for a cation (M = 3,929), and from Burnette and Marrero (1986) for a neutral peptide (M = 362.42), illustrating the effect of current on electroosmotic flow. (b) Data from Green et al. (1992a) for an anionic tripeptide (M = 344).

this case, however, the influence of the electroosmotic flux was not significant because the ionic strength remained unchanged.

A number of researchers have shown iontophoretic permeability to be a linear function of current density (Bellantone et al., 1986; Burnette and Marrero, 1986; Wearley et al., 1989; Lelawongs et al., 1989; Green et al., 1992a; Kumar et al., 1992) (Figure 8a and 8b). This effect can be related to the transport number of the ion in question, as the flux of the ion is described by

$$J_i = \frac{t_i I_T}{z_i F}, \tag{19}$$

in which t_i is the ion transport number, and I_T is the total current density applied. Note that neutral solutes can also experience an increase in flux with increasing current density (Burnette and Marrero, 1986) (Figure 8a) as the electroosmotic solvent volume flow increases with current density. As a means of increasing flux, however, the current density can only be increased to a certain extent before it causes pain and discomfort. This is clearly a drawback of this technology. The pain is caused by burns resulting from pH changes in the subcutaneous tissue (Sanderson et al., 1989). Sanderson et al. found that the maximum tolerable current, I_{max}, in terms of the subcutaneous tissue concentration of solute, clearance from the location of application, and area over which the current is administered, A, is given by

$$I_{max} = \frac{A(28.6\,\text{mA/cm}^2)}{48.3\,\text{cm}^2 + A}. \tag{20}$$

For ionic solutes with low transport numbers then, the iontophoretic flux can be increased by increasing the application area. Nevertheless, the nature of the expression in Eq. 20 suggests that for some compounds, an adequate flux may not be possible.

It has also been reported that the normalized electroosmotic flux of amino acids and tripeptides through hairless mouse skin was independent of lipophilicity, reinforcing the idea that flux occurs in aqueous pores, and decreased as molecular weight increased (Green et al., 1992b; Yoshida and Roberts, 1992). The latter result can be explained by noting that for relatively small solutes iontophoretic transport is dependent on the solute diffusivity (Eq. 18). The effect of molecular weight on solute diffusivity in the stratum corneum has been explained in terms of the Stokes–Einstein theory, free-volume theory, and a pore restriction model (Yoshida and Roberts, 1993).

In the Stokes–Einstein theory, the solute is considered to be a sphere moving through a continuum under an applied electric field. The molecular weight of the solute determines its Stokes hydrodynamic radius, which in turn affects its diffusivity in the aqueous environment of the pore. Solute diffusivity is related to molecular weight as,

$$D = \kappa M^{\theta}, \tag{21}$$

in which κ and θ are constants and M is molecular weight. The normalized flux of a solute can then be expressed as,

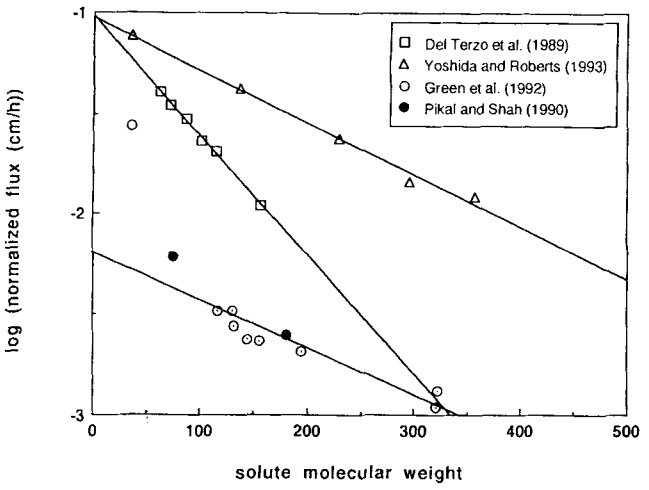
$$\log(J/C) = \log(B) + \theta \log(M), \tag{22}$$

in which B is a constant. Recall that in the free-volume theory, the solute moves through the medium when a hole (or free volume) in the medium is created adjacent to the solute molecule. Solute diffusivity is given by Eq. 11, therefore the solute normalized flux in this case is expressed as

$$\log(J/C) = \log(B') - \frac{M}{aV_{av}}, \tag{23}$$

in which B' is a constant.

Normalized anionic solute flux through various excised skin samples taken from the literature is plotted against the logarithm of solute molecular weight in Figure 9a and against solute molecular weight in Figure 9b. With the exception of the chloride ion data (M = 36) reported by Yoshida and Roberts (1993) and Green et al. (1992b), it appears that ei-



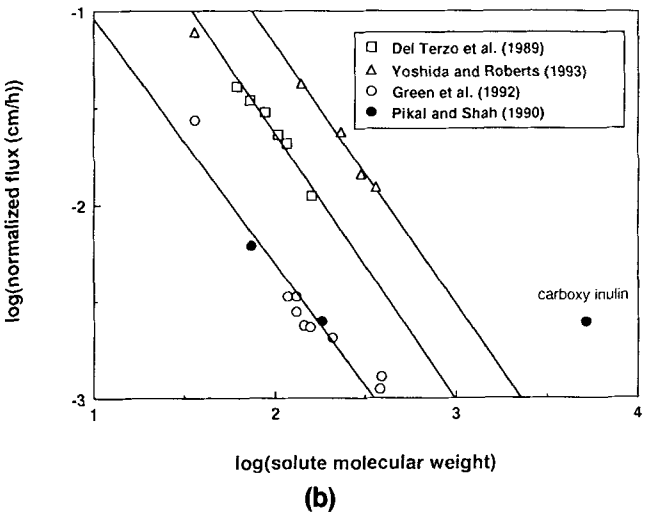


Figure 9. Normalized iontophoretic flux vs. solute molecular weight. The data of Green et al. (1992b) and Pikal and Shah (1990b) is for anions permeating through nude mouse skin, that of del Terzo et al. (1989) is for anions through rat skin, and that of Yoshida and Roberts (1993) is for anions through human skin.

(a) Based on Stokes–Einstein equation. (b) Based on free volume theory.

ther relationship works equally well over a range of molecular weight. This similarity in predictive behavior can be explained by noting that expansion of Eq. 22 as a Taylor's series results in it being mathematically similar over a small range of molecular weights as Eq. 23. The good fit of the relationships to the data indicates that the skin can be modeled using theories developed for solute transport in polymer films. At higher molecular weights, for example, that of carboxy inulin (M = 3,929) (Pikal and Shah, 1990b), the theories break down (Figure 9a). Carboxy inulin has a normalized flux equal to that of a solute of approximately 1/26 its molecular weight. Therefore, it is being transported by a different driving force. The carboxy inulin point illustrates the importance of electroosmotic flux for larger permeants.

Pore-restriction models have also been used to describe the decrease in solute diffusivity as it moves through the pore (Yoshida and Roberts, 1992, 1993). In these models, a further decrease in solute diffusivity arises from steric hindrance and other interactions between the solute and the pore wall materials and from increased drag on the solute molecule due to the existence of the pore walls (Deen, 1987). Without knowing the nature of the aqueous pores it would be difficult to determine which model applies best. It is likely that both free-volume or Stokes–Einstein and restricted transport effects are present.

The fact that most electrically facilitated transport probably occurs in aqueous pores is potentially clinically important. The focusing of the applied electric field to these pores leads to high current densities and possibly cell damage from heating (Burnette and Ongpipattanakul, 1988). Also, the drug becomes concentrated in the pores, resulting in potential irritation and allergic reaction at relatively low donor solution concentrations (Cullander and Guy, 1991). There is evidence that formation of these pores in excised skin may not be reversible. For example, Burnette and Ongpipattanakul (1988) found that the passage of clinically acceptable levels of current (0.16 mA/cm^2 for 1 h) through excised human skin resulted in a decrease in its electrical resistance. Similarly, Bagniefski and Burnette (1990) found that skin impedance decreased after iontophoretic treatment (0.235 mA/cm^2 for 9 h). Green et al. (1991) discovered that time-dependent changes in the properties of hairless mouse skin occurred during iontophoresis (0.36 mA/cm^2 for 24 h) and that these changes were not reversible after 24 h. Chien et al. (1990) determined that 40 min of iontophoretic delivery of vasopressin across excised hairless rat skin at a current density of 0.22 mA/cm^2 was the upper limit before the reversibility of skin permeation was affected. These authors concluded that the skin morphology had been altered. The amount of current applicable to the skin is limited, which restricts iontophoresis to short-term peptide or protein delivery regimens, that is, to delivery periods of minutes to hours but not for days. More evidence is required to demonstrate the applicability of this delivery method *in vivo* as well.

Alternating current, direct current, and pulsed current have all been investigated as driving forces. The principle behind the use of alternating or pulsed current is that the application of a steady electrical field polarizes the stratum corneum, causing it to behave like a capacitor whose impedance changes with the frequency of the applied electric field (Okabe et al., 1986; Chien et al., 1990). The polarization acts against the applied electric field, decreasing the effective current across the skin, thereby reducing the efficiency of iontophoretic delivery. The use of a pulsed current was proposed to allow the stratum corneum to become depolarized, improving the iontophoretic efficiency, and reducing electrically induced skin damage. In practice, the applied current must be pulsed slowly to avoid muscle stimulation (Ranade, 1991). Nevertheless, it has been demonstrated that steady-state pulsed current iontophoretic transdermal flux is not significantly different from the flux at an equivalent constant current, except at high frequencies where the pulsed current exhibits a reduced flux (Bagniefski and Burnette, 1990; Kumar et al., 1992). Propo-

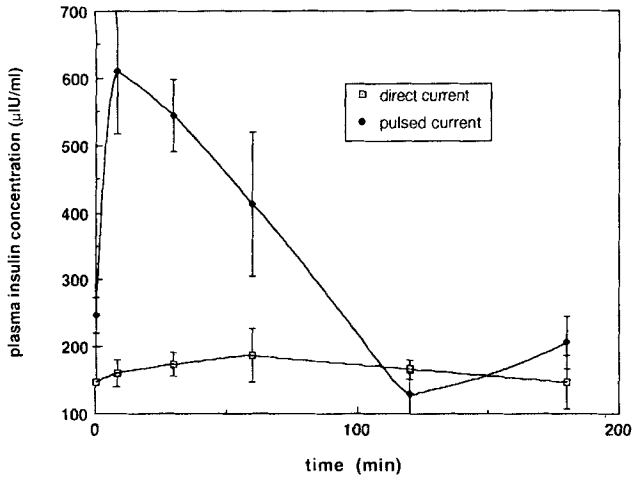


Figure 10. Pulsed vs. continuous direct current for the iontophoretic delivery of insulin to diabetic rabbits, both at 1 mA.

Data from Chien et al. (1990).

nents of the theory have also observed a similar phenomenon with insulin delivery via pulsed iontophoresis. However, before steady state was reached, the pulsed iontophoresis protocol resulted in higher initial transdermal fluxes of insulin to hairless rats (Chien et al., 1990) (Figure 10). The flux peaked after 10 min and rapidly fell to approximately the same steady-state rate as that of direct current. It appears that the skin eventually reaches the same state of polarization regardless of the method of current application. The only advantage of pulsed over continuous delivery is that a higher initial dosage is attained. For short delivery regimens, pulsed current iontophoresis is best.

There is a discrepancy in the literature as to whether relatively large molecular weight solutes can be made to pass through human skin using iontophoresis. For example, Srinivasan et al. (1989, 1990) found that leuprolide, insulin, and a cholecystokinin-8 analogue did not permeate full thickness excised human skin *in vitro*, although other *in vitro* and *in vivo* studies with animal skin indicate that molecules of molecular weight as high as 69,000 can be passed through the skin using iontophoresis (Table 4). Also, Turner et al. (1994) found that 12 h of iontophoresis was required to permeate poly-L-lysine of molecular weight 21,000 through hairless mouse skin. Yoshida and Roberts (1993) found that iontophoresis did not significantly enhance the permeation of cyclosporin *A* (M = 1201) and inulin (M = 6000) through excised human skin. However, from the data reported, the passive permeation coefficients of these solutes are quite high (1 and 3.64 (10^{-3}) cm/h, respectively) as compared to other compounds (approximately 2 (10^{-5}), Table 2), which may explain their results.

It is instructive to compare iontophoretically obtained polypeptide drug delivery dosages with their recommended daily dosages. Based on the conditions recommended earlier (a delivery time of 40 min and a current density less than 0.30

Table 4. Examples of Peptide Drugs Delivered Transdermally by Iontophoresis

Peptide	Molec. Mass	Charge	Current Density (mA/cm^2)	Flux ($\mu g/cm^2 h$)	Skin Source	Reference
Threonine-lysine-proline	344	+1	0.18	0.038	Hairless rat	Green et al. (1992a)
			0.27	0.062		
			0.36	0.093		
Thyrotropin releasing hormone	362.42	+1	0.31	2.68	Hairless mouse	Burnette and Marrero (1986)
			0.16	1.74		
		0	0.16	3.62		
			0.31	7.97		
			0.47	10.3		
Vasopressin	1,084	+	0.5	0.116	Hairless rat	Lelawongs et al. (1989)
			1.0	0.178		
		+	0.62	1.56	Hairless rat	Banga and Chien (1993)
Cholecystokinin-8 analogue	1,150	> −1	n/a	~ 0.0	Human	Srinivasan et al. (1990)
Leutenizing Hormone Releasing hormone (LHRH)	1,182	+	0.2	~ 0.148	Porcine	Heit et al. (1993)
Leuprolide acetate (an LHRH	1,269	n/a	0.003	n/a	Human (*in vivo*)	Meyer et al. (1988, 1990)
analogue)	1,209	+1	n/a	~ 0.0	Human (*in vitro*)	Srinivasan et al. (1990)
Calcitonin	3,527	+	0.33	0.025	Hairless rat	Thysman et al. (1994)
Growth hormone releasing factor	3,929	+1	0.098	0.0218	Hairless guinea	Kumar et al. (1992)
			0.14	0.0447	pig	
			0.23	0.0568		
Carboxy inulin	5,200	−1	0.32	2.2	Hairless mouse	Pikal and Shah (1990b)
Insulin	5,808	+	n/a	n/a	Human	Shapiro et al. (1975)
			n/a	n/a	Pig	Stephen et al. (1984)
			< 0.16	n/a	Rabbit	Kari (1986)
			0.67	n/a	Hairless rat	Siddiqui et al. (1987)
			n/a	~ 0.0	Human	Srinivasan et al. (1989)
			0.62	0.089	Hairless rat	Banga and Chien (1993)
Bovine serum albumin	69,000	−0.35	0.32	0.52	Hairless mouse	Pikal and Shah (1990b)

Table 5. Iontophoretically Delivered Dosages vs. Required Adult Dosages

Peptide	Current Density (mA/cm^2)	Delivered Dosage (μg)	Required Adult Dosage* ($\mu g/d$)	Reference
Thyrotropin releasing hormone	0.31	267	500	Burnette and Marrero (1986)
Vasopressin	0.50	3.9	2–4	Lelawongs et al. (1989)
LHRH	0.20	5.0	1,000	Heit et al. (1990)
LHRH analogue	0.30	440	1,000	Miller et al. (1990)
Calcitonin	0.33	0.84	250	Thysman et al. (1994)
Insulin	0.62	3.0	$\leq$ 3,000	Banga and Chien (1993)

*From *Martindale The Extra Pharmacopoeia* (1993).

mA/cm^2) and an application site of 50 cm^2 the data of Table 5 were derived. From these data it is apparent that only one drug is delivered at an adequate rate (vasopressin) with a single iontophoretic application; however, this was accomplished at greater than the recommended charge density. Other drugs, such as vasopressin and an LHRH analogue, have the potential of being successfully delivered, although they may require multiple applications. An important caveat is that none of the studies were done with human skin. Clearly, iontophoresis has potential in facilitating the delivery of some peptide drugs through the skin, and in fact clinical trials in humans have begun (Wallace and Lasker, 1993); however, more work is required before viable delivery systems will be available.

Iontophoresis also has the disadvantages of potentially irreversibly damaging skin permeability, being difficult to control from person to person due to variation in electrical and physicochemical skin properties such as its electrical resistance and capacitance (Cranne-van Hinsberg et al., 1991), yielding inconsistent rates of delivery for molecules with molecular weights greater than 8,000–12,000 (Parasrampuria and Parasrampuria, 1991), passing extraneous ions through the skin, delivering only a small percentage of the drug (Burnette and Marrero, 1986) therefore being questionable for extremely expensive drugs, and being limited in its duration of application. The latter characteristic restricts its use to pulsatile or short-term delivery. There are a number of unresolved issues in iontotherapeutic delivery, such as whether the pores suggested by the model of Pikal (1990) are present in the skin; how the application of the applied field increases skin permeability; the relative impact on flux of electrophoresis, electroosmosis, and skin structural changes; and whether peptides and proteins can be made to pass through human skin and if so, what route they take in passage. For chronic care, the effects of long-term use of iontophoresis have yet to be demonstrated.

Combined chemical enhancement and iontophoresis

The use of chemical enhancer pretreatment of skin followed by iontophoresis has also been investigated (Srinivasan et al., 1989, 1990; Francoeur and Potts, 1991). Srinivasan et al. (1990) were able to obtain a passive permeation rate of leuprolide, an LHRH analogue (MW = 1,209.4), and a cholecystokinin-8 analogue (MW = 1,150.17) by contacting excised human skin with ethanol for 2 h. Before ethanol treatment, no permeation of these compounds was detected. The application of iontophoresis resulted in further increased permeation rates. As a word of caution, however, there is no evidence to suggest that the enhancement effect of iontophoresis is always additive, especially if the skin becomes permanently altered by the chemical enhancer used. For example, Hirvonen et al. (1993b) found that combining iontophoresis with the use of Azone did not increase the permeation of sotalol over that of chemical enhancement or iontophoresis alone.

Electroporation

Over the last decade there has been extensive use of electroporation in many different fields such as cell biology, molecular biology, plant genetics, and hybridoma technology. Electroporation describes the phenomenon in which permeation through lipid bilayers is increased as a result of a pulsed applied electric field. It is believed that the field creates transient pores in cell and lipid lamellae membranes, although the existence of these pores has yet to be proved. During the lifespan of these pores, membrane permeability to foreign, exterior macromolecules becomes markedly increased. This technique has been used to introduce foreign DNA (Whitney et al., 1993), antibodies (Chakrabarti et al., 1989), and proteins (Hashimoto et al., 1989) into living cells in suspension and intact tissue *in vivo* (Titomirov et al., 1991; Prausnitz et al., 1993), and *in vitro* (Powell et al., 1989; Prausnitz et al., 1992, 1993; Tamada et al., 1993). Recently it has also been demonstrated to be a promising means of delivering peptides through the skin (Prausnitz et al., 1992, 1993; Tamada et al., 1993).

Electroporation differs from iontophoresis in the duration and intensity of current application, and probably on its effect on skin structure. Electroporation involves the application of high-voltage electric pulses, whereas iontophoresis uses a relatively constant low-voltage electric field. The driving force for flux for both techniques is electroosmosis and electrostatic repulsion (Weaver and Barnett, 1992; Sowers, 1992; Prausnitz et al., 1993). During electroporation, a transmembrane potential is induced by an externally applied electric field, usually a relatively short direct current pulse. The pulse can be a square wave with a duration of less than 100 μs, or it can be an exponentially decaying capacitive discharge with durations in the millisecond range (Chang et al., 1992). It is widely assumed that this applied electric field induces pore formation in lipid lamellae membranes. The dynamics of pore formation and resealing are not completely understood and are currently a focus of active investigation. Presumably the pores initially evolve as small hydrophobic

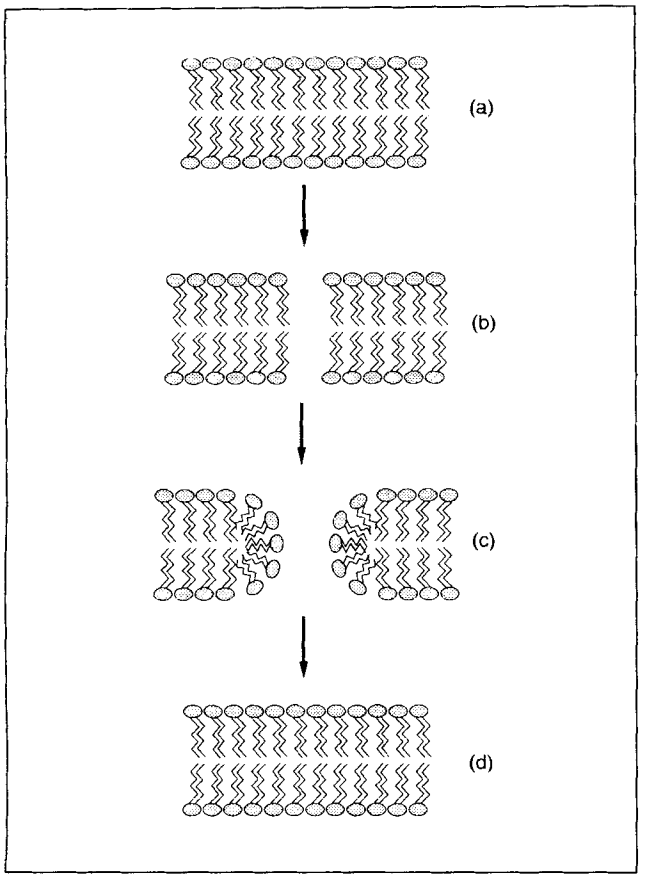


Figure 11. Proposed method of development of electropores in lipid bilayer.

(a) Initial lipid bilayer before onset of electric field. (b) After onset of electric field small hydrophobic pores are created. (c) If radius of pore is large enough, hydrophilic pores will form due to thermodynamic favorability. (d) After electric field is removed complete resealing of the pores occurs within seconds to minutes.

pores (radius < 0.5 nm) as a result of lateral thermal fluctuations of the lipid molecules in the lamellae. If the radius of the hydrophobic pore formed exceeds a critical value (for which it is energetically favorable for hydrophilic pores to form), then the lipid molecules reorient themselves to form a water-filled hydrophilic pore (Figure 11). The properties of these temporary pores depend on pulse duration and amplitude. For cells, in general, the longer the pulse duration, the lower the amplitude required to induce electroporation and for the same pulse amplitude, the larger the diameter and number of pores formed (Chernomordik, 1992). Complete resealing of the formed pores once the electric field is removed varies from seconds to minutes, so that lamella membrane permeability remains increased for a short duration after the applied pulse.

The application of electroporation to the transdermal delivery of peptide drugs involves placing a solution containing the peptide on the skin and applying a short, pulsed electric field. Prausnitz et al. (1992, 1993), who pioneered this transdermal delivery strategy, have determined that, across excised human stratum corneum, flux enhancement of fluorescent polar molecules (such as calcein, molecular weight 623,

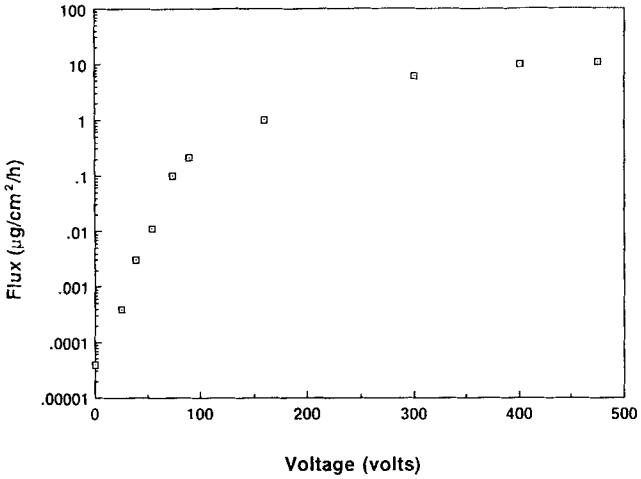


Figure 12. Effect of applied electroporation voltage on the transdermal flux of calcein (M = 623) through human skin. (From Prausnitz et al., 1993.)

charge -4) increases with the applied voltage and is reversible until approximately 100 V, after which the flux increases weakly as a function of the applied voltage and is only partially reversible (Figure 12). In these experiments, an exponential decay pulse (decay time = 1–1.3 ms) was applied every 5 s for 1 h. At the cessation of pulsing, flux dropped off rapidly (Figure 13). Comparing electrically equivalent direct current iontophoresis and electroporation revealed that electroporation caused fluxes 3 orders of magnitude larger than iontophoresis (Figure 14). This result indicates that skin structural changes occur during electroporation that are more dramatic and probably much different than those induced by iontophoretic conditions. Whether electroporation generates transient pores in the lipid lamellae and/or the cells of the stratum corneum, which appears to be the likely mechanism, has yet to be demonstrated.

Prausnitz et al. (1993) also applied an electroporation pulsed field to full thickness excised skin and observed that

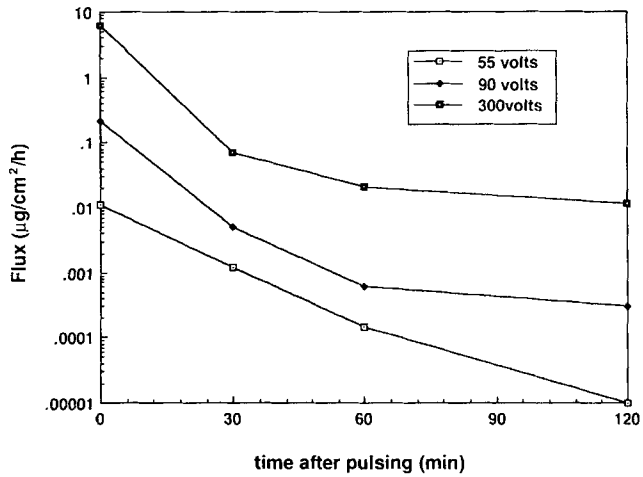


Figure 13. Reduction in skin permeability following electroporation at different voltages.

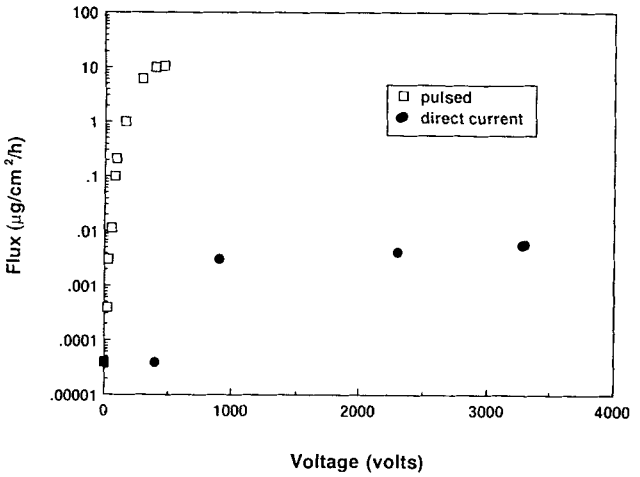


Figure 14. Electroporation permeation enhancement of calcein vs. that of electrically equivalent iontophoretic enhancement through human skin.

From Prausnitz et al. (1993).

the fluxes were enhanced, but not to such a degree as those observed for the stratum corneum alone, and had a lag time of about one hour. These results indicate that diffusional resistances occurred in the viable epidermis and dermis and that electroporation effects did not extend much beyond the stratum corneum. Since the stratum corneum presents the largest electrical resistance, most of the applied charge should be concentrated within it. Therefore, most of the morphological changes should occur within the stratum corneum. The more dramatic effect of electroporation on skin structure results in greater fluxes.

Electroporation has also been used in conjunction with iontophoresis. Transdermal iontophoretic fluxes of leutenizing hormone-releasing hormone, vasopressin, and neurotensin were three to eight times greater after the application of a pulsed field (300–400 V for 5–10 ms) than without the pulsed field (Tamada et al., 1993). The fluxes returned to close to baseline levels within hours after the application of the pulse. This work demonstrates another possible peptide delivery method.

There are a number of unknowns involved with electroporating viable skin. These include the route of entry (transcellular or via the lipid lamellae), the maximum molecular weight of the delivered molecule, the extent of the skin that is electroporated, the maximum amount of current that can be safely applied, and the reversibility of the pores generated in cells. Since cell membranes are more difficult to electroporate than lipid lamellae (Chang, 1992), and since the cells of the stratum corneum have very thick cell envelopes, the pores are probably formed in the continuous lipid lamellae of the stratum corneum. Thus, peptide flux probably occurs through this region. Prausnitz et al. (1992) assert that the viable cells in the lower epidermis should be minimally affected; however, this has not been verified. Although pore formation in lipid lamellae is reversible, there is evidence that pores formed in cells may not be reversible (Chang, 1992). This possibility may affect the health of skin being electroporated.

The primary advantage of electroporation is that it does not involve puncturing the skin while resulting in high levels of flux enhancement. However, it is useful only as a means of one-time application of peptide drugs. It removes the complication involved with injections, but still requires care and supervision, and so is not a candidate for prolonged delivery of peptide drugs. The mechanism for electroporation of skin is neither well-understood nor sufficiently controlled as yet to determine its potential in controlled release. Although electroporation shows promise, *in vivo* tests on rat skin showed erythema (inflammation of the skin) and edema (tissue swelling) occurring at relatively low (150 V) applied fields, and a two order of magnitude increase in transdermal flux of calcein above the detection limit, which did not increase with increasing voltage (Prausnitz et al., 1993). This result implies that another rate-limiting step other than transport across the stratum corneum exists. Long-term studies on its effects on living skin tissue are required to assess the viability of this technique.

Ultrasound

Another method being investigated to increase the transport of peptide drugs through the stratum corneum is the use of ultrasound. Ultrasound has been used extensively in medical diagnostics and physical therapy for the last three decades, and investigated by a variety of researchers as a means of increasing the diffusive flux of a number of drugs through the skin (Skauen and Zentner, 1984; Tyle and Agrawal, 1989; Levy et al., 1989; Benson et al., 1989; Bommannan et al., 1992a). An illustration of a possible ultrasound transdermal delivery device is given in Figure 15.

Ultrasound is defined as any sound having a frequency greater than 16 kHz. These sound waves cause compression and expansion of the skin tissue through which they travel, resulting in pressure variations. These pressure variations can cause cavitation, time-independent fluid flow, mixing (which reduces the boundary layer thickness), and an increase in temperature (Kost, 1993). In order for ultrasound to be successful it must act primarily on the stratum corneum to increase the permeation of peptide drugs through it. The impact each of the preceding effects has on the increased diffusive flux of drugs through the skin has yet to be decisively determined. Heating is not considered to be a likely cause because the stratum corneum was not found to increase in temperature appreciably during the application of ultrasound (Levy et al., 1989; Bommannan, 1992a).

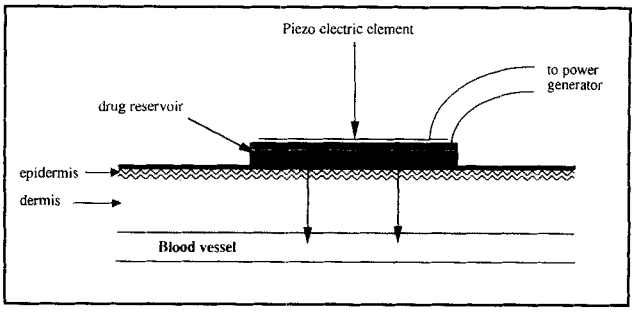


Figure 15. Sonophoresis delivery device.

It has been suggested that ultrasound may interact with the extracellular lipid lamellae structure (Miyazaki et al., 1991), although the exact means have not been described. Support for this hypothesis is given by the findings of Bommannan et al. (1992b), who found that an ultrasound permeated water-soluble tracer was concentrated in the hydrophilic region of the lipid lamellae. The probable pathway for ultrasound-delivered water-soluble drugs then appears to be through the extracellular lipid lamellae, although it is possible that the follicles and sweat ducts may also be important.

Recently ultrasound was demonstrated to increase the diffusive flux of insulin through the skin of hairless mice (Tachibana and Tachibana, 1991) and shaved rabbits (Tachibana, 1992). In each case, the blood glucose concentration of the diabetic animals dropped, indicating successful insulin delivery. These researchers found that the higher the ultrasound energy input, the greater the drop in the glucose level. This finding was also reported by Bommannan et al. (1992a) for the transdermal delivery of salicylic acid using ultrasound. They found that the use of higher frequencies (10 and 16 MHz, as compared to the normal 1 to 3 MHz for medical use) resulted in greater salicylic acid flux. Greater energy depositions of the applied beam into the stratum corneum apparently increases its permeability to the diffusing drug. However, high-energy depositions over prolonged periods (16 MHz for 20 minutes) altered epidermal morphology (Bommannan et al., 1992b), leading to unresolved questions concerning the reversibility of the action of the ultrasound on the skin. The route and mechanisms of entry of the compound into the skin have to be determined before this mode of delivery can become useful. Whether ultrasound enhances transdermal flux by direct action on the permeant, and/or by convection, and/or by altering skin morphology are issues that remain to be resolved.

From the discussion so far, it has been shown that in designing a transdermal drug delivery device a number of factors must be considered, such as the physicochemical properties of the drug and its routes of entry. What is equally important in the safe and effective use of any drug is the determination of the drug's pharmacokinetics. The majority of the studies done on transdermal drug delivery are done *in vitro*. The resulting release kinetics are usually not reproduced *in vivo*, nor are the determined kinetic rate constants. The input rate of the drug is also an important consideration. A constant input rate does not necessarily translate into a constant plasma level as time-dependent effects, such as binding and metabolism (Figure 3), may occur in the skin, and especially for peptides and proteins, the drug's physiological and pharmacological activities can involve complex feedback interactions with target organ receptors (Mazer, 1990). As a means of determining *in vivo* parameters based on absorption experiments, mathematical pharmacokinetic models have been developed to aid in designing transdermal drug delivery systems.

Pharmacokinetics

In pharmacokinetic models, the excretion rate of the permeant is solved for and related to its physicochemical and kinetic properties. Two types of pharmacokinetic models have been used: diffusion models (Chandrasekaran et al., 1978; Berner, 1985; Parry et al., 1990) based on Fick's Laws, and compartment models (Guy and Hadgraft, 1985, 1987b; Kubota, 1991; Williams and Riviere, 1993). In diffusional models, non-steady-state diffusion through each skin layer plus the device along with the permeant elimination kinetics in the tissue must be considered. This makes the problem very involved, necessitating the use of simplifying assumptions. The assumptions usually made are that the dermal side acts as an infinite sink, elimination reactions are first order, permeant diffusivity is constant in each layer, partition equilibrium exists at each boundary layer, the device is an infinite drug reservoir that is well-stirred (no concentration boundary layer exists), and Fickian diffusion occurs. The resulting equations are complicated and not easy to use. For example, Chandrasekaran et al. (1978) developed an equation for the urinary excretion rate, R_E,

$$R_E = \frac{4}{\pi}\alpha e^{-k_E t}\sum_{n=1}^{\infty}(-1)^{n-1}(2n-1)\left[\frac{G}{\alpha(2n-1)^2}\left(\frac{e^{k_E t}}{k_E}-\frac{e^{(k_E-\alpha(2n-1)^2)t}}{k_E-\alpha(2n-1)^2}-\frac{1}{k_E}+\frac{1}{k_E-\alpha(2n-1)^2}\right)+\frac{H}{\alpha(2n-1)^2-h}\left(\frac{e^{(k_E-h)t}}{k_E-h}-\frac{e^{(k_E-\alpha(2n-1)^2)t}}{k_E-\alpha(2n-1)^2}-\frac{1}{k_E-h}+\frac{1}{k_E-\alpha(2n-1)^2}\right)\right], \quad (24)$$

in which k_E is the elimination rate constant, $\alpha = [\pi^2 D/(4x^2(r+1))]$, D is permeant diffusivity, r is a constant, and G, H, and h are constants related to the input rate. The products from such a rigorous approach, however, are direct insights into the properties of the permeating molecule that influence its absorption rate.

A more pragmatic approach may be the use of compartment models. In this technique, each layer is a compartment and first-order kinetic equations describe the mass balance of permeant within that compartment (Figure 16). The resulting linear first-order differential equations are solved by fitting rate constants to experimental data, although some rate constants can be solved *a priori* with knowledge of partition coefficients and diffusivities of the permeant in the various layers (Guy and Hadgraft, 1985). Compartment models cannot be used for parameter estimation, as small changes in any of the estimated parameters may cause large changes in the fitted parameters, but it can be used for prediction purposes. Although simplistic in approach, compartment models have more potential for use in device design. Compartment models have been used to illustrate the effects of penetration enhancers (Guy and Hadgraft, 1987b) and iontophoresis (Williams and Riviere, 1993) on small molecular weight perme-

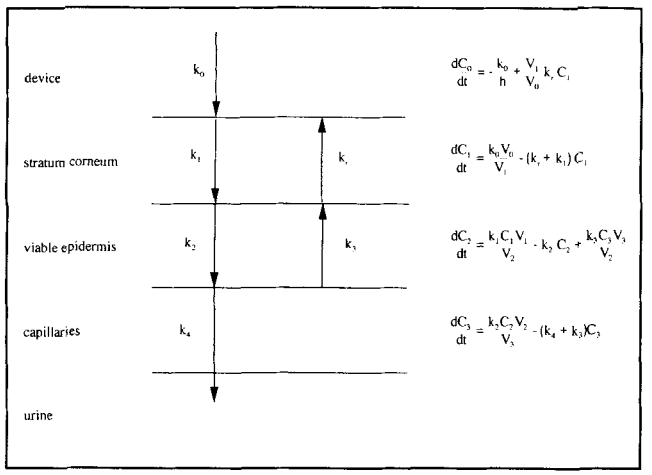


Figure 16. Example of a compartment model for transdermal delivery pharmacokinetics.
Guy and Hadgraft (1985).

ants. Their use has yet to be extended to the examination of the pharmacokinetics of peptide and protein transdermal drug delivery, where they would be useful in determining limitations and advantages of the various delivery schemes.

Concluding Remarks

Considerable work has been undertaken to understand solute mass transport mechanisms through the skin, as well as developing a means of facilitating the permeation of peptide and polypeptide drugs through this barrier. The stratum corneum represents the main resistance to the permeation of these drugs. A more detailed knowledge of the morphology of the lamellar region of this layer and the degree to which transport through the hair follicles and sweat glands is important are issues which, when resolved, will lead to a greater understanding of the means of bypassing this barrier.

Although passive diffusion of some peptides through the skin occurs, the rates are too slow to be effective. This has led to the investigation of methods of increasing these permeation rates. The ability of chemical permeation enhancers and prodrugs to facilitate the delivery of peptides or proteins is limited and they are probably not viable alternatives for peptide and protein drugs. Iontophoretic delivery appears promising. A number of issues of this technique, however, remain to be resolved. These issues include: whether positive, negative, and neutral pores are present in the skin; how the application of the applied field increases skin permeability; the extent to which electrophoresis, electroosmosis, and skin structural changes affect permeant flux; and whether peptides and proteins can be made to pass through human skin and if so, what route they take in passage. A relatively new method, electroporation, has resulted in apparent high levels of flux enhancement. Long-term studies on the effects of both electroporation and iontophoresis on living skin tissue are required to assess their viability. Ultrasound has unresolved questions concerning the reversibility of its action on the skin and its mechanism of action. Like iontophoresis and electroporation, it is useful only as a one-time application of peptide drugs. These strategies are not candidates for prolonged controlled transdermal delivery of peptide drugs, as long-term delivery would require moving the application to different sites on the skin. This would result in a pulsatile delivery regimen, as a lag time is induced with every move.

It is clear that much work still is needed in this area before marketable transdermal delivery devices for peptide and protein drugs become available. Chemical engineers, by virtue of their background, have the ability to make a notable impact in the development of these delivery devices. We hope that this article stimulates interest in this exciting and lucrative field.

Acknowledgments

We would like to thank Dr. Gary Murray of Pharma Patch, Toronto, Ont., Canada for his valuable input, and the Ontario Centre of Materials Research for supplying funding through a Cooperative Scholarship.

Literature Cited

Abramson, H. A., "Skin Reactions X: Pre-seasonal Treatment of Hay Fever by Electrophoresis of Ragweed Pollen Extracts into the Skin. Preliminary Report," *J. Allergy*, **12**, 169 (1941).

Albery, J. W., and J. Hadgraft, "Percutaneous Absorption: Theoretical Description," *Pharm. Pharmacol.*, **31**, 129 (1979).

Al-Saidan, S. M. H., A. J. Winfield, and A. B. Selkirk, "Effect of Pre-heating on the Permeability of Neonatal Rat Stratum Corneum to Alkanols," *J. Invest. Dermatol.*, **89**, 430 (1987).

Amsden, B. G., and Y.-L. Cheng, "A Generic Protein Delivery System Based on Osmotically Rupturable Monoliths," *J. Controlled Release*, **33**, 9 (1995).

Anderson, R. L., and J. M. Cassidy, "Variations in Physical Dimensions and Chemical Composition of Human Stratum Corneum," *J. Invest. Dermatol.*, **61**, 30 (1973).

Bagniefski, T. M., and R. R. Burnette, "A Comparison of Pulsed and Continuous Current Iontophoresis," *J. Controlled Release*, **11**, 113 (1990).

Baker, R. W., and H. K. Lonsdale, "Controlled Release: Mechanisms and Rates," *Controlled Release of Biologically Active Agents*, A. C. Tanquary and R. E. Lacey, eds., Plenum, New York (1974).

Banerjee, P. S., and W. A. Ritschel, "Transdermal Permeation of Vasopressin: I. Influence of pH, Concentration, Shaving and Surfactant on in vitro Permeation," *Int. J. Pharm.*, **49**, 189 (1989).

Banga, A. K., and Y. W. Chien, "Hydrogel-Based Iontotherapeutic Delivery Devices for Transdermal Delivery of Peptide/Protein Drugs," *Pharm. Res.*, **10**, 697 (1993).

Barry, B. W., "The Transdermal Route for the Delivery of Peptides and Proteins," *NATO ASI Ser. A*, **125**, 265 (1986).

Bellantone, N. H., S. Rim, M. L. Francoeur, and B. Rasadi, "Enhanced Percutaneous Absorption via Iontophoresis I. Evaluation of an in vitro System and Transport of Model Compounds," *Int. J. Pharm.*, **30**, 63 (1986).

Benson, H. A., J. C. McElnay, and R. Harland, "Use of Ultrasound to Enhance Percutaneous Penetration of Benzydamine," *Phys. Ther.*, **69**, 113 (1989).

Berner, B., "Pharmacokinetics of Transdermal Drug Delivery," *J. Pharm. Sci.*, **74**, 718 (1985).

Berner, B., and E. R. Cooper, "Models of Skin Permeability," *Transdermal Delivery of Drugs*, Vol. II, A. F. Kydonieus and B. Berner, eds., CRC Press, Boca Raton, FL (1987).

Bissett, D. L., "Anatomy and Biochemistry of the Skin," *Transdermal Delivery of Drugs*, Vol. I, A. F. Kydonieus and B. Berner, eds., CRC Press, Boca Raton, FL (1987).

Blank, I. H., J. Moloney III, G. E. Emslie, I. Simon, and C. Apt, "The Diffusion of Water Across the Stratum Corneum as a Function of Its Water Content," *J. Invest. Dermatol.*, **82**, 188 (1984).

Boddé, H. E., J. C. Verhoef, and M. Ponec, "Transdermal Peptide Delivery," *Biochem. Soc. Trans.*, **17**, 943 (1989a).

Boddé, H. E., M. A. M. Kruithof, J. Brussee, and H. K. Koerten, "Visualisation of Normal and Enhanced $HgCl_2$ Transport through Human Skin in vitro," *Int. J. Pharm.*, **53**, 13 (1989b).

Boddé, H. E., I. van den Brink, H. K. Koerten, and F. H. N. de Haan, "Visualization of in vitro Percutaneous Penetration of Mercuric Chloride; Transport through Intercellular Space versus Cellular Uptake through Desmosomes," *J. Controlled Release*, **15**, 227 (1991).

Bommannan, D., H. Okuyama, P. Stauffer, and R. H. Guy, "Sonophoresis: I. The Use of High-Frequency Ultrasound to Enhance Transdermal Drug Delivery," *Pharm. Res.*, **9**, 559 (1992a).

Bommannan, D., H. Okuyama, P. Stauffer, and R. H. Guy, "Sonophoresis: II. Examination of the Mechanism(s) of Ultrasound-Enhanced Transdermal Drug Delivery," *Pharm. Res.*, **9**, 1043 (1992b).

Bouwstra, J. A., M. A. de Vries, G. S. Gorris, W. Bras, J. Brussee, and M. Ponec, "Thermodynamic and Structural Aspects of the Skin Barrier," *J. Controlled Release*, **15**, 209 (1991).

Bundgaard, H., "The Utility of the Prodrug Approach to Improve Peptide Absorption," *J. Controlled Release*, **21**, 63 (1992).

Burnette, R. R., "Iontophoresis," *Transdermal Drug Delivery — Development Issues and Research Initiatives*, J. Hadgraft and R. H. Guy, eds., Marcel Dekker, New York (1989).

Burnette, R. R., and D. Marrero, "Comparison Between the Iontophoretic and Passive Transport of Thyrotropin Releasing Hormone across Excised Nude Mouse Skin," *J. Pharm. Sci.*, **75**, 738 (1986).

Burnette, R. R., and B. Ongpipattanakul, "Characterization of the Pore Transport Properties and Tissue Alteration of Excised Human Skin during Iontophoresis," *J. Pharm. Sci.*, **77**, 132 (1988).

Burnette, R. R., and B. Ongpipattanakul, "Characterization of the Permselective Properties of Excised Human Skin during Iontophoresis," *J. Pharm. Sci.*, **76**, 765 (1987).

Chakrabarti, R., D. E. Wylie, and S. M. Schuster, "Transfer of Monoclonal Antibodies into Mammalian Cells by Electroporation," *J. Biol. Chem.*, **264**, 15494 (1989).

Chandrasekaran, S. K., P. S. Campbell, and A. S. Michaels, "Effect of Dimethyl Sulfoxide on Drug Permeation Through Human Skin," *AIChE J.*, **23**, 810 (1977).

Chandrasekaran, S. K., W. Bayne, and J. E. Shaw, "Pharmacokinetics of Drug Permeation Through Human Skin," *J. Pharm. Sci.*, **67**, 1370 (1978).

Chandrasekaran, S. K., P. S. Campbell, and T. Watanabe, "Application of the 'Dual Sorption' Model to Drug Transport Through Skin," *Poly. Eng. Sci.*, **20**, 36 (1980).

Chang, D. C., J. A. Saunders, B. M. Chassy, and A. E. Sowers, "Overview of Electroporation and Electrofusion," *Guide to Electroporation and Electrofusion*, D. C. Chang, J. A. Saunders, B. M. Chassy, and A. E. Sowers, eds., Academic Press, New York (1992).

Chang, D. C., "Structure and Dynamics of Electric Field-Induced Membrane Pores as Revealed by Rapid-Freezing Electron Microscopy," *Guide to Electroporation and Electrofusion*, D. C. Chang, J. A. Saunders, B. M. Chassy, and A. E. Sowers, eds., Academic Press, New York (1992).

Charman, W. N., and V. J. Stella, *Lymphatic Transport of Drugs*, CRC Press, London (1992).

Chernomordik, L. V., "Electropores in Lipid Lamellaes and Cell Membranes," *Guide to Electroporation and Electrofusion*, D. C. Chang, J. A. Saunders, B. M. Chassy, and A. E. Sowers, eds., Academic Press, New York (1992).

Chien, Y. W., P. Lelawongs, O. Siddiqui, Y. Sun, and W. M. Shi, "Facilitated Transdermal Delivery of Therapeutic Peptides and Proteins by Iontophoretic Delivery Devices," *J. Controlled Release*, **13**, 263 (1990).

Chien, Y. W., O. Siddiqui, W.-M. Shi, P. Lelawongs, and J.-C. Liu, "Direct Current Iontophoretic Transdermal Delivery of Peptide and Protein Drugs," *J. Pharm. Sci.*, **78**, 376 (1989).

Choi, H. K., G. L. Flynn, and G. L. Amidon, "Transdermal Delivery of Bioactive Peptides: The Effect of n-Decyl Methyl Sulfoxide, pH, and Inhibitors on Enkaphalin Metabolism and Transport," *Pharm. Res.*, **7**, 1099 (1990).

Coapman, S. D., J. L. Lichtin, A. Sakr, and J. R. Schiltz, "Studies of the Penetration of Native Collagen, Collagen Alpha Chains, and Collagen Cyanogen Bromide Peptides Through Hairless Mouse Skin *in vitro*," *J. Soc. Cosmet. Chem.*, **39**, 275 (1988).

Cohen, M. H., and D. Turnbull, "Molecular Transport in Liquids and Gases," *J. Chem. Phys.*, **31**, 111 (1959).

Cooper, E. R., "Increased Skin Permeability for Lipophilic Molecules," *J. Pharm. Sci.*, **73**, 1153 (1984).

Cooper, E. R., and B. Berner, "Penetration Enhancers," *Transdermal Delivery of Drugs*, Vol. II, A. F. Kydonieus and B. Berner, eds., CRC Press, Boca Raton, FL (1987).

Crank, J., and G. S. Park, *Diffusion in Polymers*, 2nd ed., Clarendon, Oxford (1989).

Cranne-van Hinsberg, W. H. M., H. E. Boddé, C. J. Verhoef, L. J. Bax, and H. E. Junginger, "Evaluation of a Pulsed Current and High-Frequency Voltage Sampling System Designed to Study Transdermal Peptide Iontophoresis and Skin Impedance," *Proc. Int. Symp. Contr. Rel. Bioact. Mater.*, **18**, 107 (1991).

Cullander, C., and R. H. Guy, "Site of Iontophoretic Current Flow into the Skin: Identification and Characterization with the Vibrating Probe Electrode," *J. Invest. Dermatol.*, **97**, 55 (1991).

Cullander, C., "What Are the Pathways of Iontophoretic Current Flow Through Mammalian Skin?," *Adv. Drug Del. Rev.*, **9**, 119 (1992).

Dalvi, U. G., and J. L. Zatz, "Effect of Skin Binding on Percutaneous Transport of Benzocaine from Aqueous Suspensions and Solutions," *J. Pharm. Sci.*, **71**, 824 (1982).

Dawson, B. V., M. E. Hadley, N. Levine, K. L. Kreutzfeld, S. Don, T. Eytan, and V. J. Hruby, "*In vitro* Transdermal Delivery of a Melanotropic Peptide through Human Skin," *J. Invest. Dermatol.*, **94**, 432 (1990).

Deen, W. M., "Hindered Transport of Large Molecules in Liquid-Filled Pores," *AIChE J.*, **33**, 1409 (1987).

Delgado-Charro, M. B., and R. H. Guy, "Characterization of Convective Solvent Flow during Iontophoresis," *Pharm. Res.*, **11**, 929 (1994).

Del Terzo, S., C. R. Behl, R. A. Nash, N. H. Bellantone, and A. W. Malick, "Evaluation of the Nude Rat as a Model: Effects of Short-Term Freezing and Alkyl Chain Length on the Permeabilities of *n*-Alkanols and Water," *J. Soc. Cosmet. Chem.*, **37**, 297 (1986).

De Young, H. G., "Controlled-Release Delivery Advances Bode Well for Biopharmaceutical Manufacturers," *Genetic Eng. News*, **21**, 56 (1993).

Elias, J. J., "The Microscopic Structure of the Epidermis and Its Derivatives," *Percutaneous Absorption*, R. L. Bronaugh and H. Maibach, eds., Marcel Dekker, New York (1989).

Elias, P. M., "Epidermal Lipids, Membranes, and Keratinization," *Int. J. Dermatol.*, **20**, 1 (1981).

Elias, P. M., and B. E. Brown, "The Mammalian Cutaneous Permeability Barrier: Defective Function in Essential Fatty Acid Deficiency Correlates with Abnormal Intercellular Lipid Deposition," *Lab. Invest.*, **39**, 574 (1978).

Elias, P. M., E. R. Cooper, A. Korc, and B. E. Brown, "Percutaneous Transport in Relation to Stratum Corneum Structure and Lipid Composition," *J. Invest. Dermatol.*, **75**, 297 (1981).

Elias, P. M., and D. S. Friend, "The Permeability Barrier in Mammalian Epidermis," *J. Cell. Biol.*, **65**, 180 (1975).

Emberry, G., and P. Dugard, "The Isolation of Dimethyl Sulfoxide Soluble Components from Human Epidermal Preparations: A Possible Mechanism of Action of Dimethyl Sulfoxide in Effecting Percutaneous Migration Phenomena," *J. Invest. Dermatol.*, **57**, 308 (1971).

Flynn, G. L., "Mechanism of Percutaneous Absorption from Physicochemical Evidence," *Percutaneous Absorption*, R. L. Bronaugh and H. Maibach, eds., Marcel Dekker, New York (1989).

Flynn, G. L., E. E. Linn, T. Kurihara-Bergstrom, S. K. Govil, and S. Y. E. Hou, "Parameters of Skin Condition and Function," *Transdermal Delivery of Drugs*, Vol. II, A. F. Kydonieus and B. Berner, eds., CRC Press, Boca Raton, FL (1987).

Francoeur, M. L., and R. O. Potts, "Transdermal Flux Enhancers in Combination with Iontophoresis in Topical Administration of Pharmaceuticals," EP 435436 A2 (1991).

Gangarosa, L. P., N. Park, A. A. Wiggins, and J. M. Hill, "Increased Penetration of Nonelectrolytes into Mouse Skin During Iontophoretic Water Transport," *J. Pharmacol. Exp. Ther.*, **212**, 377 (1980).

Golden, G., J. E. McKie, and R. O. Potts, "Role of Stratum Corneum Lipid Fluidity in Transdermal Drug Flux," *J. Pharm. Sci.*, **76**, 25 (1987).

Goodman, M., and B. W. Barry, "Action of Penetration Enhancers on Human Stratum Corneum as Assessed by Differential Scanning Calorimetry," *Percutaneous Absorption*, R. L. Bronaugh and H. I. Maibach, eds., Marcel Dekker, New York (1989).

Goodman, M., and B. W. Barry, "Differential Scanning Calorimetry (DSC) of Human Stratum Corneum: Effect of Azone," *J. Pharm. Pharmacol.*, **37**, 80P (1985).

Grayson, S., and P. M. Elias, "Isolation and Lipid Biochemical Characterization of Stratum Corneum Membrane Complexes: Implications for the Cutaneous Permeability Barrier," *J. Invest. Dermatol.*, **78**, 128 (1982).

Green, P. G., R. S. Hinz, A. Kim, F. C. Szoka, and R. H. Guy, "Iontophoretic Delivery of a Series of Tripeptides Across the Skin *in vitro*," *Pharm. Res.*, **8**, 1121 (1991).

Green, P. G., B. Shroot, F. Bernerd, W. R. Pilgrim, and R. H. Guy, "*In vitro* and *in vivo* Iontophoresis of a Tripeptide Across Nude Rate Skin," *J. Controlled Release*, **20**, 209 (1992a).

Green, P. G., R. S. Hinz, A. Kim, C. Cullander, F. C. Szoka, and R. H. Guy, "Transdermal Iontophoresis of Amino Acids and Peptides in vitro," *J. Controlled Release*, **21**, 187 (1992b).

Grimnes, S., "Pathways of Ionic Flow Through Human Skin *in vivo*," *Acta. Derm. Venereol. (Stockholm)*, **64**, 93 (1984).

Guy, R. H., and J. Hadgraft, "The Prediction of Plasma Levels of Drugs Following Transdermal Application," *J. Controlled Release*, **1**, 177 (1985).

Guy, R. H., and J. Hadgraft, "Drug Parameters Important for Transdermal Delivery," *Transdermal Delivery of Drugs*, Vol. III, A. F. Kydonieus and B. Berner, eds., CRC Press, Boca Raton, FL (1987c).

Hadgraft, J., "Penetration Enhancers in Percutaneous Absorption," *Pharm. Int.*, **5**, 252 (1984).

Hashimoto, K., N. Tatsumi, and K. Okuda, "Introduction of Phalloidin Labelled with Fluorescein Isothiocyanate into Living Polymorphonuclear Leukocytes by Electroporation," *J. Biochem. Biophys. Methods*, **19**, 143 (1989).

Heit, M. C., P. L. Williams, F. L. Layes, S. K. Chang, and J. E. Riviere, "Transdermal Iontophoretic Delivery: *in vitro* and *in vivo* Studies with Lutenizing Hormone Releasing Hormone," *J. Pharm. Sci.*, **82**, 240 (1993).

Higuchi, W. I., and C. Yu, "Prodrugs in Transdermal Delivery," *Transdermal Delivery of Drugs*, Vol. III, A. F. Kydonieus and B. Berner, eds., CRC Press, Boca Raton, FL (1987).

Hirvonen, J., R. Sutinen, P. Paronen, and A. Urtti, "Transdermal Penetration Enhancers in Rabbit Pinna Skin: Duration of Action, Skin Irritation and *in vivo/in vitro* Comparisons," *Int. J. Pharm.*, **99**, 253 (1993a).

Hirvonen, J., K. Konturri, L. Murtomaki, P. Paronen, and A. Urtti, "Transdermal Iontophoresis of Sotalol and Salicylate; The Effect of Skin Charge and Penetration Enhancers," *J. Controlled Release*, **26**, 109 (1993b).

Hoogstraate, A. J., J. Verhoef, J. Brussee, A. P. Ijzerman, F. Spies, and H. E. Boddé, "Kinetics, Ultrastructural Aspects and Molecular Modelling of Transdermal Peptide Flux Enhancement by *N*-Alkylazacycloheptanones," *Int. J. Pharm.*, **76**, 37 (1991).

Hoogstraate, A. J., V. Srinivasan, S. M. Sims, and W. I. Higuchi, "Iontophoretic Enhancement of Peptides: Behaviour of Leuprolide versus Model Permeants," *J. Controlled Release*, **31**, 41 (1994).

Howes, D., "The Percutaneous Absorption of Some Anionic Surfactants," *J. Soc. Cosmet. Chem.*, **26**, 29 (1975).

Illel, B., and H. Schaeffer, "Transfollicular Percutaneous Absorption —Skin Model for Quantitative Studies," *Acta Dermatol. Venereol.*, **68**, 426 (1988).

Illel, B., H. Schaeffer, J. Wepierre, and O. Doucet, "Follicles Play an Important Role in Percutaneous Absorption," *J. Pharm. Sci.*, **80**, 424 (1991).

Jadoul, A., J. Doucet, D. Durand, and V. Preat, "Modifications Induced on Stratum Corneum Structure After Iontophoresis: ATR-FTIR and X-Ray Diffraction Studies," *Proc. Int. Symp. Contr. Rel. Bioact. Mater.*, **21**, 367 (1994).

Kari, B., "Control of Glucose Levels in Alloxan-Diabetic Rabbits by Iontophoresis of Insulin," *Diabetes*, **35**, 217 (1986).

Kastin, A. J., A. Arimura, and A. V. Schally, "Topical Absorption of Polypeptides with Dimethylsulfoxide," *Arch. Derm.*, **93**, 471 (1966).

Kasting, G. B., R. L. Smith, and E. R. Cooper, "Effect of Lipid Solubility and Molecular Size on Percutaneous Absorption," *Pharmacology and the Skin, Skin Pharmacokinetics*, Vol. I, B. Shroot and H. Schaefer, eds., Karger, Basel (1987).

Keister, J. C., and G. B. Kasting, "The Use of Transient Diffusion to Investigate Transport Pathways Through the Skin," *J. Controlled Release*, **4**, 111 (1986).

Kligman, A. M., "Topical Pharmacology and Toxicology of Dimethyl Sulfoxide: I," *JAMA*, **193**, 140 (1965).

Kost, J., "Ultrasound Induced Delivery of Peptides," *J. Controlled Release*, **24**, 247 (1993).

Kubota, K., "A Compartment Model for Percutaneous Drug Absorption," *J. Pharm. Sci.*, **80**, 502 (1991).

Kumar, S., H. Char, S. Patel, D. Piemontese, K. Iqbal, A. W. Malick, E. Neugroschel, and C. R. Behl, "Effect of Ionotophoresis on *in vitro* Skin Permeation of an Analogue of Growth Hormone Releasing Factor in the Hairless Pig Model," *J. Pharm. Sci.*, **81**, 635 (1992).

Kurihara-Bergstrom, T., G. L. Flynn, and W. I. Higuchi, "Physicochemical Study of Percutaneous Absorption by Dimethyl Sulfoxide: Kinetic and Thermodynamic Determinants of Dimethyl Sulfoxide Mediated Mass Transfer of Alkanols," *J. Pharm. Sci.*, **75**, 479 (1986).

Lampe, M. E., A. L. Burlingame, J. Whitney, M. L. Williams, B. E. Brown, E. Roitman, and P. M. Elias, "Human Stratum Corneum Lipids: Characterization and Regional Variations," *J. Lipid. Res.*, **24**, 120 (1983).

Langer, R., "Bioavailability of Macromolecular Drugs and Its Control," *Controlled Drug Bioavailability*, **3**, 307 (1985).

Lashmar, U. T., J. Hadgraft, and N. Thomas, "Topical Administration of Penetration Enhancers to the Skin of Nude Mice: A Histological Study," *J. Pharm. Pharmacol.*, **41**, 118 (1989).

Lee, V. H. L., "Peptide and Protein Drug Delivery Systems," *Biopharm. Manuf.*, **1**, 24 (1988).

Lelawongs, P., J.-C. Liu, O. Siddiqui, and Y. W. Chien, "Transdermal Iontophoretic Delivery of Arginine-Vasopressin (I): Physicochemical Considerations," *Int. J. Pharm.*, **56**, 13 (1989).

Levy, D., J. Kost, Y. Meshulman, and R. Langer, "Effect of Ultrasound on Transdermal Drug Delivery to Rats and Guinea Pigs," *J. Clin. Invest.*, **83**, 2074 (1989).

Mackay, M., "Delivery of Recombinant Peptide and Protein Drugs," *Biotechnol. Genet. Eng. Rev.*, **8**, 251 (1990).

Martindale The Extra Pharmacopoeia, 30th ed., J. E. F. Reynolds, ed., The Pharmaceutic Press, London (1993).

Mazer, N. A., "Pharmacokinetic and Pharmacodynamic Aspects of Polypeptide Delivery," *J. Controlled Release*, **11**, 343 (1990).

Meyer, B. R., W. Kreis, J. Eschbach, V. O'Mara, S. Rosen, and D. Sibalis, "Successful Transdermal Administration of Therapeutic Doses of a Polypeptide to Normal Human Volunteers," *Clin. Pharmacol. Ther.*, **44**, 607 (1988).

Meyer, B. R., W. Kreis, J. Eschbach, V. O'Mara, S. Rosen, and D. Sibalis, "Transdermal versus Subcutaneous Leuprolide: A Comparison of Acute Pharmacodynamic Effect," *Clin. Pharmacol. Ther.*, **48**, 340 (1990).

Michaels, A. S., S. K. Chandrasekaran, and J. E. Shaw, "Drug Permeation though Human Skin: Theory and *in vitro* Experimental Measurement," *AIChE J.*, **21**, 985 (1975).

Miller, L. L., C. J. Kolaskie, G. A. Smith, and J. Riviere, "Transdermal Iontophoresis of Gonadotropin Releasing Hormone (LHRH) and Two Analogues," *J. Pharm. Sci.*, **79**, 490 (1990).

Miyazaki, S., H. Mizuoka, M. Oda, and M. Takada, "External Control of Drug Release and Penetration: Enhancement of the Transdermal Absorption of Indomethacin by Ultrasound Irradiation," *J. Pharm. Pharmacol.*, **43**, 204 (1991).

Monteiro-Riviere, N. A., A. O. Inman, and J. E. Riviere, "Identification of the Pathway of Iontophoretic Delivery: Light and Ultrastructural Studies Using Mercuric Chloride in Pigs," *Pharm. Res.*, **11**, 251 (1994).

Móss, J., and H. Bundgaard, "Prodrugs of Peptides. 7. Transdermal Delivery of Thyrotropin Releasing Hormone (TRH) via Prodrugs," *Int. J. Pharm.*, **66**, 39 (1990).

Munk, P., *Introduction to Macromolecular Science*, Wiley, Toronto (1989).

Noonan, P. K., and R. C. Wester, "Cutaneous Metabolism of Xenobiotics," *Percutaneous Absorption*, Marcel Dekker, New York (1989).

Novak, E., and S. F. Francom, "Inflammatory Response to Sodium Lauryl Sulfate in Aqueous Solutions Applied to the Skin of Normal Human Volunteers," *Contact Dermatitis*, **10**, 101 (1984).

Okabe, K., H. Yamaguchi, and Y. Kawai, "New Iontophoretic Transdermal Administration of the Beta-Blocker Metropropol," *J. Controlled Release*, **4**, 79 (1986).
Okumara, M., K. Sugibayashi, K. Ogawa, and Y. Morimoto, "Skin Permeability of Water-Soluble Drugs," *Chem. Pharm. Bull.*, **37**, 1404 (1989).
Pannatier, P. J., B. Testa, and J. C. Etter, "The Skin as a Drug Metabolising Organ," *Drug Metab. Rev.*, **8**, 319 (1978).
Parasrampuria, D., and J. Parasrampuria, "Percutaneous Delivery of Proteins and Peptides Using Iontophoretic Techniques," *J. Clin. Pharm. Ther.*, **16**, 7 (1991).
Parry, G. E., A. L. Bunge, G. D. Silcox, L. K. Pershing, and D. W. Pershing, "Percutaneous Absorption of Benzoic Acid Across Human Skin: I. *In vitro* Experiments and Mathematical Modelling," *Pharm. Res.*, **7**, 230 (1990).
Pikal, M. J., "Transport Mechanisms in Iontophoresis: I. A Theoretical Model for the Effect of Electroosmotic Flow in Flux Enhancement in Transdermal Iontophoresis," *Pharm. Res.*, **7**, 118 (1990).
Pikal, M. J., "The Role of Electroosmotic Flow in Transdermal Iontophoresis," *Adv. Drug Delivery Rev.*, **9**, 201 (1992).
Pikal, M. J., and S. Shah, "Transport Mechanisms in Iontophoresis: II. Electroosmotic Flow and Transfer Number Measurements for Hairless Mouse Skin," *Pharm. Res.*, **7**, 213 (1990a).
Pikal, M. J., and S. Shah, "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 (1990b).
Potts, R. O., and R. H. Guy, "Predicting Skin Permeability," *Pharm. Res.*, **9**, 663 (1992).
Potts, R. O., R. H. Guy, and M. Francoeur, "Routes of Ionic Permeability Through Mammalian Skin," *Solid State Ionics*, **53–56**, 165 (1992).
Powell, K. T., A. W. Morganthaler, and J. C. Weaver, "Tissue Electroporation: Observation of Reversible Electrical Breakdown in Viable Frog Skin," *Biophys. J.*, **56**, 1163 (1989).
Prausnitz, M. R., V. G. Bose, R. Langer, and J. C. Weaver, "Transdermal Drug Delivery by Electroporation," *Proc. Int. Symp. Contr. Rel. Bioact. Mat.*, **19**, 232 (1992).
Prausnitz, M. R., V. G. Bose, R. Langer, and J. C. Weaver, "Electroporation of Mammalian Skin: A Mechanism to Enhance Transdermal Drug Delivery," *Proc. Nat. Acad. Sci. USA*, **90**, 10504 (1993).
Ranade, V. V., "Drug Delivery Systems: 6. Transdermal Drug Delivery," *J. Clin. Pharmacol.*, **31**, 401 (1991).
Rhein, L. D., C. R. Robbins, K. Fernsee, and R. Cantore, "Surfactant Structure Effects on Swelling of Isolated Human Stratum Corneum," *J. Soc. Cosmet. Chem.*, **37**, 125 (1986).
Ruland, A., and J. Kreuter, "Transdermal Permeability and Skin Accumulation of Amino Acids," *Int. J. Pharm.*, **72**, 149 (1991).
Saltzman, M. W., "Antibodies for Treating and Preventing Disease: The Potential Role of Polymeric Controlled Release," *Clin. Rev. Ther. Drug Carrier Sys.*, **10**, 111 (1993).
Sanderson, J. E., S. D. Riel, and R. Dixon, "Iontophoretic Delivery of Nonpeptide Drugs: Formulation Optimization for Maximum Skin Permeability," *J. Pharm. Sci.*, **78**, 361 (1989).
Sarpotdar, P. P., and C. R. Daniels, "Use of Polymeric Buffers to Facilitate Iontophoretic Transport of Drugs," *Pharm. Res.*, **7**, S185 (1990).
Scheuplein, R. J., "Mechanism of Percutaneous Absorption: I. Routes of Penetration and the Influence of Solubility," *J. Invest. Dermatol.*, **45**, 334 (1965).
Scheuplein, R. J., "Mechanism of Percutaneous Absorption: II. Transient Diffusion and the Relative Importance of Various Routes of Skin Penetration," *J. Invest. Dermatol.*, **48**, 79 (1967).
Scheuplein, R. J., *The Physiology and Pathophysiology of the Skin*, Vol. 5, A. Jarret, ed., Academic Press, London (1978).
Scheuplein, R. J., and I. H. Blank, "Permeability of the Skin," *Physiol. Rev.*, **51**, 702 (1971).
Scheuplein, R. J., and P. H. Dugard, "Effects of Ionic Surfactants on the Permeability of the Human Epidermis: An Electrometric Study," *J. Invest. Dermatol.*, **60**, 263 (1973).
Scheuplein, R. J., and L. Ross, "Effects of Surfactants and Solvents on the Permeability of Epidermis," *J. Soc. Cosmet. Chem.*, **21**, 853 (1970).
Scott, E. R., A. I. Laplaza, H. S. White, and J. B. Phipps, "Transport of Ionic Species in Skin: Contribution of Pores to the Overall Skin Conductance," *Pharm. Res.*, **10**, 1699 (1993).
Shapiro, B. L., T. V. Spence, and W. J. Warwick, "Insulin Iontophoresis in Cystic Fibrosis," *Proc. Soc. Exp. Biol. Med.*, **149**, 592 (1975).
Sharata, B., and R. R. Burnette, "Percutaneous Absorption of Electron-Dense Ions Across Normal and Chemically Perturbed Skin," *J. Pharm. Sci.*, **77**, 27 (1989).
Sheretz, E. F., K. B. Sloan, and R. G. McTiernan, "Use of Theoretical Partition Coefficients Determined from Solubility Parameters to Predict Permeability Coefficients for 5-Fluorouracil," *J. Invest. Dermatol.*, **89**, 147 (1987).
Siddiqui, O., Y. Sun, J.-C. Liu, and Y. W. Chien, "Facilitated Transdermal Transport of Insulin," *J. Pharm. Sci.*, **76**, 341 (1987).
Sims, S. M., and W. I. Higuchi, "Baseline Studies on Iontophoretic Transport in Hairless Mouse Skin: The Effect of Applied Voltage Drop and pH on the Iontophoresis of a Model Weak Electrolyte," *J. Mem. Sci.*, **49**, 305 (1990).
Skauen, D. M., and G. M. Zentner, "Phonophoresis," *Int. J. Pharm.*, **2**, 235 (1984).
Sloan, K. B., "Prodrugs for Dermal Delivery," *Adv. Drug Del. Rev.*, **3**, 67 (1989).
Sowers, A. E., "Mechanisms of Electroporation and Electrofusion," *Guide to Electroporation and Electrofusion*, D. C. Chang, J. A. Saunders, B. M. Chassy, and A. E. Sowers, eds., Academic Press, New York (1992).
Srinivasan, V., W. I. Higuchi, S. M. Sims, A. H. Ghanem, and C. R. Behl, "Transdermal Iontophoretic Drug Delivery: Mechanistic Analysis and Application to Polypeptide Delivery," *J. Pharm. Sci.*, **78**, 370 (1989).
Srinivasan, V., M.-H., Su, W. I. Higuchi, and C. R. Behl, "Iontophoresis of Polypeptides: Effect of Ethanol Pretreatment of Human Skin," *J. Pharm. Sci.*, **79**, 588 (1990).
Stephen, R. L., T. J. Petelenz, and S. C. Jacobsen, "Potential Novel Methods for Insulin Administration: I. Iontophoresis," *Biomed. Biochim. Acta*, **43**, 553 (1984).
Stoughton, R. B., "Enhanced Percutaneous Penetration with 1-Dodecyl-azacycloheptan-2-one," *Arch. Dermatol.*, **118**, 474 (1982).
Stoughton, R. B., and W. O. McClure, "Azone: A New Non-Toxic Enhancer of Cutaneous Penetration," *Drug Dev. Ind. Pharm.*, **9**, 725 (1983).
Tachibana, K., "Transdermal Delivery of Insulin to Alloxan-Diabetic Rabbits by Ultrasound Exposure," *Pharm. Res.*, **9**, 952 (1992).
Tachibana, K., and S. Tachibana, "Transdermal Delivery of Insulin by Ultrasonic Vibration," *J. Pharm. Pharmacol.*, **43**, 270 (1991).
Tamada, J., J. Sharifi, D. Bommi Bommannan, L. Leung, N. Azimi, W. Abraham, and R. Potts, "Effect of Electroporation of the Iontophoretic Delivery of Peptides in vitro," *Proc. Nat. Acad. Sci. USA*, **90**, 10504 (1993).
Tanford, C., *Physical Chemistry of Macromolecules*, Wiley, New York (1961).
Tauber, U., "Drug Metabolism in the Skin: Advantages and Disadvantages," *Transdermal Drug Delivery*, Marcel Dekker, New York (1987).
Thysman, S., C. Hanchard, and V. Preat, "Human Calcitonin in Rats by Iontophoresis," *J. Pharm. Pharmacol.*, **46**, 725 (1994).
Titomirov, A. V., S. Sukharev, and E. Kistanova, "*In vivo* Electroporation and Stable Transformation of Skin Cells of Newborn Mice by Plasmid DNA," *Bioch. Biophys. Acta*, **1088**, 131 (1991).
Tojo, K., "Mathematical Model of Iontophoretic Transdermal Drug Delivery," *J. Chem. Eng. Japan*, **22**, 512 (1989).
Tojo, K., "Random Brick Model for Drug Transport across Stratum Corneum," *J. Pharm. Sci.*, **76**, 889 (1987).
Turner, N., L. Ferry, C. Cullander, and R. H. Guy, "Iontophoresis of Poly-L-lysines: Transport as a Function of Molecular Weight Measured by Confocal Microscopy," *Proc. Int. Symp. Contr. Rel. Bioact. Mater.*, **21**, 393 (1994).
Tyle, P., and P. Agrawal, "Drug Delivery by Phonophoresis," *Pharm. Res.*, **6**, 355 (1989).
Vaidyanathan, R., V. Rajadhyaksha, B. Kim, and J. J. Anisko, "Azone," *Transdermal Delivery of Drugs*, Vol. II, A. F. Kydonieus and B. Berner, eds., CRC Press, Boca Raton, FL (1987).
Valia, K. H., and Y. W. Chien, "Effect of Stratum Corneum Hydration. How Serious Is It?" *Drug Dev. Ind. Pharm.*, **12**, 265 (1986).

Van Duzee, B. F., "Thermal Analysis of Human Stratum Corneum," *J. Invest. Dermatol.*, **65**, 404 (1975).
Wallace, B. M., and J. S. Lasker, "Stand and Deliver: Getting Peptide Drugs into the Body," *Science*, **260**, 912 (1993).
Walters, K. A., "Penetration Enhancers and Their Use in Transdermal Therapeutic Systems," *Transdermal Drug Delivery*, J. Hadgraft and R. H. Guy, eds., Marcel Dekker, New York (1987).
Wearley, L., "Recent Progress in Protein and Peptide Delivery by Noninvasive Routes," *Crit. Rev. Ther. Drug Carrier Sys.*, **8**, 331 (1991).
Wearley, L., L.-C. Chiu, and Y. W. Chien, "Iontophoresis-Facilitated Transdermal Delivery of Verapamil. I. In vitro Evaluation and Mechanistic Studies," *J. Controlled Release*, **8**, 237 (1989).
Wearley, L., K. Tojo, and Y. W. Chien, "A Numerical Approach to Study the Effect of Binding on the Iontophoretic Transport of a Series of Amino Acids," *J. Pharm. Sci.*, **79**, 992 (1990).
Weaver, J. C., and A. Barnett, "Progress Toward a Theoretical Model for Electroporation Mechanism: Membrane Electrical Behavior and Molecular Transport," *Guide to Electroporation and Electrofusion*, D. C. Chang, J. A. Saunders, B. M. Chassy, and A. E. Sowers, eds., Academic Press, New York (1992).
Weiner, N. D., M. Y. Lu, and M. Rosoff, "Interactions of Dimethyl Sulfoxide with Lipid and Protein Monolayers," *J. Pharm. Sci.*, **61**, 1098 (1972).
Wertz, P. W., and D. T. Downing, "Stratum Corneum: Biological and Biochemical Considerations," *Transdermal Drug Delivery*, J. Hadgraft and R. H. Guy, eds., Marcel Dekker, New York (1987).
Wester, R. C., J. M. Melendres, L. Sedik, and H. I. Maibach, "Percutaneous Absorption of Azone Following Single and Multiple Doses to Human Volunteers," *J. Pharm. Sci.*, **83**, 124 (1994).
Whitney, G. K., A. J. Daugulis, and B. N. White, "Transformation of *Bacillus Brevis* 47 by Electroporation," *Biotech. Tech.*, **7**, 47 (1993).
Williams, P. L., and J. E. Riviere, "Model Describing Transdermal Iontophoretic Delivery of Lidocaine Incorporating Consideration of Microvascular State," *J. Pharm. Sci.*, **82**, 1080 (1993).
Wood, D. C. F., and F. R. Bettley, "The Effect of Various Detergents on Human Epidermis," *Br. J. Derm.*, **84**, 320 (1971).
Wotton, P. K., B. Møllgaard, J. Hadgraft, and A. Hoelgaard, "Vehicle Effect on Topical Drug Delivery: III. Effect of Azone on the Cutaneous Permeation of Metronidazole and Propylene Glycol," *Int. J. Pharm.*, **24**, 19 (1985).
Yoshida, N. H., and M. S. Roberts, "Structure-Transport Relationships in Transdermal Iontophoresis," *Adv. Drug Delivery Rev.*, **9**, 239 (1992).
Yoshida, N. H., and M. S. Roberts, "Solute Molecular Size and Transdermal Iontophoresis across Excised Human Skin," *J. Controlled Release*, **25**, 177 (1993).

Manuscript received Dec. 6, 1993, and revision received Oct. 19, 1994.